



Relative Abundance of *Xiphophorus* Fishes and Its Effect on Sexual Communication

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Abstract

As major river drainages separated and stream capture occurred, some populations of *Xiphophorus* fishes became geographically isolated from one another, while others came into contact with new *Xiphophorus* species. Sexual communication and mechanisms of reproductive isolation were likely influenced by the variation in community structure each population experienced. One swordtail species, *Xiphophorus birchmanni*, occurs both in streams where other *Xiphophorus* species are rare, or more typically with large populations of congeners. Using the *X. birchmanni* system, we tested the theory that species recognition should be favored when the opportunity for hybridization is high. We found that at a site where the congener, *X. variatus*, is the predominant species, female *X. birchmanni* have a strong preference for conspecific cues; however, female *X. birchmanni* from a location where *X. variatus* are rare do not discriminate between conspecific and heterospecific cues. Moreover, we show evidence that chemosignals used by male *X. birchmanni* to attract females are unique between the two populations that differ in community structure. Our findings suggest that not only can the presence or absence of a congener influence sexual communication, both in terms of female preference and male trait, but the abundance of congeners may also be an equally important source of interpopulation variation.

Introduction

Populations often show striking differences between one another, particularly in terms of male sexual traits and female preferences (Hill 1994; Endler & Houde 1995; Verrell 1999). In particular, the ecological community an individual is exposed to can have a great impact on its behavior. The proportion of predators, for example, can increase risk of sexual signaling and impose constraints on mate traits and female preferences, as seen in the guppy, *Poecilia reticulata* (Stoner & Breden 1988; Godin & Briggs 1996). Sympatric congeners can also influence sexual communication since selec-

tion to avoid hybridization may favor signal divergence (Howard 1993; Servedio & Noor 2003; Coyne & Orr 2004). For example, it is often observed that sympatric species partition acoustic communication channels (Drewry & Rand 1983; Duellman & Pyles 1983; Wilczynski et al. 1993). Similarly, in Lake Victoria, sympatrically living, closely related haplochromine male cichlids often differ by color (Seehausen et al. 1997). Furthermore, sympatric heterospecifics may influence receiver behavior, in addition to signal evolution, by altering female preferences for conspecifics (e.g., Gerhardt 1994; Höbel & Gerhardt 2003; Pfenning 2000; Saetre et al. 1997).

Selection for communication divergence is greatest in sympatry when the cost of hybridization is high (Howard 1993; Coyne & Orr 2004). Reproductive character displacement predicts that geographic variation among conspecific populations will result from the distinctive community to which each population is exposed (Brown & Wilson 1956; Butlin 1987; Howard 1993; Coyne & Orr 2004). This theory can be further extended, beyond comparing mechanisms of reproductive isolation between conspecific and heterospecific populations in sympatry and allopatry, and looked at on a continuous scale of sympatric-heterospecific abundance. Selection for reproductive isolation between sympatric species is greatest when the costs incurred from hybridization are high (Peterson et al. 2005); moreover, the cost of indiscriminate mating is relative to the number of potential mis-matings. We hypothesize, therefore, that as the abundance of sympatric heterospecifics increase, and thus the potential for hybridization, the divergence in sexual communication also will increase.

Xiphophorus birchmanni and *X. variatus* represent an ideal system to test questions of communication divergence in relation to relative population density. Both congeners occur in the southeast portion of Río Pánuco basin in Northeastern Mexico in the state of Hidalgo. *Xiphophorus birchmanni* is a member of the nine-species clade of northern swordtails, and often occurs in sympatry with a *X. variatus*, a member of the five-species clade of southern platys. Although phylogenetic relationships within the major clades (northern swordtails, southern swordtails, northern platys, and southern platys) remain ambiguous, the major clades are clearly differentiated based on morphology, pigmentation, electrophoretic characters (Rauchenberger et al. 1990), and DNA sequence data (Marcus & McCune 1999).

When *X. birchmanni* and *X. variatus* occur in sympatry, the relative density of the two species can vary greatly among sites. In the Río Garces, at the town of Garces, *X. variatus* are the predominant species, outnumbering *X. birchmanni* by approx. 4:1; In contrast, in the Río Conzintla at Huiznopal *X. birchmanni* outnumber *X. variatus* at a similar frequency (see Results). These two habitats, however, are quite similar. Both sampling sites are broad, warm lowland streams, with *X. birchmanni* inhabiting rocky areas at the stream margins and in backwaters. Community composition is nearly identical at both sites: *X. birchmanni* and *X. variatus* are the only *Xiphophorus* species, potential predators include birds, aquatic snakes, cichlids, characids, and eleotrids, and poten-

tial competitor including cyprinids, ictalurids, and several other genera of poeciliids.

It is not unusual for *Xiphophorus* species to co-occur with closely related congeners (Rauchenberger et al. 1990), and thus mate choice is important in this group of fishes that lack mechanisms of post-mating reproductive isolation (Constantz 1989). Reproductive isolation is heavily mediated by chemical communication in swordtails. For example, female *Xiphophorus* often show robust mating preferences for chemical cues produced by conspecific males (Crapon de Caprona & Ryan 1990; McLennan & Ryan 1997, 1999) despite showing preferences for the visual cues of heterospecifics (Ryan & Wagner 1987; Crapon de Caprona & Ryan 1990; Basolo 1995; Hankison & Morris 2003). In this study, we show evidence for communication divergence, in terms of male chemosignals and female mate choice, in two populations (Garces and Huiznopal) of *X. birchmanni* that differ in abundance relative to the sympatric congener, *X. variatus*.

Methods

Sexually mature *X. birchmanni* and *X. variatus* adults were collected from the Río Garces, at Garces, Hidalgo, Mexico (Rauchenberger et al. 1990), and from the Río Conzintla at Huiznopal, Hidalgo, Mexico (Rosenthal et al. 2003). We used baited minnow traps and seine nets to collect fish, and focused our collection efforts on mature adults of the targeted population, immediately releasing by-catch at the point of capture. We collected 300 adult fish at both locations to perform a population census, individuals were all sexually mature and were easily identified to species.

Sexually mature females were isolated from males for at least 2 wks prior to testing and were housed in single-population, single-species, 40 l aquaria with vegetation. Animals were maintained on a 12:12 h light: dark cycle and were fed TetraMin flakes and live brine shrimp. All fish were fed the same diet to control for the possibility that individuals maintained on different diets may elicit different responses (Fisher & Rosenthal 2006b), and standard length was measured to control for size differences. Fish were housed and tested at Boston University, Boston, MA in Feb.–July 2005.

Female Preference Tests

Stimulus preparation and preference tests closely followed published methods (Fisher & Rosenthal

Table 1: Summary of female preference tests on male chemical cues from two different populations, stimulus A and B

Focal female	Stimulus A	Stimulus B	Female preference
<i>Xiphophorus birchmanni</i> –Garces	<i>X. birchmanni</i> –Garces	<i>X. variatus</i> –Garces	<i>X. birchmanni</i> –Garces
<i>X. birchmanni</i> –Garces	<i>X. birchmanni</i> –Garces	<i>X. birchmanni</i> –Huiznopal	None
<i>X. birchmanni</i> –Garces	<i>X. birchmanni</i> –Huiznopal	<i>X. variatus</i> –Garces	<i>X. birchmanni</i> –Huiznopal
<i>X. birchmanni</i> –Huiznopal	<i>X. birchmanni</i> –Huiznopal	<i>X. birchmanni</i> –Garces	None
<i>X. birchmanni</i> –Huiznopal	<i>X. birchmanni</i> –Huiznopal	<i>X. variatus</i> –Huiznopal	None
<i>X. variatus</i> –Garces	<i>X. variatus</i> –Garces	<i>X. birchmanni</i> –Garces	<i>X. variatus</i> –Garces
<i>X. variatus</i> –Garces	<i>X. variatus</i> –Garces	<i>X. birchmanni</i> –Huiznopal	None

Female preference indicates that focal female associated significantly more ($p < 0.05$) with a particular stimulus.

2006a,b; Fisher et al. 2006; McLennan & Ryan 1997, 1999. See McLennan & Ryan 1997 for diagram of experimental setup). Stimulus water for female mate choice experiments was prepared by moving five males (standard length 48 ± 2 mm) into a single 20 l collection aquarium, which was adjacent to a 20 l aquarium containing five conspecific females to provide them visual stimulation. Males remained in the collection aquarium for 3 h. Preference tests were conducted in an aquarium (length \times width \times height = $75 \times 30 \times 30$ cm) divided lengthwise into three equal sections by lines drawn on the sides of the tank, and filled with 40 l of water. Each test tank had two stimulus delivery systems located at either end of the tank carrying stimulus water from two different populations. Each stimulus delivery system consisted of 2.5 mm silicone tubing fixed to the bottom of a collection aquarium with a suction cup on one end, and 1 cm above the waterline of the test tank on the other end. The collection aquarium was raised above the test tank to aid the flow of stimulus water into the test tank, and a valve controlled the flow to a rate of 5 ml/min.

Each female was acclimatized for 30 min in the test tank containing testing water; stimulus flow was then initiated. A preference test began once the female had passed into both side compartments, and continued for 600 s. If the female did not visit both compartments within 300 s, the trial was ended and the data was not used in the analysis. We scored association time as the total time spent within 25 cm of stimulus outflow. Association time is widely used in studies of mate choice in fishes, and is the standard measure of mating 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). Association time is thus a meaningful estimate of mating preference.

We tested female preference with individuals from all three populations: *X. birchmanni* and *X. variatus* collected from Garces, and *X. birchmanni* collected from Huiznopal. Eighteen *X. birchmanni* females were tested in each trial and were not recycled between trials (total $N_{\text{Garces}} = 54$, $N_{\text{Huiznopal}} = 36$). Twenty-five *X. variatus* females were each tested under two different testing conditions, with the stimuli presented in a randomized fashion to control for order effects. Females that did not respond to both stimuli within 300 s were removed from the analysis. Stimuli and focal females used in each treatment are described in Table 1. To assess female preference for the two stimuli presented within each set of trials, we analyzed the association time data using paired, two-tailed *t*-tests.

Results

Approximately 81% of fish collected at Garces were identified as *X. variatus*, and 19% as *X. birchmanni* ($N_{\text{birchmanni}} = 57$, $N_{\text{variatus}} = 243$). At Huiznopal, 23% of fish were identified as *X. variatus* and 77% as *X. birchmanni* ($N_{\text{birchmanni}} = 231$, $N_{\text{variatus}} = 69$).

In mate preference tests, we found that female *X. birchmanni* collected from Garces were more attracted to male chemical cues from conspecific males than sympatric *X. variatus* cues, whether the conspecific males were from the same population (*t*-test: $t = 2.77$, $df = 13$, $p = 0.016$; Fig. 1), or an allopatric population at Huiznopal (*t*-test: $t = 2.19$, $df = 16$, $p = 0.044$; Fig. 1). However, these females did not discriminate between allopatric and sympatric conspecific male cues (*t*-test: $t = 1.31$, $df = 17$, $p = 0.207$; Fig. 1).

Xiphophorus birchmanni females collected from Huiznopal also did not discriminate between male cues from the two *X. birchmanni* populations (*t*-test: $t = 0.009$, $df = 7$, $p = 0.931$; Fig. 1). Nor did Huiznopal females discriminate between cues from sympatric conspecifics and *X. variatus* (*t*-test: $t = 0.429$, $df = 14$, $p = 0.674$; Fig. 1).

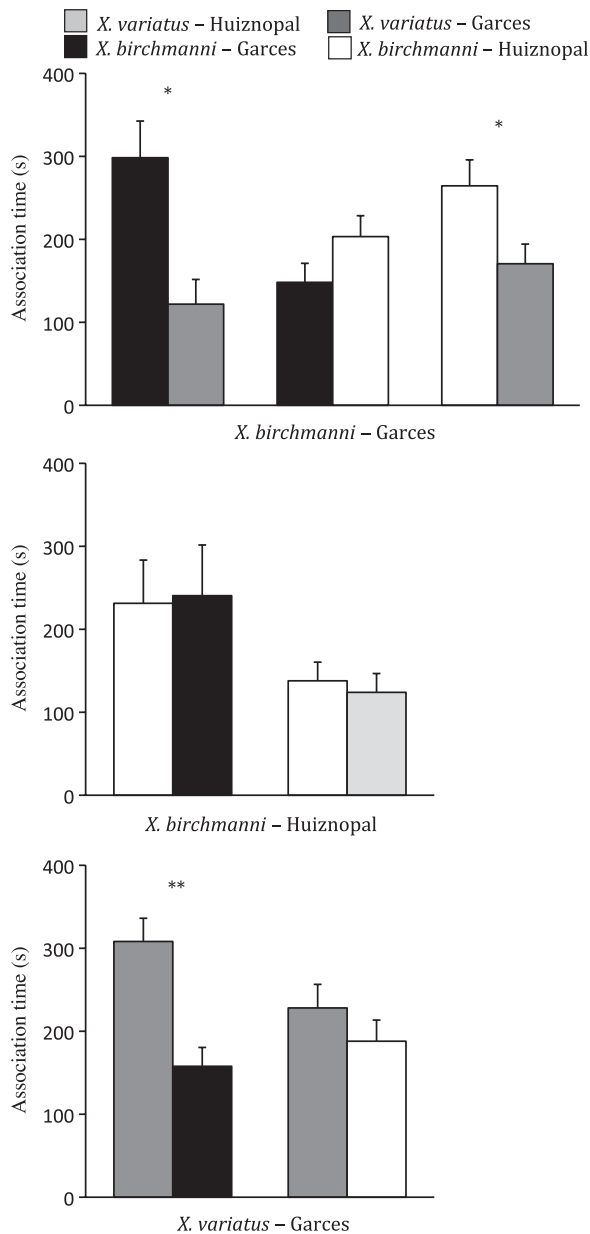


Fig. 1: Mean + SE association time of focal females from three different populations (*Xiphophorus birchmanni* from Garces, *X. birchmanni* from Huiznopal and *X. variatus* from Garces) in a simultaneous choice tests with male chemical stimuli from two different populations, as indicated by the color of the bar. * $p < 0.05$, ** $p < 0.005$.

Finally, *X. variatus* females associated significantly more with cues from conspecific males when the alternative was sympatric *X. birchmanni* (t -test: $t = 3.16$, $df = 21$, $p = 0.005$; Fig. 1), but did not discriminate when the alternative was *X. birchmanni* from Huiznopal (t -test: $t = 0.887$, $df = 22$, $p = 0.384$; Fig. 1).

Discussion

Our results show that *X. birchmanni* females from a population outnumbered by the congener *X. variatus* (Garces) prefer conspecific male cues over cues of sympatric-heterospecific males, even if the conspecific males are from an allopatric population. In contrast, *X. birchmanni* females collected from a site where congeners are more rare (Huiznopal) did not discriminate against cues from heterospecific males. Further, we found that *X. variatus* females avoid cues of sympatric *X. birchmanni* males, but do not discriminate between conspecific and allopatric *X. birchmanni* cues. Taken together, these findings suggest three things: 1) when congeners are abundant, there is stronger selection for females to avoid heterospecific males, 2) females are under greater selection pressure to avoid sympatric-heterospecific mates than allopatric heterospecifics, and 3) the *X. birchmanni* mating signal differs between populations, even if *X. birchmanni* females do not attend to the difference.

We hypothesize that the behavioral differences exhibited by *X. birchmanni* females from the two populations, Garces and Huiznopal, reflects the relative chance of mating with a heterospecific in each location. When there is a higher risk of mating with a heterospecific male, selection should favor individuals who discriminate against heterospecific mates if hybrid offspring result in lower fitness (Liou & Price 1994). Such is the case in studies of other *Xiphophorus* crosses: hybrid offspring can develop and die of diseases such as melanoma (Kraehn et al. 1995; Kazianis & Walter 2002), and suffer reduced fitness under conditions of environmental stress (Mitchell et al. 2004). Although *X. birchmanni* readily hybridize with their sympatric sister species *X. malinche* (Rosenthal et al. 2003), evidence suggest that hybridization is recent (Rauchenberger et al. 1990) and is mediated by environmental disturbance (Fisher et al. 2006). *Xiphophorus variatus* are more distantly related to *X. birchmanni* than *X. malinche*, and therefore negative fitness consequences are likely to be greater (Coyne & Orr 2004). Since mating with *X. variatus* is likely to be more costly for *X. birchmanni* females, this may impose greater constraints on them in terms of discriminating against the more closely related *X. malinche*.

The costs of non-selective mating by females in areas where congeners outnumber conspecifics can be high, particularly in *Xiphophorus*, which is known for little, if any, post-zygotic reproductive isolation (Constantz 1989). Our findings, however, are in contrast to *X. nigrensis*, another northern swordtail,

which is allopatric to all other *Xiphophorus* species, and is more reproductively isolated based on olfactory cues than two more widespread species (McLennan & Ryan 1999). However, as pointed out by McLennan & Ryan (1999), other selective pressures may be acting on signal evolution in this species. Unlike the other swordtails examined in their study, and *X. birchmanni* in this study, which all occur in rocky habitat, *X. nigrensis* is found in sandy to muddy habitats with extensive vegetation. Since habitat affects signal transmission (Bradbury and Vehrencamp, 1988), olfactory signaling in *X. nigrensis* has evolved under a different selective regime than the species that occur in rocky habitat. Thus, the finding that an allopatric species has the most derived communication system may be influenced by habitat differentiation, as well as interaction, or lack thereof, of closely related species.

The preference tests conducted on *X. variatus* from Garces suggest that either females do not recognize *X. birchmanni* males from Huiznopal as a heterospecifics, or do not attend to the difference between conspecific and allopatric-heterospecific cues. In contrast, *X. variatus* females avoided sympatric *X. birchmanni* cues. The cues emitted by *X. birchmanni* males, therefore, must differ between populations. The interpopulation difference in cues, or cue complexes (McLennan & Ryan 1999), in *X. birchmanni* may be as a result of divergence in composition, relative concentration, or both. Although it is unclear what compounds females respond to, the simplest explanation is that the 'bouquet' used by each population differs by one or more components, or component concentration (Wyatt 2003).

Despite how *X. birchmanni* signals differ between populations, our data suggest that *X. variatus* females only avoid *X. birchmanni* cues from sympatric males. As *X. variatus* females from Garces do not encounter *X. birchmanni* males from Huiznopal, the chance of hybridization with them is effectively zero, and thus selection to avoid allopatric males is extremely weak. This may indicate that *X. birchmanni* from Huiznopal use a less derived signaling mechanism. As opportunities for interspecific mating are more rare at Huiznopal, *X. birchmanni* signals are under less selective constraints to differ from *X. variatus* signals. Our data supports the notion that as risk of hybridization decreases, interspecific difference in the cue-receiver systems should decrease as well (Ryan et al. 2003).

The idea that greater differences in mate attraction and choice should exist between sympatric taxa than those in allopatry is not without some controversy. It is disputed that if two sympatric populations or

species produce hybrid offspring with high fitness, then a hybrid swarm will result; but if hybrid fitness is low, the rarer species will be driven to extinction before reinforcement occurs (Liou & Price 1994). Evolution of divergent communication systems is therefore unlikely unless the rarer species is prevented from going extinct, which is possible if the two species occupy different ecological niches. *Xiphophorus variatus* tends to occupy stream edges and have a greater tolerance to low dissolved oxygen levels than swordtails (Borowsky 1990). *Xiphophorus birchmanni* prefer rocky habitat (Rauchenberger et al. 1990) with faster moving water, and are found throughout the width of the stream channel (Fisher H. S., personal observation). The two species, therefore, occupy distinct microhabitats, thus the chances that the rarer species would go extinct due with low hybrid fitness is low.

Overall, our results are consistent with reproductive character displacement. When a cost is incurred through a reduction in reproductive fitness from hybrid mating, selection should favor mechanisms of reproductive isolation between two sympatric species (Howard 1993; Andersson 1994; Servedio & Noor 2003; Coyne & Orr 2004). Although we did not measure fitness costs to hybrid mating between *X. birchmanni* and *X. variatus*, we expect relative hybrid fitness to be low given evidence for other *X. variatus*/swordtail crosses (Mitchell et al. 2004), and the low degree of relatedness between the two species (Marcus & McCune 1999). As the abundance of closely related sympatric species increase, the chance of hybridizing, and thus the cost of indiscriminate mating, increase. Reproductive character displacement, in terms of both male trait and female preference, should therefore not only reflect differences observed in reproductive isolating mechanisms in sympatry and allopatry, but also differences in heterospecific abundance. Our results support this notion and show that the relative number of congeneric species can influence mate recognition and discrimination. Heterospecific abundance, not just presence, may therefore be an important source of variation among conspecific populations.

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