



Female swordtails, *Xiphophorus continens*, prefer the scent of heterospecific males

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(Received 9 April 2007; initial acceptance 7 July 2007;
final acceptance 3 October 2007; published online 18 March 2008; MS. number: A10739R)

Xiphophorus continens is one of two northern swordtail species that has only small, noncourting, sneaker males. In this study we discovered that female *X. continens* were strongly attracted to the scent of courting males of their close relative *X. montezumae* and that they preferred the odour of those males to the scent of conspecific males. The heterospecific attraction is asymmetric; female *X. montezumae* were not attracted to the scent of *X. continens*. These results suggest that the olfactory cue is in fact multicomponent, transmitting information about both the species identification (conspecific versus not a conspecific) and sexual status (sneaker male versus courting male) of the sender. These two 'bits' of information can interact in either a mutually reinforcing or an antagonist manner depending upon the choice being offered. *Xiphophorus montezumae* females choosing between *X. montezumae*/*X. continens* males are faced with mutually reinforcing cue components: 'conspecific + courting male' versus 'not a conspecific + sneaker male'. *Xiphophorus continens* females making the same choice, however, are faced with conflicting cue components: 'conspecific + sneaker male' versus 'not a conspecific + courting male'. We suggest that the strength of attraction to the conspecific based upon olfactory cues in these fishes represents the complex outcome of the female's attraction to the different components in the male-based cue.

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Keywords: asymmetric mate preference; heterospecific mate preference; mate choice; olfactory cue; swordtail fish; *Xiphophorus continens*; *Xiphophorus cortezi*; *Xiphophorus montezumae*; *Xiphophorus nigrensis*

Olfactory cues are important in fish communication, transmitting information in interactions from parental care through predation and alarm signalling to foraging, schooling and migration. More importantly, from a mating system perspective, many fishes use olfactory cues to detect conspecifics, discriminate between conspecifics and heterospecifics, recognize their own offspring and differentiate between familiar and unfamiliar shoalmates (for references see McLennan 2003, 2006). Olfactory cues have traditionally been classified as long-distance signals in moving water (Dusenbery 1992), so a role for olfaction in mate recognition is not surprising. Such signals allow a female to detect the 'I am here' message in the olfactory modality before the male can be detected in

the visual modality. Studies of interactions between temporally displaced signals indicate that the first cue (in this case chemical) functions to alert the receiver to the presence of the second cue (visual), increasing the probability of its detection and recognition (Rowe 1999). In addition to transmitting information about species identity and sex, male-based chemical cues are involved in the cycling of female receptivity. So, for example, female African catfish, *Clarias gariepinus* (Resink et al. 1989), and zebra danios, *Brachydanio rerio* (Chen & Martinich 1975; van den Hurk et al. 1987), require olfactory input from male conspecifics to ovulate consistently, whereas female angelfish, *Pterophyllum scalare* (Chien 1973), achieve their highest spawning rates only when exposed to both visual and chemical stimuli from males. The long-distance alerting and stimulating aspects of olfactory signals may combine to decrease the costs incurred by a female during the mate selection process and increase her probability of finding a mate during her period of peak receptivity (Real 1990; Jumper & Baird 1991).

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Northern swordtail species in the genus *Xiphophorus* are an excellent system for studying the importance of olfactory cues in the male–female mating dialogue. Seven of the nine species contain two types of males: large sworded courters and small swordless sneakers. Courting males use conspicuous displays to persuade a female to collaborate in mating; sneakers use coercion, approaching the female from below and behind, swinging their modified anal fin (gonopodium) forward, and attempting to copulate rapidly (Rosen & Tucker 1961; Franck 1964; Ryan & Causey 1989; Zimmerer & Kallman 1989; Ryan & Rosenthal 2001). Females show consistent behavioural responses to the different male tactics, cooperating with courtship-based intromissions and fleeing from sneak copulation attempts as soon as they detect the male (Ryan & Causey 1989; *X. multilineatus* (formerly described as *X. nigrensis*): Zimmerer & Kallman 1989). When given a choice between the scent of a conspecific versus a heterospecific male, females from most species strongly prefer the scent of the conspecific, regardless of how closely related the heterospecific alternative is (*X. nigrensis*: Crapon de Caprona & Ryan 1990; McLennan & Ryan 1999; *X. cortezi*: McLennan & Ryan 1997; *X. montezumae*: McLennan & Ryan 1999; *X. birchmanni*: Fisher et al. 2006).

The role for olfactory cues is less straightforward in *X. pygmaeus*, one of two northern swordtail species that contains only sneaker males. The males in these species are similar to the small males in sworded species in that they are not only small but also shallow-bodied, lack a conspicuous extension of the lower rays of the caudal fin (a sword) and are more likely to chase and attempt to force copulations with a female than to court (Ryan & Causey 1989; Morris et al. 2005). *Xiphophorus pygmaeus* females strongly prefer the scent of conspecific males when offered the odour from sworded *X. cortezi* as the alternative (Hankison & Morris 2003), but show only a weak preference for conspecifics when offered scent from the potentially more closely related (Rauchenberger et al. 1990; but see Meyer et al. 1994) *X. nigrensis* (significantly more lunges to the source of the conspecific odour: $P = 0.018$), but a nonsignificant preference for time associated with the conspecific cue ($P = 0.071$; Crapon de Caprona & Ryan 1990). This latter result is unlike that seen in females from sworded species, which raises the intriguing possibility that the olfactory cue is transmitting more information than just species identification and sex in these fishes. It might also be transmitting information about the sexual status (courting male versus sneaker male) of the sender.

The reactions of *X. pygmaeus* cannot be readily generalized to the other sneaker-only species, *X. continens*. For example, unlike other swordtails, these females do not show a preference for a large courting male based on visual cues when closely related heterospecifics are offered (Morris et al. 2005). How do they respond to conspecific scent in a choice situation? Will they, like *X. pygmaeus* females, display only a weak preference for the conspecific under some conditions or will they be more like their close relative *X. montezumae*, whose females always show a strong preference for conspecific males? To answer this question, we presented female *X. continens* with olfactory cues from conspecific and *X. montezumae* males. We also investigated the reactions of

X. montezumae females to the odour of *X. continens* males to determine whether there was any asymmetry in the interaction between the two species. If the olfactory cue does contain information about both sexual status and species, we predicted that *X. montezumae* females would respond less strongly to the scent of *X. continens* males than expected given their close phylogenetic relationship because 'courting male' will not be part of the *X. continens* message.

METHODS

Study Animals

The experiments were originally conducted in June 1995. At that time we were able to collect data from only 12 female *X. continens*, so we repeated the tests 10 years later in April 2005. We did not repeat the experiment with *X. montezumae* females in 2005 because there was no difference in the response to the conspecific cue by females in June 1995 ($N = 13$) and different females from the same population used in another experiment conducted in April 1995 ($N = 16$: total time: 250.53 s (April) versus 242.61 s (June); paired t test: $t = 0.65$, $P = 0.52$; % time interacting: 34.10 (April) versus 39.93 (June); $t = 0.87$, $P = 0.39$; time hovering: 24.44 s (April) versus 26.32 s (June); $t = 0.17$, $P = 0.86$). The similarity indicated that we were gathering reliable estimates of female behaviour with our sample size in 1995.

Xiphophorus montezumae individuals were collected from Río Gallinas, Agua Buena, Mexico in April 1995 and March 2005 to supplement the breeding colony of northern swordtails, which consists of 34 separate, species-specific, flow-through, raised 3000-litre outdoor ponds containing abundant vegetation at Brackenridge Field Lab, Austin, Texas, U.S.A. *Xiphophorus continens* subjects were progeny of field-captured animals collected from Río Ojo Frio, El Quince, Mexico. Whenever possible, we used individuals bred in captivity rather than wild-caught fish. Research was in accordance with Animal Care protocol no. 5120201 to M.J.R.

Fish were maintained in 227-litre stock tanks in the laboratory during May–July 1995 and April 2005. Holding tanks in the laboratory were enriched with floating and rooted plants, rocks and polyvinyl chloride pipes for shelter. Lights were set on a 12:12 h light:dark cycle and temperature fluctuated 20–22°C. Individual test females were housed in 4.5-litre jars, covered with coarse nylon mesh and placed within water-filled 22-litre tanks (four jars to a tank). Filters in each tank ensured that the water circulating throughout and around the jars was aerated, cleaned and temperature controlled. The females could see each other but were visually isolated from other fish. All fish were fed ad libitum on live brine shrimp nauplii and Tetra Min flakes twice daily. The jars were emptied and refilled every day to remove excess food.

Producing the Stimulus

Xiphophorus continens and *X. montezumae* males were used as stimulus fish in the olfactory experiments. Because

X. continens are much smaller, we adjusted the number of stimulus fish based on differences in snout–vent length to correct for any potential bias in olfactory cue production based solely upon body size. This is, of course, only a coarse-grained compensation that can be modified in future studies once the chemical composition of the olfactory cue is determined. The cue was thus produced using groups of three *X. continens* (25.6, 27.9, 28.3 mm; 22.1, 26.9, 24.8 mm) or two *X. montezumae* (45.5, 47.4 mm; 43.2, 44.6 mm) males.

Stimulus males were placed in a 19-litre tank filled with 15 litres of water that had been aerated and charcoal-filtered for 24 h and covered with glass to prevent contamination from airborne odours. A larger tank containing five female conspecifics was placed beside the 19-litre tank to behaviourally stimulate the males. These females were not used in any of the experimental trials. Opaque dividers outside the tanks prevented males of one species from seeing males and females of another species. Males remained, fasting, in the stimulus tank for 24 h and then were moved to a holding tank. The stimulus water was used as needed for a maximum of 2 weeks before being discarded. Preliminary tests revealed no decline in female response to the stimulus over that period. We used the same group of males for each species to produce stimulus as required. Control water was produced following the pattern for stimulus water: 15 litres of water was aerated and charcoal-filtered in a glass-covered tank for 24 h, allowed to sit unperturbed for another 24 h, used for 2 weeks and then discarded.

Experimental Procedure

The experimental apparatus consisted of two 80-litre tanks (test tanks; 60 × 31 × 43 cm) and four 4.5-litre jars (stimulus jars). The stimulus jars were placed above and behind the test tanks. A stimulus delivery system was constructed by attaching a 12 mm glass pipette to a piece of 2.5 mm silicone tubing. The tip of the pipette sat 0.5 cm above the water surface. Fish, fed in stock tanks by injecting a large pipette full of brine shrimp into the tank, had learned to respond quickly to any surface disturbance. Dripping the stimulus above the water mimicked that disturbance, prompting the female to respond quickly and to move between the two sides. After each trial, the tank was scrubbed vigorously with a 3% solution of hydrogen peroxide and soap, rinsed with an intense jet of water concentrated along the silicone seams and dried thoroughly (details in McLennan & Ryan 1997).

The stimulus was assigned randomly to the left or the right side in each trial. A female was placed gently in the test tank, allowed to familiarize herself with her new surroundings for 30 min and then videotaped for 5 min with a double water drip (pretrial period). The test cues were then dripped into the tank and the female was allowed to interact for 5 min once she had passed within 10 cm of both stimulus pipettes. We chose the 5 min period for all of our studies because we have observed that females often change their initial responses over time. Olfactory cues are generally used to locate conspecifics

or food from a distance (Dusenbery 1992). Individuals should thus have an expectation of locating the stimulus source after a certain period of 'tracking time', especially when the cue is concentrated in a small area as it was in these experiments. Test fish showed all the signs of tracking an odour at the beginning of the trial, interacting most strongly where the cue was the most intense and spending most of their time near the source of the cue, but, despite all their attempts, they never visually located the source. There thus appeared to be a 'giving up' point in each trial and increasing the length of the trial increased the probability of incorporating that giving up point, plus associated changes in behaviour, into the experimental results. Based on our experience, we believe that tests longer than 5 min may be subject to errors generated by females giving up their pursuit of the more preferred cue and either moving away (expanding their search area) or moving towards the less preferred cue.

Trials in both years followed the sequence 'water versus water (pretrial), stimulus 1 versus water (recognition), stimulus 2 versus water (recognition), stimulus 1 versus stimulus 2 (preference)'. In half of the trials the conspecific cue was offered first; in the other half the heterospecific cue was offered first. The positions of the stimuli were reversed in the choice test from their position in the 'stimulus versus water' trials. In 1995 the female was returned to her holding jar at the end of each trial and allowed 1 day of rest. In 2005 the female was given all the tests in 1 day and allowed to rest for 1 h between each trial in a tank filled with plants. We changed the original experimental design to keep female sexual motivation as consistent as possible across each individual.

Variables scored

Trials were numbered on the videotapes so the scorer did not know either the type of test being conducted or the location (right or left side of the tank) of the stimuli. Videotapes were scored at a later time for the following variables.

Total time. Time spent within 20 cm of each outflow pipette. Association time is a reliable indicator of mating preference in other poeciliids (*X. nigrensis*: Ryan et al. 1990; Morris et al. 1992; *Gambusia holbrooki*: Bisazza et al. 2001; Cummings & Mollaghan 2006).

Percentage of time interacting. Time spent engaged in diving + nudging behaviour on stimulus side/total time spent on stimulus side × 100. Diving involved swimming rapidly up the side of the tank towards the stimulus outflow, turning and swimming rapidly (angle between 10–45°) towards the bottom. During nudging, the female made contact with the side of the test aquarium in a series of butts against the glass. A bout of nudging involved a series of rapid butts interspersed with prolonged swimming, nose in contact with the glass and all fins flared. This behaviour was measured because changes in interaction in previous experiments indicated that the

female recognized the presence of a cue even when she was not attracted to it (McLennan & Ryan 1997, 1999).

Time spent hovering. Sexually motivated females stop moving to facilitate male gonopodial thrusting and may even move backwards into the thrust (e.g. Basolo 1990). We thus used the amount of time that a female spent hovering beneath the cue as a marker of sexual interest. Resting on the ground, either at the end of intense activity or as a result of the female being frightened, was not recorded as 'hovering'.

Statistical Analysis

Paired and nonpaired *t* tests were used to evaluate the null hypothesis that there was no difference in the female's response to different cues. Given the relatively large number of tests being conducted, a sequential Bonferroni correction at $\alpha \leq 0.05$ was applied to decrease the probability of committing a type I error by chance alone (Peres-Neto 1999).

RESULTS

Xiphophorus continens

Two females in 1995 and three females in 2005 were eliminated from the analysis because they spent more than 10% of the time in one or more trials resting on the ground or because they spent a trial fluttering (swimming rapidly up and down in one area, respiration rate elevated, attempting to escape). There was no difference in any measured variable between females in 1995 and 2005, so the data were combined for both years ($N = 24$).

Females showed no side bias in these tests (Fig. 1). They responded more strongly to the stimulus than to the control water in both recognition trials (paired *t* test: time interacting: conspecific: $t_{23} = 5.59$, $P < 0.0001$; heterospecific: $t_{23} = 5.75$, $P < 0.0001$; time hovering: conspecific: $t_{23} = 2.67$, $P < 0.01$; heterospecific: $t_{23} = 4.00$, $P < 0.0006$; time near: Fig. 1). When offered a choice between the two stimuli, females spent more time near (Fig. 1) and more time hovering under (paired *t* test: $t_{23} = 4.38$, $P < 0.0002$) the heterospecific cue but did not alter the intensity of their interactions (paired *t* test: $t_{23} = 1.07$, $P = 0.30$) between the two scents. They spent less time near both stimuli in the preference trials than in each of the recognition tests (paired *t* test: conspecific: $t_{23} = 6.681$, $P < 0.0001$; heterospecific: $t_{23} = 3.74$, $P < 0.001$; Fig. 1).

Xiphophorus montezumae

Females showed no side bias in these tests (Fig. 2). They responded more strongly to the stimulus from conspecific males than to the control water (paired *t* test: percentage of time interacting: $t_{12} = 6.78$, $P < 0.0001$; time hovering: $t_{12} = 3.23$, $P < 0.007$; time near: Fig. 2). They were not attracted to the cue from *X. continens* males (paired *t* test: percentage of time interacting: $t_{12} = 1.05$, $P = 0.31$; time

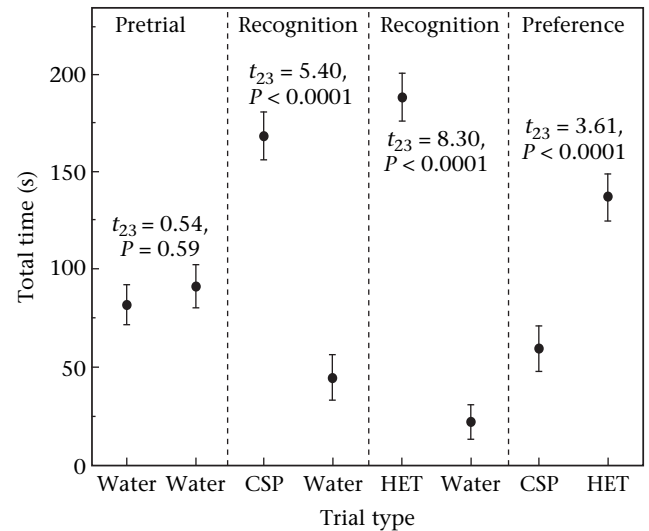


Figure 1. Time (mean \pm SE) that female *Xiphophorus continens* spent within 20 cm of each outflow pipette. CSP: conspecific cue; HET: heterospecific cue (*X. montezumae* males).

hovering: $t_{12} = 1.00$, $P = 0.34$; time near: Fig. 2), although they did increase their activity level (paired *t* test: versus pretrial: $t_{12} = 2.99$, $P < 0.01$). When offered a choice between the two stimuli, females spent more time near (Fig. 2), more time interacting with (paired *t* test: $t_{12} = 6.11$, $P < 0.0001$) and more time hovering under (paired *t* test: $t = 3.07$, $P < 0.01$) the conspecific cue. They spent the same amount of time near the conspecific cue (paired *t* test: $t_{12} = 0.97$, $P = 0.35$) but less time near the heterospecific cue (paired *t* test: $t_{12} = 4.97$, $P < 0.0003$) in the preference trials than in the recognition tests (Fig. 2).

Female *X. montezumae* responded more strongly to the scent of conspecific males than did female *X. continens*

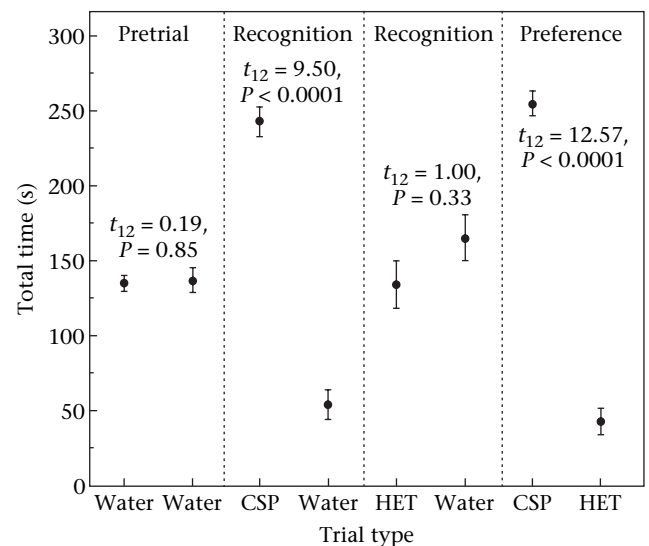


Figure 2. Time (mean \pm SE) that female *Xiphophorus montezumae* spent within 20 cm of each outflow pipette. CSP: conspecific cue; HET: heterospecific cue (*X. continens* males).

(unpaired t test: total time: 246.98 s versus 201.26 s, $t_{12} = 3.54$, $P = 0.0009$; time spent hovering: 25.29 s versus 8.19 s, $t = 2.63$, $P = 0.01$). There was no difference in the percentage of time spent interacting (unpaired t test: 36.72% versus 42.66%, $t_{12} = 0.97$, $P = 0.34$). *Xiphophorus continens* females responded as strongly to the scent of *X. montezumae* males as did *X. montezumae* females (unpaired t test: time spent hovering: 22.20 s versus 25.29 s, $t_{12} = 0.40$, $P = 0.69$; percentage of time spent interacting: 39.54% versus 36.72%, $t_{12} = 0.50$, $P = 0.62$), with the exception of total time spent near (unpaired t test: 220.82 s versus 246.98 s, $t_{12} = 2.07$, $P = 0.04$).

DISCUSSION

Xiphophorus continens females display a unique mate choice pattern based upon olfactory cues compared with other northern swordtail species. Females of species with large courting males always strongly prefer conspecific scent, regardless of the phylogenetic distance between the sender and the receiver (*X. nigrensis*: Cragon de Caprona & Ryan 1990; McLennan & Ryan 1999; *X. cortezi*: McLennan & Ryan 1997; *X. montezumae*: McLennan & Ryan 1999; *X. birchmanni*: Fisher et al. 2006; this study). Females of a species with only small sneaker males, *X. pygmaeus*, also show a preference for conspecific scent, but the strength of that preference is more influenced by phylogeny, being weak when the alternative male is a close relative (*X. nigrensis*: Cragon de Caprona & Ryan 1990) and strong when the male is more distantly related (*X. cortezi*: Hankison & Morris 2003). In this study we showed that the attraction of *X. continens* females to the scent of a close relative (*X. montezumae*) was so strong that it eliminated the expected preference for the conspecific. That this attraction reflects sexual motivation is evidenced by the appearance of hovering, a female receptive behaviour, especially in response to the heterospecific scent. This is the first demonstration that females of a northern swordtail prefer a heterospecific male based upon olfactory cues alone (for a similar dynamic in northern swordtails based on male mate choice see Wong et al. 2005).

Of all senses, odour is one of the most difficult to study because it requires a substantial investment of time and funds to delineate the active components in the cue. For example, it was 68 years after von Frisch (1938) discovered alarm substance in minnows before Brown et al. (2000, 2001) showed that the nitrogen oxide functional group was the prime molecular trigger in that substance. Researchers working with olfaction are thus often forced to rely upon changes in receiver behaviour alone to demonstrate the efficacy of a cue rather than on manipulating the cue's composition and then tracking changes in behaviour. We know nothing about the structure of the olfactory cue in swordtails, but we do know that male-based sexual pheromones in fish are generally composed of conjugated and free steroid metabolites and/or prostaglandins (e.g. Sørensen & Stacey 1999 and references therein) that 'leak' from individuals in their urine. The stimulatory effect of these substances depends upon their structure,

relative concentrations and/or overall concentration, implying that detailed information about the sexual status of the sender may be transmitted by a multicomponent olfactory cue (syntactic coding: Colombo et al. 1982). Hormonal profiles differ between courting and sneaker males in all teleost species examined to date; courting males have elevated levels of keto-testosterone (Brantley et al. 1993). If the sexual status-based component of the olfactory cue is carried in metabolites of gonadal steroids, it is possible that a difference in the steroid mixture between courting and sneaker males may be partially responsible for the pattern of female responses detected in this and other studies.

What is really at issue here is the relative contributions of cue components transferring information about sexual status (sneaker versus courter) and species identification (conspecific versus not a conspecific). It would appear that the 'sexual' and 'species' parts of the message are not interacting in a simple fashion. So, for example, in the *Xiphophorus montezumae* female–*X. montezumae*/*X. continens* male pairing, information transmitted in the olfactory cue is mutually reinforcing; the 'conspecific' component is paired with 'courting male' while 'not a conspecific' is paired with 'sneaker male'. Given the general preferences of *Xiphophorus* females for large bodied, sworded, courting males, it is likely that female *X. montezumae* would find the small *X. continens* males completely unattractive based on visual cues. In this interaction, then, the receiver's response to visual cues is reinforced by her response to chemical ones, females are not attracted to (total time) or sexually motivated by (time spent hovering) the scent of *X. continens* males. They are, however, aware of the male's presence. In our tests females increased the amount of time spent moving around the tank when exposed to the odour of *X. continens* males versus control water. This movement was not associated with fear-based fluttering behaviour, so it may reflect the erratic swimming activity that females use to evade attempted copulations of sneaker males. Whatever the explanation, both visual and chemical cues transmit mutually reinforcing information that allows *X. montezumae* females to strongly reject *X. continens* males as potential mates.

The situation is more complicated in the *X. continens* female–*X. continens*/*X. montezumae* male interaction because the information transmitted by the male-based cues appears to be arrayed in antagonistic combinations. So, male *X. continens* transmit 'conspecific, sneaker male' while the *X. montezumae* signal is interpreted as 'not a conspecific but a courting male'. The presence of 'courting male' appears to compensate for the absence of species-specific information in the *X. montezumae* cue when *X. continens* females are the receiver. Because the two species are closely related, we can assume that the components of the signal transmitting information about species identity will overlap somewhat, so the presence of 'courting male' plus the presence of some similarity in species identification combine to produce a strong preference for the heterospecific. The asymmetry in responses of female *X. montezumae* and *X. continens* based on chemical cues is similar to that reported for the *X. pygmaeus*–*X. nigrensis* pairing based on visual cues (Ryan & Wagner 1987).

This, of course, raises the question of why female *X. pygmaeus* do not show a similar strong preference for the scent of courting males from a close relative (*X. nigrensis*). Females were ostensibly faced with the same antagonistic pairing of male messages as *X. continens* ('I am a conspecific but I am a sneaker male' versus 'I am not a conspecific but I am a courting male'), so why the difference? There are a number of potential answers to this question. For example, the *X. pygmaeus* population tested by [Crapon de Caprona & Ryan \(1990\)](#) may have been in contact with a heterospecific long enough for selection to begin shifting the female's attraction away from the scent of courting males. Such a dynamic has been reported for female *X. pygmaeus* that show a reduced visually based preference for large body size in areas of sympatry with the sworded *X. cortezi* (G. G. Rosenthal & M. J. Ryan, unpublished observations). On the other hand, the cue may have been slightly too concentrated in the *X. pygmaeus* study (flow rate: 8.5 ml/s for *X. pygmaeus* versus 1.2 ml/s for *X. continens*), causing females to move away from the source of the sneaker-based odour (a relationship between increasing concentration and avoidance has been noted for zebrafish, *Brachydanio rerio*, ictaluriid catfish and Arctic char, *Salvelinus alpinus*: [Lambert & Resink 1991](#)).

It is also possible that the result of the conflict between the 'sexual status' and the 'species' components of the olfactory cue in the choice tests will depend upon the strength of the female's attraction to the different components. This, in turn, will depend upon (1) the degree of overlap between the 'species' message in the *X. pygmaeus*–*X. nigrensis* and *X. continens*–*X. montezumae* pairings, (2) the exact chemical profiles signalling 'sneaker male' and 'courting male' in the four species (e.g. perhaps a *X. pygmaeus* male is less strongly identified as a 'sneaker' than is an *X. continens* male, or perhaps an *X. montezumae* male is more strongly identified as a 'courter' than is an *X. nigrensis* male) and (3) the sensitivities of females from each species to the cue components.

Overall, then, just like the colour pattern in threespine sticklebacks ([McLennan 2006](#) and references therein), the olfactory cue in northern swordtails is multicomponent, containing information about the species identification (I am a conspecific versus I am not a conspecific), sex (I am a male) and sexual status (I am a courter versus I am a sneaker) of the sender. Female choice in these fishes thus represents the outcome of a complicated, context-dependent interaction between multicomponent and multimodal cues. This interaction appears to have followed different evolutionary trajectories in the two 'sneaker-only' species in the group. Strength of preference for the conspecific in *X. pygmaeus* females is the outcome of a preference for large courting males based on visual stimuli ([Ryan & Wagner 1987](#)), something that is widespread in fish ([McLennan, in press](#) and references therein) and thus is probably plesiomorphic for swordtails ([Ryan & Wagner 1987](#); [Morris & Ryan 1995](#); [Morris et al. 1996](#)), plus an interaction between components of the olfactory cue signalling species identity and male sexual status in which the response to 'courting male' is antagonistic to but does not override the response to 'conspecific' ([Crapon de Caprona & Ryan 1990](#)). The response of

X. continens females, on the other hand, reflects a decrease in the preference strength for large courting males based on visual stimuli ([Morris et al. 2005](#)) plus an interaction between components of the olfactory cue signalling species identity and male sexual status in which the response to 'courting male' overrides the response to 'conspecific'. The different female responses to the olfactory cue in these two species indicate that the outcome of a conflict between cue components depends on the strength of the receiver's attraction to each component. These results support [Crapon de Caprona & Ryan's \(1990\)](#) proposal that multimodal cues need not evolve in a coordinated fashion and further highlight the need to delineate the structure of the male-based cue in the northern swordtail clade, matching that structure with variance in female response to components signalling 'sexual status' and 'degree of relatedness (species)'.

Acknowledgments

We thank the Mexican government for permission to collect fish in their country, C. Hubbs for providing space in his laboratory in 1995, J. Bull, L. Dries, C. Hubbs and G. Rosenthal for animated discussions during the course of the experiments and D. Brooks and J. Ward for comments on the manuscript. This research was funded in part by a Natural Sciences and Engineering Research Council of Canada (NSERC) Postdoctoral Fellowship, followed by an NSERC Discovery Research grant to D.A.M. and a National Science Foundation grant to M.J.R.

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