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Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*

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Abstract. Female *Xiphophorus cortezi* responded to olfactory cues from both conspecific males and heterospecific *X. nigrensis* and *X. montezumae* males when given a choice between the stimulus and water. When given a choice between the conspecific and heterospecific cues, however, females demonstrated a strong preference for the conspecific stimulus. Of the two heterospecific species, females responded more strongly to their close relative *X. nigrensis* than they did to the more distantly related *X. montezumae*. Mate recognition in northern swordtails is evidently not a simple process based upon a response to one variable, but the outcome of complex responses to information from at least both visual and olfactory cues.

Animals are immersed in a whirlwind of sensory stimuli. Every species, however, responds to only a fraction of that whirlwind, allowing conspecifics to recognize and locate one another in what might otherwise be a chaotic universe. The intuition that sensory stimuli were partitioned in a species-specific manner was a critical prerequisite to formulating concepts of reproductive isolation, mate recognition and sexual selection (Andersson 1994). Although this intuition was based largely upon data for visual and acoustical signals, evidence that chemical cues transmit species-specific information has also begun to accumulate over the past three decades (e.g. moths: Löfstedt 1993; cryptic or mimetic butterflies: Vane-Wright & Boppré 1993; *Drosophila*: Ewing & Manning 1967; Narda 1968; plethodontid salamanders: Uzendoski & Verrell 1993; mammals: Ferkin & Johnston 1995).

During that time, only three representative clades from the most species-rich vertebrate group on earth, the teleost fishes, have been studied, and the results of those studies have been ambiguous. With the exception of one species, the snakeskin gourami, *Trichogaster pectoralis*, male belontiids demonstrated no specificity in their response to female-based chemical cues (McKinnon & Liley 1987 and references therein). Similarly, Cardwell et al. (1992) reported that two sympatric species of suckers, *Catostomus commersoni* and *C. catostomus*, did not differ in their olfactory-receptor sensitivity to various components of putative cypriniform sexual pheromones. The authors cautioned that further behavioural and physiological testing was required before a role for chemical cues in the reproductive isolation of the two suckers was abandoned. Finally, female *Xiphophorus nigrensis*, a species from the northern swordtail clade, displayed a strong preference for olfactory cues from their own males versus cues from the closely related *X. pygmaeus*. *Xiphophorus pygmaeus* females also showed a strong attraction to their own males versus heterospecific ones in terms of number of lunges, but not total time (Crapon de Caprona & Ryan 1990). Overall, these three studies suggest that odour might not be as important for piscine conspecific mate recognition and reproductive isolation as are cues transmitted through visual and electromagnetic sensory channels.

These results are counterintuitive given the general importance of chemical cues in almost every aspect of a fish's life, from parental care, through predation and alarm signalling, to foraging, schooling and migration (reviewed in Hara 1986; Reebs 1994). More importantly from a mating system perspective, numerous fish are capable of recognizing conspecifics of the opposite sex (summarized in Liley 1982; Hara 1994; Sørenson

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& Scott 1994), and some species are even capable of differentiating among individuals, based solely upon olfactory stimuli (e.g. Brown & Smith 1994). Given these abilities, it is intriguing that ostensibly so little information about species identity should be contained within the chemical cues. Since only three groups have been studied, however, this begs the question of just how general this pattern is among fish.

To address this question, we began a comparative investigation of the response by females to olfactory cues produced by males for representative species from the three lineages within the northern swordtail clade. In this paper, we present the results from the first experiment in our comparative study: the response by *X. cortezi* females to olfactory cues produced by conspecific, *X. montezumae* and *X. nigrensis* males. During this investigation we asked four questions. (1) Do females respond to olfactory cues produced by male conspecifics? (2) Do females respond to olfactory cues produced by male heterospecifics? (3) Do females discriminate between olfactory cues produced by male conspecifics and heterospecifics? (4) Do females respond differently to olfactory cues from different heterospecific species?

MATERIAL AND METHODS

Study Animals

We collected *X. montezumae* and *X. cortezi* individuals from Río Gallinas, Agua Buena, Mexico and Río Huichihuayán, Huichihuayán, Mexico respectively in April, 1995. *Xiphophorus nigrensis* subjects were progeny of field-captured animals, collected from Río Choy, Mexico and raised in outdoor ponds at Brackenridge Field Lab, Austin. Fish were maintained in 227-litre stock tanks in the laboratory during May–July 1995. Lights were set on a 12:12 h light:dark cycle. Temperature fluctuated between 20 and 22°C in the stock tanks. We fed fish ad libitum on live brine shrimp nauplii and Tetra Min flakes twice daily. Standard length (tip of snout to base of caudal peduncle) of all individuals used in the experiments was measured with dial calipers.

Housing Test Females

We housed individual test females 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 isolated from all other fish. We fed them ad libitum on brine shrimp nauplii at 0700 hours and 1700 hours daily. The jars were emptied and refilled every day to remove excess food. Three of the 18 females we tested initially were unresponsive, so data analysis was based upon 15 females.

Producing the Stimulus

We placed 10 sworded males, representing most of the male size range in the population (*X. cortezi*: 25.9–43.9 mm; *X. montezumae*: 39.7–57.4 mm; *X. nigrensis*: 28.8–38.3 mm), into a 45-litre stimulus tank filled with 32 litres of water and covered it with glass to prevent contamination from air-borne odours. The water had been aerated and charcoal-filtered for 24 h prior to adding the males. We placed a larger holding tank, well planted and containing female conspecifics, beside the stimulus tank to arouse the males during their sojourn. Males remained, fasting, in the stimulus tank for 24 h, then were moved to the holding tank. The stimulus water was used as needed for a maximum of 48 h 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: 32 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 as required for 48 h, then discarded.

Experimental Set-up

The experimental apparatus consisted of two 70-litre tanks (test tanks; 60 × 26 × 30 cm) and four 4.5-litre jars (stimulus jars). We placed the stimulus jars above and behind the test tanks (Fig. 1). A stimulus delivery system was constructed by attaching a 125-mm glass pipette to a piece of 2.5-mm silicone tubing. The system was secured by slipping the open end of the silicone tubing through a piece of 6.0-mm tubing attached to the bottom of the stimulus jar, and slipping the pipette through a piece of 6.0-mm tubing attached

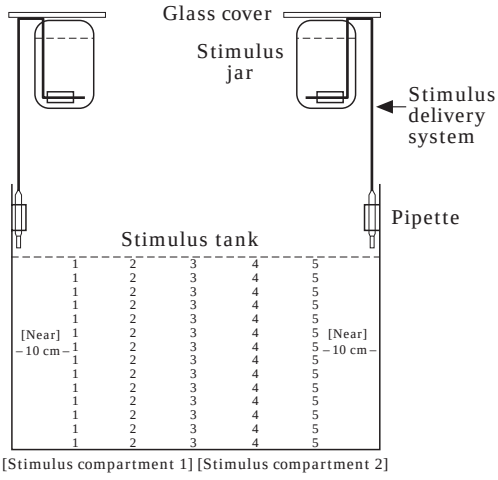

Figure 1. Diagram of experimental set-up.

to the side of the test tank. Each stimulus delivery system was used for only one stimulus type. 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, rather than below the surface, mimicked that disturbance. Preliminary tests showed that this prompted the female to respond quickly and to move between the two sides. Provoking this response is particularly critical in an olfactory experiment because, unlike visual cues, the female must physically move throughout the entire test tank to guarantee exposure to both stimuli.

Stimulus flow was initiated each day by injecting water from the test tank into the stimulus delivery system, placing the open end of the tubing in the stimulus jar, and allowing the stimulus water to flow, via gravity, through the pipette into a drain for 60 s. Flow rate, controlled by the use of metal screw clamps, was set at 1.2 ml/min. At the end of each trial, the pipette was placed back in the stimulus jar so the flow rate rarely needed to be adjusted throughout the test period. Observation of water dyed with food colouring showed that the stimulus flow within each tank was symmetrical, and that, in the absence of a test fish, the currents on either side of the tank had not met after 30 min. To control for side biases, the stimulus was assigned randomly to the left or the right side in each trial.

The test tank was divided into six equal 10-cm compartments with numbered lines on the outside of the tank to pinpoint the female's position during video analysis (Fig. 1). After each trial, the tank was drained, washed with soap, rinsed and dried. Preliminary observations indicated, however, that the olfactory cue bonded to the silicone used to seal the glass panes of the test tank despite these precautions (during the pre-trial period, females responded to the side of the newly cleaned tank that had been the stimulus side in the previous trial). Eventually we discovered that scrubbing the tank vigorously with a 3% solution of hydrogen peroxide and soap, rinsing it with an intense jet of water concentrated along the silicone seams, and drying it thoroughly appeared to remove all vestigial traces of the stimulus.

Experimental Procedure

A female was placed in the test tank, allowed to familiarize herself with her new surroundings for 30 min, then videotaped for 5 min (pre-trial period). Stimulus flow was initiated by simultaneously slipping the pipettes into their respective holders on either side of the tank. We videotaped the trial as soon as the female had passed through both near compartments (Fig. 1). At the end of the trial, we returned the female to her holding jar and allowed her to rest for one day. Females were thus tested every other day. The presentation of test stimuli followed the pattern: conspecific versus control water (days 1–2); heterospecific 1 (*X. montezumae*) versus water (days 3–4); heterospecific 2 (*X. nigrensis*) versus water (days 5–6); conspecific versus heterospecific 1 (days 7–8); conspecific versus heterospecific 2 (days 9–10); heterospecific 1 versus heterospecific 2 (days 11–12). To determine whether the female's behaviour changed across the 12-day experimental period, we videotaped pre-trial tests before the conspecific versus water trials (PT 1: first two days of the experiment) and before the heterospecific 1 versus heterospecific 2 trials (PT 2: last two days of the experiment).

Variables Scored

We analysed the videotapes at a later date, and recorded the following variables.

(1) Total time: the time spent in each stimulus compartment (Fig. 1).

(2) Percentage of time near: the time spent within 10 cm of the stimulus outflow (the time spent in the near compartment/the total time spent in the stimulus compartment; Fig. 1).

(3) Percentage of time interacting: the time spent engaged in lunge/dive + nudging behaviour/ the total time spent in the stimulus compartment. Lunge/dive involved swimming rapidly up the side of the tank towards the stimulus outflow, turning and diving down towards the bottom. The angle of the turn varied from very sharp, at which point the female practically retraced her upwards swim during the dive, to approximately 45°, at which point the dive terminated near the 20 cm boundary. 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. Very intense nudging bouts generally terminated in a bite.

(4) Time spent engaged in head-down behaviour: the fish orients at approximately 90° to the substrate, then swims rapidly around the tank, all fins flared, nose in contact with the substrate. In other fish, head-down behaviour is thought to represent the outcome of an ambivalent motivational state hovering between tendencies to attack and to flee (Tinbergen 1952). It is difficult to assign a particular motivation to this behaviour because the female does not remain in one area, nor does she orient towards any particular point. From watching the fish, head-down in this system appeared to reflect an increased level of agitation in response to an introduced stimulus. Preliminary analysis of the data indicated that there was no difference between the two stimulus compartments in terms of time spent engaged in head-down behaviour. This result confirmed our impression that females tended to move rapidly and stochastically around the tank once they embarked upon a head-down bout. We therefore combined the times from both compartments to get a measure of overall agitation following the detection of an introduced stimulus (or stimuli). The time engaged in head-down behaviour was recorded as a percentage of the 300-s trial period.

Other behaviours observed sporadically were as follows.

(1) Bite: a lunge followed by a jaw snap, generally directed towards the glass side of the tank or toward the stimulus inflow.

(2) Back-up: the female orients horizontally with the substrate, facing away from the stimulus inflow, fins depressed, hovers, then moves backwards in a short, jerky motion towards the stimulus. The movement was repeated several times, followed by a prolonged hover. This behaviour may be homologous with the back-up behaviour signalling sexual receptivity described for female *X. helleri* (Basolo 1990).

(3) Nod: the female, moving horizontally, tilts forward slightly, to no more than a 45° angle with the substrate, then returns to horizontal position. Motion is very rapid and resembles a nod or a bow. This behaviour occurs (a) at the end of a swim-approach (female approaches one side, nods, turns, swims rapidly to other side) and (b) at the end of a lunge/dive (female dives towards substrate, pulls up to a horizontal position, nods, turns and returns to the lunge/dive side).

Statistics

The non-parametric Wilcoxon signed-ranks test was used to evaluate the null hypothesis that there was no difference in the female's response to different cues. Data were analysed using the software package Statview II v. 4.5 (Abacus Concepts, Berkeley, California).

RESULTS

Pre-trial

The females showed no side bias in (1) the total time spent in each stimulus compartment (Fig. 2a), (2) the percentage of time spent near (Fig. 3a), or (3) the percentage of time spent interacting on either side of the tank (Fig. 4a). Females spent approximately 9% of their time engaged in lunge/dive and nudge behaviours during the pre-trial periods. They appeared to be responding to their own reflections in the glass sides of the aquarium; these behaviours were shown in tanks that had never been used for olfactory tests. Some of the variability in the females' test responses may thus have arisen from the conflicting messages they were receiving based upon the chemical (male) and visual (female) information. There was no difference between pre-trial 1 (PT 1) and pre-trial 2 (PT 2) in any of the preceding variables, indicating that the females did not adjust these aspects of

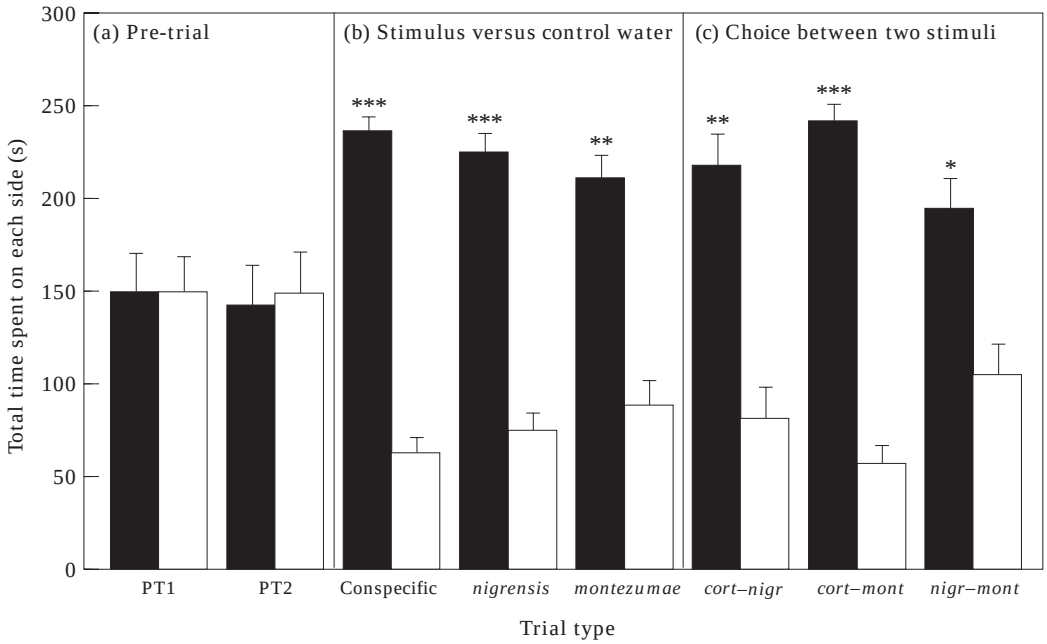

Figure 2. Mean + SE total time that the female *X. cortezi* spent in both stimulus compartments. (a) Pre-trial: ■ = left side, □ = right side. (b) Responses to stimulus (■) versus control water (□). (c) Responses to choice between two stimuli. The species listed first in the choice trial type is represented by a solid bar. PT 1 = pre-trial at beginning of experimental period (days 1–2); PT 2 = pre-trial at end of experimental period (days 11–12); *cort* = *X. cortezi*; *mont* = *X. montezumae*; *nigr* = *X. nigrensis*. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 (see Table I).

their behavioural response across the 12-day experimental session. Head-down bouts were virtually absent during pre-trials (PT 1: $\bar{X} = 0.46$ s; PT 2: $\bar{X} = 0.36$ s).

Stimulus versus Control Water

The results of the statistical analyses are presented in Table I. Females spent significantly more time in the compartment associated with the male odour than they did in the compartment associated with the control water for all three test species (Fig. 2b). While in each compartment, females spent a greater percentage of their time near the conspecific and *X. nigrensis* stimulus than they did near the control water, but there was no significant difference in the percentage of time spent near the male odour versus the control water for the *X. montezumae* stimulus (Fig. 3b). Females interacted more intensely with the stimulus than they did with the control water for all three test species (Fig. 4b). The percentage of time spent engaged in head-down behaviour significantly increased in all trials versus the pre-trial period (conspecific: $\bar{X} = 2.43$ s, $z = -2.04$, $P = 0.05$; *X. nigrensis*: $\bar{X} = 3.11$ s, $z = -2.12$, $P = 0.04$; *X. montezumae*: $\bar{X} = 2.78$ s, $z = -2.12$, $P = 0.03$). There was no significant difference between the trials (conspecific versus *X. nigrensis*: $P = 0.75$; conspecific versus *X. montezumae*: $P = 0.48$; *X. nigrensis* versus *X. montezumae*: $P = 0.92$).

Conspecific versus Heterospecific Stimuli

Females (1) spent significantly more time in the conspecific compartment than they did in the heterospecific compartment (Fig. 2c), (2) spent a greater percentage of time in the compartment near the conspecific stimulus than they did near the heterospecific stimulus (Fig. 3c), and (3) interacted more intensely with the conspecific stimulus than they did with the heterospecific stimulus (Fig. 4c). The percentage of time spent engaged in head-down behaviour increased significantly

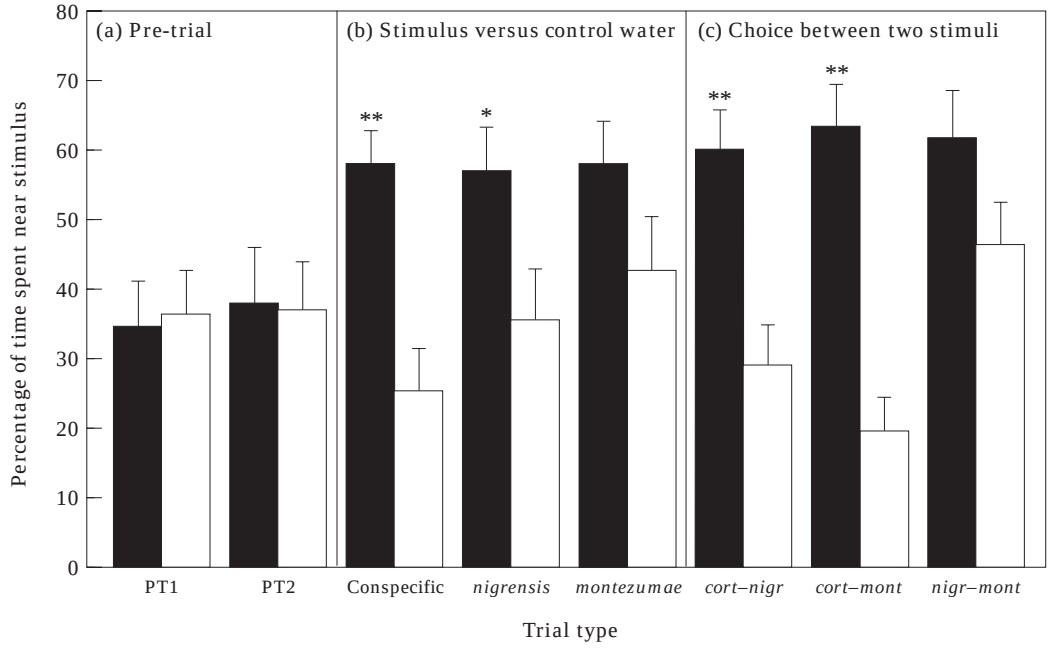

Figure 3. Mean + SE % time that the female *X. cortezi* spent near both stimuli. (a) Pre-trial: ■ = left side, □ = right side. (b) Responses to stimulus (■) versus control water (□). (c) Responses to choice between two stimuli. The species listed first in the choice trial type is represented by a solid bar. PT 1 = pre-trial at beginning of experimental period (days 1–2); PT 2 = pre-trial at end of experimental period (days 11–12); *cort* = *X. cortezi*; *mont* = *X. montezumae*; *nigr* = *X. nigrensis*. $*P < 0.05$; $**P < 0.01$ (see Table I).

in both trials over the pre-trial period (conspecific versus *X. nigrensis*: $\bar{X} = 4.88$ s, $z = -3.11$, $P = 0.002$; conspecific versus *X. montezumae*: $\bar{X} = 2.72$ s, $z = -2.35$, $P = 0.02$). There was no significant difference between the two trials ($P = 0.10$).

Response to Heterospecifics

Females showed a significant attraction to the stimulus versus the control water in the conspecific and both heterospecific tests. Based upon these results, we tested the null hypothesis that the strength of the female's response did not differ among the three species (mate recognition sensu, Ryan & Rand 1993). Response strength was measured as the relative attraction to the stimulus versus the control water for each of the three behavioural variables (y), and was calculated as: [(time engaged in y on stimulus side − time engaged in y on control side)/(time engaged in y on stimulus side + time engaged in y on control side)] × 100 (Fig. 5). There was no difference in the strength of a female's response to olfactory cues from conspecific males versus *X. nigrensis* males for any of the three variables. There was also no difference in the strength of the female's response to olfactory cues from conspecific males versus *X. montezumae* males in terms of the total time spent in the stimulus compartment. *Xiphophorus cortezi* females, however, demonstrated a significantly weaker response to *X. montezumae* males than to conspecific males in terms of the time actually spent near ($z = -2.21$, $P = 0.03$; a in Fig. 5) and time spent interacting ($z = -2.21$, $P = 0.03$; a in Fig. 5) with the *X. montezumae* stimulus. The difference in recognition strength based upon the per cent of time interacting was also significant in the *X. nigrensis* versus *X. montezumae* comparison ($z = -1.92$, $P = 0.05$; b in Fig. 5).

Differences in the female's response to heterospecifics shown in the stimulus versus control water trials were not detected during the conspecific versus heterospecific choice trials. There was, however, a non-significant trend towards a weaker response to the *X. montezumae* cue than to

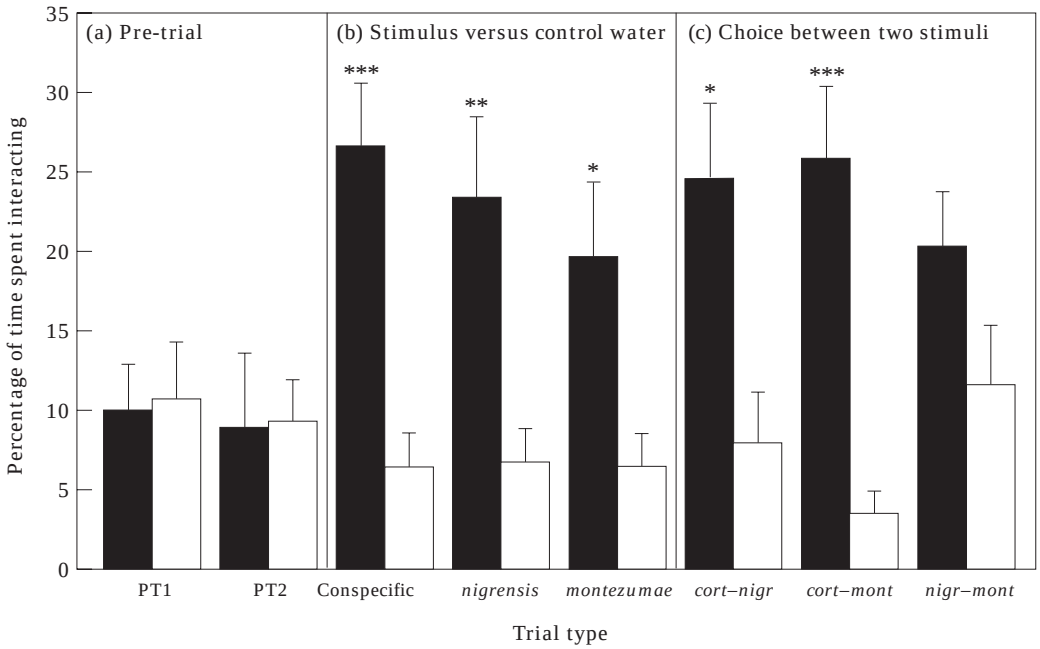

Figure 4. Mean + SE % time that the female *X. cortezi* spent interacting with both stimuli. (a) Pre-trial: ■ = left side, □ = right side. (b) Responses to stimulus (■) versus control water (□). (c) Responses to choice between two stimuli. The species listed first in the choice trial type is represented by a solid bar. PT 1 = pre-trial at beginning of experimental period (days 1–2); PT 2 = pre-trial at end of experimental period (days 11–12); *cort* = *X. cortezi*; *mont* = *X. montezumae*; *nigr* = *X. nigrensis*. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 (see Table I).

Table I. Results of statistical analysis of olfactory data

	Trial type					
	Stimulus versus control water ($N = 15$)			Choice between two stimuli ($N = 15$)		
	Conspecific versus water	*montezumae* versus water	*nigrensis* versus water	Conspecific versus *montezumae*	Conspecific versus *nigrensis*	*montezumae* versus *nigrensis*
Total time						
z	− 3.41	− 3.18	− 3.41	− 3.41	− 2.78	− 2.22
P	0.0007	0.002	0.0007	0.0007	0.006	0.03
Percentage of time near						
z	− 2.95	NS	− 2.56	− 3.24	− 2.90	NS
P	0.004	0.43	0.02	0.002	0.004	0.19
Percentage of time interacting						
z	− 3.41	− 2.04	− 2.86	− 3.35	− 2.23	NS
P	0.0007	0.05	0.005	0.0007	0.03	0.09

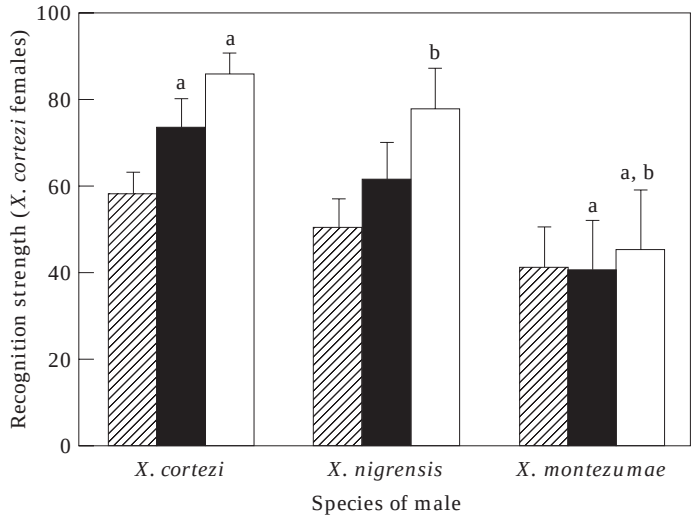

Figure 5. Strength of *X. cortezi* females' attraction to the olfactory cue from conspecific and heterospecific males. ▨ = Total time; ■ = percentage of time spent near; □ = percentage of time spent interacting. a: Response to *X. montezumae* was significantly less than response to conspecific for the behavioural variable; b: response to *X. montezumae* was significantly less than response to *X. nigrensis* for the behavioural variable (see text).

the *X. nigrensis* cue (Figs 2–4c). In the choice trial between the two heterospecific stimuli, females spent significantly more time overall on the *X. nigrensis* side than they did on the *X. montezumae* side (Fig. 2c); however, there was no difference in the percentage of time that the female spent near each stimulus (Fig. 3c), nor was there any difference in the intensity of her response to the two heterospecific stimuli (Fig. 4c).

DISCUSSION

Female *X. cortezi* are capable of detecting and responding to olfactory cues from conspecific and closely related heterospecific males, and of discriminating between those cues. This result indicates that the olfactory stimulus is transmitting information about species identity that is useful to a female in the initial stages of mate recognition. The olfactory cue, however, does not appear to play a role in mate preference (sensu Ryan & Rand 1993: an individual's ability to differentiate among the subset of potential mating partners). Although female *X. nigrensis* show a strong preference for large versus small males based upon visual cues (Ryan et al. 1990), they do not differentiate between male size classes based upon chemical cues (Crapon de Caprona & Ryan 1990). There are two possible explanations for this lack of discrimination; either not enough information is being transmitted to permit the evolution of intraspecific mate preference based upon olfaction in these fish, or the olfactory cue functions as an aggregating signal and is not sex-specific. To examine the second explanation more closely, we need to determine whether female *X. cortezi* respond to olfactory cues from conspecific females, and whether they differentiate between cues from males and females. The observation, however, that female *X. cortezi* are capable of distinguishing between the heterospecific and conspecific cue even though they respond very strongly to both heterospecific cues, hints that the olfactory cue may be transmitting a more complex message than either 'aggregate with conspecifics' or 'aggregate with congenerics'.

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 from the male before she can actually see him. 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 (Wiley 1994). Thus, female angelfish, *Pterophyllum scalare*, achieve their highest spawning rates only when exposed to both chemical and visual stimuli from males (Chien 1973). The long-distance and reinforcing aspects of olfactory signals combine to decrease the costs incurred by a female during the mate selection process and increase her probability of finding a mate (Real 1990; Jumper & Baird 1991). The importance of the chemical cue to mate location in *Xiphophorus* is underscored by observations that female swordtails bear their smallest broods during the rainy season (Morris & Ryan 1992). Since females who experience fewer mating attempts might have smaller broods (Borowsky & Kallman 1976; Travis 1989), this seasonal effect may in part reflect problems associated with tracking an olfactory cue through turbulent, murky waters.

If the olfactory cue is sex-specific, then it may play two more subtle roles in the swordtail breeding system. First, olfactory stimuli from conspecific males influence the physiology of female teleost receptivity, including gonadotrophin release, induction of ovulation, and the appearance of courtship behaviour (e.g. Van den Hurk et al. 1987; Resink et al. 1989b). The male cue is, in effect, acting as a primer of female mating behaviour in many fish (and vice versa for female-based olfactory cues and male behaviour). Second, we discovered that females moved away from the conspecific stimulus when it was dripped rapidly into the tank (a relationship between increasing concentration and avoidance has been noted for zebrafish, *Brachydanio rerio*, ictaluriid catfish, and Arctic charr, *Salvelinus alpinus*: Lambert & Resink 1991). High population densities adversely affect female fecundity in poeciliids (Warren 1973; Schoenherr 1977; Dahlgren 1979), as well as male growth rate in various *Xiphophorus* species (Borowsky 1987a, b). Male size, in turn, influences female choice of a mating partner (Ryan et al. 1990), male–male competition for access to females (Morris et al. 1992) and male swimming endurance (Ryan 1988). The concentration of the olfactory cue may thus function to disperse conspecifics when population density gets too high in a particular area, reducing the negative effects of overcrowding. It seems probable, therefore, that olfactory stimuli produced by male swordtails play a role in the physiological priming of the female, in mate recognition and location, and in reinforcing the information provided by visual signals. The results of this study are congruent with those reported for two other members of the northern swordtail clade, *X. nigrensis* and, to a lesser extent, *X. pygmaeus* (Crapon de Caprona & Ryan 1990). Evidence is thus beginning to accumulate supporting the proposal that olfactory cues are as important in mate recognition and reproductive isolation in at least one group of fish as they are in a variety of terrestrial systems.

Given the existence of species-specific chemical cues in northern swordtails, what is their likely source? Conjugated steroids (steroid glucuronides) from the seminal vesicle, testis and urogenital tract, and amino acids from the seminal vesicle, urine and skin mucus of conspecific males act as powerful attractants in a variety of teleost species (e.g. Lambert & Resink 1991; Moore & Scott 1991; Becker et al. 1992). In the African catfish, *Clarias gariepinus*, the level of the stimulatory effect of steroid glucuronides depends upon their structure and their relative concentrations in the seminal fluid, implying that the blend of these substances can transmit species-specific information. The complex nature of the cue allows a more intricate message to be encoded than would be possible based solely upon the structurally conservative, non-conjugated gonadal steroids (Laumen et al. 1974; Resink et al. 1989a). In the northern swordtail species investigated to date, that message may contain at least two bits of information: 'I am a male' (gonadal steroid-based component) and 'I am a member of species *y*' (either a particular blend of conjugated gonadal steroids or a protein-based component).

The manner in which a female ultimately responds to a male represents the outcome of higher-level signal processing (Resink et al. 1989c). For example, feedback from the protein component could directly affect the response to the gonadal steroid fraction; as the protein becomes less recognizable to the female's sensory system, the response to the gonadal steroid decreases. This hypothesis may explain why female *X. cortezi* respond less strongly to *X. montezumae* males than they do to *X. nigrensis* males. Both species provoke some level of response based upon the fraction of the chemical cue that is transmitting the plesiomorphic

message. 'I am a male'. *Xiphophorus cortezi*, however, is more closely related to *X. nigrensis* than it is to *X. montezumae* (Rauchenberger et al. 1990; Meyer et al. 1994), so the most parsimonious explanation of this result is that *X. cortezi* and *X. nigrensis* share an apomorphic change in the cue-receiver system that is absent in *X. montezumae*. We will pursue the proposal that the female's response to heterospecific olfactory cues is influenced by phylogenetic distance (e.g. Ryan & Rand 1995) in studies with *X. montezumae* and *X. nigrensis*.

The signal-receiver system mediating mate recognition is not perfect in these fish: females did make mistakes in the choice trials. The number of those mistakes, however, was in the direction predicted by genealogy: females made no mistakes in the conspecific-*X. montezumae* choice test and only two mistakes in the conspecific-*X. nigrensis* choice test. The females who made mistakes or approached a no-choice pattern during the conspecific-*X. nigrensis* tests (females 2, 3, 6, 7) were not the same females as those who approached a no-choice pattern in the conspecific-*X. montezumae* trials (females 6, 10, 13, 15). We also found no correlation between the intensity of a female's preference for the heterospecific cue during the stimulus versus control water trials and the intensity of her preference for that cue in the conspecific–heterospecific choice tests. The female's response during the choice test, therefore, was not predictable based upon the strength of her response to the heterospecific cue alone. Finally, when the two errant females from the conspecific-*X. nigrensis* choice trials were retested the following day, both showed a strong preference for the conspecific cue. Taken together, these results imply that the female's response to a particular male-based olfactory cue is context-dependent. Although the majority of the female's responses tend towards the conspecific when averaged over her life, at any point she may be attracted to the olfactory cue from a heterospecific male. It thus seems unlikely that inter-female variability in mate recognition based upon olfactory stimuli is genetically based in the traditional Mendelian sense. Rather, it seems more plausible that females differ in terms of the probability that they will respond to an inappropriate olfactory cue during their lifetime.

Whatever the ultimate identity of the chemical cue, the results from this study indicate that both the chemical cue, and the female's ability to detect and preferentially respond to it, are evolving rapidly in the northern swordtails. At first glance, this rapid evolution is intriguing because it has occurred, to a large extent, in allopatry. Upon closer examination, a more intriguing possibility emerges: members of the northern swordtail clade are generally allopatric with respect to their sister species, but are often sympatric with the more distantly related congener, the platyfish *X. variatus* (or the *X. variatus* clade: Rauchenberger et al. 1990). Speciation in the northern swordtail clade has been largely vicariant, occurring during the separation of major river drainages (Rosen 1979; Rauchenberger et al. 1990), so changes in the olfactory cue did not initiate speciation in this group. Once an ancestral species had been subdivided geographically, however, the olfactory cue's evolution, and its subsequent effects on mate recognition, may have been influenced by the presence of congeneric species, and this, in turn, may have contributed to the completion of the speciation process. Given that the platyfish and at least one of the northern swordtails, *X. nezahualcoyotl*, are capable of hybridization (Rauchenberger et al. 1990), the concept of premating isolating mechanisms need not be restricted to zones of secondary contact between differentiating sister species. In some cases, both geographical isolation and interactions with closely related, but non-sister, congeners may play mutually reinforcing roles in the speciation process.

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