

Copyright

by

Pamela Margaret Willis

2011

**The Dissertation Committee for Pamela Margaret Willis Certifies that this is the  
approved version of the following dissertation:**

**Mate Choice and Hybridization within Swordtail Fishes (*Xiphophorus*  
spp.) and Wood Warblers (family Parulidae)**

**Committee:**

---

Michael J. Ryan, Supervisor

---

Daniel I. Bolnick

---

Ulrich G. Mueller

---

Gil G. Rosenthal

---

Michael C. Singer

**Mate Choice and Hybridization within Swordtail Fishes (*Xiphophorus*  
spp.) and Wood Warblers (family Parulidae)**

**by**

**Pamela Margaret Willis, B.S., M.S.**

**Dissertation**

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

**Doctor of Philosophy**

**The University of Texas at Austin**

**May, 2011**

## **Dedication**

To Sparky

## **Acknowledgements**

I am deeply indebted to my advisor, Mike Ryan, for his insight, guidance, patience, good humor, and unwavering support.

I am also much indebted to Gil Rosenthal, who advised me as if I were one of his own students. I am very grateful.

I had an excellent thesis committee - in addition to Mike and Gil, Dan Bolnick, Ulrich Mueller, and Mike Singer gave excellent advice and feedback.

Karin Akre was my grad school support buddy in the last years of my PhD. I could not have done it without her.

The Ecology, Evolution, and Behavior Program has provided a stimulating and supportive environment. Kim Hoke, Chad Smith, Nichole Bennett, Eben Gering, Barrett Klein, Ray Engezer, Frank Stearns, Frank Bronson, Hans Hoffman, and members of the Ryan lab deserve special mention.

Thanks to my collaborators Beckie Symula and Irby Lovette on the warbler research. Luke Harmon, Wesley Hochachka, Richard Glor, Pierre Legendre, and John Sullivan contributed in a variety of ways to the phylogenetic comparative analyses.

Thanks to the many undergraduate students that assisted me in studying swordtails: Gina Calabrese, James Cheng, Farees Masood, Sharon Monsivais, Jackie Moran, and Stephen Stapleton.

Financial support was provided by The University of Texas (Zoology Scholarship Endowment for Excellence Fellowship), the Society for Integrative and Comparative Biology, the Southwestern Association of Naturalists, the American Livebearer Association, and the PADI Foundation.

**Mate Choice and Hybridization within Swordtail Fishes (*Xiphophorus* spp.) and Wood Warblers (family Parulidae)**

Publication No. \_\_\_\_\_

Pamela Margaret Willis, Ph.D.

The University of Texas at Austin, 2011

Supervisor: Michael J. Ryan

Behavioral isolation is an important barrier to gene flow, contributing to the formation and maintenance of animal species. Nevertheless, hybridization occurs more commonly than is generally recognized, occurring in over ten percent of animal species in the wild. Although the genetic consequences of hybridization are of considerable interest given their evolutionary implications, the reasons that animals choose to mate with other species are less clear. I apply mate choice theory to the question of hybridization, using wood warblers (family Parulidae) and swordtail fishes (genus *Xiphophorus*) as study systems.

Over half of the 45 species of North American wood warbler have produced hybrids. Using comparative methods, I address the questions: Do ecological and demographic factors predict hybridization in this family? Similarly, how do phylogeny, song similarity, and sympatry with congeners correlate with hybridization? As with North American wood warblers, behavioral isolation is also considered of primary importance

in isolating sympatric species of swordtail fishes. Two species, *X. birchmanni* and *X. malinche*, hybridize in several locations in the wild. Through experimentation with these and other *Xiphophorus* species, I investigate some of the factors that cause female mate choice to vary, possibly contributing to hybridization. Specifically, I address the following questions: Do females become less choosy when predation risk is high, or encounter rates with conspecifics are low? Are female preferences for conspecifics innate, or can they be modified by experience? And, do female preferences for conspecifics vary among species, populations, or experiments?

These studies illustrate the utility of treating hybridization as just another possible outcome of variation in mate choice. I find that warbler hybridization correlates with ecological and other variables, that female swordtails become more responsive to heterospecifics when mate choice is costly, and that female preferences for conspecifics are species- and context-dependent. As animal hybridization can have important evolutionary consequences, studying the factors that contribute to this variation can enhance our understanding of the evolutionary process.

## Table of Contents

List of Tables .....	x
List of Figures .....	xii
Introduction.....	1
Chapter 1: Ecology, phylogeny, and song similarity predict hybridization in North American wood warblers .....	7
ABSTRACT .....	7
INTRODUCTION .....	8
METHODS.....	11
RESULTS .....	16
DISCUSSION.....	20
Chapter 2: Predation risk alters female mate choice in a naturally hybridizing swordtail <i>Xiphophorus</i> .....	29
ABSTRACT .....	29
INTRODUCTION .....	29
METHODS.....	31
RESULTS .....	34
DISCUSSION.....	36
Chapter 3: Encounter rates with conspecific males influence female mate choice in a naturally hybridizing fish .....	38
ABSTRACT .....	38
INTRODUCTION .....	39
METHODS.....	42
RESULTS .....	46
DISCUSSION.....	49
Chapter 4: The effect of short-term experience on conspecific mate preference in the swordtail fish <i>Xiphophorus birchmanni</i> .....	52
ABSTRACT .....	52



INTRODUCTION .....	53
METHODS.....	55
RESULTS .....	59
DISCUSSION.....	62
Chapter 5: Conspecific preference in female <i>Xiphophorus</i> : variation among species, populations, and experiments .....	65
ABSTRACT .....	65
INTRODUCTION .....	66
METHODS.....	70
RESULTS .....	76
DISCUSSION.....	81
Concluding Remarks .....	88
Appendix.....	92
References .....	95

## List of Tables

TABLE 1.1:	HYBRD CROSS COMBINATIONS REPORTED SINCE 1980.....	17
TABLE 1.2:	PHYLOGENETIC PERMUTATION AND MANTEL TEST RESULTS OF CORRELATIONS BETWEEN HYBRID OCCURRENCE, PHYLOGENETIC, SONG, AND SYMPATRY MATRICES. ....	19
TABLE 1.3:	INFLUENCE OF BREEDING RANGE SIZE, BREEDING HABITAT QUALITY, AND THE NUMBER OF SYMPATRIC HETEROSPECIFICS ON SPECIES' PER-CAPITA HYBRIDIZATION RATE. P-PARAM = ONE-TAILED PARAMETRIC P-VALUE, P- PERM = ONE-TAILED PERMUTATION P-VALUE. FULL DATASET = ALL SPECIES, REDUCED DATASET = EXCLUDING SPECIES WITH ARBITRARILY- ASSIGNED HYBRID NUMBER VALUES; SEE TEXT.....	20
TABLE 2.1:	BEST LINEAR MIXED MODELS OF EFFECTS ON FEMALE ASSOCIATION TIME, IN THE PRESENCE OR ABSENCE OF MALE STIMULI. EFFECT SIZES (COEFFICIENTS) WITH 95% CREDIBILITY INTERVALS AND P-VALUES. ....	35
TABLE 3.1:	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 OR WITHOUT A CHOICE OF MALE. EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS (HPD <sub>LOWER</sub> , HPD <sub>UPPER</sub> ) AND MCMC P-VALUES. .	47
TABLE 4.1:	LINEAR MIXED MODEL OF FIXED EFFECTS ON STRENGTH OF CONSPECIFIC PREFERENCE IN LAB-REARED FEMALES FOLLOWING MALE EXPERIENCE. EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS (HPD <sub>LOWER</sub> , HPD <sub>UPPER</sub> ) AND MCMC P-VALUES. ....	61
TABLE 5.1:	. LINEAR MIXED MODELS OF FIXED EFFECTS ON ASSOCIATION TIME OF FEMALE <i>X. BIRCHMANNI</i> , <i>X. MALINCHE</i> , AND <i>X. VARIATUS</i> IN DICHOTOMOUS CHOICE TESTS. RESULTS OF TOP-RANKING MODELS ACCORDING TO AICc VALUES PRESENTED (SEE TEXT). EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS (HPD <sub>LOWER</sub> , HPD <sub>UPPER</sub> ) AND MCMC P-VALUES.....	77
TABLE 5.2:	LINEAR MIXED MODEL OF FIXED EFFECTS ON ASSOCIATION TIME OF FEMALE <i>X. BIRCHMANNI</i> IN DICHOTOMOUS CHOICE TESTS BETWEEN CONSPECIFIC AND <i>X. MALINCHE</i> MALES, OVER TWO EXPERIMENTS (1 AND 4). RESULTS OF TOP-RANKING MODEL ACCORDING TO AICc VALUES PRESENTED (SEE TEXT). EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS (HPD <sub>LOWER</sub> , HPD <sub>UPPER</sub> ) AND MCMC P-VALUES. ....	80

TABLE A.1: NORTH AMERICAN WOOD WARBLER SPECIES DATA USED IN ANALYSES OF CHAPTER 1. No_HYBRIDS = NUMBER OF HYBRIDS REPORTED SINCE 1980, SEE TEXT FOR HOW VALUES WERE ASSIGNED; SOURCES PROVIDED IN TABLE 1.1. PSZ_NA_09 = POPULATION SIZE IN NORTH AMERICA IN 2009. FROM PARTNERS IN FLIGHT LANDBIRD POPULATION ESTIMATES DATABASE VERSION 2004 ( <a href="http://rmbo.org/pif_db/laped">HTTP://RMBO.ORG/PIF_DB/LAPED</a> ; BLANCHER ET AL. 2007). BR_DENS = AVERAGE NUMBER OF BIRDS PER BBS ROUTE 1990-1999. FROM PARTNERS IN FLIGHT LANDBIRD POPULATION ESTIMATES DATABASE VERSION 2004 ( <a href="http://rmbo.org/pif_db/laped">HTTP://RMBO.ORG/PIF_DB/LAPED</a> ; BLANCHER ET AL. 2007). RANGE_SZ = BREEDING RANGE DISTRIBUTION FROM 1 (<500,000 KM, OR VERY RESTRICTED COASTAL AREAS OR INTERIOR UPLANDS) TO 5 ( $\geq 4,000,000$ KM <sup>2</sup> , OR >8,000 KM OF COAST). TRANSFORMED (ORDINAL DIRECTION REVERSED) FROM PIF SPECIES ASSESSMENT DATABASE VERSION 2005, <a href="http://www.rmbo.org/pif/pifdb.htm">HTTP://WWW.RMBO.ORG.PIF/PIFDB.HTM</a> ; PANJABI ET AL. 2005. HAB_QUAL = BREEDING HABITAT QUALITY. TRANSFORMED (ORDINAL DIRECTION REVERSED) FROM "THREATS TO BREEDING" SCORE, PIF SPECIES ASSESSMENT DATABASE VERSION 2005, <a href="http://www.rmbo.org/pif/pifdb.htm">HTTP://WWW.RMBO.ORG.PIF/PIFDB.HTM</a> ; PANJABI ET AL. 2005. No_HET_SPP = NUMBER OF HETEROSPECIFIC SPECIES BREEDING IN SYMPATRY (>0 REGIONAL CO-OCCURRENCE, SEE LOVETTE & HOCHACHKA 2006).....	92
TABLE A.2: CORNELL LAB OF ORNITHOLOGY MACAULAY LIBRARY CATALOGUE NUMBERS OF NORTH AMERICAN WOOD WARBLER SONG SAMPLES ANALYSED IN CHAPTER 1. ....	94

## List of Figures

- FIGURE 2.1: EXPERIMENTAL TANK DESIGN, DRAWN TO SCALE. SHADED REGIONS REPRESENT SHELTER (CIRCLES) AND ZONES (RECTANGLES) IN WHICH ASSOCIATION TIME WAS RECORDED. UPPER PANEL = SHELTER EQUIDISTANT FROM EITHER MALE COMPARTMENT. LOWER PANEL = SHELTER CLOSER TO THE HETEROSPECIFIC COMPARTMENT. C = CONSPECIFIC MALE COMPARTMENT, H = HETEROSPECIFIC MALE COMPARTMENT. REPRESENTATIVE PATHS OF A SUBJECT FEMALE OVER TWO CONSECUTIVE TRIALS ARE SHOWN..... 33
- FIGURE 2.2: FEMALE ASSOCIATION TIME IN EITHER ZONE IN THE (A) PRESENCE OR (B) ABSENCE OF MALE STIMULI, WITH THE SHELTER EITHER CENTERED OR OFFSET WITHIN THE TANK. IN (A), WHITE BARS = CONSPECIFIC MALE SIDE, GRAY BARS = HETEROSPECIFIC MALE SIDE. IN (B), BAR COLOR DESIGNATES OPPOSITE SIDES OF THE TANK.....35
- FIGURE 3.1: EXPERIMENTAL TANK DESIGN, AS SEEN FROM ABOVE. STIMULUS FISH ARE ISOLATED BEHIND BARRIERS (DASHED LINES). GRAY AREAS = ASSOCIATION ZONES. CENTER CIRCLE = SHELTER..... 44
- FIGURE 3.2: A) MEAN PROPORTION OF ASSOCIATION TIME SPENT WITH THE HETEROSPECIFIC MALE, AND B) NUMBER OF MALE VISITS MADE BY THE FEMALE, IN CHOICE TESTS WITH (EXPERIMENT 2) OR WITHOUT (EXPERIMENT 1) A CHOICE OF MALE. WHITE BARS = HIGH ENCOUNTER RATE TREATMENT, GRAY BARS = LOW ENCOUNTER RATE TREATMENT. ERROR BARS =  $\pm$  STANDARD ERROR.....48
- FIGURE 4.1: FEMALE ASSOCIATION TIME WITH CONSPECIFIC (WHITE BARS) AND *X. VARIATUS* (GRAY BARS) MALES, IN WILD-CAUGHT (DATA FROM CHAPTER 5) AND LAB-REARED *X. BIRCHMANNI*. ERROR BARS =  $\pm$  SE.....60
- FIGURE 4.2: FEMALE STRENGTH OF PREFERENCE FOR CONSPECIFICS BEFORE (WHITE BARS) AND AFTER (GRAY BARS) EXPERIENCE WITH EITHER CONSPECIFIC MALES, OR A COMBINATION OF CONSPECIFIC AND *X. VARIATUS* MALES, IN LAB-REARED *X. BIRCHMANNI*. PREFERENCE STRENGTHS ABOVE 0.5 (DASHED LINE) WOULD INDICATE A PREFERENCE FOR CONSPECIFICS, SCORES BELOW, A PREFERENCE FOR HETEROSPECIFICS. ERROR BARS =  $\pm$  SE.....60
- FIGURE 4.3: CONSISTENCY OF STRENGTH OF PREFERENCE IN INDIVIDUAL LAB-REARED FEMALES OVER TWO TESTS (BEFORE AND AFTER MALE EXPERIENCE).61

FIGURE 5.1: FEMALE ASSOCIATION TIME WITH CONSPECIFIC VS. HETEROSPECIFIC OR HETEROPOPULATION MALES. WHITE BARS = TIME WITH SYMPATRIC CONSPECIFIC. GRAY BARS = TIME WITH HETEROSPECIFIC OR HETEROPOPULATION MALES. ERROR BARS =  $\pm 1$  SE. A) *X. BIRCHMANNI* ASSOCIATION TIMES WITH CONSPECIFIC VS. HETEROSPECIFIC MALES, IN COMPARISONS WITH *X. MALINCHE*, SYMPATRIC *X. VARIATUS*, AND ALLOPATRIC *X. VARIATUS* AS THE HETEROSPECIFIC SPECIES. B) *X. MALINCHE* ASSOCIATION TIMES WITH CONSPECIFIC VS. *X. BIRCHMANNI* MALES. C) *X. VARIATUS* ASSOCIATION TIMES WITH SYMPATRIC CONSPECIFIC VS. *X. BIRCHMANNI* MALES, OR VS. ALLOPATRIC CONSPECIFICS. D) *X. BIRCHMANNI* ASSOCIATION TIMES WITH CONSPECIFIC VS. *X. MALINCHE* MALES OVER A SECOND EXPERIMENT. .... 78

FIGURE 5.2: FEMALE *X. VARIATUS* ASSOCIATION TIMES WITH SYMPATRIC CONSPECIFICS (HOMOPOPULATION) VS. HETEROPOPULATION MALES (EITHER SYMPATRIC *X. BIRCHMANNI* OR ALLOPATRIC CONSPECIFICS), BY TRIAL NUMBER...79

## **Introduction**

Hybridization is the production of viable offspring from the crossing of genetically distinguishable taxa (Mallet 2005). It is surprisingly common: at least 25% of plant species and 10% of animal species hybridize in nature (Mallet 2005), and rapid advances in genetic techniques are revealing ever-increasing examples. Although botanists have long recognized the diversity-generating potential of hybridization (e.g. Anderson, 1949, Stebbins 1959), it is becoming increasingly apparent that animal hybridization, too, can act not only as a limit on diversification, but as a source of evolutionary novelty (Arnold 1997; Seehausen 2004; Grant et al. 2005; Mallet 2005, 2007).

Why do animals hybridize? To answer this question, one needs to understand how individuals recognize and choose their mates. It is common to explain hybridization as a 'mistake' in mate choice, because hybrids are often less fit than purebred individuals. However, it can be fruitful to distinguish between the causes of hybridization, and the consequences. To that end, I phrase the question "Why do animals hybridize?" differently, asking "What influences mate choice in actually or potentially hybridizing species?" My first three chapters address one category of influences, the direct fitness effects of mate choice. My fourth chapter addresses another category, species preferences and how they arise. Finally, chapter 5 explores how preferences for conspecifics can vary, even in closely-related taxa.

Traditionally, studies of hybridizing species have focused on the genetic (i.e. indirect) costs of hybridization, which are often substantial, and are integral to the study of reproductive isolation. However, acquiring "good genes" for offspring is only one

consequence of mate choice. While empirical studies of indirect benefits are somewhat scarce, direct benefits or other mechanisms appear at least as important as indirect benefits in influencing mate choice within species (Møller and Alatalo 1999, Cameron et al. 2003, Charmantier and Sheldon 2006, Kotiaho and Puurtinen 2007, Hetttyey et al 2010). Direct fitness effects are those that influence the chooser's survival or fecundity directly; for example, exposure to predators or access to quality breeding sites. These have prominent effects of mate choice within species (reviewed in Jennions and Petrie 1997, Cotton et al. 2006).

Although direct costs and benefits are important in intra-specific mate choice, their role in hybridizing taxa is largely unknown. Yet, where selection against hybridization is weak or absent (e.g., where hybridization itself is rare or absent, or where hybrids have non-zero fitness), direct costs and benefits may play an important role promoting or inhibiting hybridization events (e.g. Pierotti and Annett 1993, Nuechterlein and Buitron 1998, Good et al. 2000, Veen et al. 2001, Pfennig 2007, Wiley 2007). Hybridization may often be the outcome of a conditional mate choice strategy, employed in response to dynamic conditions that alter the direct costs and benefits of choice (Wilson and Hedrick 1982, Wiley et al. 2007).

In chapter 1, I examine hybridization across the North American wood-warblers. Hybridization has been reported in over half of the 45 species of wood-warbler that breed in North America. In other birds, both a limited availability of conspecifics and of quality breeding habitat have been proposed as promoting hybridization through a decline in choosiness (e.g., Nuechterlein and Buitron 1998, Jouventin and Bried 2001). Using phylogenetic comparative methods, I investigate whether species-level measures of mate availability (population size and mean breeding density) and breeding habitat availability (habitat size and quality) predict the occurrence of hybridization in each species. I also

examine the effects of phylogeny, song similarity, and extent of sympatry with heterospecifics on patterns of hybridization. I find little evidence of an influence of mate availability on warbler hybridization, but do find higher hybridization rates among species with low breeding habitat availability. I also find increasing hybridization with increasing song similarity, extent of breeding sympatry, and genetic relatedness. This study highlights the potential importance of ecology and opportunities for gene flow in facilitating animal hybridization, and demonstrates how the comparative method can be useful in the study of rare events like hybridization.

In chapter 2, I use experimental methods to focus in on hybridization within a single species pair, *Xiphophorus birchmanni* and *X. malinche*. These two species of closely-related, freshwater fishes hybridize in certain locations where their ranges meet (Rosenthal et al. 2003, Culumber et al. 2011). Hybrids are both viable and fertile, and introgression is extensive in some locations (Rosenthal et al. 2003, Culumber et al. 2011). As predation risk is a very important influence on mate choice within species (e.g., Forsgren 1992), I investigate whether a proxy for predation risk, shelter distance, can counteract female *Xiphophorus birchmanni* preference for conspecifics. I find that females are very sensitive to the presence of shelter, and conclude that females calibrate their mate choices in response to predation risk, as well as species preference. This study supports the notion that context-dependent mate choice might play an important role in animal hybridization.

Chapter 3 similarly uses the *X. birchmanni* - *X. malinche* species pair to investigate how the availability of conspecific males influences female *X. birchmanni* response to heterospecific males. In many species, low encounter rates increase the direct costs of mate sampling, leading to reduced choosiness (e.g., Alatalo et al. 1988; Milinski and Bakker 1992). I evaluate whether or not the delay between encounters with



conspecific males affects female response to heterospecific ones. I find that it depends: if females are offered a choice between a conspecific and heterospecific male, encounter rate has no effect; however, females with only a choice of heterospecific male are more attentive to him when encounter rates are low, rather than high. This study demonstrates that females are sensitive to changes in encounter rate with conspecifics, but that experimental design and female sampling methods will influence the observed outcome. Females also appear to increase their sampling effort in certain circumstances, a finding which further underscores the need to consider sampling behavior in the design and interpretation of choice experiments. As with chapter 2, this study highlights the potential importance of direct costs of mate choice in promoting or inhibiting hybridization between closely-related taxa.

Direct costs of mate choice are relevant to hybridization only if individuals recognize heterospecifics as potential mates. Although preferences for conspecifics often have a heritable basis, in some species they can be learned. The extent to, and mechanisms by which preferences for conspecifics are learned can influence the extent and evolution of reproductive isolation. Many studies of learning in the formation of mate preferences examine learning during early life stages (reviewed in Irwin and Price 1999). However, learning during adulthood can also be important (e.g. Haskins and Haskins 1949, Magurran and Ramnarine 2004). Remarkably, a recent study of *X. birchmanni* and *X. malinche* found opposing effects of short-term experience with heterospecifics, during adulthood, on female preferences for conspecifics (MN Verzijden, ZW Culumber, and GG Rosenthal, unpublished data). In chapter 4, I conduct a similar study with a different species pair, investigating whether female *X. birchmanni* preferences for conspecifics are influenced by short-term experience with their sympatric congener, *X. variatus*. Although wild-caught females have expressed preferences for conspecifics in previous studies (e.g.

Fisher and Rosenthal 2009), lab-reared females, which have never encountered *X. variatus*, show no preference for males of either species. This lack of species preference persists even after short-term experience with heterospecifics. Although this study does not identify how preferences are acquired, the difference between wild-caught and lab-reared females suggests that conspecific preferences are not fixed throughout life, but respond to age- or experience-related factors, which may influence the extent of sexual isolation in the wild.

Even in closely-related species pairs, female preferences for conspecifics can be highly asymmetric (e.g., Collins and Luddem 2002, McLennan and Ryan 2008). In chapter 5, I examine reciprocal preferences for conspecifics in two species pairs: *X. birchmanni* - *X. malinche*, and *X. birchmanni* - *X. variatus*. In the former pair, the two species are close relatives within the clade of northern swordtails (Rauchenberger et al. 1990, Marcus and McCune 1999). They have parapatric distributions, and hybridize in some regions of overlap (Rosenthal et al. 2003, Culumber et al. 2011). In contrast, members of the latter species pair are more distantly-related, and occur in sympatry in several regions with no reported hybridization (Rauchenberger et al. 1990, Marcus and McCune 1999, Kallman and Kazianis 2006). I find asymmetries in both species pairs, with only *X. birchmanni* exhibiting a preference for conspecifics. However, in a repeat experiment, *X. birchmanni* did not express a preference for conspecific over *X. malinche* males, a difference which is puzzling and emphasizes the importance of replication. I suggest that reproductive isolation between *X. birchmanni* and *X. variatus* could be mediated by male, as well as female, preferences, and discuss a number of factors that may influence conspecific preference, or the measurement thereof.

These studies offer insight into why animals hybridize. Animal hybridization is an "ecologically-dependent behavioural phenomenon" (Grant and Grant 2008). Approaching

the study of animal hybridization from this perspective may help us to better understand reproductive isolation, and the loss and gain of diversity.

## **Chapter 1: Ecology, phylogeny, and song similarity predict hybridization in North American wood warblers**

### **ABSTRACT**

Animal hybridization can have dramatic effects on biological diversity. Theory predicts that variation in ecological conditions, such as the density of potential mates, should influence the ease with which hybridization occurs, however, this prediction is rarely tested empirically. Using phylogenetic comparative methods, we investigated whether species-level measures of mate availability (population size and mean breeding density) and breeding habitat availability (habitat size and quality) predict the frequency of hybridization in the 45 species of North American wood warbler (family Parulidae). We also examined the effects of phylogeny, song similarity, and the extent of sympatry with heterospecifics on patterns of hybridization. Our findings suggest that species with smaller population sizes, but not those with lower mean breeding densities, have higher hybridization rates. We also find higher hybridization rates among species with smaller or poorer quality breeding habitats. Our findings support the view that wood warblers may sometimes hybridize when the availability of conspecific mates or breeding habitat constrain mate choice. In addition, we found hybridization to decrease with increasing phylogenetic distance, and increase with increasing song similarity and extent of breeding sympatry. Our study highlights the potential role ecological conditions can play in promoting or inhibiting hybridization.

## INTRODUCTION

Animal hybridization can have profound effects on biological diversity, acting as both a homogenizing force and a source of evolutionary novelty (Mayr 1963; Arnold 2006). Until recently, the importance of animal hybridization in evolution was still contentious (Dowling and Secor 1997). Molecular studies, however, provide rapidly increasing evidence of introgressive hybridization in animals and its capacity to fuel speciation and adaptive radiations (Seehausen 2004; Mallet 2005, 2007) as well as species extinctions (Rhymer and Simberloff 1996; Seehausen et al. 2008). Traditionally, most studies of hybridizing taxa have focussed on the genetic consequences, which are evolutionarily important. However, identifying both the causes as well as the consequences of animal hybridization can enhance our understanding of the loss and gain of diversity.

Animal hybridization is an "ecologically-dependent behavioral phenomenon" (Grant and Grant 2008). In some cases, ecological conditions may mask or confuse mate recognition cues (e.g. Grant and Grant 1997, Seehausen et al. 1997), or bring formerly allopatric species that lack behavioral isolation into secondary contact (e.g. Rosenfield and Kodric-Brown 2003), leading to hybridization. Ecological conditions are, however, likely to be important in a broader range of contexts. They influence the ease of finding preferred mates, the relative quality of potential mates, and other factors that, by influencing expected survival and reproductive success, cause variation in mate choice (Jennions and Petrie 1997; Widemo and Sæther 1999; Cotton et al. 2006). This variation may include the choice of heterospecifics, as some authors have argued (e.g. Wilson and Hedrick 1982, Pfennig 2007, Wiley 2007). For example, a lack of available conspecific

mates is considered one of the most common causes of animal hybridization (e.g. Hubbs 1955; Mayr 1963; McCarthy 2006). This assertion, however, is predominantly a post hoc explanation of observed patterns, and there are few formal tests of it (but see Grant and Grant 1997; Nuechterlein and Buitron 1998; Wirtz 1999; Randler 2002, 2006; Gee 2003). Studies investigating the influence of other ecological conditions on hybridization, such as breeding habitat availability, are similarly uncommon (e.g. Pfennig 2007; Wiley 2007).

Hybridization occurs regularly among birds (Grant and Grant 1992; Mallet 2005), with premating isolation acting as a primary barrier to gene flow (Price and Bouvier 2002; Fitzpatrick 2004). Hybridization has been reported for 34 of the 45 species of North American wood warblers (family Parulidae) (McCarthy 2006). The age of the family (Lovette and Bermingham 1999; Price et al. 2000) relative to the slow evolution of hybrid inviability in passerines (Price and Bouvier 2002; Fitzpatrick 2004) predicts high hybrid viability across the family, and where studied, hybrids face few intrinsic fitness disadvantages (e.g. Rohwer and Wood 1998; Pearson 2000; Vallender et al. 2007; Neville et al. 2009; but see Brelsford and Irwin 2009). In North America, most parulid species breed in sympatry with several others (Lovette and Hochachka 2006), with as many as 19 species found breeding in a 30 x 30 km area (Lemon et al. 1983). The absence of prominent post-mating isolation in the presence of extensive breeding sympatry suggests that mate choice is particularly important in limiting interspecific gene flow.

Although many factors can facilitate avian hybridization (e.g. Grant and Grant 1997; Randler 2002 2006), limited breeding opportunities with conspecifics appear to play an important role (e.g. Nuechterlein and Buitron 1998; Randler 2002, 2006). It has also been suggested that limited favorable breeding habitat can similarly constrain mate

choice and promote avian hybridization (Jouventin and Bried 2001; Randler 2002). Both of these factors may facilitate hybridization among North American wood warblers. Wood warbler hybrids are commonly found at species' range limits, or where one or both parental species are rare, and a lack of conspecific mates is frequently posited as a contributing factor (e.g. Parkes 1978; Carlson 1981; Hurley and Jones 1983; Morse 1989; Rohwer 1994; Latta and Parkes 2001). In addition, favored breeding habitat is limited in many species, and competition for territories, to which females strongly attend, can be intense (e.g. Sherry and Holmes 1989; Bayne and Hobson 2001; Martin and Martin 2001; Sillett et al. 2004). Females might therefore be more likely to pair with heterospecifics where competition for breeding sites is high.

Several other factors unrelated to mate or habitat availability could also influence hybridization in wood warblers. For example, male song is important for female mate choice in many songbirds (reviewed in Kroodsma and Miller 1996; Price 2008), and song similarity could contribute to heterospecific pairing and hybridization (e.g. Qvarnström et al. 2006). Breeding sympatry with heterospecifics offers increased opportunities for hybridization, but could also act to strengthen premating isolation between species via reinforcement (Dobzhansky 1940; Randler 2006). As related species are similar due to common descent (see Harvey and Pagel 1991), species that are closely related phylogenetically might be more prone to hybridization. Increasing phylogenetic distance might also have negative effects on hybrid viability, as occurs at larger taxonomic scales in birds (Price and Bouvier 2002). Among wood warblers, however, intergeneric hybrids have been reported as outnumbering intrageneric ones (Parkes 1978), a pattern known as "Parkes' paradox" (Gill 1998). Parkes (1978) suggested this pattern could reflect weaker premating isolation between distantly related taxa.

Most studies of the causes of animal hybridization address a particular system (but see Randler 2002, 2006). A comparative approach can complement such studies by indicating how general patterns are across taxa, and allowing several factors to be evaluated simultaneously. Here we use phylogenetic comparative methods to investigate whether measures of conspecific mate and breeding habitat availability predict hybridization in North American wood warblers. We use two measures of abundance, species' population size and mean breeding density, as estimates of the availability of conspecific mates, and breeding range size and breeding habitat quality as measures of breeding habitat availability. We likewise address the influence of song similarity, extent of sympatry with heterospecifics, and phylogeny on wood warbler hybridization.

## **METHODS**

### Data collection

We compiled hybridization data from published literature, conference abstracts, ornithological websites, and GenBank (Table 1.1). Various types of detection biases or sampling errors may have influenced the subset of natural hybrid crosses that are recognized and documented. We used a conservative approach of including only well-documented hybrids, most of which were based on specimen materials, which allowed close inspection and hence relatively robust inferences about the identities of the parental species. We assigned species names according to the most recent AOU checklist (American Ornithologists' Union 1998) and supplements. Crosses between sub-species were not considered. To maximize the relevance of current population parameters (e.g.



population size), we considered only hybridization events occurring since 1980. As most demographic data were available only for species that breed primarily or exclusively in North America north of Mexico, analyses were restricted to these 45 taxa.

We calculated three measures of hybridization: a binary measure (hybrids reported or not) of hybrid occurrence for each potential pairwise combination of species; the total number of hybrid individuals reported per species; and the per-capita species hybridization rate  $((\text{total number of hybrids} + 0.1) / \text{population size})$ . A constant of 0.1 was added to allow for log-transformation (see below). For six species pairs only a qualitative estimate of hybrid number was available (Table 1). We therefore arbitrarily assigned a value of three to crosses involving few, but at least two, hybrids; a value of 10 to yellow-throated warbler (*D. dominica*) x northern parula (*P. americana*) crosses, for which hybrid individuals are sporadically but regularly reported; and a value of 100 to the three species pairs with active and extensive hybrid zones. Excluding crosses with arbitrarily assigned values did not qualitatively change the results except for the addition of a significant predictor in one analysis, detailed below.

We used estimates of species population size and mean breeding density from the Partners in Flight (PIF) Landbird Population Estimates Database version 2004 (Blancher et al. 2007; [http://rmbo.org/pif\\_db/laped](http://rmbo.org/pif_db/laped)). The PIF Species Assessment Database (version 2005, Panjabi et al. 2005; <http://www.rmbo.org/pif/pifdb.htm>) provides an ordinal measure of breeding range size, as well as an ordinal score of threats to survival and reproduction in breeding areas (Panjabi et al. 2005). We transformed this latter score into a breeding quality score ranging from 1 (extreme deterioration in breeding conditions expected, species in danger of extirpation over large regions) to 5 (expected conditions for breeding are enhanced by widespread human activity). These data are provided in the Appendix.

As a measure of sympatry, we used regional co-occurrence indices as described in Lovette and Hochachka (2006), calculating the mean for each species pair. These indices are derived from Breeding Bird Survey census data obtained between 1997 and 2003 (Sauer et al. 2008) and range from 0 (species B is never reported on transects reporting species A) to 1 (species B is always reported on transects reporting species A). We also recorded the total number of heterospecific species breeding in sympatry (defined as  $> 0$  regional co-occurrence) with each species.

Song in wood warblers is complex and variable (Spector 1992), and a robust analysis of song similarity among species is beyond the scope of the present paper. Spectrogram cross-correlation analysis, however, provides one measure of similarity (Clark et al. 1987). Samples of male primary song (used in mate attraction; Spector 1992) archived in the Cornell Lab of Ornithology Macaulay Library ([www.macaulaylibrary.org](http://www.macaulaylibrary.org); catalogue numbers in the Appendix) were high-pass filtered at 500 Hz and subjected to amplitude-normalized cross-correlation analysis using SIGNAL 5.10.28 (Engineering Design 2008).

## Statistical analyses

Wood warbler species do not represent independent data points because of their shared phylogenetic history (Harvey and Pagel 1991), necessitating phylogenetic comparative methods where appropriate. We used a robust maximum-likelihood phylogenetic hypothesis derived from multiple nuclear and mtDNA loci (Figure 5 in Lovette et al. 2010). The incorporation of multiple nuclear loci reduces potential biases in the topology arising from mtDNA introgression due to past hybridization.

In this study, we were interested in two general questions. First, are pairwise distance measures (e.g. song similarity) correlated with the occurrence of hybridization of each species pair? Second, what is the relationship between species-level parameters (e.g. population size, see below) and either the number of hybrids reported for that species or its per-capita hybridization rate? In both cases, data are not independent: two species are represented in 1) all pairwise measures, 2) each species' hybrid number, and 3) each species' hybridization rate. This non-independence exists in addition to any arising from shared phylogenetic history. We therefore performed two types of analyses to accommodate the type of data (pairwise or not) and the two sources (phylogenetic or not) of non-independence in data: a phylogenetic permutation test of pairwise distance matrices (Lapointe and Garland 2001), and permutation tests of regression coefficients (including, where appropriate, independent contrasts, Garland et al. 1992; Legendre and Desdevises 2009).

To examine the relationship between hybridization, song similarity, and sympatry, we conducted a phylogenetic permutation test. The phylogenetic permutation test corrects for the autocorrelation of the data arising due to phylogenetic structure (Lapointe and Garland 2001), accommodating both sources of non-independence in the data. We converted the hybrid occurrence, extent of sympatry, and song similarity matrices to dissimilarity matrices before analysis (9 999 permutations) with the 'ape' package of the statistical package R (<http://cran.r-project.org>). We were also interested in whether hybridization was more or less likely among closely-related species pairs. We therefore created a patristic distance matrix from the phylogeny, and tested for correlations with the hybrid dissimilarity matrix using a Mantel test (9 999 permutations).

To determine whether conventional or phylogenetically-informed regression analyses were appropriate, we tested for phylogenetic signal in species-level variables using the 'multiPhylosignal' function of the 'picante' package in R.

We investigated whether species with smaller population sizes produce proportionately more hybrids using a negative binomial generalized linear model, with 9 999 permutations. As no phylogenetic signal was detected for these variables, we conducted standard statistical analyses.

We next examined the relationship between species per-capita hybridization rate and four predictor variables: mean breeding density, breeding range size, breeding habitat quality, and number of heterospecific species breeding in sympatry. To do so, we derived independent contrasts and conducted a permutation test of linear multiple regression through the origin (Legendre and Desdevises 2009). One advantage of the independent contrasts method is that contrasts can be derived for phylogenetically independent traits using a neutral star phylogeny (e.g. Perry and Garland 2002; Rheindt et al. 2004). Multiple regression can then be carried out on contrasts of both phylogenetically dependent and independent variables. To obtain appropriately standardized contrasts values for the only measure exhibiting phylogenetic signal, hybridization rate, we log-transformed the data and branch lengths (see Garland et al. 1992). We calculated contrasts using the 'pic' function of the 'ape' package in R. At each step of model fitting we tested the robustness of the model by removing any influential contrasts. We selected the best subset model using stepwise automatic variable selection based on Akaike's Information Criteria, provided by the 'stepAIC' function of the 'MASS' package of R. We performed permutation of the regression coefficients (9 999 permutations) using the 'lmargin' package in R.

## RESULTS

Twenty-four of 45 species produced at least one reported hybrid, involving 20 combinations of species crosses (Table 1.1). Phylogenetic permutation and Mantel test results are given in Table 1.2. Hybridizing species pairs had significantly shorter patristic (phylogenetic) distances (mean  $\pm$  1 SE:  $0.110 \pm 0.015$  vs.  $0.189 \pm 0.001$ ) and greater song similarity (mean correlation coefficients  $\pm$  1SE:  $0.290 \pm 0.027$  and  $0.216 \pm 0.004$ ) than non-hybridizing pairs. Overall, hybridizing species pairs shared significantly greater levels of breeding sympatry than did non-hybridizing pairs (mean co-occurrence values  $\pm$  1SE:  $0.353 \pm 0.052$  vs.  $0.196 \pm 0.007$ , respectively). Considering only those species that at least occasionally overlap (i.e., have  $> 0$  co-occurrence values), the same trend was observed (mean co-occurrence values  $\pm$  1SE:  $0.372 \pm 0.051$  hybridizing pairs,  $0.279 \pm 0.008$  non-hybridizing pairs), although this difference was not significant (Two-sample permutation test:  $z = -1.889$ ,  $N_1 = 17$ ,  $N_2 = 611$ ,  $P = 0.063$ ). There was no correlation between patristic distance and extent of sympatry. Song similarity and breeding range overlap, accounting for phylogeny, were not significantly correlated with each other. Taking into account both phylogeny and the extent of breeding range overlap, song similarity was weakly but significantly positively correlated with the occurrence of hybridization.

There was no correlation between the number of hybrids reported and population size (pseudo  $R^2 = 0.007$ , permutation  $P = 0.094$ ).

TABLE 1.1: HYBRID CROSS COMBINATIONS REPORTED SINCE 1980.

parental cross	no. hybrids reported	sources
<i>Dendroica caerulescens</i> x <i>Dendroica petechia</i> x <i>Vermivora ruficapilla</i>	1 1	Ducharme and Lamontange 1992 Dunn and Garrett 1997; Parkes pers. comm., cited in Williams 1996
<i>Dendroica castanea</i> x <i>Dendroica fusca</i>	1	Hurley and Jones 1983
<i>Dendroica cerulea</i> x <i>Parula americana</i>	>2	Wright 2007; <a href="http://www.ohiobirds.org/news.php?News_ID=97">http://www.ohiobirds.org/news.php?News_ID=97</a> ; <a href="http://www.birds.cornell.edu/WSB/letter">http://www.birds.cornell.edu/WSB/letter</a> ; <a href="http://birding.typepad.com/youngbirders/2009/05/index.html">http://birding.typepad.com/youngbirders/2009/05/index.html</a>
<i>Dendroica coronata</i> x <i>Dendroica magnolia</i> x <i>Dendroica tigrina</i> x <i>Dendroica townsendi</i>	1 1 2	Latta et al. 1998 B. Marks and D. Willard, unpublished data <sup>1</sup> Dunn and Garrett 1997; McCaskie 1984
<i>Dendroica dominica</i> x <i>Parula americana</i>	several	Ulrich and Ulrich 1981; Stevenson and Anderson 1994
<i>Dendroica fusca</i> x <i>Dendroica kirtlandii</i> x <i>Mniotilta varia</i>	1 2	Latta and Parkes 2001 K. C. Parkes, unpublished data <sup>2</sup> ; Bain 1996
<i>Dendroica nigrescens</i> x <i>Dendroica occidentalis</i> x <i>Dendroica townsendi</i>	>2 1	Rohwer and Wood 1998; Rohwer et al. 2000 Rohwer 1994
<i>Dendroica occidentalis</i> x <i>Dendroica townsendi</i>	extensive hybridization	Pearson 2000; Rohwer et al. 2000
<i>Dendroica petechia</i> x <i>Geothlypis trichas</i>	1	J. Griffiths, J. Crowley, and W. Messier, unpublished data <sup>3</sup> ; GenBank record EU340248
<i>Dendroica townsendi</i> x <i>Dendroica virens</i>	1	Rohwer 1994

TABLE 1.1 - CONTINUED

<i>Mniotilta varia</i>	x	<i>V. chrysoptera</i>	1	<a href="http://amazilia.net/images/Birds/NewWarblers/Hybrid_Warbler.htm">http://amazilia.net/images/Birds/NewWarblers/Hybrid_Warbler.htm</a>
<i>Oporornis formosus</i>	x	<i>Oporornis philadelphia</i>	1	Bonter and Lovette 2007; <a href="http://www.birds.cornell.edu/AllAboutBirds/mysteryfiles/answer">http://www.birds.cornell.edu/AllAboutBirds/mysteryfiles/answer</a>
<i>Oporornis philadelphia</i>	x	<i>Oporornis tolmiei</i>	extensive hybridization	Irwin et al. 2009
<i>Vermivora chrysoptera</i>	x	<i>Vermivora pinus</i>	extensive hybridization	Confer and Tupper 2000; Vallender et al. 2007
<i>Vermivora peregrina</i>	x	<i>Vermivora ruficapilla</i>	1	Dunn and Garrett 1997; Parkes pers. comm, cited in Williams 1996

<sup>1</sup>Marks, B. and Willard, D. 1996. Two new intrageneric warbler hybrids. Abstracts of the 114th meeting of the American Ornithologists' Union, Boise State University, 13-17 August 1996. Boise, Idaho.

<sup>2</sup>Parkes, K. C. 1983. Three additional hybrid combinations in North American birds. Proceedings of the 101st meeting of the American Ornithologists' Union, 26-30 September 1983. New York.

<sup>3</sup>Griffiths, J., Crowley, J. and Messier, W. 2008. An intergeneric hybrid warbler (*Dendroica* x *Geothlypis*) captured in Big Sur, California. Abstracts of the AOU/COS/SCO 2008 Meeting, 4-9 August 2008. Portland, Oregon.  
<http://www.pdxbirds08.org/viewabstract2.asp?AbstractID=4414>

response variable	predictor	z-stat	<i>r</i>	<i>P</i>
hybrid occurrence	patristic distance*	183.27	0.186	<0.001
	sympatry, holding song constant	3.14	0.116	<0.001
	song, holding sympatry constant	1.55	0.106	0.003
song	patristic distance*	145.47	0.135	0.020
	sympatry	568.24	0.010	0.466
sympatry	patristic distance*	137.56	-0.022	0.599

\*Mantel test results

TABLE 1.2: PHYLOGENETIC PERMUTATION AND MANTEL TEST RESULTS OF CORRELATIONS BETWEEN HYBRID OCCURRENCE, PHYLOGENETIC, SONG, AND SYMPATRY MATRICES.

The results of the regression analyses and permutation tests of independent contrasts of hybridization rate and ecological predictors are provided in Table 1.3. In this analysis, exclusion of those species with arbitrarily-assigned values of hybrid number (see Methods) yielded qualitatively different results. For both the full and reduced datasets, the best model chosen by the stepwise AIC-based procedure explained approximately half (50% full, 54% reduced) of the variance in hybridization rate contrasts. Contrasts of breeding density, although negatively correlated with those of hybridization rate in univariate models (full:  $b = -1.629$ , permutation  $P = <0.001$ ; reduced:  $b = -1.291$ , permutation  $P = 0.005$ ), did not contribute significantly to hybridization rate contrasts in the multivariate models using either dataset. For both datasets, contrasts of breeding range size were significantly negatively correlated with contrasts of hybridization rate, whereas a significant positive correlation was observed between contrasts of the number of heterospecifics species in sympatry and hybridization rate. Contrasts of breeding habitat quality were significantly negatively correlated with those of hybridization rate only in the reduced dataset. These results suggest that



hybridization might be facilitated by limited or low quality breeding habitat, and sympatry with multiple heterospecific species.

predictor	coefficient	<i>P</i> -param	<i>P</i> -perm
<i>full dataset</i>			
breeding range size	-0.262	<0.001	<0.001
no. of sympatric species	0.012	0.02	0.022
<i>reduced dataset</i>			
breeding range size	-0.194	<0.001	0.001
breeding habitat quality	-0.148	0.034	0.032
no. of sympatric species	0.015	0.012	0.012

TABLE 1.3: INFLUENCE OF BREEDING RANGE SIZE, BREEDING HABITAT QUALITY, AND THE NUMBER OF SYMPATRIC HETEROSPECIFICS ON SPECIES' PER-CAPITA HYBRIDIZATION RATE. P-PARAM = ONE-TAILED PARAMETRIC P-VALUE, P-PERM = ONE-TAILED PERMUTATION P-VALUE. FULL DATASET = ALL SPECIES, REDUCED DATASET = EXCLUDING SPECIES WITH ARBITRARILY-ASSIGNED HYBRID NUMBER VALUES; SEE TEXT.

## DISCUSSION

Premating isolation is a primary mechanism limiting gene flow between sympatric bird species (Price and Bouvier 2002; Fitzpatrick et al. 2004). In North American wood warblers, behavioral isolation is likely to be the predominant barrier to hybridization among co-occurring species. We found that smaller populations appear to produce proportionately more hybrids than larger ones, and that hybridization rates are higher in species with smaller breeding ranges and lower-quality breeding habitats. These findings support the view that warblers are sensitive to mate and breeding habitat availability during mate choice, and that heterospecific pairing and hybridization might

sometimes result when the availability of either is limited. We did not find support, however, for our hypothesis that hybridization rate increases as average breeding density declines. As with any comparative analysis, our study identified correlations only. Our analyses were also limited to the data available to us, including measures which were either indirect, or were subject to measurement error we cannot account for. For these reasons, a conservative interpretation of our results and consideration of potential confounding variables are warranted; we attempt both below.

#### Influence of conspecific mate availability

Several empirical and comparative studies in birds indicate that a limited availability of mates is often involved in hybridization events (e.g. Nuechterlein and Buitron 1998; Randler 2002 2006). We found that the number of hybrids reported did not scale with population size. As a positive relationship is expected if small and large populations produce the same proportion of hybrids, our result suggests that, on average, smaller populations produce proportionately more hybrids. A low abundance of mates may promote reduced choosiness and facilitate heterospecific pairing. Indirect evidence also suggests that mate availability is often a factor in warbler hybridization. For example, recurring hybrids of *D. dominica* and *P. americana* ("Sutton's warbler") occur primarily where both species are uncommon (Carlson 1981; Morse 1989). Other hybrids are often found near species range limits, or observed between species that are rare or have narrowly-overlapping breeding ranges (e.g. Parkes 1978; Carlson 1981; Hurley and Jones 1983; Morse 1989; Rohwer 1994; Latta and Parkes 2001). The Montréal Biodôme provides an artificial example: within a simulated forest ecosystem, two lone representatives of their species, the black-and-white warbler (*Mniotilta varia*) and the

yellow-rumped warbler (*D. coronata*), paired and produced 3 hybrids (Vallender et al. 2009).

We found no influence of mean breeding density on hybridization rate. This suggests that breeding density either plays little role in driving rare hybridization, or that our density metric is a poor measure of mate availability. Our inability to detect a density effect might be because our measure is a species average, and average conditions are unlikely to be those under which hybridization occurs. The fact that density predicts hybridization rate in the absence of other predictors also suggests the possibility that a density effect exists, but that we have insufficient statistical power to detect it. Within-species studies of hybridization rates with varying levels of mate density (or other measures of mate availability) will be welcome additions to comparative studies such as this one.

#### Influence of breeding habitat availability

We found an influence of breeding range size and, in the reduced dataset, breeding habitat quality on hybridization rate. Territory or nest site quality can be an important, even predominant, influence on female mate choice in wood warblers and other birds (e.g. Ficken and Ficken 1966; Alatalo et al. 1986; Bart and Earnst 1999; Pearson 2000; Bayne and Hobson 2001). Female warblers limited by favorable breeding habitat might become less discriminating and pair heterospecifically, as proposed for other examples of avian hybridization (e.g. Nuechterlein and Buitron 1998; Jouventin and Bried 2001).

Hybridization in both plants and animals has long been associated with disturbed habitats (Hubbs 1955, Mayr 1963). While habitat disturbance is a predisposing, not

causal, factor (Grant and Grant 1997), it can create conditions where both preferred mates and favored breeding areas are limited, and can also bring formerly isolated species into secondary contact. All three conditions can facilitate hybridization (e.g. Lamb and Avise 1986; Rhymer and Simberloff 1996; Wirtz 1999; Scribner et al. 2000).

Breeding habitat quality was a significant predictor of hybridization rate only in the reduced dataset, which excluded those ten species for which only qualitative estimates of hybrid number were available. All six species forming hybrid zones were therefore absent from the reduced dataset, leaving only species that (reportedly) only rarely or never hybridize. Although we can only speculate on why the two datasets should yield different results, it is possible that the factors facilitating the extensive hybridization within hybrid zones are different from those contributing to rare hybridization events. For example, both the golden-winged x blue-winged warbler (*V. chrysoptera* x *V. pinus*) and mourning x MacGillivray's warbler (*O. philadelphia* x *O. tolmiei*) hybrid zones have arisen only recently, following recent secondary contact (Gill 1980; Irwin et al. 2009). Perhaps the quality of breeding habitat, or other ecological influences on choice, are relatively insignificant in promoting heterospecific pairing in species that already lack substantial mating isolation.

#### Influence of song similarity

We found hybridization to be weakly correlated with song similarity. Hybridization in other avian species has been observed in individuals that have imprinted on heterospecifics, or that produce mixed song (e.g. Lemaire 1977; Grant and Grant 1997; Baker and Boylan 1999; Qvarnström et al. 2006). Whether or not mis-imprinting contributes to heterospecific pairing in wood warblers is unknown. Ficken and Ficken

(1968) observed pairings of *V. chrysoptera* and *V. pinus* and noted that, while imprinting errors occurred they appeared to contribute little to mixed pairings. Similar song likely plays a role in attracting potential partners, however pairing might arise only conditionally in response to other factors, such as plumage (e.g. Thusius et al. 2001), male territory (e.g. Pearson 2000) or mate availability.

### Influences of phylogeny and sympatry with heterospecifics

We found hybridization to be more common among closely-related species, but did not find a correlation between extent of sympatry and phylogenetic distance. This implies that closely-related species are not more prone to hybridization simply because they are more likely to co-occur. The pattern of greater hybridization among close relatives is consistent with decreasing hybrid viability with increasing phylogenetic distance as observed at larger taxonomic scales (e.g. Price and Bouvier 2002). Our result is, however, inconsistent with "Parkes' paradox": the observation that intergeneric wood warbler hybrids outnumber intrageneric ones (Parkes 1978). This difference might result from our larger sample size and a phylogeny informed by molecular data. Most traditionally recognized parulid genera are not monophyletic (Lovette and Bermingham 2002; Klein et al. 2004, Lovette et al. 2010) such that some intergeneric crosses (e.g. *P. americana* x *D. dominica*) occur between close relatives.

The small but significant correlation between the number of species breeding in sympatry and the incidence of hybridization suggests that any selection acting to strengthen behavioral isolation (e.g. through reduced hybrid viability) is weak, as expected where hybridization is rare. Such selection should be weakest for species with little opportunity for gene flow, such as those that breed in allopatry. It follows that

hybridization that occurs at breeding range limits could reflect intrinsically weaker behavioral isolation between parapatric taxa (Parkes 1978; Randler 2006), rather than a low availability of conspecific mates. A study of hybrid avian taxa from the western Palearctic (Randler 2006) supports such an interpretation: sympatric species pairs hybridized less than parapatric ones. Among species that at least sometimes co-occur (i.e. occur in parapatry or sympatry), however, we found a different pattern: hybridizing pairs shared the same levels of sympatry as non-hybridizing ones. This finding suggests that hybridization in wood warblers can not be explained solely by any intrinsic weaker premating isolation between parapatric taxa. In wood warblers, it would appear that sympatry does not breed contempt, although our study can not address whether reproductive isolation is enhanced with respect to allopatric species. Behavioral isolation could also be expected to be weaker among species that have only recently come into secondary contact (e.g. *V. chrysoptera* and *V. pinus*), as is seen in many instances of hybridization following species introductions (Rhymer and Simberloff 1996). Most hybridizing wood warbler species have, however, long shared some level of breeding sympatry (Mengel 1967; Lovette and Bermingham 1999; Rohwer and Martin 2007).

#### Other potential influences

As in other birds (e.g. Randler 2002, 2006), a number of additional factors likely influence hybridization in wood warblers, or the reporting thereof. Plumage signals are an important mate choice criterion in many birds (reviewed in Price 2008), and are correlated with male mating success in some wood warbler species (e.g. Thusius et al. 2001; Leichty and Grier 2006; Reudink et al. 2009; but see Chiver et al. 2008). Plumage similarity could therefore facilitate heterospecific pairing. The plumage patterns of wood

warbler species are, however, strikingly diverse, and the parental species of hybrids often differ dramatically in plumage characteristics (Gill and Murray 1972; Morse 1989). This latter observation suggests that plumage similarity is not a prerequisite for heterospecific pairing. Another potential influence on the number and type of hybrids reported is differential sampling or detection bias. Randler (2004) found an influence of detectability on the number of avian hybrids at higher taxonomic levels, but not within orders or families, although this hypothesis has not been tested within the Parulidae.

## Implications

Hybrids are very rarely observed in most wood warbler species. Presumably hybrids occur more frequently than are considered here, as the number of unverified reports (e.g. via birder websites) suggests. Hybridization and introgression can even be extensive despite a paucity of reports (e.g. Irwin et al. 2009). Regardless, rare does not mean insignificant: rare hybridization events can have major impacts on the origin and evolution of lineages (Arnold 1997, 2006; Dowling and Secor 1997; Schwenk et al. 2008). In birds as in other taxa, hybridization has played a creative role, introducing phenotypic novelty and fueling adaptive radiations (Grant et al. 2005; Price 2008). It has also produced many examples of genetic swamping and species extinction (Rhymer and Simberloff 1996; Price 2008) and is a well-recognized conservation concern. The extensive hybridization between *V. pinus* and *V. chrysoptera* is such an example (e.g. Confer and Tupper 2000). Identifying the contexts that lead to hybridization, rare or otherwise, can broaden our understanding of the processes that increase or decrease diversity.

Traditionally, studies of hybridizing species have emphasized the indirect, or genetic, costs of hybridization: the reduced fitness of hybrids, relative to purebred crosses. However, direct costs and benefits (i.e., those that affect the chooser's fitness directly) have prominent effects on mating decisions within species (reviewed in Jennions and Petrie 1997; Widemo and Sæther 1999; Cotton et al. 2006), and can influence those of hybridizing individuals as well (e.g. Nuechterlein and Buitron 1998). In wood warblers, constraints on mate choice might arise through limited availability of mates, competition for breeding sites, declines in fecundity over the breeding season, or other factors that make continued mate search costly. A decline in choosiness may be all that is necessary for the occasional heterospecific mating to occur. Heterospecific pairing may be more likely if females obtain direct benefits, such as superior breeding sites or enhanced provisioning from males (e.g. Pierotti and Annett 1993; Good et al. 2000; Wiley et al. 2007), or are able to offset some of the indirect costs of hybridization, if present, through extra-pair copulations with conspecifics (e.g. Veen et al. 2001). Direct costs and benefits of mate choice could therefore be important in promoting or inhibiting reproductive isolation of sympatric wood warbler species.

Although rarely considered, direct costs and benefits of mate choice are likely to influence hybridization in a broad range of taxa. A rarity of mates makes mate choice more costly, and probably accounts for many hybridization events both in captivity and in the wild (Wirtz 1999). A variety of conditions, however, might contribute to either a decline in choosiness leading to hybridization, or the preferential choice of a heterospecific. For example, female spadefoot toads, *Spea bombifrons*, are more accepting of *S. multiplicata* males when water levels are low, giving the faster-metamorphosing hybrid tadpoles better odds of survival than their pure species counterparts (Pfennig 2007). Changes in the environment therefore have the potential to



promote hybridization between formerly reproductively isolated species (e.g. Lehman et al. 1991), or create "merge-and-diverge oscillations" (Grant et al. 2004) over time (Wilson and Hedrick 1982, Grant et al. 2004, Taylor et al. 2006). Animal hybridization is increasingly recognized as an important evolutionary phenomenon, but our understanding of the role of mate choice in promoting hybridization is poor. While hybridization can result from mistakes in species recognition, it may often be the outcome of context-dependent mate choice.

## **Chapter 2: Predation risk alters female mate choice in a naturally hybridizing swordtail *Xiphophorus***

### **ABSTRACT**

Mate choice is context-dependent, but the importance of context to interspecific mating is largely unexplored. We investigated how variation in predation risk, which has prominent effects on choosiness, influences mate choice in a naturally hybridizing swordtail, *Xiphophorus birchmanni*. Using distance to shelter as a proxy for perceived predation risk, we investigated whether females attend to the risk of predation, and whether predation risk can counteract female preference for conspecifics. Females were sensitive to shelter distance independent of the presence of males. When conspecific and heterospecific males were equally distant from shelter, females preferentially associated with conspecifics. When heterospecific males were closer than conspecific males to shelter, females no longer exhibited a preference, suggesting that females calibrate their mate choices in response to predation risk. Our findings suggest that context-dependent mate choice could play an important role in animal hybridization.

### **INTRODUCTION**

Mate choice is context-dependent (Jennions and Petrie 1997). Internal and environmental conditions influence the costs and benefits individuals accrue from their mate preferences, and the expression thereof. Consequently, mate choice often varies according to attributes of the environment (e.g., predation risk, mate density) or the

chooser themselves (e.g., age, condition). This context-dependent variation is important because it alters the form and strength of sexual selection.

Less appreciated is the influence of context on the potential for hybridization. Certain conditions can, by changing or constraining mate choice, make hybridization more likely (e.g., Gerlai, 2007; Nuechterlein and Buitron, 1998; Pfennig, 2007; Wiley et al., 2007; Wilson and Hedrick, 1982). For example, in hybridizing populations of grebes (*Aechmophorus occidentalis* and *A. clarkii*), males increasingly pursue heterospecific females as conspecific partners become scarce over the breeding season (Nuechterlein and Buitron 1998). If context-dependent mate choice is important in hybridization, then it can not only alter the form and magnitude of sexual selection, it can also affect the origin, loss, and fate of evolutionary lineages through interspecific gene flow (see Arnold 2006).

A prominent influence on mate choice is predation risk (Jennions and Petrie 1997). For many species, mate sampling entails the risk of predation (e.g., Pockington and Dill 1995). Individuals often become less choosy when predation risk is high. For example, female sand gobies (*Pomatoschistus minutus*) prefer large colorful males, but become indiscriminate around predators (Forsgren 1992). Predation risk might similarly decrease choosiness in potentially hybridizing individuals, although this hypothesis has not been tested.

Here we investigate whether variation in perceived predation risk influences female mate choice in *Xiphophorus birchmanni*. Hybridization occurs between *X. birchmanni* and *X. malinche* in several tributaries of the Río Pánuco basin in Hidalgo, Mexico (Rosenthal et al. 2003). The two species inhabit shallow rocky streams subject to seasonal flooding and drought (Rauchenberger et al. 1990), and likely experience considerable variation in predation pressure. Previous studies have shown that female *X. birchmanni* prefer the cues of conspecific males over those of *X. malinche* (Fisher et al.

2006, Wong and Rosenthal 2006). We experimentally tested female sensitivity to predation risk, and whether it influences their choice of conspecifics over heterospecifics.

## METHODS

We collected *X. birchmanni* from Garces (20°56'24"N, 98°16'54"W), and *X. malinche* from Chicayotla (20°55'26"N, 98°34'35"W), in 2008 and 2009. Subjects and stimuli, all sexually-mature, were either wild-caught or first-generation descendents. Females were isolated from males for at least two weeks before testing.

### Experimental design

Water was conditioned and carbon-filtered before use. Two filtered 500 W halogen lamps provided downwelling irradiance (UV/visible) comparable to that of natural *Xiphophorus* habitat (Cummings et al. 2003). The sides of the test aquarium (76 x 30 x 30 cm) were lined with Teflon overlain with filter gels, providing diffused horizontal irradiance (Cummings et al. 2003).

Females were individually offered the choice between conspecific and heterospecific males, presented at opposite ends of the test tank behind clear, UV-transmittant, porous barriers (Fig. 2.1). The barriers allowed access to visual and olfactory cues, both important in *X. birchmanni* mate choice (Fisher et al. 2006, Wong and Rosenthal 2006). Males were presented in randomly-assigned groups of three per side to reduce stress (as can occur when males are presented individually, P.M.W. pers. obs.) and allow females access to within-species phenotypic variation. Each pair of male

groups served as stimuli for three to five females. One *X. malinche* male died during the experiment, and was replaced by a similarly-sized male.

Seeking shelter is a common antipredator tactic in fishes (Allouche 2002), and *X. birchmanni* display this behaviour immediately when startled (P.M.W., pers. obs.). We used distance to shelter as a proxy for predation risk. Females spontaneously took cover underneath a central shelter (a 7.5 cm diameter sponge filter) upon introduction to the tank. Emergence from beneath the shelter, followed by visits to both sides of the tank, initiated a 3 min acclimation period. We then conducted two consecutive, 5 min trials: one with the shelter equidistant from either male compartment, and one with it closer to the heterospecific side (Fig. 2.1). Trial order was alternated between females. Trials in which the female either hid or was inactive for over half the trial were declared void. We recorded association time with each male stimulus, which predicts mate choice and reproductive success in *Xiphophorus* (e.g., Cummings and Mollaghan 2006, Ryan et al. 1990, Walling et al. 2010). We collected data using the automated EthoVision XT video tracking system (version 5.0, Noldus Information Technology, Wageningen, Netherlands). Tanks were emptied, rinsed, and dried between females.

The shelter was visible to males as well as females, thus males could also respond to changes in shelter position. We controlled for potential shelter-male interactions in two ways. First, we recorded two uncorrelated measures of male behaviour: the number of aggressive events among males within the group (bite attempts and lateral displays, (Morris et al. 1995), and overall group activity (total time during which at least one male is actively moving). This allowed us to account for any variation in female behaviour arising due to changes in these (or other correlated) male behaviours (see 'Statistical analyses'). Second, we repeated the experiment without males. This allowed us to observe the influence of shelter position alone on female behaviour.

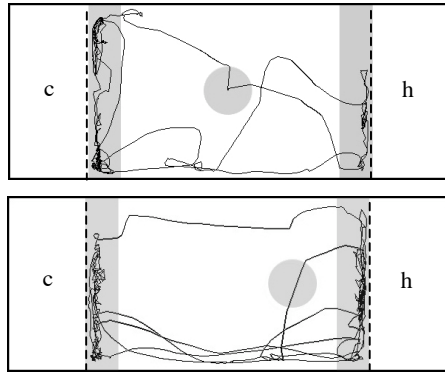


FIGURE 2.1: EXPERIMENTAL TANK DESIGN, DRAWN TO SCALE. SHADED REGIONS REPRESENT SHELTER (CIRCLES) AND ZONES (RECTANGLES) IN WHICH ASSOCIATION TIME WAS RECORDED. UPPER PANEL = SHELTER EQUIDISTANT FROM EITHER MALE COMPARTMENT. LOWER PANEL = SHELTER CLOSER TO THE HETEROSPECIFIC COMPARTMENT. C = CONSPECIFIC MALE COMPARTMENT, H = HETEROSPECIFIC MALE COMPARTMENT. REPRESENTATIVE PATHS OF A SUBJECT FEMALE OVER TWO CONSECUTIVE TRIALS ARE SHOWN.

## Statistical analyses

We used linear mixed models to examine the influence of shelter distance, male species (or side, for trials with no male stimuli), and their interaction on female association time. In the presence of a significant interaction, we performed nested contrasts to evaluate the difference in association time between male species (or side) within each treatment. We used a maximum likelihood protocol implemented by the *lme4* package of R. Female ID and male group were treated as random effects, and treatment order as a covariate. For trials including male stimuli, male aggression and overall activity were included as covariates. We used the second-order Akaike Information

Criterion (AICc) for model selection (Burnham and Anderson, 2002), using the *MuMIn* package in R.

Conventional significance testing of fixed effects in mixed models is a contentious issue (see Baayen et al. 2008). We used Markov chain Monte Carlo sampling (10000 samples) of the posterior distribution of the parameters to generate 95% posterior density credibility intervals and *p*-values (Baayen et al., 2008) using the *pvals.fnc* function in the *languageR* library of R.

## RESULTS

Females preferentially associated with conspecific males when the shelter was equidistant from either species (nested contrast,  $p_{\text{MCMC}} = 0.018$ ; Figure 2.2a). When the shelter was closer to the heterospecific side, females no longer exhibited a preference (nested contrast,  $p_{\text{MCMC}} = 0.123$ ; linear mixed model, significant male species by shelter position interaction; Table 2.1, Figure 2.2a). A significant effect of male species was also detected, with females associating more with conspecifics (Table 2.1). The two measures of male behaviour, aggression and activity, decreased model fit considerably ( $\Delta\text{AIC} = 7.249$ ), and were not retained in the final model. No other significant effects were detected (Table 2.1).

In the absence of males, and with the shelter equidistant from either side, females spent their time equally on either side (nested contrast,  $p_{\text{MCMC}} = 0.225$ ; Figure 2.2b). With the shelter offset, however, females spent more time on the side nearest the shelter (nested contrast,  $p_{\text{MCMC}} = 0.005$ ; Figure 2.2b), resulting in a significant side by shelter position interaction (Table 2.1). No other significant effects were detected (Table 2.1).

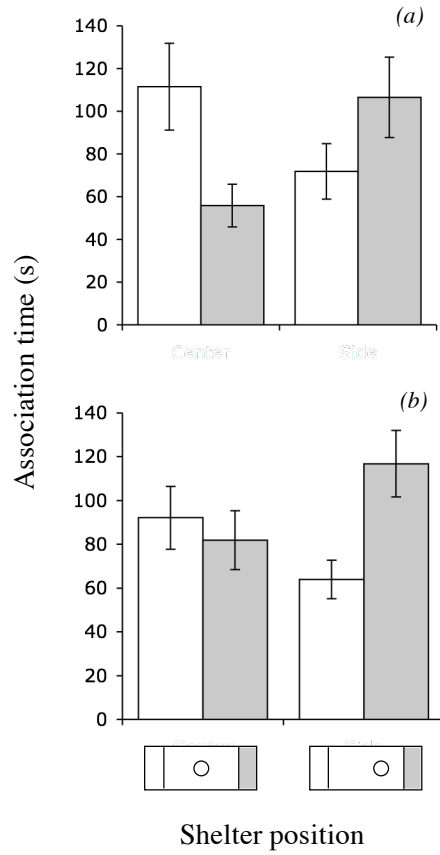


FIGURE 2.2: FEMALE ASSOCIATION TIME IN EITHER ZONE IN THE (A) PRESENCE OR (B) ABSENCE OF MALE STIMULI, WITH THE SHELTER EITHER CENTERED OR OFFSET WITHIN THE TANK. IN (A), WHITE BARS = CONSPECIFIC MALE SIDE, GRAY BARS = HETEROSPECIFIC MALE SIDE. IN (B), BAR COLOR DESIGNATES OPPOSITE SIDES OF THE TANK.

Experiment	Parameter	Coefficient estimate	HPD <sub>lower</sub>	HPD <sub>upper</sub>	$p_{\text{MCMC}}$
males present	male species	-55.67	-102.37	-13.73	0.015
	shelter position	-39.67	-85.26	2.79	0.073
	treatment order	14.97	-17.86	47.75	0.372
	male species x shelter position	90.33	28.44	153.45	0.006
males absent	side	-10.21	-48.76	28.83	0.599
	shelter position	-28.43	-65.40	9.82	0.138
	treatment order	6.41	-23.14	35.39	0.677
	side x shelter position	63.12	10.26	116.46	0.020

TABLE 2.1: BEST LINEAR MIXED MODELS OF EFFECTS ON FEMALE ASSOCIATION TIME, IN THE PRESENCE OR ABSENCE OF MALE STIMULI. EFFECT SIZES (COEFFICIENTS) WITH 95% CREDIBILITY INTERVALS AND P-VALUES.



## DISCUSSION

As with most other species, when conspecific males and those of a sympatric, heterospecific species are presented in otherwise similar contexts, female *X. birchmanni* prefer conspecifics. Such preferences are likely to be important in limiting gene flow in sympatry. Females also appear sensitive to perceived predation risk, as they were reluctant to stray far from shelter. Our results are consistent with field observations of *X. birchmanni*, who rarely venture far from cover (Rauchenberger et al. 1990).

Individuals can benefit both from being choosy and from avoiding predators; however, these are often conflicting activities (Pocklington and Dill, 1995). We have shown that females are sensitive to the perceived risk of predation, and that they adjust their mate choices accordingly. In the wild, seasonal flooding and drought can isolate *Xiphophorus* fishes in small pools (e.g., Gordon 1953). Along with reducing the number of potential mates (which can also lead to reduced choosiness, Jennions and Petrie 1997), this may expose individuals to increased risk of predation, particularly by birds, which are among the main predators of *X. birchmanni* (G.G.R., unpub. dat.). Such elevated risk may constrain female mate choice and increase the likelihood of hybridization. This may be particularly true in areas of anthropogenic disturbance, where discrimination of species-specific olfactory cues is thought to be compromised (Fisher et al. 2006).

Plasticity in mate choice in response to predation risk is widespread (Jennions and Petrie 1997), and can influence the form and strength of sexual selection (e.g., Godin and Briggs 1996). The present study is the first for any animal to demonstrate that predation risk can oppose conspecific mate preferences. The implication is that context-dependent mate choice has the potential to influence not only the form and intensity of sexual selection, but also the fate of evolutionary lineages by introducing genetic novelty into

populations. Discussions of hybridization are often centered upon the genetic consequences. We suggest that further studies of the behavioural causes of interspecific mating can broaden our understanding of the processes promoting or suppressing diversity.

### **Chapter 3: Encounter rates with conspecific males influence female mate choice in a naturally hybridizing fish**

#### **ABSTRACT**

Mate choice can vary in response to environmental or other 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 encounter rates increase sampling costs, favoring reduced choosiness. We investigated the influence of encounter rate on female mate choice in a naturally hybridizing swordtail, *Xiphophorus birchmanni*. We exposed females to conspecific males, followed by either no delay or a long (24 hr) delay before their next male encounter. In the next encounter, females were offered either a choice of only a heterospecific *X. malinche* male or a choice between a conspecific and heterospecific male. When not given a choice, females spent more time with the heterospecific male following the long delay between encounters than they did following 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 changes in male encounter rate, but also employ a sample-based (versus standard-based) comparison tactic, which could make hybridization less likely. We also found that females in the no-choice environment made more visits to the male following the long-delay treatment than they did following the no-delay treatment, which may indicate an increase in sampling

effort. Our study highlights the potential importance of context-dependent mate choice in animal hybridization.

## 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), and quality (e.g., 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 choosiness, their mate preferences, their sampling strategy, or some combination thereof. For example, female fiddler crabs (*Uca annulipes*) becomes less selective as time constraints on successful reproduction increase (Backwell and Passmore 1996); female green swordtails (*Xiphophorus 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 between them. For example, male western grebes (*Aechmophorus occidentalis*) increasingly pursue female Clark's grebes (*A. 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 towards heterospecific (*Spea 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 is a significant source of evolutionary change, capable of promoting, inhibiting, or reversing diversification (Seehausen 2004; Mallet 2005, 2007).

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

encounter rate on heterospecific mate choice, however, has not been investigated experimentally.

Here we investigate the influence of encounter rate on female mate choice in a naturally hybridizing swordtail, *Xiphophorus birchmanni*. *Xiphophorus* fishes belong to the internally fertilizing, live-bearing family Poeciliidae, and range from northeastern Mexico to Honduras (Kallman and Kazianis 2006). Despite general preferences for conspecific cues (Fisher et al. 2006; Wong and Rosenthal 2006), *X. birchmanni* occasionally hybridizes with *X. malinche* at intermediate elevations of the Río Pánuco basin where their ranges meet, allowing introgression to occur (Rosenthal et al. 2003). The two species 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 either 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 two 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 two sets of experiments designed to reveal the sampling tactic females use.

## METHODS

*Xiphophorus birchmanni* were collected from the Río Garces (20°56'24"N, 98°16'54"W), Hidalgo, Mexico and *X. malinche* from the Arroyo Xontla (20°55'26"N, 98°34'35"W) near Chicayotla, Hidalgo (Culumber et al. 2011) between 2008 and 2009. Females used in this study were either wild-caught individuals or their first-generation descendants. We group-housed females in isolation from males for at least 2 weeks before testing (e.g., Morris et al. 1996).

We used a 76 x 30 x 30 cm aquarium for conducting 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., Georgia, USA) and carbon-filtered before use. The aquarium was emptied, rinsed, and dried between trials.

## *Experiment 1*

Before testing, each subject female ( $n = 18$ ) was individually placed into an "encounter tank" (61 x 31 x 51 cm), containing seven randomly-chosen, conspecific males. The same seven 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 hrs, females were removed and tested either immediately, or after 24 hrs in their home (female-only) tank. These two delay periods (0 or 24 hrs) simulated variation in conspecific encounter rate, and are hereafter referred to as the "high encounter rate" and "low encounter rate" treatments.

Upon testing, females were presented with a heterospecific *X. malinche* male and a conspecific female, each isolated at opposite ends of the experiment tank behind a clear porous barrier (Fig. 3.1). This 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 three zones: two 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. Trials in which the female spent over half the time hidden beneath the shelter were excluded from analysis. 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 two 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, Netherlands) for data collection.

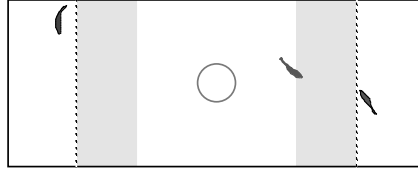


FIGURE 3.1: EXPERIMENTAL TANK DESIGN, AS SEEN FROM ABOVE. STIMULUS FISH ARE ISOLATED BEHIND BARRIERS (DASHED LINES). GRAY AREAS = ASSOCIATION ZONES. CENTER CIRCLE = SHELTER.

Each female was tested twice, once at each delay period, with at least 7 days between treatments. The same stimulus pair was used for both tests; however, no stimulus fish was used with more than one subject. Treatment order was randomized across females.

### *Experiment 2*

The second experiment was conducted in the same manner as Experiment 1, except that an unfamiliar conspecific male replaced the female conspecific stimulus, allowing subject females ( $n = 17$ ) a choice between two males: one conspecific, and one heterospecific. Because of a limited number of male stimulus fish, each stimulus pair was used to test two 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).

### Statistical analysis

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 measures. We included trial number (i.e., whether it was a female's first or second trial) as a covariate. Here and elsewhere, we selected *a priori* the model considered most appropriate, rather than use information-theoretic or other statistical methods of model selection. Diagnostic plots of model residuals were used to examine model assumptions. In analysis of each experiment, two 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) 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 three 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 two 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 (10000 samples) to generate *P*-values and 95% posterior density credibility intervals from the posterior distribution of parameter estimates (Baayen et al. 2008). Modeling and significance testing were conducted using the *lme4* and *languageR* libraries of the programming language R (R Development Core Team 2010).

## RESULTS

### *Experiment 1*

Females significantly increased both the proportion of time spent with (Table 3.1; Fig. 3.2a), and the number of visits made to (Table 3.1, Fig. 3.2b), the heterospecific male after experiencing a 24 hr delay between male encounters compared to when they

encountered conspecific males immediately preceding testing. There was no significant effect of trial order in either analysis.

Choice of male(s)	Dependent Variable	Parameter	Coefficient Estimate	HPD <sub>lower</sub>	HPD <sub>upper</sub>	$P_{\text{mcmc}}$
Heterospecific only (Experiment 1)	Proportion of time spent with the heterospecific	encounter rate trial order	0.1925 0.0315	0.0467 -0.1761	0.3378 0.1134	0.009 0.666
	Number of male visits (square-root transformed)	encounter rate trial order	2.009 -0.705	0.101 -2.539	3.932 1.358	0.043 0.482
Conspecific or heterospecific (Experiment 2)	Proportion of time spent with the heterospecific	encounter rate trial order	-0.053 0.087	-0.204 -0.050	0.084 0.234	0.472 0.211
	Number of male visits (square-root transformed)	encounter rate trial order	-0.280 0.035	-0.759 -0.466	0.194 0.496	0.247 0.967

TABLE 3.1: 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 OR WITHOUT A CHOICE OF MALE. EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS (HPD<sub>LOWER</sub>, HPD<sub>UPPER</sub>) AND MCMC P-VALUES.

### *Experiment 2*

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:  $224.9 \pm 18.7$  vs.  $167.0 \pm 18.0$ ; Table 3.2). There was no influence of

encounter rate treatment or trial order on either the proportion of time females spent associating with the heterospecific (Table 3.1, Fig. 3.2a), or the number of visits made to the males (Table 3.1, Fig. 3.2b).

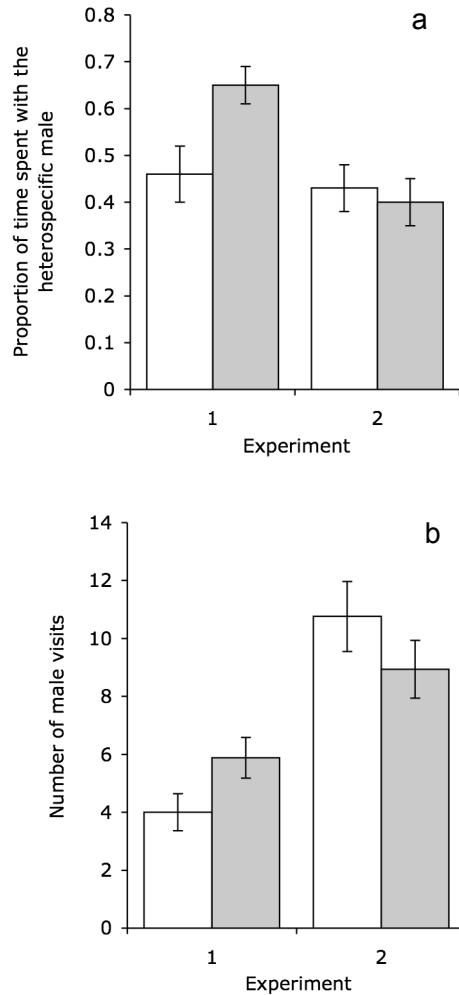


FIGURE 3.2: A) MEAN PROPORTION OF ASSOCIATION TIME SPENT WITH THE HETEROSPECIFIC MALE, AND B) NUMBER OF MALE VISITS MADE BY THE FEMALE, IN CHOICE TESTS WITH (EXPERIMENT 2) OR WITHOUT (EXPERIMENT 1) A CHOICE OF MALE. WHITE BARS = HIGH ENCOUNTER RATE TREATMENT, GRAY BARS = LOW ENCOUNTER RATE TREATMENT. ERROR BARS =  $\pm$  STANDARD ERROR.

## 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-hr 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). Seasonal flooding and drought dramatically alter *Xiphophorus* habitat (e.g., Gordon 1953; 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. 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 ([author names removed for anonymity] unpublished data), low encounter rates with conspecifics may increase the probability of hybridization.

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. Whether they 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.

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 perceived risk of predation, male and female amphipods (*Gammarus duebeni*) both 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 no choice of male available, females made more visits to the heterospecific male following the low-encounter rate treatment than they did after the high-encounter rate treatment. 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 two measures are uncorrelated. The increase in visits could suggest that females without recent encounters with conspecifics 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 low rate treatment, which might also be expected if females were more motivated. Perhaps females were prepared to invest more in sampling following the low-rate 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 low-encounter rate treatment, relative to the high-rate treatment. Future studies are needed to determine how environmental influences on mate choice affect female sampling effort.

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 can not 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. Studying the factors that contribute to variation in mate choice may broaden our understanding of the processes contributing to the loss or gain of diversity.



## **Chapter 4: The effect of short-term experience on conspecific mate preference in the swordtail fish *Xiphophorus birchmanni***

### **ABSTRACT**

Preferences for conspecifics as mates contribute to the reproductive isolation of many closely-related species in sympatry. The extent to, and process by which such preferences are learned have important consequences for reproductive isolation and speciation; however, the role of learning by adult females in shaping conspecific preferences is largely unknown. We investigated female conspecific preference in *X. birchmanni* with respect to males of their sympatric congener, *X. variatus*. In contrast to wild-caught females, lab-reared females lacking prior experience with *X. variatus* showed no preference for conspecific over *X. variatus* males. Subsequent short-term (8-day) experience of lab-reared females with either conspecific males or males of both species had no influence on female preference. Although we found no effect of short-term experience on preference, the differences observed between wild-caught and lab-reared females suggest that conspecific preference in this species are not necessarily fixed through life, but may depend on experience or other age-related factors. Despite a lack of consensus preference in lab-reared females, individuals were weakly consistent in their preferences, which suggests that they can discriminate between males of either species. Our results suggest that the strength of female conspecific preference in this species is variable, which may influence the extent of interspecific mating in the wild.

## INTRODUCTION

Preferences for conspecific mates are integral to the sexual isolation of many closely-related, sympatric species (Mayr 1963, Seehausen et al. 1997, Wirtz 1999). These preferences often have a heritable basis (Bakker and Pomiankowski 1995), and most models of mate preference in reproductive isolation and speciation are explicitly genetic (e.g. Liou and Price 1994, Servedio 2000). Conspecific preferences can also, however, be learned, and the mechanism by which learning occurs can influence evolution, for example, by facilitating or impeding speciation (Laland 1994, Breden et al. 1995, Irwin and Price 1999, Verzijden et al. 2005, Servedio et al. 2009).

Most studies of the acquisition of mate preferences through learning focus on sexual imprinting, wherein early experience leads to preferences for familiar, typically parental, phenotypes (Lorenz 1935, Bateson 1966, Baker and Cunningham 1985). However, learning during adulthood, although less well-understood, can be particularly important in species discrimination among species that lack parental care, such as many insects and fish (e.g., Haskins and Haskins 1949, Magurran and Ramnarine 2004, Svensson et al. 2010). Moreover, preferences for conspecifics can be learned, not only through experience with familiar (usually conspecific) individuals, but through experience with heterospecifics. Until recently, this latter form of learning in adult individuals was known only in males (e.g. Haskins and Haskins 1949; Baker 1994; Dukas 2004, 2008, 2009; Magurran and Ramnarine 2004); however, recent studies reveal its occurrence in females as well (Kozak and Boughman 2009; delBarco-Trillo et al. 2010; Svensson et al. 2010; MN Verzijden, ZW Culumber, and GG Rosenthal, unpublished data).

Conspecific mate preferences are likely to be of primary importance in the maintenance of closely-related, syntopic species (Haskins and Haskins 1949, Seehausen et al. 1997, Verzijden et al. 2008), including members of the genus *Xiphophorus* (Hankison and Morris 2003, Fisher et al. 2006, Fisher and Rosenthal 2010). These freshwater, live-bearing fishes range from northeastern Mexico to Honduras with most species having very restricted ranges. Although hybrids are readily produced in the lab (Rosen 1960, 1979; Kallman and Kazianis 2006), hybridization in the wild is uncommon, with the exception of the *X. birchmanni* - *X. malinche* species pair (Rosenthal et al. 2003, Culumber et al 2010). Sexual imprinting is unlikely in this genus, due to the lack of parental care. However, intra-specific preferences can be learned, or modified, through experience with males of different phenotypes, (e.g. Walling et al. 2008, Tudor and Morris 2009). Recently, Verzijden and colleagues (MN Verzijden, ZW Culumber, and GG Rosenthal, unpublished data) identified opposing effects of short-term (4-7 day) experience with heterospecifics on the conspecific preferences of adult females within the hybridizing *X. birchmanni* - *X. malinche* species pair. Remarkably, female *X. malinche* learned to discriminate against the olfactory cues of heterospecific males after only a few days experience with them; however, *X. birchmanni* females exhibited the opposite pattern, showing increasing preferences for familiar individuals, regardless of species.

*Xiphophorus birchmanni* is parapatric with *X. malinche*, but is syntopic with *X. variatus* throughout much of its range (Rauchenberger et al. 1990, Fisher and Rosenthal 2010). *X. birchmanni* individuals therefore have ample opportunity to interact with *X. variatus*, yet show no evidence of hybridization. Female *X. birchmanni* show a preference for conspecific over *X. variatus* olfactory cues only where the latter species are common, a pattern which is consistent with represent reproductive character

displacement arising in response to selection against hybridization (Fisher and Rosenthal 2010). Alternatively, females could learn to discriminate through experience with heterospecifics.

Here we investigate whether short-term, adult experience with conspecific and *X. variatus* males affects female preference for conspecifics. Previously, we found that wild-caught females prefer conspecific over *X. variatus* males (see Chapter 5). In the present study, we investigated whether lab-reared adult females lacking any experience with *X. variatus* exhibited similar preferences, and whether those preferences were altered by short-term (8-day) experience with either conspecific or *X. variatus* males.

## METHODS

Lab-reared *X. birchmanni* females included first-generation descendents of females collected from the Río Garces (20°56'24"N, 98°16'54"W) and the Río Coacuilco (21°05'49"N, 98°35'21"W) between 2007 and 2009; *X. variatus* are common at both sites (Fisher and Rosenthal 2010, H Kindsvater, pers. comm.). Unlike their wild counterparts, lab-born females were raised to sexual maturity in isolation from *X. variatus*. Due to uncontrolled rearing conditions, females differed in the extent of social and/or mating experience with conspecific males preceding testing; however, all were naïve to *X. variatus*. Males of both species were wild-caught individuals collected from the Río Garces between 2007 and 2009. We isolated females from males at least two weeks before their first tests (e.g., Morris et al. 1996).

We divided 32 females evenly into two treatment groups, consisting of two replicates each. Females descended from the two different source populations (Garces,

Coacuilco) were distributed equally across groups. Female size did not differ between replicates (Kruskal-Wallis chi-square = 2.454,  $df = 3$ ,  $p = 0.482$ ).

Prior to treatment, females in each replicate underwent the first of two dichotomous choice tests. Tests were conducted in 76 x 30 x 30 cm aquarium filled with conditioned, carbon-filtered water. The aquarium was lined with Teflon overlain with filter gels, lit from above with filtered 500W halogen lamps. These lighting conditions provided downwelling and horizontal irradiance similar to their native stream habitat (see Cummings et al. 2003). Upon testing, each female was offered the choice of a conspecific or *X. variatus* male, each isolated behind a clear, UV-transmittant, permeable barrier at either end of the test aquarium. The center chamber was divided transversely into three zones: two narrower "association" zones adjacent to each male compartment, and a wider, central "neutral" zone. A 5 min acclimation period began following female emergence from beneath a central shelter and visits to both association zones. We then recorded time spent in each association zone for 10 min. Association time predicts mate choice and mating success in other species of *Xiphophorus* (Ryan et al. 1990; Morris et al. 1992, 2010; Cummings and Mollaghan 2006; Walling et al. 2010). Four males of each species were used as stimuli for one of the two replicate groups in each treatment, blocked such that each male was used to test 2 females, and each female was tested with a unique species pair combination ( $n = 16$ ). A different set of four males of each species was used for the second replicate group in each treatment.

After their initial test, the eight females in each replicate were housed with eight unfamiliar males in a 208 l aquarium for eight days, with the average size of males across replicate groups held constant (Kruskal-Wallis chi-square = 2.895,  $df = 3$ ,  $p = 0.408$ ). In one treatment group, females were housed with only conspecifics. In the other, females were housed with a 1:1 mix of conspecific and heterospecific males. We chose a mixed-

species male group for the latter treatment, rather than a group composed exclusively of *X. variatus*, because in some species exposure to multiple phenotypes is necessary for, or enhances, learned discrimination (e.g. Rosenqvist and Houde 1997; Dukas 2008, 2009). Additionally, if females avoid mating or reproducing with heterospecifics, then females exposed to only conspecific males may subsequently have a different reproductive status than those exposed only to heterospecifics, confounding reproductive status with treatment. After eight days, females underwent a second round of choice tests, using a pair of unfamiliar males (one of each species) from their original set of stimulus males. Randomization of side biases stuff

We collected data using the video tracking system Ethovision XT 5.0 (Noldus Information Technology, Wageningen, Netherlands). As female *X. birchmanni* attend to male size in intraspecific mate choice (Fisher et al. 2009), and as intraspecific preferences have been shown to vary with female size in some studies (e.g. Morris et al. 2006, Rios-Cardenas et al. 2007, Robinson and Morris 2010), we recorded male and female standard length.

We were interested both in whether females preferred conspecifics over heterospecifics, and whether the strength of (any) preference varied with treatment (conspecific or mixed-species male exposure) or treatment stage (pre- or post-treatment). To assess the former, we constructed a linear mixed model (REML estimation) implemented with the *lme4* package in the R programming language (R Development Core Team 2009). Association time was the dependent variable, and male species, treatment, stage, and their interaction were fixed effects. Male and female length, and female source population, were included as covariates. Female subject ID and replicate group were included as random effects. Due to the large number of predictors, we used the second-order Akaike Information Criterion (AICc) to identify the top-fitting model(s)

( $\Delta\text{AICc} < 2$ , Burnham and Anderson 2002) using the package *MuMIn*; the full model was identified as the single best model. Conventional significance testing in mixed model analysis is a contentious issue, largely because it is unclear how to approximate the degrees of freedom (Pinheiro and Bates 2000). As an alternative, we used Markov Chain Monte Carlo sampling to derive 95% posterior density intervals and associated  $p$ -values (Baayen et al. 2008), implemented with the package *languageR*. Diagnostic tests identified one observation as influential, and visualization of the residuals suggested the possibility of overdispersion. However, analysis with the influential observation removed, nor with transformation to improve residual variance, qualitatively changed the results. As a measure of model goodness-of-fit, we calculated an  $R^2$  statistic as described by Edwards et al (2008).

To evaluate changes in the strength of preference, we first calculated strength as the proportion of association time spent near the conspecific male. We then constructed a full model using linear mixed model procedures as described previously, with preference strength as the dependent variable, and stage, treatment, and their interaction as fixed effects. The difference in length between the male *X. birchmanni* and male *X. variatus* was included as a covariate, with female length, source population, and random effects specified as before. Models were then ranked according to AICc scores. The best model identified fit no fixed effect or interaction terms, with no other model ranking highly (all  $\Delta\text{AICc} > 3$ ), obviating the need for significance testing.

We also investigated whether individual females were consistent in their preferences across their two tests (pre- and post-treatment). We constructed a linear mixed model with strength of preference post-treatment as the dependent variable, and pre-treatment preference strength as a main effect. Additional predictors were treatment,

source population, and female length. Replicate group was included as a random effect. All model assumptions were met.

Our previous analysis of the preferences of wild-caught *X. birchmanni* females for conspecific over *X. variatus* males (see Chapter 5) did not include female length as a covariate. As with the lab-reared females, we were interested in whether larger (or smaller) wild-caught females exhibited weaker (or stronger) preferences. We therefore re-analyzed our earlier data with a linear model, with preference strength as the dependent variable, and female length and the difference in length between the two male stimuli (conspecific - heterospecific) as predictors. All model assumptions were met. We calculated an  $R^2$  statistic as described above.

Finally, we tested whether wild-caught and lab-reared females differed in length using a Wilcoxon rank sum test.

## RESULTS

Unlike wild-caught females (see Chapter 5), lab-reared females exhibited no preference for males of either species, with neither stage, treatment, female length, nor any other predictor influencing association time (mixed model  $R^2 = 0.04$ , all  $p_{\text{mcmc}}$ -values  $> 0.19$ ; Fig. 4.1). Accordingly, the best model of conspecific preference strength included no fixed effect or interaction terms, with all ( $>99\%$ ) of the explained variance due to by-female and by-replicate variability (mixed model  $R^2 = 0.71$ , Fig. 4.2).

Consistent with the results from lab-reared females, our re-analysis of the data from wild-caught revealed no effect of female length, nor length difference between male stimuli, on the strength of preference (linear model  $R^2_{\text{adj}} = 0.04$ ,  $p = 0.305$ ). However,



wild-caught females were significantly larger than lab-reared females (mean  $\pm$  SE: wild-caught,  $1.55 \pm 0.03$ ; lab-reared,  $1.44 \pm 0.04$ ;  $W = 353$ ,  $p = 0.035$ ).

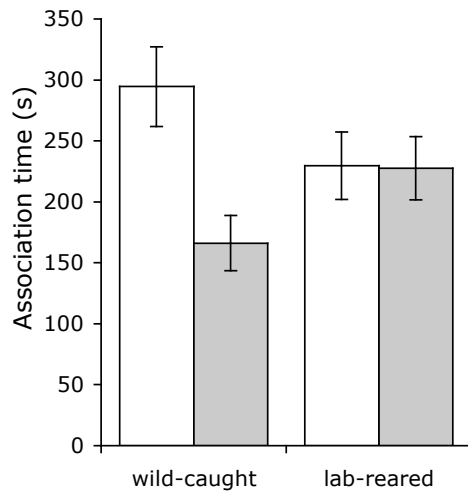


FIGURE 4.1: FEMALE ASSOCIATION TIME WITH CONSPECIFIC (WHITE BARS) AND *X. VARIATUS* (GRAY BARS) MALES, IN WILD-CAUGHT (DATA FROM CHAPTER 5) AND LAB-REARED *X. BIRCHMANNI*. ERROR BARS =  $\pm$  SE.

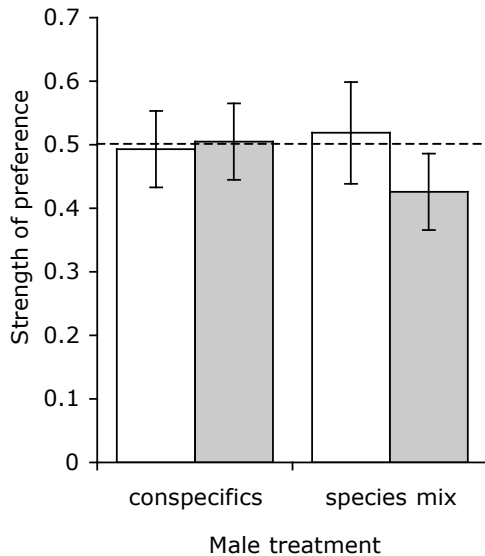


FIGURE 4.2: FEMALE STRENGTH OF PREFERENCE FOR CONSPECIFICS BEFORE (WHITE BARS) AND AFTER (GRAY BARS) EXPERIENCE WITH EITHER CONSPECIFIC MALES, OR A COMBINATION OF CONSPECIFIC AND *X. VARIATUS* MALES, IN LAB-REARED *X. BIRCHMANNI*. PREFERENCE STRENGTHS ABOVE 0.5 (DASHED LINE) WOULD INDICATE A PREFERENCE FOR CONSPECIFICS, SCORES BELOW, A PREFERENCE FOR HETEROSPECIFICS. ERROR BARS =  $\pm$  SE.

Within lab-reared females, we detected a weak but significant effect of strength of preference pre-treatment on strength of preference post-treatment (mixed model  $R^2 = 0.24$ , Table 4.1; Fig. 4.3), indicating a low level of consistency. Neither treatment, source population, nor female length had significant effects on post-treatment preference strength.

Predictor	Coefficient Estimate	HPD <sub>lower</sub>	HPD <sub>upper</sub>	$p_{\text{mcmc}}$
strength of preference pre-treatment	0.544	0.093	0.958	0.016
experience treatment	0.045	-0.255	0.328	0.685
female source population	0.012	-0.369	0.376	0.945
female length	-0.242	-1.043	0.582	0.538

TABLE 4.1: LINEAR MIXED MODEL OF FIXED EFFECTS ON STRENGTH OF CONSPECIFIC PREFERENCE IN LAB-REARED FEMALES FOLLOWING MALE EXPERIENCE. EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS (HPD<sub>LOWER</sub>, HPD<sub>UPPER</sub>) AND MCMC P-VALUES.

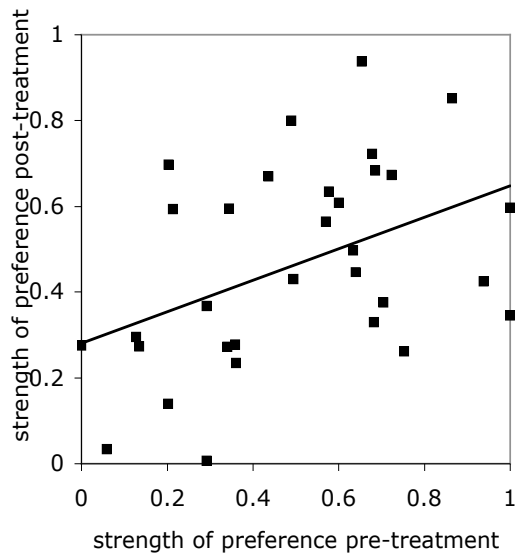


FIGURE 4.3: CONSISTENCY OF STRENGTH OF PREFERENCE IN INDIVIDUAL LAB-REARED FEMALES OVER TWO TESTS (BEFORE AND AFTER MALE EXPERIENCE).

## DISCUSSION

We found that lab-reared *X. birchmanni* females showed no species preference. These results are inconsistent with previous studies of wild-caught females (Fisher and Rosenthal 2010, Chapter 5). Moreover, short-term experience with males of either species had no effect on preference. In contrast, wild-caught *X. birchmanni* exposed to heterospecific *X. malinche* for a similar duration displayed reduced aversion towards the olfactory cues heterospecific males, indicative of a positive familiarity effect on preference (MN Verzijden, ZW Culumber, and GG Rosenthal, unpublished data). Evidently, the development or expression of conspecific preference in female *X. birchmanni* is not straight-forward, and may depend a multitude of factors, including the early experience of females, the attributes of the heterospecific species (e.g. phenotype, phylogenetic distance, extent of sympatry; e.g. Kozak and Boughman 2009, Svensson et al. 2010), and the cues available to females (e.g. Hankison and Morris 2002, Fisher et al. 2006, Seehausen et al. 2007). We are currently investigating how longer-term experience with males of either species, both before and after reaching sexual maturity, influences species preference in lab-reared females.

Although we are unable to identify any particular mechanism in the formation of conspecific preference, we can conclude that species preferences are not necessarily fixed throughout life. In addition, as individuals exhibited some consistency between trials, with some females tending to prefer conspecifics, and others, heterospecifics, we can also conclude that the lack of a consensus species preference is not due to an inability of lab-reared females to discriminate between males of each species.

One potentially important influence on conspecific preference is female age. Several studies of *Xiphophorus* have found either size or age (which are correlated;

Kallman 1989, Morris and Ryan 1990, Marcus and McCune 1999) to influence the strength of intraspecific preference, although not consistently in a positive manner (e.g. Rios-Cardenas et al. 2007; Morris et al. 2006, 2010; Walling et al. 2008; Robinson and Morris 2010). We found no influence of female length within any of our analyses; however, wild-caught females were larger, on average, than the lab-reared ones, which means we cannot rule out a size/age component to preference. It is also possible that early experience with either conspecific or heterospecific males is necessary for the formation of conspecific preference in *X. birchmanni*, at least with respect to *X. variatus* (e.g., Breden et al. 1995, Rosenqvist and Houde 1997, Hebets 2003, Dukas 2005, Hebets and Vink 2007). Similarly, the reproductive statuses may have differed between the wild-caught and lab-reared females we studied, although in *X. nigrensis*, neither receptivity nor intraspecific preference varies over the reproductive cycle (Ramsey et al. 2011). Female *X. birchmanni* are continuous breeders, although reproductive effort in wild females appears highest at the end of the dry season (Urbano-Amilpa 2006). Our current experiments took place slightly later in the year than the original experiment with wild-caught females, which suggests that any seasonal effects on preference could contribute to the different outcomes. Finally, the lab environment differs from natural *X. birchmanni* habitat in many ways other than in social environment. Differences in the strength of preferences between wild-caught and lab-reared females, while not usually studied directly, are nonetheless apparent from studies of other species (e.g. Morris et al. 2006 vs. Tudor and Morris 2009; Kozak and Boughman 2009 vs. Kozak et al. 2009).

Despite the importance of mating interactions on the subsequent expression of intraspecific preferences in some taxa (e.g. van Gossum et al. 2001, Hebets 2003, Dukas 2005, Hebets and Vink 2007), few studies have investigated the effect of adult experience on species preference (e.g. Haskins and Haskins 1949, 1950; Dukas 2004, 2008, 2009;

Magurran and Ramnarine 2004). Of these, only four address such learning by females (delBarco-Trillo et al. 2010; Svensson et al. 2010; MN Verzijden, ZW Culumber, and GG Rosenthal, unpublished data; present study). In many species, females are under stronger selection than males to avoid heterospecific matings (Parker 1979, Parker and Partridge 1998, Gage et al. 2002), and are often considered the "gatekeepers" of sexual isolation (e.g. Wirtz 1999). Learning of conspecific preferences by adult females, through experience with heterospecifics, or through interactions with conspecifics, may be much more widespread than currently recognized. The extent and manner in which experience shapes species preference will affect species-assortative mating and hybridization, and therefore has the potential to ease or impede introgression and speciation (Irwin and Price 1999, Verzijden et al. 2005, Servedio et al. 2009). Further investigation of learned conspecific preference can enhance our understanding of these processes.

## Chapter 5: Conspecific preference in female *Xiphophorus*: variation among species, populations, and experiments

### ABSTRACT

Mating preferences for conspecifics are a major component of reproductive isolation between sympatric species. Such preferences are often stronger in females than in males, however, female preferences for conspecifics are often highly asymmetric between closely-related species. We examined reciprocal female preferences for conspecifics in two species pairs of *Xiphophorus*, each pair differing with respect to the geographical overlap, genetic distance, and frequency of hybridization. In the sympatric species pair (*X. birchmanni* - *X. variatus*), we also evaluated female response to males of an allopatric population of *X. variatus*. Finally, we conducted a second test of female *X. birchmanni* preferences for conspecific over *X. malinche* males, to evaluate the repeatability of our original result. We found asymmetries in both species pairs, with only *X. birchmanni* exhibiting conspecific preferences. However, female *X. birchmanni* preferred conspecifics over *X. malinche* in only the first of the two experiments, a difference we could not account for, although female size-related factors may play a role. Females of the *X. birchmanni* - *X. variatus* did not appear to discriminate between allopatric and sympatric *X. variatus*, although our tests were conservative. Despite the lack of apparent species or population preferences in *X. variatus*, females preferred heteropopulation (*X. birchmanni* or allopatric conspecific) males their first trial, and sympatric conspecifics their second. This finding highlights how experimental design components such as test duration or repeat testing can affect measures of preference. Our

results suggest that sexual isolation in these species pairs may be mediated not only through female preference, but male preference as well. In addition, our findings emphasize the importance of evaluating different preference assays, the repeated testing of females, and study replication in identifying and understanding variation in female preference.

## **INTRODUCTION**

Mating preferences are a primary component of reproductive isolation in many sympatric, closely-related species (Mayr 1963, Coyne and Orr 2004). The extent to which individuals recognize and accept heterospecifics as mates can shape subsequent premating isolation, trait evolution, and the direction and rate of diversification (e.g., Sætre et al. 1997, Seehausen et al. 1997, Stein and Uy 2006, Richmond and Jockusch 2007, Grant and Grant 2008). Selection for conspecific preferences varies with the opportunity for gene flow and the fitness costs of mating heterospecifically. For example, sympatry, by providing opportunities for heterospecific encounters, can increase the strength of conspecific preference, either through learning, reinforcement (i.e., selection against hybrid offspring; Dobzhansky 1940, Blair 1955), or direct selection on preferences (reviews in Brown and Wilson 1956, Irwin and Price 1999, Servedio and Noor 2003). The strength of conspecific preference also often increases with phylogenetic distance (e.g. Ryan and Rand 1995, Strecker and Kodric-Brown 1999); frequently, both the degree of sympatry and phylogenetic distance contribute to the strength of species assortative preferences (e.g. Coyne and Orr 1989, de Kort and ten Cate 2001, Wong et al. 2004).

Within species, the strength of conspecific preference frequently differs by sex (Wirtz 1999). Females are often the more discriminating sex (Bateman 1948, Trivers 1972), and are considered to be under stronger selection to resist heterospecific matings (Parker 1979, Parker and Partridge 1998). However, even within closely-related, sympatric species pairs, female preferences are often asymmetric (e.g., insects: Shapiro 2001, Deering and Scriber 2002, Gray 2005; amphibians: Secondi et al. 2010; fish: Strecker and Kodric-Brown 1999, Rafferty and Boughman 2006, McLennan and Ryan 2008, Kozak and Boughman 2009, Egger et al. 2010; mammals: Smadja and Ganem 2005, birds: Collins and Luddem 2002). Such asymmetries in preference might arise in response to other species asymmetries, for example, in hybrid fitness (Pfennig and Simovitch 2002, Smadja and Gamen 2005), size (Karl et al. 1995), ecology (Wirtz 1999, Yoshimura and Starmer 1997, Secondi et al. 2010), behavior (Kozak and Boughman 2009), and male attractiveness (Ryan and Wagner 1987, Boughman 2001, Collins and Ludem 2002). The strength and direction of asymmetries in species preference can in turn impact the extent and direction of heterospecific mating and hybridization (Shapiro 2001, Stein and Uy 2006).

Although variation in conspecific preference is usually studied at the population or species level, preferences for conspecifics might also vary within individuals or populations, depending on factors such as female age, experience, and environment (Lynch et al. 2005, Grant and Grant 1997, Wirtz 1999). Within- or between-individual variation in species preference is often not the target of experimental studies, however, studies sometimes unexpectedly yield different results, often not attributable to any one factor (e.g. Rundle and Schluter 1998, Kozak et al. 2009). The challenges of animal behavior studies make repetitive testing of females and replication non-trivial endeavors; however, such endeavors are necessary in order to fully understand female preference



variation (Wagner 1998), as is the unbiased dissemination of positive and negative results (Palmer 2000, Lortie et al. 2007).

Preferences for conspecifics as mates are considered of primary importance in the reproductive isolation of sympatric species of fishes of the genus *Xiphophorus* (Hankison and Morris 2002, Fisher et al. 2006, Kallman and Kazianis 2006, Fisher and Rosenthal 2010). This genus of live-bearing, freshwater fishes ranges from northeastern Mexico to Honduras, with most species occupying limited ranges along the eastern slopes of the Sierra Madre Oriental and continuing mountain ranges (Rauchenberger et al. 1990, Kallman and Kazianis 2006). Intrinsic post-mating isolation is weak to non-existent between species (Rosen 1960, Kazianis et al. 1996, Kallman and Kazianis 2006); however, although many species occur in sympatry (Rauchenberger et al. 1990, Kallman and Kazianis 2006) hybridization in the wild is uncommon (reviews in Kallman and Kazianis 2006, Scharl 2008).

Female *Xiphophorus* respond to a multitude of visual, olfactory, and behavioral cues during mate recognition and choice (e.g., Ryan and Wagner 1987, Basolo 1990, Morris et al. 1995, Rosenthal et al. 1996, Fisher et al. 2006). These cues can have reinforcing or opposing effects (e.g., Hankison and Morris 2002, 2003; Crapon de Caprona and Ryan 1990; McLennan and Ryan 2008, Fisher et al. 2009). Assessment of multiple cues may permit females to more accurately evaluate both species status and conspecific attractiveness (Pfennig 1998, Hankison and Morris 2003, Secondi et al. 2010).

Natural hybridization is most extensive between the *X. birchmanni* - *X. malinche* species pair (Rosenthal et al. 2003, Culumber et al. 2011). The two species have parapatric distributions, with *X. malinche* occurring in headwaters at higher elevations (Rauchenberger et al. 1990, Rosenthal et al. 2003). Although there is no consensus

regarding their phylogenetic relationship, all hypotheses place them within the clade of northern swordtails (Rauchenberger et al. 1990, Marcus and McCune 1999, Morris et al. 2001, Gutiérrez-Rodríguez et al. 2007, Culumber et al. 2011), which are thought to have diversified in allopatry (Rauchenberger et al. 1990, Kallman and Kazianis 2006, Gutiérrez-Rodríguez et al. 2007). As observed in other *Xiphophorus* species pairs (e.g. Ryan and Wagner 1987; Crapon de Caprona and Ryan 1990; McLennan and Ryan 1999, 2008), females of this species pair exhibit asymmetries in species preference for specific cues. Female *X. birchmanni* prefer the olfactory and visual cues of conspecifics (Wong and Rosenthal 2006, Fisher et al. 2009), whereas *X. malinche* females exhibit a conspecific preference only for olfactory cues (GGR, unpublished data). Remarkably, their olfactory preferences shift in opposite directions after short-term exposure to heterospecifics (MN Verzijden, ZW Culumber, GG Rosenthal, unpublished data).

Unlike *X. malinche*, *X. birchmanni* is sympatric with another congener, *X. variatus* (Rauchenberger et al. 1990, Kallman and Kazianis 2006). This congener is a more distantly-related species belonging to the clade of southern platys (Rauchenberger et al. 1990, Marcus and McCune 1999, Meyer et al. 2006). Despite the co-occurrence of *X. birchmanni* and *X. variatus* in several streams, no hybridization has been reported between them. There is some evidence to suggest that opportunities for heterospecific encounters may contribute to preferences for the olfactory cues of conspecifics: Fisher and Rosenthal (2010) found that female *X. birchmanni* preferred the cues of conspecific over *X. variatus* males, but only where the latter species are common; *X. variatus* females preferred the conspecific cues over those of sympatric, but not allopatric, heterospecifics.

Here we investigate female species preferences within the two species pairs, *X. birchmanni* - *X. malinche*, and *X. birchmanni* - *X. variatus*. By using live males as stimuli, we were able to observe female response to a complex of cues presented

simultaneously. We also investigated whether *X. birchmanni* females responded differently depending on whether the *X. variatus* males were from sympatric or allopatric populations. In addition, we investigated whether *X. variatus* females expressed a preference for either sympatric or allopatric conspecifics. Finally, we evaluated whether the mean response by female *X. birchmanni* to conspecific and *X. malinche* males persisted over a second experiment with different individuals.

## METHODS

Mating pref *X. birchmanni* and *X. variatus* were collected from the Río Garces (20°56'24"N, 98°16'54"W), and *X. malinche* from the Arroyo Xontla near Chicayotla (20°55'26"N, 98°34'35"W), during multiple trips to the Río Pánuco basin in Hidalgo, Mexico between 2006 and 2009. *X. variatus* males were also obtained from a lab colony established from individuals collected near Agua Fria, Tamaulipas (23°12'08"N, 98°58'05"W) in 2005. The sexes were isolated for a minimum of four weeks before testing, communally housed in 75 and 208 l aquaria. Fish were fed fish flake (TetraMin Pro) twice daily, and brine shrimp nauplii once a day.

To assay female preference, we conducted dichotomous choice tests in a 76 x 30 x 30 cm aquarium. We lined the sides of the aquarium with Teflon overlain with filter gels, using overhead and ambient filtered halogen lighting to approximate the downwelling and horizontal irradiance of their native stream habitat (see Cummings et al. 2003). Clear, UV-transmittant, porous screens separated the male compartments at each end from the larger (46 x 30 x 30 cm) center compartment. An artificial plant at the center provided shelter. We conditioned (Prime, Seachem Laboratories Inc., Georgia,

USA) and carbon-filtered the water before use, and emptied, rinsed, and dried the aquarium between trials.

Upon testing, females were individually presented with a conspecific and heterospecific male, one in each end compartment. Males of each species were chosen at random for each trial. Females were initially placed in the center of the test tank for a 5 min acclimation period, restricted to the region of the central shelter by a cylindrical screen. Following the remote removal of the cylindrical screen via pulley, the trial was initiated when the female emerged from the shelter, and ran for ten minutes. We recorded the time the female spent in each of three equally-sized zones: one center zone and one adjacent to each male compartment. Association time was used as our assay of preference as it predicts mate choice and reproductive success in *Xiphophorus* (Ryan et al. 1990; Morris et al. 1992, 2010; Cummings and Mollaghan 2006; Walling et al. 2010). Trials during which the female did not visit both sides of the tank within the first five minutes, or spent over five minutes beneath the shelter, were aborted. We used the video tracking software Ethovision Pro 2.2 (Experiments 1-3) and Ethovision XT 5.0 (Experiment 4) (Noldus Information Technology, Wageningen, Netherlands) for data collection.

#### Experiment 1. *X. birchmanni*

Each of 16 females underwent three choice tests, varying the species or population identity of the heterospecific: *X. malinche*; *X. variatus* from the same river (hereafter denoted *X. variatus*<sub>sym</sub>); and *X. variatus* from Agua Fria (hereafter denoted *X. variatus*<sub>allo</sub>). The compartment (left or right) in which each species was presented was varied (at least once, but otherwise) at random across a female's three trials, as was the species of heterospecific, blocked such that each female received a unique combination

of conspecific side and heterospecific presentation order. Each heterospecific male was used for only one trial, with the exception of one *X. malinche*, which was used three times. Due to the large number of trials, conspecific males were chosen at random with replacement, with each of 27 males used an average of  $1.74 \pm 1.20$  times, and no male used to test the same female more than once.

#### Experiment 2. *X. malinche*

Nineteen females were offered the choice between a conspecific and *X. birchmanni* male, with the compartment in which each species was presented alternated between females. Fifteen male *X. birchmanni* and 11 male *X. malinche* were used as stimuli (used an average of  $1.67 \pm 1.11$  and  $2.27 \pm 1.19$  times, respectively).

#### Experiment 3. *X. variatus*

Eighteen females each underwent two choice tests, one offering the choice between a *X. variatus*<sub>sym</sub> and *X. birchmanni* male, and one offering the choice between a *X. variatus*<sub>sym</sub> and *X. variatus*<sub>allo</sub> male. The compartment in which each stimulus was presented, and the order of tests, was randomized among females. A unique *X. variatus*<sub>sym</sub> was used for each test, with 10 *X. birchmanni* and 14 *X. variatus*<sub>allo</sub> males used as stimuli (used an average of  $1.70 \pm 0.95$  and  $1.21 \pm 0.43$  times, respectively).

#### Experiment 4. *X. birchmanni*

We conducted another experiment with a different set of 18 *X. birchmanni* females, with only *X. malinche* as the heterospecific species. All males were different than those in Experiment 1. Seventeen male *X. birchmanni* and *X. malinche* were used as stimuli (used an average of  $1.06 \pm 0.56$  and  $1.05 \pm 0.90$  times, respectively).

## Statistical analyses

All statistical analyses were conducted using the R programming language (R Core Development Team 2009) and the additional packages *lmer*, *languageR*, and *MuMIn*. We constructed linear mixed models to assess the influence of male species status (conspecific or heterospecific) on female association time. In the case of *X. variatus* trials, we instead assessed the influence of male population status, either homo- or heteropopulation, on female association time, where heteropopulation males (i.e., males from a different population, Hosken et al. 2002) included either allopatric conspecifics, or sympatric *X. birchmanni*. Model residuals were examined to verify model assumptions were met.

For analysis of female *X. birchmanni* data from Experiment 1, we included the interaction of male species status (conspecific or heterospecific) and heterospecific population (*X. malinche*, *X. variatus*<sub>sym</sub>, or *X. variatus*<sub>allo</sub>) as a predictor to investigate whether the strength of (any) species bias exhibited by female *X. birchmanni* varied with the type of heterospecific presented. We also included male standard length as a covariate, as *X. birchmanni* females have been shown to attend to male body size in within-species comparisons (Fisher et al. 2009). We also included calendar date, trial number (i.e., whether it was a female's first, second, or third time tested), and the interaction of each with male species status, to control for any potential seasonal or

repeated-testing effect on female association time or strength of preference. Female subject ID and male stimulus ID were included as random effects. Due to the large number of predictors in the full model, we used the second-order Akaike Information Criterion (AICc) for mixed models (Vaida and Blanchard 2005) for final model selection, identifying the top models as those with  $\Delta\text{AICc} < 2$  (Burnham and Anderson 2002); only one model fit this criterion. As conventional significance testing using mixed models is complicated by the issue of approximating degrees of freedom (Pinheiro and Bates 2000), we conducted Markov Chain Monte Carlo sampling (10000 samples, locally uniform priors for fixed effects, locally non-informative priors for random effects) to construct 95% posterior density intervals (similar to confidence intervals) and associated  $p$ -values (Baayen et al. 2008). To obtain overall tests of the fixed effects with more than two levels (i.e., heterospecific population and its interaction with male species status), we used the *aovlmer* function.

To analyze female *X. malinche* data (Experiment 2), we constructed a model with male species (conspecific vs. *X. birchmanni*), male length, and the interaction of male species and calendar date as predictors, and female association time as the dependent variable. Female and male ID were included as random effects as before. Two top models were identified based on AICc ranks; we chose the fuller of the two, which included the main effect of calendar date, for significance testing (following the testing procedures described for Experiment 1). A chi-square test was conducted to examine whether females were more likely to end the trial on the conspecific than heterospecific male side.

We analyzed female *X. variatus* association time data (Experiment 3) by constructing a model with male population status (homo- or heteropopulation), type of comparison (*X. variatus*<sub>sym</sub> versus *X. birchmanni*, or *X. variatus*<sub>sym</sub> versus *X. variatus*<sub>allo</sub>), and their interaction as fixed effects. We further included calendar date, trial number, and

the interaction of each with male population status. Male length was included as a fixed effect, and female and male ID as random effects. AICc ranking identified two top models; we selected the fuller of the two, which included calendar date, for significance testing, conducted as described above.

To analyze *X. birchmanni* association time data from Experiment 4, we constructed a model with male species (conspecific vs. *X. malinche*), male length, calendar date, and the male species x calendar date interaction as fixed effects, including female and male ID as random effects. One model was identified as the best model based on AICc ranks, analyzed as described for the previous experiments.

Females responded differently in Experiment 1 and Experiment 4 (see Results). To make statistical comparisons between experiments, and to explore other potential covariates of association time, we combined the data from the conspecific vs. *X. malinche* trials in Experiment 1 with the data of Experiment 4, and analyzed these data together. We constructed a model with experiment number (1 or 4), female length, calendar date on which the trial was conducted, days since the female was collected from the wild, and the interaction of each of these main effects with male species (conspecific vs. *X. malinche*). Male length, and the random effects of female and male ID, were also included. One top model was identified according to AICc rank. Wilcoxon rank sum tests (calendar date, days since collected) and t-test (female length) were conducted to evaluate whether these variables differed between the two experiments; similarly, an ANOVA was used to test whether male length differed between experiments or species.

Lastly, we tested whether calendar test date, or the number of days since females were collected from the field, differed across all 4 experiments, using Kruskal-Wallis rank sum tests and Siegel and Castellan's (1988) method of multiple comparisons. No



accurate collection date data were available for the *X. malinche* females, excluding these species from the corresponding analysis.

## RESULTS

Female *X. birchmanni* spent significantly more time in association with conspecific males than with heterospecific ones (Table 5.1, Fig. 5.1a). Females spent a similar amount of time with each male when the heterospecific was from an allopatric population (Fig. 5.1a), however, this was not statistically significant (Table 5.1). There was no effect of type of heterospecific presented, male length, or trial number on female association time (Table 5.1).

There was no effect of male species, male length, or calendar date on female *X. malinche* association time (Table 5.1, Fig. 5.1b). More females, however, ended the trial on the conspecific rather than heterospecific side ( $X^2 = 4.167$ ,  $df = 1$ ,  $p = 0.041$ ).

Female *X. variatus* spent a similar amount of time with either stimulus in both comparisons (Fig. 5.1c). Taking all other predictors into account, a significant effect of population type (homo- or heteropopulation) was detected, with heteropopulations having a positive effect on association time (Table 5.1). Trial number also had a positive effect on association time. In addition, there was a significant effect of the interaction between population type and trial number, such that females preferentially associated with heteropopulation males their first trial, and their own population (*X. variatus*<sub>sym</sub>) the second, independent of whether the heteropopulation was *X. birchmanni* or *X. variatus*<sub>allo</sub> (Table 5.1, Fig. 5.2; median time between trials = 2.03 days). There was no significant effect of heteropopulation type (i.e., *X. birchmanni* or *X. variatus*<sub>allo</sub>), male length,

TABLE 5.1: . LINEAR MIXED MODELS OF FIXED EFFECTS ON ASSOCIATION TIME OF FEMALE *X. BIRCHMANNI*, *X. MALINCHE*, AND *X. VARIATUS* IN DICHOTOMOUS CHOICE TESTS. RESULTS OF TOP-RANKING MODELS ACCORDING TO AICc VALUES PRESENTED (SEE TEXT). EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS ( $HPD_{LOWER}$ ,  $HPD_{UPPER}$ ) AND MCMC *P*-VALUES.

Experiment	Predictor	Coefficient estimate	$HPD_{lower}$	$HPD_{upper}$	$P_{mcmc}$
1. <i>X. birchmanni</i>	male species status (conspecific / heterospecific)	-104.0	-184.3	-23.4	0.009
	heterospecific population	-	-	-	0.272
	male length	-19.0	-138.9	92.4	0.726
	trial number	-4.3	-33.8	25.4	0.790
2. <i>X. malinche</i>	male species status x heterospecific species	-	-	-	0.520
	male species	35.6	-39.5	117.8	0.332
	male length	-128.3	-339.5	120.3	0.334
	calendar date tested	0.3	-1.3	1.5	0.942
3. <i>X. variatus</i>	male population type (homo / heteropopulation)	274.5	134.8	416.0	<0.001
	heteropopulation type ( <i>X. birchmanni</i> / <i>X. variatus</i> <sub>allo</sub> )	33.8	-31.8	96.4	0.309
	male length	36.5	-172.2	230.0	0.731
	trial number	84.9	23.7	149.0	0.008
	calendar date tested	0.4	-0.1	0.9	0.114
	male population type x heteropopulation type	-34.4	-129.9	60.5	0.476
4. <i>X. birchmanni</i>	male population type x trial number	-170.1	-263.9	-85.5	<0.001
	male species	38.3	-4.9	80.9	0.086
	male length	17.5	-140.8	165.5	0.842

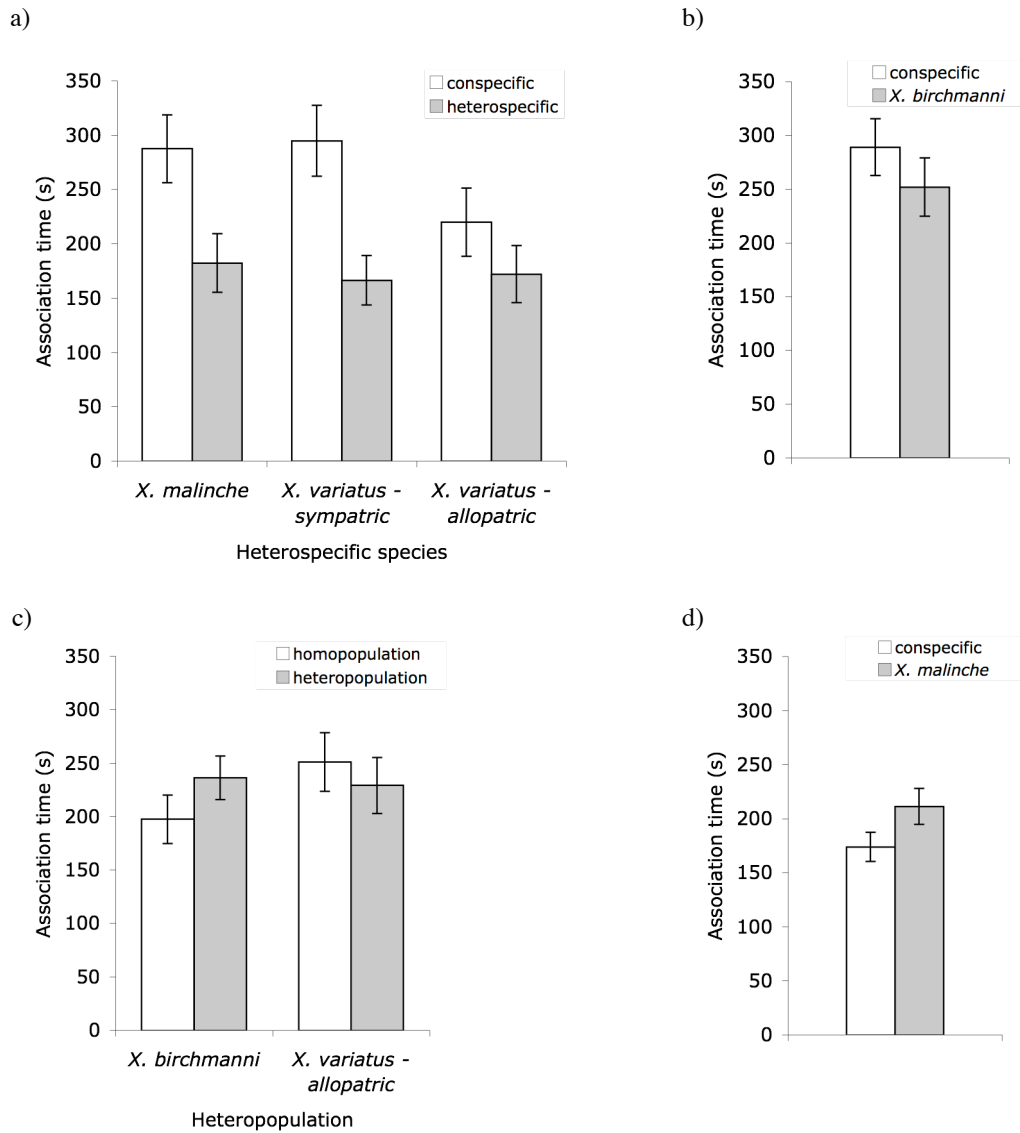


FIGURE 5.1: FEMALE ASSOCIATION TIME WITH CONSPECIFIC VS. HETEROSPECIFIC OR HETEROPOPULATION MALES. WHITE BARS = TIME WITH SYMPATRIC CONSPECIFIC. GRAY BARS = TIME WITH HETEROSPECIFIC OR HETEROPOPULATION MALES. ERROR BARS =  $\pm 1$  SE. A) *X. BIRCHMANNI* ASSOCIATION TIMES WITH CONSPECIFIC VS. HETEROSPECIFIC MALES, IN COMPARISONS WITH *X. MALINCHE*, SYMPATRIC *X. VARIATUS*, AND ALLOPATRIC *X. VARIATUS* AS THE HETEROSPECIFIC SPECIES. B) *X. MALINCHE* ASSOCIATION TIMES WITH CONSPECIFIC VS. *X. BIRCHMANNI* MALES. C) *X. VARIATUS* ASSOCIATION TIMES WITH SYMPATRIC CONSPECIFIC VS. *X. BIRCHMANNI* MALES, OR VS. ALLOPATRIC CONSPECIFICS. D) *X. BIRCHMANNI* ASSOCIATION TIMES WITH CONSPECIFIC VS. *X. MALINCHE* MALES OVER A SECOND EXPERIMENT.

calendar date, or the interaction between population and heteropopulation types on female association time (Table 5.1).

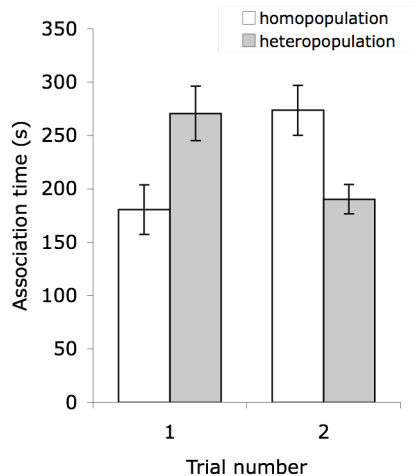


FIGURE 5.2: FEMALE *X. VARIATUS* ASSOCIATION TIMES WITH SYMPATRIC CONSPECIFICS (HOMOPOPULATION) VS. HETEROPOPULATION MALES (EITHER SYMPATRIC *X. BIRCHMANNI* OR ALLOPATRIC CONSPECIFICS), BY TRIAL NUMBER.

In contrast to the results of Experiment 1, in Experiment 4 there was no significant effect of male species on female association time (Table 5.1, Fig. 5.1d). No effect of male length was detected. Association time overall was less in Experiment 4 than in the corresponding (conspecific versus *X. malinche*) trials of Experiment 1 (Figs. 5.1a and b; Table 5.2). Considering the data from Experiment 1 and 4 together, and accounting for the other variables in the model, there was a significant positive effect of *X. malinche* male species on female association time. There was also a significant experiment number by male species interaction (Table 5.2), reflecting the difference in the apparent strength of conspecific preference between the two experiments (Figs. 5.1a and b). Female length had no significant effect on association time, however there was a significant effect of its interaction with male species (Table 5.2). Females in the second experiment were significantly larger than those in the first (Experiment 1, mean  $\pm$  SD:

1.55  $\pm$  0.10, Experiment 2, mean  $\pm$  SD: 1.70  $\pm$  0.17;  $t = -3.131$ ,  $df = 1$ ,  $p = 0.004$ ), a finding which, on its own, could suggest that this mean length difference explains the different outcomes of the two experiments. However, the difference in preference between experiments is still significant even accounting for length differences, which suggests female length alone is insufficient in explaining the different experimental outcomes, and some other unaccounted-for source of variation is also present. No other significant effects were detected.

Predictor	Coefficient estimate	HPD <sub>lower</sub>	HPD <sub>upper</sub>	$p_{\text{mcmc}}$
male species	603.3	67.1	1217.4	0.036
experiment	120.3	51.3	198.8	0.001
male length	30.2	-99.1	177.0	0.630
female length	101.8	-142.3	349.5	0.409
male species X experiment	-188.7	-302.1	-95.7	<0.001
male species X female length	-335.7	-694.0	-15.5	0.049

TABLE 5.2: LINEAR MIXED MODEL OF FIXED EFFECTS ON ASSOCIATION TIME OF FEMALE *X. BIRCHMANNI* IN DICHOTOMOUS CHOICE TESTS BETWEEN CONSPECIFIC AND *X. MALINCHE* MALES, OVER TWO EXPERIMENTS (1 AND 4). RESULTS OF TOP-RANKING MODEL ACCORDING TO AIC<sub>C</sub> VALUES PRESENTED (SEE TEXT). EFFECT SIZES (COEFFICIENTS) WITH BAYESIAN 95% CREDIBILITY INTERVALS (HPD<sub>LOWER</sub>, HPD<sub>UPPER</sub>) AND MCMC P-VALUES.

We looked for other differences between the two experiments that could potentially explain the different outcomes, but identified none. Male length did not differ by species or experiment ( $F = 1.721$ ,  $3/64$  df,  $p = 0.172$ ). The average calendar date on which conspecific versus *X. malinche* trials were conducted did not differ between experiments (Experiment 1, mean  $\pm$  SD: 220.0  $\pm$  61.4 (~ mid-August), Experiment 4

mean  $\pm$  SD:  $247.7 \pm 32.9$  (~ early September);  $W = 169.5$ ,  $p = 0.3882$ ). Nor did the days since subject females were collected from the field differ between the two experiments (Experiment 1, mean  $\pm$  SD:  $164.8 \pm 45.5$ , Experiment 4 mean  $\pm$  SD:  $201.4 \pm 96.3$ ;  $W = 176.0$ ,  $p = 0.2767$ ).

Considering all four experiments, we found that female *X. variatus* were, on average, tested earlier in the year (~ early May) than females of the other experiments (~ mid-August and early September; Kruskal-Wallis  $X^2 = 50.91$ ,  $df = 3$ ,  $p < 0.0001$ ; pairwise  $p$ -values  $< 0.05$ ), and had, on average, been collected more recently from the field (Kruskal-Wallis  $X^2 = -15.53$ ,  $df = 2$ ,  $p = 0.0004$ ; pairwise  $p$ -values  $< 0.05$ ). There were no significant differences in mean test or collection date between the remaining experiments.

## DISCUSSION

We have shown that, in at least two species, female *Xiphophorus* can discriminate between males of different species, and, in the case of *X. variatus*, different populations. However, the extent to which they preferentially associate with one type of male over another can vary. We found that some of the observed variation correlated with factors such as female species and trial number, which invite speculation about some of the biologically-meaningful factors that might contribute to this variation. We also found variation between experiments, however, that we cannot account for, which underscores the importance of considering both biological as well as potential experimental influences.

In our first experiment with *X. birchmanni*, females preferred to associate with conspecifics. This finding is consistent with previous studies (Fisher et al. 2006, Wong

and Rosenthal 2006, Fisher and Rosenthal 2010). We detected no influence of the type of heterospecific compared. There was a trend suggesting that females might discriminate less against allopatric than sympatric *X. variatus*, a pattern observed in the reciprocal cross (Fisher and Rosenthal 2010). Future studies comparing conspecific preference in sympatric and allopatric populations, and in both sexes, would allow tests of reproductive character displacement in this species pair.

Sympatry and genetic relatedness can both contribute to the development and strength of conspecific preference (e.g. Coyne and Orr 1989, Wong et al. 2004, Kozak and Boughman 2009). We might expect female *X. birchmanni* to discriminate more strongly against the sympatric, more distantly-related *X. variatus* than their parapatric, more closely-related congener, but such a pattern did not emerge. A robust test of this prediction would require a larger sample size than obtained in the present study. However, if male *X. variatus* do not recognize *X. birchmanni* females as mates, selection for female discrimination would be reduced. Whether male *X. variatus* recognize *X. birchmanni* females as mates is not known.

We observed no species preferences in *X. malinche* females, at least with respect to association time. This result is largely consistent with previous work: females expressed no preferences with respect to visual cues in one study (GGR, unpublished data), and evidence of preferences for the olfactory cues of conspecifics is mixed (GGR, unpublished data; MN Verzijden, ZW Culumber, GG Rosenthal, unpublished data). In the present study, any preferences for visual and/or behavioral cues of heterospecifics may have conflicted with any olfactory preferences for conspecifics (e.g., Hankison and Morris 2002, 2003). Unlike female *X. birchmanni*, which are downstream from *X. malinche* and sympatric with *X. variatus*, female *X. malinche* have upstream distributions, and no sympatric congeners, characteristics which should relax selection for

conspecific preference. Alternatively, it is possible that the apparent asymmetry in female conspecific preference reflects a difference in sampling behavior, not preference (Wagner 1998): for example, female *X. malinche* might sample for longer periods before selecting a stimulus. The fact that females were more often with the conspecific male at trial's end is consistent with this hypothesis, and suggests that preference studies consider sampling behavior when designing, and comparing among, experiments.

Female *X. variatus* exhibited no overall preferences, whether for conspecifics over sympatric *X. birchmanni*, or sympatric conspecifics over allopatric ones. In fact, when controlling for all other variables, a bias towards males from different populations (either allopatric conspecifics, or sympatric *X. birchmanni*) was detected. These results are inconsistent with those of Fisher and Rosenthal (2010), who, studying the same population at the same time of year, found a preference for the olfactory cues of conspecifics over those of sympatric *X. birchmanni*. As postulated above, the cues presented may matter. Females of many species of *Xiphophorus* exhibit robust preferences for conspecific olfactory cues (e.g., McLennan and Ryan 1997, 1999), but these can conflict with preferences for the visual and/or behavioral cues of heterospecifics (e.g. Cragon de Caprona and Ryan 1990; Hankison and Morris 2002, 2003; McLennan and Ryan 2008). Alternatively, the manner in which cues are delivered may influence preference, for example, whether the cues are actively delivered to females. The lack of obvious conspecific preference in the present study could contribute to heterospecific mating and possibly hybridization, and suggests that additional premating barriers may act in the wild (e.g., tactile cues, Svensson et al. 2010). One possible barrier is male mate recognition: male *X. birchmanni* prefer the olfactory cues of conspecific over *X. variatus* females, and may not recognize *X. variatus* as potential mates (Wong et al. 2005). If so, selection, and/or learned opportunities, for female



discrimination against heterospecifics may be weak. Experiments evaluating the response of males of both species to a suite of female cues are needed to identify sex asymmetries in species preferences and the degree of behavioral isolation between species.

Despite a lack of obvious population or species preferences, *X. variatus* females did discriminate between males: they associated more with heteropopulation males (whether sympatric *X. birchmanni* or allopatric conspecifics) their first trial, and with sympatric conspecifics their second. This result is not an experimental artifact, as we controlled for potential side biases and order effects. Perhaps females, upon their initial introduction to the test arena, are attending preferentially to novel components of their environment, but upon subsequent testing have habituated to the experimental conditions, and require less sampling time before choosing their preferred stimulus. If so, then this illustrates how design components, such as acclimation time and trial duration, can influence measures of preference. An unanticipated trial effect was also observed by Tudor and Morris (2009), where, despite a lack of population-level preference, individual females "switched" their preference between trials. The results of these studies highlight the importance of repetitive tests in elucidating within- and between-female variation in preference.

In contrast to the *X. birchmanni* in the first experiment, *X. birchmanni* females in the subsequent experiment exhibited no obvious species preference. These latter females also spent less time associating with males, which could suggest a lower interest in them. Our inability to replicate our results limits our ability to make generalizations, however, they illustrate the importance of replication. When attempting to understand biological patterns, a different picture can emerge following replication, and consideration of negative results (Palmer 2000, Lortie et al. 2007). We consider possible sources of the unanticipated variation in *X. birchmanni* behavior below.

### *Female size/age*

Female *Xiphophorus* show indeterminate growth, such that older females tend to be larger (Kallman 1989, Marcus and McCune 1999). In *Xiphophorus* and many other taxa, females preferences can vary with size and/or age (e.g., Ryan et al. 1992, Kodric-Brown and Nicoletto 2001, Moore and Moore 2001, Coleman et al. 2004, Morris et al. 2006). *Xiphophorus birchmanni* females were significantly larger in the second experiment than in the first, which suggests that female conspecific preference strength might decline with age, or age-related factors, although we found no apparent role of length within any of our experiments. Accounting for length alone was insufficient to explain the different outcomes of the two *X. birchmanni* experiments, which indicates that some other factor was also at play.

### *Season*

In some species, mating preferences can also vary with seasonal changes in reproductive effort (e.g. Qvarnström et al. 2000, Borg et al. 2006). Female *Xiphophorus* breed continuously, however reproductive effort is lowest during the rainy season (approximately May through September (Morris and Ryan 1992, Urbano-Amilpa 2006)). It has been hypothesized that the turbid conditions during the rainy season may make mating more difficult (Morris and Ryan 1992, McLennan and Ryan 1997); it is possible that female preferences may change by season as well. In the present study, the two *X. birchmanni* experiments were conducted at approximately the same time of year, which suggests that seasonal influences were not responsible for the observed differences. The

*X. malinche* trials were also conducted at the same time of year as the *X. birchmanni* trials, which should also have controlled for (any) seasonal effects. Like female size or age, however, potential season influences should be considered in the design of future experiments with these species.

#### *Male size*

Several studies in *Xiphophorus* have documented a female preference for larger males (e.g. Ryan and Wagner 1987, Ryan et al. 1990, Morris et al. 1996, Hankison and Morris 2002, Cummings and Mollaghan 2006, Fisher et al. 2009). We found no effect of male size in any of our experiments, nor did male size differ between the two *X. birchmanni* experiments, which suggests that male size is not contributing to species preferences. The fact that female *X. birchmanni* prefer large males in intra-specific mate choice (Fisher et al. 2009), but that we found no such influence in the present study, suggests that females might emphasize different cues in mate recognition than they do in mate choice (e.g., Boake et al. 1997), potentially minimizing conflict between these two processes (e.g. Hankison and Morris 2003).

#### *Other influences*

In some species, preference strength can decline over time in captivity (Gibbons 1994). In the present study, the average number of days in captivity did not differ between females of the two *X. birchmanni* experiments, controlling for this potential influence. Other factors that we cannot account for, but that might have affected the outcomes of the two *X. birchmanni* experiments include: random chance, differences in

the two samples (e.g. in female experience or condition, Fisher and Rosenthal 2006, Hebets et al. 2008, Holveck and Riebel 2010), differences in the male stimuli (e.g. Ryan and Wagner 1987, Morris et al. 1996, Fawcett and Bleay 2009), and differences in water quality (e.g. Borowsky and Diffley 1981, Heubel and Schlupp 2006, Fisher et al. 2006, McLennan and Ryan 1999, Reynolds and Jones 1999).

## Conclusions

The extent and direction of sex and species asymmetries in conspecific preference has a wide range of evolutionary implications, affecting the strength of premating isolation, sexual conflict, the direction of hybridization and introgression, the movement of hybrid zones, the direction of species replacement, and the direction and rate of speciation (Rhymer and Simberloff 1996, Parker and Partridge 1998, Wirtz 1999, Shapiro 2001, Seehausen et al. 2008). We found species asymmetries in female *Xiphophorus* conspecific preference, but not consistently, which emphasizes the importance of replication in studies of mate preference. Even before replication, consideration of potential confounds and artifacts in the design of experiments, in statistical tests, and in the exposition of results, will accelerate our ability to identify biological patterns, as will the unbiased reporting of positive and negative results. Embracing such practices in the study of sex, population, and species asymmetries in preference will enhance our understanding of sexual selection and the evolution of reproductive isolation.

## Concluding Remarks

Traditionally, studies of hybridization have focused primarily on the genetic consequences of hybridization, because of the important evolutionary consequences. Much less is known about why animals hybridize. Understanding both the causes and consequences of hybridization will enhance our understanding of how diversity is lost and gained. Below I outline a short categorization of the ways ecology can influence hybridization behavior, avenues for future research, and some comments on how I expect the study of hybridization to change in the future.

### Hybridization as an ecologically-dependent, behavioral phenomenon

I concur with Grant and Grant (1997, 2004) that animal hybridization is an "ecologically-dependent behavioral phenomenon", and can be understood within a mate choice framework. Ecological conditions can influence mate choice in actually or potentially hybridizing species in several ways. I offer the following categorization of these ways in the hopes that helps conceptualize the relationship between ecology, mate choice, and hybridization under one framework:

1. *Bringing allopatric species into contact.* Hybridization often occurs when formerly allopatric species that lack complete behavioral isolation are brought into contact through secondary contact or species introductions (e.g., Rosenfield and Kodric-Brown 2003). Although hybridization in such contexts are well-recognized, they are rarely studied within a mate-choice context.

2. *Masking cues assessed in mate recognition.* Ecological conditions can interfere with the production or perception of mate recognition cues, leading to hybridization (e.g. Seehausen et al. 1997).

3. *Disrupting the species-typical expression or recognition of cues.* In species with learned mate recognition, learning of (e.g., Grant and Grant 1997) or deprivation from heterospecific cues (e.g., Kozak and Boughman 2009) can contribute to hybridization events.

4. *Influencing the costs and benefits of mate choice.* The same dynamic conditions that, by changing the costs and benefits of mate choice, produce variation in mate choice within species can also promote or inhibit mating between them (e.g. Nuechterlein and Buitron 1998, Pfennig 2007).

#### Avenues for future research

With respect to the studies described in this dissertation, there are several avenues that could complement this work. In swordtails, a promising next step would be to measure hybridization itself. For example, by exposing females to different treatments (such as different encounter rates with conspecifics and/or heterospecifics), and allowing them to breed freely, one could see the outcome of treatments on the frequency of hybridization. Another complementary direction for future research would be to correlate environmental conditions relevant to choice (e.g. predation risk) with levels of hybridization in the wild. For studies with wood warblers, it would be fruitful to focus on

one or two species in the wild, and see whether the patterns observed here hold when using more fine-scale measures of mate choice and hybridization rates.

More generally, mate choice theory may be useful, not only in understanding why hybridization happens, but the population-level outcomes that arise. For example, it can help explain hybrid zone dynamics, and the rate of speciation (e.g. Wilson and Hedrick 1982, Kopp and Hermisson 2008). A particularly interesting area for future research is the "merge-and-diverge" (Grant et al. 2004) oscillations in the isolation of species predicted by changing ecological conditions. Speciation or species collapse are well-recognized potential outcomes of hybridization following species introductions or secondary contact; however, even among sympatric taxa, changing ecological conditions may be expected to increase or decrease isolation between species. The best-known example of this is found in hybridizing Darwin's finches (e.g. Grant et al. 2004); however, such oscillations are likely elsewhere. The sudden species collapse in Enos Lake sticklebacks (Taylor et al. 2006), coincident with the introduction of crayfish, may be such an example, a possibility that awaits further study.

### Shifting paradigms

I end my concluding remarks with a couple of examples of the changes I anticipate in how we view animal hybrids and hybridization.

*Deconstructing the "purity" of species.* Hybrids were discussed extensively by Darwin (1959), who recognized the fluid nature of species. Although botanists have long recognized the prevalence and evolutionary importance of introgression in plants,

zoologists have tended towards almost eugenic views about animal species (see Paterson 1985, Arnold 1997, and Mallet 2005 for discussions). The bias against hybrids in favor of "good" species encourage the intuitive view that hybrids represent reproductive "mistakes", and therefore are deviations from, not consistent with, expectations from mate choice theory. As evidence of animal hybridization and introgression continue to accumulate (reviews in Mallet 2005, Schwenk et al. 2008), the prevalence of these phenomena and the non-discrete nature of species will be increasingly recognized.

*Challenging the assumption of reproductive "mistakes".* In many cases, hybrid offspring are less fit than parental species, and therefore often assumed to be the result of decision error. However, direct costs and benefits have prominent effects on mate choice (Jennions and Petrie 1997). Employing a conditional mate choice strategy that responds to both the direct and indirect costs and benefits of choice may enhance lifetime fitness, even if some less fit hybrids are produced. Of course, hybrids can also be fitter than parental species (reviewed in Arnold 1997). It might be helpful to stop using terms such as "mistake" or "error" in studies of interspecific mate choice, and just focus on the causes and consequences of variation in mate choice themselves.



## Appendix

TABLE A.1: NORTH AMERICAN WOOD WARBLER SPECIES DATA USED IN ANALYSES OF CHAPTER 1. NO\_HYBRIDS = NUMBER OF HYBRIDS REPORTED SINCE 1980, SEE TEXT FOR HOW VALUES WERE ASSIGNED; SOURCES PROVIDED IN TABLE 1.1. PSZ\_NA\_09 = POPULATION SIZE IN NORTH AMERICA IN 2009. FROM PARTNERS IN FLIGHT LANDBIRD POPULATION ESTIMATES DATABASE VERSION 2004 ([HTTP://RMBO.ORG/PIF\\_DB/LAPED](http://rmbo.org/pif_db/laped); BLANCHER ET AL. 2007). BR\_DENS = AVERAGE NUMBER OF BIRDS PER BBS ROUTE 1990-1999. FROM PARTNERS IN FLIGHT LANDBIRD POPULATION ESTIMATES DATABASE VERSION 2004 ([HTTP://RMBO.ORG/PIF\\_DB/LAPED](http://rmbo.org/pif_db/laped); BLANCHER ET AL. 2007). RANGE\_SZ = BREEDING RANGE DISTRIBUTION FROM 1 (<500,000 KM, OR VERY RESTRICTED COASTAL AREAS OR INTERIOR UPLANDS) TO 5 ( $\geq 4,000,000$  KM<sup>2</sup>, OR >8,000 KM OF COAST). TRANSFORMED (ORDINAL DIRECTION REVERSED) FROM PIF SPECIES ASSESSMENT DATABASE VERSION 2005, [HTTP://WWW.RMBO.ORG.PIF/PIFDB.HTM](http://www.rmbo.org/pif/pifdb.htm); PANJABI ET AL. 2005. HAB\_QUAL = BREEDING HABITAT QUALITY. TRANSFORMED (ORDINAL DIRECTION REVERSED) FROM "THREATS TO BREEDING" SCORE, PIF SPECIES ASSESSMENT DATABASE VERSION 2005, [HTTP://WWW.RMBO.ORG.PIF/PIFDB.HTM](http://www.rmbo.org/pif/pifdb.htm); PANJABI ET AL. 2005. NO\_HET\_SPP = NUMBER OF HETEROSPECIFIC SPECIES BREEDING IN SYMPATRY (>0 REGIONAL CO-OCCURRENCE, SEE LOVETTE & HOCHACHKA 2006).

Species	No_ hybrids	Psz_NA_09	BR_dens	Range_sz	Hab_qual	No_het_spp
<i>Dendroica caerulescens</i>	2	2000000	0.030	3	3	35
<i>Dendroica castanea</i>	1	3000000	0.032	3	3	26
<i>Dendroica cerulea</i>	3	560000	0.011	2	2	31
<i>Dendroica chrysoparia</i>	0	21000	0.008	1	1	6
<i>Dendroica coronata</i>	4	130000000	0.413	5	4	41
<i>Dendroica discolor</i>	0	1400000	0.020	3	3	28
<i>Dendroica dominica</i>	10	1600000	0.024	3	3	26
<i>Dendroica fusca</i>	4	5900000	0.069	4	3	35
<i>Dendroica graciae</i>	0	1000000	0.055	3	3	9
<i>Dendroica kirtlandii</i>	1	2100	0.001	1	2	18
<i>Dendroica magnolia</i>	1	30000000	0.169	5	4	36
<i>Dendroica nigrescens</i>	3	2900000	0.048	3	3	15
<i>Dendroica occidentalis</i>	100	2400000	0.125	1	2	9
<i>Dendroica palmarum</i>	0	20000000	0.176	4	4	27
<i>Dendroica pensylvanica</i>	0	9400000	0.087	4	4	37
<i>Dendroica petechia</i>	2	30000000	0.084	5	4	42
<i>Dendroica pinus</i>	0	11000000	0.107	3	4	35
<i>Dendroica striata</i>	0	20000000	0.105	4	3	29
<i>Dendroica tigrina</i>	1	3000000	0.031	4	3	26
<i>Dendroica townsendi</i>	100	12000000	0.137	3	2	19
<i>Dendroica virens</i>	1	10000000	0.069	4	3	35
<i>Geothlypis trichas</i>	1	32000000	0.085	5	4	42
<i>Helmitheros vermivorus</i>	0	700000	0.017	3	3	27
<i>Limnothlypis swainsonii</i>	0	84000	0.003	2	2	22
<i>Mniotilta varia</i>	3	14000000	0.077	4	4	36
<i>Oporornis agilis</i>	0	1200000	0.014	3	3	29
<i>Oporornis formosus</i>	1	1100000	0.017	3	3	27
<i>Oporornis philadelphia</i>	1	7000000	0.054	3	4	36
<i>Oporornis tolmiei</i>	0	5400000	0.042	3	4	22
<i>Parula americana</i>	10	7300000	0.053	4	4	36
<i>Protonotaria citrea</i>	0	1800000	0.027	3	3	24
<i>Seiurus aurocapillus</i>	0	24000000	0.110	4	4	39
<i>Seiurus motacilla</i>	0	260000	0.004	4	3	29
<i>Seiurus noveboracensis</i>	0	13000000	0.053	5	4	35
<i>Setophaga ruticilla</i>	0	25000000	0.103	5	4	38
<i>Vermivoria celata</i>	0	80000000	0.297	5	4	32
<i>Vermivoria chrysoptera</i>	100	210000	0.005	2	2	33
<i>Vermivoria luciae</i>	0	900000	0.047	1	2	7
<i>Vermivoria peregrina</i>	1	60000000	0.300	4	4	29
<i>Vermivoria pinus</i>	100	390000	0.007	3	3	31
<i>Vermivoria ruficapilla</i>	2	34000000	0.229	4	4	37
<i>Vermivoria virginiae</i>	0	400000	0.019	2	3	10
<i>Wilsonia canadensis</i>	0	1400000	0.012	4	3	36
<i>Wilsonia citrina</i>	0	4000000	0.064	4	3	28
<i>Wilsonia pusilla</i>	0	40000000	0.140	5	3	32

TABLE A.2: CORNELL LAB OF ORNITHOLOGY MACAULAY LIBRARY CATALOGUE NUMBERS  
OF WOOD WARBLER SONG SAMPLES ANALYSED IN CHAPTER 1.

Species	Catalogue number
<i>Dendroica caerulescens</i>	27208
<i>Dendroica castanea</i>	9168
<i>Dendroica cerulea</i>	113504
<i>Dendroica chrysoparia</i>	109434
<i>Dendroica coronata</i>	111142
<i>Dendroica discolor</i>	110248
<i>Dendroica dominica</i>	9557
<i>Dendroica fusca</i>	9593
<i>Dendroica graciae</i>	45144
<i>Dendroica kirtlandii</i>	73997
<i>Dendroica magnolia</i>	94359
<i>Dendroica nigrescens</i>	22811
<i>Dendroica occidentalis</i>	40644
<i>Dendroica palmarum</i>	93798
<i>Dendroica pensylvanica</i>	74913
<i>Dendroica petechia</i>	107321
<i>Dendroica pinus</i>	84747
<i>Dendroica striata</i>	94355
<i>Dendroica tigrina</i>	10022
<i>Dendroica townsendi</i>	47707
<i>Dendroica virens</i>	10039
<i>Geothlypis trichas</i>	94345
<i>Helmitheros vermivorus</i>	26270
<i>Limnothlypis swainsonii</i>	10392
<i>Mniotilta varia</i>	53172
<i>Oporornis agilis</i>	69841
<i>Oporornis formosus</i>	94335
<i>Oporornis philadelphia</i>	73995
<i>Oporornis tolmiei</i>	119421
<i>Parula americana</i>	107309
<i>Protonotaria citrea</i>	74000
<i>Seiurus aurocapillus</i>	73935
<i>Seiurus motacilla</i>	84757
<i>Seiurus noveboracensis</i>	131464
<i>Setophaga ruticilla</i>	10672
<i>Vermivoria celata</i>	111023
<i>Vermivoria chrysoptera</i>	107379
<i>Vermivoria luciae</i>	118635
<i>Vermivoria peregrina</i>	130981
<i>Vermivoria pinus</i>	93774
<i>Vermivoria ruficapilla</i>	44893
<i>Vermivoria virginiae</i>	45121
<i>Wilsonia canadensis</i>	107387
<i>Wilsonia citrina</i>	76731
<i>Wilsonia pusilla</i>	111024

## References

- Alatalo RV, Lundberg A, Glynn C, 1986. Female pied flycatchers choose territory quality and not male characteristics. *Nature* 323:152-153.
- Alatalo RV, Carlson A, Lundberg A, 1988. The search cost in mate choice of the pied flycatcher. *Anim Behav* 36:289-291.
- Allouche S, 2002. Nature and functions of cover in riverine fish. *Bull Fr Pêche Piscic* 365-366:297-324.
- American Ornithologists' Union, 1998. Check-list of North American Birds, 7th edn. American Ornithologists' Union, Washington, DC.
- Anderson E, 1949. Introgressive hybridization. New York: John Wiley and Sons.
- Andersson M, 1994. Sexual selection. Princeton, NJ: Princeton University Press.
- Arnold ML, 1997. Natural hybridization and evolution. Oxford: Oxford University Press.
- Arnold ML, 2006. Evolution through genetic exchange. 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.
- Bain M, 1996. A mystery warbler in southern Ontario. *Birders Journal* 5:134-135.
- Baker M, Boylan JT, 1999. Singing behavior, mating associations and reproductive success in a population of hybridizing Lazuli and Indigo buntings. *Condor* 101:493-504.
- Baker MC, Cunningham MA, 1985. The biology of bird song dialects. *Behav Brain Sci* 8:85-100.
- Bakker TCM, Pomiankowski, A, 1995. The genetic basis of female mate preferences. *J Evol Biol* 8:129-171.

- Bart J, Earnst SL, 1999. Relative importance of male and territory quality in pairing success of male rock ptarmigan (*Lagopus mutus*). Behav Ecol Sociobiol 45:355-359.
- Basolo AL, 1990. Female preference for male sword length in the green swordtail, *Xiphophorus helleri* (Pisces: Poeciliidae). Anim Behav 40:332-338.
- Bateson PPG, 1966. The characteristics and context of imprinting. Biol Rev 41:177-220.
- Bayne EM, Hobson KA, 2001. Effects of habitat fragmentation on pairing success of ovenbirds: importance of male age and floater behavior. Auk 118:380-388.
- Berglund A, 1995. Many mates make male pipefish choosy. Behaviour 132:213-218.
- Blancher PJ, Rosenberg KV, Panjabi AO, Altman B, Bart J, Beardmore CJ, Butcher GS, Demarest D, Dettmers R, Dunn EH, Easton W, Hunter WC, Iñigo-Elias EE, Pashley DN, Ralph CJ, Rich TD, Rustay CM, Ruth JM, Will TC, 2007. Guide to the Partners in Flight Population Estimates Database. Version: North American Landbird Conservation Plan 2004. Partners in Flight Technical Series No 5. <http://www.partnersinflight.org>
- Boake C, DeAngelis MP, Ward RD, 1997. Is sexual selection and species recognition a continuum? Mating behavior of the stalk-eyed fly *Drosophila heteroneura*. Can J Fish Aquat Sci 45:2035-2041.
- Bonter D, Lovette I, 2007. Name that warbler. Living Bird Spring:32-35.
- 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, Diffley J, 1981. Synchronized maturation and breeding in natural populations of *Xiphophorus variatus* (Poeciliidae).
- Boughman JW, 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944-948.
- Breden F, Novinger, D, Schubert, A, 1995. The effect of experience on mate choice in the Trinidad guppy, *Poecilia reticulata*. Env Biol Fish 42:323-328.
- Brelsford A, Irwin DE, 2009. Incipient speciation despite little assortative mating: the yellow-rumped warbler hybrid zone. Evolution 63:3050-3060.
- Brown WL, Wilson EO, 1956. Character displacement. Syst Zool 5:49-64  
Burley NT, Foster VS, 2006. Variation in female choice of mates: condition influences selectivity. Anim Behav 72:713-719.

- Burnham KP, Anderson DR, 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. New York: Springer-Verlag.
- 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.
- Cameron E, Day T, Rowe L, 2003. Sexual conflict and indirect benefits. *J Evol Biol* 16:1055-1060.
- Carlson CW, 1981. The Sutton's warbler - a critical review and summation of current data. *Atlant. Nat.* 34:1-11.
- Charmantier A, Sheldon BC, 2006. Testing genetic models of mate choice evolution in the wild. *Trends Ecol Evol* 21:417-419.
- Chiver I, Stutchbury BJM, Morton ES, 2008. Do male plumage and song characteristics influence female off-territory forays and paternity in the hooded warbler? *Behav Ecol Sociobiol* 62:1981-1990.
- Clark C, Marler P, Beeman K, 1987. Quantitative analysis of animal vocal phonology: an application to swamp sparrow song. *Ethology* 76:101-115.
- Coleman SW, Patricelli GL, Borgia G, 2004. Variable female preferences drive complex male displays. *Nature* 428:742-745.
- Collins SA, Luddem ST, 2002. Degree of male ornamentation affects female preference for conspecific versus heterospecific males. *Proc R Soc Lond B* 269:111-117.
- Confer JL, Tupper SK, 2000. A reassessment of the status of golden-winged and blue-winged warblers in the Hudson highlands of southern New York. *Wilson Bulletin* 112:544-546.
- Constanz GD, 1989. Reproductive biology of poeciliid fishes. In: Meffe GK, Snelson, F.F, editor. *Ecology and evolution of livebearing fishes (Poeciliidae)* Englewood Cliffs: Prentice Hall. p. 33-50.
- Cotton S, Small J, Pomiankowski A, 2006. Sexual selection and condition-dependent mate preferences. *Current Biol* 16:R755-R765.
- Coyne JA, Orr HA, 1989. Patterns of speciation in *Drosophila*. *Evolution* 43:362-381.
- Coyne JA, Orr HA, 2004. *Speciation*. Sunderland, MA: Sunderland Associates Inc.

- Crapon de Caprona MD, Ryan MJ, 1990. Conspecific mate recognition in swordtails, *Xiphophorus nigensis* and *X. pygmaeus* (Poeciliidae): olfactory and visual cues. *Anim Behav* 39:290-296.
- 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, Barber, PH, Sorenson, MD, 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 nigrens*. *Anim Behav* 72:217-224.
- Cummings ME, Rosenthal GG, Ryan MJ, 2003. A private ultraviolet channel in visual communication. *Proc R Soc Lond B* 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.
- Darwin C. 1859. The origin of species by means of natural selection or the preservation of favoured races in the struggle for life. London: John Murray.
- de Kort SR, ten Cate C, 2001. Response to interspecific vocalizations is affected by degree of phylogenetic relatedness in *Streptopelia* doves. *Anim Behav* 61:239-247.
- Deering MD, Scriber JM, 2002. Field bioassays show heterospecific mating preference asymmetry between hybridizing North American *Papilio* butterfly species (Lepidoptera: Papilionidae). *J Ethol* 20:25-33.
- delBarco-Trillo J, McPhee, ME, Johnston, RE, 2010. Adult female hamsters avoid interspecific mating after exposure to heterospecific males. *Behav Ecol Sociobiol* 64:1247-1253.
- Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. *Am. Nat.* 74:312-332.
- Dowling TE, Secor CL, 1997. The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics* 28:593-619.

- Ducharme C, Lamontagne J, 1992. Un cas d'hybridation chez les parulines? *Dendroica caerulescens* x *Dendroica petechia*. Quebec Oiseaux 4:6.
- Dukas R, 2004. Male fruit flies learn to avoid interspecific courtship. Behav Ecol 15:695-698.
- Dukas R, 2005. Learning affects mate choice in female fruit flies. Behav Ecol 16:800-804.
- Dukas R, 2008. Learning decreases heterospecific courtship and mating in fruit flies. Biol Lett 4:645-647.
- Dukas R, 2009. Dynamics of learning in the context of courtship in *Drosophila persimilis* and *D. pseudoobscura*. Anim Behav 77:253-259.
- Dunn J, Garrett K, 1997. A field guide to the warblers of North America. Houghton Mifflin, Boston.
- 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.
- Edwards LJ, Muller KE, Wolfinger RD, Qaqish BF, Schabenberger O, 2008. An  $R^2$  statistic for fixed effects in the linear mixed model. Statist Med 27:6137-6157.
- Egger B, Mattersdorfer K, Sefc KM, 2010. Variable discrimination and asymmetric preferences in laboratory tests of reproductive isolation between cichlid colour morphs. J Evol Biol 23:433-439.
- Fawcett TW, Bleay C, 2009. Previous experiences shape adaptive mate preferences. Behav Ecol 20:68-78.
- Fawcett TW, Johnstone RA, 2003. Mate choice in the face of costly competition. Behav Ecol 14:771-779.
- Ficken, M. S, and Ficken, R. W. 1966. Notes on mate and habitat selection in the yellow warbler. Wilson Bulletin 78:232-233.
- Ficken MS, Ficken RW, 1968. Reproductive isolating mechanisms in the blue-winged warbler-golden-winged warbler complex. Evolution 22:166-179.
- Fisher HS, Mascuch SJ, Rosenthal GG, 2009. Multivariate male traits misalign with multivariate female preferences in the swordtail fish, *Xiphophorus birchmanni*. Anim Behav 78:265-269.



- Fisher HS, Rosenthal GG, 2006. Hungry females show stronger mating preferences. *Behav Ecol* 17:979-981.
- Fisher HS, Rosenthal GG, 2009b. Relative abundance of *Xiphophorus* fishes and its effect on sexual communication. *Ethology* 116:32-38.
- 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* 273:1187-1193.
- Fitzpatrick BM, 2004 Rates of evolution of hybrid inviability in birds and mammals. *Evolution* 58:1865-1870.
- 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.
- Gabor CR, Halliday TR, 1997. Sequential mate choice by multiply mating smooth newts: females become more choosy. *Behav Ecol* 8:162-168.
- Gabor CR, Page R, 2003. Female preference for large males in sailfin mollies, *Poecilia latipinna*: the importance of predation pressure and reproductive status *Acta Ethol* 6:7-12.
- Gage MJG, Parker GA, Nylin S, Wiklund C, 2002. Sexual selection and speciation in mammals, butterflies and spiders. *Proc Roy Soc B* 269:2309-2316.
- Garland T, Harvey PH, Ives AR, 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* 41:18-32.
- Gee JM, 2003. How a hybrid zone is maintained: behavioral mechanisms of interbreeding between California and Gambel's quail (*Callipepla californica* and *C. gambelii*). *Evolution* 57:2407-2415.
- Gerlai R, 2007. Mate choice and hybridization in Lake Malawi cichlids, *Sciaenochromis fryeri* and *Cynotilapia afra*. *Ethology* 113:673-685.
- Gibbons EF, 1994. Naturalistic environments in captivity for animal behavior research. Albany: State University of New York Press.

- Gill FB, 1980. Historical aspects of hybridization between blue-winged and golden-winged warblers. *Auk* 97:1-18.
- Gill FB, 1998. Hybridization in birds. *Auk* 115:281-283.
- Gill FB, Murray BG, 1972. Discrimination behavior and hybridization of the blue-winged and golden-winged warblers. *Evolution* 26:282-293.
- Godin JGJ, Briggs SE, 1996. Female mate choice under predation risk in the guppy. *Anim Behav* 51:117-130.
- Good TP, Ellis JC, Annett CA, Pierotti R, 2000. Bounded hybrid superiority in an avian hybrid zone: effects of mate, diet, and habitat choice. *Evolution* 54:1774-1783.
- Gordon M, 1953. The ecological niche of the pygmy swordtail, *Xiphophorus pygmaeus*, in the Río Axtla, Mexico. *Copeia* 1953:148-150.
- Grant BR, Grant PR, 2008. Fission and fusion of Darwin's finches populations. *Phil Trans R Soc B* 363:2821-2829.
- Grant PR, Grant BR, 1992. Hybridization of bird species. *Science* 256:193-197.
- Grant PR, Grant BR, 1997. Hybridization, sexual imprinting, and mate choice. *Am Nat* 149:1-28.
- Grant PR, Grant BR, Markert JA, Keller LF, Petren K, 2004. Convergent evolution of Darwin's finches caused by introgressive hybridization and selection. *Evolution* 58:1588-1599.
- Grant PR, Grant BR, Petren K, 2005. Hybridization in the recent past. *Am Nat* 166:56-67.
- Gray DA, 2004. Does courtship behavior contribute to species-level reproductive isolation in field crickets? *Behav Ecol* 16:201-206.
- Gutiérrez-Rodríguez C, Shearer AE, Morris MR, de Queiroz K, 2007. Phylogeography and monophyly of the swordtail fish species *Xiphophorus birchmanni* (Cyprinodontiformes, Poeciliidae) *Zool Scripta* 37:129-139
- 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.

- Hankison SJ, Morris, M.J, 2003. Avoiding a compromise between sexual selection and species recognition: female swordtail fish assess multiple species-specific cues. *Behav Ecol* 14:282-287.
- Harvey P, Pagel M. 1991. The comparative method in evolutionary biology. Oxford University Press, New York.
- Haskins CP, Haskins EF, 1949. The role of sexual selection as an isolating mechanism in three species of poeciliid fishes. *Evolution*:160-169.
- Haskins CP, Haskins, EF, 1950. Factors governing sexual selection as an isolating mechanism in the poeciliid fish *Lebistes reticulatus*. *Proc Natl Acad Sci USA* 36:464-476.
- Hebets EA, 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. *Proc Natl Acad Sci USA* 100:13390-13395.
- Hebets EA, Vink CJ, 2007. Experience leads to preference: experienced females prefer brush-legged males in a population of syntopic wolf spiders. *Behav Ecol* 18:1010-1020.
- Hebets EA, Wesson J, Shamble PS, 2008. Diet influences mate choice selectivity in adult female wolf spiders. *Anim Behav* 76:355-365.
- Hedrick AV, Dill LM, 1993. Mate choice by female crickets is influenced by predation risk. *Anim Behav* 46:193-196.
- Helfman GS, Collette BB, Facey DE, 1997. The diversity of fishes. Oxford: Blackwell Science.
- Hettyey A, Hegy G, Puurtinen M, Hoi H, Török J, Penn DJ, 2010. Mate choice for genetic benefits: time to put the pieces together. *Ethology* 116:1-9.
- Heubel KU, Schlupp I, 2006. Turbidity affects association behaviour in male *Poecilia latipinna*. *J Fish Biol* 68:555-568.
- Holveck M-J, Riebel K, 2010. Low-quality females prefer low-quality males when choosing a mate. *Proc R Soc B* 277:153-160.
- Hosken DJ, Blanckenhorn WU, Garner TWJ, 2002. Heteropopulation males have a fertilization advantage during sperm competition in the yellow dung fly (*Scathophaga stercoraria*). *Proc R Soc Lond B* 269:1701-1707.

- Hovi M, Rätti O, 1994. Mate sampling and assessment procedures in female pied flycatchers (*Ficedula hypoleuca*). *Ethology* 96:127-137.
- Hubbs CL, 1955. Hybridization between fish species in nature. *Syst. Zool.* 4:1-20.
- Hurley G, Jones J. 1983. A presumed mixed Bay-breasted x Blackburnian warbler nesting in West Virginia. *Redstart* 50:108-111.
- Irwin DE, Price, T, 1999. Sexual imprinting, learning and speciation. *Heredity* 82:347-354.
- Irwin DE, Brelsford A, Toews, DPL, MacDonald C, Phinney M, 2009. Extensive hybridization in a contact zone between MacGillivray's warblers *Oporornis tolmiei* and mourning warblers *O. philadelphia* detected using molecular and morphological analyses. *J Avian Biol* 40:539-552.
- 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.
- Jouventin P, Bried J, 1997. The effect of mate choice on speciation in snow petrels. *Biol Rev* 72:283-327.
- Kallman KD, 1989. Genetic control in size at maturity in *Xiphophorus*. In: Meffe GK, Snelson, F.F, editor. *Ecology and evolution of livebearing fishes (Poeciliidae)* Englewood Cliffs: Prentice Hall. p. 163-184.
- Kallman KD, Kazianis SD, 2006. The genus *Xiphophorus* in Mexico and Central America. *Zebrafish* 3:271-285.
- Karl SA, Bowen BW, Avise JC, 1995. Hybridization among the ancient mariners: characterization of marine turtle hybrids with molecular genetic assays. *J Hered* 86:262-268.
- 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 Research* 6:280-289.

- Klein N, Burns KJ, Hackett SJ, Griffiths CS, 2004. Molecular phylogenetic relationships among the wood warblers (Parulidae) and historical biogeography in the Caribbean basin. *J Carib Ornith* 17(Special Issue):3-17.
- Kodric-Brown A, Nicoletto PF, 2001. Age and experience affect female choice in the guppy (*Poecilia reticulata*). *Am Nat* 157:316-323.
- Kopp M, Hermisson, J. 2008. Competitive speciation and costs of choosiness. *J. Evol. Biol.* 21:1005-1023.
- Kokko H, Rankin DJ, 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Phil Trans R Soc B* 361:319-334.
- Kotiaho JS, Puurtinen M, 2007. Mate choice for indirect genetic benefits: scrutiny of the current paradigm. *Funct Ecol* 21:638-644.
- Kozak GM, Boughman JW, 2009. Learned conspecific mate preference in a species pair of sticklebacks. *Behav Ecol* 20:1282-1288.
- Kozak GM, Reisland M, Boughman JW, 2009. Sex differences in mate recognition and conspecific preference in species with mutual mate choice. *Evolution* 63:353-365.
- Kroodsma DE, Miller EH, 1996. Ecology and evolution of acoustic communication in birds. Cornell University Press, Ithaca, NY.
- 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.
- Laland KN, 1994. On the evolutionary consequences of sexual imprinting. *Evolution* 48:477-489.
- Lamb T, Avise, JC., 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. *Proc Natl Acad Sci USA* 83:2526-2530.
- Lapointe F, Garland J, 2001. A generalized permutation model for the analysis of cross-species data. *J Classif* 18:109-127.
- Latta SC, Parkes KC, 2001. A possible *Dendroica kirtlandii* hybrid from Hispaniola. *Wilson Bull* 113:378-383.
- Latta SC, Parkes KC, Wunderle JM, 1998. A new intrageneric *Dendroica* hybrid from Hispaniola. *The Auk* 115:533-537.

- Legendre P, Desdevises Y, 2009. Independent contrasts and regression through the origin. *J Theor Biol* 259:727-743.
- Lehman, N, Eisenhawer, A, Hansen, K, Mech, L. D, Peterson, R. O, Gogan, P. J. P, and Wayne, R.K. 1991. Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. *Evolution* 45:104-119.
- Leichty E, Grier, J, 2006. Importance of facial pattern to sexual selection in golden-winged warbler (*Vermivora chrysoptera*). *Auk* 123:962-966.
- Lemaire F, 1977. Mixed song, interspecific competition and hybridisation in the reed and marsh warblers (*Acrocephalus scirpaceus* and *palustris*). *Behaviour* 63:215-240.
- Lemon RE, Struger J, Lechowicz MJ, 1983. Song features as species discriminants in American warblers (Parulidae). *Condor* 85:308-322.
- Leonard AS, Hedrick AV, 2009. Male and female crickets use different decision rules in response to mating signals. *Behav Ecol* 20:1175-1184.
- Liou LW, Price, TD, 1994. Speciation by reinforcement of premating isolation. *Evolution* 48:1451-1459.
- Lorenz K, 1937. The companion in the bird's world. *Auk* 54:245-273.
- Lortie CJ, Aarssen LW, Budden AE, Koricheva JK, Leimu R, Tregenza T, 2007. Publication bias and merit in ecology. *Oikos* 116:1247-1253.
- Lovette IJ, Bermingham E, 1999. Explosive speciation in the New World *Dendroica* warblers. *Proc R Soc B* 266:1629-1636.
- Lovette IJ, Bermingham E, 2002. What is a wood-warbler? Molecular characterization of a monophyletic Parulidae. *Auk* 119:695-714.
- Lovette IJ, Hochachka WM, 2006. Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. *Ecology* 87(7 Suppl):S14-28.
- Lovette IJ, Pérez-Emán JL, Sullivan J, Banks RC, Fiorentino I, Córdoba-Córdoba S, Echevvery-Galvis M, Barker FK, Burns KJ, Klicka J, Lanyon SM, Bermingham, E, 2010. A comprehensive multilocus phylogeny for the wood-warblers and a revised classification of the Parulidae (Aves). *Mol Phylogenet Evol* 57:753-770.
- Lynch KS, Rand AS, Ryan MJ, Wilczynski W, 2005. Plasticity in female mate choice associated with changing reproductive states. *Anim Behav* 69:689-699.

- Magurran AE, Ramnarine, IW, 2004. Learned mate recognition and reproductive isolation in guppies. *Anim Behav* 67:1077-1082.
- Magurran AE, Ramnarine, IW, 2005. Evolution of mate discrimination in a fish. *Current Biol* 15:R867-R868.
- 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.
- Martin PR, Martin TE, 2001. Ecological and fitness consequences of species coexistence: a removal experiment with wood warblers. *Ecology* 82:189-206
- Mayr E, 1963. *Animal species and evolution*. Cambridge: Belknap Press.
- McCarthy E., 2006. *Handbook of avian hybrids of the world*. Oxford University Press, New York.
- McCaskie G, 1984. Southern Pacific coast region. *Amer Birds* 38:245-248.
- McLennan DA, Ryan MJ, 1997. Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. *Anim Behav* 54:1077-1088.
- McLennan DA, Ryan MJ, 1999. Interspecific recognition and discrimination based upon olfactory cues in northern swordtails. *Evolution* 53:880-888.
- McLennan DA, Ryan, M.J, 2008. Female swordtails, *Xiphophorus continens*, prefer the scent of heterospecific males. *Anim Behav* 75:1731-1737.
- Mengel RM, 1964. The probable history of species formation in some northern wood warblers (Parulidae). *Living Bird* 2:9-43.
- Meyer A, Salzburger W, Scharl M, 2006. Hybrid origin of a swordtail species (Teleostei: *Xiphophorus clemenciae*) driven by sexual selection. *Mol Ecol* 15:721-730.
- Milinski M, Bakker TCM, 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proceedings of the Royal Society B: Biological Sciences* 250:229-233.
- Møller AP, Alatalo RV, 1999. Good-genes effects in sexual selection. *Proc Biol Sci* 266:85-91.

- Moore PJ, Moore AJ, 2001. Reproductive aging and mating: the ticking of the biological clock in female cockroaches. *Proc Natl Acad Sci USA* 98:9171-9176
- Morris MR, Ryan MJ, 1990. Age at sexual maturity of male *Xiphophorus nigrensis* in nature. *Copeia* 3:747-751.
- Morris MR, Batra P, Ryan, MJ, 1992. Male-male competition and access to females in the swordtail *Xiphophorus nigrensis*. *Copeia* 1992:980-986.
- Morris MR, de Queiroz K, Morizot DC, 2001. Phylogenetic relationships among populations of northern swordtails (*Xiphophorus*) as inferred from allozyme data. *Copeia* 2001:65-81.
- Morris MR, Gass L, Ryan MJ, 1995a. Assessment and individual recognition of opponents in the pygmy swordtails *Xiphophorus nigrensis* and *X. multilineatus*. *Behav Ecol Sociobiol* 37:303-310.
- Morris MR, Mussel M, Ryan MJ, 1995b. Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. *Behav Ecol* 6:274-279.
- Morris MR, Rios-Cardenas O, Brewer J, 2010. Variation in mating preference within a wild population influences the mating success of alternative mating strategies. *Anim Behav* 79:673-678.
- Morris MR, Rios-Cardenas O, Tudor MS, 2006. Larger swordtail females prefer asymmetrical males. *Biol Lett* 2:8-11.
- Morris MR, Wagner WE Jr, Ryan MJ, 1996. A negative correlation between trait and mate preference in *Xiphophorus pygmaeus*. *Anim Behav* 52:1193-1203.
- Morse D, 1989. American warblers: an ecological and behavioral perspective. Harvard University Press, Cambridge, Massachusetts.
- Neville K, Vallender R, Robertson R, 2009. Nestling sex ratio of golden-winged warblers *Vermivora chrysoptera* in an introgressed population. *J Avian Biol* 39:599-604.
- Nuechterlein GL, Buitron D, 1998. Interspecific mate choice by late-courting male western grebes. *Behav Ecol* 9:313-321.
- Palmer AR, 2000. Quasireplication and the contract of error: lessons from sex ratios, heritabilities and fluctuating asymmetry. *Ann Rev Ecol Syst* 31:441-480.
- Palokangas P, Alatalo RV, Korpimäki E, 1992. Female choice in the kestrel under different availability of mating options. *Anim Behav* 43:659-665.



- Panjabi AO, Dunn EH, Blancher PJ, Hunter WC, Altman B, Beardmore J, Berlanga H, Butcher GS, Davis SK, Demarest DW, Easton W, Gomez de Silva Garza H, Iñigo-Elias EE, Pashley DN, Rich TD, Rosenberg KV, Rustay CM, Ruth JM, Wendt JS, Will TC. 2005. The Partners in Flight handbook on species assessment. Version 2005. Flight Technical Series No. 3. Rocky Mountain Bird Observatory. <http://www.rmbo.org/pubs/downloads/Handbook2005.pdf>
- Parker GA, 1979. Sexual selection and sexual conflict. In: Blum MS, Blum NB, editors. Sexual selection and reproductive competition in insects New York: Academic Press.
- Parker GA, 1983. Mate quality and mating decisions. In: Bateson P, editor. Mate choice Cambridge: Cambridge University Press. p. 141-164.
- Parker GA, Partridge L, 1998. Sexual conflict and speciation. Phil Trans R Soc Lond B 353:261-274.
- Parkes KC, 1978. Still another Parulid intergeneric hybrid (*Mniotilta* × *Dendroica*) and its taxonomic and evolutionary implications. Auk 95:682-690.
- Pearson SF, 2000. Behavioral asymmetries in a moving hybrid zone. Behav Ecol 11:84-92.
- Perry G, Garland T Jr, 2002. Lizard home ranges revisited: effects of sex, body size, diet, habitat, and phylogeny. Ecology 83:1870-1885.
- Pfennig KS, 1998. The evolution of mate choice and the potential for conflict between species and mate-quality recognition. Proc R Soc Lond B 265:1743-1748.
- Pfennig KS, 2007. Facultative mate choice drives adaptive hybridization. Science 318:965-967.
- Pfennig KS, Simovich MA, 2002. Differential selection to avoid hybridization in two toad species. Evolution 56:1840-1848.
- Pierotti R, Annett CA, 1993. Hybridization and male parental investment in birds. The Condor 95:670-679.
- Pinheiro JC, Bates DM, 2000. Mixed-effects models in S and S-PLUS. New York: Springer.
- Pocklington R, Dill LM, 1995. Predation on females or males: who pays for bright male traits? Anim Behav 49:1122-1124.
- Price T, 2008. Speciation in Birds. Roberts and Company, Greenwood Village, Colorado.

- Price TD, Bouvier MM, 2002. The evolution of F1 postzygotic incompatibilities in birds. *Evolution* 56:2083-2089.
- Price T, Lovette I, Bermingham E, Gibbs HL, Richman AD, 2000. The imprint of history on communities of North American and Asian warblers. *Am Nat* 156:354-367.
- Qvarnström A, Pärt T, Sheldon BC, 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. *Nature* 405:344-347.
- Qvarnström A, Haavie J, Sæther SA, Eriksson D, Pärt T, 2006. Song similarity predicts hybridization in flycatchers. *J Evol Biol* 19:1202-1209.
- R Development Core Team, 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Rafferty NE, Boughman JW, 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. *Behav Ecol* 17:965-970.
- Ramsey ME, Wong, RY, Cummings, ME, 2010. Estradiol, reproductive cycle and preference behavior in a northern swordtail. *Gen Comp Endocr* 170:381-390
- Randler C, 2002. Avian hybridization, mixed pairing and female choice. *Anim Behav* 63:103-119.
- Randler C, 2004. Frequency of bird hybrids: does detectability make all the difference? *J Ornithol* 145:123-128.
- Randler C. 2006. Behavioural and ecological correlates of natural hybridization in birds. *Ibis* 37:1-5.
- Rauchenberger M, Kallman KD, Morizot DC, 1990. Monophyly and geography of the Río Pánuco basin swordtails (genus *Xiphophorus*) with descriptions of four new species. *Amer Mus Novit* 2975:1-41.
- Reudink MW, Marra PP, Boag PT, Ratcliffe LM. 2009. Plumage coloration predicts paternity and polygyny in the American redstart. *Anim Behav* 77:495-501.
- 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.
- Rheindt, F, Grafe, T, and Abouheif, E. 2004. Rapidly evolving traits and the comparative method: how important is testing for phylogenetic signal? *Evol Ecol Res* 6:377-396.

- Rhymer JM, Simberloff D, 1996. Extinction by hybridization and introgression. *Ann Rev Ecol Syst* 27:83-109.
- Richmond J, Jockusch E, 2007. Body size evolution simultaneously creates and collapses species boundaries in a clade of scincid lizards. *Proc R Soc B* 274:1701-1708.
- 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.
- Robinson DM, Morris MR, 2010. Unraveling the complexities of variation in female mate preference for vertical bars in the swordtail *Xiphophorus cortezi*. *Behav Ecol Sociobiol* 64:1537-1545.
- Rohwer S, 1994. Two new hybrid *Dendroica* warblers and new methodology for inferring parental species. *Auk* 111:441-449.
- Rohwer S, Wood C, 1998. Three hybrid zones between hermit and Townsend's warblers in Washington and Oregon. *Auk* 115:284-310.
- Rowher S, Martin PR, 2007. Time since contact and gene flow may explain variation in hybrid frequencies among three *Dendroica townsendi* x *D. occidentalis* (Parulidae) hybrid zones. *Auk* 124:1347-1358.
- Rohwer S, Wood C, Bermingham, E, 2000. A new hybrid warbler (*Dendroica nigrescens* x *D. occidentalis*) and diagnosis of similar *D. townsendi* x *D. occidentalis* recombinants. *Condor* 102:713-718.
- Rosen DE, 1960. Middle-American poeciliid fishes of the genus *Xiphophorus*. *Bulletin of the Florida State Museum of Biological Sciences* 5:57-242.
- Rosen DE, 1979. Fishes from the uplands and intermontane basins of Guatemala: revisionary studies and comparative geography. *Bulletin of the American Museum of Natural History* 162:268-375.
- Rosenfield JA, Kodric-Brown A, 2003. Sexual selection promotes hybridization between Pecos pupfish, *Cyprinodon pecosensis* and sheepshead minnow, *C. variegatus*. *J Evol Biol* 18:595-606.
- Rosenqvist G, Houde, A, 1997. Prior exposure to male phenotypes influences mate choice in the guppy, *Poecilia reticulata*. *Behav Ecol* 8:194-198.
- 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.
- Rosenthal GG, Evans CS, Miller WL, 1996. Female preference for dynamic traits in the green swordtail, *Xiphophorus helleri*. *Anim Behav* 51:811-820.
- Rosenthal GG, Wagner, W.E.J, Ryan, M.J, 2002. Secondary loss of preference for swords in the pygmy swordtail *Xiphophorus nigrensis* (Pisces: Poeciliidae). *Anim Behav* 63:37-45.
- Rundle HD, Schluter D, 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. *Evolution* 52:200-208.
- Rundle HD, Schluter D, 2004. Natural selection and ecological speciation in sticklebacks. *Adaptive speciation*: Cambridge University Press. p. 192-209.
- Ryan MJ, Rand AS, 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* 47:647-657.
- Ryan MJ, Rand AS, 1995. Female responses to ancestral advertisement calls in the túngara frog. *Science* 269:390-392.
- Ryan MJ, Wagner WE, 1987. Asymmetries in mating preferences between species: female swordtails prefer heterospecific males. *Science* 236:595-597.
- 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.
- Ryan MJ, Perrill SA, Wilczynski W, 1992. Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans*. *Am Nat* 139:1370-1383.
- Sætre G-P, Moum, T, Bures, S, Král, M, Adamjan, M, Moreno, J, 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* 387:589-592.
- Sauer JR, Hines JE, Fallon J, 2008. The North American Breeding Bird Survey, Results and Analysis 1966 - 2007. Version 5.15. USGS Patuxent Wildlife Research Center, Laurel, MD. <http://www.mbr-pwrc.usgs.gov/bbs/>
- 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. *Phil Trans R Soc B* 363:2805-2811.
- Scribner KT, Page KS, Bartron M, 2001. Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. *Rev Fish Biol Fish* 10:293-323.
- Secondi J, Johanet A, Pays O, Cazimajou F, Djalout Z, Lemaire C, 2010. Olfactory and visual species recognition in newts and their role in hybridization. *Behaviour* 147:1693-1712.
- Seehausen O. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277:1808-1811.
- Seehausen O, 2004. Hybridization and adaptive radiation. *Trends Ecol Evol* 19:198-207.
- Seehausen O, van Alphen JJM, Witte F, 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277:1808-1811.
- Seehausen O, Takimoto G, Roy D, Jokela J, 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol Ecol* 17:30-44.
- Servedio MR, 2000. Reinforcement and the genetics of nonrandom mating. *Evolution* 54:21-29.
- Servedio MR, Noor MAF, 2003. The role of reinforcement in speciation: theory and data. *Ann Rev Ecol Evol Syst* 34:339-364.
- Servedio MR, Sæther SA, Sætre GP, 2009. Reinforcement and learning. *Evol. Ecol.* 23:109-123.
- Shapiro LH, 2001. Asymmetric assortative mating between two hybridizing *Orchelimum* katydids (Orthoptera: Tettigoniidae). *Am Midl Nat* 145:423-427.
- Shelly TE, Bailey WJ, 1992. Experimental manipulation of mate choice by male katydids: the effect of female encounter rate. *Behav Ecol Sociobiol* 30:277-282.
- Sherry TW, Holmes RT, 1989. Age-specific social dominance affects habitat use by breeding American redstarts (*Setophaga ruticilla*): a removal experiment. *Behav Ecol Sociobiol* 25:327-333.
- 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.

- Siegel S, Castellan NJJ, 1988. Nonparametric statistics for the behavioural sciences, 2nd ed. ed. New York: McGraw-Hill.
- Sillett TS, Rodenhouse NL, Holmes RT, 2004. Experimentally reducing neighbor density affects reproduction and behavior of a migratory songbird. *Ecology* 85:2467-2477.
- Simcox H, Colegrave N, Heenan A, Howard C, Braithwaite VA, 2005. Context-dependent male mating preferences for unfamiliar females. *Anim Behav* 70:1429-1437.
- Smadja C, Ganem G, 2005. Asymmetrical reproductive character displacement in the house mouse. *J Evol Biol* 18:1485-1493.
- Spector D, 1992. Wood-warbler song systems: a review of paruline singing behaviors. *Current Ornith* 9:199-238.
- Stebbins GL, 1959. The role of hybridization in evolution. *Proc Am Phil Soc* 103:231-251.
- Stein A, Uy A, 2006. Unidirectional introgression of a sexually selected trait across an avian hybrid zone: a role for female choice? *Evolution* 60:1476-1485.
- Stevenson H, Anderson B, 1994. Birdlife of Florida. University Press of Florida, Gainesville, Florida.
- Strecker U, Kodric-Brown A, 1999. Mate recognition systems in a species flock of Mexican pupfish. *J Evol Biol* 12:927-935.
- Svensson EI, Eroukhmanoff F, Karisson K, Runemark A, Brodin A, 2010. A role for learning in population divergence of mate preferences. *Evolution* 64:3101-3113.
- Tatarenkov A, Healy CIM, Avise JC, 2010. Microgeographic population structure of green swordtail fish: genetic differentiation despite abundant migration. *Mol Ecol* 19:257-268.
- Taylor EB, Boughman JW, Groenenbroom M, Sniatynski M, Schluter D, Gow JL. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Mol Ecol* 15:343-355.
- Thusius KJ, Peterson KA, Dunn PO, Whittingham LA, 2001. Male mask size is correlated with mating success in the common yellowthroat. *Anim Behav* 62:435-446.

- Trivers RL, 1972. Parental investment and sexual selection. In: Campbell B, editor. Sexual selection and the descent of man 1871 - 1971 Chicago, IL: Aldine
- Tudor MS, Morris, M.R, 2009. Experience plays a role in female preference for symmetry in the swordtail fish *Xiphophorus malinche*. *Ethology* 115:812-822.
- 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.
- Ulrich D, Ulrich S, 1981. Observations of the 1980 Indiana Sutton's warbler. *Atlan Nat* 34:12-13.
- Urbano-Amilpa H, 2006. Estrategia reproductiva de tres especies del género *Xiphophorus* (Pisces: Poeciliidae) en la cuenca del río Atlapexco, México Querétaro: Universidad Autónoma de Querétaro.
- Uy JAC, Patricelli GL, Borgia G, 2001. Complex mate searching in the Satin Bowerbird *Ptilonorhynchus violaceus*. *Am Nat* 158:530-542.
- Vaida F, Blanchard S, 2005. Conditional Akaike information for mixed-effects models. *Biometrika* 92:351-370.
- Vallender R, Friesen V, Robertson R, 2007. Paternity and performance of golden-winged warblers (*Vermivora chrysoptera*) and golden-winged x blue-winged warbler (*V. pinus*) hybrids at the leading edge of a hybrid zone. *Behav Ecol Sociobiol* 61:1797-1807.
- Vallender R, Gagnon J, Lovette I., 2009. An intergeneric wood-warbler hybrid (*Mniotilta varia* × *Dendroica coronata*) and use of multilocus DNA analyses to diagnose avian hybrid origins. *Wilson J Ornith* 12:298-305.
- van Gossum H, Stoks, R, De Bruyn, L, 2001. Reversible frequency-dependent switches in male mate choice. *Proc Roy Soc B* 268:83-85.
- Veen T, Borge T, Griffith SC, Saetre GP, Bures S, Gustafsson L, Sheldon BC, 2001. Hybridization and adaptive mate choice in flycatchers. *Nature* 411:45-50.
- Verzijden MN, Lachlan, R. F, Servedio, M.R, 2005. Female mate choice behavior and sympatric speciation. *Evolution* 59:2097-2108.
- Wagner WE, 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.
- Walling CA, Royle NJ, Lindström J, Metcalfe NB, 2008. Experience-induced preference for short-sworded males in the green swordtail, *Xiphophorus helleri*. *Anim Behav* 76:271-276.
- Widemo F, Sæther SA, 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. *Trends Ecol Evolution* 14:26-31.
- 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.
- Williams J, 1996. Nashville warbler (*Vermivora ruficapilla*). in A. Poole, ed. *The Birds of North America Online*. Cornell Lab of Ornithology, Ithaca, New York.  
<http://bna.birds.cornell.edu/bna>
- 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, Fisher HS, Rosenthal GG, 2005. Species recognition by male swordtails via chemical cues. *Behav Ecol* 16:818-822.
- Wong BBM, Keogh JS, Jennions MD, 2004. Mate recognition in a freshwater fish: geographical distance, genetic differentiation, and variation in female preference for local over foreign males *J Evol Biol* 17:701-708.
- 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 swordtail fish. *Am Nat* 167:136-140.
- Wright R, 2007. Sightings: early May to early June 2007. *Birding July/August*:30-31.
- Yoshimura J, Starmer WT, 1997. Speciation and evolutionary dynamics of asymmetric mating preference. *Res Popul Ecol* 39:191-200.