ECOLOGY LETTERS

Ecology Letters, (2014) **17**: 203–210 doi: 10.1111/ele.12220

LETTER

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

Darren Rebar* and Rafael L. Rodríguez

Behavioral and Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin–Milwaukee, Lapham Hall, 3209 N Maryland Ave, Milwaukee, WI, 53201, USA

**Correspondence: E-mail: dwrebar@uwm.edu*

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 compositions of arthropod faunas. We demonstrate that such cross-trophic interactions also influence sexually selected traits. We used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae) to ask whether male mating signals are influenced by host plant genetic variation. We reared a random sample of the treehoppers on potted replicates of a sample of host plant clone lines. We found that treehopper male signals varied according to the clone line on which they developed, showing that genetic variation in host plants affects male treehoppers' behavioural phenotypes. This is the first demonstration of cross-trophic indirect genetic effects on a sexually selected trait. We discuss how such effects may play an important role in the maintenance of variation and within-population phenotypic differentiation, thereby promoting evolutionary divergence.

Keywords

Developmental plasticity, indirect genetic effects, laser vibrometry, plant–insect interactions, vibrational signals.

Ecology Letters (2014) **17**: 203–210

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 (West-Eberhard 2003), 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 (West-Eberhard 1983; Hereford *et al*. 2004), and experience with the behaviour of other individuals is often an important cause of variation in phenotypes (West-Eberhard 2003; Verzijden *et al*. 2012; Rodríguez *et al*. 2013b). Similarly, many species spend considerable portions of their lives on other 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.

A 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 (Wolf *et al*. 1999; Shuster *et al*. 2006; Hughes *et al*. 2008). 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 (Danielson-François *et al*. 2009; Bailey & Moore 2012; Rebar & Rodríguez 2013). 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 (Moore *et al*. 1997). Empirical research on IGEs is only just beginning, but there is evidence that they are taxonomically widespread (Kent *et al*. 2008; Bleakley & Brodie 2009; Danielson-François *et al*. 2009) and that they affect important fitness-related traits, such as maternal provisioning behaviour, fecundity and mate preferences (Wade 2000; Agrawal *et al*.2001; Rebar & Rodríguez 2013).

When dealing with environments that are not social, but instead involve heterospecific individuals, researchers refer to interspecific indirect genetic effects (IIGEs; Rowntree *et al*. 2011). Exploration of IIGEs has revealed diverse effects on so-called community phenotypes (Whitham *et al*. 2006; Hughes *et al*. 2008; Bailey *et al*. 2009). There is, e.g. 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 (Johnson *et al*. 2006; Zytynska *et al*. 2011; Moreira & Mooney 2013).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 (Khudr *et al*. 2013). 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, we 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.

We 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. We used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae), a group in which speciation has involved colonisation and adaptation to novel host plant species and divergence of their communication systems (Wood 1993; Cocroft *et al*. 2008). These treehoppers spend their entire lives on their host plants (Wood 1993) and communicate with plant-borne vibrational signals (Cocroft *et al*. 2008). Males produce mating signals, and females exhibit strong mate preferences on the basis of the features of those signals, particularly length and signal frequency – the latter being the most divergent feature of adult phenotypes in the clade (Rodríguez *et al*. 2004, 2006; Cocroft *et al*. 2010). Although in this study we describe variation in mating signals and not in reproductive success, there is evidence that male mating signals are an important determinant of reproductive success (Sullivan-Beckers & Cocroft 2010). 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 (Rodríguez *et al*. 2006; McNett & Cocroft 2008). They are also an important determinant of behavioural reproductive isolation between the members of the complex (Wood 1980; Rodríguez *et al*. 2004).

Our 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. We used a quantitative genetics experimental design in which clone lines of a sample of host plant genotypes formed the background environment (Lynch & Walsh 1998), and randomly collected insect individuals were reared on those environments. We described the signals of those insects and estimated the variation due to among- and within-clone line components.

We test two hypotheses about the role of cross-trophic interactions in shaping the phenotypes of individuals influenced by those interactions. First, we 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, we test the hypothesis that genetic variation in the host plants influences male mating signals (i.e. we 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

We used one of the two members of the *E. binotata* complex that live on the host plant *Viburnum lentago* (Caprifoliaceae) in our 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 (Rodríguez *et al*. 2004; Hamilton & Cocroft 2009; Cocroft *et al*. 2010). We used the high-frequency species found on *V. lentago* (dominant frequency = 312 Hz), and we kept voucher specimens in 95% EtOH.

Our experiment consisted of a rearing phase and a signal-recording phase. During the rearing phase, we 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). We then recorded the mating signals of those males.

Rearing

We established replicated plant clone lines to determine within- and among-clone line effects on the tree hoppers. *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 (Niering *et al*. 1986). The suckers remain connected to the parent plant and each other through lateral roots. We took advantage of this growth feature by digging up evenly sized suckers (0.5 m) surrounding a parental plant from the University of Wisconsin-Milwaukee (UWM) Field Station (Saukville, WI, USA) in Fall 2011. We ensured that the suckers were clones of one another by verifying that they were connected by lateral roots. We placed the suckers in moistened peat moss and stored them over winter in a dark cold room maintained at 4 °C. The following March 2012, we potted each sucker into a one gallon plastic pot using Fafard 3B mix (Conrad Fafard Inc., Agawam, MA, USA). We then moved the potted plants into a greenhouse to promote the onset of budding and subsequent development.

We obtained treehopper individuals by randomly collecting newly emerged nymphs from a large population located at Tendick Nature Park 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 minimise the likelihood of relatedness on the same plant or within a clone line (Fig. 1). Individuals were reared together on each plant from the time they were first instars until their adult moult. We recorded signals from all males 2–3 weeks after the adult moult. We were thus able to partition variation in male signal traits among components due to clone lines and within-clone line replicates (Fig. 1).

Signal recording and analysis

We used a single recording plant individual for all males, which was a different genotype from any of the rearing plants.

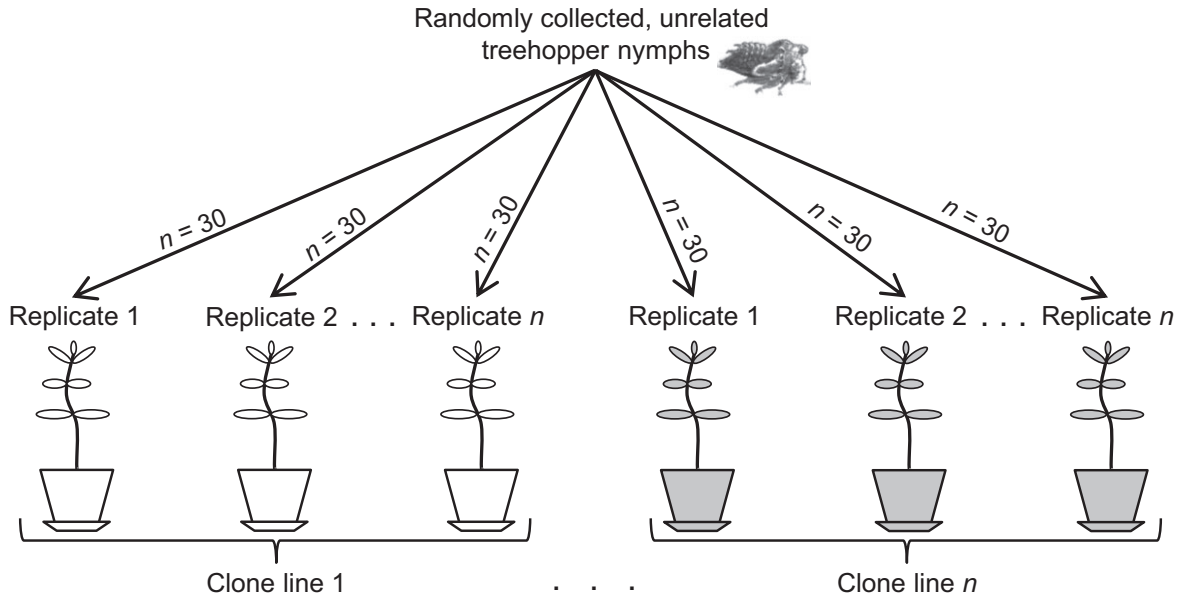

Figure 1 Experimental design to test if genetic variation in host plants influences the mating signals of treehopper individuals reared on them. Clones were used as the genetic component, with at least three plant individuals as replicates for each clone. Randomly collected, unrelated treehopper individuals were reared on those plants, and we assessed variation in their mating signals according to among- and within-clone components.

We used only one recording plant to minimise the potential for plant signal-transmission features to influence our measures of signal variation and any other potential influences on the treehoppers' behaviour. We 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 (Sattman & Cocroft 2003; Cocroft *et al.* 2006; Rodríguez *et al.* 2008).

We placed each male at the same site on the recording stem, and we 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 (model AE0505D16; Thorlabs, Newton, NJ, USA). The actuator was controlled by a piezo controller (model MDT694A; Thorlabs) from an iMac computer at an amplitude of 0.10 mm s^{-1}. We recorded male signals with a laser vibrometer (model CLV-2534; Polytec Inc., Auburn, MA, USA). We focused the laser beam onto a small piece of reflective tape (*c.* 2 mm^2) placed on the plant stem. Males were within 10 cm of the reflective tape when they signalled. 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, USA) at 60 Hz. The output was sent to an iMac computer through an Edirol UA-25 USB interface (Roland Corporation, Hamamatsu, 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. We monitored male signals with a Hameg HM 504-2 50 MHz oscilloscope (Hameg Instruments, Mainhausen, Germany). To isolate the set-up from noise due to building vibrations, the recording plant was placed on shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY, USA) on top of an iron plank (*c.* 135 kg) resting on partially inflated bicycle inner tubes on top of a slate table (*c.* 1 × 2 m). We also placed vibration dampening pads (model 3291-22-PM-50; Polymer Dynamics, Inc., Allentown, PA, USA) under the table legs to further isolate the entire setup. We randomised recording across and within clone lines over the course of this phase in an attempt to minimise 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. 2). We standardised our 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, we measured the last signal in the bout (n = 55 of 324 males). Male signals consist of a whine portion followed by several pulses (Fig. 2; Rodríguez *et al.* 2006). We analysed variation in seven signal traits that differ among species in the *E. binotata* complex. We measured the interval between signals, length of the whine portion, number and length of the pulses, the pulse rate and the dominant frequency (Fig. 2). We measured frequency from the last 10 cycles of the whine portion of the waveform because male signals are relatively pure tone. We conducted all analyses with AUDACITY.

Statistical analysis

We were interested in analysing 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 (Rodríguez *et al.* 2006; Cocroft *et al.* 2008; Sullivan-Beckers & Cocroft 2010). However, this approach increases the chance of spurious significance (Rice 1989), while measures that reduce this

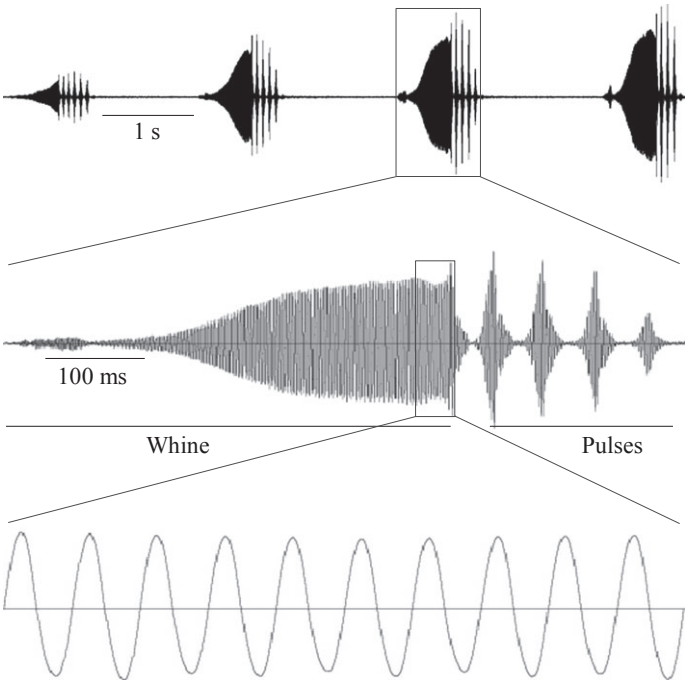

Figure 2 Recording of a bout consisting of four signals that increase in amplitude, along with close-ups of the waveform of a signal produced by male *Enchenopa binotata*.

risk also reduce statistical power (Moran 2003; Nakagawa 2004). To deal with this problem, we assessed the degree of non-independence in our data with a principal component analysis on the seven signal traits. This analysis yielded four axes with eigen values > 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 the four axes only 70% of the total variation in male signals was accounted for. This indicates that, in our study, variation in each of the original signal traits was very poorly correlated with variation in the other traits. To confirm this result, we 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, we consider that analysing the original signal traits separately is justified, as well as evolutionarily relevant. Nevertheless, to allay concerns about spurious significance, we also report the results of the analysis with the four PCA axes.

The aim of our 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 among and within clone lines. We 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. We initially included temperature as a covariate, but it was non-significant for all signal traits and we 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), we estimated broad-sense heritability for genetic variation among host plants in the induction of variation in the treehoppers' mating signals. We denote this estimate as H^2_{IIGE}, and obtained it as follows: $H^2_{\mathrm{IIGE}} = \sigma^2_{\mathrm{clone}}/(\sigma^2_{\mathrm{clone}} + \sigma^2_{\mathrm{residual}})$. We 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 ($\sigma^2_{\mathrm{clone}}$; Lynch & Walsh 1998). This among-clone component of variation contains both additive and non-additive (dominance, epistasis and common environmental effects) genetic variation, and therefore likely overestimates narrow-sense heritability (Lynch & Walsh 1998). Significance for the test of the hypothesis that $H^2_{\mathrm{IIGE}} > 0$ is provided by the clone term in the above linear-mixed models. In addition, we calculated the standard error for each H^2_{IIGE} estimate. As there is no precedent to follow, we adopted the procedure for typical broad-sense heritability with weighted clone line samples (Roff 1997, p. 42). We performed all statistical analyses in JMP v. 7.0 (SAS Institute Inc., Cary, NC, USA).

We only included in our 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 our analysis was $n = 324$.

RESULTS

We found a cross-trophic component of variation to male tree hopper 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. 3, Table 1). That is to say, we 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 2). 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 1). In particular, the signal traits that most contribute to mate choice decisions (whine length and signal frequency; Rodríguez *et al.* 2006; Cocroft *et al.* 2010) were influenced by these IIGEs (Fig. 3, Table 1).

We 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. 3, Tables 1 and 2).

DISCUSSION

Here, we 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, we were able to ask whether variation in the mating signals of insect

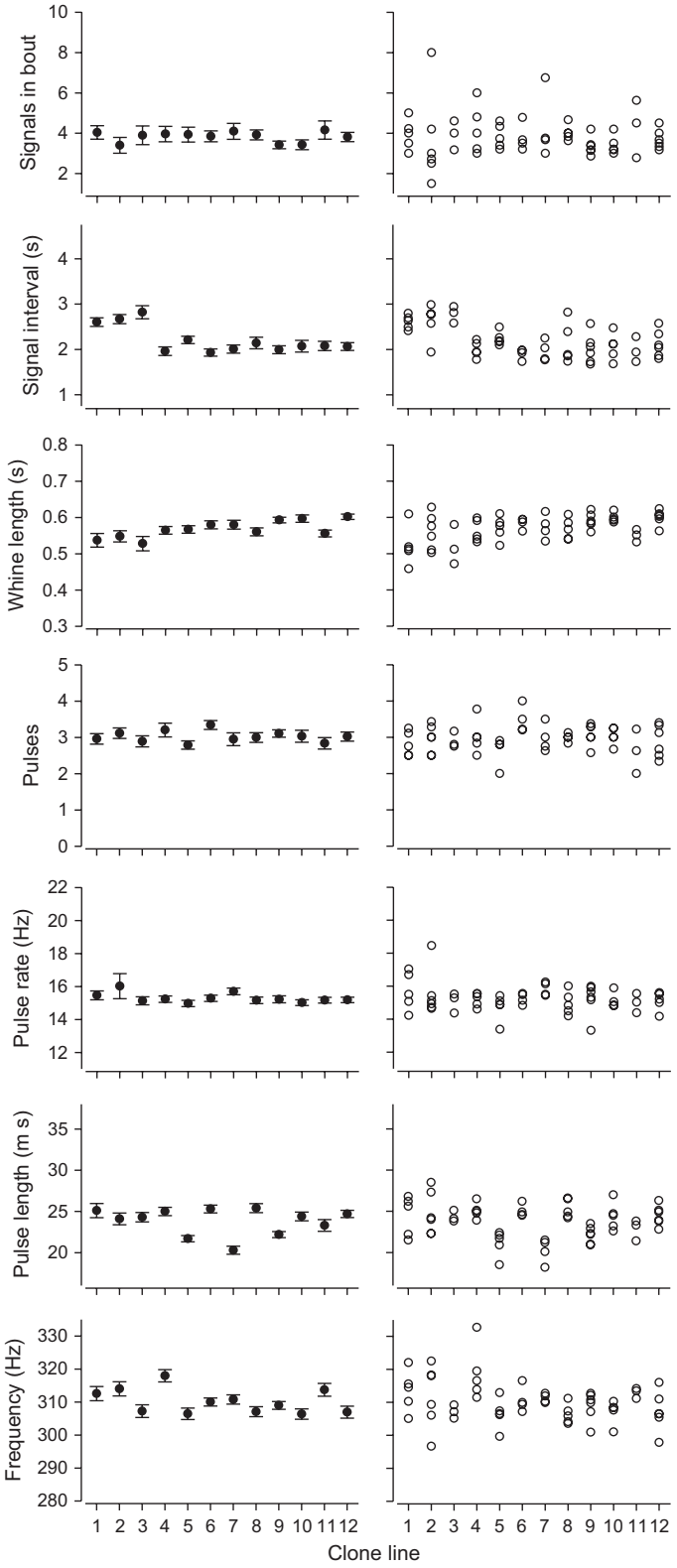

Figure 3 Clone mean ± 1 SE (left column, closed circles) and replicate means (right column, open circles) for the seven treehopper male signal traits analysed across the 12 clone lines. The *y*-axis represents the range of phenotypic variation in each male trait in the study population.

Table 1 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}

Trait	Factor	d.f.	*F*	*P*	Var. Comp.	$H^2_{\mathrm{IIGE}} \pm$ 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	

Significant tests and estimates are in bold.

Table 2 Principal components analysis of the seven measured *Enchenopa* male signal traits, and variation in each component attributed to differences among-clone lines and within-clone lines (replicates)

Trait	Eigenvalue	PVE	Factor	d.f.	*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

PVE denotes the per cent of variance explained by the corresponding principal component. Significant tests and estimates are in bold.

individuals that developed on the plants was influenced by among-clone differences or other environmental effects. We demonstrate that among-clone variation in cross-trophic interactions influences several signal traits, with additional within-clone components of variation. Although we 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 (Sullivan-Beckers & Cocroft 2010), and that they have evolved under strong selection arising from mate choice (Rodríguez *et al.* 2004, 2006). Consequently, the cross-trophic IIGEs on mating signals that we 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 we detect? Are plant defensive compounds involved? Are plants selected to induce such variation? (Given our 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 we 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 (Rodríguez *et al*. 2008). 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 (Astles *et al*. 2005).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 (Rodríguez *et al*. 2006; Cocroft *et al*. 2010). 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 favouring 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 signalling 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. Bailey & Moore 2012).

More broadly, our 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 (Rundle & Nosil 2005; Schluter 2009; Nosil 2012). In the context of this study, the colonisation 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 (Coley & Barone 1996; Drès & Mallet 2002; Cocroft *et al*. 2008). Our 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, we 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 (Rebar & Rodríguez 2013) and male signalling traits (D Rebar unpublished data), along with plasticity in mate preferences arising from social experience (Fowler-Finn & Rodríguez 2012a,b; Rodríguez *et al*. 2013a). 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 we 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

We thank D. Cruz and T. Schuck for help with plant care and D.A. Gray for the idea of using naturally occurring clones. We also thank E. Latch for fruitful discussions and F. Barbosa for helpful feedback on the manuscript. This study 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.

AUTHOR CONTRIBUTION

D.R. and R.L.R. designed the study, D.R. performed the study and collected the data, and D.R. and R.L.R. analysed the data. D.R. wrote the first draft of the manuscript, and R.L.R. contributed significantly to the revisions.

REFERENCES

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

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.

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.

Bailey, J.K., Schweitzer, J.A., Úbeda, F., Koricheva, J., LeRoy, C.J., Madritch, M.D. *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.

Bleakley, B.H. & Brodie, E.D., III (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.

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.

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.). University of California Press, Berkeley, CA, pp. 88–100.

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.

Coley, P.D. & Barone, J.A. (1996). Herbivory and plant defenses in tropical forests. *Annu. Rev. Ecol. Syst.*, 27, 305–335.

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

Drès, M. & Mallet, J. (2002). Host races in plant–feeding insects and their importance in sympatric speciation. *Philos. Trans. Roy. Soc. B.*, 357, 471–492.

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

Fowler-Finn, K.D. & Rodríguez, R.L. (2012b). Experience-mediated plasticity in mate preferences: Mating assurance in a variable environment. *Evolution*, 66, 459–468.

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.

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

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.

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.

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.

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.

Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.

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

Moore, A.J., Brodie, E.D., III & Wolf, J.B. (1997). Interacting phenotypes and the evolutionary process: i. Direct and indirect genetic effects of social interactions. *Evolution*, 51, 1352–1362.

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

Moreira, X. & Mooney, K.A. (2013). Influence of plant genetic diversity on interactions between higher trophic levels. *Biol. Lett.*, 9, 20130133.

Nakagawa, S. (2004). A farewell to Bonferroni: The problems of low statistical power and publication bias. *Behav. Ecol.*, 15, 1044–1045.

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.

Nosil, P. (2012). *Ecological Speciation*. Oxford University Press, Oxford.

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

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution*, 43, 223–225.

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.

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.

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.

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

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

Roff, D.A. (1997). *Evolutionary Quantitative Genetics*. Chapman & Hall, New York, NY.

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.

Rundle, H.D. & Nosil, P. (2005). Ecological speciation. *Ecol. Lett.*, 8, 336–352.

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.

Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science*, 323, 737–741.

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.

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.

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.

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, E.D., III & Wade, M.J.). Oxford University Press, New York, pp. 213–231.

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

West-Eberhard, M.J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press, New York.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J. *et al*. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat. Rev. Genet.*, 7, 510–523.

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

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

Wood, T.K. (1993). Speciation of the *Enchenopa binotata* complex (Insecta: homoptera: Membracidae). In: *Evolutionary Patterns and Processes* (eds Lees, D.R. & Edwards, D.). Academic Press, New York, pp. 299–317.

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.

Editor, David Westneat
Manuscript received 7 October 2013
First decision made 11 October 2013
Manuscript accepted 18 October 2013