

Original Article

Opposite effects of learning cause asymmetric mate preferences in hybridizing species

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How do females decide which males to accept or reject as potential mates when the individuals encountered are unfamiliar and may be either heterospecifics or conspecifics? Learning often influences the development of mate preferences. Experience with particular phenotypes often positively biases preference for that phenotype. However, experience can also induce aversion. We studied the effect of short-term experience with unfamiliar conspecific, heterospecific, or hybrid males on mate preferences of females of 2 swordtail fish species with native habitats, which differ in both ecology and effective population size. After exposure to males for a week, we tested the females' preferences for male olfactory cues. Both species shifted their mate preferences, but in opposite directions. Female *Xiphophorus birchmanni*, living in larger populations, increased their preference for familiar phenotypes, whereas female *X. malinche*, from smaller, island-like populations, showed an inverse effect of familiarity, namely a decreased preference for newly familiarized males. The pattern of opposite effects of learning on mate choice mirrors with that seen in the evolution of reinforcement of genetic preferences in continental and island populations. Diametrically opposed shifts in preference can thus arise from the same social experience, causing asymmetry in the species' conspecific mate preferences. **Keywords:** hybrid zone, mate choice, olfactory communication, reproductive isolation, *Xiphophorus*. [*Behav Ecol*]

INTRODUCTION

One of the decisions an animal faces is which individuals to accept or reject as potential mates. In many populations, especially after a dispersal event, a female will have to choose among males she has not met before, including males of her own and other species. Understanding how individuals make such choices is important for our understanding of how species become and remain reproductively isolated and how sexual selection operates (Laland 1994; Verzijden et al. 2005; ten Cate and Rowe 2007; Servedio et al. 2009). Female response to heterospecific males often differs sharply between closely related species, a phenomenon referred to as asymmetric mate preferences (Ryan and Wagner 1987; Arnold et al. 1996; Shine et al. 2002). This can have important consequences for the occurrence and direction of natural hybridization (Pearson 2000; Shapiro 2001) and the evolution of reproductive isolation (Kaneshiro 1980). Current models explaining asymmetry in the degree of rejection of heterospecific individuals between species, or incipient species, are based on the assumption that mate preferences are genetically based (Kaneshiro 1980; Arnold et al. 1996; Kirkpatrick and Servedio 1999). This assumption invokes different evolutionary histories of mate preferences.

An alternative hypothesis is that preference asymmetries could arise from learning, when females of different species assign different subjective value (positive or negative) to novel versus familiar phenotypes. Moreover, both the

tendency to learn and the direction of the subjective value of learned preferences may differ among species due to different evolutionary histories (Magurran and Ramnarine 2005; Izzo and Gray 2011).

A growing body of evidence suggests that learning plays a ubiquitous role in shaping mating preferences (Hebets 2003; Magurran and Ramnarine 2004; Verzijden and ten Cate 2007; Dukas 2008; Svensson et al. 2010; Kozak et al. 2011). However, theoretical models of learning and mate choice invariably assume that familiarity with a phenotype will always increase the preference for that phenotype (Kirkpatrick and Dugatkin 1994; Aoki et al. 2001; Lachlan and Servedio 2004; Verzijden et al. 2005; Servedio et al. 2009). Learned mate preferences are, by definition, always based on the existing variation in phenotypes within a population. This does not mean that experience with a certain phenotype also produces a preference for that phenotype. Indeed, learning can generate aversion towards certain mates. For instance, male fruit flies that have experience courting either immature, unreceptive, or heterospecific females subsequently reduce courtship towards them (Dukas 2008). Male guppies from populations allopatric with a closely related congener learn to discriminate against heterospecific females over the course of a few days of interaction with them (Magurran and Ramnarine 2004). Thus, learned preferences can vary in direction and could cause a pattern of asymmetric mate preferences.

Learning to discriminate may be especially important after a dispersal event that causes previously allopatric populations to overlap with closely related species. This places the choosing individual among unfamiliar potential mates, which might be either conspecific or heterospecific. In order to understand how preferences are structured in hybridizing populations of closely related species, it is therefore important to understand how recent experience influences mating decisions of mature individuals from wild populations. Here we examine the shifts in preference

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that can arise from new social experience in adult females in 2 closely related, naturally hybridizing (Rosenthal et al. 2003) species of swordtail fish. We compared the effects of exposure to male phenotypes on female *Xiphophorus birchmanni* and *X. malinche*. These are 2 closely related species (Culumber et al. 2011) that inhabit the same tributaries of the Río Pánuco drainage in the mountains of the Sierra Madre in Mexico. *X. birchmanni* live in large populations in sympatry with several other fish, including the more distantly related congener *X. variatus* (Fisher and Rosenthal 2010). By contrast, *X. malinche* live in smaller populations, fragmented among highland pools, upstream from *X. birchmanni*, where they are often the only fish species. Populations with hybrids are found at intermediate elevation with varying frequencies of backcrosses and F2 and later hybrids, indicating incomplete reproductive isolation (Culumber et al. 2011). The geographic distribution and population sizes of the 2 species is similar to an island–mainland setting, where one of the species is more rare and thus more likely to be affected by occasional migrants from the more abundant population (Kirkpatrick and Servedio 1999).

Mimicking a scenario where females are occasional migrants from one population to another, we address the question of what the effect is of recent experience with unfamiliar males on mate preferences. In particular, we test if short-term experience with heterospecific or hybrid males affects adult female preferences differently than conspecific males. To this end, we exposed wild caught adult females of the 2 swordtail fish species to either conspecific males, the males of the parapatric species or hybrids for a week, and then tested their preferences for olfactory cues of these 3 types of males. We assume that wild caught females had experience with males from their native population and that this experience shaped their preferences (Walling et al. 2008; Verzijden and Rosenthal 2011; Willis et al. 2011). Female *X. birchmanni* learn to prefer the species of male they have had experience with during ontogeny, and this effect is more consistent in their olfactory preferences than in visual preferences (Verzijden and Rosenthal, 2011). Moreover, female preferences for olfactory cues in *Xiphophorus* species are more often species specific than preferences for visual cues (e.g. Ryan and Wagner 1987; Crapon de Caprona and Ryan 1990; McLennan and Ryan 1999; Rosenthal et al. 2002), and olfactory preferences can override visual preferences (Crapon de Caprona and Ryan 1990; Morris et al. 2005). Thus, olfactory preferences are likely more conservative. Here we experimentally mimic a scenario of occasional migration between *X. birchmanni* and *X. malinche* populations, akin to a secondary contact scenario, and test the hypothesis that experience with unfamiliar males can affect the female's preferences for olfactory cues. We predicted that experience with heterospecific males would strengthen the female's preferences for their own species, and that this effect would be stronger in *X. malinche* than in *X. birchmanni*.

METHODS

Study populations

All fish were collected from the Río Pánuco basin in the state of Hidalgo, Mexico. We collected from multiple populations to ensure that females could be exposed to individual males with whom they had no prior experience. In total, we collected 86 females and 60 male *X. birchmanni*, 48 female and 60 male *X. malinche*, and 50 hybrid males. Female *X. birchmanni* were collected from the Río Huazalingo at San Pedro Huazalingo, Hidalgo, Mexico (20°57'3"N 98°31'26"W) and from the Río Tlacolula at Crisolco (20°52'3"N 98°20'36"W).

Male *X. birchmanni* were collected at these 2 sites plus the Río Garces (20°56'24"N 98°16'54"W). *X. malinche* males and females were collected from the Arroyo Xontla near Chicayotla (20°55'30"N 98°34'36"W), and males were also collected from a tributary of the Río Xochicoatlán near Malila (20°43'58"N 98°43'12"W). *X. malinche* × *X. birchmanni* hybrids were collected from the Río Calnali at Aguazarca (20°53'48"N 98°36'9"W). All females and males used in this study were sexually mature, as diagnosed from fully developed gonopodia (males) and brood spots (females). All fish were used within 1–5 days of collection from the wild. *X. birchmanni* females were longer than *X. malinche* ($F(1,88) = 8.55$, $P = 0.004$; mean SL *X. birchmanni* 45.0 mm ± 0.8 SE (standard error); *X. malinche* 41.3 mm ± 0.8 SE), but female size was similar among exposure treatments within species (*X. malinche* $F(2,34) = 0.083$, $P = 0.921$; *X. birchmanni* $F(2,50) = 0.473$, $P = 0.626$) and between the 2 populations of *X. birchmanni* (Mann–Whitney $U = 762$, $n_1 = 44$, $n_2 = 28$, $P = 0.091$).

Exposure

Experiments were conducted at the Centro de Investigaciones Científicas de las Huastecas “Aguazarca” (CICHAZ), Calnali, Hidalgo, Mexico. Outdoor concrete tanks (2 × 1 × 1 m) were filled with carbon-filtered municipal tap water, and a continuous, flow-through trickle was maintained throughout the experiment. Wire mesh covered the top to prevent access by birds or other fish predators. In each tank, we placed 8 females and 8 males, where they remained for 5–7 days. The females were exposed to one of the following types of males: *X. birchmanni*, *X. malinche* or *X. birchmanni*–*malinche* hybrids. In other words, females were either exposed to conspecific, heterospecific or hybrid males. Females that were exposed to conspecific males were exposed to males from a different population than their own, in order to eliminate any possibility of prior experience.

Subjects were removed from the tank and placed in 80-l aquaria at least 18 h before testing, and all females from a particular exposure tank remained isolated from other individuals until the time of testing.

Testing

We tested females for their preferences for olfactory cues of the males by measuring the association time of the females with the cues. Association time is a standard measure of preferences in poeciliids, including swordtails (see Wong et al. 2005 and references therein). In a closely related species, *X. nigrensis*, association time in laboratory trials is a strong predictor of association in open-field trials and of observed mate choices in the wild (Ryan et al. 1992) and is highly repeatable and correlated with displays in the courtship sequence (Cummings and Mollaghan 2006). In another species, *X. helleri*, association time also predicts mating decisions (Walling et al. 2010). Male of swordtails court females using both the visual and olfactory signals, but only olfactory signals are necessary for species or population recognition (Crapon de Caprona and Ryan 1990; McLennan and Ryan 1997; Fisher et al. 2006; Fisher and Rosenthal 2010). Wild caught *X. birchmanni* females prefer the scent of conspecific males from their own population over males of the other species but do not distinguish between conspecific cues of different populations (Fisher et al. 2006; Fisher and Rosenthal 2010). Olfactory preference tests closely followed the methodology of previous studies (McLennan and Ryan 1999; Fisher et al. 2006). To produce stimulus water, groups of 4 males were put in an aquarium filled with 20-l water and were visually exposed to females of the same population in an adjacent, identical tank.

Males and females were allowed to visually interact for at least 2 h. Visual contact significantly increases the release of olfactory stimuli (Rosenthal et al. 2011). Previous work has shown that, under this experimental protocol, the dilution of male olfactory cues does not significantly decrease female association time with that cue (Rosenthal et al. 2011). In total, 39 hybrid males, 49 *X. malinche* males, and 47 *X. birchmanni* males were used to produce stimulus water. Females were never tested with stimulus water produced by individual males from their own exposure tanks.

Every female was tested in 3 successive trials. We paired olfactory stimuli of males from a female's native population with olfactory stimuli from males from populations used in the exposure treatments, that is, (1) conspecifics (from a different population than the female's), (2) hybrids, and (3) heterospecifics. Trial order and stimulus side (see below) were systematically varied across females to control for order effects and side biases. By testing each of the olfactory stimuli from the non-native populations against the native olfactory stimulus, we obtained a referenced preference score for each of the non-native populations, allowing us to directly compare the effect of the experimental treatments on preferences for those olfactory cues. We refer to the relative preferences for the 3 stimuli as a preference pattern.

Each female was introduced to the middle of a test aquarium (length \times width \times height = 75 \times 30 \times 30 cm), which was divided into 3 equal zones along the length of the tank and filled with clean, carbon-filtered municipal tap water. At the 2 far ends, the olfactory stimuli entered and were classified as *preference zones*, whereas the middle part of the tank was a neutral zone. The female was allowed to acclimatize to the tank for at least 10 min prior to the start of the trial. To start the trial, we initiated flow of stimulus water on each end of the tank through silicone tubes that drew water from the stimulus water holding tank. The flow rate was approximately 5 ml/min. A trial started when stimulus water started dripping. The moment a female had visited each zone after the trial started, we started the observation time, 5 min, during which we measured the amount of time the female spent in each zone. Females that failed to visit all 3 zones within 5 min after the addition of stimulus water were operationally defined as unresponsive. The test aquarium was refilled with clean water, and tubes carrying stimulus water were flushed between each trial. After testing a female on all 3 stimulus pairs, we measured her standard length.

We obtained data from 78 *X. birchmanni* females (27 females exposed to *X. malinche*; 21 females exposed to hybrids; 30 females exposed to *X. birchmanni*) and 48 *X. malinche* females (16 exposed to *X. malinche*, 16 exposed to hybrids, 16 exposed to *X. birchmanni*). Two *X. birchmanni* females were unresponsive in all 3 trials.

Ethical note

We collected the fish with permission of the Mexican federal government (DGOPA.07311.130709-2261) using baited minnow traps to catch them and transported to the nearby field station (CICHAZ), where the experiments took place. We did this using isolated containers with air stones filled with the native river water and StressCoat (Aquarium Pharmaceuticals, Chalfont, PA, USA). Survival rate of these collection trips was near 100%. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Texas A&M University (Animal Use Protocol 2009-25).

Statistical analyses

We only analyzed data from females that responded in all 3 trials: 53 *X. birchmanni* females (17 exposed to *X. birchmanni*,

20 exposed to *X. malinche*, 16 exposed to hybrids) and 37 *X. malinche* females (12 exposed to *X. birchmanni*, 12 exposed to hybrids, 13 exposed to *X. malinche*). 76% of females responded in all 3 trials. They had a response latency of 105 s (mean, \pm 6.6 SE). *X. malinche* females responded later than *X. birchmanni* females ($F(1,268) = 12.9$, $P < 0.001$; *X. birchmanni* 86.4 s \pm 6.3 SE; *X. malinche* 133.7 s \pm 12.9 SE), but response latency was similar between exposure treatments within species (*X. malinche* $F(2,108) = 1.071$, $P = 0.346$, *X. birchmanni* $F(2,156) = 1.63$, $P = 0.199$).

We subtracted the time spent with the non-native stimulus from the time spent with the native stimulus, and dividing this by the total duration of the time, we recorded the female's behaviour (300 s). Values more than 0 thus indicate that the female spent more time with the native stimulus than with the non-native stimulus. The resulting dataset fulfilled the assumptions of constancy of variance, and the distribution of the residuals was checked for each separate model. We tested for differences in the preferences for the olfactory cues between females from different treatments and different species. To this end, we compared the relative preferences of each female for the 3 olfactory stimuli in a repeated measures ANOVA in PASW 18.0. The net preference times from the 3 trials were the dependent variable and test stimuli (3 levels: conspecific, heterospecific, and hybrid), exposure (3 levels: *X. birchmanni*, hybrid, and *X. malinche*), species of the subject (2 levels: *X. birchmanni* and *X. malinche*) were explanatory variables.

Because we found a 3-way interaction between stimuli, species and exposure (see Results) we performed 3 repeated measures ANOVAs, 1 per treatment, to further investigate the interaction between stimuli and species. Additionally, we also evaluated whether individual preferences deviated from chance expectations (significantly different from zero) with one-sample *t*-tests for each species \times treatment \times stimulus combination (18 tests) then corrected for multiple testing with sequential Bonferroni adjustment.

RESULTS

The results of the one-way sample *t*-tests for significant preferences of each treatment group and species for each stimulus are reported in Table 1. After correction for multiple testing, none of the preferences remained significant.

Full model Females in each exposure treatment showed a distinct preference pattern for the 3 olfactory cues, and the 2 species showed opposite effects of exposure treatment on the direction of these preference patterns. We found a 3-way interaction between the preferences for the 3 stimuli, treatment and species (stimulus \times exposure \times female species $F(4,168) = 5.568$, $P < 0.001$). The two-way interaction between treatment and stimuli was also significant (interaction between the 3 stimuli and exposure $F(4,168) = 4.973$, $P = 0.001$), whereas the two-way interaction between stimuli and species was not ($F(2,168) = 1.413$, $P = 0.246$), nor was there a significant main effect of stimulus ($F(2,168) = 0.495$, $P = 0.610$).

Because the factors, exposure and species, had a significant interaction with the stimuli, we tested for the interaction between the 3 different stimuli and species holding treatment constant.

Conspecific exposed After exposure to non-native conspecific males, the preference pattern for the 3 different stimuli (heterospecific, hybrid, and non-native conspecific) of the *X. malinche* females differed from those of *X. birchmanni* females, where *X. malinche* females showed no preference in the 3 tests and *X. birchmanni* female preferences varied in their preferences across the 3 tests (Figure 1a)

Table 1
Means and one-sample *t*-test statistics of female preferences

Species	Exposure treatment	Stimulus	Preference mean	<i>t</i>	Degree of freedom	<i>P</i>
<i>X. birchmanni</i>	Conspecific	Heterospecific	0.217	2.600	16	0.019
		Conspecific	-0.215	-3.072	16	0.007
		Hybrid	0.0412	0.756	16	0.461
	Heterospecific	Heterospecific	-0.019	-0.304	19	0.764
		Conspecific	-0.066	-1.295	19	0.211
		Hybrid	-0.082	-1.271	19	0.219
	Hybrid	Heterospecific	-0.065	-0.701	15	0.494
		Conspecific	0.067	0.900	15	0.384
		Hybrid	0.079	-1.394	15	0.184
<i>X. malinche</i>	Conspecific	Heterospecific	-0.128	-0.972	12	0.350
		Conspecific	-0.035	-0.282	12	0.783
		Hybrid	-0.108	-0.103	12	0.920
	Heterospecific	Heterospecific	0.301	2.987	11	0.012
		Conspecific	0.087	0.862	11	0.407
		Hybrid	-0.141	-1.504	11	0.161
	Hybrid	Heterospecific	-0.326	-2.549	11	0.027
		Conspecific	-0.136	-0.917	11	0.379
		Hybrid	0.232	3.303	11	0.007

Female preferences were tested for significant difference from zero (indifference). After correction for multiple testing, none of the preferences remained significant. Uncorrected values are reported.

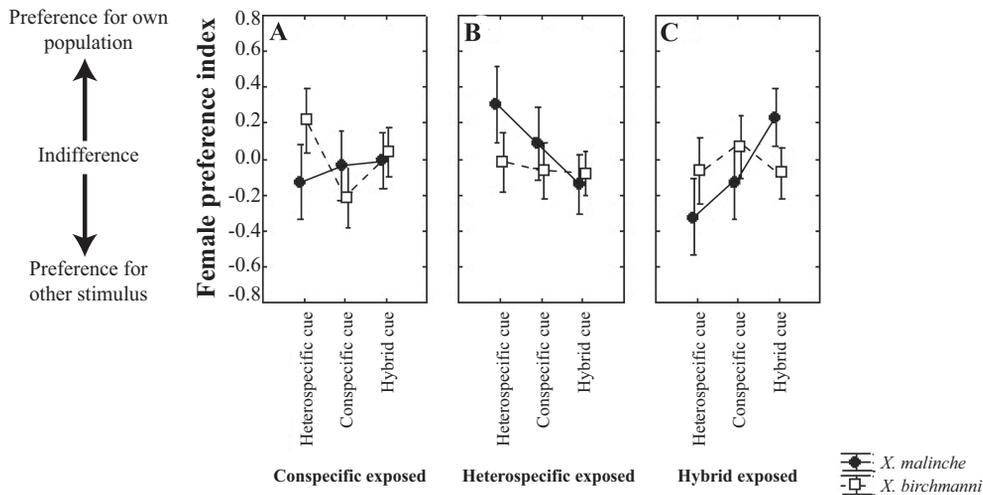


Figure 1

Preferences. Female preferences on a -1 to 1 scale, where 1 translates to a preference for the stimuli of males of their native population and -1 a preference for the other stimulus in the preference test. Each preference test consisted of olfactory cues from males of the females' native population versus either cues from conspecific males from another population, heterospecific males or hybrid males. Panel A shows the preferences of females that were exposed to conspecific males from a different population. Panel B shows the preferences of females that were exposed to heterospecific males. Panel C shows the preferences of females that were exposed to hybrid males. Closed circles and solid lines represent *X. malinche* females, open squares and dotted lines represent *X. birchmanni* females. The interaction between the 2 species in the preferences for the 3 stimuli is significant. Note that *X. birchmanni* females show preferences in panel A, after exposure to conspecific males, but are indifferent after exposure to heterospecific or hybrid males, although this pattern is the opposite in *X. malinche*.

(interaction between species and stimuli $F(2,56) = 3.275$, $P = 0.045$, but the main effect of stimuli was not significant ($F(2,56) = 1.551$, $P = 0.221$)). For *X. malinche*, the 95% CI of the preferences for the 3 stimuli all overlapped with 0, i.e. these females did not prefer any one of the stimuli more than another. For *X. birchmanni*, however, the preferences varied across the 3 stimuli, with a decrease in preference for heterospecific cues, and a preference for non-native conspecific cues.

Heterospecific exposed In this treatment, the species showed an opposite pattern to that of the conspecific exposure

treatment. Although *X. birchmanni* females showed indifference between their native stimulus and the other stimuli, *X. malinche* females showed a marked decrease in preference for heterospecific cues (i.e., a preference for native conspecific cues) and indifference to the other 2 stimuli (Figure 1b). There was a significant interaction between test stimulus and species on preference: $F(2,60) = 3.359$, $P = 0.041$. The main effect of stimulus was also significant ($F(2,60) = 5.951$, $P = 0.004$). The preferences of the 2 species thus show the opposite pattern after exposure to novel heterospecific males versus novel conspecific males.

Hybrid exposed In this treatment, as in the heterospecific exposure treatment, both the interaction between preference and stimulus ($F(2,52) = 5.695, P = 0.006$) and the main effect of stimulus ($F(2,52) = 4.298, P = 0.019$) were significant. As with the preferences after exposure to heterospecific males, and opposite to the preferences after exposure to conspecific males, *X. birchmanni* females showed indifference to the stimuli, whereas *X. malinche* showed a decrease in preferences for the hybrid stimulus and a preference for the heterospecific stimulus (Figure 1c).

DISCUSSION

Short-term exposure to males changes female preferences

As in other species (Nagle and Kreutzer 1997; Rosenqvist and Houde 1997; Dukas 2008; Izzo and Gray 2011;), short-term exposure of sexually mature females to male phenotypes had a pronounced effect on mating preferences. This was true in both *X. birchmanni* and *X. malinche*, but remarkably, and contrary to our prediction, familiarity changed mating preferences in opposite directions. *X. birchmanni* females shifted their preference in favor of the population to which they had been exposed, that is, newly familiarized phenotypes. *X. malinche* females, by contrast, showed a decreased preference to the newly familiarized males, which is consistent with an aversion to these stimuli. *X. birchmanni* exposed to conspecifics showed strong preferences for conspecific over heterospecific signals, whereas those exposed to heterospecifics showed no preference. *X. malinche* showed the opposite pattern: no preference after exposure to conspecifics, and conspecifics were more preferred after exposure to heterospecific males (Figure 1a and 1b).

The opposite directions of shifts in preferences in the 2 species for newly acquired familiarity were consistent across treatments. Whereas hybrid-exposed *X. birchmanni* females showed indifference between conspecifics and hybrids, hybrid-exposed *X. malinche* showed a preference for conspecifics. Experience with conspecific males from a different population by female *X. birchmanni* resulted in a preference for the foreign population, whereas *X. malinche* remained indifferent.

Several mechanisms could account for opposite effects of learning in the 2 species. First, opposite effects could arise from different past experiences operating on a conserved learning mechanism. During ontogeny, *X. birchmanni* females learn to prefer the visual and olfactory cues of *X. birchmanni* or *X. malinche* males if they have exposure to adults of that species (Verzijden and Rosenthal, 2011). *Xiphophorus birchmanni* female subjects are allopatric from *X. malinche*, having experience with not only conspecific males but also males of another species, *X. variatus* (Fisher and Rosenthal, 2010). *Xiphophorus malinche* females, in contrast, are naïve to all heterospecific *Xiphophorus*. These differences in prior experience of the wild caught females may have resulted in opposite effects in how the females responded to short-term exposure. The difference in the 2 species in this study may thus reflect differences in prior experience in the wild, rather than a genetic difference in the valence assigned to experience.

Finally, the females may have had qualitatively different experiences with males during exposure. Although males of both species court females of both species, male *X. birchmanni* prefer olfactory cues of *X. malinche* over those of conspecific females (Wong et al., 2005). If *X. malinche* were more likely to be courted and harassed by males in our experiment, this might have produced a negative association with familiar males. Male behavior would then directly influence species differences in female mate preferences.

Female *X. birchmanni* preferred the scent of conspecific males from a different population after exposure to them, indicating that there might be variation in olfactory cues across populations within a species. In a previous study, however, *X. birchmanni* females did not discriminate between conspecific populations (Fisher and Rosenthal 2010).

Asymmetric mate preferences

The effects of familiarity with heterospecific males in secondary contact may cause asymmetric mate preferences. The current distribution of natural hybrids does not, however, provide good evidence for asymmetric hybridization (Culumber et al., 2011). Yet, the pattern of opposite effects of learning on mate choice mirrors as that seen with the evolution of reinforcement in continental and island populations. Reinforcement occurs more strongly on small island populations than on large continental ones, because islanders are more likely to encounter continental migrants than vice versa (Kirkpatrick and Servedio, 1999). Similarly, ecological differences may have selected for opposite effects of familiarity between *X. malinche* and *X. birchmanni*. The former live in the freshwater equivalent of small islands, in small, isolated populations near tributary headwaters, upstream from *X. birchmanni* (Culumber et al., 2011). Any new incoming males are likely to be from a foreign downstream population, and therefore possibly heterospecific. *X. malinche* may therefore have evolved to be more conservative when choosing between newly familiarized males and those from their native population. By contrast, *X. birchmanni* populations are much larger (Rosenthal et al., 2003). Unfamiliar males are therefore most likely to represent novel conspecific phenotypes.

Interestingly, a study on 2 species of field crickets showed very similar opposite effects of recent experience with heterospecific males on female mate preferences (Izzo and Gray, 2011). Again, experience with heterospecific males causes females of 1 species of field cricket, until recently relatively rare (i.e. island species), to prefer heterospecific males, whereas females of another closely related species with larger population sizes (i.e. mainland species) preferred conspecifics or showed indifference after experience with heterospecific males. Thus, in swordtail fish and in field crickets, the valence effects of familiarity are markedly different between 2 closely related species, showing a pattern of asymmetric mate preferences similar to island-mainland species pairs in secondary contact.

Hybridization

Whereas learned preference for phenotypes from the native population, as shown in a previous study in *X. birchmanni* (Verzijden and Rosenthal 2011), can facilitate reproductive isolation (Verzijden et al. 2005; Servedio et al. 2009), indifference to novel phenotypes as shown in this study by *X. birchmanni* females can promote hybridization and facilitate genetic exchange. The learned disinclination to associate with novel heterospecific phenotypes as shown by *X. malinche*, by contrast, would provide a barrier to hybridization. These differences in the valence of familiar stimuli, as shown by the 2 species in this study, may yield dynamic effects with respect to natural hybridization and sexual selection. Furthermore, females showed indifference to hybrid scent across treatments; across all 3 exposure treatments, they were just as attractive to females as conspecifics. The notable exception, consistent with *X. malinche* learning more conservative preferences, was a marked reduction in the association with hybrids in *X. malinche* females that were exposed to hybrids. The interplay between hybrid

signals and the asymmetric effects of experience might play an important role in the evolution of these natural hybrid zones (Culumber et al. 2011).

So far, theoretical models of mate preferences that are learned or genetically inherited have, to our knowledge, provided predictions that are qualitatively similar. Models of both the learned mate preferences and the evolution of reproductive isolation have not allowed for between-species differences in the effects of learning (Laland 1994; Verzijden et al. 2005; Servedio et al. 2009). Our study shows that social experience may alter mate preferences to either a preference for the new phenotypes or a distinct disinclination to associate with the new phenotypes, depending on the species and experience. Incorporating this phenomenon in formal models may aid in distinguishing differential effects of learned versus genetic mate preferences. Moreover, behavioral studies of reproductive isolation measuring mate preferences in females (or males) naïve to individuals from heterospecific or hybrid populations may not be indicative of the behavior of wild animals in secondary contact.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

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