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Architecture of Mate Choice Decisions in Enchenopa Treehoppers

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ARCHITECTURE OF MATE CHOICE DECISIONS IN ENCHENOPA TREEHOPPERS

by

Bretta Lynn Vrieze Speck

A Dissertation Submitted in
Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy
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ABSTRACT

ARCHITECTURE OF MATE CHOICE DECISIONS IN ENCHENOPA TREEHOPPERS

by

Bretta Lynn Vrieze Speck

The University of Wisconsin-Milwaukee, 2022
Under the Supervision of Professor Rafael Lucas Rodriguez

Mate selection is one of the most important choices a female can make for herself and her offspring. Variation in mate choice decisions has consequences for the maintenance of and the diversity within a population and the promotion of divergence between populations. Mate choice decisions arise from the interaction of two main components: “mate preferences” (the relative attractiveness of a potential mate) and “choosiness” (the effort put into procuring a preferred mate). My dissertation analyzes the relationship between the components involved in female mate choice decisions in *Enchenopa binotata* treehoppers. I take a three-pronged approach. First, I investigated how *E. binotata* females process a male mating signal with multiple elements. I tested the hypothesis of basic combinatorial processing against two competing hypotheses: beginning rule and no-ordering rule. This was done by presenting females with different arrangements of signal elements and recording the female responses. I found support for combinatorial processing, meaning that female treehoppers use rules for acceptable element combinations, which allows them to process complex signals when selecting mates. Second, I investigated how mate preferences and choosiness adaptively change over the lifetime of a female. Mate preference functions are described using 4 traits: tolerance, strength, responsiveness, and peak. Hypotheses were tested for all four preference function traits along with choosiness using vibrational playback experiments. All showed significant changes that

allowed females to expand the pool of preferred mate types to procure a mating from the diminishing pool of available males. Females also increased their choosiness putting more effort into securing a preferred mate. Third, I looked at variation in mate choice decisions; testing whether individual female differences or the social context of what options are available contribute more to mate selection. We found considerable individual differences in preferred mate types, but found that our manipulation of the immediate social context had no effect. My dissertation deepens the breadth of knowledge about how mate choice decisions are made, which in turn helps us understand the consequences of variation in such decisions for the maintenance of population diversity and the promotion of speciation.

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Dedicated to:

My parents Don & Peggy Vrieze
who instilled in me a love of nature, curiosity, and learning.

My children Athanasius, Jael, and Eunice
who bring me so much joy and love. I am truly blessed to be your Mom.

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TABLE OF CONTENTS

***LIST OF FIGURES*..... vii**

***LIST OF TABLES* viii**

***ACKNOWLEDGEMENTS*..... Error! Bookmark not defined.ix**

***Introduction*..... 1**

 References4

***Chapter One: Combinatorial Signal Processing in an Insect* 9**

 Introduction10

 Methods14

 Results18

 Discussion.....21

 References25

Chapter Two: Adaptive lifetime variation in the mate preferences and choosiness in female

***Enchenopa treehoppers*..... 31**

 Introduction31

 Methods37

 Results43

 Discussion.....45

 References48

Chapter Three: Variation in preferred mate types: individual differences matter more than the

***immediate context of mate choice in Enchenopa treehoppers* 53**

 Introduction54

 Methods56

 Results62

 Discussion.....64

 References67

***Appendix A: Chapter 1 Raw Data*..... 72**

***Appendix B: Chapter 3 Raw Data* 78**

***Curriculum Vitae* 89**

LIST OF FIGURES

Figure #	Figure title	Page #
Figure 1.1	The two-element male advertisement signal of <i>Enchenopa</i> treehoppers	9
Figure 1.2	Results of the test for combinatorial processing	15
Figure 2.1	Mate preference function traits illustration	33
Figure 2.2	Choosiness illustration	34
Figure 2.3	Female <i>E.binotata</i> with pronotums painted for individual identification	37
Figure 2.4	Lifetime variation in preference function traits and choosiness	42
Figure 2.5	Lifetime variation in mass	44
Figure 3.1	<i>Enchenopa</i> female duetting response to a vibrational playback stimulus	58
Figure 3.2	Vibrational playbacks presented in relation to mate preference function	59
Figure 3.3	Female responses to treatment and focal stimuli by the treatments	63

LIST OF TABLES

Table #	Table title	Page #
Table 1.1	Examples of basic and hierarchical combinatorial processing in animals	8
Table 1.2	Hypotheses that analyze processing of signal element combinations and their predictions	11
Table 1.3	Analysis of variation in female response to stimuli	16
Table 2.1	Analysis of variation in fixed terms for lifetime preference function traits, choosiness, & mass	43
Table 2.2	Analysis of variation in random terms for lifetime preference function traits, choosiness, & mass	44
Table 3.1	Analysis of variation in response to stimuli that alter the immediate social context	62

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INTRODUCTION

Mate choice is an important cause of variation in individual fitness for males and females, and the cause of strong natural and sexual selection on communication systems (Darwin 1871; Andersson 1994; Andersson and Simmons 2006; Rosenthal 2017). Therefore, mate choice has important evolutionary consequences: it explains the evolution of extreme or elaborate ornaments; it may promote divergence and speciation or it may sustain within-population variation; and it may facilitate or hinder local adaptation (Darwin 1871; West-Eberhard 1983; Lorch et al. 2003; Coyne and Orr 2004; Greenfield and Rodriguez 2004; Servedio and Bürger 2014). Which of these consequences results from the action of mate choice, depends in part on the patterns of individual- and population-level variation in mate choice decisions (Jennions and Petrie 1997). Consequently, understanding the evolutionary consequences of mate choice requires analyzing variation in the components that generate mate choice decisions.

Mate choice decisions arise from the interaction of two main components: mate preferences, and choosiness (Jennions and Petrie 1997). Mate preferences are functions that relate variation in ornament features to variation in the perceived attractiveness of potential mates (Ritchie 1996; Jennions and Petrie 1997; Kilmer et al. 2017). Choosiness is the effort invested in assessing the quality of and acquiring of a preferred mate type (Jennions and Petrie 1997; Neelon et al. 2019).

Variation in mate choice decisions may arise from changes along the course of mating seasons. As mating seasons progress, individuals age, which may bring about changes in behavior (e.g., overall levels of activity; Carey et al. 2006), as well as fecundity (Arnold and Duvall 1994). There may also be changes in the composition of available mates; e.g., when males take the initiative to seek a mate by moving about and advertising themselves, they often

incur elevated risks of predation and suffer shorter life expectancies (Andersson 1994; Kotiaho et al. 1998; Candolin 2003). Further, demographic changes along the mating season may place a time constraint on female mate choice decisions and put a premium on balancing the essential task of securing a mating with obtaining preferred mate types (cf. (Fowler-Finn and Rodríguez 2012)). Consequently, some changes in mate choice decisions over the mating season may constitute adaptive adjustments to mate preferences and choosiness.

There has also been much interest in how different signal features interact to determine attractiveness. Advertisement signals are often highly complex, having many elements in different perceptual modalities (West-Eberhard 1983; Hebets and Papaj 2004; Hebets et al. 2016; Rosenthal 2017). We do not understand how preferences for such varied features may interact, although there is clear evidence that they do in ways that influence signal attractiveness (Brooks et al. 2005; Bentsen et al. 2006; Gerhardt and Brooks 2009).

In some cases, signals are so complex (e.g., (Girard et al. 2011; Elias et al. 2012)) that it is puzzling how animal brains are able to even process them at all. One potential solution is “combinatorial signal processing” in which there are rules regarding the combinations of individual signal elements that grouped together into combinations that are viewed as single units and are acceptable to receivers. In human language, this capacity groups sound phonemes into syllables, syllables into words, words into phrases, and so on. Some authors have referred to this as phonology (Fitch 2010) or as finite state grammar (Zuberbühler 2019). Many vertebrate species have been shown to use combinatorial processing (Hailman et al. 1987; Marler and Peters 1988; Kanwal et al. 1994; Dahlin and Wright 2009; Ouattara et al. 2009; Kershenbaum et al. 2012; Casar et al. 2013; Hedwig et al. 2015; Soma and Mori 2015; Heimbauer et al. 2018; Engesser et al. 2019) with some going beyond combinatorial and generating hierarchical

structuring as well (Marler and Peters 1988; Gentner et al. 2006; Dahlin and Wright 2009; Hedley et al. 2017; Bergman et al. 2019; Dutour et al. 2019; Suzuki et al. 2019), but hasn't been studied in invertebrates until this dissertation. Determining if this processing is possible in insects will help us understand how animals with tiny brains determine what is attractive.

In addition, attractiveness may not be solely based on a signal fitting any given preference or processing rule; females may have variation in mate choice decisions based on individual differences between choosing individuals or based on the immediate social context of available mates to choose from. Even at such a seemingly narrow scale, these causes of variation may have important consequences. Individual differences arise from genetic and developmental-social inputs (West-Eberhard 2003, 2005; Nussey et al. 2007; Chenoweth and McGuigan 2010; Dingemanse et al. 2010; Hebets and Sullivan-Beckers 2010; Verzijden et al. 2012; Rodríguez et al. 2013). Such individual variation may help explain the maintenance of variation under selection due to mate choice. For instance, the form and strength of selection suggested by population-level analysis is often revealed, by examination of individual differences in preferences, to allow for success for a broader range of mate types than would be expected otherwise (e.g., (Wagner et al. 1995; Hedrick and Weber 1998; Murphy and Gerhardt 2000; Neelon et al. 2019)). Differences in the options available at the time of mate choice may also have drastic effects, leading in the extreme to preference reversals (Kirkpatrick et al. 2006; Regenwetter et al. 2021).

For my dissertation, I use a “cognitive phenotype” approach to analyze variation in the components of mate choice decisions. Cognitive phenotypes are components of decision-making processes expressed in animal brains — they vary between individuals, have genetic and environmental components of variation, and can therefore respond to selection (Mendelson et al.

2016). Mate preferences, choosiness, and combinatorial processing rules are prime examples of cognitive phenotypes that determine mate choice decisions, and analyzing them in these terms will help understand how their variation contributes to the fitness of the individuals that express them, as well as to the evolutionary processes that they shape.

In my study of the architecture of mate choice decisions, I take a three-pronged approach. I first look at how females combine and process signals, specifically looking at responsiveness to different arrangements of signal elements to determine which signal element combinations are considered acceptable (Chapter 1). Second, I look at how preferences and choosiness change over a female's lifetime (Chapter 2). Third I look at how individual female differences and the immediate social context influence relative attractiveness (Chapter 3).

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CHAPTER ONE: COMBINATORIAL SIGNAL PROCESSING IN AN INSECT

Abstract: Human language is combinatorial: phonemes are grouped into syllables, syllables into words, and so on. The capacity for combinatorial processing is present to different degrees in some mammals and birds. We used a vibrational insect, *Enchenopa* treehoppers, to test the hypothesis of basic combinatorial processing against two competing hypotheses: beginning rule (where the early signal portions play a stronger role in acceptability); and no ordering rule (where the order of signal elements plays no role in signal acceptability). *Enchenopa* males use plant-borne vibrational signals that consist of a whine followed by pulses (WP). We tested the above hypotheses with vibrational playback experiments in which we presented *Enchenopa* females with stimuli varying in signal element combinations. We monitored female responses to these playbacks with laser vibrometry. We found strong support for combinatorial processing in *Enchenopa*: in brief, females preferred natural-combination signals regardless of the beginning element and discriminated against reverse-order signals or individual elements. Finding support for the combinatorial rule hypothesis in an insect suggests that this capability represents a common solution to the problems presented by complex communication.

Keywords: *Enchenopa binotata*, insect communication, mate choice, signal evolution, signal processing, phonology, syntax, vibrational signaling

INTRODUCTION

Many aspects of human communication and cognition are built on the basis of mental processing that gives rise to combinatorial and hierarchical structure. In language, this capacity groups sound phonemes into syllables, syllables into words, words into phrases, and so on — and it also creates structure in music, mathematics and thinking (Pinker 1994; Hauser and Chomsky 2002; Pinker and Jackendoff 2005; Fitch 2010; Corballis 2011; Fitch and Martins 2014).

Combinatorial processing is hypothesized to have originated prior to modern humans, and to be present in different degrees in other lineages (Fitch 2010). A key distinction is whether processing is only combinatorial, or whether it is also hierarchical and recursive. “Basic” combinatorial processing involves rules regarding the specific combinations (or orderings) of discrete signal elements that are acceptable to receivers. In human language, for instance, processing at this level combines sound phonemes to form words; e.g., in English the sounds /k/, /æ/ and /t/ are combined to form the word “cat” (Bowling and Fitch 2015). Some authors refer to such processing as “phonology” (Fitch 2010: 94) or “finite state grammar” (Zuberbühler 2019: 2). Examples of this basic combinatorial processing can be found in some primates, bats, hyraxes, and birds (table 1). Combinatorial processing may go beyond the above basic level, and generate hierarchical structuring by recursive embedding of combinations of signal elements (Pinker 1994; Hauser and Chomsky 2002; Pinker and Jackendoff 2005; Fitch 2010; Fitch and Martins 2014). In human language, processing at this level combines words to form phrases (e.g., “cats purr) that can be further combined with other phrases and embedded in sentences and more complex structures (Bowling and Fitch 2015). Some authors refer to this type of processing as “syntax” (Fitch 2010: 100) or “phrase structure grammar” (Zuberbühler 2019: 2). Examples of

this more complex combinatorial-hierarchical processing can be found in some whales and birds (table 1) (Bergman et al. 2019; Suzuki et al. 2019)).

Table 1.1 Examples of basic and hierarchical combinatorial processing in animals.

SPECIES	METHOD OF TESTING	REFERENCES
Basic Combinatorial Processing		
Birds		
Black-capped chickadee (<i>Parus atricapillus</i>)	Structure of naturally occurring calls	(Hailman et al. 1987)
Swamp sparrow (<i>Melospiza georgiana</i>)	Ability to learn test sequences	(Marler and Peters 1988)
Yellow-naped amazons (<i>Amazona auropalliata</i>)	Structure of naturally occurring calls	(Dahlin and Wright 2009)
Java sparrows (<i>Lonchura oryzivora</i>)	Structure of spontaneously occurring calls in tutored and non-tutored males	(Soma and Mori 2015)
Chestnut-crowned babbler (<i>Pomatostomus ruficeps</i>)	Habituation	(Engesser et al. 2019)
Great tit (<i>Parus major</i>)	Innate response to playbacks	(Dutour et al. 2019)
Coal tit (<i>Periparus ater</i>)	Innate response to playbacks	(Dutour et al. 2019)
Common chaffinch (<i>Fringilla coelebs</i>)	Innate response to playbacks	(Dutour et al. 2019)
Primates		
Wedge-capped capuchins (<i>Cebus olivaceus</i>)	Structure of naturally occurring calls	(Robinson 1984)
Cotton-top tamarins (<i>Saguinus Oedipus</i>)	Habituation	(Fitch and Hauser 2004)
Chimpanzee (<i>Pan troglodytes</i>)	Structure of naturally occurring calls	(Crockford and Boesch 2005)
Putty-nosed monkey (<i>Cercopithecus nictitans</i>)	Innate response to playbacks	(Arnold and Zuberbühler 2008)
Campbell's monkeys (<i>Cercopithecus campbelli campbelli</i>)	Structure of naturally occurring calls & Innate response to playbacks	(Ouattara et al. 2009)
Red-capped mangabeys (<i>Cercocebus torquatus</i>)	Structure of naturally occurring calls in captivity	(Bouchet et al. 2010)
Titi monkey (<i>Callicebus nigrifrons</i>)	Structure of naturally occurring calls	(Cäsar et al. 2013)
Western gorilla (<i>Gorilla gorilla</i>)	Structure of naturally occurring calls	(Hedwig et al. 2015)
Mountain gorilla (<i>Gorilla beringei beringei</i>)	Structure of naturally occurring calls	(Hedwig et al. 2015)
Geladas (<i>Theropithecus gelada</i>)	Innate response to playbacks	(Gustison and Bergman 2016)
Bonobos (<i>Pan paniscus</i>)	Structure of naturally occurring calls	(Schamberg et al. 2016)
Rhesus macaques (<i>Macaca Mulatta</i>)	Ability to learn test sequences	(Heimbauer et al. 2018)
Other mammals		
Mustached bat (<i>Pteronotus parnellii parnellii</i>)	Structure of naturally occurring calls	(Kanwal et al. 1994)
rock hyrax (<i>Procapra capensis</i>)	Innate response to playbacks	(Kershenbaum et al. 2012)
Hierarchical Combinatorial Processing		
Birds		
Song sparrow (<i>Melospiza melodia</i>)	Ability to learn test sequences	(Marler and Peters 1988)
European starlings (<i>Sturnus vulgaris</i>)	Habituation	(Gentner et al. 2006)
Yellow-naped amazons (<i>Amazona auropalliata</i>)	Structure of naturally occurring calls	(Dahlin and Wright 2009)
Cassin's Vireos (<i>Vireo cassinii</i>)	Innate response to playbacks	(Hedley et al. 2017)
Other Mammals		
Humpback whales (<i>Megaptera novaeangliae</i>)	Structure of naturally occurring calls	(Allen et al. 2019)

Similarities in the capacity for combinatorial processing across different species may represent common descent or convergent solutions to the problems that animals face in complex environments and complex communication systems (Fitch 2010). Consequently, understanding the evolution of combinatorial processing in animals, as well as the course it followed in our lineage, requires exploring the diversity and taxonomic distribution of combinatorial processing capabilities among animals.

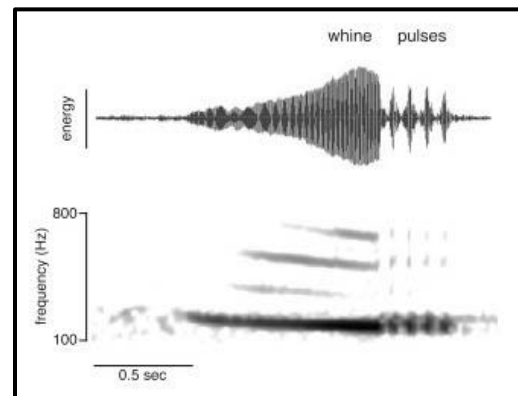
Here we report basic combinatorial processing in the communication system of an insect, a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera:

Membracidae). *Enchenopa* treehoppers are phloem-feeding insects that communicate with plant-borne vibrational signals (Cocroft and Rodríguez 2005; Cocroft et al. 2008). Males produce advertisement signals consisting of two elements: a whine (W) followed by a series of pulses (P) (Cocroft et al. 2010) (Fig. 1). This “WP” signal structure is highly conserved in the *E. binotata*

complex, while other signal features (especially dominant frequency) are strongly species-specific and covary with divergent female preferences (Rodríguez et al. 2006; Cocroft et al. 2008, 2010). This conservation of basic signal structure against the background of strong divergence in other traits suggests either strong stabilizing selection on signal

structure or strong signal-production constraints. While “PW” variants are observed only rarely in the *E. binotata* complex (R. B. Cocroft, pers. comm.), reversals in signal structure are common across species in treehoppers and other vibrational insects (Cocroft 2003; Percy et al. 2006, 2008). This observation argues against production constraints, and we therefore focus here

Figure 1.1 The two-element male advertisement signal of *Enchenopa* treehoppers, shown as an oscillogram (top) and



on testing for a female combinatorial processing rule for signal structure in *Enchenopa* treehoppers.

We tested the “combinatorial rule” hypothesis in terms of the *Enchenopa* communication system and the two-element (WP) structure of male advertisement signals (predictions [i]-[iii] in table 2). We tested this hypothesis against two competing alternatives. First, signal processing might not be truly combinatorial; instead, earlier elements may simply have stronger effects on signal acceptability than later signal elements. There is evidence in support of this hypothesis from studies in some insects and frogs, which have referred to the “temporal-order effect” hypothesis (Gerhardt et al. 2007; Reichert et al. 2017). We use the term “beginning rule” to emphasize that this hypothesis focuses on the strength of the effect of the beginning of signals rather than on the combination of elements *per se* (prediction [iv] in table 2). The second competing hypothesis is the null or “no ordering rule” hypothesis, whereby many possible arrangements of signal elements are acceptable (prediction [v] in table 2). There is evidence in support of this hypothesis from studies in some frog species (Wilczynski et al. 1999; Oliva et al. 2018).

We also contrasted the response to the natural-combination stimulus (WP) against the single element stimuli (W and P). These tests do not directly address the logic of the hypotheses, but inform us of the relative importance of the presence of each signal element *per se*.

We tested the above predictions (table 2) with a vibrational playback experiment in which we presented *Enchenopa* females with synthetic stimuli varying in signal element composition to assess their acceptability. The combinatorial processing literature includes a very broad range of methods, from analysis of the structure of naturally-occurring signals to experiments requiring sophisticated learning abilities in the animals to test the hypotheses (table

1). Our experiment tests for differences in female responsiveness to stimuli varying in signal element combinations. It makes no assumptions about whether those differences are innate or acquired (although the former seems likely). The tests involve potential effects from preceding signal elements on subsequent signal elements (“combinatorial rule” and “beginning rule” hypotheses). We therefore presented each female with only one stimulus. Our experiment thus assessed variation in the responses of females across stimuli to test for population-level rules regarding signal element combinations.

Table 1.2 Hypotheses that analyze processing of signal element combinations in *Enchenopa* treehoppers, and their predictions.

Hypothesis	Predictions: females should:	Summary
Combinatorial rule	(i) prefer the natural-combination stimulus over a reverse-order stimulus	WP > PW
	(ii) make no distinction between the natural-combination stimulus and a stimulus beginning with the wrong element but containing the natural combination stimulus	WP = PWP
	(iii) prefer a stimulus beginning with the wrong element but containing the natural combination stimulus over a reverse-order stimulus	PWP > PW
Beginning rule	(iv) prefer stimuli with the natural-beginning element over stimuli beginning with the wrong element [†]	WP > PW WP > PWP W > PW W > PWP
No ordering rule	(v) make no distinction between stimuli with different signal element combinations	WP = PW = PWP

^{*} We did not contrast the response to W and P stimuli because this comparison might depend not only on the “beginning” of the P stimulus but also on it being insufficient on its own (see below)

METHODS

We worked with one of the two *E. binotata* species that live on *Viburnum lentago* (Adoxaceae) bushes and trees in Wisconsin, USA. Most of the species in the complex have not yet been formally described (Hamilton and Cocroft 2009), but they can be distinguished by their host plant, nymph coloration, and the dominant frequency of adult male signals (Wood, 1980;

Wood, 1993; Rodríguez, Sullivan, & Cocroft, 2004; Cocroft et al., 2008, 2010) . We used the species that has male signals with a dominant frequency of 185 Hz.

We conducted the study over the summers of 2016 and 2017. In 2016 we collected the treehoppers in June as late instar nymphs at the University of Wisconsin-Milwaukee's Downer Woods and then reared them to maturity on potted *V. lentago* plants in the UWM greenhouse. In 2017, we used nymphs reared from eggs we hatched ahead of the summer season at the greenhouse.

We separated adult females from males 1-3 days after their final molt. Separation was essential to prevent the females from mating, thus keeping them sexually receptive and responsive to playbacks. We began our trials when the females reached sexual maturity, four weeks after the adult molt.

We created vibrational playback stimuli on an iMac computer using MATLAB (v.7.5.0.338) (The Mathworks, Inc., Natick, MA, USA; www.mathworks.com). The playback stimuli varied in the combination of signal elements presented to females, as follows: stimuli had either the natural whine-pulse (WP) signal element combination; the reverse pulse-whine (PW) element combination; a pulse-whine-pulse (PWP) element combination; a single whine (W) element; or a single pulse (P) element. We set all other stimulus features to the population mean (185 Hz dominant frequency, 950 ms whine length, 3 pulses, 38 ms pulse length, and 21 Hz pulse rate; R.L. Rodríguez unpublished data.), except that we used only one signal per stimulus rather than the typical signal bout structure (Cocroft et al. 2010) in order to avoid possible confounding effects of preceding stimuli on subsequent stimuli (a possibility under the combinatorial and beginning rule hypotheses).

We presented each female with a single, randomly assigned playback stimulus (i.e., we tested each female only once). Thus, responses to each stimulus by each female are fully independent of each other. Further, this was necessary in our study because of the potential for confounding effect of preceding stimuli on subsequent stimuli (see rationale above).

To present females with a playback stimulus, we placed each female singly on a small potted *V. lentago* plant and allowed her to roam and settle (a minimum of 30 seconds and maximum of 10 minutes) before presenting her with a single, randomly selected stimulus. *Enchenopa* females that find a male's signal attractive respond with their own single-element signals establishing a duet that lasts until mating begins (Rodríguez and Cocroft 2006; Cocroft et al. 2008; Rodríguez et al. 2012). Our assay of stimulus acceptability took advantage of this aspect of the *Enchenopa* communication system: we noted whether a female produced a duetting signal in response to the stimulus. If the female did not respond to the stimulus, we presented her with a playback of a recording of a male signal to confirm that she was sexually receptive and that she did not respond to the experimental stimulus because it was unattractive to her. If she did not respond to the playback of the recorded male, we retested her 1-3 days later. We tested $n = 40$ females for each of the five stimuli ($n = 20$ females per stimulus per year).

We imparted the playback stimuli onto the test plant with a piezo-electric controller and actuator (Thorlabs, Newton, NJ, USA) attached to its stem with wax. We delivered all stimuli at a peak amplitude of 0.15 mm/s.

We recorded the stimuli and female responses with a laser vibrometer (Polytec PLV-100; Polytec Inc., Auburn, MA, USA). We sent the output of the laser vibrometer through a band-pass filter (40-4000 Hz; Krohn-Hite 3202; Krohn-Hite Corp., Brockton, MA, USA) and digitized it on

an iMac computer with a sampling rate of 44.1 kHz using the computer program AUDACITY (v.2.1.2)(www.audacityteam.org).

After testing, we returned females to the lab colony or to the field (Downer Woods).

Statistical Analysis

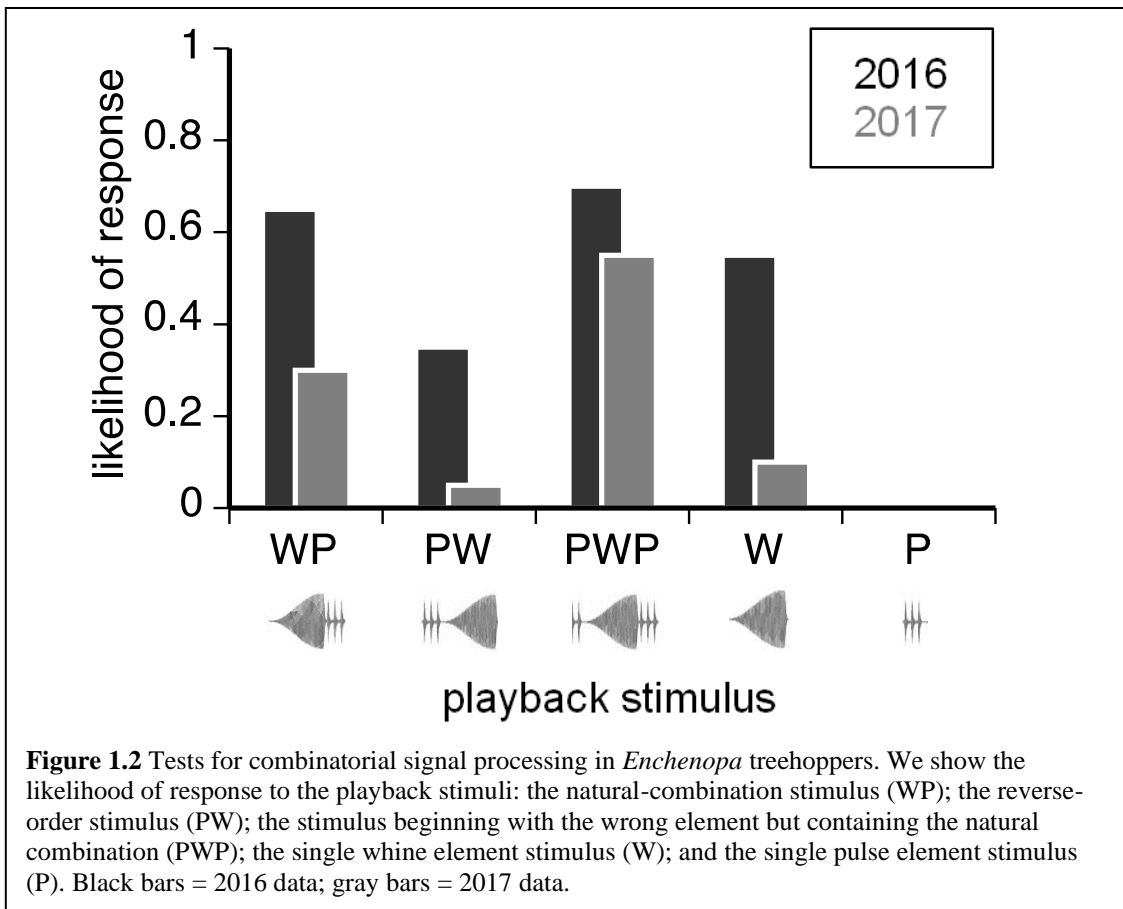
We conducted the statistical analysis in two steps. We first tested for variation in the likelihood of female response to the different stimuli. The model had female response (0 for no response, 1 for response) as the dependent variable. The error structure was a binomial distribution. The explanatory variables were: stimulus (i.e., signal element combination), year, and their interaction. Due to complete separation in the data (0% response for one stimulus; see below), the maximum likelihood estimates of the regression coefficients could not be estimated as they do not exist (Albert and Anderson 1984). We therefore used a bayesian generalised linear model with a Cauchy prior (Gelman et al. 2008). We fit the model in R using the function *bayesglm* of the lme4 package (v. 1.10-1; (Gelman, A., and Su 2018)). We checked model stability by excluding data points one at a time from the data set, and found that all the coefficient estimates were stable. We derived variance inflation factors (Field 2009) with the *vif* function of the car package (version 3.0-3; (Fox and Weisberg 2011)). We found no collinearity between fixed effects.

The second step of our analysis was to test each of the predictions specifically (table 2). To this end, we used Tuckey post-hoc tests to compare the response to each of the relevant stimulus pairs. We obtained these comparisons from a model with only the main terms (stimulus and year), as their interaction was not significant (see below).

RESULTS

Enchenopa females varied in their likelihood to respond to the playback stimuli according to signal element combinations (significant stimulus term in table 3; Fig. 2). The term for year was also significant, with females tested in 2016 being overall more responsive than those tested in 2017 (table 3; Fig. 2). However, the stimulus \times year interaction was not significant (table 3), indicating that the patterns of response were similar across years (Fig. 2).

On the basis of the above overall effect of stimulus signal element combination on female responses, we used post-hoc tests to pinpoint the comparisons specified by the hypotheses' predictions (table 2).



In support of the “combinatorial rule” hypothesis, *Enchenopa* females were significantly more likely to respond to the natural-combination stimulus (WP) than to the reverse-order stimulus (PW) (table 4; Fig. 2) (supporting prediction [i]). Females were also about as likely to respond to the natural-combination stimulus (WP) as to the stimulus containing the natural-combination but beginning with the wrong element (PWP): the difference in response was small and non-significant (table 4; Fig. 2) (supporting prediction [ii]). Females were also significantly more likely to respond to the natural-combination beginning with the wrong element (PWP) than to the reverse-order stimulus (PW) (table 4; Fig. 2) (supporting prediction [iii]).

Table 1.3 Analysis of variation in the likelihood of *Enchenopa* female response to vibrational playback stimuli varying in element composition.

term	df	z	p
Stimulus	4	55.74*	<< 0.001*
Year	1	19.85*	0.0013*
Stimulus × year	4	1.22	0.87

Note: We used a Bayesian generalised linear model with a binomial error distribution to test the effect of the playback treatments, year of testing, and the playback × year interaction (model explanation in text). The main terms remain nearly identical after removing the non-significant interaction (stimulus: $z = 54.51$, $p << 0.0001$; year: $z = 18.63$, $p << 0.0001$).

*Significant term

The comparison between the natural-combination stimulus (WP) to the reverse-order stimulus (PW) would also support the “beginning rule” hypothesis (table 4; Fig. 2) (prediction [iv]). However, in opposition to prediction (iv) we found the following results: *Enchenopa* females were not significantly more likely to respond to the natural-combination stimulus (WP) than the stimulus containing the natural-combination but beginning with the wrong element (PWP) (table 4; Fig. 2); they were not more likely to respond to the single whine element stimulus (W) than to the reverse-order stimulus (PW) (table 4; Fig. 2); and they were

significantly more likely to respond to the natural-combination stimulus beginning with the wrong element (PWP) than to the single whine element stimulus (W) (table 4; Fig. 2).

Table 1.4 Comparison of the likelihood of *Enchenopa* female response according to stimulus pairings that address the predictions of the hypotheses (see Table 2). We show the results of the post-hoc tests from the bayesian generalised linear model that included only the main terms for stimulus and year (see text and Table STATS 1). Significant comparisons indicated in bold.

Stimulus comparison*	<i>z</i>	<i>p</i>	Predictions supported	Predictions rejected
WP - PW	2.71	0.0497	(i), (iv)	(v)
WP - PWP	-1.44	0.59	(ii)	(iv)
PWP - PW	3.92	< 0.0001	(iii)	(iv), (v)
W - PW	1.34	0.65		(iv)
W - PWP	-2.82	0.037		(iv), (v)
WP - W	1.46	0.58		
WP - P	4.22	< 0.0001		(v)
W - P	3.30	0.0078		(v)

* There are two additional comparisons that we do not emphasize because they do not address any specific prediction; reported here for completeness: PW-P: $z = 2.34$, $p = 0.13$; PWP-P: $z = 5.03$, $p < 0.001$.

Additionally, the overall significant effect of the stimulus term (table 3) and the predominance of significant comparisons supporting the “combinatorial rules” hypothesis (table 4; Fig. 2) reject prediction (v) of the null “no ordering rules” hypothesis.

Finally, *Enchenopa* females tended to be more likely to respond to the natural-combination stimulus (WP) than to either of the single-element stimuli (W or P), but only significantly so against the P stimulus (table 4; Fig. 2).

DISCUSSION

We tested for basic combinatorial signal processing in *Enchenopa* treehoppers with playback experiments varying signal element structure. We found that *Enchenopa* females were more likely to respond to the natural-combination stimulus (WP) than to the reverse combination (PW) stimulus. Interestingly, they were not more likely to respond to the natural-combination stimulus (WP) than to the natural-combination stimulus with the wrong element tacked in front (PWP), but were more likely to respond to the latter than to the reverse combination (PW) stimulus. They also were more likely to respond to the natural-combination stimulus with the wrong beginning element (PWP) than to the single whine element (W) stimulus, but were not more likely to respond to the single whine element (W) stimulus than to the reverse-order stimulus (PW). These results thus strongly support the combinatorial rule hypothesis, and strongly oppose the beginning rule and no ordering rule hypotheses (table 2).

A potential confounding factor in these tests is that some stimuli varied not only in element combination but also in length, potentially influencing female responses because of the overall strength of the stimulation provided (e.g., the WP stimulus may have stimulated females more strongly than the single element stimuli, and the PWP stimulus may have stimulated females more strongly than the WP stimulus or the W stimulus). We consider, however, that this is not an important confounding factor in our results. First, *Enchenopa* mate preferences for signal length favor intermediate values (with the preferred length varying across species), rather than longer values (Rodríguez et al. 2004, 2006). Thus, a simple response to stronger stimulation in longer stimuli is unlikely to have an influence in our data. Further, in a crucial test (WP versus PW), the competing stimuli had the same length but varied in acceptability: this pin-points element combination as the key factor. Additionally, some non-significant comparisons involved

stimuli of different lengths (WP versus PWP, W versus PW, and WP versus W), with the pattern in one of those being of greater response to the shorter stimulus (W versus PW) (table 4; Fig. 2). We therefore conclude that any effect of stimulus length was at best secondary to the effect of signal element combinations.

Variation in female responsiveness over the two years of testing may be due to females in the second year being slightly younger than in the first year (or to additional variables arising from working with field-collected vs greenhouse-reared treehoppers; e.g., early development effects). Nevertheless, the patterns of signal acceptability that we detect were robust to those differences. We therefore interpret our results in terms of the effect of signal element composition.

Thus, our results support the combinatorial rule hypothesis and reject the competing beginning rule and no ordering rule hypotheses: signal processing in *Enchenopa* includes rules about the combination of the two elements of male signals. If these rules are conserved across the *E. binotata* complex, they may explain the highly-conserved structure of male advertisement signals in spite of remarkable signal-preference co-divergence in continuous signal features (especially dominant signal frequency; (Rodríguez et al. 2006; Cocroft et al. 2008, 2010). However, structure reversal across different species is widespread in animals that communicate with substrate-borne vibrational signals (e.g., (Cocroft 2003; Percy et al. 2006, 2008). This observation suggests that the rules that govern the combinations of signal elements that are acceptable to receivers also diverge frequently.

We might have initially expected combinatorial rules to be categorical; i.e., to render signal element combinations either acceptable or completely unacceptable. However, although stimuli with the wrong combinations considerably lowered female responsiveness, only a few

stimuli were consistently rejected by a large majority of females (Fig. 2). It will therefore be interesting to explore the nature of the relationship between combinatorial rules and preferences for continuous signal traits (such as signal frequency), and the different forms that this relationship may take across different species.

We note that our experiment may not have probed the limit of *Enchenopa* combinatorial capabilities; it remains to be tested whether they may have more sophisticated hierarchical capabilities. Some insects and spiders have far more elaborate multi-element signals than *Enchenopa*. Some jumping spiders, for instance, have multi-modal signals that rival the most elaborate displays of vertebrates in complexity (e.g., Elias, Maddison, Peckmezian, Girard, & Mason, 2012; Girard, Kasumovic, & Elias, 2011). Higher-level combinatorial processing than we have documented here may be involved in such cases.

Shared combinatorial processing capabilities across vertebrates and invertebrates most likely represents convergence, rather than common descent, as the last common ancestor of these groups likely had a simple neural system (Feinberg and Mallatt 2016). Further studies will be required to see how widespread this capability is in invertebrates. We conclude, however, with the suggestion that combinatorial processing may represent a common solution to the problems presented by complex communication in a complex world.

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ETHICS. All our procedures adhere to the legal requirements of the country in which the work was carried out, and all institutional guidelines.

DATA ACCESSABILITY. Female Simple Combinatorial Processing Data files are available as supplementary materials and in Dryad: Speck, Bretta; Rodriguez, Rafael (2020), Female Simple Combinatorial Processing , Dryad, Dataset, <https://doi.org/10.5061/dryad.r4xgxd28v>

AUTHOR CONTRIBUTIONS. BS participated in the design of the study, coordinated and carried out field and lab work, participated in data analysis, participated in statistical analysis, and drafted the manuscript; SS carried out field and lab work, and participated in data analysis. SB carried out field and lab work and participated in data analysis; SJ carried out field and lab work and participated in data analysis; CC carried out field and lab work and participated in data analysis; CD conducted the statistical analysis, and helped write the manuscript; RLR conceived of the study, designed the study, coordinated the study, carried out statistical analysis, and critically revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

COMPETING INTERESTS. We declare we have no competing interests.

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CHAPTER 2: ADAPTIVE LIFETIME VARIATION IN MATE PREFERENCES AND CHOOSINESS IN FEMALE *ENCHENOPA* TREEHOPPERS (HEMIPTERA: MEMBRACIDAE)

I. INTRODUCTION

Mate choice is an important cause of natural and sexual selection (Darwin 1871; Andersson 1994; Andersson and Simmons 2006*a*; Rosenthal 2017). Although the process of mate choice seems simple—favor some mate types over others—it creates a broad range of consequences. Mate choice may promote the evolution of extreme or elaborate ornaments and lead to rapid divergence and speciation; but it may also promote the maintenance of variation under selection; and it may facilitate or hinder local adaptation (Darwin 1871; West-Eberhard 1983*a*; Lorch et al. 2003; Coyne and Orr 2004; Greenfield and Rodriguez 2004*a*; Servedio and Bürger 2014*a*).

Variation in the consequences that arise from mate choice depends in part on within-population variation in mate preferences (Jennions and Petrie 1997; Andersson and Simmons 2006*a*; Neelon et al. 2019). For instance, the individual-level variation preferred mate types and mate preference strengths that often underlies population-level patterns may help sustain variation in ornaments (Wagner et al. 1995; Jennions and Petrie 1997; Neelon et al. 2019).

Variation in mate choice decisions may arise from changes along the course of mating seasons. As mating seasons progress, individuals age, which may bring about changes in behavior (e.g., overall levels of activity; Carey et al. 2006), as well as fecundity (Arnold and Duvall 1994). There may also be changes in the composition of available mates; e.g., when males take the initiative to seek a mate by moving about and advertising themselves, they often incur elevated

risks of predation and suffer shorter life expectancies (Andersson 1994; Kotiaho et al. 1998; Candolin 2003).

Changes in behavior and group composition along the mating season may alter ornament-mate preference relationships and result in changes in the strength and form of selection resulting from mate choice. Further, demographic changes along the mating season may place a time constraint on female mate choice decisions and put a premium on balancing the essential task of securing a mating with obtaining preferred mate types (cf. (Fowler-Finn and Rodríguez 2012a)).

Consequently, some changes in mate choice decisions over the mating season may constitute adaptive adjustments.

Here we test hypotheses regarding adaptive change in mate choice decisions along the mating season. We analyze variation in mate choice decisions in terms of two main components of the decision-making apparatus in mate choice—two main "cognitive phenotypes"(Mendelson et al. 2016; Rosenthal 2017): mate preference functions and choosiness (Jennions and Petrie 1997).

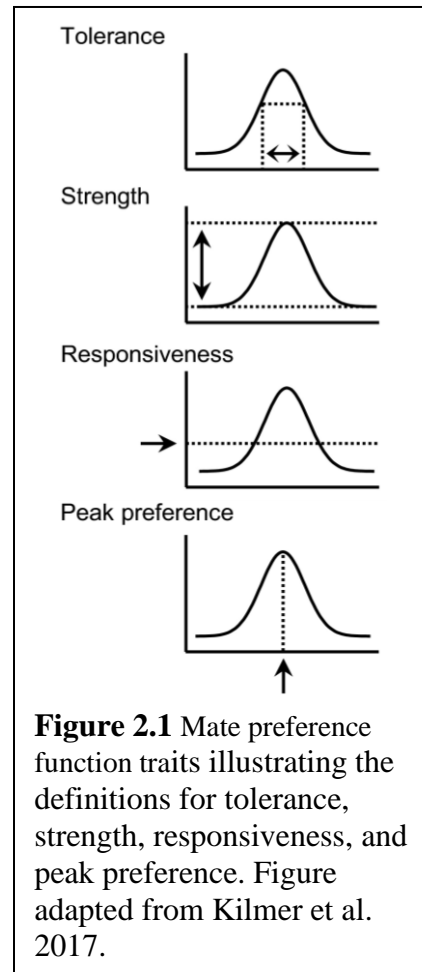
Mate preference functions relate variation in display features to variation in the attractiveness of potential mates bearing those displays (Ritchie 1996; Jennions and Petrie 1997; Wagner 1998; Kilmer et al. 2017). Describing mate preference functions at the individual level is useful for characterizing genetic and environmental causes of variation (Chenoweth and McGuigan 2010; Rodríguez et al. 2013b; Kilmer et al. 2017). Further, when described at population levels or higher, mate preference functions constitute hypotheses about the form and strength of selection on displays due to mate choice; and these hypotheses can be tested against other hypothesized

causes of selection, or used in comparative studies by assessing their relationship with the distribution of ornament (Ritchie 1996; Rodríguez et al. 2006, 2013c).

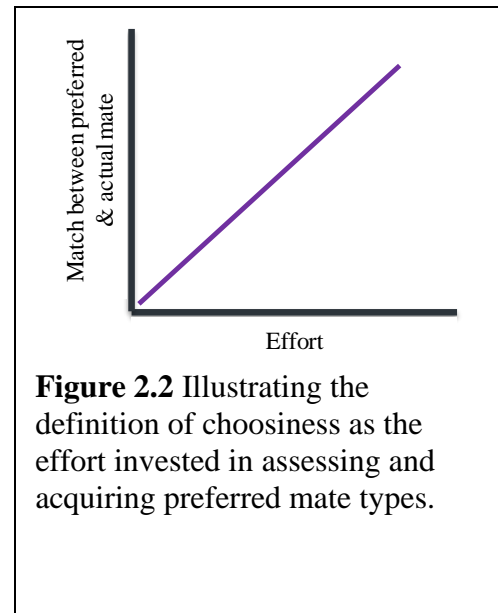
Mate preference functions can be analyzed in terms of four basic traits (Kilmer et al. 2017): “tolerance” (the range of display trait values over which the function remains relatively high),

“strength” (how steeply the function drops as displays deviate from the peak), “responsiveness” (the mean overall elevation of the function), and the “peak preference” (the most preferred display trait value)

(Figure 2.1). Here, we use this framework to ask if mate preference function traits change as adult females age. In general, and in our study species in particular, tolerance, strength and responsiveness tend to be correlated with each other and to vary independently of peak preference (Fowler-Finn and Rodríguez 2012b, 2012a; Neelon et al. 2019). Nevertheless, we analyze these traits separately because the hypotheses make different predictions for each (see below).



Choosiness is the effort invested in assessing/searching for and acquiring preferred mate types (Jennions and Petrie 1997; Kilmer et al. 2017) (Figure 2.2). Rather than influence the form of selection on displays, choosiness determines how similar a female's actual mate choice is to her preferred mate types, and hence it contributes to the strength of selection (Jennions and Petrie 1997). Females may have similar preference functions, but end up with different mates if they differ in choosiness.



There is debate on whether mate preference functions and choosiness are distinct traits or whether choosiness is a feature of the preference functions (Reinhold and Schielzeth 2014; Edward 2015; Kopp et al. 2017). However, these traits are defined as independent in principle (Jennions & Petrie 1997)—what an individual prefers, and by how much, can well differ from how hard that individual strives to obtain it. Further, there is evidence that mate preference functions and choosiness are in fact distinct traits that are influenced by different variables and vary independently (Neelon et al. 2019; Feagles & Höbel in review; Speck & Rodríguez in prep.).

The basic rationale for our hypotheses is the challenge females face to balance obtaining preferred mate types with ensuring mating. First, we test the hypothesis that mate preference functions change adaptively with age along the mating season. This hypothesis makes the

following predictions: as females age and the availability of mates decreases, there should be: (i) an increase in tolerance; (ii) a decrease in strength; and (iii) an increase in responsiveness.

Regarding peak preference, there is evidence of adaptive change in preferred mate types according to seasonal and environmental changes in which mate types are beneficial (Lesna and Sabelis 1999; Pfennig 2007; Chaine and Lyon 2008). But we know of no reason why purely demographic changes should alter the mate types that it is adaptive to mate with. However, in populations where the mean peak preference does not match the mean display—i.e., where mate preferences exert directional selection on displays, as is the case in our study species (Fowler-Finn et al. 2017)—it might be adaptive for individual females to shift their peak preferences towards the ornament mean. Consequently, we expect that: (iv) as females age there should either be no change in peak preferences or a shift towards the mean display in the population.

We also test the hypothesis that choosiness changes adaptively with age along the mating season. Previous studies claiming lifetime changes in insect “choosiness” have yielded mixed results (Moore and Moore 2001; Judge et al. 2010; Atwell and Wagner 2014; Travers et al. 2016). However, these studies have been inconsistent in their definitions of “choosiness”, with measures closer to “pickiness” or preference strength. We follow Jennions and Petrie (1997): the effort invested in assessing/searching for and securing a preferred mate type. Accordingly, we predict that (v) as females age, choosiness should increase.

One consideration for testing the above predictions is that patterns of adaptive change may not necessarily span an entire season or the entire lifespan of females. Once the available options have become very rare or absent (e.g., when very few or no males remain in the population),

there may no longer be selection favoring adaptive adjustments in mate preferences or choosiness. Further, progressively fewer females may remain as the season progresses, weakening the contribution of later stages to selection on mate choice decisions (Cotton & Day 2021). We therefore apply a temporal qualifier to the predictions (see below).

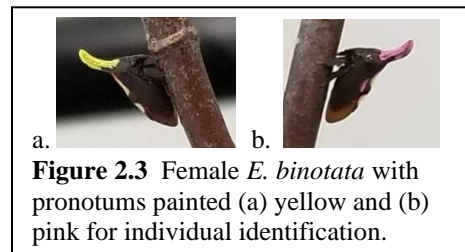
We tested the above hypotheses with a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). These phloem-feeding insects are common across the eastern United States, and our study species is native to Wisconsin and lives on *Viburnum lentago* (Adoxaceae) nannyberry trees. As many treehoppers and other plant-feeding insects, *E. binotata* communicate by sending vibrational signals through the branches or stems of their host plant (Cocroft and Rodríguez 2005; Cocroft et al. 2008). Males that are searching for mates fly from plant to plant and produce advertisement signals. Sexually receptive females that find the male's signals attractive produce a response signal and establish a duet that continues until they find each other on a tree branch and begin copulation (Rodríguez et al. 2004, 2006; Rodríguez and Cocroft 2006). Males in the *E. binotata* complex experience higher and earlier mortality than females along the mating season, and by the later summer most if not all males are dead, whereas females oviposit throughout the fall, only dying with the first frosts (Wood and Guttman 1981). Specifically, by about 6 weeks after the adult molt only 40-60% of males survive, and 1-2 weeks later, very few if any remain (Sullivan-Beckers 2008 Dissertation (Sullivan-Beckers and Cocroft 2010). Once no males remain, there can be no selection for adaptive adjustments of mate choice decisions. Consequently, our analyses below feature the full extent of female longevity, but we focus adaptive interpretation focus on the first several weeks of the mating season, when there are diminishing males but still some that remain.

METHODS

Study species and rearing

As most members of the *E. binotata* complex, our study species has not yet been formally described (Hamilton and Cocroft 2009). However, the different species in the complex can be readily distinguished by their host plant species, nymph coloration, and adult signal frequencies (Cocroft et al. 2008, 2010). Our study species was one of two that live on *Viburnum lentago* (Adoxaceae) nannyberry host plants in Wisconsin. It has distinctive nymph gray coloration and a male advertisement signal dominant frequency at ca. 165 Hz (Rodríguez et al. 2018). We kept voucher specimens in 70% EtOH.

We collected nymphs along the Oak Leaf Trail in Milwaukee, Wisconsin during the month of June in 2018, and reared them at the UWM Greenhouse on potted *V. lentago* plants. We separated males and females within 1-3 days of their final molt (late June-early July) to ensure that females did not gain experience with male signals (males begin signaling approximately 2 weeks after the adult molt), and to prevent mating after which they become unresponsive to male signals (Cocroft et al. 2008). We kept females in groups of 8-9 individuals per plant. We gave each female a unique number (1-60) and painted markings on the pronotum with using Apple Barrel quick drying acrylic paint (Figure 2.3).



Playback trials

When females became sexually receptive in late July, approx. 4 weeks after the adult molt, we assessed their mate preference functions and choosiness weekly until they died. We also weighed females weekly to the nearest 0.00X mg weekly with a microbalance (Mettler Toledo XP26; Greifensee, Switzerland).

The strongest female mate preference in the *E. binotata* complex is for the dominant frequency of male advertisement signals, which is the most distinctive feature of adult phenotypes in the complex (Rodríguez et al. 2006, 2013a; Coccoft et al. 2010). We therefore focused on varying signal frequency for the playbacks, and set all other stimulus features to the population mean (950 ms whine length, 3 pulses, 38 ms pulse length, and 21 Hz pulse rate; R.L. Rodríguez unpublished data.), with 3 signals per bout

We used vibrational playbacks to assess female mate preferences and choosiness. Our assay of response to the playbacks was based on *Enchenopa* females' selective duetting with synthetic playback stimuli of male advertisement signals. We created the playback stimuli on an iMac computer using MATLAB (v.7.5.0.338) (The Mathworks, Inc., Natick, MA, USA; www.mathworks.com) using custom-written code. We imparted the playback stimuli from an Intel Core Duo iMac computer to the stem of a playback test plant with a piezo-electric controller and actuator (Thorlabs MDT694A; Thorlabs, Newton, NJ, USA) attached to its stem with accelerometer wax. The piezo-electric stack imparts the signals as vibrations to the stem. We delivered all stimuli at a peak amplitude of 0.15 mm/s. (calibrated with a Rohde & Schwarz

HMO 1002 oscilloscope; Rohde & Schwarz, Columbia, MD, USA). Females remained within a few centimeters from the piezo-electric stack during trials. We isolated this setup from building vibrations by placing it on a ca 135-kg iron plank that rested on partially inflated bicycle inner tubes on a table. The plant was isolated from the iron plank by shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY).

Each trial began with a preliminary test to check if the female was responsive to playbacks. We presented females with a playback of a single recorded male signal with features near the population mean. If the female responded to the preliminary test, then we began the playback experiments to assess mate preferences and choosiness. If the female failed to respond for 3 minutes, we returned her to her host plant for testing the following week.

We recorded the playbacks and female responses with a laser vibrometer (Polytec PLV-100; Polytec Inc., Auburn, MA, USA). We band-pass filtered the output of the laser vibrometer (40-4000 Hz; Krohn-Hite 3202; Krohn-Hite Corp., Brockton, MA, USA) and sent it to an iMac computer through a USB audio interface (Edirol UA-25; Roland, Corp. Hamamatsu, Japan). We digitized it with a sampling rate of 44.1 kHz using the computer program AUDACITY (v.2.1.2)(www.audacityteam.org).

To describe female mate preference functions, we presented each female with 14 synthetic playbacks in random order. The playbacks ranged in frequency from 140-240 Hz. We tested each female each with two rounds of these playbacks. Each round lasted just under 5 minutes and were separated by 30 seconds.

One min after the mate preference playback rounds, we began the choosiness trials. We assessed choosiness by comparing the time a female would continue to duet with attractive (185 Hz) versus unattractive (220 Hz) playbacks. Each week, we presented each female with each of these stimuli, alternating which we presented first with each female. We presented each female with each stimulus every 20 seconds until she failed to respond for three minutes or 60 minutes had passed.

Bioacoustic analysis:

We analyzed the recordings of the playback trials with the program Audacity (v.2.1.2) (www.audacityteam.org). For preference function recordings, we tallied female responses to signals in the bout (0-3) for each playback stimuli and then averaged the responses in the two rounds for each stimulus. For choosiness recordings we measured the length of time spent duetting with the attractive (185 Hz) and unattractive stimulus (220 Hz).

Describing mate preference functions:

We described female mate preference functions with the program PFunc (Kilmer et al. 2017). PFunc uses cubic spline non-parametric regressions to generate individual preference functions from the response data. This method makes no assumptions about the shapes of functions other than that they are somewhat smooth (Kilmer et al. 2017). From each of these individual preference functions (for each female, each week), we extracted values for the following traits:

peak, tolerance, strength, and responsiveness. We then averaged these individual values to give us a weekly value for each female. We used these individual weekly values to test for changes in the preference function traits throughout the lifetime of the treehoppers.

Assessing choosiness:

We measured choosiness as the difference in the time females expend in duetting with the attractive (185 Hz) and unattractive stimulus (220 Hz). With this metric, a female with high choosiness is one that expended much more time (effort) duetting with the stimulus she prefers compared to the one she dislikes. We therefore estimated choosiness as the absolute value of the difference in duetting with the two stimuli.

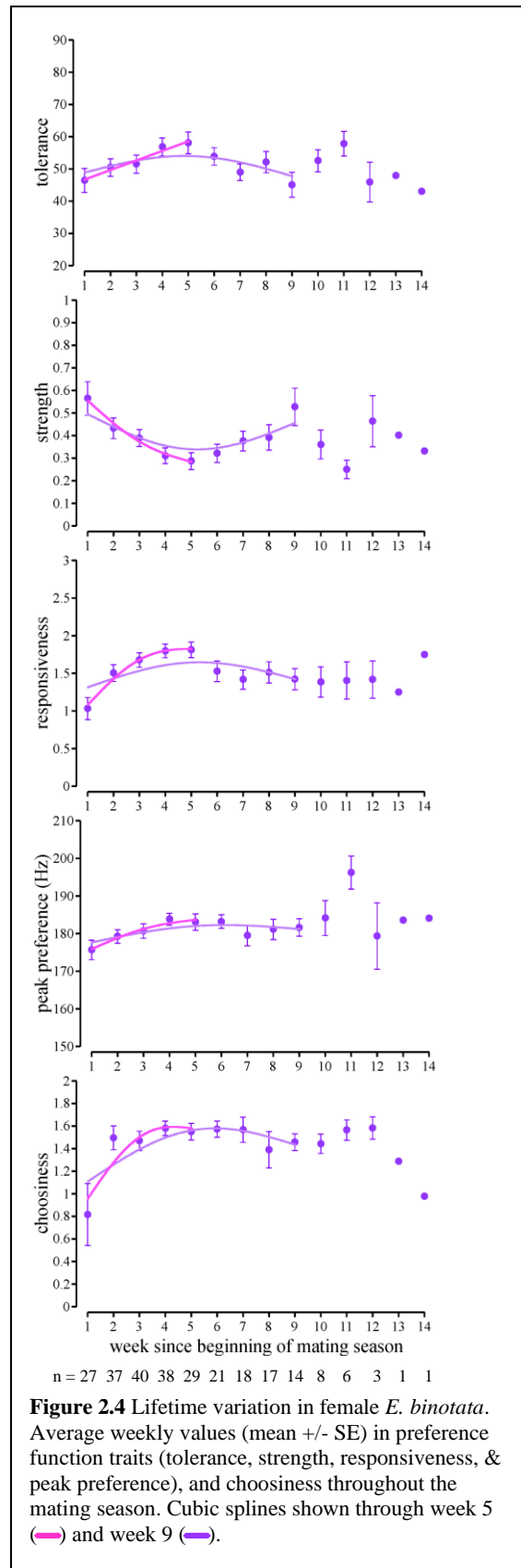
Statistical analysis:

We conducted all analyses with JMP Pro (v 16.0.0; SAS Institute). To test for change in the components of mate choice decisions over the mating season, we fit linear mixed models with each preference function trait (e.g., preference strength), choosiness, or mass as the dependent variable. We fit separate models for each of the dependent variables. The models had the following explanatory terms: linear and quadratic terms for week (with week 1 being the week of onset of sexual receptivity in the season). These terms test for linear or curvilinear change over the mating season. The model also had female identity and its interaction with the linear and quadratic week terms as random terms. The main term for female identity tests for overall

individual differences along the season (differences in individual "intercepts"); and the interaction terms test for individual differences in the patterns of change over the mating season.

As noted above, adaptive interpretation can only be applied to a portion of the entire season, because as males progressively dwindle, there comes a time when there can be no selection for adaptive adjustments of mate choice decisions.

Additionally, our sample of females also declined throughout the experiment, and not all females responded to playbacks each week. Of 60 females we marked and tested, 54 responded in at least 1 week. Over the first 9 weeks, our weekly sample sizes ranged from 14-40 females (Figure 2.4). The final 5 weeks (weeks 10-14) there were fewer females surviving, with week 12 having only 3 females and weeks 13-14 having only a single female. We therefore report the results through the first 5 and 9 weeks of the mating season, as this is the adaptively relevant portion of their reproductive lifespan.



RESULTS

We found that all the components of mate choice decisions in *Enchenopa* females varied throughout their adult reproductive lifetime.

In the analysis over the first 5 weeks, there was mainly linear increase in tolerance, responsiveness, and choosiness, and mainly linear decrease in preference strength (Figure 2.4; the linear term for week was always significant, and the quadratic term for week was less often significant; Table 2.1). We also detected a mainly linear increase in peak preference (Table 2.1; Figure 2.4).

Table 2.1 Analysis of variation in the lifetime preference function traits, choosiness, and mass in *E.binotata* treehoppers. Fixed linear and quadratic terms for age test for changes in preference traits and choosiness over the females' lifetime.

Trait	Fixed Terms	Week 1-5		Week 1-9	
		F	P	F	P
Peak Preference	Week	9.44	0.004	7.72	0.007
	Week ²	3.11	0.08	11.54	0.001
Tolerance	Week	16.86	0.0002	0.83	0.37
	Week ²	0.52	0.47	0.007	0.94
Strength	Week	23.38	<0.0001	5.43	0.03
	Week ²	0.49	0.48	11.31	0.002
Responsiveness	Week	63.00	<0.0001	19.62	0.0002
	Week ²	7.09	0.01	40.61	<0.0001
Choosiness	Week	6.47	0.01	11.57	0.0008
	Week ²	4.93	0.03	13.74	0.0003
Mass	Week	0.02	0.09	.30	.59
	Week ²	1.54	0.22	.02	.90

* Significant terms in bold.

The analysis over the first 9 weeks detected similar patterns for all traits, but with stronger curvilinearity (quadratic term for week more often significant; Table 2.1) and an inflexion point ca. weeks 5-6 (Figure 2.4).

The above changes in the mate preference function traits and choosiness were not related to changes in females' mass, as that measure did not vary throughout the season (non-significant linear and quadratic terms in Table 2.1; Figure 2.5).

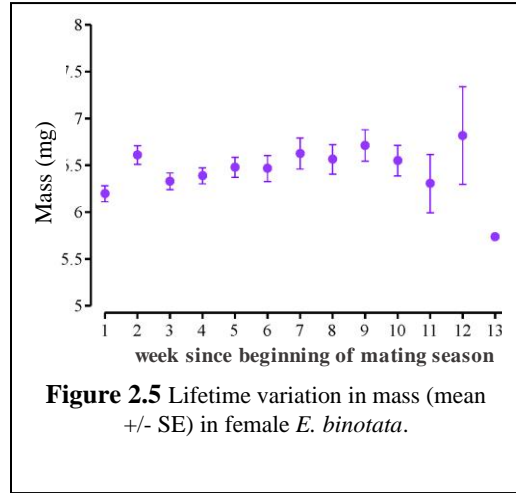


Table 2.2 Analysis of variation in the lifetime preference function traits (tolerance, strength, responsiveness, and peak), choosiness, and mass in *E. binotata* treehoppers. For random terms (Female), we report the 95% confidence interval (CI) of the variance component, Wald P-values, and the repeatability estimates obtained from the percentage variance components.

Trait	Week 1-5				Week 1-9		
	Random Terms	95% CI	Wald-P	r	95% CI	Wald-P	r
Tolerance	Female	69.69-221.80	0.0002	51.56	63.79-197.01	0.0001	32.78
	Female x Week	-5.65-21.71	0.25	2.84	-1.65-4.88	0.33	0.40
Strength	Female	0.002-0.03	0.02	24.89	0.01-1.03	0.003	21.62
	Female x Week	-0.002-0.006	0.36	2.91	-0.0003-0.002	0.13	1.31
Responsiveness	Female	0.12-0.35	<0.0001	59.05	0.12-0.33	<0.0001	53.09
	Female x Week	-0.01-0.02	0.51	1.28	-0.001-0.012	0.09	1.36
Peak	Female	18.43-80.72	0.002	39.26	21.50-64.58	<0.0001	23.40
	Female x Week	-3.60-10.07	0.35	2.56	-0.51-1.26	0.41	0.20
Choosiness	Female	-0.07-0.04	0.57	0.00	-0.02-0.07	0.33	5.83
	Female x Week	-0.01-0.06	0.15	7.49			
Mass	Female	0.13-0.32	<0.0001	65.33	0.14-0.35	<0.0001	75.99
	Female x Week	-0.01-0.004	0.35	0.00	-0.001-0.001	0.95	0.00

* Significant terms in bold.

In addition, we included random terms of individual female identity and the interaction between female and week in our model. Female identity was significant for all preference function traits and mass, through all measurements, while female identity was never significant for choosiness

(Table 2.2). The repeatabilities were highly detectable and of medium and large effect size for all values with the exception of choosiness (Table 2.2.)

DISCUSSION

We tested hypotheses regarding adaptive changes in mate preference functions and choosiness over the mating season. Working with *Enchenopa* females. We found changes in all preference function traits (peak, tolerance, strength, and responsiveness) as well as in choosiness. As adaptive adjustments cannot be expected beyond the interval when some males and females remain in the population (cf. Cotton & Day 2021), we focused on the first several weeks of the season. The pattern over the first 5 weeks provides strong support for the hypotheses—females showed the predicted increase in tolerance, responsiveness, and choosiness, and the predicted decrease in preference strength. Interestingly, further into the season, there were inflexion points that reversed the patterns, with decreases in tolerance, responsiveness, and choosiness, and increases in preference strength.

These results provide support for the hypotheses that *E.binotata* females adaptively adjust the cognitive phenotypes that regulate their of mate choice decisions in order to balance securing preferred mate types with ensuring a mating. In simple terms, as females age and males decrease, they become more tolerant of a wider variety of males, decrease their strength of preference for preferred males, and increase responsiveness to a variety of males, while increasing efforts to secure preferred mate types when present.

By contrast, the results for peak preference do not support the hypotheses. We had expected either no change or a shift towards the mean display in the population (which for our study species would mean a shift downward (Fowler-Finn et al. 2017)). However, we found mainly an increase in peak preference. We note, however, that this change in peak preference was small compared to the other traits, spanning a smaller portion of the season-long range of variation (y-axes in Figure 2.4).

Previous studies on lifetime changes in choosiness have shown contradictory results with some showing a decrease (Moore and Moore 2001; Atwell and Wagner 2014), while others show an increase (Judge et al. 2010; Travers et al. 2016). Those studies use “choosiness” to mean pickiness or strength of preference, and measure choosiness as how long it takes a female to evaluate a male before she puts forth effort to copulate. We take the approach of measuring how much effort a female puts forth to get a preferred mating (Jennions and Petrie 1997) and therefore feel that our study is distinctive from this previous work.

The changes in behavior (female mate preferences and choosiness) along with changes in group composition (declining male availability) that we observe suggest concomitant changes in the form and strength of selection on signals along the mating season. For most of the traits we measured, the patterns should weaken the strength of selection and therefore contribute to the maintenance of variation within the population (Jennions & Petrie 1997). By contrast, the changes in peak preference, albeit weak, increase the distance between the mean peak preference and the mean signal frequency in the population, thus likely leading to more directional selection.

These adaptive changes in preferences and choosiness are important for individual females to ensure a mating along with sustaining population-level variation in male signaling. This study deepens the breadth of knowledge about how mate choice decisions are made as females age and as the availability of mate options dwindle. We conclude that understanding how mate preferences and choosiness change in relation to age, advances our understanding of mate choice as a cause of selection.

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CHAPTER 3: VARIATION IN MATE CHOICE DECISIONS: INDIVIDUAL DIFFERENCES MATTER MORE THAN OPTIONS AVAILABLE IN *ENCHENOPA* TREEHOPPERS (HEMIPTERA: MEMBRACIDAE)

Abstract: The evolutionary consequences of mate choice depend on the patterns of within- and between-population variation in mate choice decisions. Two basic sources of variation may contribute to these patterns: variation in the composition of the individuals making mate choice decisions; and the composition of the options in the context of mate choice. We compared the relative contribution of these two aspects with a member of the *Enchenopa binotata* species complex of treehoppers—insects that communicate with plant-borne vibrational signals, and in which females express their mate preferences with selective duetting with males. We used vibrational playbacks to manipulate the immediate social context of mate choice with treatments consisting of an initial presentation of attractive/unattractive stimuli, followed by focal intermediately attractive/unattractive stimuli, and we monitored females' responses with laser vibrometry. We found considerable individual differences in preferred mate types, but no effect of our manipulation of the immediate social context. Either of these factors may help maintain variation under selection due to mate choice, and we conclude that comparative work analyzing their relative impact will help understand the evolutionary consequences of mate choice.

KEY WORDS

Enchenopa binotata, individual variation, mate choice, plasticity, social context, vibrational signals

INTRODUCTION

In evolution, interactions between individuals influence the patterns of variation in the traits under selection, as well as the form and strength of selection (Moore et al. 1997; Bailey et al. 2018; Rodríguez et al. 2019). In mate choice, for instance, evolution is influenced by two kinds of feedback. There are feedbacks between the causes of variation in traits and the traits on which variation is induced—an individual's behavior may vary according to the other individuals in the population, as well as induce variation in those other individuals' behavior (Moore et al. 1997; Hebets and Sullivan-Beckers 2010; Verzijden et al. 2012; Rodríguez et al. 2013*b*; Bailey et al. 2018). And there are coevolutionary feedbacks between the traits under selection (e.g., courtship displays) and the causes of selection—the selective environment constituted by the displays and mate preferences of the other individuals in the population changes with the targets of selection (West-Eberhard 1983, 2014).

Analysis of individual variation in mate choice decisions brings insight into the evolution and evolutionary consequences of mate choice (Jennions and Petrie 1997; Andersson and Simmons 2006; Rodríguez et al. 2013*b*; Mendelson et al. 2016; Neelon et al. 2019). This includes explaining the range of outcomes that can arise from mate choice—from whether it sustains within-population variation to promote divergence, and whether variation in mate choice represents adaptive adjustments according to changing benefits (Jennions and Petrie 1997; Lesna and Sabelis 1999; Chaine and Lyon 2008; Hebets and Sullivan-Beckers 2010; Verzijden et al. 2012; Rodríguez et al. 2013*b*).

Here we focus on the immediate context of mate choice to analyze variation in mate choice decisions in terms of two potential causes: (i) differences between choosing individuals;

and (ii) differences in the options available for choice. Even at such a seemingly narrow scale, these causes of variation may have important consequences. Individual differences arise from genetic and developmental-social inputs (West-Eberhard 2003, 2005; Nussey et al. 2007; Chenoweth and McGuigan 2010; Dingemanse et al. 2010; Hebets and Sullivan-Beckers 2010; Verzijden et al. 2012; Rodríguez et al. 2013*b*). Such individual variation may help explain the maintenance of variation under selection due to mate choice. For instance, the form and strength of selection suggested by population-level analysis is often revealed, by examination of individual differences in preferences, to allow for success for a broader range of mate types than would be expected otherwise (e.g., (Wagner et al. 1995; Hedrick and Weber 1998; Murphy and Gerhardt 2000; Neelon et al. 2019)). Differences in the options available at the time of mate choice may also have drastic effects, leading in the extreme to preference reversals (Kirkpatrick et al. 2006; Regenwetter et al. 2021).

As causes of variation in mate choice decisions, the two factors that we test (differences between choosing individuals and in the options available for choice) are not exclusive; both could be important. Our aim was therefore to assess their relative magnitude. Accordingly, we tested predictions that most variation in preferred mate types will be due to differences between choosing individuals or to the available options, respectively.

We tested these hypotheses with a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Enchenopa* are plant-feeding insects that communicate with plant-borne vibrational signals (Cocroft and Rodríguez 2005; Cocroft et al. 2008; Hill 2008; Rodríguez RL & Desjonquères D. 2019). Prior work with our study species has found support for genetic and developmental-social inputs that underlie individual differences in mate choice decisions. This includes broad-sense heritability in preferred mate preferences

(Rodríguez et al. 2013a); as well as developmental and social plasticity in mate preferences arising from inputs such as the composition of juvenile and young adult social groupings (Fowler-Finn and Rodríguez 2012a, 2012b; Rebar and Rodríguez 2013; Rodríguez et al. 2013b; Fowler-Finn et al. 2017; Desjonquères et al. 2019a, 2019b, 2021) Desjonquères et al. in review).

We used a playback experiment in which we presented females with treatment male signal stimuli to set the social context. Treatments consisted of highly attractive or unattractive stimuli at peak and bottom positions on the population-level mate preference function for our study species (see below). We then presented females with relatively attractive and unattractive focal stimuli and assessed whether the treatment stimuli altered the response to the focal stimuli. We compared this effect against individual differences in females' preferred mate types.

In prior work we have focused on variation in mate preference functions, which are assessed with multiple playback stimuli spanning a range of signal trait values (Kilmer et al. 2017). In the current experiment, the effect of our treatment could take the form of a "sequence effect". To avoid this potential confound, we used only two focal stimuli to test for the predicted effects of differences between choosing individuals and the options available on preferred signal values, rather than use full preference functions.

MATERIALS AND METHODS

Study species and collecting

Most members of the *E. binotata* complex have not been formally described (Hamilton and Coccoft 2009). However, they can be readily distinguished by their host plant species, nymph coloration, and adult signal frequencies (Coccoft et al. 2008). Our study species is one of

the two that live on *Viburnum lentago* (Adoxaceae) nannyberry trees in Wisconsin. It is distinguished by the grey coloration of the nymphs, and the ca. 165 Hz dominant frequency of the adult male signals (as opposed to black-and-white nymph coloration and ca. 315 Hz male signals for the other species)(Rodríguez et al. 2018).

In early June of 2020, we collected 2nd and 3rd instar nymphs from the Oak Leaf Trail along the Milwaukee River, near the UWM campus. We reared them on potted *V. lentago* plants in the UWM Greenhouse. Within 1-4 days of molting to adulthood, we separated males and females onto different rearing plants to ensure that the females did not gain experience with male signals (males begin signaling ca. 2 weeks after the adult molt), and that they did not mate, after which they become sexually unreceptive and unresponsive to male signals (Cocroft et al. 2008). When females reached reproductive maturity (based on the onset of responsiveness to male signals), we began our playback experiment (ca. 5 weeks post adult molt).

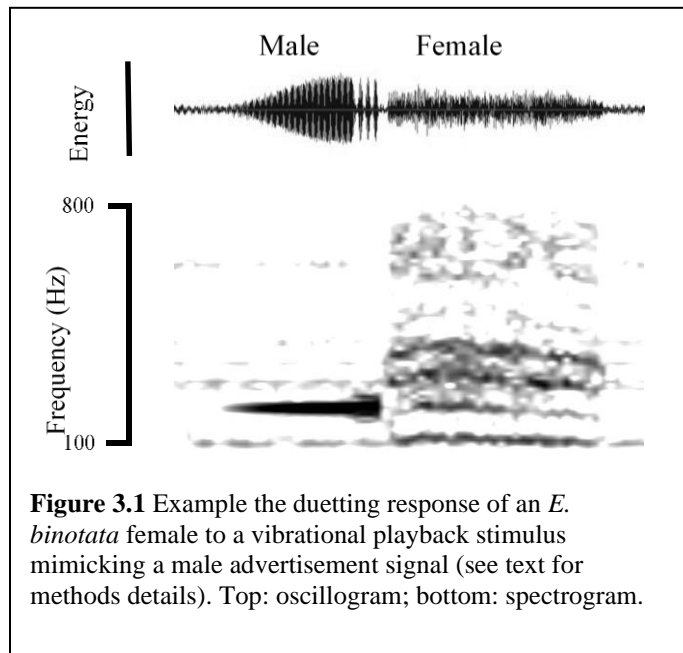
Playback experiment

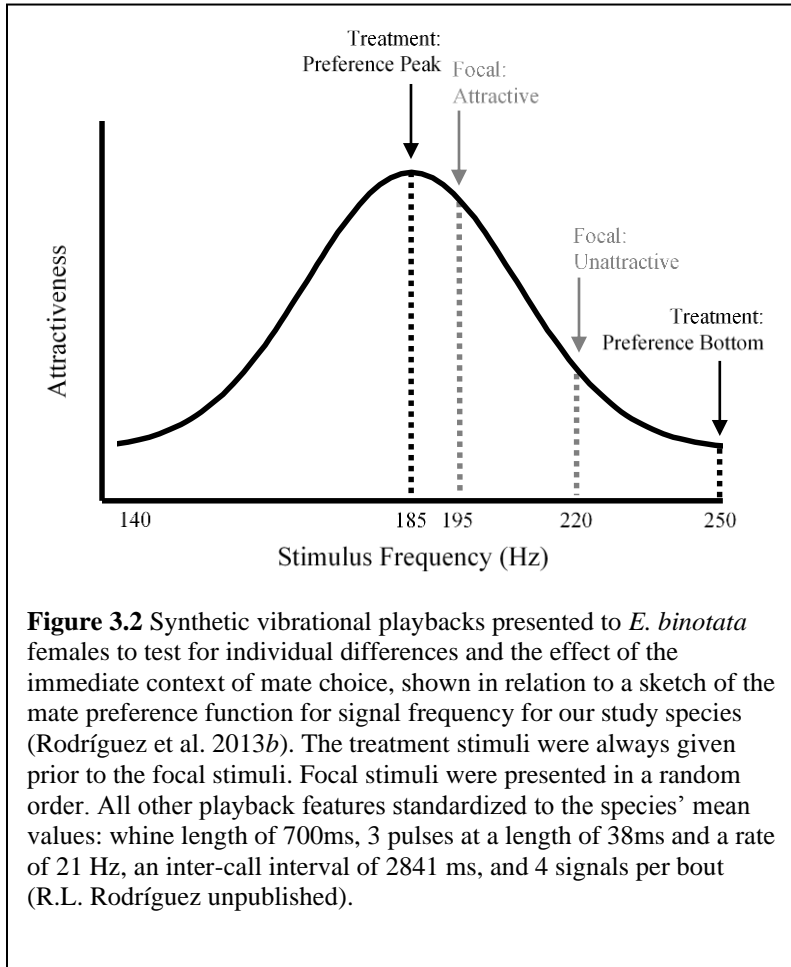
Enchenopa males in search of a mate fly from plant to plant producing vibrational advertisement signals. Receptive females that find a male's signals attractive produce response signals and establish a duet that continues until the males finds them and copulation begins (Cocroft et al. 2008) (Figure 1). Females express their mate preferences by selective duetting with males (Rodríguez et al. 2004, 2006; Cocroft et al. 2008). We therefore used female duetting

behavior—the number of responses to signals in the stimulus bouts (0-4)—as our assay of preference.

The strongest female mate preferences in the *E. binotata* complex are for the dominant frequency of male advertisement signals, and this signal feature is the most distinctive aspect of the phenotype of the adults in the complex (Rodríguez et al. 2004, 2006;

Cocroft et al. 2008, 2010). We thus used stimuli differing in frequency (each stimulus having a single frequency, consisting of an amplitude-modulated sine wave to simulate the whine-pulses structure of male signals). We created the vibrational playback stimuli using custom-written code in MATLAB (v. 9.4.0) (The Mathworks, Inc., Natick, MA, USA; www.mathworks.com). We used two treatment stimuli (attractive and unattractive, at the peak and bottom of the preference function, respectively) to set the social context, and two focal stimuli (relatively attractive and relatively unattractive) to assess for a shift in preference—prior work with our study species has found a population mean for the female preferred signal frequency values at 185 Hz, with some responsiveness sustained over approximately 140-250 Hz (Fowler-Finn and Rodríguez 2012b, 2012a; Rodríguez et al. 2013a) (Figure 2).





In each trial, we placed females individually on a potted *V. lentago* playback plant. We used the same plant for all trials to avoid any potential (albeit unlikely; (Cocroft et al. 2006)) effects of differences in plant transmission features. The plant was surrounded on three sides by a mesh tent to hinder the treehopper from escaping. We allowed each female to find a place to settle before the start of the experiment (minimum 30

seconds).

We administered the vibrational playbacks at an amplitude of 0.15 mm/s (calibrated with a Rohde & Schwarz HMO 1002 oscilloscope; Rohde & Schwarz, Columbia, MD, USA). We sent the signals from an Intel Core Duo iMac computer using MATLAB to a piezo-electric stack attached to the stem of the plant, driven by a piezo-controller (Thorlabs MDT694A; Thorlabs, Newton, NJ, USA.). The piezo-electric stack imparts the signals as vibrations to the stem. We coupled the piezo-electric stack to the stem of the playback plant (ca. 3 mm-wide) with accelerometer wax. Females remained within a few centimeters from the piezo-electric stack during trials. We isolated this setup from building vibrations by placing it on a ca 135-kg iron

plank that rested on partially inflated bicycle inner tubes on a table. The plant was isolated from the iron plank by shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY).

We randomly assigned each female to one of three treatments: control (no initial treatment stimulus); a single bout of the attractive treatment stimulus (corresponding to the peak of the preference function); or a single bout of the unattractive treatment stimulus (corresponding to the bottom of the preference function) (Figure 2). We presented the treatment stimuli first to establish the social context. We then presented both the attractive and unattractive focal stimuli to each female in random sequence (a single bout of each). Thus, we presented each female with one treatment stimulus and two focal stimuli. In each trial, treatment and focal stimulus bouts were separated from each other by 15 seconds.

We recorded female duetting responses to the treatment and focal playbacks using a portable laser Doppler vibrometer (Polytec PLV-100; Polytec Inc. Auburn, MA, U.S.A.). We focused the laser beam on a piece of reflective tape (approx. 5 mm²) adhered to the stem of the recording plant, 2 cm from the piezo-electric stack. We sent the laser signal through a band-pass filter (40-4000 Hz; Krohn-Hite 3202; Krohn- Hite Corp., Brockton, MA, U.S.A.) to an iMac computer through a USB audio interface (Edirol UA-25; Roland, Corp. Hamamatsu, Japan). We recorded the female signals with the program Audacity (v.2.1.3 <http://audacity.sourceforge.net/>) at a sampling rate of 44.1 Hz. We tested 50 females per treatment (initial total sample: $n = 150$).

Statistical Analysis

We conducted all analyses with JMP (v. 15.2.1; SAS Institute). To confirm that the treatment attractive and unattractive stimuli were in fact favored and disfavored, respectively, we

used a linear model with female response (0-4) as the dependent variable, and treatment stimulus as the explanatory term.

To ask whether female response to the focal stimuli varied with treatment stimuli, we fit a linear mixed model with female response (0-4) as the dependent variable, and the following explanatory variables: treatment and focal stimuli, and their interaction. The main terms test for differences in overall response across treatment and focal stimuli; the treatment \times focal stimulus interaction tests for changes in preferred signal values due to treatment.

To ask about individual variation, we also included in the above linear mixed model random terms for female identity and its interaction with focal stimulus. The female identity term tests for individual differences in overall response levels (responsiveness; (Kilmer et al. 2017)). The variance component of this term provides an estimate of short-term repeatability (consistency of individual differences) in responsiveness (Bell et al. 2009; Nakagawa and Schielzeth 2010). The female identity \times focal stimulus interaction tests for individual differences in preferred signal values.

To assess the relative magnitude of the potential effects of the hypotheses, we complemented significance testing with effect size estimates. We used the metric r , whereby $r < 0.3$ corresponds to small effect sizes; $0.3 < r < 0.5$ to medium effects; and $r > 0.5$ to large effects (Nakagawa and Cuthill 2007). To help interpret cases where we detect no effect through significance testing, we also calculated the statistical power ($1 - \beta$) of our tests, whereby $1 - \beta > 0.80$ corresponds to adequate power (Nakagawa and Cuthill 2007).

We only included in our analyses females that responded to at least one of the focal stimuli, as females that responded to neither provided no information regarding their preferred signal values. The final total sample size for the analysis was $n = 107$ (Figure 3).

RESULTS

Female responses to the treatment stimuli confirmed that the attractive treatment playback was strongly preferred over the unattractive treatment playback ($F_{1,98} = 49.42$, $P < 0.0001$; Fig. 3A). Females also overall preferred the focal relatively attractive stimulus over the focal relatively unattractive stimulus (significant focal stimulus term: Table 1; Figure 3B).

Table 3.1 Analysis of variation in female responses to relatively attractive and unattractive focal stimuli according to treatments (attractive versus unattractive) that alter the immediate social context of mate choice.

Term	df	<i>F</i>	<i>P</i>
treatment	2, 104	0.68	0.51
focal stimulus	1, 104	13.25	0.0004
treatment x focal stimulus	2, 104	0.23	0.80
Random Terms	95% confidence interval of variance component	Wald <i>P</i>	<i>r</i>
female	0.30-1.23	0.0013	0.33
female × focal stimulus	1.20-2.07	< 0.0001	0.67

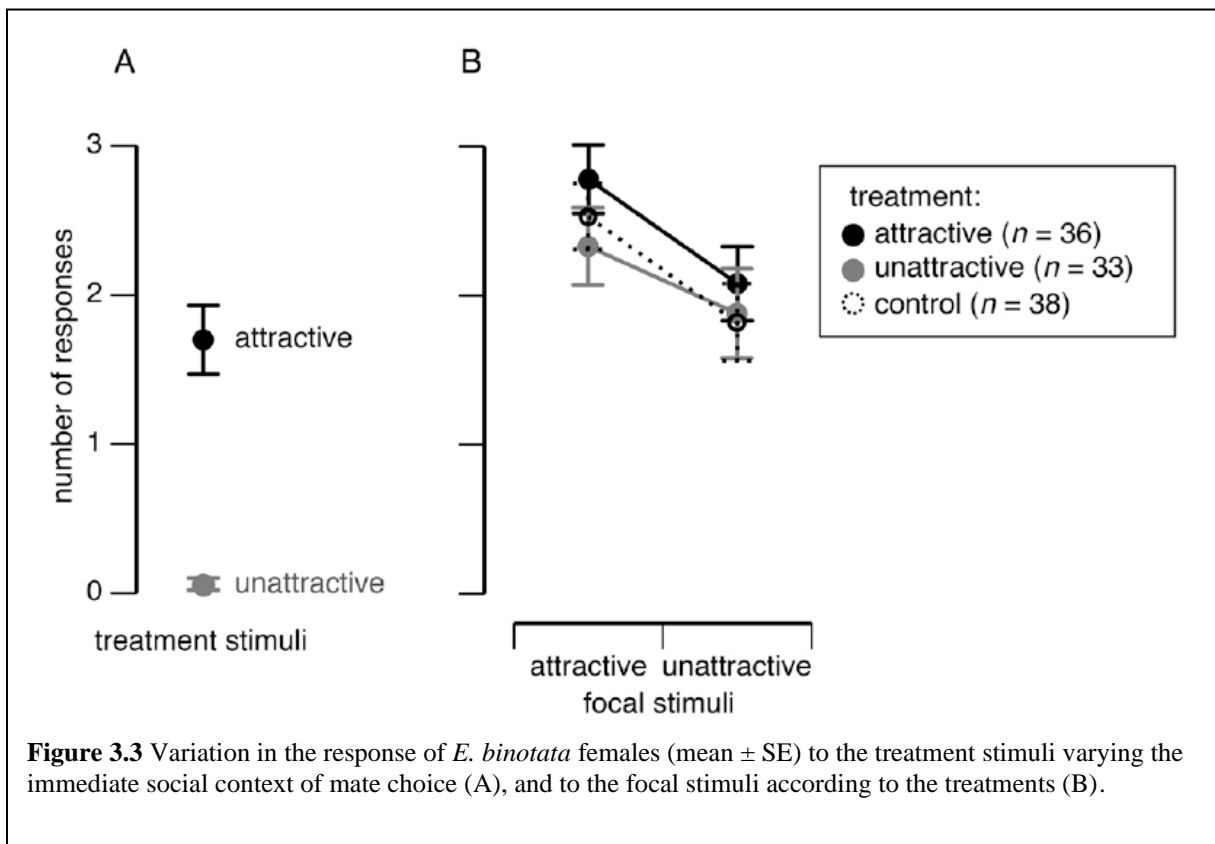
Note: For these terms, we report *F*-ratio tests from the model (see Statistical Analysis for details). The random terms assess individual female differences in overall responsiveness (main female identity term) and in preferred signal values (female × focal stimulus interaction term). For these random terms, we report the 95% confidence interval of the variance component, Wald *P*-values, and effect size (*r*) estimates obtained from the percentage variance components in the model (see Statistical Analysis for details).

*Significant terms in bold.

There was an indication of a treatment effect on responses to the focal stimuli—female responsiveness was higher overall, and there was stronger attractive-unattractive focal stimulus distinction, with the attractive rather than the unattractive treatment (Figure 3B). However, those effects were not detectable (the treatment and treatment × focal stimulus interaction terms were not significant; Table 1), and they were very small—e.g., for the between-treatment difference in

the response to the attractive focal stimulus (Figure 3), the effect size was $r = 0.09$. Thus, there was no indication of a change in preferred signal values according to the immediate social context of mate choice. With $n = 33-38$ per treatment and the observed overall variation in female response (Figure 3B), this test had adequate power ($1 - \beta > 0.80$) to detect small effect size differences ($r \geq 0.21$; i.e, an overall difference of ≥ 0.6 responses between stimuli), and encompasses what we would consider biologically relevant.

By contrast, individual differences in overall responsiveness and in preferred signal



values were highly detectable (significant individual and individual \times focal stimulus interaction terms; Table 1), and of medium and large effect size, respectively (r estimates in Table 1).

DISCUSSION

We analyzed variation in female mate choice decisions in a member of the *E. binotata* complex of treehoppers in terms of two aspects of the immediate context of mate choice: differences between females, and differences in the options available to those females. We found that the former (differences between females) contributed much more strongly to variation in preferred mate types than the latter (the options available). Indeed, the largest effect size we detected corresponds to individual females differences in preferred mate types.

Our results suggest that the evolutionary consequences of variation in mate choice decisions, in terms of the types of mates that are favored, may depend more strongly on the composition of *females* in the population—of the individuals exerting mate choice (Jennions and Petrie 1997; Neelon et al. 2019). Specifically, although at the population level there is an overall preference for a given mate type, there are many individual females that would accept the less-preferred type. Such underlying individual variation in mate choice decisions may contribute to the maintenance of variation under selection due to mate choice.

The maintenance of variation under selection due to mate choice could also result from effects of the composition of the options available at the time of mate choice (e.g., if there arise preference reversals; (Kirkpatrick et al. 2006; Regenwetter et al. 2021)). We did not find support for this hypothesis. It would be interesting, however, to ask whether there are individual differences in the response to such potential effects (cf. (Dingemanse et al. 2010; Stamps and Groothuis 2010; Dingemanse and Wolf 2013; Regenwetter et al. 2021), and what patterns of variation in mate choice decisions may arise from such differences.

We conclude by emphasizing that complex ecological and social environments constitute not only problems for animal decision making, but also causes of variation of evolutionary importance. Our understanding of the selection that arises from animal decision making will improve by consideration of the patterns of individual variation that subtend population-level patterns (Jennions and Petrie 1997; Moore et al. 1997; Dingemanse and Wolf 2013; Rodríguez et al. 2013*b*, 2019; Bailey et al. 2018; Neelon et al. 2019).

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Author Contributions

BS and RLR designed the study. BS coordinated and carried out field and lab work, and carried out bioacoustic analysis. SS helped with field and lab work. BS and RLR conducted statistical analysis and wrote the manuscript. All authors contributed to the manuscript, have given approval for publication, and agree to be held accountable for the work performed therein.

Data availability

Our data is available in Appendix C.

Competing Interests

We declare we have no competing interests.

Ethics

All our procedures adhere to the legal requirements of the country in which the work was carried out and all institutional guidelines.

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Appendix A: Raw Data for Chapter 1 Combinatorial Signal Processing in an Insect

FEMALE COMBINATORIAL PROCESSING DATA SHEET						
Species: <i>Enchenopa binotata</i> ' <i>Viburnum lentago</i> ' low frequency						
Population location: UWM Downer Woods						
Researchers: Speck (2016-2017), Rodriguez (2016-2017), Belo (2016), Johnson (2016), Conley (2017), Seidita (2017)						
		Year	Rearing Method		Response	
Recording	Female #	2016 or 2017	Natural or Jump started	Stimulus	1 or 0	Length
2EbLentWILoFreqFemPhonF1	1	2016	N	P	0	
2EbLentWILoFreqFemPhonF2	2	2016	N	W	1	0.2311
2EbLentWILoFreqFemPhonF3	3	2016	N	PW	0	
2EbLentWILoFreqFemPhonF4	4	2016	N	WP	0	
2EbLentWILoFreqFemPhonF5	5	2016	N	PWP	0	
2EbLentWILoFreqFemPhonF6	6	2016	N	PW	0	
2EbLentWILoFreqFemPhonF7	7	2016	N	P	0	
2EbLentWILoFreqFemPhonF8	8	2016	N	PWP	1	0.9937
2EbLentWILoFreqFemPhonF9	9	2016	N	W	0	
2EbLentWILoFreqFemPhonF10	10	2016	N	WP	0	
2EbLentWILoFreqFemPhonF11	11	2016	N	P	0	
2EbLentWILoFreqFemPhonF12	12	2016	N	WP	1	0.9847
2EbLentWILoFreqFemPhonF13	13	2016	N	PW	0	
2EbLentWILoFreqFemPhonF14	14	2016	N	W	1	1.2321
2EbLentWILoFreqFemPhonF15	15	2016	N	PWP	0	
2EbLentWILoFreqFemPhonF16	16	2016	N	WP	0	
2EbLentWILoFreqFemPhonF17	17	2016	N	PW	1	8.2914
2EbLentWILoFreqFemPhonF18	18	2016	N	P	0	
2EbLentWILoFreqFemPhonF19	19	2016	N	PWP	1	0.6148
2EbLentWILoFreqFemPhonF20	20	2016	N	W	0	
2EbLentWILoFreqFemPhonF21	21	2016	N	PWP	1	0.9416
2EbLentWILoFreqFemPhonF22	22	2016	N	W	0	
2EbLentWILoFreqFemPhonF23	23	2016	N	P	0	
2EbLentWILoFreqFemPhonF24	24	2016	N	PW	1	0.6666
2EbLentWILoFreqFemPhonF25	25	2016	N	WP	1	1.2179
2EbLentWILoFreqFemPhonF26	26	2016	N	PWP	1	0.4579
2EbLentWILoFreqFemPhonF27	27	2016	N	W	1	0.8053
2EbLentWILoFreqFemPhonF28	28	2016	N	P	0	
2EbLentWILoFreqFemPhonF29	29	2016	N	PW	0	
2EbLentWILoFreqFemPhonF30	30	2016	N	WP	0	
2EbLentWILoFreqFemPhonF31	31	2016	N	W	0	
2EbLentWILoFreqFemPhonF32	32	2016	N	PWP	0	

2EbLentWILoFreqFemPhonF33	33	2016	N	WP	1	1.029
2EbLentWILoFreqFemPhonF34	34	2016	N	PW	0	
2EbLentWILoFreqFemPhonF35	35	2016	N	P	0	
2EbLentWILoFreqFemPhonF36	36	2016	N	W	0	
2EbLentWILoFreqFemPhonF37	37	2016	N	WP	1	0.5911
2EbLentWILoFreqFemPhonF38	38	2016	N	P	0	
2EbLentWILoFreqFemPhonF39	39	2016	N	PWP	1	0.9598
2EbLentWILoFreqFemPhonF40	40	2016	N	PW	1	1.0101
2EbLentWILoFreqFemPhonF41	41	2016	N	P	0	
2EbLentWILoFreqFemPhonF42	42	2016	N	W	1	0.9867
2EbLentWILoFreqFemPhonF43	43	2016	N	PWP	0	
2EbLentWILoFreqFemPhonF44	44	2016	N	PW	1	0.8447
2EbLentWILoFreqFemPhonF45	45	2016	N	WP	1	1.089
2EbLentWILoFreqFemPhonF46	46	2016	N	PWP	1	1.3072
2EbLentWILoFreqFemPhonF47	47	2016	N	P	0	
2EbLentWILoFreqFemPhonF48	48	2016	N	W	1	1.3504
2EbLentWILoFreqFemPhonF49	49	2016	N	PW	0	
2EbLentWILoFreqFemPhonF50	50	2016	N	WP	1	1.2633
2EbLentWILoFreqFemPhonF51	51	2016	N	PW	0	
2EbLentWILoFreqFemPhonF52	52	2016	N	P	0	
2EbLentWILoFreqFemPhonF53	53	2016	N	W	0	
2EbLentWILoFreqFemPhonF54	54	2016	N	PWP	1	0.7779
2EbLentWILoFreqFemPhonF55	55	2016	N	WP	0	
2EbLentWILoFreqFemPhonF56	56	2016	N	P	0	
2EbLentWILoFreqFemPhonF57	57	2016	N	W	1	0.811
2EbLentWILoFreqFemPhonF58	58	2016	N	PW	0	
2EbLentWILoFreqFemPhonF59	59	2016	N	WP	1	1.1887
2EbLentWILoFreqFemPhonF60	60	2016	N	PWP	1	1.037
2EbLentWILoFreqFemPhonF61	61	2016	N	PW	0	
2EbLentWILoFreqFemPhonF62	62	2016	N	P	0	
2EbLentWILoFreqFemPhonF63	63	2016	N	PWP	1	1.0522
2EbLentWILoFreqFemPhonF64	64	2016	N	W	1	0.9493
2EbLentWILoFreqFemPhonF65	65	2016	N	WP	1	1.3504
2EbLentWILoFreqFemPhonF66	66	2016	N	P	0	
2EbLentWILoFreqFemPhonF67	67	2016	N	WP	1	1.0195
2EbLentWILoFreqFemPhonF68	68	2016	N	PW	1	0.5977
2EbLentWILoFreqFemPhonF69	69	2016	N	W	0	
2EbLentWILoFreqFemPhonF70	70	2016	N	PWP	1	1.1771
2EbLentWILoFreqFemPhonF71	71	2016	N	WP	1	0.4108
2EbLentWILoFreqFemPhonF72	72	2016	N	PW	0	
2EbLentWILoFreqFemPhonF73	73	2016	N	P	0	

2EbLentWILoFreqFemPhonF74	74	2016	N	PWP	1	0.9996
2EbLentWILoFreqFemPhonF75	75	2016	N	W	1	1.0796
2EbLentWILoFreqFemPhonF76	76	2016	N	P	0	
2EbLentWILoFreqFemPhonF77	77	2016	N	W	0	
2EbLentWILoFreqFemPhonF78	78	2016	N	PWP	1	0.3298
2EbLentWILoFreqFemPhonF79	79	2016	N	PW	0	
2EbLentWILoFreqFemPhonF80	80	2016	N	WP	1	0.6519
2EbLentWILoFreqFemPhonF81	81	2016	N	PWP	0	
2EbLentWILoFreqFemPhonF82	82	2016	N	P	0	
2EbLentWILoFreqFemPhonF83	83	2016	N	W	0	
2EbLentWILoFreqFemPhonF84	84	2016	N	PW	1	0.896
2EbLentWILoFreqFemPhonF85	85	2016	N	WP	1	1.2983
2EbLentWILoFreqFemPhonF86	86	2016	N	PWP	1	1.26
2EbLentWILoFreqFemPhonF87	87	2016	N	W	1	1.0423
2EbLentWILoFreqFemPhonF88	88	2016	N	P	0	
2EbLentWILoFreqFemPhonF89	89	2016	N	PW	1	0.6112
2EbLentWILoFreqFemPhonF90	90	2016	N	WP	0	
2EbLentWILoFreqFemPhonF91	91	2016	N	W	1	1.1958
2EbLentWILoFreqFemPhonF92	92	2016	N	PWP	0	
2EbLentWILoFreqFemPhonF93	93	2016	N	WP	0	
2EbLentWILoFreqFemPhonF94	94	2016	N	WP	1	0.2032
2EbLentWILoFreqFemPhonF95	95	2016	N	PW	0	
2EbLentWILoFreqFemPhonF96	96	2016	N	PWP	1	1.0172
2EbLentWILoFreqFemPhonF97	97	2016	N	P	0	
2EbLentWILoFreqFemPhonF98	98	2016	N	W	1	1.5822
2EbLentWILoFreqFemPhonF99	99	2016	N	P	0	
2EbLentWILoFreqFemPhonF100	100	2016	N	PW	0	
EbLentWILoFreqFemPhon101	101	2017	JS	WP	1	1.2033
EbLentWILoFreqFemPhon102	102	2017	JS	P	0	
EbLentWILoFreqFemPhon103	103	2017	JS	PWP	1	1.0227
EbLentWILoFreqFemPhon104	104	2017	JS	W	0	
EbLentWILoFreqFemPhon105	105	2017	JS	PW	0	
EbLentWILoFreqFemPhon106	106	2017	JS	PWP	1	1.2251
EbLentWILoFreqFemPhon107	107	2017	JS	W	0	
EbLentWILoFreqFemPhon108	108	2017	JS	P	0	
EbLentWILoFreqFemPhon109	109	2017	JS	PW	0	
EbLentWILoFreqFemPhon110	110	2017	JS	WP	1	1.1991
EbLentWILoFreqFemPhon111	111	2017	JS	PWP	0	
EbLentWILoFreqFemPhon112	112	2017	JS	WP	0	
EbLentWILoFreqFemPhon113	113	2017	JS	W	0	
EbLentWILoFreqFemPhon114	114	2017	JS	P	0	

EbLentWILoFreqFemPhon115	115	2017	JS	PWP	1	0.0867
EbLentWILoFreqFemPhon116	116	2017	JS	PW	0	
EbLentWILoFreqFemPhon117	117	2017	JS	P	0	
EbLentWILoFreqFemPhon118	118	2017	JS	WP	0	
EbLentWILoFreqFemPhon119	119	2017	JS	W	0	
EbLentWILoFreqFemPhon120	120	2017	JS	PW	0	
EbLentWILoFreqFemPhon121	121	2017	JS	W	0	
EbLentWILoFreqFemPhon122	122	2017	JS	WP	0	
EbLentWILoFreqFemPhon123	123	2017	JS	WP	0	
EbLentWILoFreqFemPhon124	124	2017	JS	PW	0	
EbLentWILoFreqFemPhon125	125	2017	JS	PWP	1	1.0424
EbLentWILoFreqFemPhon126	126	2017	JS	P	0	
EbLentWILoFreqFemPhon127	127	2017	JS	PW	0	
EbLentWILoFreqFemPhon128	128	2017	JS	PWP	1	0.762
EbLentWILoFreqFemPhon129	129	2017	JS	P	0	
EbLentWILoFreqFemPhon130	130	2017	JS	P	0	
EbLentWILoFreqFemPhon131	131	2017	JS	WP	0	
EbLentWILoFreqFemPhon132	132	2017	JS	PWP	0	
EbLentWILoFreqFemPhon133	133	2017	JS	W	1	0.5859
EbLentWILoFreqFemPhon134	134	2017	JS	PW	0	
EbLentWILoFreqFemPhon135	135	2017	JS	PWP	0	
EbLentWILoFreqFemPhon136	136	2017	JS	W	0	
EbLentWILoFreqFemPhon137	137	2017	JS	P	0	
EbLentWILoFreqFemPhon138	138	2017	JS	PW	0	
EbLentWILoFreqFemPhon139	139	2017	JS	WP	1	0.5087
EbLentWILoFreqFemPhon140	140	2017	JS	PWP	1	0.8797
EbLentWILoFreqFemPhon141	141	2017	JS	WP	1	0.4174
EbLentWILoFreqFemPhon142	142	2017	JS	W	0	
EbLentWILoFreqFemPhon143	143	2017	JS	P	0	
EbLentWILoFreqFemPhon144	144	2017	JS	PWP	0	
EbLentWILoFreqFemPhon145	145	2017	JS	PW	0	
EbLentWILoFreqFemPhon146	146	2017	JS	P	0	
EbLentWILoFreqFemPhon147	147	2017	JS	WP	0	
EbLentWILoFreqFemPhon148	148	2017	JS	W	0	
EbLentWILoFreqFemPhon149	149	2017	JS	PW	0	
EbLentWILoFreqFemPhon150	150	2017	JS	W	0	
EbLentWILoFreqFemPhon151	151	2017	JS	WP	0	
EbLentWILoFreqFemPhon152	152	2017	JS	WP	0	
EbLentWILoFreqFemPhon153	153	2017	JS	PWP	1	1.0237
EbLentWILoFreqFemPhon154	154	2017	JS	WP	0	
EbLentWILoFreqFemPhon155	155	2017	JS	W	0	

EbLentWILoFreqFemPhon156	156	2017	JS	P	0	
EbLentWILoFreqFemPhon157	157	2017	JS	PWP	0	
EbLentWILoFreqFemPhon158	158	2017	JS	PW	0	
EbLentWILoFreqFemPhon159	159	2017	JS	P	0	
EbLentWILoFreqFemPhon160	160	2017	JS	WP	1	0.3295
EbLentWILoFreqFemPhon161	161	2017	JS	W	0	
EbLentWILoFreqFemPhon162	162	2017	JS	PW	0	
EbLentWILoFreqFemPhon163	163	2017	JS	W	0	
EbLentWILoFreqFemPhon164	164	2017	JS	WP	0	
EbLentWILoFreqFemPhon165	165	2017	JS	PW	0	
EbLentWILoFreqFemPhon166	166	2017	JS	PWP	1	0.8888
EbLentWILoFreqFemPhon167	167	2017	JS	P	0	
EbLentWILoFreqFemPhon168	168	2017	JS	P	0	
EbLentWILoFreqFemPhon169	169	2017	JS	WP	0	
EbLentWILoFreqFemPhon170	170	2017	JS	PWP	0	
EbLentWILoFreqFemPhon171	171	2017	JS	W	0	
EbLentWILoFreqFemPhon172	172	2017	JS	PW	0	
EbLentWILoFreqFemPhon173	173	2017	JS	PWP	0	
EbLentWILoFreqFemPhon174	174	2017	JS	PWP	0	
EbLentWILoFreqFemPhon175	175	2017	JS	W	1	0.523
EbLentWILoFreqFemPhon176	176	2017	JS	P	0	
EbLentWILoFreqFemPhon177	177	2017	JS	PW	1	0.5294
EbLentWILoFreqFemPhon178	178	2017	JS	P	0	
EbLentWILoFreqFemPhon179	179	2017	JS	WP	0	
EbLentWILoFreqFemPhon180	180	2017	JS	PWP	1	0.6491
EbLentWILoFreqFemPhon181	181	2017	JS	W	0	
EbLentWILoFreqFemPhon182	182	2017	JS	PW	0	
EbLentWILoFreqFemPhon183	183	2017	JS	PW	0	
EbLentWILoFreqFemPhon184	184	2017	JS	PWP	0	
EbLentWILoFreqFemPhon185	185	2017	JS	W	0	
EbLentWILoFreqFemPhon186	186	2017	JS	P	0	
EbLentWILoFreqFemPhon187	187	2017	JS	PW	0	
EbLentWILoFreqFemPhon188	188	2017	JS	P	0	
EbLentWILoFreqFemPhon189	189	2017	JS	WP	0	
EbLentWILoFreqFemPhon190	190	2017	JS	W	0	
EbLentWILoFreqFemPhon191	191	2017	JS	PW	0	
EbLentWILoFreqFemPhon192	192	2017	JS	W	0	
EbLentWILoFreqFemPhon193	193	2017	JS	WP	0	
EbLentWILoFreqFemPhon194	194	2017	JS	PW	0	
EbLentWILoFreqFemPhon195	195	2017	JS	PWP	1	0.2886
EbLentWILoFreqFemPhon196	196	2017	JS	P	0	

EbLentWILoFreqFemPhon197	197	2017	JS	WP	1	0.7272
EbLentWILoFreqFemPhon198	198	2017	JS	PWP	1	0.5532
EbLentWILoFreqFemPhon199	199	2017	JS	W	0	
EbLentWILoFreqFemPhon200	200	2017	JS	P	0	

Appendix B: Raw Data for Chapter 3

2020 Female Mate Choice Immediate Context Data						
Location: Oak Leaf Trail, Milwaukee, Wisconsin						
Year: 2020						
Researchers: Speck, Seidita, Rodriguez						
3 treatments (Decoy): Control(No Playback), High Quality Decoy(Preference Peak), & Low Quality Decoy(Preference Bottom)						
3 Stimuli: D-Decoy (treatment stimuli), P-Preferred (attractive), L-Less Preferred (unattractive)						
					Responses	
Recording	Female	Decoy	Stimulus	Sequence	1 or 0	# of
EbLentWILoFreTranUni08072020F001	1	HQ	D		1	1
	1	HQ	P	1	0	0
	1	HQ	L	2	0	0
EbLentWILoFreTranUni08072020F002	2	LQ	D		0	0
	2	LQ	P	1	0	0
	2	LQ	L	2	0	0
EbLentWILoFreTranUni08072020F003	3	N	P	1	1	4
	3	N	L	2	0	0
EbLentWILoFreTranUni08072020F004	4	HQ	D		1	4
	4	HQ	L	1	1	2
	4	HQ	P	2	1	4
EbLentWILoFreTranUni08072020F005	5	LQ	D		0	0
	5	LQ	L	1	0	0
	5	LQ	P	2	1	1
EbLentWILoFreTranUni08072020F006	6	N	L	1	1	1
	6	N	P	2	1	3
EbLentWILoFreTranUni08072020F007	7	LQ	D		0	0
	7	LQ	P	1	1	2
	7	LQ	L	2	1	2
EbLentWILoFreTranUni08072020F008	8	HQ	D		1	3
	8	HQ	P	1	1	4
	8	HQ	L	2	1	1
EbLentWILoFreTranUni08072020F009	9	N	P	1	1	4
	9	N	L	2	0	0
EbLentWILoFreTranUni08072020F010	10	LQ	D		0	0
	10	LQ	L	1	0	0
	10	LQ	P	2	0	0
EbLentWILoFreTranUni08072020F011	11	HQ	D		1	4
	11	HQ	L	1	1	3
	11	HQ	P	2	1	4

EbLentWILoFreTranUni08072020F012	12	N	L	1	0	0
	12	N	P	2	1	2
EbLentWILoFreTranUni08072020F013	13	N	P	1	1	3
	13	N	L	2	1	4
EbLentWILoFreTranUni08072020F014	14	LQ	D		0	0
	14	LQ	P	1	0	0
	14	LQ	L	2	0	0
EbLentWILoFreTranUni08072020F015	15	HQ	D		1	1
	15	HQ	P	1	0	0
	15	HQ	L	2	0	0
EbLentWILoFreTranUni08072020F016	16	N	L	1	1	1
	16	N	P	2	1	1
EbLentWILoFreTranUni08072020F017	17	LQ	D		0	0
	17	LQ	L	1	0	0
	17	LQ	P	2	0	0
EbLentWILoFreTranUni08072020F018	18	HQ	D		1	4
	18	HQ	L	1	1	2
	18	HQ	P	2	1	4
EbLentWILoFreTranUni08072020F019	19	HQ	D		0	0
	19	HQ	P	1	0	0
	19	HQ	L	2	0	0
EbLentWILoFreTranUni08072020F020	20	LQ	D		0	0
	20	LQ	P	1	0	0
	20	LQ	L	2	0	0
EbLentWILoFreTranUni08072020F021	21	N	P	1	1	2
	21	N	L	2	1	3
EbLentWILoFreTranUni08072020F022	22	HQ	D		1	4
	22	HQ	L	1	1	4
	22	HQ	P	2	1	4
EbLentWILoFreTranUni08072020F023	23	LQ	D		0	0
	23	LQ	L	1	0	0
	23	LQ	P	2	0	0
EbLentWILoFreTranUni08072020F024	24	N	L	1	1	1
	24	N	P	2	0	0
EbLentWILoFreTranUni08072020F025	25	LQ	D		0	0
	25	LQ	P	1	1	2
	25	LQ	L	2	0	0
EbLentWILoFreTranUni08072020F026	26	HQ	D		1	2
	26	HQ	P	1	1	2
	26	HQ	L	2	0	0
EbLentWILoFreTranUni08072020F027	27	N	P	1	1	3

	27	N	L	2	1	3
EbLentWILoFreTranUni08072020F028	28	LQ	D		0	0
	28	LQ	L	1	0	0
	28	LQ	P	2	0	0
EbLentWILoFreTranUni08072020F029	29	HQ	D		0	0
	29	HQ	L	1	1	4
	29	HQ	P	2	1	1
EbLentWILoFreTranUni08072020F030	30	N	L	1	1	3
	30	N	P	2	1	4
EbLentWILoFreTranUni08072020F031	31	N	P	1	1	1
	31	N	L	2	0	0
EbLentWILoFreTranUni08072020F032	32	LQ	D		0	0
	32	LQ	P	1	1	2
	32	LQ	L	2	0	0
EbLentWILoFreTranUni08072020F033	33	HQ	D		1	3
	33	HQ	P	1	1	1
	33	HQ	L	2	0	0
EbLentWILoFreTranUni08082020F034	34	N	L	1	1	1
	34	N	P	2	1	1
EbLentWILoFreTranUni08082020F035	35	LQ	D		0	0
	35	LQ	L	1	1	1
	35	LQ	P	2	1	4
EbLentWILoFreTranUni08082020F036	36	HQ	D		0	0
	36	HQ	L	1	0	0
	36	HQ	P	2	0	0
EbLentWILoFreTranUni08082020F037	37	HQ	D		0	0
	37	HQ	P	1	0	0
	37	HQ	L	2	0	0
EbLentWILoFreTranUni08082020F038	38	LQ	D		1	2
	38	LQ	P	1	0	0
	38	LQ	L	2	1	1
EbLentWILoFreTranUni08082020F039	39	N	P	1	1	3
	39	N	L	2	1	3
EbLentWILoFreTranUni08082020F040	40	HQ	D		0	0
	40	HQ	L	1	0	0
	40	HQ	P	2	0	0
EbLentWILoFreTranUni08082020F041	41	LQ	D		0	0
	41	LQ	L	1	1	1
	41	LQ	P	2	1	2
EbLentWILoFreTranUni08082020F042	42	N	L	1	0	0
	42	N	P	2	0	0

EbLentWILoFreTranUni08082020F043	43	LQ	D		0	0
	43	LQ	P	1	1	4
	43	LQ	L	2	1	4
EbLentWILoFreTranUni08082020F044	44	HQ	D		1	2
	44	HQ	P	1	0	0
	44	HQ	L	2	0	0
EbLentWILoFreTranUni08082020F045	45	N	P	1	1	3
	45	N	L	2	0	0
EbLentWILoFreTranUni08082020F046	46	LQ	D		0	0
	46	LQ	L	1	1	1
	46	LQ	P	2	0	0
EbLentWILoFreTranUni08082020F047	47	HQ	D		1	4
	47	HQ	L	1	1	2
	47	HQ	P	2	1	1
EbLentWILoFreTranUni08082020F048	48	N	L	1	1	4
	48	N	P	2	1	3
EbLentWILoFreTranUni08082020F049	49	N	P	1	1	1
	49	N	L	2	0	0
EbLentWILoFreTranUni08082020F050	50	LQ	D		0	0
	50	LQ	P	1	0	0
	50	LQ	L	2	0	0
EbLentWILoFreTranUni08082020F051	51	HQ	D		0	0
	51	HQ	P	1	0	0
	51	HQ	L	2	0	0
EbLentWILoFreTranUni08082020F052	52	N	L	1	1	4
	52	N	P	2	1	3
EbLentWILoFreTranUni08082020F053	53	LQ	D		0	0
	53	LQ	L	1	0	0
	53	LQ	P	2	1	1
EbLentWILoFreTranUni08082020F054	54	HQ	D		0	0
	54	HQ	L	1	1	3
	54	HQ	P	2	1	2
EbLentWILoFreTranUni08082020F055	55	HQ	D		1	2
	55	HQ	P	1	0	0
	55	HQ	L	2	0	0
EbLentWILoFreTranUni08082020F056	56	LQ	D		0	0
	56	LQ	P	1	0	0
	56	LQ	L	2	0	0
EbLentWILoFreTranUni08082020F057	57	N	P	1	0	0
	57	N	L	2	0	0
EbLentWILoFreTranUni08082020F058	58	HQ	D		1	4

	58	HQ	L	1	0	0
	58	HQ	P	2	1	3
EbLentWILoFreTranUni08082020F059	59	LQ	D		0	0
	59	LQ	L	1	0	0
	59	LQ	P	2	0	0
EbLentWILoFreTranUni08082020F060	60	N	L	1	0	0
	60	N	P	2	0	0
EbLentWILoFreTranUni08082020F061	61	LQ	D		1	1
	61	LQ	P	1	1	4
	61	LQ	L	2	1	1
EbLentWILoFreTranUni08082020F062	62	HQ	D		1	3
	62	HQ	P	1	1	2
	62	HQ	L	2	0	0
EbLentWILoFreTranUni08082020F063	63	N	P	1	1	4
	63	N	L	2	1	4
EbLentWILoFreTranUni08082020F064	64	LQ	D		0	0
	64	LQ	L	1	0	0
	64	LQ	P	2	1	1
EbLentWILoFreTranUni08082020F065	65	HQ	D		1	4
	65	HQ	L	1	1	4
	65	HQ	P	2	1	4
EbLentWILoFreTranUni08092020F066	66	N	L	1	1	4
	66	N	P	2	1	4
EbLentWILoFreTranUni08092020F067	67	N	P	1	1	2
	67	N	L	2	0	0
EbLentWILoFreTranUni08092020F068	68	LQ	D		0	0
	68	LQ	P	1	1	3
	68	LQ	L	2	1	3
EbLentWILoFreTranUni08092020F069	69	HQ	D		0	0
	69	HQ	P	1	0	0
	69	HQ	L	2	1	1
EbLentWILoFreTranUni08092020F070	70	N	L	1	1	3
	70	N	P	2	1	2
EbLentWILoFreTranUni08092020F071	71	LQ	D		0	0
	71	LQ	L	1	0	0
	71	LQ	P	2	0	0
EbLentWILoFreTranUni08092020F072	72	HQ	D		1	1
	72	HQ	L	1	1	3
	72	HQ	P	2	1	2
EbLentWILoFreTranUni08092020F073	73	HQ	D		1	2
	73	HQ	P	1	1	2

	73	HQ	L	2	0	0
EbLentWILoFreTranUni08092020F074	74	LQ	D		0	0
	74	LQ	P	1	1	3
	74	LQ	L	2	1	4
EbLentWILoFreTranUni08092020F075	75	N	P	1	1	3
	75	N	L	2	1	1
EbLentWILoFreTranUni08092020F076	76	HQ	D		0	0
	76	HQ	L	1	1	4
	76	HQ	P	2	1	4
EbLentWILoFreTranUni08092020F077	77	LQ	D		0	0
	77	LQ	L	1	0	0
	77	LQ	P	2	1	1
EbLentWILoFreTranUni08092020F078	78	N	L	1	1	3
	78	N	P	2	0	0
EbLentWILoFreTranUni08092020F079	79	LQ	D		0	0
	79	LQ	P	1	1	2
	79	LQ	L	2	0	0
EbLentWILoFreTranUni08092020F080	80	HQ	D		0	0
	80	HQ	P	1	0	0
	80	HQ	L	2	1	3
EbLentWILoFreTranUni08102020F081	81	N	P	1	0	0
	81	N	L	2	0	0
EbLentWILoFreTranUni08102020F082	82	LQ	D		0	0
	82	LQ	L	1	1	3
	82	LQ	P	2	1	4
EbLentWILoFreTranUni08102020F083	83	HQ	D		0	0
	83	HQ	L	1	1	3
	83	HQ	P	2	1	4
EbLentWILoFreTranUni08102020F084	84	N	L	1	0	0
	84	N	P	2	1	4
EbLentWILoFreTranUni08102020F085	85	N	P	1	0	0
	85	N	L	2	0	0
EbLentWILoFreTranUni08102020F086	86	LQ	D		0	0
	86	LQ	P	1	1	3
	86	LQ	L	2	1	4
EbLentWILoFreTranUni08102020F087	87	HQ	D		1	3
	87	HQ	P	1	1	4
	87	HQ	L	2	1	2
EbLentWILoFreTranUni08102020F088	88	N	L	1	1	1
	88	N	P	2	1	4
EbLentWILoFreTranUni08102020F089	89	LQ	D		0	0

	89	LQ	L	1	1	4
	89	LQ	P	2	1	4
EbLentWILoFreTranUni08102020F090	90	HQ	D		1	1
	90	HQ	L	1	1	4
	90	HQ	P	2	1	4
EbLentWILoFreTranUni08102020F091	91	HQ	D		1	3
	91	HQ	P	1	1	4
	91	HQ	L	2	0	0
EbLentWILoFreTranUni08102020F092	92	LQ	D		0	0
	92	LQ	P	1	0	0
	92	LQ	L	2	1	4
EbLentWILoFreTranUni08102020F093	93	N	P	1	0	0
	93	N	L	2	0	0
EbLentWILoFreTranUni08102020F094	94	HQ	D		0	0
	94	HQ	L	1	0	0
	94	HQ	P	2	0	0
EbLentWILoFreTranUni08102020F095	95	LQ	D		0	0
	95	LQ	L	1	0	0
	95	LQ	P	2	0	0
EbLentWILoFreTranUni08102020F096	96	N	L	1	0	0
	96	N	P	2	1	2
EbLentWILoFreTranUni08102020F097	97	LQ	D		0	0
	97	LQ	P	1	1	3
	97	LQ	L	2	1	4
EbLentWILoFreTranUni08102020F098	98	HQ	D		1	3
	98	HQ	P	1	1	4
	98	HQ	L	2	1	3
EbLentWILoFreTranUni08102020F099	99	N	P	1	1	4
	99	N	L	2	0	0
EbLentWILoFreTranUni08102020F100	100	LQ	D		0	0
	100	LQ	L	1	0	0
	100	LQ	P	2	1	2
EbLentWILoFreTranUni08102020F101	101	HQ	D		1	4
	101	HQ	L	1	1	4
	101	HQ	P	2	1	3
EbLentWILoFreTranUni08102020F102	102	N	L	1	1	2
	102	N	P	2	1	1
EbLentWILoFreTranUni08102020F103	103	N	P	1	1	4
	103	N	L	2	1	3
EbLentWILoFreTranUni08102020F104	104	LQ	D		0	0
	104	LQ	P	1	0	0

	104	LQ	L	2	0	0
EbLentWILoFreTranUni08102020F105	105	HQ	D		1	3
	105	HQ	P	1	1	3
	105	HQ	L	2	1	4
EbLentWILoFreTranUni08102020F106	106	N	L	1	0	0
	106	N	P	2	0	0
EbLentWILoFreTranUni08102020F107	107	LQ	D		0	0
	107	LQ	L	1	0	0
	107	LQ	P	2	1	4
EbLentWILoFreTranUni08102020F108	108	HQ	D		0	0
	108	HQ	L	1	1	4
	108	HQ	P	2	1	2
EbLentWILoFreTranUni08102020F109	109	HQ	D		1	1
	109	HQ	P	1	1	4
	109	HQ	L	2	1	1
EbLentWILoFreTranUni08102020F110	110	LQ	D		0	0
	110	LQ	P	1	1	4
	110	LQ	L	2	1	3
EbLentWILoFreTranUni08102020F111	111	N	P	1	0	0
	111	N	L	2	0	0
EbLentWILoFreTranUni08102020F112	112	HQ	D		0	0
	112	HQ	L	1	0	0
	112	HQ	P	2	0	0
EbLentWILoFreTranUni08102020F113	113	LQ	D		0	0
	113	LQ	L	1	1	2
	113	LQ	P	2	1	4
EbLentWILoFreTranUni08102020F114	114	N	L	1	0	0
	114	N	P	2	0	0
EbLentWILoFreTranUni08102020F115	115	LQ	D		0	0
	115	LQ	P	1	0	0
	115	LQ	L	2	1	3
EbLentWILoFreTranUni08102020F116	116	HQ	D		1	1
	116	HQ	P	1	1	1
	116	HQ	L	2	1	1
EbLentWILoFreTranUni08102020F117	117	N	P	1	0	0
	117	N	L	2	1	2
EbLentWILoFreTranUni08102020F118	118	LQ	D		0	0
	118	LQ	L	1	0	0
	118	LQ	P	2	1	1
EbLentWILoFreTranUni08102020F119	119	HQ	D		1	1
	119	HQ	L	1	1	1

	119	HQ	P	2	0	0
EbLentWILoFreTranUni08102020F120	120	N	L	1	0	0
	120	N	P	2	1	1
EbLentWILoFreTranUni08112020F121	121	N	P	1	1	4
	121	N	L	2	1	4
EbLentWILoFreTranUni08112020F122	122	LQ	D		0	0
	122	LQ	P	1	0	0
	122	LQ	L	2	0	0
EbLentWILoFreTranUni08112020F123	123	HQ	D		1	1
	123	HQ	P	1	1	4
	123	HQ	L	2	1	4
EbLentWILoFreTranUni08112020F124	124	N	L	1	1	4
	124	N	P	2	1	4
EbLentWILoFreTranUni08112020F125	125	LQ	D		0	0
	125	LQ	L	1	1	4
	125	LQ	P	2	1	4
EbLentWILoFreTranUni08112020F126	126	HQ	D		1	3
	126	HQ	L	1	1	1
	126	HQ	P	2	1	1
EbLentWILoFreTranUni08112020F127	127	HQ	D		1	4
	127	HQ	P	1	1	4
	127	HQ	L	2	1	2
EbLentWILoFreTranUni08112020F128	128	LQ	D		0	0
	128	LQ	P	1	1	4
	128	LQ	L	2	1	4
EbLentWILoFreTranUni08112020F129	129	N	P	1	1	3
	129	N	L	2	1	4
EbLentWILoFreTranUni08112020F130	130	HQ	D		1	4
	130	HQ	L	1	1	3
	130	HQ	P	2	1	4
EbLentWILoFreTranUni08112020F131	131	LQ	D		0	0
	131	LQ	L	1	1	4
	131	LQ	P	2	1	4
EbLentWILoFreTranUni08112020F132	132	N	L	1	1	4
	132	N	P	2	1	4
EbLentWILoFreTranUni08112020F133	133	LQ	D		0	0
	133	LQ	P	1	0	0
	133	LQ	L	2	0	0
EbLentWILoFreTranUni08112020F134	134	HQ	D		1	4
	134	HQ	P	1	1	3
	134	HQ	L	2	1	2

EbLentWILoFreTranUni08112020F135	135	N	P	1	1	3
	135	N	L	2	0	0
EbLentWILoFreTranUni08112020F136	136	LQ	D		0	0
	136	LQ	L	1	0	0
	136	LQ	P	2	0	0
EbLentWILoFreTranUni08112020F137	137	HQ	D		1	1
	137	HQ	L	1	0	0
	137	HQ	P	2	0	0
EbLentWILoFreTranUni08112020F138	138	N	L	1	0	0
	138	N	P	2	0	0
EbLentWILoFreTranUni08112020F139	139	N	P	1	0	0
	139	N	L	2	0	0
EbLentWILoFreTranUni08112020F140	140	LQ	D		0	0
	140	LQ	P	1	1	3
	140	LQ	L	2	1	4
EbLentWILoFreTranUni08122020F141	141	HQ	D		0	0
	141	HQ	P	1	0	0
	141	HQ	L	2	0	0
EbLentWILoFreTranUni08122020F142	142	N	L	1	0	0
	142	N	P	2	0	0
EbLentWILoFreTranUni08122020F143	143	LQ	D		0	0
	143	LQ	L	1	1	1
	143	LQ	P	2	0	0
EbLentWILoFreTranUni08122020F144	144	HQ	D		0	0
	144	HQ	L	1	0	0
	144	HQ	P	2	0	0
EbLentWILoFreTranUni08122020F145	145	HQ	D		0	0
	145	HQ	P	1	1	3
	145	HQ	L	2	0	0
EbLentWILoFreTranUni08122020F146	146	LQ	D		0	0
	146	LQ	P	1	0	0
	146	LQ	L	2	0	0
EbLentWILoFreTranUni08122020F147	147	N	P	1	1	2
	147	N	L	2	1	1
EbLentWILoFreTranUni08122020F148	148	HQ	D		0	0
	148	HQ	L	1	0	0
	148	HQ	P	2	1	4
EbLentWILoFreTranUni08122020F149	149	N	L	1	1	1
	149	N	P	2	0	0
EbLentWILoFreTranUni08122020F150	150	LQ	D		0	0
	150	LQ	L	1	0	0

	150	LQ	P	2	1	1
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Education

2015-Present	University of Wisconsin-Milwaukee Ph.D. Candidate in Behavioral Ecology	GPA: 4.00
1999-2003	Bethel University , St. Paul, MN B.S. Biological Sciences	GPA: 3.53

Research Description

Mate selection is one of the most important choices a female can make for herself and her offspring. Variation in mate choice decisions has consequences for maintenance of and the diversity within a population and the promotion of divergence between populations. Mate choice decisions arise from the interaction of two main components: “mate preferences” (the relative attractiveness of a potential mate) and “choosiness” (the effort put into procuring a preferred mate). Previous work in the Rodriguez lab has identified many of the mate preferences in *Enchenopa* treehoppers (a local Wisconsin insect.) My study builds upon that knowledge to further investigate female mate choice decisions. My study takes a three-pronged approach. First, I investigated how *Enchenopa* females process a male mating signal with multiple elements. This was done by presenting females with different arrangements of signal elements and recording the female responses. I found that female treehoppers use rules for acceptable element combinations, which allows them to process complex signals when selecting mates. Second, I looked at how the social context may influence or alter the attractiveness of a male when he is compared to competitors. To investigate this, I compared female responsiveness to an average male grouped with either a highly attractive male or an unattractive male. I found that female responsiveness to an average male increased when he is in the social context of attractive males. Third, I am currently investigating how mate preferences and choosiness relate to each other and how they change over the lifetime of a female, due to her desire to ensure mating success. This dissertation deepens the breadth of knowledge about how mate choice decisions are made, which in turn helps us understand the consequences of variation in such decisions for the maintenance of population diversity and the promotion of speciation.

Publications

- Desjonquères, C., R. R. Holt, B. Speck, and R. L. Rodríguez. 2020. The relationship between a combinatorial processing rule and a continuous mate preference function in an insect. *Proceedings of the Royal Society B: Biological Sciences* 287:20201278.
- Speck, B., S. Seidita, S. Belo, S. Johnson, C. Conley, C. Desjonquères, and R. L. Rodríguez. 2020. Combinatorial Signal Processing in an Insect. *The American Naturalist* 196:406–413.
- Desjonquères, C., B. Speck, and R. L. Rodríguez. 2019. Signalling interactions during ontogeny are a cause of social plasticity in *Enchenopa* treehoppers (Hemiptera: Membracidae). *Behavioural Processes* 166:103887.
- Desjonquères, C., J. Maliszewski, E. N. Lewandowski, B. Speck, and R. L. Rodríguez. 2019. Social ontogeny in the communication system of an insect. *Animal Behaviour* 148:93–103.
- Eberhard, W. G., R. L. Rodríguez, B. A. Huber, B. Speck, H. Miller, B. A. Buzatto, and G. Machado. 2018. Sexual Selection and Static Allometry: The Importance of Function. *The Quarterly Review of Biology* 93:207–250.

In Review:

- Speck B, Seidita S, & Rodríguez RL. (In review). Variation in mate choice decisions: individual differences matter more than options available in *Enchenopa* treehoppers (Hemiptera: Membracidae). *Journal of Evolutionary Biology*.
- Desjonquères C, Speck B, Seidita S, Cirino LA, Escalante I, Sergi C, Maliszewski J, Wiese C, Hoebel G, Bailey NW & Rodríguez RL. (In review). Social plasticity in signals and preferences may create or enhance signal-preference co-divergence. *Proceedings of the Royal Society B*.

Presentations

- Speck, B. (November 2021). Architecture of mate choice decisions in *Enchenopa* treehoppers (Hemiptera: Membracidae). Oral Presentation at UWM's Biological Sciences Colloquium.
- Speck, B. (July 2021). Rationality of Mate Choice Decisions in *Enchenopa binotata* Treehoppers. Virtual Presentation at the 2021 Animal Behavior Society Meeting
- Speck, B. (November 2019). Lifetime Variation in the Mate Preference Functions of Female *Enchenopa binotata* treehoppers (Hemiptera: Membracidae). Poster Presentation at Riveredge Nature Center's Student Research Symposium

- Speck, B. (July 2019). Lifetime Variation in the Mate Preference Functions of Female *Enchenopa binotata* treehoppers (Hemiptera: Membracidae). Poster Presentation at the 2019 Animal Behavior Society Meeting
- Speck, B. (November 2018). Insect Phonology: Combinatorial Signal Processing in *Enchenopa* Treehoppers (Hemiptera, Membracidae). Oral Presentation at Riveredge Nature Center's Student Research Symposium
- Speck, B. (August 2018). Insect Phonology: Combinatorial Signal Processing in *Enchenopa* Treehoppers (Hemiptera, Membracidae). Oral Presentation at the 2018 Animal Behavior Society Meeting
- Speck, B. (April 2018). Insect Phonology: Combinatorial Signal Processing in *Enchenopa* Treehoppers (Hemiptera, Membracidae). Oral Presentation at the University of Wisconsin – Milwaukee Biological Sciences Research Symposium
- Speck, B. (2017). Transitive Mate Choice in *Enchenopa binotata* Treehoppers (Hemiptera, Membracidae). Oral Presentation at the University of Wisconsin – Milwaukee Biological Sciences Research Symposium
- Speck, B. (2016). Pick-up lines for treehoppers *Enchenopa binotata* (Hemiptera, Membracidae). Oral Presentation at EEB Journal Club at University of Wisconsin – Milwaukee
- Speck, B. (2016). Allometry of non-genitalic courtship structures in *Polistes fuscatus* wasps (Hymenoptera: Vespidae) Poster Presentation at the University of Wisconsin – Milwaukee Biological Sciences Research Symposium
- Speck, B. (2015). Phonology of treehoppers *Enchenopa binotata* (Hemiptera, Membracidae). Oral Presentation at EEB Journal Club at University of Wisconsin – Milwaukee
- Vrieze, B (2003). Habitat Quality of Grassland Reconstruction Using Small Mammals as an Indicator. Oral Presentation at Bethel University Biological Sciences Research Symposium
- Vrieze, B (2003). Habitat Quality of Grassland Reconstruction Using Small Mammals as an Indicator. Oral Presentation at Tri-Beta National Biological Honor Society Research Symposium

Awards & Honors

- UWM's R1 Distinguished Dissertator Fellowship (2021)
- Clifford H. Mortimer Scholarship (2021)
- Ruth I. Walker Memorial Scholarship (2021 & 2019)
- Chancellor's Graduate Student Award (2020, 2019, & 2018)
- Best Poster Presentation at Riveredge Nature Center Student Symposium (2019)

- Best oral Presentation at Riveredge Nature Center Student Symposim (2018)
- James J. Magnino Scholarship (2016)

Public Outreach

- Guest Lecturer at Concordia University Wisconsin's Ecology Course. Topic: Behavioral Ecology & Research. October 2021
- Research featured in the UWM College of Letters and Science In Focus Magazine. "Little insects, big brains?" October 2020 - Volume 10, No. 10.
- Nature Walks with Mrs. Speck at Elm Grove Lutheran School. Fall 2020
- Guest Lecturer at Concordia University Wisconsin's Ecology Course. Topic: Behavioral Ecology. November 2019
- Animal Behavior Society-Outreach Fair. Discover Treehoppers Table. August 2018
- Science Olympiad Ecology Event Supervisor. State Tournament. April 2018
- Doors Open Milwaukee. Treehopper Research in the UWM Greenhouse. September 2017
- UWM Graduate School Booth. Wisconsin State Fair. August 2017
- Bugs in your backyard presentation to Elm Grove Lutheran Preschool. May 2016