# Geographic variation in female mate-copying in the species complex of a unisexual fish, *Poecilia formosa*

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(Accepted: 19 February 2008)

## Summary

The Amazon molly, *Poecilia formosa*, is a gynogenetic, all-female fish. Its mating system relies on heterospecific matings with males of closely related sexual species. In mixed populations, males mate with conspecific sexual females and heterospecific asexual females. Such matings are not isolated dyadic interactions but rather elements of a communication network, because multiple females can observe these interactions. This is the only known case of heterospecific mate-copying and, thus, a system in which the potential for mate-copying could be influenced by the presence of another species. Here we show that mate-copying is exhibited by the sexual species *P. mexicana* and *P. latipinna*, and the asexual *P. formosa*. Both sexual and asexual females copy each other's mate choice decisions in sympatry, but females from allopatric populations do not show heterospecific mate-copying. Males benefit from heterospecific matings with Amazon mollies because these increase their attractiveness to the conspecific sexual females. In mixed shoals, mate-copying potentially imposes costs as it increases a male's attractiveness to heterospecific females. We argue that the net-effect of mate-copying is beneficial to males because the relative strength of mate-copying is lower in Amazon mollies. We hypothesize that an added benefit to males lies in the signal value of copulations.

*Keywords*: sexual-asexual coexistence, association preferences, visual communication, video playback, evolution of mate-copying, female choice, male mating behaviour.

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*Behaviour 145, 1041-1064*
Also available online - www.brill.nl/beh

## Introduction

Mate choice is often influenced by the social environment. Mate-copying is one example of socially influenced mate choice in which individuals copy the mate choice of others (Losey et al., 1986; Brooks, 1998; Westneat et al., 2000; Witte, 2006). Studies on mate-copying place mate choice in a social context rather than viewing it as isolated events (Emlen & Oring, 1977; Gibson & Höglund, 1992; Pruett-Jones, 1992; Andersson, 1994; Höglund & Alatalo, 1995; Dugatkin, 1996; Galef & White, 2000; Ophir & Galef, 2004). Mate-copying is especially prevalent in fishes, where females copy conspecific females (Dugatkin & Godin, 1992; Briggs et al., 1996; Witte & Ryan, 1998, 2002), males copy males (Schlupp & Ryan, 1997; Widemo, 2006), and sneaker-males copy females (Gonçalves et al., 2003).

Although mate-copying typically occurs among conspecifics, there is a system in which it mediates heterospecific matings in the complex of unisexual mollies (*Poecilia formosa*) and their sexual counterparts. In nature, the gynogenetic *P. formosa* must obtain sperm from one of their close relatives, either *P. latipinna* or *P. mexicana*. Schlupp et al. (1994) showed that heterospecific mate-copying may contribute to the maintenance of this system because male *P. latipinna* increase their attractiveness to conspecific females by mating with the gynogenetic female *P. formosa*. Male behaviour in general seems to play an important role in this mating system (Schlupp, 2005; Riesch et al., 2008, Kokko et al., 2008).

Amazon mollies, *P. formosa*, are all-female gynogenetic fish of the live-bearing family Poeciliidae (Hubbs & Hubbs, 1932). Gynogenesis is a special form of parthenogenesis in which sperm of a host species serve as a physiological stimulus to trigger embryogenesis. The sperm normally is not incorporated into the genome of the offspring (Schlupp et al., 1998; Schlupp, 2005). In the case of *P. formosa*, sperm is usually provided by males of one of two species, *P. mexicana* or *P. latipinna* (Hubbs & Hubbs, 1932; Schlupp et al., 1998). The Amazon molly is probably derived from a single hybridisation event of a *P. mexicana* female and a *P. latipinna*-like male (Turner, 1982; Avise et al., 1991; Schartl et al., 1995). Amazon mollies range from southeast Texas to northeast Mexico. *P. formosa* is sympatric with *P. latipinna* in Texas and a few areas in northeast Mexico, while it is sympatric with *P. mexicana* in Mexico (Darnell & Abramoff, 1968; Schlupp et al., 2002).

### *Scenarios of mate-copying*

To study mate-copying in this species complex, we took advantage of a natural experiment in Central Texas, USA. In the 1930s *P. latipinna* was introduced from Louisiana (Brown, 1953) into the San Marcos River. Twenty years later *P. formosa* was introduced (Hubbs, 1953). Thus, the duration of sympatry for those populations can be dated exactly. The first documentation of heterospecific mate-copying by Schlupp et al. (1994) occurred not more than four decades after the introduction of Amazon mollies into the drainage. Assuming two to three generations per year (Hubbs, 1964), there is a maximum of 80–100 generations of sympatry and hence opportunity for heterospecific mate-copying to arise in the San Marcos River drainage. Thus, if mate copying is absent in allopatry but present in sympatry this population offers some insight into the time-scale over which this behaviour evolved.

Schlupp et al. (1994) argued that males gain an advantage by mating with Amazon mollies through heterospecific mate-copying. Their study provided the first evidence that it might be adaptive for males to serve as sperm donors for heterospecific females: *P. latipinna* males became more attractive to females when the *P. latipinna* female has had an opportunity to observe the male sexually interacting with *P. formosa*. The full complexity of potential interactions in the natural situation, however, is more complicated. Groups of these fishes in sympatry always comprise males, sexual females and asexual females (Schlupp & Ryan, 1996; Witte & Ryan, 2002). Thus, any male–female interaction can be observed by either of the two species of females, leading to four possible situations with different payoffs to males: (1) a sexual female observing a sexual female, (2) a sexual female observing an asexual female, (3) an asexual female observing a sexual female and (4) an asexual female observing an asexual female. While the first scenario of conspecific mate-copying in sexuals is obviously the most beneficial to males, because they can reap both the immediate benefit of a conspecific mating and the indirect benefit of potential future mating success resulting from other females' tendencies to copy, the second scenario provides only a fraction of the opportunities for sexual reproduction to males. In the last scenario of Amazons copying Amazons, males only increase their attractiveness to other asexuals. In this case, males do not gain any benefit from female mate-copying; indeed, this might be costly to males. In a recent study Hill & Ryan (2006) showed that *P. latipinna* copy stronger, when the model female is another *P. latipinna*, indicating that the quality of the model can be an important modifier for mate copying.

The Amazon molly has two main host species: *P. latipinna* serves as sperm donor in the northern part of its biogeographic range, *P. mexicana* in the southern part. Several studies on mate choice indicate that *P. mexicana* is less discriminating than *P. latipinna* (Balsano et al., 1985; Schlupp et al., 1991; Ryan et al., 1996). Therefore, it is crucial to differentiate between these two different systems within the Amazon molly mating complex (Schlupp et al., 2002), and address the four possible behavioural interactions for the two mating systems. Furthermore, to understand the pattern of heterospecific mate-copying, it is important also to include individuals from populations that are allopatric with *P. formosa* (Table 1). Allopatric populations resemble a situation before the arrival of Amazon mollies (Gabor & Ryan, 2001).

For the present paper, we combined and compared data from several experiments investigating mate-copying, using both live stimuli and video playback. We tested for presence of heterospecific mate-copying behaviour in both sexual host species of *P. formosa* and measured its relative strength in females from an allopatric and a sympatric population.

## Materials and methods

### *Study organisms*

We used fishes from populations in which the sexual species *P. latipinna* and *P. mexicana* were either sympatric or allopatric with the asexual *P. formosa* (Schlupp et al., 2002) (Table 1).

All fish used in experiments 1a, 3a, 4, 5 and 6 originated from randomly outbred laboratory populations (Schlupp et al., 1991; Schlüter et al., 1998) and were maintained under standard aquarium conditions at the University of Hamburg, Germany. The founder fishes of these stocks were collected in 1993, 1995 and 1996 from the San Marcos River, TX, USA, from the Rio Purificacion, Nuevo Leon, Mexico, from water bodies near Tampico, Tamaulipas, Mexico and Arroyo de Solpho, Tapijulapa, Tabasco, Mexico (Table 1). All animals used in these experiments were returned to stock tanks at the University of Hamburg at the end of the study. All fish used in experiments 1b, 2 and 3b were collected in the field from populations in Central Texas, USA (Comal River, San Marcos River) a few weeks prior to

**Table 1.** Methodological details of different mate-copying experiments.

| Experiment | Conspecific/ heterospecific | Setup | Tested population | SL female (mm, mean ± SD) | Sym-/allopatry | Male population | Male size diff. (mm, mean ± SD) | Model population | SL model (mm, mean ± SD) | tank size (l) | mean RI (%) | *N* total | *N* side biased | *N* initial pref. for smaller male | *N* RI < 0.5 | Single test unit (min) | Presentation (min) | Control experiments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | hetero | Video | *lat* SM | 36 ± 2 | sym | *lat* SM | 15 | *for* BT | 55 | 80 | 75 | 23 | 8 | 5 | 2 | 5 | 3 | 1, 2 |
| 1b[1] | hetero | Live | *lat* SM | 52 ± 8 | sym | *lat* SM | 13 ± 4 | *for* SM | 57 ± 4 | 180 | 92 | 70 | 22 | 4 | 3 | 10 | 20 | 1, 2 |
| 2 | hetero | Live | *lat* AS | 31 ± 2 | allo | *lat* AS | 7 ± 3 | *for* SM | 33 ± 4 | 80 | 87 | 57 | 30 | 9 | 0 | 5 | 5 | – |
| 3a | con | Video | *for* BT | 44 ± 4 | sym | *lat* SM | 15 | *for* BT | 55 | 80 | 83 | 34 | 20 | 4 | 0 | 5 | 3 | 1, 2 |
| 3b | con | Live | *for* SM | 47 ± 5 | sym | *lat* CS | 9 ± 4 | *for* SM | 48 ± 5 | 180 | 81 | 80 | 34 | 20 | 0 | 5 | 5 | 1, 2 |
| 4 | hetero | Live | *mex* TAM | 34 ± 5 | sym | *mex* TAM | 4 ± 1 | *for* TAM | 32 ± 2 | 100 | 83 | 38 | 11 | 9 | 3 | 5 | 5 | 1, 2 |
| 5 | hetero | Live | *mex* PS0 | 40 ± 4 | allo | *mex* PS0 | 9 ± 4 | *for* SM | 33 ± 4 | 180 | 92 | 85 | 63 | 3 | 3 | 10 | 20 | 1 |
| 6 | con | Video[2] | *mex* TAM | 39 ± 5 | sym | *mex* TAM | 17 | *mex* TAM | 38 | 200 | 82 | 45 | 8 | 5 | 3 | 5 | 5 | – |

Abbreviations: *lat*, *Poecilia latipinna*; *mex*, *P. mexicana*; *for*, *P. formosa*; SM, San Marcos River near Martindale, TX, USA (Schlupp et al., 1994); AS, Aquarena Springs, Spring head, San Marcos River, San Marcos, TX, USA. Like other authors, we considered this population allopatric with *P. formosa* (Gabor & Ryan, 2001). Recently, very few Amazon mollies have been collected from this site (Schlupp et al., 2002). CS, near spring head Comal River, New Braunfels, TX, USA (Witte & Ryan, 2002). BT, VI/17, Rio Purificacion, Barretal, Nuevo Leon, Mexico (Körner et al., 1999). PS0, Arroyo de Solpho, Tapijulapa, Tabasco, Mexico (Parzefall, 2001). TAM III/2, III/13, III/14, IV/5, near Tampico, Tamaulipas, Mexico (Schlüter et al., 1998; Körner et al., 1999). RI, response index. Control 1, without opportunity for copying in phase two between first and second preference test. Control 2, shoaling control using all females controlling for social effects. Monitors used for video playback: Mitsubishi CT-15 MS 1 ETX (experiments 1a and 3a), Saba M5520C Colour TV and BW 15″ CCTV CEM 15 A (c).

[1] From Schlupp et al. (1994).

[2] Animated slides.

testing (Table 1). They were maintained at the University of Texas at Austin in large stock tanks in the laboratory or in large outdoor breeding tanks at Brackenridge Field Laboratory at University of Texas in Austin. Animals were transferred to large stock tanks at the Brackenridge Field Laboratory at the end of the study.

In the laboratory, all fishes were maintained in 25–600 l tanks at 24–29°C on a 14/12 h artificial illumination cycle. All fishes were fed daily with commercially available flake food and *Artemia* nauplii, *Daphnia*, or *Tubifex* worms as occasional supplementary food. Individuals from sympatric and allopatric populations were kept under the same housing conditions, i.e., typically without contact to the other species prior to testing. Measurements of standard length (the distance from the snout to the base of the caudal peduncle) were taken from all individuals after testing.

### *General experimental procedure*

Each test consisted of three different phases: (1) an initial preference test that determined which male a focal female preferred, (2) a presentation in which the focal female had the opportunity to observe the initially less preferred male interacting with another (model) female and, finally, (3) a second preference test in which the focal female's preference was again measured. Mate copying took place if in this final test the female increased her preference for the previously non-preferred male.

We used a standard preference-testing paradigm: each test tank was divided into three equal zones by lines drawn vertically on the front side as visual markings. The central compartment was defined as a neutral zone, the two side-sections as preference zones. The test female was able to move freely among the three zones. On the right and the left end of the experimental tank, adjacent to the preference zones we visually displayed the respective stimuli. In experiments using video-playback (Oliveira et al., 2000), we placed two video-monitors as close as possible next to the preference zones, so that the choosing females could view the displayed video images. This technique has been successfully used before in this species complex (Körner et al., 1999; Landmann et al., 1999; Gonçalves et al., 2000). In experiments with live stimuli we utilized a test tank with two outer compartments separated by clear Plexiglas dividers from the preference zones (Schlupp et al., 1994). The dividers were fitted tightly to reduce flow of water and chemical cues between the compartments. Water level and temperature in the test tank were stable during the experiments. The bottom of the tank was covered with gravel. The back and the sides of the tank were covered with light blue cardboard or grey Teflon foil (unless we used monitors for playback) as a neutral homogenous background.

Females were separated from males prior to testing for at least one day. Unless stated otherwise, all fishes had prior experience with the opposite sex. Test females and stimulus males never originated from the same tank to exclude individual familiarity. Only adult females with a body size of >28 mm standard length were used in the experiments and most females displayed a gravity spot. Female body sizes are summarised in Table 1. This procedure makes it unlikely that late maturing males were inadvertently tested as females (Parzefall, 1969).

Preference tests of phase one and three were initiated by introducing a test female into a clear cylinder (>12 cm diameter) in the centre of the neutral zone of the experimental tank. Two males, differing at least 10% (23 ± 8.4%) in size, were then introduced into the stimulus compartments. We randomly started with either the larger or smaller fish at the left or the right side of the tank. Absolute mean size differences of male pairs are presented in Table 1. Stimulus males have only been used once in the final data set. Individuals may have been used previously in a terminated test (e.g., when a side-bias occurred). After an acclimatisation period we gently removed the cylinder and immediately started recording the time the female spent in each of the three sections (Table 3). Then the positions of the stimulus males (live or video playback) were swapped and the experimental unit was repeated to detect a potential side bias. Thus, each preference test consisted of two units. We decided a priori that a side bias occurred when a female spent more than 80% of her time on the same side of the tank after swapping stimuli in the preference tests. In such cases females did not perform consistent preferences for stimuli. Those trials were excluded from further analysis.

As another measure of female responsiveness to the stimuli, we calculated a response index as the percentage of the total observation period females spent outside the neutral zone (Schlüter et al., 1998). Response indices compare the extent of reactions to stimuli in different experimental procedures (Table 1). We assumed these side-biased or non-reacting females to be unmotivated to choose. Those individuals have not been used in the analysis.

We also decided a priori not to continue with phase 2 of a mate-copying trial when females did not show an initial preference for the larger male in the first preference test (phase one). Thereby, we checked whether the actual female association preferences are in accordance with the average overall population preference for larger males (see Table 2). By excluding those females that did not show an initial preference for the larger male, we acknowledged the fact that females do not have random mating preferences. It is known from several studies that the population level preference of females is for larger males (Marler & Ryan, 1997; Ptacek & Travis, 1997; Gabor, 1999). Consequently, if a female initially chooses a small male and then modifies her preferences towards the larger male, it is difficult to determine exactly why this change has occurred. Any change towards the generally more likely preference for the larger male in the second test might not be due to copying and would result in a false positive data point. If all females have the same preference for a large male with a normal distribution around it, then sometimes a female will choose a small male by chance. If she does, then next time she is likely to change her preference to the large male, but this is not really a 'change'. It is, therefore, conservative to exclude such trials. In Table 1 we report the number of terminations and exclusions according to our set criteria. Sample sizes for initial size preferences are presented in Table 2, and for mate-copying and control experiments in Table 3.

After the initial preference test, we maintained the focal female in a clear Plexiglas cylinder in the neutral zone. In phase two, we then gave the female the opportunity to observe the smaller, initially less-preferred male consorting with a model female. In experiments with live stimuli we covered half of the tank dividers with an opaque screen and additionally divided the stimulus tanks parallel to the long axis of the tank into two separate chambers by clear Plexiglas panes (Schlupp et al., 1994). We added a model female to the newly created compartments of the stimulus tank. Thus, males and the added model female could interact visually. The model female added to the larger, initially preferred male was not visible to the test female in the central cylinder. On the side with the smaller, initially less preferred stimulus male we removed the opaque divider. During the presentation phase the test female could observe the initially less preferred male interacting with a model female and the initially preferred larger male without visible female company. After the presentation period we removed the model females and additional dividers and repeated the first preference test in phase three. Methodological details and minor differences among protocols of the different experiments are summarised in Table 1.

In this experimental design, the relevant unit of data analysis was a complete preference test (including side swapping). Each preference test consisted of the two test units in order to detect side biases. A separate consistency criterion had not been adopted. Nevertheless, due to our exclusion criteria (>80% side bias, reaction index <50%, initial preference for smaller males) we expect only very few cases in which female preferences for individual males may not be consistent. In most experiments separate control trials without opportunity for copying had been carried out to control for female consistency in preferences for individual males (Table 1).

### *Controls*

To ensure our results were due to mate-copying, we controlled for consistency in female mate preferences. We conducted the same experiment (using live or video stimuli, respectively) without opportunity for copying. In experiments using live stimuli, both model females remained visually isolated by opaque dividers from the test female during the presentation phase. Video tapes in the trials controlling for consistency of female preferences did not show model females.

To control for shoaling effects, we also conducted the same experiment with all-female stimuli (live or video). This procedure ensured that sexual and not social motivation of the test female's behaviour led to the results. A direct comparison of copying scores (the relative difference in time spent with the initially less preferred male after minus before the opportunity for copying) between control and treatment is presented in Table 3.

### *Creation of video stimuli*

Videotapes of stimuli were produced from recordings of the behaviour of one small and one large *P. latipinna* male and a *P. formosa* model female interacting with the smaller male (Table 1). Recordings were made using a Panasonic AG 450 S-VHS video camera from 1 m distance in a 50 × 25 × 10 cm Plexiglas tank to facilitate keeping the fish in focus. Sides and background of the tank were covered with light blue cardboard identical to the tank background in the experiments. We used two 500 W light bulbs (3200 K) as a light source coming mainly from above the tank. We chose video sequences of 0.24–4.15 min as stimuli for preference tests, opportunity for copying and control experiments. No bottom, water surface or tank sides were visible in the video sequences. The recordings were edited and looped to a final duration sufficient to perform the experiments. After 20 trials, a new set of tape copies was made to maintain a stable good quality of the playbacks. The method of videotaping, editing, and playback was as described in Landmann et al. (1999) and Körner et al. (1999).

Conclusions drawn from video-playback experiments using only one stimulus pair can be problematic. Therefore, we chose to use both approaches (video and live) in experiments 1a, 1b, 3a and 3b. In both experiments, trials using live and video stimuli gave similar results.

### *Playback with animated slides*

Video stimuli for conspecific mate-copying in sympatric *P. mexicana* females (experiment 6) were created by editing a digitised slide of a *P. mexicana* male interacting with a *P. mexicana* female. In Adobe Photoshop 4.0.1 we replaced all background with light blue colour matching the cardboard used as cover for the preference tank and rescaled the sizes of the fish. We had a set of three files showing (1) a male downscaled to small size (25 mm) and the female *P. mexicana*, (2) the downscaled male alone and (3) an enlarged male alone. The small and the large *P. mexicana* male pictures had a size difference of 40%, which is equivalent to size differences found in nature (Heubel, unpublished data). With this technique we created a pair of stimuli that differed only in size. We animated and looped the set of pictures in Adobe Premiere 4.2 and recorded these loops on SVHS tapes showing the fish appearing and moving forward from left to right, disappearing and reappearing and moving from right to left and so forth. The video did not show the fish turning.

### *Statistical analysis*

We compared copying scores, the relative time spent with the initially less preferred stimulus fish of the preference tests, after minus before we gave the opportunity to copy. Positive copying scores indicate a presence of mate-copying. Negative scores indicate absence of mate-copying and strengthening of the initial preference. All statistical tests were non-parametric. Unless noted otherwise, we used Wilcoxon tests for planned comparisons. All $p$ values are two-tailed. Where appropriate, post-hoc pairwise comparisons were corrected using the Dunn-Šidák Bonferroni method (Sokal & Rohlf, 1995) and, thus, significance thresholds refer to $\alpha'$. Data presented in experiment 1b have been published previously (Schlupp et al., 1994). We reanalysed the data and calculated copying scores to confirm compliance with experimental setup, exclusion criteria and statistical analysis used in the other experiments.

## Results

### *Initial preference for larger males*

Females used in our study had an initial preference for larger males. Out of 225 trials testing female association times with larger versus smaller males (phase 1), 153 preferred the larger stimulus and hence continued the mate-copying trials. Analysing all the data for phase 1 of the six different experiments, there is only one experiment (experiment 6, this is the control experiment testing for conspecific mate-copying within *Poecilia mexicana* using animated slides as stimuli) in which we do not have an initial preference for larger males (Table 2).

### *Patterns of mate-copying*

#### Experiment 1: Trials with sympatric *P. latipinna* as test females and *P. formosa* as models

*P. latipinna* exhibited mate-copying in these experiments. After opportunity to copy, *P. latipinna* spent significantly more time in the preference zone adjacent to the (a) video playback and (b) live stimulus (data reanalysed from Schlupp et al., 1994) of the initially less preferred and smaller *P. latipinna* male (Table 3).

**Table 2.** Initial female preference for larger males.

| Experiment | Mean time with larger male (s) | Mean time with smaller male (s) | $N$ | $z$ | $p$ (Wilcoxon test) |
|---|---|---|---|---|---|
| 1 | 832.7 | 280.5 | 31 | −4.106 | 0.0001 |
| 2 | 313.8 | 213.3 | 32 | −2.048 | 0.0406 |
| 3 | 271.1 | 210.1 | 67 | −2.220 | 0.0264 |
| 4 | 299.5 | 186.4 | 44 | −2.894 | 0.0038 |
| 5 | 768.4 | 370.6 | 22 | −2.127 | 0.0334 |
| 6 | 222.3 | 280.2 | 29 | −0.876 | 0.3812 |

**Table 3.** Results of different mate-copying experiments.

| Experiment | Type | Conspecific/ heterospecific | Tested population | Sym-/allopatry | Assocation time small male before median (IQR) (s) | Association time small male after median (IQR) (s) | $z$ | $N$ | $^{b}p$ (Wilcoxon tests) | Copying score median (IQR) (%) | $z$ (test vs. control) | $^{b}p$ (Mann–Whitney $U$-test) (test vs. control) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | test | hetero | *lat* SM | sym | 132 (99) | 299 (197) | −2.38 | 8 | **0.017** | 23.5 (36) | | |
| | control 1 | | | | 67 (110) | 0 (133) | −0.41 | 7 | 0.686 | 0.0 (16) | −1.62 | 0.105 |
| | control 2 | | | | 0 (130) | 0 (147) | −0.14 | 7 | 0.893 | 0.0 (45) | −0.81 | 0.418 |
| 1b[a] | test | hetero | *lat* SM | sym | 256 (209) | 376 (494) | −2.64 | 16 | **0.008** | 17.2 (45) | | |
| | control 1 | | | | 206 (211) | 61 (222) | −1.27 | 11 | 0.203 | −2.43 (9) | −2.27 | **0.023** |
| | control 2 | | | | 48 (149) | 42 (287) | −1.27 | 11 | 0.203 | 2.56 (27) | −1.40 | 0.160 |
| 2 | test | hetero | *lat* AS | allo | 137 (66) | 110 (116) | −0.24 | 18 | 0.811 | −2.0 (26) | | |
| 3a | test | con | *for* BT | sym | 66 (124) | 438 (300) | −2.40 | 10 | **0.017** | 77.1 (72) | | |
| | control 1 | | | | 82 (73) | 67 (122) | −0.31 | 7 | 0.753 | 0.0 (12) | −2.15 | **0.032** |
| | control 2 | | | | 49 (45) | 98 (73) | −1.69 | 7 | 0.09 | 9.0 (24) | −1.37 | 0.172 |
| 3b | test | con | *for* SM | sym | 119 (92) | 185 (117) | −2.57 | 25 | **0.01** | 18.6 (34) | | |
| | control 1 | | | | 157 (29) | 190 (109) | −1.41 | 12 | 0.158 | 14.3 (22) | −0.36 | 0.721 |
| | control 2 | | | | 187 (68) | 166 (148) | −1.33 | 15 | 0.182 | −8.0 (28) | −2.70 | **0.007** |

**Table 3.** (Continued).

| Experiment | Type | Conspecific/ heterospecific | Tested population | Sym-/allopatry | Assocation time small male before median (IQR) (s) | Association time small male after median (IQR) (s) | $z$ | $N$ | $^b p$ (Wilcoxon tests) | Copying score median (IQR) (%) | $z$ (test vs. control) | $^b p$ (Mann–Whitney $U$-test) (test vs. control) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | test | hetero | *mex* TAM | sym | 142 (67) | 246 (186) | −2.56 | 15 | **0.011** | 25.5 (41) | | |
| | control 1 | | | | 113 (123) | 171 (201) | −0.37 | 10 | 0.721 | −1.4 (33) | −1.91 | 0.056 |
| | control 2 | | | | 226 (122) | 221 (185) | −0.05 | 10 | 0.959 | 0.6 (30) | −1.89 | 0.059 |
| 5 | test | hetero | *mex* PS0 | allo | 197 (263) | 207 (321) | −1.71 | 13 | 0.087 | 6.0 (44) | | |
| | control 1 | | | | 277 (159) | 259 (305) | $x = 2$ | 5 | $>0.8^c$ | −2.1 (16) | −0.84 | 0.402 |
| 6 | test | con | *mex* TAM | sym | 95 (66) | 236 (188) | −2.37 | 7 | **0.018** | 21.5 (40) | | |

For abbreviations of populations, see legend of Table 1. IQR, interquartile range. Control 1, without opportunity for copying between first and second preference test. Control 2, shoaling control using all females controlling for social effects. Copying scores, percentages shown are relative differences in time spent with initially less preferred male after and before opportunity for copying.

[a] From Schlupp et al. (1994).

[b] Significant results are in boldface.

[c] Sign test.

### Experiment 2: Allopatric *P. latipinna* as test females and *P. formosa* as models

This experiment did not provide evidence that *P. latipinna* females from allopatry show mate-copying when *P. formosa* is the model. We could not reject the null-hypothesis that there is no difference in time allopatric *P. latipinna* females spent in the preference zone in front of the initially less preferred and smaller *P. latipinna* male before and after opportunity for mate-copying of *P. formosa*'s mate choice (Table 3).

### Experiment 3: Conspecific mate-copying in *P. formosa*

*P. formosa* in these experiments with (a) video stimuli and (b) live stimuli showed mate-copying, as they significantly increased the relative time they spent with the initially less preferred *P. latipinna* male before and after opportunity for copying (Table 3).

In our experiment on conspecific mate-copying in *P. formosa* using video playback (experiment 3a) we were limited to a very small sample size. The control for shoaling was close to being statistically significant (Table 3). We, therefore, decided to analyse our data conservatively and excluded these data when comparing copying scores (Figure 1).

### Experiment 4: Sympatric *P. mexicana* as test females, using *P. formosa* as models

*P. mexicana* exhibited mate-copying with *P. formosa* as the model. Females spent significantly more time with the initially less preferred smaller *P. mexicana* male after observing the smaller male interacting with a heterospecific *P. formosa* model (Table 3).

### Experiment 5: Allopatric *P. mexicana* as test females, using *P. formosa* as models

Allopatric *P. mexicana* females did not exhibit statistically significant mate-copying, although there was a trend in that direction. We were unable to reject the null-hypothesis that there is no difference in time *P. mexicana* females spent in the preference zone in front of the initially less preferred smaller *P. mexicana* male before and after opportunity to copy the mate choice decision of a *P. formosa* (Table 3). Nevertheless, unlike the results with allopatric *P. latipinna* (experiment 2) there is a non-significant trend for allopatric *P. mexicana* females to exhibit heterospecific copying.

### Experiment 6: Conspecific mate-copying in *P. mexicana*

We also determined if there is conspecific mate-copying behaviour in *P. mexicana* by testing sympatric *P. mexicana* with *P. mexicana* model females with video playback (animated slides). There was a significant difference in time *P. mexicana* females spent in the preference zone near the initially less preferred smaller *P. mexicana* male stimulus before and after opportunity for mate-copying of conspecific mate choice (Table 3).

In all control experiments for consistent preferences without opportunity for copying, there was no significant difference in preferences for stimuli between the first and third test phases (Table 3). In all experiments controlling for female social shoaling behaviour, there was no significant difference in time spent with female stimuli before and after opportunity for copying.

In summary, we found mate-copying in all experiments testing *P. formosa* or sympatric sexual females. We did not detect heterospecific mate-copying in sexual females originating from allopatric populations (experiments 2 and 5), although in *P. mexicana* there was a trend towards copying. We also showed that there was conspecific mate-copying behaviour in *P. mexicana* and *P. formosa*. This is the first evidence for mate-copying in these two species.

### *Comparison of copying scores*

The relative extent of mate-copying behaviour was independent of the experimental protocol used. We found no relationship between presentation time in which females had the opportunity to observe model females interacting with a male and copying scores (Spearman rank correlation: $r_s = -0.099$, $z = -1.081$, $N = 121$, $p = 0.28$). In addition, graphical analysis of scatter plots did not reveal any impact of presentation duration on copying scores.

We compared copying scores between allopatric and sympatric populations and conspecific versus heterospecific models. Sympatric and allopatric *P. latipinna* differ in the heterospecific copying score (experiment 1 vs. 2: Mann–Whitney $U$-test: $U = 142$, $U' = 362$, $z = -2.48$, $N_1 = 18$, $N_2 = 28$, $\alpha' = 0.017$, $p = 0.013$), while *P. mexicana* do not show such a difference (experiment 4 vs. 5: Mann–Whitney $U$-test: $U = 80$, $U' = 115$, $z = -0.81$, $N_1 = 13$, $N_2 = 15$, $\alpha' = 0.017$, $p = 0.420$, Figure 1).

There is no significant difference in the copying scores between experiments in which heterospecific *P. formosa* or a conspecific *P. mexicana* were

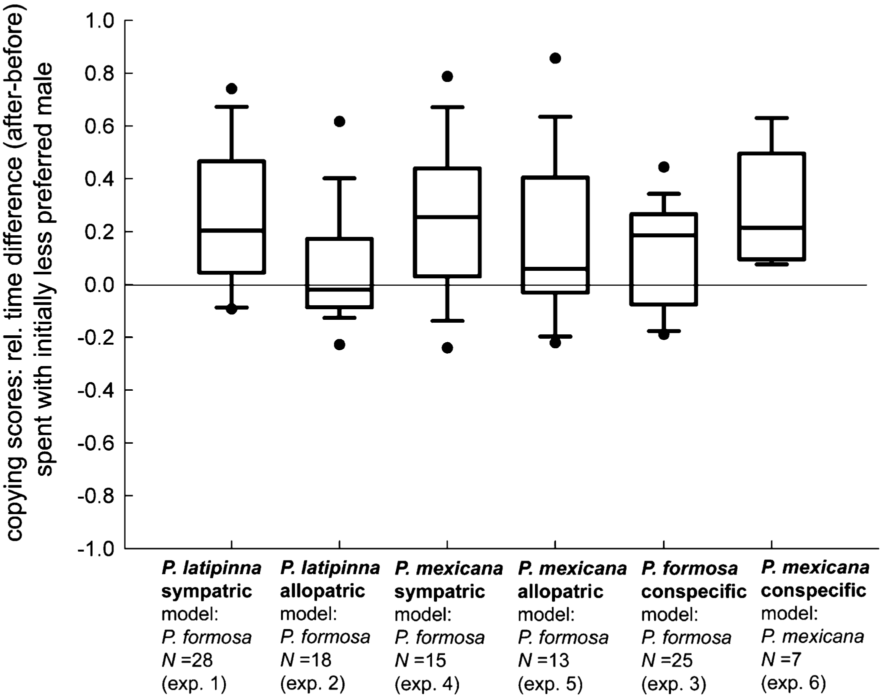

**Figure 1.** Mate-copying in the mating complex of *P. formosa*. Box plots of copying scores, the relative time differences (before-after opportunity for copying) in allopatric and sympatric populations of *P. latipinna* and *P. mexicana*. First two boxes: *P. latipinna* females from sympatric and allopatric populations with heterospecific model females. Two middle boxes: *P. mexicana* females from sympatric and allopatric populations with heterospecific models. Last two boxes: conspecific models. The lower boundary of the box indicates the 25th percentile and the upper boundary the 75th percentile, the solid line in the centre represents the median. Whiskers indicate the 90th and 10th percentiles. Points show 5th and 95th percentiles. As a reference, a horizontal line at zero is included. Positive data above the line indicate presence of mate-copying, copying scores around zero represent consistency of the initial preference for a male.

used as model females (experiment 4 vs. 6: Mann–Whitney $U$-test: $U = 50$, $U' = 55$, $z = -0.176$, $N_1 = 7$, $N_2 = 15$, $\alpha' = 0.017$, $p = 0.860$, Figure 1).

## Discussion

### *Female association preference for larger males*

Our data show that females generally prefer to associate with larger males. Nevertheless, there are individual differences between females not only among but also within a population regarding mate choice. Among the presented experiments and populations, there is only one experiment (experiment 6, this is the control experiment testing for conspecific mate-copying within *Poecilia mexicana* using animated slides as stimuli) in which we unexpectedly were unable to detect an initial preference for larger males (Table 2). This may partly be due to the animated slides. Using such simplified stimuli may lead to generally weaker responses and, hence, higher proportions of excluded trials. Therefore, such results should be interpreted cautiously. Our data, however, show similar response indices (RI) and proportions of terminated or excluded trials for experiments using live, video, or animated stimuli. We do not base any major conclusion on this specific experiment 6; rather it serves as a control treatment that shows (1) even severely simplified models such as animated slides may work sufficiently as stimuli and (2) this experiment serves as a general control indicating that not only *Poecilia latipinna* and *P. formosa* but also *P. mexicana* copies mate preferences when the model is conspecific. Nevertheless, we are fully aware of the potential drawbacks using such highly simplified stimuli.

### *Patterns of mate-copying*

We show that the extent of heterospecific mate-copying is highly variable among populations. Mate-copying occurs in sympatric populations of both sexual host species of *P. formosa*. Thus, *P. latipinna* and *P. mexicana* males that had interacted visually with *P. formosa* model females increased their attractiveness to conspecific females. Several studies reported conspecific mate-copying for *P. latipinna* (Witte & Ryan, 1998, 2002; Witte & Noltemeier, 2002; reviewed in Witte, 2006), indicating the importance of mate-copying in this species.

In our first experiment (1a), we repeated an earlier study (Schlupp et al., 1994) but used video playbacks instead of live stimuli with the same result. The use of video stimuli made it less likely that mate-copying could have been due to cryptic changes in male behaviour, motivation or status that occurred during the presentation phase due to interactions with the test female, the model female, or the other male. This potential confounding effect can now be excluded.

The validity of video-based experiments is somewhat limited because it is more difficult to generalize from using a limited number of stimuli (Rosenthal, 1999; Oliveira et al., 2000; Tobler et al., 2006). However, even though using a single exemplar as stimulus, the paired design of the study allows us to most likely attribute all detected differences in response to differences between the treatments. Nevertheless, we chose to carry out experiments using both approaches (video and live) in experiment 1a, 1b, 3a and 3b. In these experiments, trials using live and video stimuli gave similar results.

In summary, we found the same pattern of mate-copying behaviour in both host species systems. While sympatric populations showed strong heterospecific mate-copying, allopatric populations did not. Thus, our results support the hypothesis that heterospecific mate-copying is beneficial for males in sympatric populations, at least for the specific case of this mating complex. In general, the adaptive value of mate-copying behaviour is not known yet. Our results indicate that mate-copying in *P. latipinna* (Witte & Ryan, 1998, 2002; Witte & Noltemeier, 2002) is not a non-specific result with any female model. The absence of heterospecific mate-copying in females from allopatry suggests that mate-copying is a population specific response relative to the model female used, not a broad response that is present in all fishes. This interpretation is consistent with previous studies showing that *P. latipinna* females can discriminate between *P. formosa* and *P. latipinna* females (Schlupp & Ryan, 1996).

Our use of a population from very recent sympatry allows a glimpse at the evolution of mate-copying. We know that sympatry in the San Marcos river drainage has existed for only about 50 years (Brown, 1953), i.e., approximately 100–150 generations (Hubbs, 1964). Therefore, the evolution of heterospecific mate-copying must have been rapid on an evolutionary time scale (Berthold, 1992). While sympatric *P. latipinna* originating from the San Marcos river (SM) showed a copying score of 20.4%, those from allopatric *P. latipinna* originating from the same river (AS) and only 10 km upstream had a negative copying score (Table 1). The rapid acquisition (whether evolved or learned) of this behaviour suggests heterospecific mate-copying is an adaptation.

Alternatively, mate-copying might be due to individual learning and sexual females might learn that Amazon mollies are adequate models. At least for large male body size (Marler & Ryan, 1997), Amazon mollies have sexual preferences identical to those of the sexual females, rendering them adequate models. These interpretations need to be considered cautiously, because we only studied one population of *P. latipinna* in allopatry and one in sympatry. Gabor & Ryan (2001) showed there was substantial variation in male mate preferences of *P. latipinna* within the range of both sympatry and allopatry, suggesting caution in any strong conclusion from restricted sampling. Furthermore, in *P. mexicana*, there was no significant difference in the copying scores between the sympatric and the allopatric population, although the pattern was similar to *P. latipinna* (Table 3). To argue that mate-copying behaviour has been influenced by the presence/absence of *P. formosa* requires study of additional populations.

### *Benefit to males*

The unisexual/bisexual mating complex of the Amazon molly is a unique system to study female heterospecific mate-copying. In such a system, (sexual and asexual) female preferences relate to immense fitness consequences for the males. Thus, we specifically studied and discussed female mate-copying and its consequences for male fitness in this complex.

In a purely sexual population, mate-copying situations provide males with immediate and delayed benefits. The immediate benefit is due to access to the (model) female. Interacting (and mating) with a conspecific female provides a fitness benefit since it directly increases a male's fitness. The delayed benefit derives from mate-copying. It increases future probabilities of gaining mates. In populations with Amazon mollies, males mating with Amazons gain only a delayed benefit due to increased attractiveness to sexual conspecifics. In sympatry, sexual interactions of males will be observed by both conspecific and heterospecific females. Thus, matings might result in males being more attractive to additional heterospecifics. Therefore, the male's benefit derived from conspecific mate-copying, might simultaneously incur a cost via increased attractiveness to Amazon females. In mixed mating situations with sexual and asexual females, *P. formosa* actively block sexual females from mating with males (Foran & Ryan, 1994). Hence, it is crucial to differentiate between female choice and male choice. Female mate-copying affects male mate choice decisions only by altering males' encounter rates with potential mates.

But increased encounter rates with Amazon mollies need not lead to more matings for Amazon mollies since males can in theory always forsake matings with Amazons, or prime and transfer less sperm (Aspbury & Gabor, 2004; Schlupp & Plath, 2005; Riesch et al., 2008). In various studies, it has been shown that males of the sexual host species *P. latipinna* and *P. mexicana* can discriminate between conspecific females and heterospecific Amazons (Schlupp et al., 1991; Ryan et al., 1996; Schlupp & Ryan, 1997; Gabor & Ryan, 2001). This way they might selectively exploit their increased attractiveness with conspecifics and consequently reduce the ‘cost’ of increased attraction for *P. formosa*.

To what degree the male’s benefit from increased attractiveness to conspecific females could be offset by a cost due to his increased attractiveness to heterospecific females depends on several variables. If being more attractive to Amazons means that males actually mate more frequently with Amazons and if mating is costly, males might not gain a net-benefit through mate copying. If such were the case, the net-benefit to males of heterospecific mate-copying will depend partly on the relative strength — the copying scores — of heterospecific and conspecific mate-copying.

In our experiments the copying score in Amazons copying conspecifics (experiment 3b) is slightly lower than copying scores in sympatric experiments with sexual and asexual females as models (Table 3). Also, the cost of increased attractiveness to Amazons depends on the ratio of sexual and asexual females in a population. It is not yet clear, however, how strong this effect will be, for example, if Amazons outnumber the sexuals. The proportion of *P. formosa* may range from 0 to 100% in the field (Hubbs, 1964; Balsano et al., 1989; Heubel, 2004). In a population with far more unisexual Amazons than sexual conspecific females, it is more likely that a male increases its attractiveness to other close-by observing Amazon mollies than to sexual females.

### *Copulations as cue*

From the male’s point of view, another benefit of heterospecific matings that has been overlooked so far is the exploitation of the signal function of this behaviour. Like an ornament, attempted and actual copulations can be considered as behaviour with signal value (Bradbury & Vehrencamp, 1998; Negro & Grande, 2001). It may indicate the presence and quality of a male. This approach places mate-copying in the context of a communication network (McGregor & Peake, 2000; Matos & Schlupp, 2005). Assuming a copulation per se is a cue, then even a copulation with a female of a different species, such as the Amazon molly or with another male (Tobler et al., 2005), may be a sexual signal and in itself beneficial to perform. This aspect of heterospecific or homosexual mating attempts raises the question why those copulations seem so rare in nature. They may have been undetected in the field and underestimated in the laboratory in many species. Especially in populations with low frequencies of Sailfin mollies, heterospecific copulations might be a way to attract the few remaining sexual females.

Our data indicate absence of heterospecific mate-copying in allopatry. If copulations indicate future copulations, one would expect presence of heterospecific female mate-copying in allopatric populations. It remains to be studied whether species recognition is stronger and, thus, avoidance of heterospecific copulations and mate-copying is stronger in allopatric populations.

Although it seems counterintuitive, we argue that males benefit from matings with heterospecific females, both via the quantitative increase of attractiveness to conspecific females, and the qualitative signalling function of this behaviour. By courting and copulating with a model female, a male is signalling its property of being a male.

## Acknowledgements

We thank A. Taebel-Hellwig, A. Schlüter, K. Körner, S. Repka, S. Keaton, N. Parvaze, K. Chmielowiec, T. Kellermann, M. Irentschiuk, C. Puls and the team of the Aquarium for animal care, help with field work and conducting the experiments. We thank the Brackenridge Field Laboratory at the University of Texas at Austin for use of their facilities. This study was supported by DAAD HSPIII. Funding was also provided by the Academy of Finland (K.U.H.), and by DFG (Heisenberg Fellowship and SCHL 344/7-1) (I.S.). We thank B. Hellriegel and P. Hammerstein for discussions. M. Buck, P. Gienapp, B. Kempenaers, M. Plath, T. Tregenza and four anonymous referees provided comments on earlier versions of the manuscript. We thank the Mexican government for permission 210696-213-03 and DOO 750-1576 to collect fishes. The experiments comply with German and European Union legislation on animal experimentation and with current laws in the USA. Animals were cared for following UT Animal Protocol 10960202.

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