

Original Article

Encounter rates with conspecific males influence female mate choice in a naturally hybridizing fish

Pamela M. Willis,^a Michael J. Ryan,^a and Gil G. Rosenthal^b

^aSection of Integrative Biology, University of Texas at Austin, 1 University Station C0930, Austin, TX 78712, USA and ^bDepartment of Biology, Texas A&M University, 3258 TAMU, College Station, TX 77843, USA

Mate choice can vary in response to internal or external conditions that alter the costs and benefits of being choosy. The relationship between mate choice and hybridization, however, is not well understood. An important influence on mate choice is the rate at which potential mates are encountered: low rates increase sampling costs, favoring reduced choosiness. We investigated the influence of conspecific encounter rate on female choice in a naturally hybridizing species of swordtail, *Xiphophorus birchmanni*. We exposed females to conspecific males, followed by either no delay or a long (24 h) delay before their next male encounter. In this second encounter, females were offered the choice of a heterospecific (*X. malinche*) male only or a choice between a conspecific and heterospecific male. When not given a choice between 2 males, females spent more time with the heterospecific following a long delay between male encounters than after no delay, suggesting a decrease in choosiness. When offered a choice between males, however, females preferentially associated with the conspecific, regardless of the time between male encounters. These results suggest that females are sensitive to conspecific encounter rate but may employ a sample-based (vs. standard-based) comparison tactic, which could make hybridization less likely. We also found that, contrary to our expectations, females with only the heterospecific male to choose from visited him more frequently following a long delay between male encounters than following no delay, possibly indicating an increase in sampling effort. Our study highlights the potential importance of context-dependent mate choice in animal hybridization. **Key words:** encounter rate, hybridization, mate choice, mate density, sampling tactic, *Xiphophorus*. [*Behav Ecol* 22:1234–1240 (2011)]

INTRODUCTION

Individuals can gain a variety of benefits from choosing among potential mates (Andersson 1994). Mate choice, however, is also costly, imposing time, energy, and/or survival costs on choosy individuals. The costs and benefits of mate choice depend on the chooser's attributes and circumstance; as a result, variation in mate choice can arise in response to factors intrinsic to the chooser, such as age or experience (e.g., Kodric-Brown and Nicoletto 2001; Coleman et al. 2004), reproductive state (e.g., Lynch et al. 2005; Uetz and Norton 2007), condition (e.g., Burley and Foster 2006; Hebets et al. 2008; Holveck and Riebel 2010), as well as social and environmental factors including the quality (e.g., Kvarnemo and Simmons 1999), density (e.g., Shine et al. 2006), or familiarity of potential mates (e.g., Simcox et al. 2005), the operational sex ratio (e.g., Berglund 1994; Jirotkul 1999), competition for mates (e.g., Fawcett and Johnstone 2003), predation risk (e.g., Forsgren 1992; Hedrick and Dill 1993), habitat quality (e.g., Reynolds and Jones 1999; Hale 2008), and season (e.g., Qvarnström et al. 2000; Borg et al. 2006).

In many species, individuals respond to changes in the costs and benefits of mate choice by adjusting their level of choosi-

ness, their mate preferences, their sampling strategy, or some combination thereof. For example, female fiddler crabs (*Uca annulipes*) become less selective as time constraints on successful reproduction increase (Backwell and Passmore 1996); female green swordtails (*X. helleri*) change their preference from long-sworded to short-sworded (less risky) males under greater perceived risk of predation (Johnson and Basolo 2003); and female pronghorns (*Antilocapra americana*) reduce their sampling effort, traveling shorter distances and visiting fewer males when they are in poorer condition (Byers et al. 2006).

Typically, mate choice takes place among conspecifics; however, this is not always the case. The same dynamic conditions that produce variation in mate choice within species can also promote or inhibit mating among different species. For example, male western grebes (*Aechmophorus occidentalis*) increasingly pursue female Clark's grebes (*Ae. clarkii*) as opportunities to pair with conspecifics decline over the breeding season (Nuechterlein and Buitron 1998), and female spadefoot toads (*Spea bombifrons*) become more permissive toward heterospecific (*S. multiplicata*) males when water levels are low, with low water levels giving the faster metamorphosing hybrid tadpoles a higher chance of survival (Pfennig 2007). Such studies are uncommon but important because, as is becoming increasingly apparent, animal hybridization can be a significant source of evolutionary change, capable of promoting, inhibiting, or reversing diversification (Seehausen 2004; Mallet 2005, 2007). Identifying how mate choice inhibits or facilitates hybridization is therefore important to our understanding of how diversity is lost or gained.

Address correspondence to P.M. Willis. E-mail: pamwillis@utexas.edu.

Received 16 November 2010; revised 9 May 2011; accepted 10 May 2011.

An important influence on mate choice is the rate at which potential mates are encountered (reviewed in Jennions and Petrie 1997). Low encounter rates increase the costs of sampling, often resulting in a decline in choosiness (e.g., Alatalo et al. 1988; Milinski and Bakker 1992; Palokangas et al. 1992; Shelly and Bailey 1992; Berglund 1995). Among hybridizing species, low encounter rates might promote the acceptance of heterospecifics. Indeed, a scarcity of conspecifics is considered a common cause of hybridization (e.g., Mayr 1963), supported by the many observations of hybridization where one or both species are rare (e.g., Wirtz 1999; Randler 2002). The influence of conspecific encounter rate on heterospecific mate choice, however, has not been investigated experimentally in any species.

Here, we investigate the influence of encounter rate on female mate choice in a naturally hybridizing species of sword-tail, *X. birchmanni*. *Xiphophorus* fishes belong to the internally fertilizing live-bearing family Poeciliidae. The genus ranges from northeastern Mexico to Honduras, and many species occur in sympatry (Rauchenberger et al. 1990; Kallman and Kazianis 2006). Although there is little to no intrinsic postzygotic isolation between species (Rosen 1960; Kazianis et al. 1996; Kallman and Kazianis 2006), hybridization in the wild is uncommon (reviews in Kallman and Kazianis 2006; Schartl 2008), and preferences for conspecifics as mates are a primary barrier to gene flow (Hankison and Morris 2002; Fisher et al. 2006; Kallman and Kazianis 2006; Fisher and Rosenthal 2010).

An exception to the rarity of hybridization observed elsewhere is the *X. birchmanni*–*X. malinche* species pair. These species occasionally hybridize at intermediate elevations of the Río Pánuco basin where their ranges meet, allowing for introgression across several broad hybrid zones (Rosenthal et al. 2003; Culumber et al. 2011). Although females of each species are morphologically similar, males of each species differ with respect to several sexually dimorphic traits (Rauchenberger et al. 1990; Rosenthal et al. 2003), for example, in the presence (*X. malinche*) or absence (*X. birchmanni*) of a long “sword” on the caudal fin. Males court by swimming in parallel with the female while raising their dorsal fin (Ryan and Causey 1989; Fisher and Rosenthal 2007) and will actively court females of either species (Rosenthal GG, personal observation). *Xiphophorus birchmanni* exhibit preferences for conspecific over *X. malinche* olfactory and visual cues (Fisher et al. 2006; Wong and Rosenthal 2006), and *X. malinche* females have been shown to prefer the olfactory cues of conspecifics over those of *X. birchmanni* (Rosenthal GG, unpublished data), although choice tests with live males, allowing females access to large suite of male traits simultaneously, are lacking. As female *X. birchmanni* appear to act primarily as the maternal parent in hybrid crosses (Rosenthal et al. 2003), we focus on mate choice in this species.

Xiphophorus birchmanni and *X. malinche* inhabit small shallow streams subject to seasonal flooding and drought (Rauchenberger et al. 1990), which are likely to introduce substantial variation in female *X. birchmanni* encounter rates with both species. We predicted that as encounter rates with conspecific males decreased, females would become less choosy, discriminating less against heterospecific males. We were also interested in whether encounter rate affected female sampling effort, independent of choosiness. In addition to, or as a consequence of, declining choosiness, individuals facing high costs of sampling may reduce their sampling effort; for example, reducing the time, energy, and/or number of potential mates involved (e.g., Dale et al. 1992; Byers et al. 2006; Dunn et al. 2008; but see Hovi and Rätti 1994). We therefore investigated whether females experiencing low encounter rates with conspecific males subsequently made fewer male visits than when encounter rates were high, which could suggest reduced investment in sampling effort.

The extent to which encounter rates with conspecific males influence female acceptance of heterospecific mates may depend on female sampling strategy. Sampling tactics fall into 2 general classes (reviewed in Uy et al. 2001; Leonard and Hedrick 2009): sample-based, where females compare among males, and standard-based, where females compare males against an internal standard. If female *X. birchmanni* use a sample-based tactic, then they might only accept heterospecifics when preferred (conspecific) mates are unavailable. If, however, females use a standard-based tactic, then heterospecifics that meet that standard will be acceptable regardless of whether conspecifics are also sampled. We therefore conducted 2 sets of experiments designed to reveal the sampling tactic females use.

MATERIALS AND METHODS

Xiphophorus birchmanni of both sexes were collected from the same location within the Río Garces (lat 20°56'24"N, long 98°16'54"W), Hidalgo, Mexico and *X. malinche* from the Arroyo Xontla (lat 20°55'26"N, long 98°34'35"W) near Chicayotla, Hidalgo (Culumber et al. 2011) between 2008 and 2009. Females used in this study, all sexually mature, were either wild-caught individuals ($n = 20$) or their first-generation descendants ($n = 12$). We did not identify relatedness of subject females, and some first-generation descendants may have been sisters or daughters of wild-caught subjects. Females were group housed in isolation from males for at least 2 weeks before testing to standardize prior experience of females and encourage receptivity (e.g., Morris et al. 1996). In many taxa, including other poeciliids (e.g., Kodric-Brown and Nicoletto 2001; Morris et al. 2006), female choice can vary according to female age, condition, reproductive status, or experience (reviewed in Jennions and Petrie 1997). In *Xiphophorus*, females breed continuously (e.g., Ryan et al. 1990; Urbano-Amilpa 2006), and, in *X. nigrensis*, neither receptivity nor preference varies over the reproductive cycle (Ramsey et al. 2011). To control for the unknown variation among females in reproductive status and other potentially influential factors both within and between experiments, we assigned females randomly to each experiment and used within-subject designs. In addition, female length (a correlate of age in *Xiphophorus*, Kallman 1989; Marcus and McCune 1999) did not differ significantly between experiments (Experiment 1: 41.56 ± 3.06 mm, Experiment 2: 41.12 ± 4.20 mm; 2-sample *t*-test, $t = 0.356$, $P = 0.734$), controlling for size/age differences between experiments.

We used a single $76 \times 30 \times 30$ cm aquarium for conducting mate choice tests. Following Cummings et al. (2003), we created a light environment approximating that of natural *Xiphophorus* habitat. Two filtered 500 W halogen lamps provided downwelling light, and the sides of the aquarium, lined with Teflon and filter gels, provided diffused horizontal irradiance. Aquarium water was conditioned (Prime; Seachem Laboratories Inc., Madison GA) and carbon-filtered before use. The aquarium was emptied, rinsed, and dried between trials.

Experiment 1—“no choice”

Before testing, each subject female ($n = 18$) was individually placed into an “encounter tank” ($61 \times 31 \times 51$ cm), containing 7 randomly chosen conspecific males. The same 7 males were used throughout the study to standardize the exposure of all the females. A clear porous barrier between the sexes permitted olfactory and visual contact while preventing physical interactions. After 24 h, females were removed and tested either immediately or after 24 h in their home (female-only) tank. These 2 delay periods (0 or 24 h) simulated variation in encounter rate with conspecifics.

On testing, individual females were presented with a heterospecific *X. malinche* male and a conspecific female, each selected at random. Each stimulus fish was isolated at opposite ends of the experiment tank behind a clear porous barrier, allowing the subject female access to both visual and olfactory cues. This design (hereafter referred to as the “no choice” design) allowed us to evaluate female response to a heterospecific male in the absence of any conspecific male. The added presence of a conspecific female stimulus, however, controlled for female motivation to shoal (see Wong and Rosenthal 2005). Females could traverse through 3 zones: 2 adjacent to each stimulus fish compartment (“association zones”) and a middle “neutral” zone containing a small central shelter. Following a 3-min acclimation period, we recorded female association time with each stimulus fish for 10 min. One trial was excluded from analysis because the female spent over half the time hidden beneath the shelter. Association time is a reliable measure of mate choice and reproductive success in *Xiphophorus* (Ryan et al. 1990; Cummings and Mollaghan 2006; Walling et al. 2010). A change in the proportion of time spent with the heterospecific male over the 2 encounter rate treatments can be interpreted as a change in female choosiness. We also recorded the number of visits made to the male, calculated as the number of transits from the female to male association zone, as a measure of sampling effort. We used the automated video tracking system Ethovision XT (version 5.0, Noldus Information Technology, Wageningen, The Netherlands) for data collection.

Each female was tested twice, once at each delay period, with at least 7 days between treatments. This within-subjects design allowed us to control for unknown variation among females. The same stimulus pair was used for both tests (i.e., held constant within females), controlling for variation among stimuli in attributes independent of species that could potentially influence choice (e.g., condition); however, no stimulus fish was used with more than one subject. Treatment order was randomized across females to control for order effects.

Experiment 2—“choice”

The second experiment was conducted in the same manner as Experiment 1, except that an unfamiliar randomly chosen conspecific male replaced the female conspecific stimulus. This allowed subject females ($n = 18$, 4 of which had also been subjects in Experiment 1) a choice between 2 males: 1 conspecific, and 1 heterospecific. Because of a limited number of male stimulus fish, each stimulus pair was used to test 2 subject females. In this experiment, number of visits was calculated as the number of transits between association zones in either direction (i.e., from either male to the other). In 3 cases, females spent 1 of their 2 trials hidden beneath the shelter for over half of the trial; these 3 trials were excluded from analysis.

Statistical analyses

To investigate changes in choosiness, we calculated the proportion of total association time spent with the heterospecific male. We used linear mixed models, fitted using restricted maximum likelihood estimation, to test for an influence of encounter rate treatment on the proportion of time spent with the heterospecific. Female subject ID and, in Experiment 2, male stimulus pair ID were included as random effects to accommodate non-independent (repeated) measures and any unmeasured but potentially influential attributes of male stimuli. We included trial number (i.e., whether it was a female’s first or second trial) as a covariate. Here and elsewhere, in considering the biological relevance and low complexity of the full model, we

chose to present the full model rather than use information-theoretic or other statistical methods of model selection, although selection via the Akaike information criterion does not change the overall interpretation. Diagnostic plots of model residuals were used to examine model assumptions. In analysis of each experiment, 2 observations had high influence (Cook’s $D > 4/(n-k-1)$; Fox 1997); however, refitting the model with the influential observations excluded did not qualitatively change the results. We therefore report the results of analyses with the influential observations included.

In Experiment 2, we also tested whether females exhibited a conspecific male preference (independent of any changes in choosiness with treatment; see above). To do so, we constructed a linear mixed model with association time (square-root transformed to satisfy the model assumption of residual homogeneity of variance; Zar 1974) as the dependent variable and male species and encounter rate treatment as fixed effects. Trial number and random effects (female subject ID, male stimulus ID) were included as before. Refitting the model excluding 3 influential observations did not qualitatively change the results.

We next investigated the influence of encounter rate on the number of male visits made by the female. In both experiments, the number of visits was uncorrelated with the proportion of time spent with the heterospecific male (Pearson correlation coefficients = -0.11 and 0.04), which indicates that the independent analysis of these 2 variables is appropriate. We modeled number of male visits (square-root transformed) using linear mixed model procedures, specification of covariates and random effects, and significance testing as described above.

As conventional significance testing is problematic in mixed model analysis (primarily due to uncertainty regarding degrees of freedom, see Pinheiro and Bates 2000), we used Markov Chain Monte Carlo sampling (10 000 samples) to generate P values and 95% posterior density credibility intervals from the posterior distribution of parameter estimates (Baayen et al. 2008). To test the significance of random effects, we implemented exact restricted likelihood ratio (RLR) tests. Modeling and significance testing were conducted using the lme4, languageR, and RLRsim libraries of the programming language R (R Development Core Team 2010).

RESULTS

Experiment 1—“no choice”

Females significantly increased both the proportion of time spent with (Table 1; Figure 1a), and the number of visits made to (Table 1; Figure 1b), the heterospecific male after experiencing a 24-h delay between male encounters compared with when they encountered conspecific males immediately preceding testing. There was no significant effect of trial order (Table 1) nor female subject ID (RLR tests, all $P > 0.370$) in either analysis.

Experiment 2—“choice”

When both a conspecific and heterospecific male were presented to females, females spent significantly more time with the conspecific (mean association time (s) \pm standard error: 224.9 ± 18.7 vs. 167.0 ± 18.0 ; Table 2). There was no influence of encounter rate treatment or trial order on either the proportion of time females spent associating with the heterospecific (Table 1; Figure 1a) or the number of visits made to the males (Table 1; Figure 1b). Female subject ID and male stimulus pair ID were not significant in any analysis (RLR tests, all $P > 0.368$).

Table 1

Linear mixed models of parameter effects on proportion of association time spent with the heterospecific male and number of visits made to the male(s) in choice tests with only a heterospecific male to choose from ($n = 18$) or with a choice of conspecific or heterospecific male ($n = 18$)

Choice of male(s)	Dependent variable	Parameter	Coefficient estimate	HPD _{lower}	HPD _{upper}	P _{MCMC}
Heterospecific only	Proportion of time spent with the heterospecific	Encounter rate	0.193	0.047	0.338	0.009
		Trial order	0.032	-0.176	0.113	0.666
	Number of male visits (square-root transformed)	Encounter rate	2.009	0.101	3.932	0.043
		Trial order	-0.705	-2.539	1.358	0.482
Conspecific or Heterospecific	Proportion of time spent with the heterospecific	Encounter rate	-0.053	-0.204	0.084	0.472
		Trial order	0.087	-0.050	0.234	0.211
	Number of male visits (square-root transformed)	Encounter rate	-0.280	-0.759	0.194	0.247
		Trial order	0.035	-0.466	0.496	0.967

Effect sizes (coefficients) with Bayesian 95% credibility intervals (HPD_{lower}, HPD_{upper}) and Markov Chain Monte Carlo (MCMC) P values.

DISCUSSION

When females had only the heterospecific male available for evaluation, they devoted a greater proportion of their time to the male following a 24-h delay between male encounters than they did when conspecific males had been encountered immediately preceding testing. This is consistent with other theoretical and empirical studies that document a decrease in choosiness with decreasing mate densities or encounter rates (e.g., Wilson and Hedrick 1982; Crowley et al. 1991; Milinski and Bakker 1992; Palokangas et al. 1992; Shelly and Bailey 1992; Berglund 1995; Kokko and Rankin 2006). Unlike previous studies however, we have shown that encounter rates with conspecifics can influence female choice in the context of hybridization. These results lend support to the view that hybridization may often be the outcome of a conditional mate choice strategy (e.g., Wilson and Hedrick 1982; Pfennig 2007; Wiley et al. 2007).

The decline in choosiness apparent in the first experiment was not observed in the second, when both the conspecific and heterospecific male were simultaneously assessable by females. Females preferentially associated with the conspecific regardless of when conspecifics had last been encountered. Taken together, the results from both experiments imply that females use, or are at least capable of, sample-based comparison of potential mates during mate sampling, as opposed to being limited to evaluation according to an internal template or threshold. A similar comparison mechanism has also been reported in *X. helleri* (Royle et al. 2008). Whether female *X. birchmanni* comparatively evaluate potential mates in the wild is not known. *Xiphophorus birchmanni* do, however, occur in high-density mixed-sex groups (Wong and Rosenthal 2005) and in many circumstances likely encounter multiple males over short periods of time and space. The capacity for sample-based comparison may therefore limit the conditions under which heterospecifics would be accepted as mates in the wild.

Female *X. birchmanni* are likely to experience substantial variation in encounter rates with conspecific and *X. malinche* males. Seasonal flooding and drought dramatically alter *Xiphophorus* habitat, greatly affecting the frequency of heterospecific interactions (e.g., Gordon 1953; Borowsky 1981; Tatarenkov et al. 2010). Females facing low encounter rates with conspecific males, for example, due to flooding, migration into heterospecific habitat, or isolation in small drought-produced pools, may become less choosy as a result. The frequency of hybrids and parental species vary substantially among stream reaches (Culumber et al. 2011). Along with other conditions that reduce female *X. birchmanni* discrimination against *X. malinche* males, such as environmental pollution (Fisher et al. 2006) and predation risk (Willis PM, Ryan MJ, Rosenthal GG, unpublished data), low encounter rates

with conspecifics may increase the probability of hybridization. Natural temporal and geographical variation in conspecific encounter rates and the frequency of hybrids provide an opportunity to test this hypothesis in the wild.

A less well-studied response to increasing costs of mate sampling is a reduction in sampling effort (in addition to, or as a consequence of, decreased choosiness). For example, under a perceived risk of predation, male and female amphipods (*Gammarus duebeni*) decrease their sampling effort, encountering fewer conspecifics, and become more permissive, rejecting fewer potential mates (Dunn et al. 2008). In contrast, we found that with only a heterospecific male available, females visited the heterospecific more frequently following a long delay after encountering conspecific males than they did after a short one. This increase in the number of visits cannot be explained by the accompanying increase in the proportion of time spent with the male, as these 2 measures are uncorrelated. The increase in visits could suggest that females without recent encounters with conspecific males were more motivated to find a male and consequently invested more effort in sampling (e.g., Hovi and Rätti 1994). This explanation, however, fails to explain the lack of a corresponding increase in visits in the second experiment, where females had a choice between males. Nor was an increase in association time with either male stimulus observed in the second experiment following the long delay treatment, which might also be expected if females were more motivated. Perhaps, females were prepared to invest more in sampling following the long delay treatment in both experiments, but this willingness was only apparent in the absence of a preferred (conspecific) male, which, once encountered, might reduce or eliminate further sampling behavior. Alternatively, it is possible that the conspecific female stimulus (introduced opposite the heterospecific male, to control for shoaling behavior), present only in the first experiment, elicited an increase in visitation behavior by females following the long delay treatment, relative to the short delay treatment. Future studies are needed to determine how environmental influences on mate choice affect female sampling effort.

Female choice in many taxa can be influenced by several factors, not all of which could be addressed in the present study. One important consideration is variation among females: for example, differences in age, condition, reproductive status, or mating experience can contribute to variation in mate choice (reviewed in Jennions and Petrie 1997). How these factors influence choice in *X. birchmanni* is unknown. In other species of *Xiphophorus*, female size/age has been shown to influence preference (e.g., Morris et al. 2006; Rios-Cardenas et al. 2007), whereas in *X. nigrensis*, reproductive cycle has not (Ramsey et al. 2011). Although individual variation among females

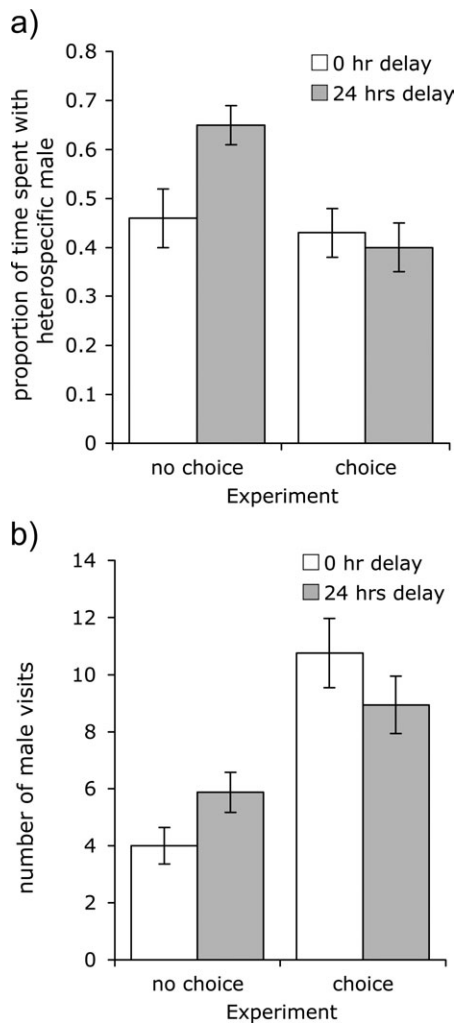


Figure 1

(a) Mean proportion of association time spent with the heterospecific male and (b) number of male visits made by females in choice tests with only a heterospecific male to choose from ("no choice," $n = 18$) or with a choice of conspecific or heterospecific male ("choice," $n = 18$). White bars = short delay between male encounters, gray bars = long delay between male encounters. Error bars = \pm standard error.

was controlled for in the present study (see "MATERIALS AND METHODS"), low samples sizes limited our ability to evaluate the effects of multiple additional variables. Studies that test the influence of multiple variables on female choice of heterospecifics are encouraged. Another limitation of the

Table 2

Linear mixed model of parameter effects on (square-root transformed) association time in tests offering the choice between a conspecific and heterospecific male. $N = 18$

Parameter	Coefficient estimate	HPD _{lower}	HPD _{upper}	P_{MCMC}
Male species	-2.191	-4.029	-0.332	0.022
Encounter rate treatment	-0.152	-2.081	1.753	0.869
Trial order	0.433	-1.534	2.363	0.650

Effect sizes (coefficients) with Bayesian 95% credibility intervals (HPD_{lower}, HPD_{upper}) and Markov Chain Monte Carlo (MCMC) P values.

present study is in the use of dichotomous-choice tests: Although they have several advantages, they also have limitations (Wagner 1998). For example, physical interactions between the sexes, or between males, are prevented, evaluation is limited to 2 nonsequential alternatives, and evaluation time is fixed; all of which may be unrealistic conditions for mate choice in the wild. These limitations highlight opportunities for future research.

In many animal species, hybridization, when present, often occurs when one or both parental species are rare (Mayr 1963; Wirtz 1999; Randler 2002). Although low encounter rates, and other factors that increase the costs of mate sampling, predict an increase in heterospecific mating among potentially hybridizing species (Wilson and Hedrick 1982), there are few theoretical and empirical studies devoted to this topic. The implications, however, are not trivial: Variation in mate choice cannot only influence trait evolution and speciation through sexual selection in the conventional sense, it might also, by introducing genetic novelty into hybridizing populations, lead to reticulate evolution, reverse speciation, hybrid speciation, or adaptive radiation (see Seehausen 2004; Mallet 2005, 2007; Schwenk et al. 2008; Seehausen et al. 2008). Although the number of individuals that actually hybridize within a species is often low, the occurrence of rare hybridization events can nonetheless greatly impact the evolutionary fate of lineages (Arnold 1997; Schwenk et al. 2008). Evidence of introgressive hybridization in animals is rapidly increasing (Mallet 2005; Schwenk et al. 2008). Studying the factors that contribute to variation in mate choice can broaden our understanding of the processes contributing to the loss or gain of diversity.

FUNDING

University of Texas (Zoology Scholarship Endowment for Excellence Fellowship to P.M.W.); the Society for Integrative and Comparative Biology; the Southwestern Association of Naturalists; the American Livebearer Association; and the PADI Foundation (grants to P.M.W.).

Thanks to Gina Calabrese, James Cheng, Farees Masood, Sharon Monsivais, Jackie Moran, and Stephen Stapleton for assistance with data collection. Four anonymous reviewers provided helpful comments on earlier versions of the manuscript.

REFERENCES

- Alatalo RV, Carlson A, Lundberg A. 1988. The search cost in mate choice of the pied flycatcher. *Anim Behav*. 36:289–291.
- Andersson M. 1994. *Sexual selection*. Princeton (NJ): Princeton University Press.
- Arnold ML. 1997. *Natural hybridization and evolution*. Oxford: Oxford University Press.
- Baayen RH, Davidson DJ, Bates DM. 2008. Mixed-effects modeling with crossed random effects for subjects and items. *J Mem Lang*. 59:390–412.
- Backwell PRY, Passmore NI. 1996. Time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, *Uca annulipes*. *Behav Ecol Sociobiol*. 38:407–416.
- Berglund A. 1994. The operational sex ratio influences choosiness in a pipefish. *Behav Ecol*. 5:254–258.
- Berglund A. 1995. Many mates make male pipefish choosy. *Behaviour*. 132:213–218.
- Borg AA, Forsgren E, Amundsen T. 2006. Seasonal change in female choice for male size in the two-spotted goby. *Anim Behav*. 72:763–771.
- Borowsky R. 1981. Tailspots of *Xiphophorus* and the evolution of conspicuous polymorphism. *Evolution*. 35:345–358.
- Burley NT, Foster VS. 2006. Variation in female choice of mates: condition influences selectivity. *Anim Behav*. 72:713–719.
- Byers JA, Byers AA, Dunn SJ. 2006. A dry summer diminishes mate search effort by pronghorn females: evidence for a significant cost of mate search. *Ethology*. 112:74–80.

- Coleman SW, Patricelli GL, Borgia G. 2004. Variable female preferences drive complex male displays. *Nature*. 248:742–745.
- Crowley PH, Travers SE, Linton MC, Cohn SL, Sih A, Sargent RC. 1991. Mate density, predation risk and the seasonal sequence of mate choices—a dynamic game. *Am Nat*. 137:567–596.
- Culumber ZW, Fisher HS, Tobler M, Mateos M, Sorenson MD, Barber PH, Rosenthal GG. 2011. Replicated hybrid zones of *Xiphophorus* swordtails along an elevational gradient. *Mol Ecol*. 20:342–356.
- Cummings M, Mollaghan D. 2006. Repeatability and consistency of female preference behaviours in a northern swordtail, *Xiphophorus nigrensis*. *Anim Behav*. 72:217–224.
- Cummings ME, Rosenthal GG, Ryan MJ. 2003. A private ultraviolet channel in visual communication. *Proc R Soc B Biol Sci*. 270:897–904.
- Dale S, Rinden H, Slagsvold T. 1992. Competition for a mate restricts mate search of female pied flycatchers. *Behav Ecol Sociobiol*. 30:165–176.
- Dunn AM, Dick JTA, Hatcher JJ. 2008. The less amorous *Gammarus*: predation risk affects mating decisions in *Gammarus duebeni* (Amphipoda). *Anim Behav*. 76:1289–1295.
- Fawcett TW, Johnstone RA. 2003. Mate choice in the face of costly competition. *Behav Ecol*. 14:771–779.
- Fisher HS, Rosenthal GG. 2007. Male swordtails court with an audience in mind. *Biol Lett*. 3:5–7.
- Fisher HS, Rosenthal GG. 2010. Relative abundance of *Xiphophorus* fishes and its effect on sexual communication. *Ethology*. 116:32–38.
- Fisher HS, Wong BBM, Rosenthal GG. 2006. Alteration of the chemical environment disrupts communication in a freshwater fish. *Proc R Soc B Biol Sci*. 273:1187–1193.
- Forsgren E. 1992. Predation risk affects mate choice in a gobiid fish. *Am Nat*. 140:1041–1049.
- Fox J. 1997. Applied regression analysis, linear models, and related methods. Thousand Oaks (CA): Sage Publications.
- Gordon M. 1953. The ecological niche of the pygmy swordtail, *Xiphophorus pygmaeus*, in the Río Axtla, Mexico. *Copeia*. 1953:148–150.
- Hale RE. 2008. Evidence that context-dependent mate choice for parental care mirrors benefits to offspring. *Anim Behav*. 75:1283–1290.
- Hankison SJ, Morris MR. 2002. Sexual selection and species recognition in the pygmy swordtail, *Xiphophorus pygmaeus*: conflicting preferences. *Behav Ecol Sociobiol*. 51:140–145.
- Hebets EA, Wesson J, Shamble PS. 2008. Diet influences mate choice selectivity in adult female wolf spiders. *Anim Behav*. 76:355–363.
- Hedrick AV, Dill LM. 1993. Mate choice by female crickets is influenced by predation risk. *Anim Behav*. 46:193–196.
- Holveck MJ, Riebel K. 2010. Low-quality females prefer low-quality males when choosing a mate. *Proc R Soc B Biol Sci*. 277:153–160.
- Hovi M, Rättö O. 1994. Mate sampling and assessment procedures in female pied flycatchers (*Ficedula hypoleuca*). *Ethology*. 96:127–137.
- Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev*. 72:283–327.
- Jirotkul M. 1999. Operational sex ratio influences female preference and male–male competition in guppies. *Anim Behav*. 58:287–294.
- Johnson JB, Basolo AL. 2003. Predator exposure alters female mate choice in the green swordtail. *Behav Ecol*. 14:619–625.
- Kallman KD. 1989. Genetic control in size at maturity in *Xiphophorus*. In: Meffe GK, Snelson FF, editors. *Ecology and evolution of livebearing fishes (Poeciliidae)*. Englewood Cliffs (NJ): Prentice Hall. p. 163–184.
- Kallman KD, Kazianis SD. 2006. The genus *Xiphophorus* in Mexico and central America. *Zebrfish*. 3:271–285.
- Kazianis S, Morizot DC, McEntire BB, Nairn RS, Borowsky RL. 1996. Genetic mapping in *Xiphophorus* hybrid fish: assignment of 43 AP-PCR/RAPD and isozyme markers to multipoint linkage groups. *Genome Res*. 6:280–289.
- Kodric-Brown A, Nicoletto PF. 2001. Age and experience affect female choice in the guppy (*Poecilia reticulata*). *Am Nat*. 157:316–323.
- Kokko H, Rankin DJ. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philos Trans R Soc B Biol Sci*. 361:319–334.
- Kvarnemo C, Simmons LW. 1999. Variance in female quality, operational sex ratio and male mate choice in a bushcricket. *Behav Ecol Sociobiol*. 45:245–252.
- Leonard AS, Hedrick AV. 2009. Male and female crickets use different decision rules in response to mating signals. *Behav Ecol*. 20:1175–1184.
- Lynch KS, Rand AS, Ryan MJ, Wilczynski W. 2005. Plasticity in female mate choice associated with changing reproductive states. *Anim Behav*. 69:689–699.
- Mallet J. 2005. Hybridization as an invasion of the genome. *Trends Ecol Evol*. 20:229–237.
- Mallet J. 2007. Hybrid speciation. *Nature*. 446:279–283.
- Marcus JM, McCune AR. 1999. Ontogeny and phylogeny in the northern swordtail clade of *Xiphophorus*. *Syst Biol*. 48:491–522.
- Mayr E. 1963. *Animal species and evolution*. Cambridge: Belknap Press.
- Milinski M, Bakker TCM. 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proc R Soc B Biol Sci*. 250:229–233.
- Morris MR, Rios-Cardenas O, Tudor MS. 2006. Larger swordtail females prefer asymmetrical males. *Biol Lett*. 2:8–11.
- Morris MR, Wagner WEJ, Ryan MJ. 1996. A negative correlation between trait and mate preference in *Xiphophorus pygmaeus*. *Anim Behav*. 52:1193–1203.
- Nuechterlein GL, Buitron D. 1998. Interspecific mate choice by late-courting male western grebes. *Behav Ecol*. 9:313–321.
- Palokangas P, Alatalo RV, Korpimäki E. 1992. Female choice in the kestrel under different availability of mating options. *Anim Behav*. 43:659–665.
- Pfennig KS. 2007. Facultative mate choice drives adaptive hybridization. *Science*. 318:965–967.
- Pinheiro JC, Bates DM. 2000. *Mixed-effects models in S and S-PLUS*. New York: Springer.
- Qvarnström A, Pärt T, Sheldon BC. 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. *Nature*. 405:344–347.
- R Development Core Team. 2010. *R: A language and environment for statistical computing*. Vienna (Austria): R Foundation for Statistical Computing.
- Ramsey ME, Wong RY, Cummings ME. 2011. Estradiol, reproductive cycle and preference behavior in a northern swordtail. *Gen Comp Endocrinol*. 170:381–390.
- Randler C. 2002. Avian hybridization, mixed pairing and female choice. *Anim Behav*. 63:103–119.
- Rauchenberger M, Kallman KD, Morizot DC. 1990. Monophyly and geography of the Río Pánuco basin swordtails (genus *Xiphophorus*) with descriptions of four new species. *Am Mus Novit*. 2975:1–41.
- Reynolds JD, Jones JC. 1999. Female preference for preferred males is reversed under low oxygen conditions in the common goby (*Pomatoschistus microps*). *Behav Ecol*. 10:149–154.
- Rios-Cardenas O, Tudor MS, Morris MR. 2007. Female preference variation has implications for the maintenance of an alternative mating strategy in a swordtail fish. *Anim Behav*. 74:633–640.
- Rosen DE. 1960. Middle-American poeciliid fishes of the genus *Xiphophorus*. *Bull Fla State Mus Biol Sci*. 5:57–242.
- Rosenthal GG, De La Rosa Reyna XF, Kazianis S, Stephens MJ, Morizot DC, Ryan MJ, De Leon FJG. 2003. Dissolution of sexual signal complexes in a hybrid zone between the swordtails *Xiphophorus birchmanni* and *Xiphophorus malinche* (Poeciliidae). *Copeia* 299–307.
- Royle NJ, Lindström J, Metcalfe NB. 2008. Context-dependent mate choice in relation to social composition in green swordtails *Xiphophorus helleri*. *Behav Ecol*. 19:998–1005.
- Ryan MJ, Causey BA. 1989. “Alternative” mating behavior in the swordtails *Xiphophorus nigrensis* and *Xiphophorus pygmaeus* (Pisces: Poeciliidae). *Behav Ecol Sociobiol*. 24:341–348.
- Ryan MJ, Hews DK, Wagner WEJ. 1990. Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. *Behav Ecol Sociobiol*. 26:231–237.
- Schartl M. 2008. Evolution of *Xmrk*: an oncogene, but also a speciation gene? *BioEssays*. 30:822–832.
- Schwenk K, Brede N, Streit B. 2008. Introduction. Extent, processes and evolutionary impact of interspecific hybridization in animals. *Philos Trans R Soc B Biol Sci*. 363:2805–2811.
- Seehausen O. 2004. Hybridization and adaptive radiation. *Trends Ecol Evol*. 19:198–207.
- Seehausen O, Takimoto G, Roy D, Jokela J. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol Ecol*. 17:30–44.
- Shelly TE, Bailey WJ. 1992. Experimental manipulation of mate choice by male katydid: the effect of female encounter rate. *Behav Ecol Sociobiol*. 30:277–282.
- Shine R, Webb JK, Lane A, Mason RT. 2006. Flexible mate choice: a male snake’s preference for larger females is modified by the sizes of females encountered. *Anim Behav*. 71:203–209.

- Simcox H, Colegrave N, Heenan A, Howard C, Braithwaite VA. 2005. Context-dependent male mating preferences for unfamiliar females. *Anim Behav*. 70:1429–1437.
- Tatarenkov A, Healy CIM, Avise JC. 2010. Microgeographic population structure of green swordtail fish: genetic differentiation despite abundant migration. *Mol Ecol*. 19:257–268.
- Uetz GW, Norton S. 2007. Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behav Ecol Sociobiol*. 61:631–641.
- Urbano-Amilpa H. 2006. Estrategia reproductiva de tres especies del género *Xiphophorus* (Pisces: Poeciliidae) en la cuenca del río Atlapexco, México [dissertation]. [Querétaro (Mexico)]: universidad Autónoma de Querétaro. p. 90.
- Uy JAC, Patricelli GL, Borgia G. 2001. Complex mate searching in the satin bowerbird *Ptilonorhynchus violaceus*. *Am Nat*. 158:530–542.
- Wagner WE Jr. 1998. Measuring female mating preferences. *Anim Behav*. 55:1029–1042.
- Walling CA, Royle NJ, Lindström J, Metcalfe NB. 2010. Do female association preferences predict the likelihood of reproduction? *Behav Ecol Sociobiol*. 64:541–548.
- Wiley C, Fogelberg N, Sæther SA, Veen T, Svedin N, Kehlenbeck JV, Qvarnström A. 2007. Direct benefits and costs for hybridizing *Ficedula* flycatchers. *J Evol Biol*. 20:854–864.
- Wilson DS, Hedrick A. 1982. Speciation and the economics of mate choice. *Evol Theory*. 6:15–24.
- Wirtz P. 1999. Mother species-father species: unidirectional hybridization in animals with female choice. *Anim Behav*. 58:1–12.
- Wong BBM, Rosenthal GG. 2005. Shoal choice in swordtails when preferences conflict. *Ethology*. 111:179–186.
- Wong BBM, Rosenthal GG. 2006. Female disdain for swords in a sword-tail fish. *Am Nat*. 167:136–140.
- Zar JH. 1974. Biostatistical analysis. Englewood Cliffs (NJ): Prentice Hall.