December 2013

Influence of Genetic Variation in the Biotic Environment on Phenotypic Variation in a Plant-feeding Insect

Darren Rebar
University of Wisconsin-Milwaukee

INFLUENCE OF GENETIC VARIATION IN THE BIOTIC ENVIRONMENT ON PHENOTYPIC VARIATION IN A PLANT-FEEDING INSECT

by

Darren Rebar

A Dissertation Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

in Biological Sciences

at

The University of Wisconsin-Milwaukee

December 2013

ABSTRACT
INFLUENCE OF GENETIC VARIATION IN THE BIOTIC ENVIRONMENT ON PHENOTYPIC VARIATION IN A PLANT-FEEDING INSECT

by

Darren Rebar

The University of Wisconsin-Milwaukee, 2013
Under the Supervision of Professor Rafael Rodríguez

While many species spend much of their lives in close association with other organisms, only recently have biologists started to explore the implications of the biotic nature of environments for their role as causes of variation in phenotypes. This means that the genotypes of individuals that constitute the biotic environment may influence the phenotypes of individuals that live in that environment. These are called indirect genetic effects (IGEs) when they occur between conspecifics, and interspecific indirect genetic effects (IIGEs) when they occur between heterospecifics. However, the impact of genetic variation in biotic environments remains largely unknown. I used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae) to assess how male mating signals and female mate preferences are influenced by genetic variation in biotic environments. I used novel implementations of classic quantitative genetics designs, with samples of full-sibling families of treehoppers (IGEs) and clone lines of a sample of host plant genotypes (IIGEs) constituting the background biotic environment.

To measure IGEs, I used full-sibling split-families as "treatment" social environments, and reared a random sample of focal females alongside each treatment family, describing the mate preferences of these focal females. With this I detected substantial genetic variation in social influence on mate preferences: the mate preferences

of focal females varied according to the treatment families along with which they grew up.

To measure IIGEs, I reared a random sample of treehoppers on potted replicates of a sample of host plant clones, describing the male signals and female mate preferences of these individuals. I found that male signals and female mate preferences varied according to the clone line on which they developed, demonstrating that genetic variation in host plants has cross-trophic consequences on sexually-selected traits at the level of the insect.

I discuss the evolutionary implications of the presence of such genetic variation in biotic environments on male signals and female mate preferences. I focus on how IGEs and IIGEs may influence the way in which selection may act within and across environments, including potential contributions to the maintenance of genetic variation and the promotion of evolutionary divergence.

TABLE OF CONTENTS

List of Figures vii

List of Tables ix

Acknowledgements x

Chapter 1: Genetic variation in social influence on mate preferences 1

Abstract 1

Introduction 2

Methods 3

Results 9

Discussion 10

Acknowledgements 13

References 13

Chapter 2: Trees to treehoppers: genetic variation in host plants contributes to variation in the mating signals of a plant-feeding insect 23

Abstract 23

Introduction 24

Methods 27

Results 32

Discussion 32

Acknowledgements 35

References 36

Chapter 3: Genetic variation in host plants contributes to phenotypic variation in the mate preferences of a plant-feeding insect 46

Abstract 46

Introduction 47

Methods 50

Results 55

Discussion 56

Acknowledgements 58

References 58

Curriculum Vitae 68

LIST OF FIGURES

Figure 1 17
Experimental design to test if the presence of genetic variation in social neighbors influences the mate preferences of focal individuals reared. A sample of full-sib families was used as the social genetic component, and each family was split onto two replicates to separate social genetic effects from environmental ones. Focal individuals were then added, and I described the mate preferences of these focal individuals.

Figure 2 18
Genetic variation in social influence on the mate preference functions of *Enchenopa* focal females. The preference functions of the two replicates of focal females that developed with 7 full-sibling treatment families are shown. The dotted line represents the mean peak preference in the population.

Figure 3 19
Example of individual preference functions of focal females from two replicates of two representative full-sibling treatment families. The dotted line represents the mean peak preference of all females tested.

Figure 4 20
Genetic variation in social influence on two traits describing the mate preference functions of *Enchenopa* focal females. The y-axis for the traits represents the phenotypic range observed in this study for each trait. Means ± SE are displayed for both replicates of focal females reared around each family.

Figure 5 41
Experimental design to test if genetic variation in host plants influences the mating signals of treehopper individuals reared on them. Clones were used as the genetic component, with at least three plant individuals as replicates for each clone. Randomly collected, unrelated treehopper individuals were reared on those plants, and I assessed variation in their mating signals according to among- and within-clone components.

Figure 6 42
Depiction of a bout consisting of four signals that increase in amplitude, along with close-ups of the waveform of a signal produced by male *E. binotata*.

Figure 7 43
Clone means ± 1 SE (left column, closed circles) and replicate means (right column, open circles) for the seven treehopper male signal traits analyzed across the 12 clone lines. The y-axis represents the range of phenotypic variation in each male trait in the study population.

Figure 8 63
Experimental design to test whether the presence of genetic variation in host plants influences the pattern of focal female mate preferences reared on them. Clones were used as the environment with a describable genetic component, with each clone line consisting of a minimum of three replicates. Randomly collected, unrelated focal individuals were then placed on each replicate in order to separate among and within-clone line effects. I described the mate preferences of these focal individuals as adults.

Figure 9 64
Genetic variation in cross-trophic influence on the mate preference functions of *Enchenopa* focal females. Each preference function depicts the mean response of all focal females measured on that replicate, and each figure shows variation in the replicates for the sampled clone line. The dotted line represents the mean peak preference of all females in the sampled population.

Figure 10 65
Genetic variation in cross-trophic influence on two traits describing the mate preference functions of *Enchenopa* focal females. The y-axis for each trait represents the phenotypic range observed in this study. The left column (solid circles) shows the mean ± SE of focal females reared on each clone line. The right column (open circles) shows the mean of focal females reared on each replicate within each respective clone line.

LIST OF TABLES

Table 1 21
Linear mixed-model testing for differences in the shape of the preference functions of focal females. Family, replicate, and individual are random terms, with replicate nested within family and individual nested within replicate and family. The family × quadratic term tests for genetic variation in social influence on the mate preference functions.

Table 2 22
Linear mixed-models of social influence on peak preference and on preference selectivity with family and replicate as random terms with replicate nested within family. Significant values are in bold.

Table 3 44
Variation in *Enchenopa* male signal traits attributed to differences among-clone lines and within-clone lines (replicates), along with estimates for the variance components and for the heritability of the influence of host plants on male signal traits, H^2_{IIGE}. Significant tests and estimates are in bold.

Table 4 45
Principal components analysis of the 7 measured *Enchenopa* male signal traits, and variation in each component attributed to differences among-clone lines and within-clone lines (replicates). PVE denotes the per cent of variance explained by the corresponding principal component. Significant tests and estimates are in bold.

Table 5 66
Linear mixed-models testing the variation in two traits of the mate preference functions of focal females attributed to differences among clone lines and within clone lines (replicates), along with estimates for H^2. Clone and replicate are random terms, with replicate nested within clone. Significant values are in bold.

Table 6 67
Linear mixed-models testing the variation in two traits of the mate preference functions of focal females attributed to differences among clone lines and within clone lines (replicates), along with estimates for H^2. Clone and replicate are random terms, with replicate nested within clone. Significant values are in bold.

ACKNOWLEDGEMENTS

I find it nearly impossible to thank all the people and experiences that have brought me to this point in my academic career, and I look forward to the people and experiences waiting for me in the future. That said, I believe the letter "C" encapsulates much of my appreciation: collaborators, community, co-conspirators, cogs, and coffee. This dissertation would not have been possible without the support of my advisor, Rafa Rodríguez, or my committee members Filipe Alberto, Gerlinde Höbel, Emily Latch, and Linda Whittingham over the years. My family and friends have also been there throughout, if only to listen to my aimless thoughts over a pint or two. Several people have been there through late nights, early mornings, and everything in-between. My bikes have been my best friends for when I have just needed to blow off some steam or have needed some time to collect my thoughts. And the bean of productivity has fuelled and inspired me along the way.

CHAPTER 1: Genetic variation in social influence on mate preferences

ABSTRACT

Patterns of phenotypic variation arise in part from plasticity due to social interactions, and these patterns contribute, in turn, to the form of selection that shapes the variation we observe in natural populations. This proximate-ultimate dynamic brings genetic variation in social environments to the forefront of evolutionary theory. However, the extent of this variation remains largely unknown. Here I use a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae) to assess how mate preferences are influenced by genetic variation in the social environment. I used full-sibling split-families as "treatment" social environments, and reared focal females alongside each treatment family, describing the mate preferences of the focal females. With this method, I detected substantial genetic variation in social influence on mate preferences. The mate preferences of focal females varied according to the treatment families along with which they grew up. I discuss the evolutionary implications of the presence of such genetic variation in social influence on mate preferences, including potential contributions to the maintenance of genetic variation, the promotion of divergence, and the adaptive evolution of social effects on fitness-related traits.

Keywords: indirect genetic effects, preference functions, vibrational signals, *Enchenopa*

INTRODUCTION

Social interactions shape the course of evolution in two main ways. First, through various forms of behavioral and developmental plasticity, social interactions are pervasive causes of variation in phenotypes [1-6]. Second, because of the nature of competition with conspecifics for mates and other resources, social interactions are strong causes of variation in fitness [7-12]. These contributions of social interactions to the evolutionary process share a key feature: in varied ways, the phenotypes of some individuals modify the phenotypes and fitness of other individuals. In such situations, genes expressed in one individual may influence the phenotypes and/or fitness of other individuals; i.e., there may arise indirect genetic effects (IGEs) [13-15].

When social interactions give rise to IGEs, theory predicts important and varied evolutionary consequences [13, 14, 16, 17]. And, because IGEs may arise from many different kinds of interactions, IGEs may be very common in nature [10, 17, 18]. Nevertheless, empirical work on IGEs has lagged behind theoretical exploration because of the difficulty of the measurements involved, although the available evidence confirms the expectation that IGEs will be taxonomically widespread [19-23]. Here I report the first measure of IGEs on mate preferences, complex behavioral traits that are sources of strong selection on sexual traits and important causes of reproduction isolation between diverging populations [7-9, 12].

I used a method that tests for IGEs according to their formal definition, as instances when the genes expressed by one individual have an effect on the phenotype of other individuals [13, 14]. My goal was to ask whether genetic variation in the social environment shapes the mate preferences of individuals placed in that social environment. I used a novel implementation of a classic quantitative genetics design: I used a sample of full-sibling split-families [24, 25] as treatment social environments for randomly-collected focal individuals. I described the mate preferences of the focal

individuals and estimated variation in the preferences due to among-treatment family and within-treatment family components. This amounts to measuring the effect of genetic variation in the social environment on the focal individuals.

I test two hypotheses about the role of social interactions. First, I test the hypothesis that the social environment influences female mate preferences. This hypothesis predicts that the mate preferences of focal females will differ across various compositions of the social environment. Second, I test the hypothesis that there is genetic variation in the influence of the social environment on female mate preferences. This hypothesis predicts that the mate preferences of focal females will differ due to the full-sib family treatments. In other words, there should be an among-treatment family effect. This result would indicate that the genetic make-up of the full-sib families is contributing to the differences in the mate preferences of the focal females.

METHODS

General methods

My study species was a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). These insects communicate with plant-borne vibrational signals, and pair formation occurs through male-female signaling duets [26]. Females exhibit strong mate preferences on the basis of male signals, mainly signal frequency, which is the most divergent adult trait among the members of this species complex [27, 28]. Female mate preferences for signal frequency are unimodal (or "closed"; i.e., they favor intermediate frequency values), and females from different species in the complex favor different signal frequencies [27, 29].

In my study site (Tendick Nature Park, Saukville, WI, USA), there are two species that live on the host plant *Viburnum lentago* (Caprifoliaceae). These species have not been formally described, but male signal frequency is a reliable trait in determining each

species, as well as others in this species complex [28-30]. I used the low-frequency species found on *V. lentago* (dominant frequency = 185Hz), and I kept voucher specimens in 95% EtOH.

My experiment consisted of a social environment manipulation phase and a testing phase. During the social environment phase, I manipulated the social environment in which focal females developed and matured sexually by rearing them with different treatment full-sibling families (i.e. an environment with a describable genetic component). During the testing phase, I described variation in female preferences for the signal frequency of male signals.

Manipulation of the social environment

To manipulate the social environment of the focal female whose mate preferences I aimed to describe, I used a full-sibling split-family (i.e., split-clutch) quantitative genetics design (Fig. 1) [24, 25]. This manipulation mimics natural variation in *Enchenopa*, where social aggregations consist of varying mixtures of broods developing together [26, 31]. These patterns are established as females aggregate during late summer and fall to lay eggs on the stems of their host plants [26, 32]. Females die at the end of the fall, and the following spring the nymph aggregations develop on the stems on which their eggs were laid [26].

To create these full-sibling treatment families, I collected mated adult females in August 2010 in the field. I note that *Enchenopa* females mate only once [31, 33]. Consequently, the broods of the females that I collected constitute full-sibling families. I placed each of these field-collected dams on an individually potted plant of *V. lentago* to allow them to lay eggs in the stem through the fall. The eggs overwintered in the plants in an outdoors facility. I moved plants into a greenhouse in April 2011 to induce budding and sap flow, which triggers development of *Enchenopa* embryos [26]. When nymphs

eclosed, I transferred them to the experimental rearing plants within a few days. I generated social groups that consisted of full-sib family members (Fig. 1). I split each family onto two potted host plant exemplars so that the social genetic effects could be separated from the environmental effects. Each of the two replicates per treatment family was composed of 20 nymphs.

To obtain the focal individuals, I randomly collected egg masses from the same population as the treatment families. I collected egg masses by cutting stems from various host plant individuals spanning a 100 meter transect in the field. I placed each stem in a water tube to trigger nymph eclosion, which occurred in late May. I timed this procedure so that focal nymphs were two weeks younger than treatment family nymphs. This manipulation allowed us to introduce an age separation between treatment and focal individuals, permitting us to track them throughout juvenile development. In nature, nymphs developing on any one stem or plant would be closer in age than this, because synchronous eclosion from eggs is prompted by the beginning of sap flow in the spring [26]. This synchrony is not perfect, however, and I have observed a lag of up to 10 days in the greenhouse and in field (D Rebar, RL Rodríguez, pers. obs.). Thus, my introduction of an age difference between treatment and focal individuals is artificial, but represents a minor alteration of the natural situation for *Enchenopa*. Upon adult molt, I painted the pronotum of all treatment individuals.

I placed 20 focal individuals with each replicate of the family treatments. Focal and treatment individuals were reared together on the plant from the time focal individuals were first instars until their adult molt. *Enchenopa* males become sexually mature and begin to signal approx. two weeks after the adult molt, and females become sexually responsive about two weeks later [26]. I therefore removed all treatment individuals when treatment males reached two weeks after the adult molt (as the focal individuals were reaching the adult molt). Focal males remained on the plant for two

weeks more. At that point, just focal females remained on each plant. Thus, the social environment phase occurred from when focal females were first instar nymphs through adulthood, and ended approx. two weeks before they became sexually receptive, at which point I described their mate preferences.

Assessing variation in mate preferences

Mate preferences are function-valued traits [34-36]. That means that a female's sexual response is a function of the signals she encounters. I described mate preferences by presenting individual females with a range of male signals and quantifying their responses to create mate preference functions [27, 37]. I presented females with these stimuli using vibrational playback experiments. Stimuli spanned the range in male signal frequency for the population, with all other features set to the population mean. Each stimulus consisted of a bout of three signals, which is the mean number of signals per bout in the population. I presented each female with a full complement of stimuli in random sequences, with each stimulus separated from the next by 15 seconds of silence. I randomized testing across treatments and replicates over the course of the testing phase to control, as best as possible, for the time between the social environment and testing phases. Signals were 2, 4, 6, 8, 10, 15, 20, 30 and 40 Hz different in each direction from the mean (185 Hz), resulting in 19 different playback stimuli being presented to each female. I created and delivered all synthetic stimuli using a custom MATLAB script (v. 7.11, Mathworks, Inc., Natick, MA; available upon request). Stimuli were delivered to the plant stem through a piezo-electric actuator attached with accelerometer wax and controlled by a piezo controller (Thorlabs, NJ, models AE0505D16 and MDT694A, respectively) from an iMac computer at an amplitude of 0.10 mm/s. I recorded the stimuli and female responses using a laser vibrometer (Polytec, Inc., Auburn, MA, model CLV-2534) connected to an iMac computer and the sound recording software

AUDACITY at a sampling rate of 44.1 kHz (v. 1.2.5; http://audacity.soundforge.net). I isolated the setup from noise due to building vibrations by placing it on shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY) on top of an iron plank (~135 kg) resting on partially inflated bicycle inner tubes on top of a slate table (~1 x 2 m). I placed vibration dampening pads (Polymer Dynamics, Inc., Allentown, PA, model 3291-22-PM-50) under the table legs for further isolation. All females were recorded from July – August 2011.

To assay female response, I took advantage of *Enchenopa* duetting behavior. Males produce an advertisement signal, and if a female finds that signal attractive, she responds with her own signal [27, 29]. A female's likelihood of responding is strongly correlated to the number of responses she gives to a signaling bout [29, 34, 35]. Thus, the number of times a female responds is a reliable indicator of male signal attractiveness. I scored the number of female responses to each stimulus from the recordings (0-3 responses).

I first tested the receptivity of each female by playing back a recording of a live male to her. If she responded to this signal, I then presented her with a sequence of the 19 signal models. Females that failed to respond to the recording of a live male were returned to their host plant and tested at a later date. Some females stopped responding during the playback sequence, which can result if a female becomes habituated. Therefore, I replayed the recording of a live male at the very end of the sequence to those females. If they responded, I considered her still receptive and she was included in the subsequent analyses. Females that failed to respond to this recording at the end were excluded from the analyses. In total, 89 females reached the testing phase of the experiment. Of those, 65 females were receptive to the live male recording, resulting in 41 females that completed the playback sequence (corresponding to n=7 full-sib treatment families) being included in the analyses.

Description of female preference functions

I constructed mate preference functions with non-parametric regression by generating cubic splines with a program created by D Schluter (glms40 cubic spline program; https://www.zoology.ubc.ca/~schluter/wordpress/software/). Cubic splines make no assumption about the shape of the preference other than it is smooth. I used the GCV statistic provided by the glms40 program to choose the smoothing value lambda for each individual preference function. In three instances I manually adjusted the lambda value to increase smoothness because these splines contained sharp angles between data points. This adjustment did not qualitatively change my results. I calculated the splines for each female using 1000 bootstraps to generate confidence intervals for each spline.

I described variation in female mate preferences in terms of peak preference and preference selectivity. Peak preference is the signal frequency that elicits the greatest response from a female. Preference selectivity describes how strongly a female disfavors signals as they deviate from her peak preference [34, 38]. Following established methods, preference selectivity was a composite derived from measurements of three aspects of the shape of the mate preference functions: responsiveness, tolerance, and strength [34, 35]. Responsiveness describes the overall elevation of the curves, and was calculated as the mean of an individual's responses. Tolerance describes the shape of the curves as they fall away from peak preference, measured as the width of the preference function at two-thirds the height of the peak preference. Strength describes the steepness of the curve's descent from peak preference, measured as the square of the coefficient of variation [34, 35, 38]. These three measurements are strongly correlated, so I performed a principal component analysis (PCA) to generate the composite trait I term preference selectivity. This first principal component, which I used in the analysis, had an eigenvalue

of 2.37 that explained 79.10 per cent of the variance, with responsiveness, tolerance, and strength loading similarly on this axis (0.58, 0.58, and -0.57, respectively).

Statistical analysis

I adopted a function-valued approach to describe variation in the female preferences [34-36]. This approach uses the entire preference function as the trait of interest, thus, each female contributes one preference function to the analysis. I performed a linear mixed-effects analysis to address differences in the shape of preference functions. Family, replicate nested within family, and focal individual nested within replicate and family were random effects. The model included linear and quadratic terms for stimulus frequency and for their interaction with family. The family term describes differences in the overall responsiveness or mean elevation of the preference functions [34, 38]. The family × quadratic stimulus frequency interaction describes differences in the shape of the preference functions. Therefore, this interaction term was of particular interest to us.

The significant family × quadratic stimulus frequency interaction (see below) prompted us to explore how the preference functions of focal females varied among treatment families. I used peak preference and preference selectivity as response variables in a linear mixed model with family and replicate as random effects and replicate nested within family. I performed all statistical analyses in JMP v. 7.0 (SAS Institute Inc., Cary, NC).

RESULTS

Social influence on mate preference functions

The social environment influenced *Enchenopa* mate preferences, and there was genetic variation in this social influence. The preference functions of focal females varied

among and within treatment full-sib families (Fig. 2, 3). The significant effect of treatment family (Table 1) indicates genetic variation in social influence on mean responsiveness of focal females; and the significant family × quadratic stimulus frequency interaction indicates genetic variation in social influence on the shape of the preference functions (Fig. 2, Table 1). There were no significant within-treatment family (i.e., replicate) differences in preference functions. Visual inspection of the preference functions indicates social influence on among-treatment family variation in the peak and overall shape of the preferences (Fig. 2).

Social influence on peak preference and on preference selectivity

The social environment influenced both traits describing the shape of *Enchenopa* mate preferences. Specifically, I found substantial and significant genetic variation in social influence on peak preference and on preference selectivity (Fig. 4, Table 2).

DISCUSSION

Here I demonstrate that the social environment influences mate preferences and that there is a substantial genetic component of variation in this social influence. Importantly, by employing a split-family design for the treatment families and rearing randomized, unrelated focal individuals with them, I am able to disentangle whether the effects are due to among-family differences or additional environmental effects. I demonstrate that these effects are due to family differences, and that the within-family component of variation was minimal, indicating the presence of consistent among-family variation in their social influence on mate preferences. Moreover, I show that two traits describing these mate preference functions, peak preference and preference selectivity, are influenced by their social neighbors. Of note is the detection of indirect genetic

effects (IGEs) on peak preference, whereas previous studies addressing plasticity due to social experience during adulthood have not resulted in shifts of peak preference [34, 35].

IGEs on mate preferences may influence the course of sexual selection in various ways. First, the presence of IGEs may help maintain genetic variation under selection [39]. Because the mate preferences of females shifted according to the genetic makeup of their social neighbors, the relative attractiveness of different males to females may vary according to the social environment. Thus, any one female genotype may favor different male phenotypes (and genotypes) according to variation in the social environment, thereby promoting the maintenance of genetic variation in male mating signals. In addition, recent work in *Enchenopa* has demonstrated direct genetic variation in mate preferences [40], social influence on mate preferences [34, 35, 40], and genetic variation in this influence [this study]. In concert with previous theoretical and empirical work highlighting genetic components of variation in both sides of social interactions [14, 20, 23, 41], genetic variation within populations may be sustained by the interplay between how plastic individuals are due to social interactions and how much influence social neighbors can exert on individual plasticity.

Second, the presence of IGEs on mate preferences may impact a population's potential to respond to selection [41, 42]. Recall that the social environment is both a determinant of reproductive success [8, 10, 14] and a cause of phenotypic plasticity in traits such as mate preferences [1-6]. This double-role of the social environment can generate feedback loops between the causes of variation in phenotypes and the causes of selection on phenotypes. That is to say, the patterns of phenotypic variation arising from the influence of the social environment are themselves sources of selection that influence the subsequent patterns of phenotypic variation of other individuals [15, 16, 43].

Third, the demonstration of IGEs on mate preferences suggests that the composition of social neighbors can influence the rate and direction of evolutionary

change. For example, variance in the social environment can promote divergent sexual selection pressures because of how mate preferences shift across these social environments [17]. These shifted female mate preferences alter how selection acts on variation in male signals, resulting in new patterns of variation in phenotypes. As a result, IGEs on mate preferences may subsequently increase the rate of trait elaboration in a particular direction and could promote Fisherian runaway processes on the basis of the IGE components of variation [17]. Furthermore, one social environment may promote preferences one way, and another social environment the other direction. Gene flow between the social groups may be restricted, and divergence between them promoted.

Finally, an important potential consequence of IGEs is that both sides of the social environment dynamic, social plasticity and influences on the plastic response to social environments, may evolve: selection may shape not only how phenotypes adjust to social environments [3434, 35, 44], but also how individual and social phenotypes influence other individuals' phenotypes. That is to say, with genetic variation in social influence on fitness-related traits such as mate preferences, selection may have the ability to shape the extent and form to which individuals exert influence on their neighbors' phenotypes. Whether this actually occurs in nature remains to be examined with theoretical and empirical work, but the potential for it to occur adds an interesting dimension to the nature of the competitive dynamics that may arise in social and sexual selection.

In short, social interactions influence the shape of mate preferences, and there can be substantial components of genetic variation to this influence. Such IGEs on mate preferences can have a broad range of evolutionary consequences, from promoting the maintenance of genetic variation to accelerating evolutionary divergence. Exploration of the patterns that IGEs take in nature will help elucidate their evolutionary consequences. For example, which aspects of social interactions are responsible for the variation that is

generated in, say, mate preferences? I find that, for *Enchenopa* females, experimental manipulation of social groupings has stronger effects than manipulation in the experience of signals alone [32, 33, this study]. What is the cause of these differences? Much can be learned from further exploring the patterns of variation that arise from these social interactions.

ACKNOWLEDGEMENTS

I thank T. Schuck for help with plant care and A. Hallett and M. Al-Wathiqui for help with playbacks. I also thank F. A. Barbosa, P. O. Dunn, K. D. Fowler-Finn, G. Höbel, J. Kilmer, D. Kim, L. A. Whittingham, and two anonymous reviewers for helpful feedback on the manuscript. R.B. Cocroft kindly provided the MATLAB script. This research was funded by NSF grant IOS-0919962 to R.L.R.; and by the Society for the Study of Evolution Rosemary Grant Award, UWM Ruth Walker and James D. Anthony awards, and a UWM Distinguished Graduate Student Fellowship to D.R.

REFERENCES

1. Greenfield M.D. 2002 *Signalers and receivers: mechanisms and evolution of arthropod communication*. New York, New York, Oxford University Press.

2. Danchin É., Giraldeau L.-A., Valone T.J., Wagner R.H. 2004 Public information: from nosy neighbors to cultural evolution. *Science* **305**, 487-491.

3. West-Eberhard M.J. 2003 *Developmental plasticity and evolution*. New York, NY, Oxford University Press.

4. Hebets E.A., Sullivan-Beckers L. 2010 Mate choice and learning. In *Encyclopedia of animal behavior* (eds. Breed M.D., Moore J.), pp. 389-393. Amsterdam, Elsevier B. V.

5. Verzijden M.N., ten Cate C., Servedio M.R., Kozak G.M., Boughman J.W., Svensson E.I. 2012 The impact of learning on sexual selection and speciation. *Trends Ecol Evol* **27**, 511-519.

6. Rodríguez R.L., Rebar D., Fowler-Finn K.D. 2013 The evolution and evolutionary consequences of social plasticity in mate preferences. *Anim Behav* **85**, 1041-1047.

7. Darwin C. 1871 *The descent of man and selection in relation to sex*. London, John Murray.

8. West-Eberhard M.J. 1983 Sexual selection, social competition, and speciation. *Q Rev Biol* **58**, 155-183.

9. Andersson M.B. 1994 *Sexual selection*. Princeton, New Jersey, Princeton University Press.

10. Lyon B.E., Montgomerie R. 2012 Sexual selection is a form of social selection. *Phil Trans Roy Soc B* **367**, 2266-2273.

11. Siepielski A.M., DiBattista J.D., Evans J.A., Carlson S.M. 2011 Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. *Proc R Soc Lond B* **278**, 1572-1580.

12. Coyne J.A., Orr H.A. 2004 *Speciation*. Sunderland, MA, Sinauer Associates.

13. Moore A.J., Brodie III E.D., Wolf J.B. 1997 Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution* **51**, 1352-1362.

14. Wolf J.B., Brodie III E.D., Cheverud J.M., Moore A.J., Wade M.J. 1998 Evolutionary consequences of indirect genetic effects. *Trends Ecol Evol* **13**, 64-69.

15. Wolf J.B., Brodie III E.D., Moore A.J. 1999 Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. *Am Nat* **153**, 254-266.

16. McGlothlin J.W., Moore A.J., Wolf J.B., Brodie III E.D. 2010 Interacting phenotypes and the evolutionary process. III. Social evolution. *Evolution* **64**, 2558-2574.

17. Bailey N.W., Moore A.J. 2012 Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the Fisher process. *Evolution* **66**, 2674-2684.

18. Bleakley B.H., Wolf J.B., Moore A.J. 2010 Evolutionary quantitative genetics of social behaviour. In *Social behaviour: genes, ecology and evolution* (eds. Székely T., Komdeur J., Moore A.J.). Cambridge, Cambridge Univ. Press.

19. Agrawal A.F., Brodie III E.D., Brown J. 2001 Parent-offspring coadaptation and the dual genetic control of maternal care. *Science* **292**, 1710-1712.

20. Kent C., Azanchi R., Smith B., Formosa A., Levine J.D. 2008 Social context influences chemical communication in *D. melanogaster* males. *Curr Biol* **18**, 1384-1389.

21. Bleakley B.H., Brodie III E.D. 2009 Indirect genetic effects influence antipredator behavior in guppies: estimates of the coefficient of interaction psi and the inheritance of reciprocity. *Evolution* **63**, 1796-1806.

22. Danielson-François A.M., Zhou Y., Greenfield M.D. 2009 Indirect genetic effects and the lek paradox: inter-genotypic competition may strengthen genotype x environment interactions and conserve genetic variance. *Genetica* **136**, 27-36.

23. Biscarini F., Bovenhuis H., van der Poel J., Rodenburg T., Jungerius A., van Arendonk J. 2010 Across-line SNP association study for direct and associative effects on feather damage in laying hens. *Behav Genet* **40**, 715-727.

24. Roff D.A. 1997 *Evolutionary quantitative genetics*. New York, NY, Chapman & Hall.

25. Lynch M., Walsh B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA, Sinauer Associates.

26. Cocroft R.B., Rodríguez R.L., Hunt R.E. 2008 Host shifts, the evolution of communication and speciation in the *Enchenopa binotata* species complex of treehoppers. In *Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. Tilmon K.J.), pp. 88-100. Berkeley, CA, University of California Press.

27. Rodríguez R.L., Ramaswamy K., Cocroft R.B. 2006 Evidence that female preferences have shaped signal evolution in a clade of specialized plant-feeding insects. *Proc R Soc Lond B* **273**, 2585-2593.

28. Cocroft R.B., Rodríguez R.L., Hunt R.E. 2010 Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biol J Linn Soc* **99**, 60-72.

29. Rodríguez R.L., Sullivan L.M., Cocroft R.B. 2004 Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Evolution* **58**, 571-578.

30. Hamilton K., Cocroft R.B. 2009 Establishing the identity of existing names in the North American *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Entomol News* **120**, 554-565.

31. Wood T.K. 1993 Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). In *Evolutionary patterns and processes* (eds. Lees D.R., Edwards D.), pp. 299-317. New York, Academic.

32. Wood T.K. 1993 Diversity in the new world Membracidae. *Ann Rev Entomol* **38**, 409-435.

33. Sullivan-Beckers L., Cocroft R.B. 2010 The importance of female choice, male–male competition, and signal transmission as causes of selection on male mating signals. *Evolution* **64**, 3158-3171.

34. Fowler-Finn K.D., Rodríguez R.L. 2012 Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* **66**, 459-468.

35. Fowler-Finn K.D., Rodríguez R.L. 2012 The evolution of experience-mediated plasticity in mate preferences. *J Evol Biol* **25**, 1855-1863.

36. Stinchcombe J.R., group F.-v.t.w., Kirkpatrick M. 2012 Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol Evol* **27**, 637-647.

37. Ritchie M.G. 1996 The shape of female mating preferences. *Proc Natl Acad Sci USA* **93**, 14628-14631.

38. Bailey N.W. 2008 Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. *Behav Ecol* **19**, 960-966.

39. Miller C.W., Moore A.J. 2007 A potential resolution to the lek paradox through indirect genetic effects. *Proc R Soc Lond B* **274**, 1279-1286.

40. Rodríguez R.L., Hallett A.C., Kilmer J.T., Fowler-Finn K.D. 2013 Curves as traits: genetic and environmental variation in mate preference functions. *J Evol Biol* **26**, 434-442.

41. Bijma P., Muir W.M., Van Arendonk J.A.M. 2007 Multilevel selection 1: Quantitative genetics of inheritance and response to selection. *Genetics* **175**, 277-288.

42. Kazancioğlu E., Klug H., Alonzo S.H. 2012 The evolution of social interactions changes predictions about interacting phenotypes. *Evolution* **66**, 2056-2064.

43. Formica V.A., McGlothlin J.W., Wood C.W., Augat M.E., Butterfield R.E., Barnard M.E., Brodie III E.D. 2011 Phenotypic assortment mediates the effect of social selection in a wild beetle population. *Evolution* **65**, 2771-2781.

44. Qvarnström A. 2001 Context-dependent genetic benefits from mate choice. *Trends Ecol Evol* **16**, 5-7.

Data deposited in the Dryad Repository: http://dx.doi.org/10.5061/dryad.nm021

Figure 1. Experimental design to test if the presence of genetic variation in social neighbors influences the mate preferences of focal individuals reared. A sample of full-sib families was used as the social genetic component, and each family was split onto two replicates to separate social genetic effects from environmental ones. Focal individuals were then added, and I described the mate preferences of these focal individuals.

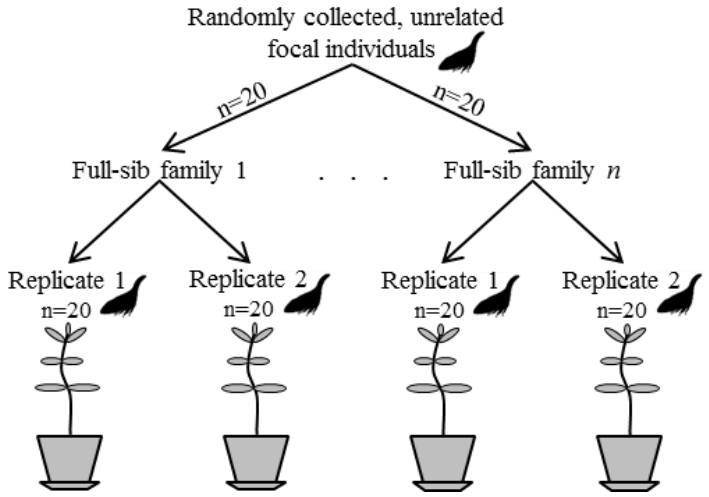

Figure 2. Genetic variation in social influence on the mate preference functions of *Enchenopa* focal females. The preference functions of the two replicates of focal females that developed with 7 full-sibling treatment families are shown. The dotted line represents the mean peak preference in the population.

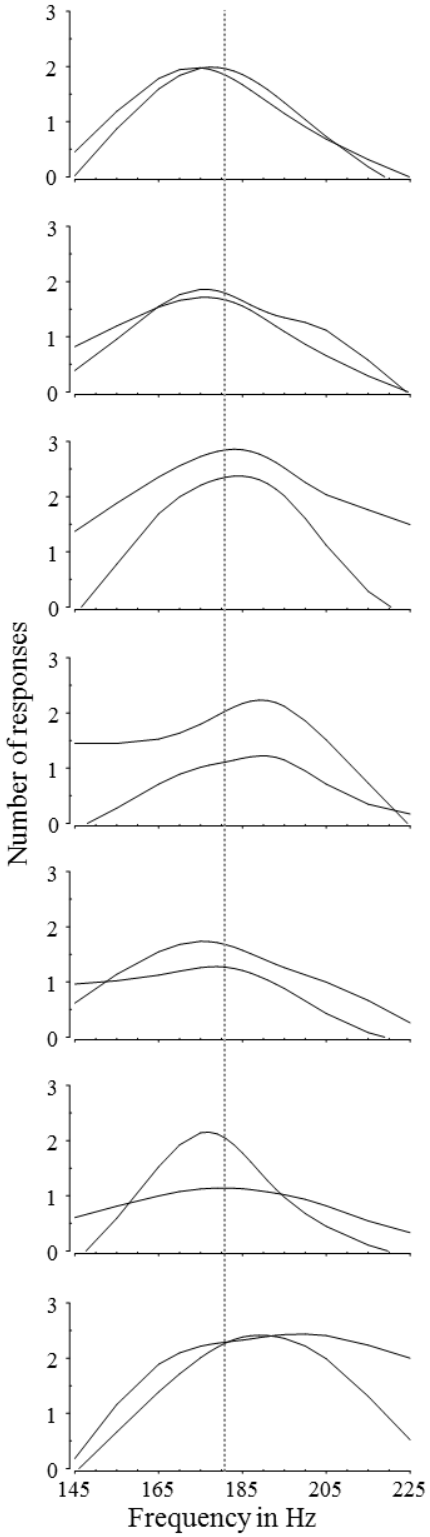

Figure 3. Example of individual preference functions of focal females from two replicates of two representative full-sibling treatment families. The dotted line represents the mean peak preference of all females tested.

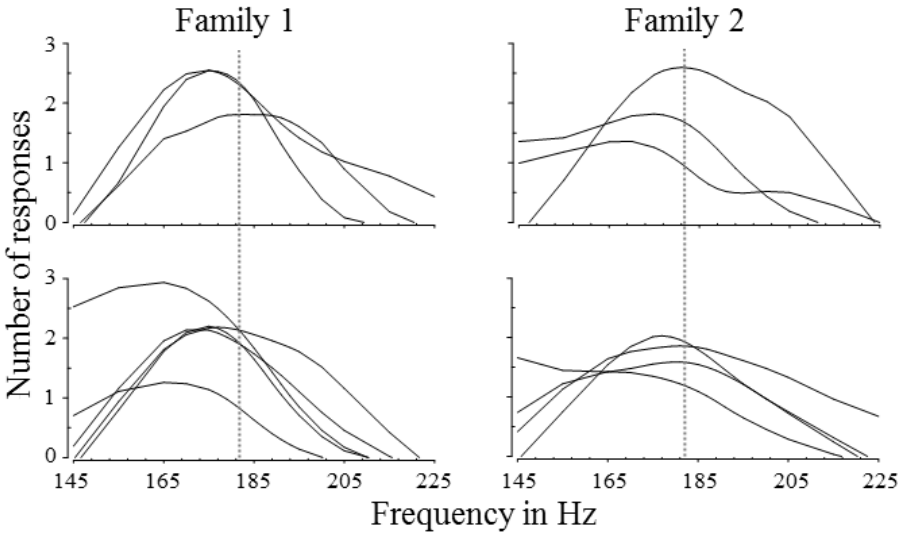

Figure 4. Genetic variation in social influence on two traits describing the mate preference functions of *Enchenopa* focal females. The y-axis for the traits represents the phenotypic range observed in this study for each trait. Means ± SE are displayed for both replicates of focal females reared around each family.

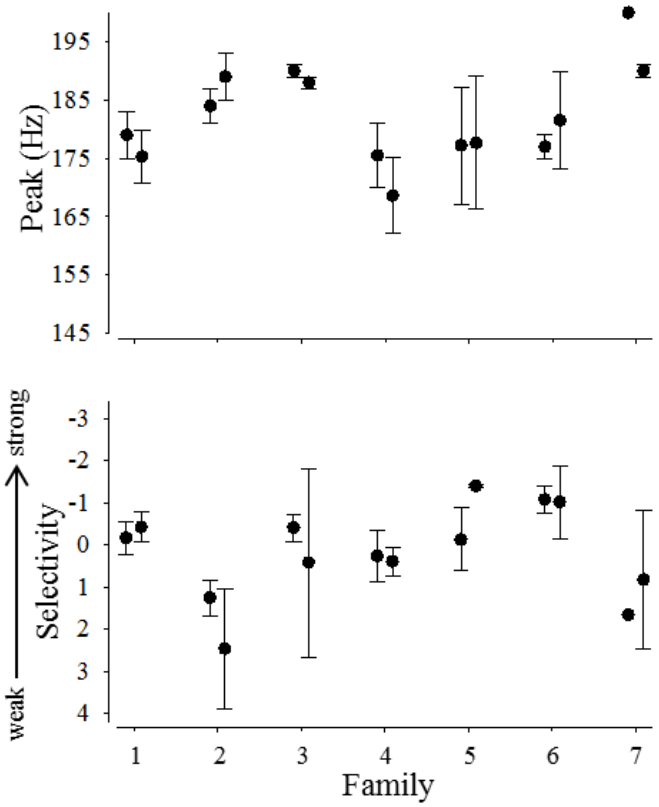

Table 1. Linear mixed-model testing for differences in the shape of the preference functions of focal females. Family, replicate, and individual are random terms, with replicate nested within family and individual nested within replicate and family. The family × quadratic term tests for genetic variation in social influence on the mate preference functions (see Methods). Significant values are in bold.

Source of Variation	df	F	P
Whole model	54, 724	**38.46**	**<0.0001**
Family	6, 7.62	**5.11**	**0.0212**
Replicate [Family]	7, 27	0.67	0.6968
Linear	1, 724	**32.51**	**<0.0001**
Family × Linear	6, 729.99	**9.64**	**<0.0001**
Quadratic	1, 6.25	**113.74**	**<0.0001**
Family × Quadratic	6, 724	**6.17**	**<0.0001**
Individual [Replicate, Family]	27, 724	**26.27**	**<0.0001**

Table 2. Linear mixed-models of social influence on peak preference and on preference selectivity with family and replicate as random terms with replicate nested within family. Significant values are in bold.

Trait	Factor	df	F	P
Peak preference	Family	6, 8.44	**7.85**	**0.0044**
	Replicate	7, 27	0.15	0.993
Preference selectivity	Family	6, 7.41	**5.12**	**0.0223**
	Replicate	7, 27	0.51	0.8192

CHAPTER 2: Trees to treehoppers: genetic variation in host plants contributes to variation in the mating signals of a plant-feeding insect

ABSTRACT

Community genetics research has demonstrated "bottom-up" effects of genetic variation within a plant species in shaping the larger community with which it interacts, such as the composition of arthropod faunas. Here I demonstrate that such cross-trophic interactions also influence sexually-selected traits. I used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). To assess how male mating signals are influenced by host plant genetic variation, I reared a random sample of treehoppers on potted replicates of a sample of host plant clone lines. I found that male signals varied according to the clone line on which they developed, showing that genetic variation in host plants affects the male treehoppers' behavioral phenotypes. This is the first demonstration of cross-trophic indirect genetic effects on sexually-selected traits. I discuss how such effects may play an important role in the maintenance of variation and within-population phenotypic differentiation, thereby promoting evolutionary divergence.

Keywords: indirect genetic effects, developmental plasticity, plant-insect interactions, vibrational signals, laser vibrometry.

INTRODUCTION

Environments are immensely important in shaping the expression of genetic and developmental variation in phenotypes. Environmental causes of phenotypic variation and novelty set the stage for evolutionary change [1], and can thus have a complex relationship with the evolutionary process. For instance, social environments (i.e. conspecific competitors and collaborators) are important sources of variation in fitness for many species [2, 3], and experience with the behavior of other individuals is often an important cause of variation in phenotypes [1, 4, 5]. Similarly, many species spend at least part of their lives on other, heterospecific organisms, as do for example many herbivores, parasites, and parasitoids. Thus, in a very real sense, most organisms' environments fully or partly consist of other organisms.

The key concept arising from the biotic nature of environmental variation is that environments can evolve as a response to direct selection on the individuals that constitute them. In doing so, they can have far-reaching consequences on other phenotypes that they influence [6-8]. For example, genetic variation at the level of the social environment can help sustain genetic variation and promote diversity at level of the phenotypes of individuals that are in that social environment [9-11]. Thus, evolution at one level can influence phenotypic diversity and evolution at another level, and the evolutionary dynamics that occur at different levels of social and ecological interaction are intimately intertwined.

To estimate the potential evolutionary importance of variation in biotic environments that are themselves causes of variation in other organisms, it is necessary to assess the presence and magnitude of genetic variation in the variation-inducing aspects of those environments. When dealing with the effect of conspecific individuals as a component of the social environment, researchers refer to indirect genetic effects (IGEs). IGEs occur when the genes expressed in one individual have an effect on the

phenotype of another conspecific individual [12]. Empirical research on IGEs is only just beginning, but there is evidence that they are taxonomically widespread [9, 13, 14] and that they affect important fitness-related traits, such as maternal provisioning behavior, fecundity, and mate preferences [11, 15, 16].

When dealing with environments that are not social, but instead involve heterospecific individuals, researchers refer to interspecific indirect genetic effects (IIGEs) [17]. Exploration of IIGEs has revealed diverse effects on so-called community phenotypes [8, 18, 19]. There is, for example, considerable evidence that genetic variation within a population of a given tree species has bottom-up effects on the diversity of the insect fauna on the trees [20-22]. Top-down IIGEs have also been detected, whereby genetic variation in parasitoid wasps influences the positioning of their aphid hosts on their host plant and whether they remain on it or not [23]. These findings suggest the question of whether there may be IIGEs on individual phenotypes with strong impacts on fitness, such as sexually-selected traits, which would have the potential to influence population-level dynamics and between-population divergence.

Here I ask whether genetic variation in host plants may influence the mating signals of a plant-feeding insect. If so, genetic variation in plants and other lower-trophic level organisms may influence not only the composition of the communities that are associated with them, but also the evolutionary dynamics of individual species living in those communities.

I develop a method that tests for IIGEs by manipulating genetic variation in a host plant and describing the mating signals of a plant-feeding insect that develops on this plant species. I used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae), a group in which speciation has involved colonization and adaptation to novel host plant species and divergence of their communication systems [24, 25]. These treehoppers spend their entire lives on their host

plants [24] and communicate with plant-borne vibrational signals [25]. Males produce mating signals, and females exhibit strong mate preferences on the basis of the features of those signals, particularly whine length and signal frequency – the latter being the most divergent feature of adult phenotypes in the clade [26-28]. Although in this study I describe variation in mating signals and not in reproductive success, there is evidence that male mating signals are an important determinant of reproductive success [29]. Male signals in the *E. binotata* complex have evolved under selection stemming from mate choice and under sensory drive related to host plant signal-transmission features [27, 30]. They are also an important determinant of behavioural reproductive isolation between the members of the complex [26, 31].

My goal was to ask whether genetic variation in the background biotic environment provided by the treehoppers' host plants contributes to variation in the mating signals of individuals that develop in that environment. I used a quantitative genetics experimental design in which clone lines of a sample of host plant genotypes formed the background environment [32], and randomly-collected insect individuals were reared on those environments. I described the signals of those insects and estimated the variation due to among- and within-clone line components.

I test two hypotheses about the role of cross-trophic interactions in shaping the phenotypes of individuals influenced by those interactions. First, I test whether host plants influence male mating signals. This hypothesis predicts that the mating signals of males will differ across individuals of the host plant. Second, I test the hypothesis that genetic variation in the host plants influences male mating signals (i.e., I test for IIGEs). This hypothesis predicts that there should be an among-clone line effect, indicating that the genetic make-up of the clone lines of host plants contributes to differences in male mating signals.

MATERIAL AND METHODS

Study species

I used one of the two members of the *E. binotata* complex that live on the host plant *Viburnum lentago* (Caprifoliaceae) in my study site (Tendick Nature Park, Saukville, WI, USA). These species have not been formally described, but male signal frequency is a reliable trait in differentiating them, as well as other species in this species complex [26, 28, 33]. I used the high-frequency species found on *V. lentago* (dominant frequency = 312Hz), and I kept voucher specimens in 95% EtOH.

My experiment consisted of a rearing phase and a signal-recording phase. During the rearing phase, I manipulated genetic variation in the developmental environment of a random sample of nymphs by rearing them on different clone lines of their host plant (i.e. by rearing them on an environment with a describable genetic component). I then recorded the mating signals of those males.

Rearing

I established replicated plant clone lines to determine within- and among-clone line effects on the treehoppers. *Viburnum lentago* plants grow in clone patches: a main plant establishes itself and sends out lateral roots that result in suckers sprouting up around the parental plant [34]. The suckers remain connected to the parent plant and each other through lateral roots. I took advantage of this growth feature by digging up evenly sized suckers (0.5 m) surrounding a parental plant from the UWM Field Station (Saukville, WI) in the Fall 2011. I ensured that the suckers were clones of one another by verifying that they were connected by lateral roots. I placed the suckers in moistened peat moss and stored them over winter in a dark cold room maintained at 4 degrees C. The following March 2012, I potted each sucker into a one gallon plastic pot using Fafard 3B

mix (Conrad Fafard, Inc., Agawam, MA). I then moved the potted plants into a greenhouse to promote the onset of budding and subsequent development.

I obtained treehopper individuals by randomly collecting newly emerged nymphs from a large population located at Tendick Nature Park (Saukville, WI) in May 2012. I collected nymphs by cutting stems from various host plants spanning a 100 meter transect. I then transferred 30 individuals onto each potted plant, distributing nymphs from each cut stem across as many clone lines and replicates as possible to minimize the likelihood of relatedness on the same plant or within a clone line (Fig. 5). Individuals were reared together on each plant from the time they were first instars until their adult molt. I recorded signals from all males 2-3 weeks after the adult molt. I was thus able to partition variation in male signal traits among components due to clone lines and within-clone line replicates (Fig. 5).

Signal recording and analysis

I used a single recording plant individual for all males, which was a different genotype from any of the rearing plants. I used only one recording plant to minimize the potential for plant signal-transmission features to influence the measures of signal variation and any other potential influences on the treehoppers' behavior. I note, however, that signal-transmission effects contribute negligible variation to recordings of treehopper male signals, and when present largely reflect the treehoppers' inclination to signal or not on the plant [27, 35, 36].

I placed each male at the same site on the recording stem, and I primed them to signal by playing a recording of a male-female duet through a piezo-electric actuator attached to the stem with accelerometer wax (Thorlabs, NJ, models AE0505D16). The actuator was controlled by a piezo controller (Thorlabs, NJ, model MDT694A) from an iMac computer at an amplitude of 0.10 mm/s. I recorded male signals with a laser

vibrometer (Polytec, Inc., Auburn, MA, model CLV-2534). I focused the laser beam onto a small piece of reflective tape (~2 mm^2) placed on the plant stem. Males were within 10 cm of the reflective tape when they signaled. The signal detected by the laser vibrometer from the stem was sent through a band-pass filter (40-4000 Hz; Krohn-Hite 3202, Krohn-Hite Corporation, Brockton, MA) at 60 Hz. The output was sent to an iMac computer through an Edirol UA-25 USB interface (Roland Corporation, Japan) and recorded with the sound recording software AUDACITY (v. 1.2.5; http://audacity.soundforge.net) at a sampling rate of 44.1 kHz. I monitored male signals with a Hameg HM 504-2 50 MHz oscilloscope (Hameg Instruments, Mainhausen, Germany). To isolate the setup from noise due to building vibrations, the recording plant was placed on shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY) on top of an iron plank (~135 kg) resting on partially inflated bicycle inner tubes on top of a slate table (~1 x 2 m). I also placed vibration dampening pads (Polymer Dynamics, Inc., Allentown, PA, model 3291-22-PM-50) under the table legs to further isolate the entire setup. I randomized recording across and within clone lines over the course of this phase in an attempt to minimize any effects of the differences in age and exposure to other males' signals. All males were recorded in July 2012.

Enchenopa males typically produce bouts of several signals (Fig. 6). I standardized measurements of male traits by selecting the bout of the highest amplitude, and measuring the third signal in the bout. If males produced less than three signals, I measured the last signal in the bout (n=55 of 324 males). Male signals consist of a whine portion followed by several pulses (Fig. 6) [27]. I analyzed variation in seven signal traits that differ among species in the *E. binotata* complex. I measured the interval between signals, length of the whine portion, number and length of the pulses, the pulse rate, and the dominant frequency (Fig. 6). I measured frequency from the last 10 cycles of the

whine portion of the waveform because male signals are relatively pure-tone. I conducted all analyses with AUDACITY.

Statistical analysis

I was interested in analyzing each signal trait separately because they are associated with differently-shaped female mate preference functions, and consequently make different contributions to mate choice decisions, to variation in male reproductive success, and to patterns of reproductive isolation among the members of the *E. binotata* complex [25, 27, 29]. However, this approach increases the chance of spurious significance [37], whilst measures that reduce this risk also reduce statistical power [38, 39]. To deal with this problem, I assessed the degree of non-independence in the data with a principal component analysis on the seven signal traits. This analysis yielded four axes with eigenvalues > 1 (1.49, 1.34, 1.12, and 1.02), each explaining a similar amount of variation in the data (21.32%, 19.11%, 16.00%, and 14.56%, respectively), and with all four axes only 70% of the total variation in male signals was accounted for. This indicates that, in this study, variation in each of the original signal traits was very poorly correlated with variation in the other traits. To confirm this result, I estimated Pearson product-moment correlations between the seven original signal traits, finding that in all cases $r < 0.24$. On the basis of these results, I consider that analyzing the original signal traits separately is justified, as well as evolutionarily relevant. Nevertheless, to allay concerns about spurious significance, I also report the results of the analysis with the four PCA axes.

The aim of the analysis was to assess the contribution of genetic variation in host plants to male signal traits. The replicated clone line design allowed us to partition variation between components for among and within clone lines. I used linear mixed-models to address variation in male signal traits among and within clones. Clone and

replicate nested within clone were random effects. The clone term describes differences in the trait of interest between males reared on the clone lines. The replicate term describes differences between males within the same clone line, and corresponds to within-clone environmental variation plus variation due to social interactions among individuals on each plant. I initially included temperature as a covariate, but it was non-significant for all signal traits and I therefore removed it from the analyses.

To provide an effect size estimate for the influence of the plant clone term on male signal traits in the above analyses (i.e., of the magnitude of the IIGEs), I estimated broad-sense heritability for genetic variation among host plants in the induction of variation in the treehoppers' mating signals. I denote this estimate as H^2_{IIGE}, and obtained it as follows: $H^2_{IIGE} = \sigma^2_{clone} / (\sigma^2_{clone} + \sigma^2_{residual})$. I obtained each of the variance component estimates from the linear mixed-models using the REML method. Note that these estimates correspond to broad-sense heritability because the calculations are based on the among-clone component of variation (σ^2_{clone}; Lynch & Walsh 1998). This among-clone component of variation contains both additive and nonadditive (dominance, epistasis and common environmental effects) genetic variation, and therefore likely overestimates narrow-sense heritability [32]. Significance for the test of the hypothesis that $H^2_{IIGE} > 0$ is provided by the clone term in the above linear-mixed models. In addition, I calculated the standard error for each H^2_{IIGE} estimate. As there is no precedent to follow, I adopted the procedure for typical broad-sense heritability with weighted clone line samples [40, p. 42]. I performed all statistical analyses in JMP v. 7.0 (SAS Institute Inc., Cary, NC).

I only included in the analysis clone lines that had at least three replicates; i.e., that were represented by at least three plant individuals on which treehoppers were reared, and from each of which at least two males were recorded. This yielded a sample of 12 clone lines, each with a mean of 4.8 replicates (range = 3-6), each of which had a

mean of 5.7 treehopper males recorded (range = 2-10). The total sample of treehopper males contributing signals to the analysis was n = 324.

RESULTS

I found a cross-trophic component of variation to male treehopper mating signals. There was significant genetic variation (among host plant clone lines) in this cross-trophic influence for four of the seven signal traits (Fig. 7, Table 3). That is to say, I detected significant cross-trophic IIGEs on the insects' mating signals. Each of the four PCA axes also showed significant genetic variation in cross-trophic influence (Table 4). The broad-sense heritability estimates for genetic variation in the influence of the host plants on those four signal traits (H^2_{IIGE}) did not overlap zero (Table 3). In particular, the signal traits that most contribute to mate choice decisions [whine length and signal frequency; 27, 28] were influenced by these IIGEs (Fig. 7, Table 3).

I also found significant variation within clone lines. In total, five of the seven measured signal traits were influenced by among-replicate within-clone variance, including three of the four signal traits for which there is an among-clone line effect (Fig. 7, Tables 3, 4).

DISCUSSION

Here I demonstrate the presence of cross-trophic IIGEs on a sexually-selected trait. By manipulating genetic variation in host plants through the use of replicated clone lines, I was able to ask whether variation in the mating signals of insect individuals that developed on the plants was influenced by among-clone differences or other environmental effects. I demonstrate that among-clone variation in cross-trophic interactions influences several signal traits, with additional within-clone components of variation. Although I did not measure reproductive success associated with this induced

variation in male signal traits, there is strong evidence that male mating signals in the *E. binotata* complex are a main determinant of mating success [29], and that they have evolved under strong selection arising from mate choice [26, 27]. Consequently, the cross-trophic IIGEs on mating signals that I detect are likely to have important consequences for the course of evolutionary processes in the treehoppers' populations.

Understanding the evolutionary impact of cross-trophic IIGEs on fitness-related traits will first require addressing the proximate causes of such effects. For instance, which aspects of the phenotype of the host plant clones vary with their genotypes in such a way as to induce the patterns of variation in male signals that I detect? Are plant defensive compounds involved? Are plants selected to induce such variation? (Given the finding of genetic variation in this induction, such selection would likely be effective.) And, are plant-feeding insects in turn adapted to compensate for such influences? Although I did not test for genotype (treehopper) × genotype (host plant) interactions in this study, there is evidence of genetic variation in the plastic response by *Enchenopa* mating signals to the developmental environment represented by different host plant species [41]. There is also evidence of genetic variation in the plastic response (by ladybird beetle predators) to indirect ecological effects (IEEs) arising from aphids reared on different host plant species and subsequently consumed by the beetles [42]. Beginning to ask such questions will illuminate how IIGEs arise and evolve under selection at different levels of trophic interactions.

Regardless of how they may arise, the presence of such IIGEs adds an important dimension to interactions between conspecifics, heterospecifics, and the environment. In the case of *Enchenopa* treehoppers, for instance, evolution in their host plants (e.g., a change in the patterns of genetic variation in the host plants as their population responds to selection) is likely to change not only their habitat but also the expression of phenotypic variation in the treehoppers' mating signals. IIGEs on male mating signals

may, in turn, influence the dynamics of sexual selection in treehopper populations in a variety of ways. They may, for instance, contribute to the maintenance of variation in traits that are under strong selection. Recall that signal frequency is the most divergent adult trait among the members of the *E. binotata* complex, and is subject to strong sexual selection due to female mate choice [27, 28]. With IIGEs influencing signal frequency (and other signal traits), females choosing a male of a given phenotype on different host plants (e.g., different clone clusters) may be favoring different underlying male genotypes. Further, as with males, genetic variation in host plants may influence the expression of female mate preferences, and further impact the dynamics of sexual selection. One result may be that genetic variation in male signaling traits is sustained, which in turn may help fuel ongoing sexual selection. Another consequence of cross-trophic IIGEs may be to influence the patterns of gene flow within and among populations. Depending on the presence and form of IIGEs on female mate preferences, gene flow between individuals developing on genetically-varied host plants may be restricted by variation in male signals due to IIGEs, potentially initiating divergence from within a population [cf. 10].

More broadly, the demonstration of bottom-up cross-trophic IIGEs on mating signals, together with their potential consequences on the dynamics of sexual selection, adds a new dimension to how biologists view the process of ecological speciation. Ecological speciation occurs when adaptation to using novel environments or resources produces not only ecological divergence but also reproductively isolated populations [43-45]. In the context of this study, the colonization of novel environments — in the form of host plant shifts — plays a major role in the process of speciation of plant-feeding insects, which constitute a large fraction of the biodiversity of many communities [25, 46, 47]. These findings suggest that not only changes in the species of host plant used by the insects, but also which plant genotypes, plant phenotypes and even plant individuals are used, may be important. Research about ecological speciation and speciation by sexual

selection will benefit from incorporating considerations of the contributions of IIGEs to the evolution of reproductive isolation.

In addition to the among-clone component of variation, I found significant within-clone effects on multiple male signal traits. This component of variation may include within-clone variation in the effects of developing on different plant individuals, as well as the effects of shared social environments for the insects developing on each plant individual. Recent work has shown social IGEs on *Enchenopa* female mate preferences [11] and male signaling traits (D Rebar unpublished data), along with plasticity in mate preferences arising from social experience [48-50]. Social and cross-trophic influences may constructively interact with one another, such as by shifting male signal traits in the same direction, thus amplifying the phenotypic variation in male traits. However, social and cross-trophic influences may also negatively interact with one another, resulting in less phenotypic variation for individuals on that plant.

In conclusion, here I show that cross-trophic interactions influence variation in the mating signals of an insect, and there is a significant component of genetic variation to this cross-trophic influence. The presence of such IIGEs has broad evolutionary implications, from the maintenance of variation to the promotion of divergence. Cross-trophic IIGEs may prove pivotal in creating and sustaining the variation upon which selection can act, and those effects may in turn be influenced by selection at other trophic levels.

ACKNOWLEDGEMENTS

I thank D. Cruz and T. Schuck for help with plant care and D.A. Gray for the idea of using naturally-occurring clones. I also thank E. Latch for fruitful discussions and F. Barbosa for helpful feedback on the manuscript. This research was funded by NSF grants IOS-0919962 to R.L.R. and IOS-1120790 to R.L.R. and K.D. Fowler-Finn; and by

a Rosemary Grant Award from the Society for the Study of Evolution, UWM Ruth Walker and James D. Anthony awards, and a UWM Dissertator Fellowship to D.R.

REFERENCES

1. West-Eberhard M.J. 2003 *Developmental plasticity and evolution*. New York, NY, Oxford University Press.

2. West-Eberhard M.J. 1983 Sexual selection, social competition, and speciation. *Q Rev Biol* **58**, 155-183.

3. Hereford J., Hansen T.F., Houle D. 2004 Comparing strengths of directional selection: how strong is strong? *Evolution* **58**, 2133-2143.

4. Verzijden M.N., ten Cate C., Servedio M.R., Kozak G.M., Boughman J.W., Svensson E.I. 2012 The impact of learning on sexual selection and speciation. *Trends Ecol Evol* **27**, 511-519.

5. Rodríguez R.L., Rebar D., Fowler-Finn K.D. 2013 The evolution and evolutionary consequences of social plasticity in mate preferences. *Anim Behav* **85**, 1041-1047.

6. Wolf J.B., Brodie III E.D., Moore A.J. 1999 Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. *Am Nat* **153**, 254-266.

7. Shuster S.M., Lonsdorf E.V., Wimp G.M., Bailey J.K., Whitham T.G. 2006 Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution* **60**, 991-1003.

8. Hughes A.R., Inouye B.D., Johnson M.T.J., Underwood N., Vellend M. 2008 Ecological consequences of genetic diversity. *Ecol Lett* **11**, 609-623.

9. Danielson-François A.M., Zhou Y., Greenfield M.D. 2009 Indirect genetic effects and the lek paradox: inter-genotypic competition may strengthen genotype x environment interactions and conserve genetic variance. *Genetica* **136**, 27-36.

10. Bailey N.W., Moore A.J. 2012 Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the Fisher process. *Evolution* **66**, 2674-2684.

11. Rebar D., Rodríguez R.L. 2013 Genetic variation in social influence on mate preferences. *Proc R Soc Lond B* **280**, 20130803.

12. Moore A.J., Brodie III E.D., Wolf J.B. 1997 Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution* **51**, 1352-1362.

13. Kent C., Azanchi R., Smith B., Formosa A., Levine J.D. 2008 Social context influences chemical communication in *D. melanogaster* males. *Curr Biol* **18**, 1384-1389.

14. Bleakley B.H., Brodie III E.D. 2009 Indirect genetic effects influence antipredator behavior in guppies: estimates of the coefficient of interaction psi and the inheritance of reciprocity. *Evolution* **63**, 1796-1806.

15. Wade M.J. 2000 Epistasis as a genetic constraint within populations and an accelerant of adaptive divergence among them. In *Epistasis and the evolutionary process* (eds. Wolf J.B., Brodie III E.D., Wade M.J.), pp. 213-231. New York, Oxford University Press.

16. Agrawal A.F., Brodie III E.D., Brown J. 2001 Parent-offspring coadaptation and the dual genetic control of maternal care. *Science* **292**, 1710-1712.

17. Rowntree J.K., Shuker D.M., Preziosi R.F. 2011 Forward from the crossroads of ecology and evolution. *Philos Trans R Soc B* **366**, 1322-1328.

18. Whitham T.G., Bailey J.K., Schweitzer J.A., Shuster S.M., Bangert R.K., LeRoy C.J., Lonsdorf E.V., Allan G.J., DiFazio S.P., Potts B.M., et al. 2006 A framework for community and ecosystem genetics: from genes to ecosystems. *Nat Rev Genet* **7**, 510-523.

19. Bailey J.K., Schweitzer J.A., Úbeda F., Koricheva J., LeRoy C.J., Madritch M.D., Rehill B.J., Bangert R.K., Fischer D.G., Allan G.J., et al. 2009 From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philos Trans Roy Soc B* **364**, 1607-1616.

20. Johnson M.T.J., Lajeunesse M.J., Agrawal A.A. 2006 Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol Lett* **9**, 24-34.

21. Zytynska S.E., Fay M.F., Penney D., Preziosi R.F. 2011 Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Philos Trans R Soc B* **366**, 1329-1336.

22. Moreira X., Mooney K.A. 2013 Influence of plant genetic diversity on interactions between higher trophic levels. *Biol Lett* **9**(3).

23. Khudr M.S., Oldekop J.A., Shuker D.M., Preziosi R.F. 2013 Parasitoid wasps influence where aphids die via an interspecific indirect genetic effect. *Biol Lett* **9**, 20121151.

24. Wood T.K. 1993 Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). In *Evolutionary patterns and processes* (eds. Lees D.R., Edwards D.), pp. 299-317. New York, Academic.

25. Cocroft R.B., Rodríguez R.L., Hunt R.E. 2008 Host shifts, the evolution of communication and speciation in the *Enchenopa binotata* species complex of treehoppers. In *Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. Tilmon K.J.), pp. 88-100. Berkeley, CA, University of California Press.

26. Rodríguez R.L., Sullivan L.M., Cocroft R.B. 2004 Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Evolution* **58**, 571-578.

27. Rodríguez R.L., Ramaswamy K., Cocroft R.B. 2006 Evidence that female preferences have shaped signal evolution in a clade of specialized plant-feeding insects. *Proc R Soc Lond B* **273**, 2585-2593.

28. Cocroft R.B., Rodríguez R.L., Hunt R.E. 2010 Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biol J Linn Soc* **99**, 60-72.

29. Sullivan-Beckers L., Cocroft R.B. 2010 The importance of female choice, male–male competition, and signal transmission as causes of selection on male mating signals. *Evolution* **64**, 3158-3171.

30. McNett G.D., Cocroft R.B. 2008 Host shifts favor vibrational signal divergence in Enchenopa binotata treehoppers. *Behav Ecol* **19**, 650-656.

31. Wood T.K. 1980 Divergence in the *Enchenopa binotata* Say complex (Homoptera: Membracidae) effects by host plant adaptation. *Evolution* **34**, 147-160.

32. Lynch M., Walsh B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA, Sinauer Associates.

33. Hamilton K., Cocroft R.B. 2009 Establishing the identity of existing names in the North American *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Entomol News* **120**, 554-565.

34. Niering W.A., Dreyer G.D., Egler F.E., Anderson J.P. 1986 Stability of a *Viburnum lentago* shrub community after 30 years. *Bull Torr Bot Club* **113**, 23-27.

35. Sattman D.A., Cocroft R.B. 2003 Phenotypic plasticity and repeatability in the mating signals of *Enchenopa* treehoppers, with implications for reduced gene flow among host-shifted populations. *Ethology* **109**, 981-994.

36. Cocroft R.B., Shugart H.J., Konrad K.T., Tibbs K. 2006 Variation in plant substrates and its consequences for insect vibrational communication. *Ethology* **112**, 779-789.

37. Rice W.R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

38. Moran M.D. 2003 Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**, 403-405.

39. Nakagawa S. 2004 A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav Ecol* **15**, 1044-1045.

40. Roff D.A. 1997 *Evolutionary quantitative genetics*. New York, NY, Chapman & Hall.

41. Rodríguez R.L., Sullivan L.M., Snyder R.L., Cocroft R.B. 2008 Host shifts and the beginning of signal divergence. *Evolution* **62**, 12-20.

42. Astles P.A., Moore A.J., Preziosi R.F. 2005 Genetic variation in response to an indirect ecological effect. *Proc R Soc Lond B* **272**, 2577-2581.

43. Rundle H.D., Nosil P. 2005 Ecological speciation. *Ecol Lett* **8**, 336-352.

44. Schluter D. 2009 Evidence for ecological speciation and its alternative. *Science* **323**, 737-741.

45. Nosil P. 2012 *Ecological speciation*. Oxford, Oxford University Press.

46. Coley P.D., Barone J.A. 1996 Herbivory and plant defenses in tropical forests. *Ann Rev Ecol Syst* **27**, 305-335.

47. Drès M., Mallet J. 2002 Host races in plant–feeding insects and their importance in sympatric speciation. *Phil Trans Roy Soc B* **357**, 471-492.

48. Fowler-Finn K.D., Rodríguez R.L. 2012 The evolution of experience-mediated plasticity in mate preferences. *J Evol Biol* **25**, 1855-1863.

49. Fowler-Finn K.D., Rodríguez R.L. 2012 Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* **66**, 459-468.

50. Rodríguez R.L., Hallett A.C., Kilmer J.T., Fowler-Finn K.D. 2013 Curves as traits: genetic and environmental variation in mate preference functions. *J Evol Biol* **26**, 434-442.

Figure 5. Experimental design to test if genetic variation in host plants influences the mating signals of treehopper individuals reared on them. Clones were used as the genetic component, with at least three plant individuals as replicates for each clone. Randomly collected, unrelated treehopper individuals were reared on those plants, and I assessed variation in their mating signals according to among- and within-clone components.

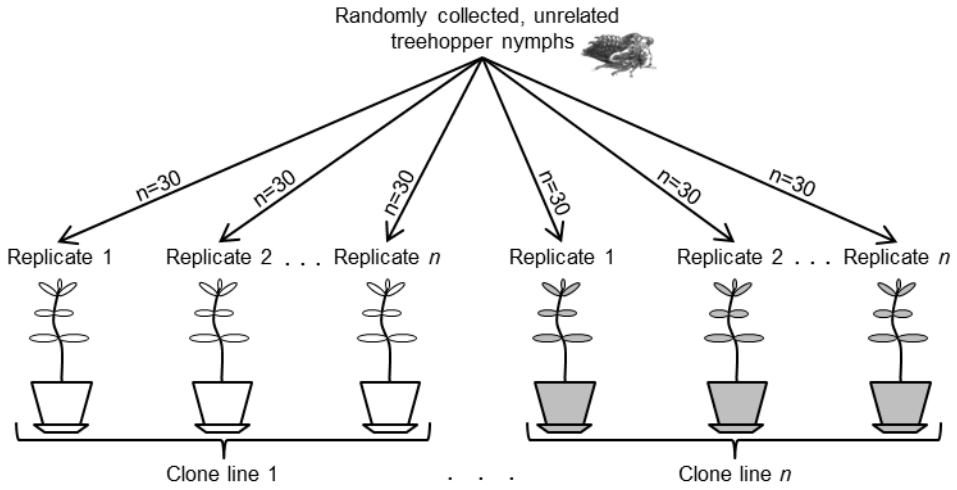

Figure 6. Depiction of a bout consisting of four signals that increase in amplitude, along with close-ups of the waveform of a signal produced by male *E. binotata*.

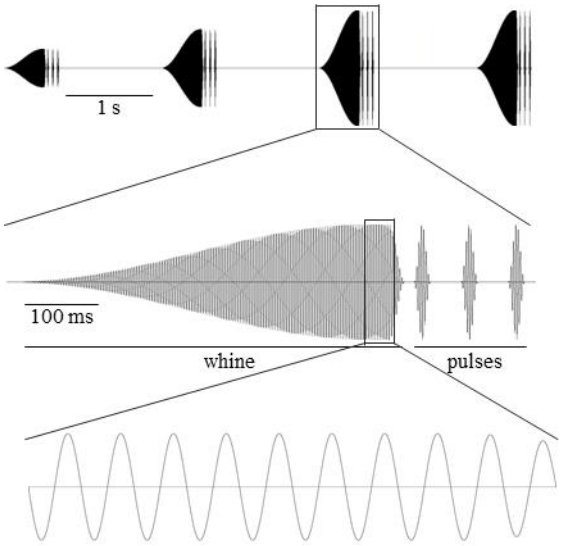

Figure 7. Clone means ± 1 SE (left column, closed circles) and replicate means (right column, open circles) for the seven treehopper male signal traits analyzed across the 12 clone lines. The y-axis represents the range of phenotypic variation in each male trait in the study population.

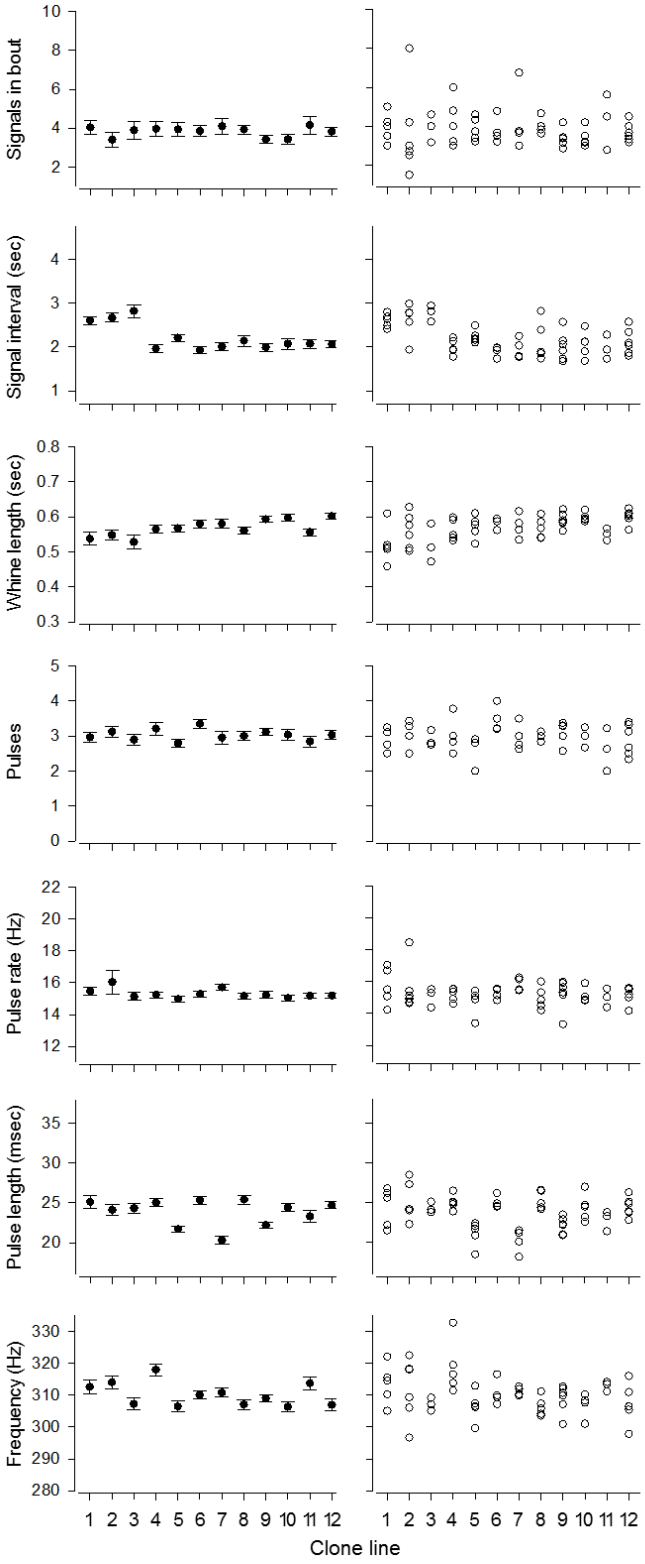

Table 3. Variation in *Enchenopa* male signal traits attributed to differences among-clone lines and within-clone lines (replicates), along with estimates for the variance components and for the heritability of the influence of host plants on male signal traits, H^2_{IIGE}. Significant tests and estimates are in bold.

Trait	Factor	df	F	P	Var. Comp.	$H^2_{IIGE} \pm$ SE
Signals in bout	Clone	11, 51.80	0.53	0.873	-0.054	-0.02 ± 0.02
	Replicate	45, 267	**1.98**	**0.0005**	**0.298**	
	Residual				2.630	
Signal interval	Clone	11, 54.30	**4.98**	**<0.0001**	**0.074**	**0.22 ± 0.11**
	Replicate	45, 267	**1.46**	**0.036**	**0.024**	
	Residual				0.260	
Whine length	Clone	11, 52.55	**2.72**	**0.0074**	**0.00036**	**0.10 ± 0.07**
	Replicate	45, 267	**1.79**	**0.0027**	**0.00048**	
	Residual				0.00337	
Pulses	Clone	11, 55.70	1.46	0.174	-0.0006	-0.001 ± 0.03
	Replicate	45, 267	1.28	0.123	0.0242	
	Residual				0.5331	
Pulse rate	Clone	11, 53.72	0.62	0.807	-0.062	-0.03 ± 0.02
	Replicate	45, 267	**1.65**	**0.0085**	**0.262**	
	Residual				2.039	
Pulse length	Clone	11, 54.91	**6.24**	**<0.0001**	**0.00000230**	**0.23 ± 0.11**
	Replicate	45, 267	1.38	0.066	0.00000038	
	Residual				0.00000771	
Frequency	Clone	11, 53.79	**2.45**	**0.0147**	**9.187**	**0.11 ± 0.07**
	Replicate	45, 267	**1.55**	**0.0197**	**5.084**	
	Residual				76.740	

Table 4. Principal components analysis of the 7 measured *Enchenopa* male signal traits, and variation in each component attributed to differences among-clone lines and within-clone lines (replicates). PVE denotes the per cent of variance explained by the corresponding principal component. Significant tests and estimates are in bold.

Trait	Eigenvalue	PVE	Factor	df	F	P
PC1	1.49	21.32	Clone	11, 266	**2.26**	**0.012**
			Replicate	45, 266	**2.48**	**<0.001**
PC2	1.34	19.11	Clone	11, 266	**5.11**	**<0.001**
			Replicate	45, 266	1.25	0.147
PC3	1.12	16.00	Clone	11, 266	**1.90**	**0.039**
			Replicate	45, 266	**1.69**	**0.006**
PC4	1.02	14.56	Clone	11, 266	**11.55**	**<0.001**
			Replicate	45, 266	1.02	0.442

CHAPTER 3: Genetic variation in host plants contributes to phenotypic variation in the mate preferences of a plant-feeding insect

ABSTRACT

Many species spend their lives in close association with other organisms, and the biotic nature of environments has important consequences for its role as a cause of variation in phenotypes. When the environment consists of organisms, genotypes expressed in individuals constituting the environment may influence the phenotypes of individuals living in that environment. When these effects are between heterospecifics, interspecific indirect genetic effects (IIGEs) occur. Several studies have detected IIGEs, but whether IIGEs contribute to variation in sexually-selected traits remains virtually unexplored. I assessed how mate preferences in a plant-feeding insect are influenced by genetic variation in their host plant. I established clone lines of a sample of host plant genotypes constituting the background biotic environment, and reared a random sample of insects on them. I found that the insects' mate preferences varied according to the clone line on which they developed, demonstrating that genetic variation in host plants has cross-trophic consequences on a trait with strong effects on fitness and inter-population dynamics such as gene flow and diversification in communication systems. I discuss how IIGEs on mate preferences may influence the way in which selection acts, including the maintenance of variation and the promotion of evolutionary divergence.

Keywords: indirect genetic effects, preference functions, plant-insect interactions, developmental plasticity, vibrational communication, laser vibrometry

INTRODUCTION

Many species spend part or all of their lives in close association with other organisms, interacting with them in various ways. Herbivores, parasites, and symbionts, for instance, spend considerable portions of their lives in intimate contact with, if not wholly on or in, the organisms that constitute their resources. Biologists have long been aware of the biotic nature of many types of environments, and of the potential for an evolutionary back-and-forth between the participants [1-4]. Only recently, however, have we started to explore the implications of the biotic nature of many environments for the role that they play as causes of variation in phenotypes and fitness. Environmental causes of variation have a prominent role in diverse evolutionary processes. They may, for instance, be highly effective in promoting and initiating divergence [5, 6], in sustaining variation under selection [7, 8], and in shaping the patterns of mate choice and reproductive isolation between populations [9-12]. As biologists explore these and other roles of biotic environments, familiar evolutionary topics acquire entirely new complexions.

When the environment consists of organisms, environmental causes of variation themselves have genetic and environmental components of variation. Consequently, the genotypes expressed in the individuals that constitute the environment may influence the phenotypes of the individuals in that environment. Such effects are termed indirect genetic effects (IGEs) when they occur among conspecifics [13-15], and interspecific indirect genetic effects (IIGEs) when they occur among heterospecifics [16-18]. The evolutionary significance of these effects is diverse. At the most basic level, the patterns of genetic variation in the environment can influence the patterns of phenotypic variation pertaining to the individuals influenced by that environment [13-15, 19]. Further, the evolutionary consequences of environmental variation can be influenced by evolutionary processes at the level of the environment. For instance, environments can

evolve as a response to selection on the constituent individuals, thereby impacting the expression of the phenotypes of the organisms in those environments, as well as their ecological and evolutionary trajectories [14].

To assess the evolutionary significance of variation in the environment and the resulting IGEs and IIGEs, a crucial question is the extent to which fitness-related traits are affected by these causes of variation. IGEs have been found in taxonomically diverse case studies and for traits such as maternal provisioning, mating signals and mate preferences, and fecundity [20-23]. IIGEs have been detected as bottom-up effects on so-called community phenotypes, such as the composition of the arthropod fauna on trees [16-18, 24]. In addition, top-down IIGEs have been detected on the behavior of aphids infected by parasitoid wasps [25]. However, a focus on sexually-selected traits has been lacking. Recently, I reported IIGEs on the mating signals of a plant-feeding insect [Chapter 2; 26]. This finding has important potential consequences for the dynamics of sexual selection and gene flow within and between populations of this species. These consequences would depend, in part, on the presence of corresponding IIGEs on mate preferences.

Here I report for the first time the finding of IIGEs on mate preferences. I implement a simple framework for disentangling whether genetic variation in the biotic environment contributes to variation in individual phenotypes. Specifically I ask if genetic variation in host plants influences the mate preferences of a plant-feeding insect. I used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Each member of this complex specializes on one species of host plant. Host plant shifts have played an integral part in the process of speciation in this complex, as well as in the divergence of their communication systems [27, 28]. These insects develop entirely on their host plants [28]. They use plant-borne vibrational signals to communicate with one another and pair formation occurs through male-female signalling

duets [27]. Females in the species complex exhibit strong preferences for some aspects of male signals, particularly signal frequency [29, 30], which is the most divergent adult trait among members of the species complex [29-31]. Female mate preferences for signal frequency are unimodal (or "closed"; i.e., they favor intermediate frequency values), and females from different species in the complex favor different signal frequencies [29, 31]. Female mate preferences for male signal frequency are thus quite important in maintaining reproductive isolation between members of the complex.

I explore these cross-trophic interactions through a novel implementation of a classic quantitative genetics design. I used clone lines of a sample of host plant genotypes as the background biotic environment [32], and reared randomly-collected insect individuals on those environments. I then described the mate preferences of those individuals, and estimated the variation in mate preferences that could be attributed to among- and within-clone line components.

I test a hypothesis about the role of cross-trophic interactions on individual phenotypes. I ask whether genetic variation in host plants influences female mate preferences (i.e., whether there are detectable IIGEs on the mate preferences of the insects that develop on those plants). This hypothesis predicts that female mate preferences will vary due to genetic differences among host plants. I should thus detect an among-clone line effect on female mate preferences, which would indicate that differences in the genetic make-up of the clone lines of host plants are contributing to differences in the female mate preferences. I first reared a random sample of female nymphs in environments with a describable genetic component: different clone lines of their host plant. This methodology allowed us to manipulate genetic variation in the developmental environment of females. I then described variation in female preferences for male signal frequency with laser vibrometry and vibrational playback experiments. I quantified their responses by creating mate preference functions [22, 33-35], and assessed

differences in the curvilinearity of the preference functions across clone lines through a clone × quadratic stimulus frequency interaction term.

METHODS

Study species

There are two members of the *E. binotata* complex that live on the host plant *Viburnum lentago* (Caprifoliaceae) at my field site (Tendick Nature Park, Saukville, WI, USA). While these species await formal description, male signal frequency is a reliable trait in identifying them. I used the high-frequency species found on *V. lentago* (dominant frequency = 312Hz), and I kept voucher specimens in 95% EtOH.

Rearing experiment

I established different replicated plant clone lines to determine within- and between-clone line effects on the mate preferences of *E. binotata* treehoppers. *Viburnum lentago* plants grow in clone patches, with an established plant sending out lateral roots just below the soil surface. These lateral roots result in new plants, known as suckers, sprouting up around the main plant, and continue to share the same root system [36]. I dug up evenly sized suckers (0.5 m) surrounding a parental plant from spatially-separated clone patches at the UWM Field Station (Saukville, WI) in the Fall 2011. I ensured that the suckers coming from each clone patch were connected via lateral roots, thus creating the replicates for each clone line. I over wintered the suckers in moistened peat moss in a dark cold room maintained at 4 degrees C. The following March 2012, I potted each sucker with Fafard 3B mix (Conrad Fafard, Inc., Agawam, MA) into a one gallon plastic pot. I moved the plants into a greenhouse to promote the onset of budding and subsequent development.

I obtained treehopper individuals by randomly collecting newly emerged nymphs from a large population of *E. binotata* located at Tendick Nature Park (Saukville, WI) in May 2012. The two species live in microallopatry at that site, and I collected nymphs by cutting stems from various host plants spanning a 100 meter transect that contains only the high frequency species. I then transferred 30 individuals onto each potted plant, distributing nymphs from each cut stem across as many clone lines and replicates as possible to minimize the likelihood of relatedness on the same plant or within a clone line (Fig. 8). Individuals were reared together on a plant from the time they were first instars until their adult molt. I removed the males two weeks later, leaving only the females on each plant. I then described the mate preferences of each female upon becoming sexually receptive, approximately 3-4 weeks after the males were removed.

Measuring variation in mate preferences

I took advantage of the duetting behavior of *Enchenopa* used for pair formation to assay female responses. Males produce a mating signal, and a female will respond with her own vibrational signal if she finds that signal attractive [29, 31]. A female's likelihood in responding is strongly correlated with the number of responses she gives in response to the signalling bout of a male [31, 34, 35, 37]. Therefore, the number of times a female responds to a male's signal is a reliable indicator of that signal's attractiveness. I described each female's mate preference by presenting her with a range of male signal frequencies, quantifying her responses to the signals across the range to create her mate preference function [29, 33]. I assessed female responses to male signal frequency, testing them beyond the natural population frequency range in both directions through playbacks of synthesized male signals. I set all other features of the signals to the population mean. I presented each signal as a bout of four, the mean number of signals for males in this population. Signals were separated by 15 seconds of silence. I randomized testing across

and within clone lines over the course of the testing phase in an attempt to minimize any effects of the differences in maturation and responsiveness to male signals by focal females. I presented females with randomly generated sequences of male signals. Signals were 2, 4, 6, 8, 10, 15, 20, 30 and 40 Hz different in each direction from the mean (312 Hz). As a result, I presented each female with 19 different playback stimuli. I created and delivered all synthetic stimuli using a custom MATLAB script (script available upon request). The stimuli were delivered to the plant stem through a piezo-electric actuator that was attached to the stem with accelerometer wax (Thorlabs, NJ, models AE0505D16). The actuator was controlled by a piezo controller (Thorlabs, NJ, model MDT694A) from an iMac computer at an amplitude of 0.10 mm/s. I recorded the stimuli and female responses using a laser vibrometer (Polytec, Inc., Auburn, MA, model CLV-2534) connected to a second iMac computer using the sound recording software AUDACITY at a sampling rate of 44.1 kHz. I isolated the setup from noise due to building vibrations by placing it on shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY) on top of an iron plank (~135 kg) resting on partially inflated bicycle inner tubes on top of a slate table (~1 x 2 m). I also placed vibration dampening pads (Polymer Dynamics, Inc., Allentown, PA, model 3291-22-PM-50) under the table legs to further isolate the entire setup. I recorded female responses using AUDACITY (v. 1.2.5; http://audacity.soundforge.net). I then listened to the recordings to verify the number of female responses to each presented signal (0-4 responses). All females were recorded from July – August 2012.

I first tested a female's receptivity by playing back a live male recording to her. Females that responded to this signal were then presented with a randomized sequence of 19 signal models. If a female failed to respond to the live male recording, I returned her to her rearing plant and tested her at a later date. Some females stopped responding during the playback sequence. I replayed the live male recording at the end of the

sequence to those females, considering a female still receptive if she responded. I thus included females that responded in the dataset, and excluded those females that failed to respond to the live recording (n=40).

I included in the final analysis only those clone lines that were represented by at least three replicates; i.e., that had at least three individual plants on which treehoppers were reared, and from which at least two females were receptive. This resulted in a sample of 10 clone lines. Each clone line had a mean of 4 replicates (range = 3-5), with each replicate having a mean of 4.0 receptive treehopper females (range = 2-9). In total, 158 treehopper females contributed one preference function each to the analysis.

Description of female preference functions

Mate preferences are function-valued traits [34, 35, 38, 39], meaning that the responses of a female are a function of the mate signals that she encounters. I constructed preference functions with non-parametric regression by generating cubic splines using the mgcv package and a custom-written script in R (v. 2.13.2; http://www.r-project.org). Cubic splines make no assumption about the shape of the preference other than it is smooth in nature. I allowed the program to choose the smoothing parameter for each individual preference function. However, for 15 females I manually adjusted the smoothing parameter to decrease smoothness because these splines appeared as almost straight lines. This adjustment did not qualitatively change my results.

I described variation in female mate preferences in terms of peak preference and preference selectivity. Peak preference represents the signal trait value (in this study, frequency) that elicits the greatest response from a female. Selectivity describes how a female disfavors male signals as they deviate from her peak preference [34, 40]. I derived preference selectivity from measurements of responsiveness, tolerance, and strength [34,

35], as follows. Responsiveness describes the overall elevation of the curves, tolerance describes the shape of the curves as they fall away from peak preference, and strength describes the steepness of the curve's descent from peak preference [34, 35, 40, 41]. These three measurements are strongly correlated, so I performed a principal component analysis (PCA) to generate the composite trait I call preference selectivity. This first principal component had an eigenvalue of 2.10 that explained 69.07 per cent of the variance, with responsiveness, tolerance, and strength loading similarly on this axis (0.53, 0.61, and -0.58, respectively).

Statistical analyses

I adopted a function-valued approach to describe variation in female preference functions [34, 35, 38, 39], a technique that uses the entire preference function as the trait of interest. I used a linear mixed-model to address differences in the shape of the preference functions. Clone, replicate nested within clone, and individual nested within replicate and clone were random effects. The model included a linear and a quadratic stimulus frequency term, a clone × linear stimulus frequency interaction, and a clone × quadratic stimulus frequency term. The clone term describes differences in the overall responsiveness or mean elevation of the preference function [34, 40] of the focal females reared on different clone lines. The clone × quadratic stimulus frequency interaction describes differences in the shape of the preference functions of females across clone lines. Therefore, this interaction term was of particular interest to us.

A significant clone x quadratic stimulus frequency interaction prompted us to explore how female preference functions varied among clone lines. I used peak preference and preference selectivity as response variables in linear mixed models with clone and replicate as random effects and replicate nested within clone.

Finally, I estimated the broad-sense heritability in peak preference and preference selectivity as: $H^2 = \sigma^2_{clone} / (\sigma^2_{clone} + \sigma^2_{residual})$. These estimates are for broad-sense heritability because the calculations are based on the among-clone component of variation (σ^2_{clone}) [32]. Therefore, this among-clone component of variation contains both additive and nonadditive (dominance, epistasis and common environmental effects) genetic variation. The test for $H^2 > 0$ is provided by the clone term in each linear mixed-model. I performed all statistical analyses in JMP v. 7.0 (SAS Institute Inc., Cary, NC).

RESULTS

Cross-trophic influence on mate preference functions

Variation in the biotic environment provided by the host plant clone lines influenced the mate preferences of *Enchenopa* females: female preference functions varied among and within clone lines (Fig. 9). There was significant genetic variation in this cross-trophic influence (Table 5). Importantly, a significant clone × quadratic stimulus frequency interaction term indicates that genetic variation among clone lines influenced the shape of the preference functions (Table 5). I did not detect a significant within-clone line replicate effect on preference functions (Fig. 9, Table 5).

Cross-trophic influence on peak preference and preference selectivity

Cross-trophic interactions influenced one of the two traits describing the shape of the mate preferences of *Enchenopa* females. There was substantial and significant genetic variation in cross-trophic influence on peak preference, but not on preference selectivity (Fig. 10, Table 6).

DISCUSSION

I focused on cross-trophic level interactions and their influence on phenotypic variation, demonstrating for the first time the presence of IIGEs on the mate preferences of a plant-feeding insect. I detected such effects by manipulating genetic variation in the insects' host plants through the use of replicated clone lines. This extension of a quantitative genetics design allowed us to ask whether variation in female mate preferences resulted from among-clone line differences or other environmental effects. I show that these among-clone cross-trophic interactions influence the peak preference of females, thus indicating the presence of cross-trophic IIGEs on female mate preferences.

The detection of female mate preferences being influenced by genetic variation in cross-trophic effects on plasticity represents a crucial step towards understanding the dynamics between individuals and the biotic environment. Patterns of variation in host plants will influence the form and shape of plasticity in mate preferences of individuals in those environments. With genetic variation in cross-trophic effects on mate preference plasticity, evolutionary changes in plants as a response to selection may yield new patterns of variation in mate preferences, and this may have both immediate and evolutionary consequences for the dynamics of sexual selection. Because female mate preferences shifted according to among-clone line genetic variation, the relative attractiveness of males to females may vary as a result of where a female developed. Different male phenotypes, and thus genotypes, may be favored by any one female genotype according to variation in host plants. Consequently, IIGEs may prove important in the maintenance of genetic variation, and help sustain sexual selection.

In addition to IIGEs, social IGEs on female mate preferences have been recently documented in *E. binotata*, and the magnitude of variation induced by such social influences is comparable to the patterns here [22]. The presence of such influences on mate preferences means that social and cross-trophic IGEs could interact with one

another. Constructive interactions could exaggerate the phenotypic variation in mate preferences. On the other hand, these interactions could counteract one another, resulting in less phenotypic variation. Nonetheless, the presence of both social IGEs and cross-trophic IIGEs may change how selection operates across and within environments because they will alter the patterns of phenotypic variation that are exposed to selection. As a result, divergence could be promoted within a population.

As well as affecting female mate preferences, cross-trophic IIGEs have also been shown to influence male signals. Genetic variation in host plants was shown to cause shifts in several signaling traits in phenotypic space according to among-plant variation [Chapter 2; 26]. The patterns of variation in male signals and female mate preferences are likely influenced by the amount and direction of dispersal among the various clusters of individuals in a population. Changes in these patterns across plants could modify the relationship between mating signals and preferences, which could have significant evolutionary consequences. Cross-trophic IIGEs could increase the potential for self-reinforcing divergence and coevolution [cf. 42]. Differences in the shifts of male signals and female mate preferences across plants could alter the genetic covariance between the sexes, allowing new patterns to be established.

For many plant-feeding insects, colonization of novel environments, as with host plant shifts for the *E. binotata* complex, are integral to the process of speciation [27, 43, 44]. Here, the findings indicate that host plants may play a continued role in diversification even after or in between host plant shifts, and the patterns of variation within host plants may be important to divergence within a species. Changes in mate preferences could restrict mating and thus subsequent gene flow between individuals developing on genetically-varied plants, particularly if IIGEs shift peak preferences in opposite directions.

In conclusion, cross-trophic interactions influence variation in female mate preferences. Furthermore, I detected a significant component of genetic variation in this cross-trophic influence on mate preferences. Such IIGEs on female mate preferences has strong implications on how selection may operate within and across environments; e.g., they may facilitate the maintenance of genetic variation under strong sexual selection, as well as promote divergence within or among populations.

ACKNOWLEDGEMENTS

I thank D. Cruz and T. Schuck for help with plant care. I also thank D.A. Gray for the idea of using naturally-occurring clones, M.D. Greenfield and E. Latch for fruitful discussions, and F. Barbosa for her helpful feedback on the manuscript. This research was funded by NSF grants IOS-0919962 to R.L.R. and IOS-1120790 to R.L.R. and K.D. Fowler-Finn; and by the Society for the Study of Evolution Rosemary Grant Award, UWM Ruth Walker and James D. Anthony awards, and a UWM Dissertator Fellowship to D.R.

REFERENCES

1. Thompson J.N. 2005 *The geographic mosaic of coevolution*. Chicago, Univ Chicago Press.

2. Ehrlich P.R., Raven P.H. 1964 Butterflies and plants: a study in coevolution. *Evolution* **18**, 586-608.

3. Anderson R.M., May R.M. 1982 Coevolution of hosts and parasites. *Parasitology* **85**, 411-426.

4. Ridley M. 2003 *The red queen: sex and the evolution of human nature*. New York, Penguin.

5. West-Eberhard M.J. 2003 *Developmental plasticity and evolution*. New York, NY, Oxford University Press.

6. West-Eberhard M.J. 2005 Developmental plasticity and the origin of species differences. *Proc Nat Acad Sci* **102**, 6543-6549.

7. Greenfield M.D., Rodríguez R.L. 2004 Genotype-environment interaction and the reliability of mating signals. *Anim Behav* **68**, 1461-1468.

8. Ingleby F.C., Hunt J., Hosken D.J. 2010 The role of genotype-by-environment interactions in sexual selection. *J Evol Biol* **23**, 2031-2045.

9. Hebets E.A., Sullivan-Beckers L. 2010 Mate choice and learning. In *Encyclopedia of animal behavior* (eds. Breed M.D., Moore J.), pp. 389-393. Amsterdam, Elsevier B. V.

10. Verzijden M.N., ten Cate C., Servedio M.R., Kozak G.M., Boughman J.W., Svensson E.I. 2012 The impact of learning on sexual selection and speciation. *Trends Ecol Evol* **27**, 511-519.

11. Rodríguez R.L., Sullivan L.M., Snyder R.L., Cocroft R.B. 2008 Host shifts and the beginning of signal divergence. *Evolution* **62**, 12-20.

12. Rodríguez R.L., Rebar D., Fowler-Finn K.D. 2013 The evolution and evolutionary consequences of social plasticity in mate preferences. *Anim Behav* **85**, 1041-1047.

13. Moore A.J., Brodie III E.D., Wolf J.B. 1997 Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution* **51**, 1352-1362.

14. Wolf J.B., Brodie III E.D., Cheverud J.M., Moore A.J., Wade M.J. 1998 Evolutionary consequences of indirect genetic effects. *Trends Ecol Evol* **13**, 64-69.

15. Wolf J.B., Brodie III E.D., Moore A.J. 1999 Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. *Am Nat* **153**, 254-266.

16. Rowntree J.K., Shuker D.M., Preziosi R.F. 2011 Forward from the crossroads of ecology and evolution. *Philos Trans R Soc B* **366**, 1322-1328.

17. Bailey J.K., Schweitzer J.A., Úbeda F., Koricheva J., LeRoy C.J., Madritch M.D., Rehill B.J., Bangert R.K., Fischer D.G., Allan G.J., et al. 2009 From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philos Trans Roy Soc B* **364**, 1607-1616.

18. Shuster S.M., Lonsdorf E.V., Wimp G.M., Bailey J.K., Whitham T.G. 2006 Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution* **60**, 991-1003.

19. Bleakley B.H., Wolf J.B., Moore A.J. 2010 Evolutionary quantitative genetics of social behaviour. In *Social behaviour: genes, ecology and evolution* (eds. Székely T., Komdeur J., Moore A.J.). Cambridge, Cambridge Univ. Press.

20. Agrawal A.F., Brodie III E.D., Brown J. 2001 Parent-offspring coadaptation and the dual genetic control of maternal care. *Science* **292**, 1710-1712.

21. Danielson-François A.M., Zhou Y., Greenfield M.D. 2009 Indirect genetic effects and the lek paradox: inter-genotypic competition may strengthen genotype x environment interactions and conserve genetic variance. *Genetica* **136**, 27-36.

22. Rebar D., Rodríguez R.L. 2013 Genetic variation in social influence on mate preferences. *Proc R Soc Lond B* **280**, 20130803.

23. Wade M.J. 2000 Epistasis as a genetic constraint within populations and an accelerant of adaptive divergence among them. In *Epistasis and the evolutionary process* (eds. Wolf J.B., Brodie III E.D., Wade M.J.), pp. 213-231. New York, Oxford University Press.

24. Whitham T.G., Bailey J.K., Schweitzer J.A., Shuster S.M., Bangert R.K., LeRoy C.J., Lonsdorf E.V., Allan G.J., DiFazio S.P., Potts B.M., et al. 2006 A framework for community and ecosystem genetics: from genes to ecosystems. *Nat Rev Genet* **7**, 510-523.

25. Khudr M.S., Oldekop J.A., Shuker D.M., Preziosi R.F. 2013 Parasitoid wasps influence where aphids die via an interspecific indirect genetic effect. *Biol Lett* **9**, 20121151.

26. Rebar D., Rodríguez R.L. *in press* Trees to treehoppers: genetic variation in host plants contributes to variation in the mating signals of a plant-feeding insect. *Ecol Lett*.

27. Cocroft R.B., Rodríguez R.L., Hunt R.E. 2008 Host shifts, the evolution of communication and speciation in the *Enchenopa binotata* species complex of treehoppers. In *Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. Tilmon K.J.), pp. 88-100. Berkeley, CA, University of California Press.

28. Wood T.K. 1993 Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). In *Evolutionary patterns and processes* (eds. Lees D.R., Edwards D.), pp. 299-317. New York, Academic.

29. Rodríguez R.L., Ramaswamy K., Cocroft R.B. 2006 Evidence that female preferences have shaped signal evolution in a clade of specialized plant-feeding insects. *Proc R Soc Lond B* **273**, 2585-2593.

30. Cocroft R.B., Rodríguez R.L., Hunt R.E. 2010 Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biol J Linn Soc* **99**, 60-72.

31. Rodríguez R.L., Sullivan L.M., Cocroft R.B. 2004 Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Evolution* **58**, 571-578.

32. Lynch M., Walsh B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA, Sinauer Associates.

33. Ritchie M.G. 1996 The shape of female mating preferences. *Proc Natl Acad Sci USA* **93**, 14628-14631.

34. Fowler-Finn K.D., Rodríguez R.L. 2012 Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* **66**, 459-468.

35. Fowler-Finn K.D., Rodríguez R.L. 2012 The evolution of experience-mediated plasticity in mate preferences. *J Evol Biol* **25**, 1855-1863.

36. Niering W.A., Dreyer G.D., Egler F.E., Anderson J.P. 1986 Stability of a *Viburnum lentago* shrub community after 30 years. *Bull Torr Bot Club* **113**, 23-27.

37. Rodríguez R.L., Haen C., Cocroft R.B., Fowler-Finn K.D. 2012 Males adjust signaling effort based on female mate-preference cues. *Behav Ecol* **23**, 1218-1225.

38. Meyer K., Kirkpatrick M. 2005 Up hill, down dale: quantitative genetics of curvaceous traits. *Proc R Soc Lond B* **360**, 1443-1455.

39. Stinchcombe J.R., group F.-v.t.w., Kirkpatrick M. 2012 Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol Evol* **27**, 637-647.

40. Bailey N.W. 2008 Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. *Behav Ecol* **19**, 960-966.

41. Schluter D. 1988 Estimating the form of natural selection on a quantitative trait. *Evolution* **42**, 849-861.

42. Bailey N.W., Moore A.J. 2012 Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the Fisher process. *Evolution* **66**, 2674-2684.

43. Drès M., Mallet J. 2002 Host races in plant–feeding insects and their importance in sympatric speciation. *Phil Trans Roy Soc B* **357**, 471-492.

44. Coley P.D., Barone J.A. 1996 Herbivory and plant defenses in tropical forests. *Ann Rev Ecol Syst* **27**, 305-335.

Figure 8. Experimental design to test whether the presence of genetic variation in host plants influences the pattern of focal female mate preferences reared on them. Clones were used as the environment with a describable genetic component, with each clone line consisting of a minimum of three replicates. Randomly collected, unrelated focal individuals were then placed on each replicate in order to separate among and within-clone line effects. I described the mate preferences of these focal individuals as adults.

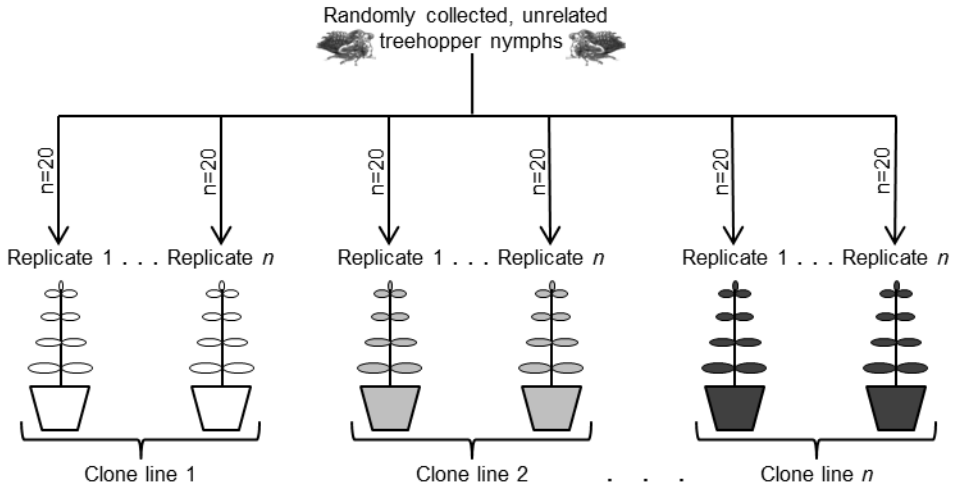

Figure 9. Genetic variation in cross-trophic influence on the mate preference functions of *Enchenopa* focal females. Each preference function depicts the mean response of all focal females measured on that replicate, and each figure shows variation in the replicates for the sampled clone line. The dotted line represents the mean peak preference of all females in the sampled population.

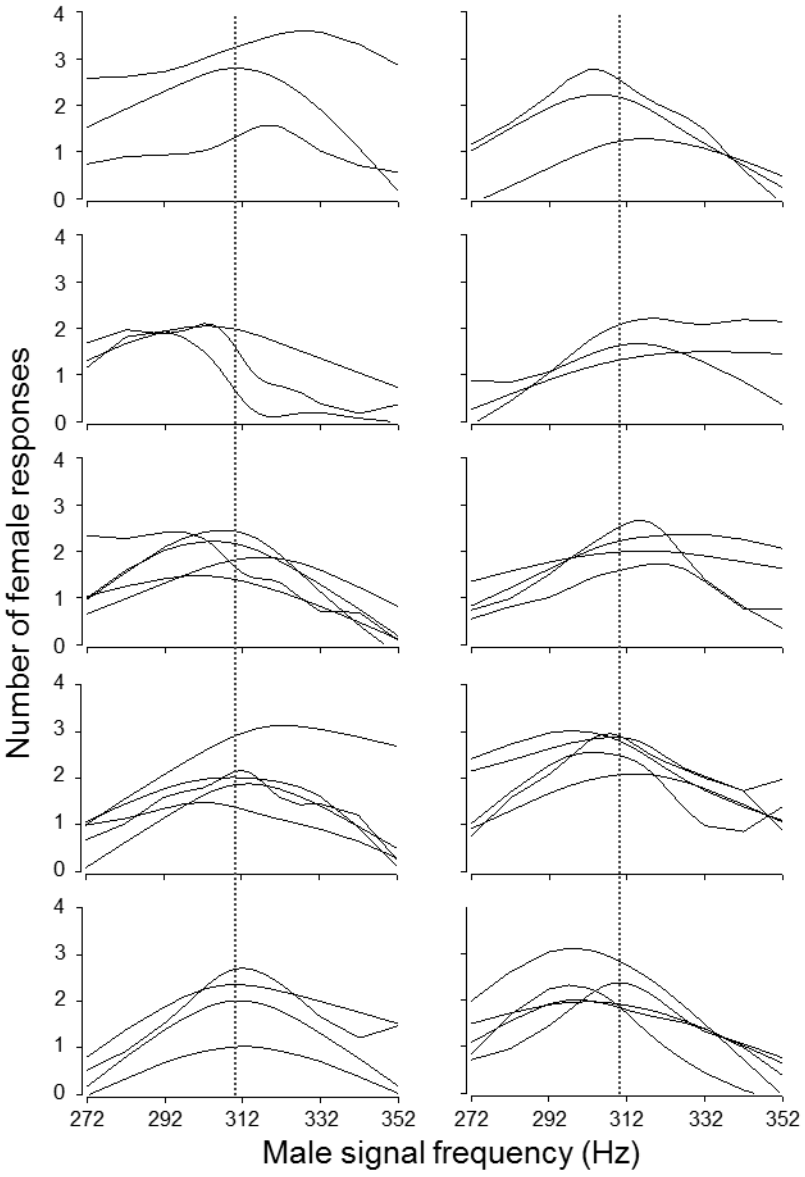

Figure 10. Genetic variation in cross-trophic influence on two traits describing the mate preference functions of *Enchenopa* focal females. The y-axis for each trait represents the phenotypic range observed in this study. The left column (solid circles) shows the mean ± SE of focal females reared on each clone line. The right column (open circles) shows the mean of focal females reared on each replicate within each respective clone line.

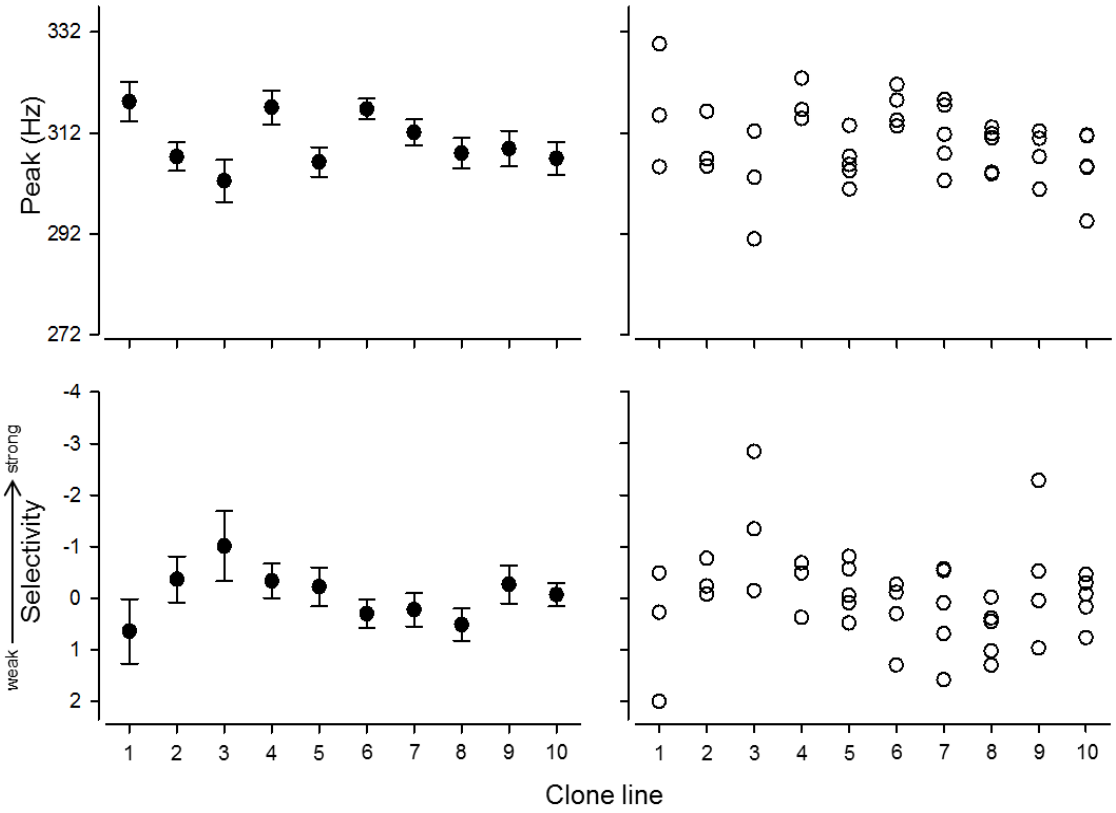

Table 5. Linear mixed-model testing for differences in the shape of *Enchenopa* female mate preference functions according to the plants on which they developed. Plant clone, plant replicate, and treehopper individual are random terms. Replicate is nested within clone and individual is nested within clone and replicate. The clone × stimulus frequency^2 term tests for genetic variation in clone lines' influence on female mate preference functions. Significant values are in bold ($p<0.05$).

Source of Variation	df	F	P
Whole model	176, 2806	**41.89**	**<0.0001**
Clone	9, 36.86	1.55	0.168
Replicate [Clone]	30, 116.99	1.10	0.346
Stimulus frequency	1, 2806	**31.45**	**<0.0001**
Clone × Stimulus frequency	9, 2811.4	**24.63**	**<0.0001**
Stimulus frequency^2	1, 2806	**257.77**	**<0.0001**
Clone × Stimulus frequency^2	9, 2806	**3.83**	**<0.0001**
Individual [Replicate, Clone]	117, 2806	**36.83**	**<0.0001**

Table 6. Linear mixed-models testing the variation in two traits of the mate preference functions of focal females attributed to differences among clone lines and within clone lines (replicates), along with estimates for H^2. Clone and replicate are random terms, with replicate nested within clone. Significant values are in bold.

Trait	Factor	df	F	P	$H^2 \pm$ SE
Peak preference	Clone	9, 38.44	**2.97**	**0.009**	**0.108 ± 0.106**
	Replicate	30, 117	0.78	0.782	
Preference selectivity	Clone	9, 35.88	1.64	0.141	0.023 ± 0.072
	Replicate	30, 117	1.11	0.342	

CURRICULUM VITAE

Darren Wade Rebar

Education

- MS, University of California, Riverside, 2009.
  Program: Evolution, Ecology, and Organismal Biology
  Advisor: Marlene Zuk

- BA, Oberlin College, 2002.
  Major: Biology, Minor: Chemistry

Dissertation title: Influence of genetic variation in the biotic environment on phenotypic variation in a plant-feeding insect

Publications: Refereed Journals

- **Rebar D**, Rodríguez RL. *In press*. Trees to treehoppers: genetic variation in host plants contributes to variation in the mating signals of a plant-feeding insect. *Ecol Lett*.
- **Rebar D**, Rodríguez RL. 2013. Genetic variation in social influence on mate preferences. *Proc R Soc B* **280**: 20130803.
- Rodríguez RL, **Rebar D**, Fowler-Finn KD. 2013. The evolution and evolutionary consequences of social plasticity in mate preferences. *Anim Behav* **85**: 1041-1047.
- Bailey NW, Fowler-Finn KD, **Rebar D**, Rodriguez RL. 2013. Green Symphonies or wind in the willows? Testing acoustic communication in plants. *Behav Ecol* **24**: 797-798.
- **Rebar D**, Höbel G, Rodríguez RL. 2012. Vibrational playback by means of airborne stimuli. *J Exp Biol* **215**: 3513-3518.
- **Rebar D**, Zuk M, Bailey NW. 2011. Mating experience in field crickets modifies pre- and postcopulatory female choice in parallel. *Behav Ecol* **22**: 303-309.
- **Rebar D**, Bailey NW, Zuk M. 2009. Courtship song's role during female mate choice in the field cricket *Teleogryllus oceanicus*. *Behav Ecol* **20**: 1307-1314.
- Zuk M, **Rebar D**, Scott SP. 2008. Courtship song is more variable than calling song in the field cricket *Teleogryllus oceanicus*. *Anim Behav* **76**: 1065-1071.

Other Publications

- **Rebar D**. 2010. Why shout when she's standing right here? *Metaleptea: The Newsletter of the Orthopterists' Society* **30** (2): 7-8.
- **Rebar D**. 2007. Comfort. The Coming Out Monologues at UC Riverside
- **Rebar D** and Allen J. 2005. The Breaking Wheel. Guitar Hero, RedOctane, Inc.

Presentations

Invited Talks

- **Rebar D**. 2013. Institut de Recherche sur la Biologie de l'Insecte (IRBI), Université François-Rabelais de Tours: Tours, France.
- **Rebar D**. 2011. Integrative Behavior Group, UW-Madison: Madison, WI.

Professional Meetings

- **Rebar D**. 2013. Royal Entomological Society: St. Andrews, Scotland, UK.
- **Rebar D**. 2012. Animal Behavior Society: Albuquerque, NM.
- **Rebar D**. 2010. Animal Behavior Society: Williamsburg, VA.
- **Rebar D**. 2009. Gradfest 2009: Riverside, CA.
- **Rebar D** and Zuk M. 2009. SICB: Boston, MA.

Funding

- 2013: UW-Milwaukee Ruth Walker grant-in-aid award, $1500
- 2013: UW-Milwaukee James D. Anthony award, $200
- 2012: UW-Milwaukee Ruth Walker grant-in-aid award, $1500
- 2012: UW-Milwaukee James D. Anthony award, $200
- 2011: UW-Milwaukee Ruth Walker grant-in-aid award, $500
- 2011: UW-Milwaukee James D. Anthony award, $200
- 2010: Society for the Study of Evolution Rosemary Grant Graduate Student Research Award, $2500
- 2010: UW-Milwaukee Ruth Walker grant-in-aid award, $1000
- 2009: UC Riverside GSA travel grant, $300
- 2008: Orthopterists' Society research grant, $800

Scholarships and Honors

- 2012 – 2013: Dissertation Fellowship, UW-Milwaukee
- 2011 – 2012: Distinguished Graduate Student Fellowship, UW-Milwaukee
- 2011 – 2012: Chancellor's graduate student award, UW-Milwaukee
- 2010 – 2011: Chancellor's graduate student award, UW-Milwaukee
- 2010: Summer Institute of Statistical Genetics scholarship, UWashington
- 2009 – 2010: Chancellor's graduate student award, UW-Milwaukee
- 2008 – 2009: Teaching Assistant fellowship, UC Riverside
- 2006 – 2007: Chancellor's distinguished fellowship, UC Riverside

Professional Memberships

- Animal Behavior Society
- International Society of Behavioral Ecology
- Society for the Study of Evolution
- Society for Integrative & Comparative Biology

Teaching and Mentoring

- 2013 UW-Milwaukee, Teaching Assistant for Introductory Biology

- 2010 – 2012 UW-Milwaukee, Graduate Mentor for Undergraduate research in Biology and Mathematics (UBM) program.
- 2010 UW-Milwaukee, Teaching Assistant for General Ecology
- 2009 UW-Milwaukee, Teaching Assistant for Introductory Biology
- 2008 – 2009 UC Riverside, Mentor Teaching Assistant for TA Development Program.
- 2008 UC Riverside, Teaching Assistant for Evolutionary Biology
- 2007 UC Riverside, Teaching Assistant for Introductory Evolution and Ecology
- 2004 – 2005 Citizen Schools, Teaching associate for 6-8th grade students, Boston, MA.
- 2002 – 2003 Desert Sun Science Center, Instructor and Head Tech, Idyllwild, CA.
- 2002 Desert Sun Science Center, Head Counselor and Instructor, Idyllwild, CA.
- 2001 – 2002 Oberlin College, EXCO Instructor, Oberlin, OH.

Service

Editorial Board

- Evolution: Education and Outreach

Referee for Peer Reviewed Journals

- Animal Behavior
- Ethology
- Journal of Insect Behavior

Other Service

- 2010 – 2011: Fundraiser, Graduate Organization of Biological Sciences, UW-Milwaukee
- 2008 – 2009: President, Biology Graduate Student Association, UC Riverside
- 2008: Graduate Student Recruitment Committee, UC Riverside