

Genetic Variation in Host Plants Influences the Mate Preferences of a Plant-Feeding Insect

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ABSTRACT: Many species spend their lives in close association with other organisms, and the environments provided by those organisms can play an important role as causes of variation in phenotypes. When this is the case, the genotypes of the 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. We assessed how mate preferences in a plant-feeding insect are influenced by the genotype of their host plant. We established clone lines of a sample of host plant genotypes constituting the background biotic environment for a random sample of insects that we reared on them. We found that the insects' mate preferences varied according to the clone line on which they developed. These results demonstrate that genetic variation in host plants has cross-trophic consequences on a trait that has strong effects on fitness and interpopulation dynamics such as diversification in communication systems. We 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

Most 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 this biotic nature of many types of environments

and of the potential for an evolutionary back-and-forth between the participants (Ehrlich and Raven 1964; Anderson and May 1982; Ridley 2003; Thompson 2005). These biotic causes of variation play a prominent role in diverse evolutionary processes. They may, for instance, be highly effective in promoting and initiating divergence (West-Eberhard 2003, 2005), in sustaining variation under selection (Greenfield and Rodríguez 2004; Bussière et al. 2008; Ingleby et al. 2010), and in shaping patterns of mate choice and reproductive isolation between populations (Rodríguez et al. 2008; Hebets and Sullivan-Beckers 2010; Verzijden et al. 2012; Rodríguez et al. 2013*b*). Recently, biologists have started to explore the consequences of the presence of genetic variation among the individuals that constitute biotic environments for the role of those environments as causes of variation in the phenotypes and fitness of individuals living in them. These consequences give familiar evolutionary topics new complexions.

When the environment consists of organisms, environmental causes of variation in individuals are themselves influenced by genetic and phenotypic 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 (Moore et al. 1997; Wolf et al. 1998, 1999) and “interspecific indirect genetic effects” (IIGEs) when they occur among heterospecifics (Shuster et al. 2006; Bailey et al. 2009; Rowntree et al. 2011). The evolutionary significance of these effects is diverse. At the most basic level, genetic variation in the environment can influence the patterns of phenotypic variation in the individuals living in that environment, thereby modifying the course of evolutionary processes in the populations pertaining to the individuals influenced by that environment (Moore et al. 1997; Wolf et al. 1998, 1999; Bleakley et al. 2010). At another level, the evolutionary consequences of environmental variation can be influenced by evolutionary processes at the level of the

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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 living on those environments, as well as their ecological and evolutionary trajectories (Wolf et al. 1998).

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. Here we focus on IIGEs on mate preferences, a fitness-related trait that is also a cause of sexual selection. IGEs have been found in taxonomically diverse case studies and for several traits such as maternal provisioning, mating signals and mate preferences, and fecundity (Wade 2000; Agrawal et al. 2001; Petfield et al. 2005; Danielson-François et al. 2009; Bailey and Zuk 2012; Rebar and Rodríguez 2013). The study of IIGEs is still nascent, however, and a focus on sexually selected traits has been lacking. IIGEs have been detected as bottom-up effects on so-called community phenotypes, such as the composition of the arthropod fauna on trees (Shuster et al. 2006; Whitham et al. 2006; Bailey et al. 2009; Rowntree et al. 2011). In addition, top-down IIGEs have been detected on the behavior of aphids infected by parasitoid wasps (Khudr et al. 2013). Recently, we reported IIGEs on the mating signals of a plant-feeding insect (Rebar and Rodríguez 2014*b*). 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. For instance, if males and females were to be influenced by IIGEs such that their signal and preference phenotypes covaried across environments, then IIGEs could promote the onset of speciation by establishing patterns of assortative mating within a population, and generating direct genetic signal-preference covariance (cf. Bailey and Moore 2012).

Here we report the finding of IIGEs on mate preferences. We implement a simple framework for disentangling whether genetic variation in the biotic environment contributes to variation in individual phenotypes. Specifically, we ask whether genetic variation in host plants influences the mate preferences of a plant-feeding insect. We used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). These insects develop entirely on their host plants (Wood 1993), and each member of the complex specializes on one host plant species. 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 (Wood 1993; Cocroft et al. 2008). All species use plant-borne vibrational signals to communicate with one another, and pair formation occurs through male-female signaling duets (Cocroft et al.

2008). Females exhibit strong preferences for some aspects of male signals, particularly signal frequency (Rodríguez et al. 2006; Cocroft et al. 2010), which is the most divergent adult trait among species within the complex (Rodríguez et al. 2004, 2006; Cocroft et al. 2010). 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 (Rodríguez et al. 2004, 2006). Female mate preferences for male signal frequency are thus quite important in maintaining reproductive isolation between members of the complex, as well as in generating divergent sexual selection resulting in signal evolution.

We explore the above cross-trophic interactions through a novel implementation of a classic quantitative genetics design. We used clone lines of a sample of host plant genotypes as the background biotic environment (Lynch and Walsh 1998) and reared randomly collected insect individuals on those environments. This methodology allowed us to manipulate genetic variation in the developmental environment provided by host plants to insect individuals. We then described variation in female preferences for male signal frequency with laser vibrometry and vibrational playback experiments. We quantified their responses by creating mate preference functions (Ritchie 1996; Fowler-Finn and Rodríguez 2012*a*, 2012*b*; Rebar and Rodríguez 2013) and assessed differences in the curvilinearity of the preference functions across clone lines through a clone \times quadratic stimulus frequency interaction term. We then estimated the variation in mate preferences that could be attributed to among- and within-clone line components.

We used the above method to test the hypothesis that genetic variation in host plants influences female mate preferences (i.e., that 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. There should thus be a detectable 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.

Methods

Study Species

There are two members of the *Enchenopa binotata* complex that live on the host plant *Viburnum lentago* (Caprifoliaceae) at our field site (Tendick Nature Park, Saukville, WI). While these species await formal description (Hamilton and Cocroft 2009), male signal frequency is a reliable

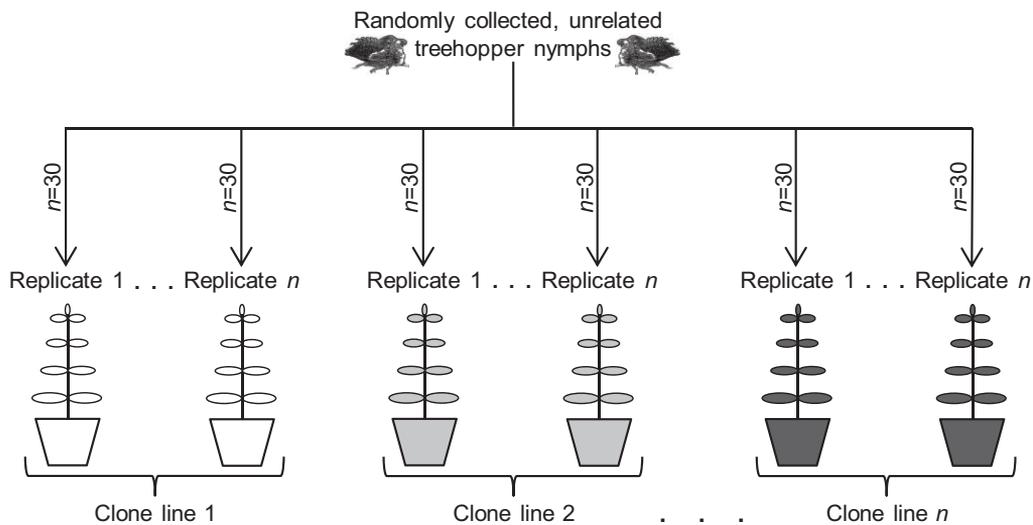


Figure 1: Experimental design to test whether the presence of genetic variation in host plants influences variation in the mate preferences of *Enchenopa* treehoppers reared on them. Plant clones were used as the environment with a describable genetic component, with each clone line consisting of a minimum of three replicates. Randomly collected, unrelated *Enchenopa* treehopper individuals were then placed on each replicate in order to separate among- and within-clone line effects. We described the mate preferences of these focal individuals as adults.

trait in identifying them (Rodríguez et al. 2004; Hamilton and Cocroft 2009). We used the high-signal-frequency species found on *V. lentago* (dominant frequency = 312 Hz), and we kept voucher specimens in 95% EtOH.

Rearing Experiment

We 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, that sprout up around the main plant and continue to share the same root system (Niering et al. 1986). We dug up evenly sized suckers (0.5 m) surrounding a parental plant from spatially separated clone patches at the University of Wisconsin–Milwaukee Field Station (Saukville, WI) in fall 2011. We ensured that the suckers coming from each clone patch were connected via lateral roots, thus creating the replicates for each clone line. We overwintered the suckers in moistened peat moss in a dark cold room maintained at 4°C, and we potted each sucker into a 1-gal plastic pot with Fafard 3B mix (Conrad Fafard, Agawam, MA) the following March 2012. We moved the plants into a greenhouse to promote the onset of budding and subsequent development.

To minimize the potential for confounding components of variation that might arise from differences in the en-

vironmental conditions experienced by the source clone patches in the field, we collected plants from an area wherein clone patches were within cruising range of the treehoppers (D. Rebar and R. L. Rodríguez, personal observation). Thus, our manipulation of host plant genetic variation represents a realistic range to which individuals could be exposed. In addition, the plants we used lacked egg mass scars and therefore had never had treehopper nymphs develop on them. We dug up all plants in the fall after they had dropped their leaves in order to overwinter them as bare root plants under identical conditions and to pot and grow them the following spring under the same controlled greenhouse environment. Nevertheless, we acknowledge that there may be a component of variation represented by an interspecific indirect ecological effect (IIEE), as has been shown, for example, in work on ladybird beetle predators (Astles et al. 2005).

We 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. We collected nymphs by cutting stems from various host plants spanning a 100-m transect. We 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. 1). Each randomly transferred group of 30 nymphs was reared together on one plant replicate from one of the clone lines from the time they were first instars until

their adult molt. We removed the males 2 wk later, leaving only the females on each plant. We then described the mate preferences of each female upon becoming sexually receptive, approximately 5–6 wk after adult molt.

Measuring Variation in Mate Preferences

We used a single commercially acquired potted recording plant individual to test all females to ensure that each one was different from the rearing plants and to minimize variation in the signal-transmission features of the playback setup and standardize the playback stimuli we presented to each female (see below).

We took advantage of the duetting behavior used in pair formation by *Enchenopa* to assay female responses. Males produce mating signals, and females respond with their own vibrational signals if they find the males' signals attractive (Rodríguez et al. 2004, 2006, 2012). Because duetting induces males to search locally for the females (rather than to fly off in search of a mate elsewhere), female responses to male signals or signal playbacks correspond to the likelihood of mating with a given male and provide a simple and relevant assay of mate preference (Rodríguez et al. 2004; Cocroft et al. 2008). We described each female's mate preference by presenting her with synthetic playbacks of male signals and noting how many response signals she produced. We assessed female responses with playback stimuli spanning beyond the population range in both directions. We set all other features of the signals to the population mean. We presented each signal as a bout of four, the mean number of signals for males in this population. Signals were separated by 15 s of silence. We 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 stimuli by focal females. We presented females with randomly generated sequences of male stimuli. The stimuli were 2, 4, 6, 8, 10, 15, 20, 30, and 40 Hz different in each direction from the mean (312 Hz). We presented each female with the 19 playback stimuli and quantified her responses to create her mate preference function (Ritchie 1996; Rodríguez et al. 2006). We created and delivered all stimuli using a custom MATLAB script (available on request). The stimuli were delivered to the plant stem through a piezoelectric actuator that was attached to the stem with accelerometer wax (Thorlabs, NJ, model AE0505D16). The actuator was controlled by a piezo controller (Thorlabs, model MDT694A) from an iMac computer at an amplitude of 0.10 mm/s. We recorded the stimuli and female responses using a laser vibrometer (Polytec, 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. We isolated

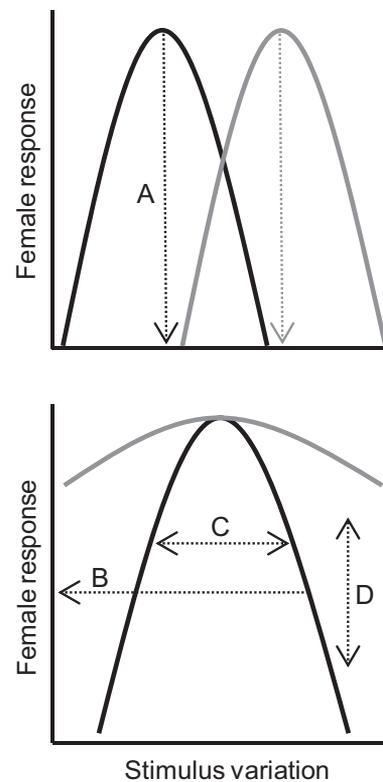


Figure 2: Two traits, peak preference and preference selectivity, that describe variation in mate preference functions. The top figure depicts two female preference functions that differ in their peak preference (*A*). The bottom figure depicts two female preference functions with the same peak preference, but the black preference function shows a female who is much more selective than the gray one across the tested stimulus range. Preference selectivity is derived from measurements of responsiveness (*B*), tolerance (*C*), and strength (*D*). These three measurements are strongly correlated and we therefore summarize them with a principal component analysis–derived trait termed “preference selectivity” (see “Methods”).

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 m × 2 m). We also placed vibration dampening pads (Polymer Dynamics, Allentown, PA, model 3291-22-PM-50) under the table legs to further isolate the entire setup. We recorded female responses using AUDACITY (v. 1.2.5; <http://audacity.sourceforge.net>). We then listened to the recordings to score the number of female responses to each playback stimulus (0–4 responses). All females were recorded from July to August 2012.

To begin each playback trial, we removed each female individually from her rearing plant and placed her at the same site on the recording plant stem. We first tested a female's receptivity by playing back a live male recording

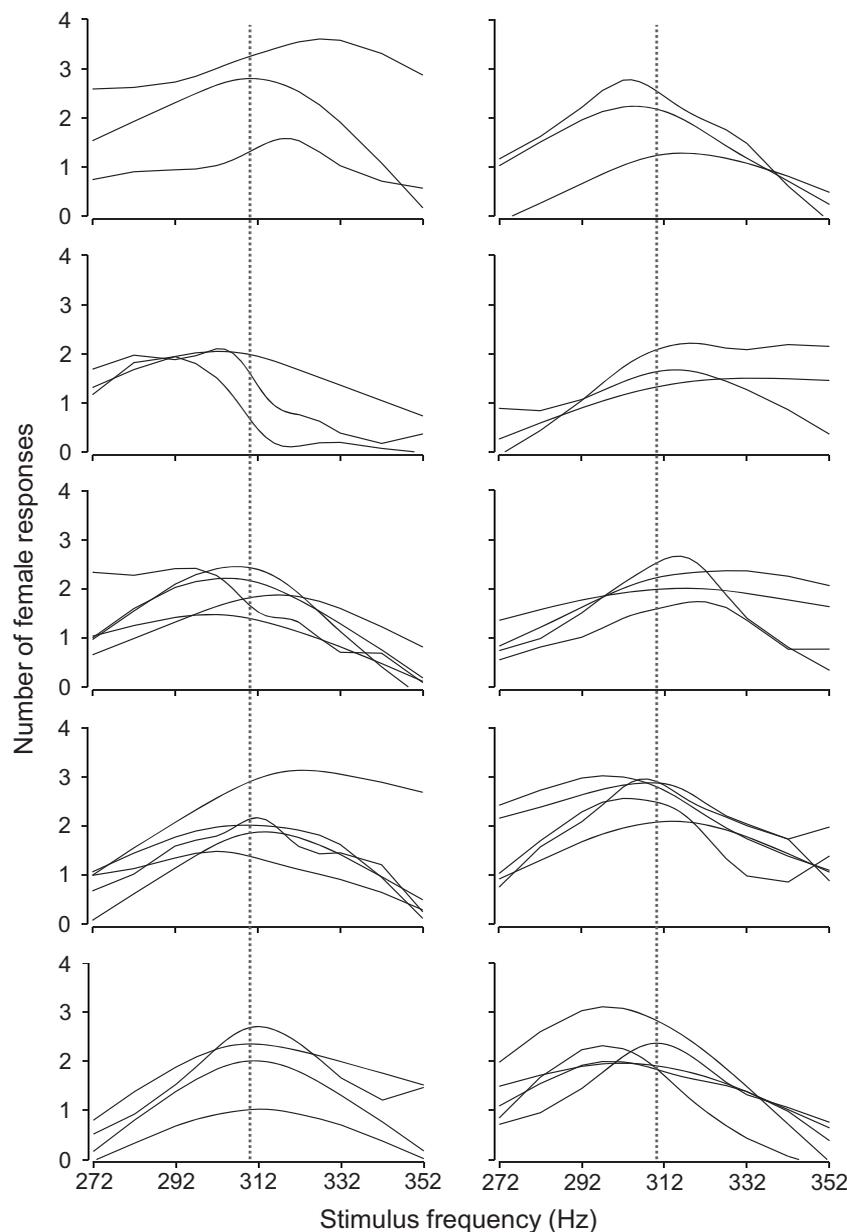


Figure 3: Genetic variation in cross-trophic influence on the mate preference functions of *Enchenopa* females. Each preference function depicts the mean response of all females measured on each clone line replicate, and each panel shows variation in the replicates for the sampled clone line. The dotted line represents the mean peak preference of all females in the sampled population.

to her. Females that responded to this signal were then presented with a randomized sequence of 19 signal models. Females that failed to respond to the live male recording were returned to their rearing plant and tested at a later date. Some females stopped responding during the playback sequence. In those cases, we replayed the live male recording, considering them still receptive if they responded or otherwise excluded them ($n = 40$).

We included in our analysis only those clone lines that were represented by at least three replicates, that is, that had at least three plant individuals 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 receptive treehopper females (range = 2–9). In total, 157 treehopper females

Table 1: Linear mixed-model testing for differences in the shape of *Enchenopa* female mate preference functions of female responses to playback stimuli according to the plants on which they developed

Source of variation	df	<i>F</i>	<i>P</i>
Whole model	176, 2,806	41.89	<.0001
Clone	9, 36.86	1.55	.168
Replicate [clone]	30, 116.99	1.10	.346
Stimulus frequency	1, 2,806	31.45	<.0001
Clone × stimulus frequency	9, 2,811.4	24.63	<.0001
(Stimulus frequency) ²	1, 2,806	257.77	<.0001
Clone × (stimulus frequency) ²	9, 2,806	3.83	<.0001
Individual [replicate, clone]	117, 2,806	36.83	<.0001

Note: Plant clone, plant replicate, and treehopper individual are random terms. Replicate is nested within clone and individual is nested within clone and replicate. The model includes a linear and quadratic stimulus frequency term, along with interactions of both with clone. The clone × (stimulus frequency)² term tests for the influence of genetic variation in clone lines on female mate preference functions. Significant values are in bold ($P < .05$).

contributed one preference function each to our analysis. All data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.f347f> (Rebar and Rodríguez 2014a).

Description of Female Preference Functions

Mate preferences are function-valued traits (Meyer and Kirkpatrick 2005; Fowler-Finn and Rodríguez 2012a, 2012b; Stinchcombe et al. 2012), meaning that the responses of a female are a function of the mate signals that she encounters. We 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. We allowed the program to choose the smoothing parameter for each individual preference function. However, for 15 females we manually adjusted the smoothing parameter to decrease smoothness because these splines appeared as almost straight lines that failed to capture the observed variation in female responses. We ran tests with both sets of spline data for these 15 females, which verified that our adjustment did not qualitatively change the results.

We described variation in female mate preferences in terms of peak preference and preference selectivity (fig. 2). Peak preference represents the signal trait value that elicits the greatest response from a female. Selectivity describes how strongly a female disfavors male signals as they deviate

from her peak preference (Bailey 2008; Fowler-Finn and Rodríguez 2012b). We derived preference selectivity from measurements of responsiveness, tolerance, and strength (Fowler-Finn and Rodríguez 2012a, 2012b), 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 (Schluter 1988; Bailey 2008; Fowler-Finn and Rodríguez 2012a, 2012b). These three measurements are strongly correlated, so we performed a principal component analysis to generate the composite trait we call "preference selectivity." This first principal component had an eigenvalue of 2.10 that explained 69.1% of the variance, with responsiveness, tolerance, and strength loading similarly on this axis (0.53, 0.61, and -0.58 , respectively).

Statistical Analyses

We adopted a function-valued approach to test for variation in female preference functions (Meyer and Kirkpatrick 2005; Fowler-Finn and Rodríguez 2012a, 2012b; Stinchcombe et al. 2012), a technique that uses the entire preference function as the trait of interest. We used a linear mixed model with female response to male stimuli as the dependent variable to test for variation in the shape of the preference functions. The model included the following random terms: clone, replicate plant nested within clone, and individual treehopper female nested within replicate and clone. Each female was tested with all playback stimuli and contributed one complete preference function to the statistical model. The model also 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 (Bailey 2008; Fowler-Finn and Rodríguez 2012b) of 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. We were unable to include a term for the interaction between replicate and quadratic stimulus frequency because the model lacked degrees of freedom. Consequently, some of the variation in mate preferences assigned to clones may correspond to replicates, and the *P* values are thus likely exaggerated in this model. However, we confirmed IIGEs on mate preferences with a subsequent analysis. We 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 as random terms,

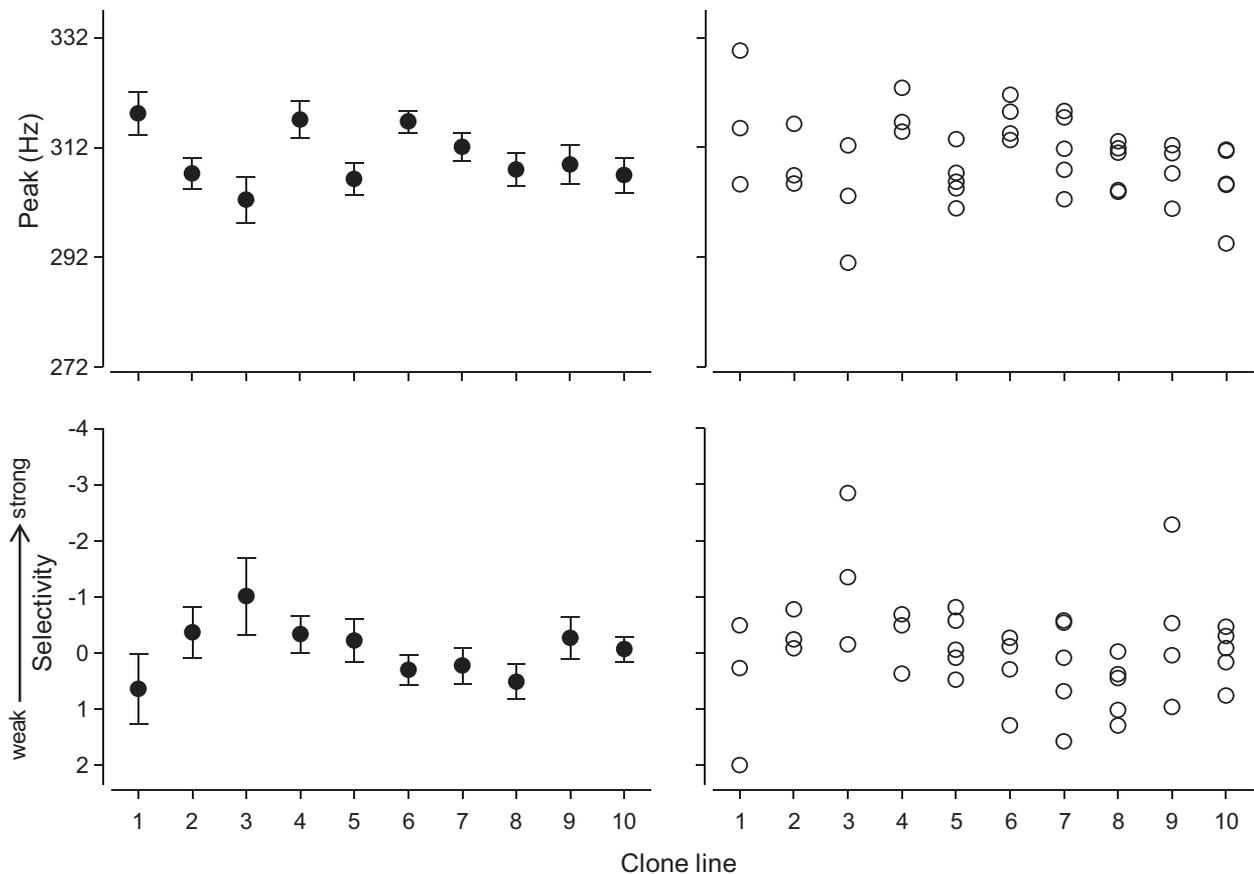


Figure 4: Genetic variation in cross-trophic influence on two traits describing the mate preference functions of *Enchenopa* females. The Y-axis for each trait represents the phenotypic range observed in this study. The left column (solid circles) shows the mean \pm SE of females reared on each clone line. The right column (open circles) shows the mean of females reared on each replicate within each respective clone line.

and acknowledge that these P values more realistic estimates of IIGEs on mate preferences.

Finally, to provide an effect size estimate for the influence of genetic variation in plant clones on female mate preferences, we estimated broad-sense heritability of the influence of genetic variation in host plants on female peak preference and preference selectivity. We designate this estimate as H_{IIGE}^2 and calculated it as follows: $H_{\text{IIGE}}^2 = \sigma_{\text{clone}}^2 / (\sigma_{\text{clone}}^2 + \sigma_{\text{residual}}^2)$. These estimates correspond to broad-sense heritability because the calculations are based on the among-clone component of variation (Lynch and Walsh 1998). We obtained these variance component estimates from the linear mixed models in the above analyses by using the restricted maximum likelihood method. The among-clone component of variation contains both additive and nonadditive (e.g., dominance variance) components of genetic variation. Significance for the test of the hypothesis that $H_{\text{IIGE}}^2 > 0$ is provided by the clone term in each linear mixed model. We also calculated the stan-

dard error for each H_{IIGE}^2 estimate by using the procedure for standard broad-sense heritability with weighted clone line samples (Roff 1997, p. 42). We performed all statistical analyses in JMP version 7.0 (SAS Institute, Cary, NC).

Results

Cross-Trophic Influence on Mate Preference Functions

Variation in the biotic environment provided by host plant clone lines influenced the mate preferences of *Enchenopa* females: female preference functions varied among and within clone lines (fig. 3). There was significant genetic variation in this cross-trophic influence (table 1). Importantly, a significant clone \times quadratic stimulus frequency interaction term suggests that genetic variation among clone lines influenced the shape of the mate preferences of *Enchenopa* females (table 1).

Table 2: Linear mixed-models testing the variation in two traits of female mate preference functions attributed to differences among clone lines and within clone lines (replicates)

Trait, factor	df	<i>F</i>	<i>P</i>	Variance component	$H_{\text{IGE}}^2 \pm \text{SE}$
Peak preference:					
Clone	9, 38.44	2.97	.009	20.032	.108 ± .106
Replicate	30, 117	.78	.782	−13.372	
Residual				166.012	
Preference selectivity:					
Clone	9, 35.88	1.64	.141	.050	.023 ± .072
Replicate	30, 117	1.11	.342	−.081	
Residual				2.142	

Note: The table also shows estimates for the variance components and for the heritability of the influence of genetic variation in host plants on female mate preference functions, H_{IGE}^2 . Clone and replicate are random terms, with replicate nested within clone. Significant values are in bold.

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. 4; table 2).

Discussion

We demonstrate the presence of IIGEs on mate preferences, detecting such effects by manipulating genetic variation in the host plants of a plant-feeding insect through the use of replicated clone lines. This extension of a classic quantitative genetics design allowed us to ask whether variation in female mate preferences resulted from among-clone line differences or other environmental effects. We 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.

In general, mate preferences are important causes of sexual selection and reproductive isolation (Andersson 1994), and in the *Enchenopa binotata* species complex, female mate preferences have been a main agent of signal diversification, especially for male signal frequency, the adult phenotype that most varies among members of the complex (Rodríguez et al. 2004, 2006; Cocroft et al. 2008, 2010). Thus, cross-trophic IIGEs on mate preferences are likely to have important consequences for evolutionary processes within and between treehopper populations.

Understanding the impact of IIGEs on evolutionary processes will require investigating the proximate causes of such effects. For example, what particular aspect of the phenotype of the clone lines causes female mate preferences to differ across them? Plants produce a variety of defensive compounds in response to herbivory, and the

amount and types of compounds produced may depend on the genetic makeup of the plants, which in turn may result in the similar responses of individuals reared on those plants. While unlikely in our experiment, differences in the environmental conditions of the developing plants may have influenced the plant phenotypes, and these phenotypic differences could have resulted in variation in mate preferences (IIEEs). To parse apart such components of variation, adoption of the trait-based framework of IIGEs (Moore et al. 1997) to quantify the strength and direction of such IIGEs may help illuminate the importance of such interactions.

How cross-trophic IIGEs on sexually selected traits may impact the course of evolution will also require assessing the interaction between genetic variation in both treehoppers and host plants. While we did not test for genotype × genotype ($G \times G$) interactions, there is evidence of genetic variation in female mate preferences, with peak preference showing high broad-sense heritability (Rodríguez et al. 2013a). In conjunction with our results here, further work on $G \times G$ interactions should provide novel insights into the interplay of genetic variation in species interactions.

In addition to IIGEs, IGEs on female mate preferences have been documented in *E. binotata*, and the magnitude of variation induced by such social influences is comparable to that of the IIGEs detected here (Rebar and Rodríguez 2013). In our experiment, IGEs were likely a source of variation among replicates within clone lines. The presence of such influences on mate preferences means that IGEs and cross-trophic IIGEs could interact with one another. Constructive interactions could exaggerate phenotypic variation in mate preferences. On the other hand, these interactions could counteract one another, resulting in less phenotypic variation. The presence of both 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. Asking how these different sources of genetic variation interact with one another will further our understanding of how IIGEs may evolve under selection across trophic levels.

As well as affecting female mate preferences, cross-trophic IIGEs have also been shown to influence male signals in *Enchenopa* treehoppers (Rebar and Rodríguez 2014b). This result, along with our detection of IIGEs on mate preferences here, greatly contributes to our understanding of the interactions between individuals and their environment. For example, patterns of variation in host plants will influence the form and shape of plasticity in mating signals and mate preferences of individuals in those environments. Further, evolution in host plants, such that the patterns of genetic variation in them change as a response to selection, will likely change the expression of phenotypic variation in male signals and female mate preferences. With genetic variation in cross-trophic effects on mating signal and mate preference plasticity, evolutionary changes in plants as a response to selection on them may yield new patterns of variation in mating signals and mate preferences, and this may have both immediate and evolutionary consequences for the dynamics of sexual selection. Because peak 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 genotypes may be favored by any one female genotype as a result of variation in host plants, according to the amount and direction of dispersal among the various patches. Consequently, one outcome may be that IIGEs prove important in the maintenance of genetic variation and help sustain sexual selection. Another outcome could be that cross-trophic IIGEs increase the potential for self-reinforcing divergence and coevolution via Fisherian selection (cf. Bailey and Moore 2012). 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.

Colonization of novel environments (e.g., host plant shifts in plant-feeding insects; Coley and Barone 1996; Drès and Mallet 2002; Cocroft et al. 2008) is often a key step in the process of speciation (Coyne and Orr 2004; Nosil 2012). Our findings indicate that host plants may play a continued role in diversification 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 distinct plants, particularly if IIGEs shift peak preferences in opposite directions. Such IIGEs on female mate preferences have strong implications on how selection may operate within and across environments; for ex-

ample, they may facilitate the maintenance of genetic variation under strong sexual selection, as well as promote divergence within or among populations.

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Left, a group of *Enchenopa binotata* nymphs, and right, an adult male *E. binotata* treehopper, on their host plant *Viburnum lentago*. Photo credit: Darren Rebar.