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Insect mating signal and mate preference phenotypes covary among host plant genotypes

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Sexual selection acting on small initial differences in mating signals and mate preferences can enhance signal–preference codivergence and reproductive isolation during speciation. However, the origin of initial differences in sexual traits remains unclear. We asked whether biotic environments, a source of variation in sexual traits, may provide a general solution to this problem. Specifically, we asked whether genetic variation in biotic environments provided by host plants can result in signal–preference phenotypic covariance in a host-specific, plant-feeding insect. We used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae) to assess patterns of variation in male mating signals and female mate preferences induced by genetic variation in host plants. We employed a novel implementation of a quantitative genetics method, rearing field-collected treehoppers on a sample of naturally occurring replicated host plant clone lines. We found remarkably high signal–preference covariance among host plant genotypes. Thus, genetic variation in biotic environments influences the sexual phenotypes of organisms living on those environments in a way that promotes assortative mating among environments. This consequence arises from conditions likely to be common in nature (phenotypic plasticity and variation in biotic environments). It therefore offers a general answer to how divergent sexual selection may begin.

KEY WORDS: **Divergence, Fisherian selection, indirect genetic effects, plant–insect interaction, runaway, vibrational communication.**

When populations of sexually reproducing organisms diverge, assortative mating helps prevent the intermingling of gene pools. The cause of assortative mating can be as simple as geographic barriers or as complex as differences in mating signals and preferences (Kirkpatrick and Ravigné 2002). Because mating signals and preferences are involved in competition for mates, they often come under the influence of sexual selection, a strong evolutionary engine that can promote rapid evolution and elaboration in mating signals and mate preferences (West-Eberhard 1983, 2014; Hoekstra et al. 2001; Kingsolver et al. 2001; Hereford et al. 2004; Kokko et al. 2006). For instance, the default mechanism of sexual selection (Fisherian selection) arises from simple initial conditions: any initial advantage to a male trait in being preferred by a female (whether or not said preference is adaptive) coupled with genetic variation in the trait and the response establish male–female genetic covariance, which in turn creates a self-reinforcing runaway process of signal–preference coevolution. This process can enhance initial differences in sexual traits and provide reproductive isolation for the diverging populations (Fisher 1915, 1958; Lande 1981; Kirkpatrick 1982; Higashi et al. 1999; Mead and Arnold 2004; Prum 2010). It remains unclear, however, how this initial variation in sexual traits arises. Current theory relies on drift or ecology to provide the initial differences in sexual traits that then recruit sexual selection in the process of divergence (Coyne and

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Orr 2004; Rodríguez 2009; Nosil 2012). This is problematic because it limits the potential for divergence to rates achievable by neutral or ecological causes of variation, which are typically slower than observed rapid rates of diversification and elaboration of sexually selected traits (West-Eberhard 1983, 2014; Eberhard 1985, 1996; Boul et al. 2007; Funk et al. 2009; Safran et al. 2012). Thus, current theory lacks a general mechanism that can account for such rapid patterns of divergence. Here, we identify a source of variation in sexual traits that is capable of fueling high rates of evolution and that arises from conditions likely to be common in natural populations early in the process of divergence.

Biologists recognize an important role for the environment in speciation, in terms of adaptation to the use of different resources (Coyne and Orr 2004; Nosil 2012). But the contribution of the environment may be much broader: environmental inputs into trait expression are likely to have a widespread role in divergence because most traits are phenotypically plastic, so that changes in the environment often result in trait differences, and variants that arise through plasticity may foster evolutionary change by exposing hidden genetic variation to selection (West-Eberhard 2003, 2005; Suzuki and Nijhout 2006; Gerhart and Kirschner 2007; Barrett and Schluter 2008; Le Rouzic and Carlborg 2008; Renn and Schumer 2013). Further, environmental inputs have greater evolutionary potential than genetic inputs because environments can influence all or most individuals in them, whereas a novel mutation can only spread from those individuals initially bearing it (West-Eberhard 2003, 2005).

When the environment is comprised of other organisms, important evolutionary contributions may arise from the biotic nature of these environments. Consider, for example, the herbivores, parasites, and symbionts that spend considerable portions of their lives on the organisms that constitute their resources (West-Eberhard 1983; Moore et al. 1997; Bleakley et al. 2010; Rowntree et al. 2011; Lyon and Montgomerie 2012). In such cases, an individual's phenotype is shaped by inputs from its genome and environment, and also by indirect inputs from the genomes and environments of the organisms that comprise its environment. The latter genetic inputs are termed indirect genetic effects (IGEs) when conspecifics are involved and interspecific IGEs (IIGEs) when heterospecifics are involved (Moore et al. 1997; Rowntree et al. 2011). These inputs have been documented in taxonomically diverse studies, and in traits as varied as maternal provisioning, mating signals, mate preferences, fecundity, and even in the composition of arthropod fauna on trees (Wade 2000; Agrawal et al. 2001; Rowntree et al. 2011; Rebar and Rodríguez 2013, 2014a,b). IGEs and IIGES can generate evolutionary feedbacks between environments and the organisms on them, such that change in the environment (including evolutionary change) influences the distribution of phenotypes of the organisms in those environments, as well as the expression of genetic variation in those organisms and their response to selection (Wolf et al. 1998; Drown and Wade 2014). Here, we show that IIGEs influence the expression of sexual phenotypes and generate signal–preference covariance that could initiate divergent sexual selection.

The hypothesis that we test was developed for IGEs arising from social environments. It posits that IGEs on signals and preferences can give rise to runaway evolution even with little or no direct genetic signal–preference covariance (Bailey and Moore 2012; and see also Drown and Wade 2014). Here, we apply this hypothesis to cross-trophic IIGEs from developmental environments on the signals and preferences of animals on those environments. In these terms, this hypothesis states that cross-trophic IIGEs can generate signal–preference phenotypic covariance among different genotypes of the organisms that constitute the biotic environment for those animals. This hypothesis makes three predictions: (1) signals should be influenced by genetic variation in the biotic environment, (2) mate preferences should be influenced by genetic variation in the biotic environment, and (3) signal and preference phenotypes should covary according to patterns of genetic variation in the biotic environment.

We tested these predictions by assessing the influence of IIGEs stemming from one species of host plant on variation in the signals and preferences of a plant-feeding insect that specializes on that plant. We worked with a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae) and its host plant, *Viburnum lentago* (Caprifoliaceae). For many animals, colonization of, and adaptation to, novel environments plays an important role in the process of speciation (Nosil 2012), a process of particular importance for plant-feeding insects such as *E. binotata* treehoppers (Drès and Mallet 2002; Cocroft et al. 2008). Speciation in the *E. binotata* complex has involved colonization and adaptation to novel host plants (host shifts) accompanied by divergence in their systems of sexual communication (Wood 1993b; Cocroft et al. 2008). Each member of this complex specializes and lives entirely on its respective host plant species (Wood 1993b). These insects use plant-borne vibrational signals and male–female signaling duets precede pair formation (Fig. 1A; Cocroft et al. 2008). The most divergent adult trait among species within the complex is male signal frequency, for which females have strong preferences (Rodríguez et al. 2004, 2006; Cocroft et al. 2010). Female preference functions for male signal frequency are unimodal (closed) in shape—that is, they rise toward a favored intermediate value, termed the peak of the preference (Fig. 1B). Females from different species have different peak preferences for male signal frequency (Rodríguez et al. 2006), and male signal frequency and female peak preference tightly covary across species in the complex (Fig. 1C; Rodríguez et al. 2004, 2006). We therefore focused on signal frequency and the corresponding mate preference.

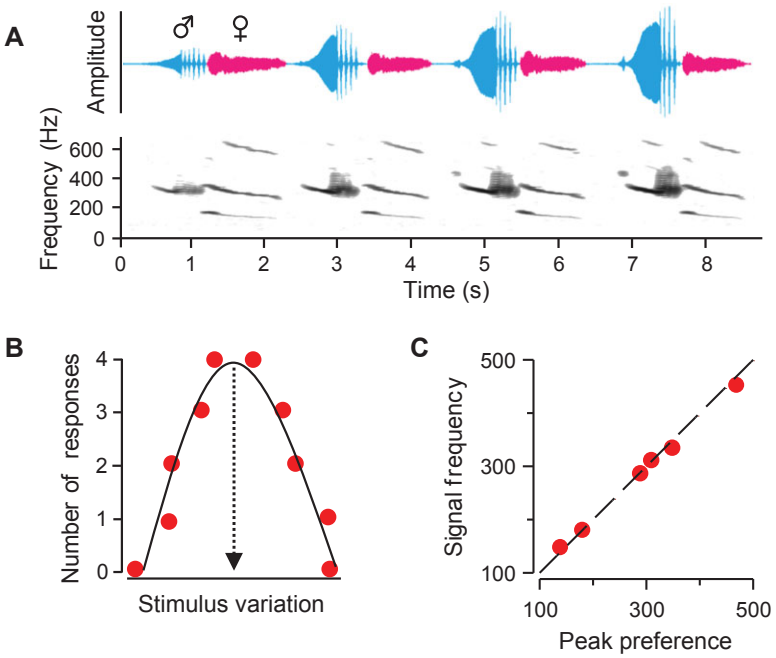

Figure 1. **(A) Waveform and spectrogram of a male–female signaling duet in the *E. binotata* species complex. Males produce signals in a bout, and each signal consists of a whine portion followed by several pulses. Females produce their own signal in response to male signals if they find them attractive (Rodríguez et al. 2004, 2006). (B) Generalized construction of a female mate preference function. Each female was tested across a range of stimuli, and the number of responses to each stimulus was used to create the preference function (see Methods). We viewed preference functions as function-valued traits (Meyer and Kirkpatrick 2005; Fowler-Finn and Rodríguez 2012a; Stinchcombe and Kirkpatrick 2012), and each female's peak preference was determined from that preference function, denoted by the dotted arrow. (C) The correspondence between mean male signal frequency and mean female peak preference for six members of the *E. binotata* species complex of treehoppers (Rodríguez et al. 2006; Rebar and Rodríguez 2013, 2014a,b). The dotted line indicates a 1:1 relationship.**

To test for host plant derived IIGEs on *Enchenopa* signals and preferences, we manipulated genetic variation in their *V. lentago* host plants, and described the mating signals and mate preferences of treehoppers that we reared on those host plants. A sample of clone lines of the treehoppers' host plants formed the background environment (Lynch and Walsh 1998), and we reared randomly collected treehoppers individuals on those plants so that any differences among clone lines could be attributed to the host plants. By using naturally occurring field-collected clones, we attempted to capture natural variation in the developmental environment provided by host plants to the treehoppers. We dug up equally sized plants that shared roots from naturally occurring clone patches to establish replicates of each clone. The replicated plant clones allowed us to estimate within- and among-plant clone effects on the treehoppers' mating signals and preferences. We then used laser vibrometry and vibrational playbacks to record male signals and describe female mate preference functions for signal frequency.

We have reported the tests for the first two predictions in prior work. We first looked at male advertisement signals, finding that male signal frequency was significantly influenced by genetic variation among host plants (Rebar and Rodríguez 2014b). We next focused on female preferences, finding that variation in the biotic environment provided by host plant genotypes influenced the mate preferences (Rebar and Rodríguez 2014a). Specifically, we found that female peak preferences were significantly influenced by genetic variation among host plants (Rebar and Rodríguez 2014a). Here, we report the test for the third, and most crucial, prediction that these signal and preference phenotypes covary among host plant genotypes.

Methods

STUDY SPECIES

There are two members of the *E. binotata* species complex that live on the host plant *V. lentago* (Caprifoliaceae) at our study site (Saukville, WI). While these species await formal description (Hamilton and Cocroft 2009), male signal frequency is a reliable trait to identify each species. We used the high-frequency species found on *V. lentago* (dominant frequency = 312 Hz), and we kept voucher specimens in 95% EtOH.

HOST PLANT CLONE LINES

We established replicated plant clone lines from field-collected *V. lentago* suckers (Rebar and Rodríguez 2014a,b). These plants

grow in clone patches in which a main plant becomes established and then produces suckers that grow up around it from lateral roots (Niering et al. 1986). We dug up six to eight evenly sized suckers (0.5 m tall) around a parental plant from 15 clone patches at the University of Wisconsin-Milwaukee (UWM) field station (Saukville, WI) in Fall 2011. We verified that plants were replicates of each clone by determining that their lateral roots were connected to one another. Each clone patch was a minimum of 50 m from any other patch to ensure genetic diversity among clone patches. We packed suckers in moistened peat moss and overwintered them in a dark cold room maintained at 4°C. The following March 2012, we potted individual suckers into one gallon plastic pots with Fafard 3B mix (Conrad Fafard Inc., Agawam, MA). Plants were moved into a greenhouse to promote budding and development. As not all plant individuals or clone lines successfully established themselves in the spring, we had three to six replicates of 14 clone lines on which we placed treehopper individuals (see below), which resulted in our final analysis of 11 clone lines with a minimum of three plant replicates ($N = 43$ plants total with a mean of 4 ± 0.9 replicates per clone line; see Statistical Analysis).

REARING

We randomly collected newly emerged nymphs from a large population of *E. binotata* treehoppers at Tendick Nature Park (Saukville, WI) in May 2012 (Rebar and Rodríguez 2014a,b). Females lay eggs in the stem of the host plant in the fall, and unrelated females aggregate to lay eggs on a single stem (Wood 1993a; Cocroft et al. 2008). Eggs overwinter until the following spring when plant budding and sap flow triggers development of *Enchenopa* embryos, and nymphs hatch synchronously (Wood 1993b; Cocroft et al. 2008). Nymphs develop on the same plant where they hatched (Wood 1993b), and we collected nymphs by cutting stems from more than 50 different plants across a 100 m transect. Thus, our sampling technique likely reflects a large amount of treehopper genetic diversity for this population, and we used field-collected individuals to capture a broad range of natural variation. We then individually transferred nymphs to our replicated clone lines, distributing nymphs from each cut stem across as many clone lines and replicates as possible to minimize the likelihood of relatedness between nymphs placed on the same plant replicate. Each host plant replicate had 30 individuals, and individuals were allowed to develop together from first instar until their adult molt. Males were removed from each plant upon reaching sexual maturity (approximately two weeks later), and we recorded their mating signals immediately thereafter on a separate plant (see below), and then placed individuals in 95% EtOH. Females remained on their respective host plants until they were sexually receptive (approximately three weeks after the males), at which time we assayed their preference for male signals, and then placed individuals in 95% EtOH. Our final sample included 250 recorded males and 165 females assayed for preferences (see below), equating to 5.8 ± 2.4 males and 3.8 ± 1.6 females (means $\pm$ SD) per plant replicate. The greater sample size for males reflects in part the greater delay before females are sexually responsive (see above) and the greater difficulty in obtaining complete playback sequences to construct female mate preference functions (see below). Nevertheless, our final sample size of 415 treehoppers reared on 43 plant replicates representing 11 plant clone lines has sufficient power to test the predictions.

MALE SIGNAL RECORDING AND ANALYSIS

We used a single commercially acquired *V. lentago* testing plant individual for all recording and playback trials. This ensured that the recording plant was distinct from all rearing plants, and minimized variation in plant signal transmission features, along with any other potential source of plant variation that could influence treehopper behavior (Rebar and Rodríguez 2014b). We randomized recording across and within clone lines over the course of the recording phase in an attempt to minimize differences in age and exposure of individuals to other males' signals.

To record male signals, we removed each male from his host plant and placed him at a standard position on the stem of the recording plant. We primed males to signal by playing a male–female duet recording through a piezo-electric actuator that was attached to the stem with accelerometer wax (model AE0505D16; Thorlabs, Newton, NJ). All males were presented with the same duet recording so that their experience was the same. We controlled the actuator with a piezo controller (model MDT694A; Thorlabs) connected to an iMac computer. The male–female duet was played at a peak amplitude of 0.10 mm s^{-1}, an average peak amplitude of *Enchenopa* male signals (D. Rebar and R. L. Rodríguez, unpubl. ms.). We recorded male signals with a laser vibrometer (model CLV-2534; Polytec Inc., Auburn, MA) whose beam was focused onto a piece of reflective tape (about 2 mm^2) that was placed on the plant stem. The male signals picked up by the laser vibrometer were sent through a band-pass filter (40–4000 Hz, Krohn-Hite 3202; Krohn-Hite Corp., Brockton, MA) at 60 Hz, and the output was sent to a second iMac computer through USB audio interface (Edirol UA-25; Roland Corp., Hamamatsu, Japan). We recorded these signals with AUDACITY (version 1.2.5; http://audacity.soundforge.net) at a sampling rate of 44.1 kHz. The setup was isolated from building vibrations by placing vibration dampening pads (model 3291–22-PM-50; Polymer Dynamics, Inc., Allentown, PA) under each leg of a slate table (1×2 m). We then placed an iron plank (about 135 kg) on top of partially inflated bicycle inner tubes on top of the table, and finally placed the recording plant on shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY) on top of the iron plank.

Males typically produce several signals per bout, and we standardized our measurement of male signal frequency by selecting the bout of highest amplitude from the 1-min recording. Signal features slightly vary along a bout, and signal amplitude increases over the first few signals of a bout (Cocroft et al. 2010). We chose the third signal in a bout as the landmark signal to analyze. For those males producing less than three signals, we used the last signal in the bout. This approach balances the benefit of having a landmark for most males (82% of males produced bouts of three or more signals) with the benefits of analyzing a high-amplitude signal within the bout. We measured signal frequency from the last 10 cycles of the whine portion of the signal. In total, we recorded 250 males across the 43 plant replicates, with 5.8 ± 2.4 males (mean $\pm$ SD) recorded per plant. All males were recorded in July 2012 and we analyzed all signals with AUDACITY.

ASSAY OF FEMALE MATE PREFERENCES

Females respond with their own vibrational signal to those males that produce an attractive mating signal (Rodríguez et al. 2004, 2006), and duetting with a male increases the likelihood of the female mating with that male (Rodríguez et al. 2004). A female's likelihood of responding is also strongly related to the number of responses she gives to the signaling bout of a male (Rodríguez et al. 2004, 2012; Fowler-Finn and Rodríguez 2012a). We took advantage of this duetting behavior used by *Enchenopa* in pair formation to assay female responses, as the number of times a female responds to a male's signal is a reliable indicator of that signal's attractiveness (Fowler-Finn and Rodríguez 2012a,b; Rebar and Rodríguez 2013, 2014a).

We described each female's mate preference by presenting her with a unique, randomly generated sequence of 19 synthesized male signals that were 2, 4, 6, 8, 10, 15, 20, 30, and 40 Hz from the population mean (312 Hz) in each direction (Rebar and Rodríguez 2014a). We set all other features of the signals to the population mean, and each signal was presented as a bout of four, the mean number of signals per bout for males in this population. Each signal bout was separated by 15 sec of silence. We quantified a female's responses to the signals across the tested range to create her mate preference function. We delivered all synthetic stimuli using a custom MATLAB script (R2010b; Mathworks, Inc., Natick, MA), and they were delivered through the same playback system as was used to prime the males, and recorded female responses using the same laser vibrometer setup.

We used the same recording plant as for the males for all of the females. We removed each female from her rearing plant and placed her at the same site on the recording plant stem. We first tested her receptivity by playing back a recording of a live male, using the same priming duet as for the males but without the female response so that females would respond to the male signal if they were receptive. Females that responded were then presented with a randomized sequence of the 19 signal models. We then replayed the recording of the live male at the end of the sequence to verify that the female was still receptive. In total, we recorded the responses of 165 females across the 43 plant replicates, with 3.8 ± 1.6 females (mean $\pm$ SD) recorded per plant.

DETERMINING PEAK PREFERENCE

Mate preferences are function-valued traits (Meyer and Kirkpatrick 2005; Fowler-Finn and Rodríguez 2012a; Stinchcombe and Kirkpatrick 2012), meaning that a female's responses are a function of the mating signals that she encounters. We first constructed a preference function by generating cubic splines using the mgcv package and a custom written script in R (version 2.13.2; http://www.r-project.org) (see online Supporting Information). We then analyzed preference functions as function-valued traits. We determined the peak preference (the signal frequency that elicited the greatest response from a female) from each female's preference function by identifying the signal stimulus value that corresponded to the highest point along the curve of her preference function (Fig. 1B; online Supporting Information lines 395–441; Rebar and Rodríguez 2013; Rodríguez et al. 2013).

STATISTICAL ANALYSIS

We included in our analysis only those host plant clones that had at least three replicates; that is, that were represented by at least three plant individuals on which treehoppers were reared, each with at least one female tested and one male recorded. We tested for signal–preference phenotypic covariance among host plant clones with a linear mixed model (following Gray and Cade 2000) that allowed us to assess variation in treehopper signals and preferences among host plant clones and within host plant replicates among clones. The model had male signal frequency and female peak preference (both in hertz) as a single dependent variable. The signal/preference nature of this variable was indicated as an explanatory fixed variable for sex (male/female) in the model. The model also included random terms for host plant clone and replicate (nested within clone) and the clone $\times$ sex interaction. This model allows a straightforward conceptualization and visualization (Table 1; Fig. 2) for signal–preference covariance among plant clones (tested by the clone term) and among plant replicates within clones (replicate term). The clone $\times$ sex interaction explicitly tests for signal–preference *mismatch* among plant clones (nonparallel lines in Fig. 2). Note that in this model, the clone term is tested over the interaction term ($F = \mathrm{MS}_{\mathrm{clone}}/\mathrm{MS}_{\mathrm{interaction}}$; Table 1), so that a significant clone term indicates not only covariance among plant clones but also near-perfect covariance (near parallel lines in Fig. 2; cf. Fry 1992). Finally, the main term for

Table 1. Test for phenotypic covariance between male signal frequency and female peak preference for *Enchenopa* treehoppers reared on different host plant clone lines (see text for explanation of the model).

Factor	df	MS	*F*, *P*
Plant clone	10, 5.94	152.87	**5.64, 0.023**
Replicate (plant clone)	32, 32	37.48	1.09, 0.403
Sex	1, 11.26	54.28	1.97, 0.187
Plant clone × sex	10, 32	27.10	0.79, 0.639
Residual		34.32	

The term for plant clone tests for differences in signals and preferences among clones above and beyond any nonparallelism (see Fig. 2). Plant clone and replicate are random terms, with replicate nested within plant clone. Significant values are in bold.

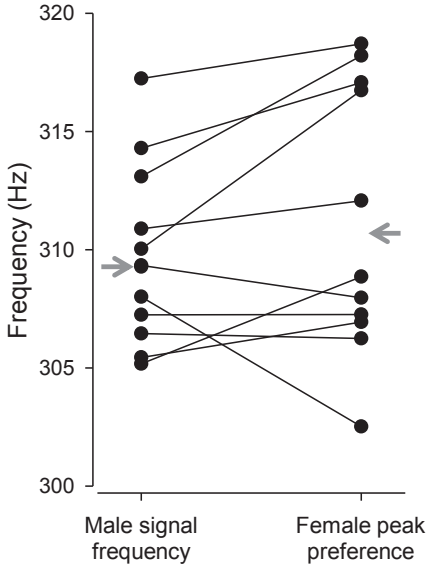

Figure 2. Visual representation of the statistical model that shows the strong phenotypic correlation between male signals and female preferences in *Enchenopa* treehoppers reared on 11 different host plant clone lines. Each line in the graph displays the mean male signal frequency and female peak preference for treehoppers reared on a given clone line. The space between the lines and their relatively parallel orientation indicate strong phenotypic differentiation of treehopper signals and preferences according to their developmental environments (plant clone term in Table 1). Arrows indicate the overall mean male signal frequency (309 Hz) and mean female peak preference (311 Hz).

sex tests for a population-level mismatch between mean signal frequency and mean peak preference. We then estimated the magnitude of the signal–preference covariance among plant clones and among plant replicates within clones with separate Pearson product-moment correlations.

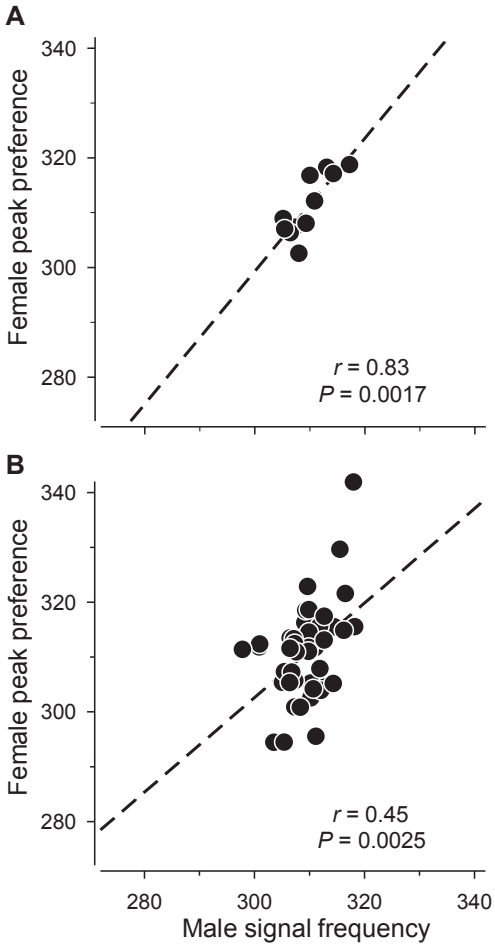

Figure 3. Correlations between male signal frequency and female peak preference in *Enchenopa* treehoppers. (A) Signal–preference correlation among host plant clones (n = 11), reflecting IIGEs. (B) Signal–preference correlation among host plant replicates (n = 43), depicting the amount of within-clone line variation. The dotted line in each panel denotes the correlation between the two traits.

Results

We found significant phenotypic covariance between male signal frequency and female mate preference across host plant clones (Figs. 2, 3, Table 1). Visual representation of mean signal and peak preference phenotypes clearly shows how remarkably signals and preferences covary among host plant clone lines, as illustrated by the fairly parallel lines with minimal nonparallelism (Fig. 2), corresponding to the significant plant clone term and nonsignificant plant clone × sex interaction (Table 1). The nonsignificant main sex term indicates population-level correspondence between mean signal and peak preference phenotypes (Table 1, Fig. 2).

We estimated the magnitude of the signal–preference phenotypic covariance among plants with Pearson product-moment correlations. We first used the mean signal frequency and mean peak

preference for the treehoppers on each host plant clone, which addresses the correlation between *Enchenopa* signals and preferences due to the host plant clones. This correlation was significant and of large effect size ($r = 0.83$, $P = 0.0017$; Fig. 3A). Second, we used the mean male signal frequency and mean female peak preference for each host plant replicate. This correlation includes not only variation due to differences between plant replicates within clones but also variation due to differences in social interactions between treehopper aggregations on the different plant replicates (including potential social IGEs; Rebar and Rodríguez 2013). This correlation was significant and of intermediate effect size ($r = 0.45$, $P = 0.0025$; Fig. 3B).

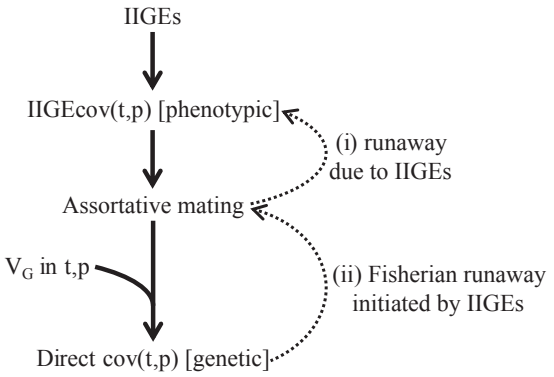

Figure 4. Heuristic model for how IIGEs on signals and preferences could initiate runaway processes. When IIGEs result in signal–preference phenotypic covariance (IIGEcov(t.p) [phenotypic]), this in turn will lead to assortative mating and generate a runaway process among environments (i) (cf. Bailey and Moore 2012). Additionally, if there is genetic variation (V_G) in both the trait and preference, the underlying genetic architecture may change, leading to direct genetic covariance between the trait and preference (direct cov(t,p) [genetic]), which in turn may kick-start the Fisherian runaway process (ii).

Discussion

We tested the hypothesis that cross-trophic IIGEs generate phenotypic covariance in the signals and mate preferences of treehoppers that developed on different host plant genotypes. In prior work, we have demonstrated cross-trophic IIGEs on male signals and mate preferences (Rebar and Rodríguez 2014a,b). Here, we demonstrate that these IIGEs generate very strong signal–preference phenotypic covariance. What is remarkable about this finding is that signal–preference phenotypic covariance arises as a by-product of standing genetic variation in the treehoppers' biotic environment. These results provide support for a key prediction of general IGE theory (Bailey and Moore 2012; tested here in terms of cross-trophic IIGEs), that IGEs can initiate a runaway process even without direct genetic signal–preference covariance. In terms of *Enchenopa* treehoppers and their host plants, there could arise a runaway process with treehoppers diverging in signals and preferences and mating assortatively among different host plant clone patches (Fig. 4, stage i). An important additional point is that, once assortative mating is generated by plant-related IIGEs, any direct genetic variation in signals and preferences would establish direct genetic signal–preference covariance, giving rise to a standard Fisherian runaway (Fig. 4, stage ii). Thus, there is a broad range of conditions that may initiate divergent sexual selection (biotic environments and the default consequences of assortative mating due to those biotic environments). Note also that the above runaway processes could be altered by changes in the originating IIGEs (say, due to evolution in the treehoppers' host plants), further promoting divergent sexual selection.

Our experimental approach offers a robust test of cross-trophic IIGEs. By distributing randomly collected, unrelated treehopper individuals across host plants, we are confident that the shifts in signals and preferences are due to inputs on signal and preference development originating from the host plant environments, and not from genetic differences in the treehoppers themselves. Further, by using replicated clone lines, we can partition variation between components due to variation among plant clone lines (IIGEs) and variation within plant clone lines. The latter component includes variation in the environments offered by different plant individuals as well as by different social dynamics in the treehopper aggregations generated by the random placing of field-collected nymphs on each plant replicate, which may involve social IGEs. In prior work, we have reported social IGEs on *Enchenopa* female mate preferences (Rebar and Rodríguez 2013). In addition, IGEs and IIGEs may interact and contribute to signal–preference variation. However, the lack of a significant replicate effect (Table 1) suggests that these inputs were not as strong as the main IIGEs, although they did weaken signal–preference phenotypic covariance (Fig. 3). Note, however, that even the weaker pattern of phenotypic covariance among replicates could give rise to the standard Fisherian runaway in stage (ii) of Figure 4 in the presence of direct genetic variation in signals and preferences.

In our experiment, we use host plant clone identity as a "black box" that captures any plant features that influence the development of treehopper signals and preferences, as long as those features differ among clones (i.e., as long as there is genetic variation in those plant features). This approach affords great power to detect IIGEs, but it comes at the cost of not knowing the aspects of the plants' phenotypes that influence the treehoppers' phenotypes (that constitute Ψ, in terms of IGE theory [Moore et al. 1997; Wolf et al. 1998]). For example, plant differences in the nutritional quality of their sap or in the mixtures of secondary compounds might be important factors. Investigating the proximate causes of such effects will help to understand the impact

of IIGEs on the evolutionary process (Bleakley and Brodie 2009; Bailey and Hoskins 2014).

Our findings offer a general solution to how divergence by sexual selection may begin, with assortative mating arising under even a broader range of conditions than previously anticipated. In particular, our findings suggest that within-environment dynamics are an important factor at play when speciation involves the colonization of novel environments (Drès and Mallet 2002; Cocroft et al. 2008; Nosil 2012). In plant-feeding insects, as in many organisms, not only are changes in the species of host plant used by the insects important to changes in sexually selected traits, but which plant genotypes, plant phenotypes, and even plant individuals are used may be important in initiating patterns of assortative mating. Within-environment dynamics are an underappreciated source of variation on the behavior and evolutionary dynamics of the organisms living in those environments that can generate assortative mating and jump-start Fisherian selection. This dynamic does not require adaptation to different environments, but it may facilitate it by providing a mechanism of reproductive isolation between populations adapting to different environments. Consideration of standing variation in the biotic environment provides a broad and simple explanation for the origin of initial differences in sexual traits that can fuel rapid diversification by sexual selection.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Annotated R script used to create individual female preference functions.