

Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects

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Mate choice is considered an important influence in the evolution of mating signals and other sexual traits, and—since divergence in sexual traits causes reproductive isolation—it can be an agent of population divergence. The importance of mate choice in signal evolution can be evaluated by comparing male signal traits with female preference functions, taking into account the shape and strength of preferences. Specifically, when preferences are closed (favouring intermediate values), there should be a correlation between the preferred values and the trait means, and stronger preferences should be associated with greater preference–signal correspondence and lower signal variability. When preferences are open (favouring extreme values), signal traits are not only expected to be more variable, but should also be shifted towards the preferred values. We tested the role of female preferences in signal evolution in the *Enchenopa binotata* species complex of treehoppers, a clade of plant-feeding insects hypothesized to have speciated in sympatry. We found the expected relationship between signals and preferences, implicating mate choice as an agent of signal evolution. Because differences in sexual communication systems lead to reproductive isolation, the factors that promote divergence in female preferences—and, consequently, in male signals—may have an important role in the process of speciation.

Keywords: vibrational signalling; sexual coevolution; phytophagous insects; Hemiptera; Membracidae

1. INTRODUCTION

Mate choice is widespread in nature and regarded as a strong and pervasive influence in the evolution of mating signals and other sexual traits (Darwin 1871; West-Eberhard 1983; Andersson 1994). The importance of mate choice in signal evolution can be evaluated by comparing signal traits with mate preferences. Preferences can be described using curves that plot attractiveness as a function of variation in signal traits, and characterizing preference functions generates hypotheses about the type of selection they exert on signals (Butlin 1993; Gerhardt 1994; Ritchie 1996; Jang & Greenfield 1998; Gerhardt *et al.* 2000; Shaw & Herlihy 2000; Ritchie *et al.* 2001; Parri *et al.* 2002; Blows *et al.* 2003; Klappert & Reinhold 2003; Brooks *et al.* 2005; Gerhardt 2005*a,b*; Bentsen *et al.* 2006). Comparison of preferences with the distribution of signal traits for closely related species or populations can reveal the extent to which signals have responded to selection exerted by preferences.

The hypothesis that mate choice is important in signal evolution makes five predictions about the preference–signal relationship: (i) for closed preferences—which favour intermediate over extreme values—mean signal values will correspond to the values preferred by females (Ritchie 1996; Shaw 2000; Mendelson & Shaw 2002; Gerhardt 2005*a,b*); (ii) preference strength will influence the degree of correspondence between preferred values and mean signal traits; (iii) preference strength will

influence the variability of signal traits; (iv) when comparing closed preferences with open preferences, which favour values in one extreme of the range, the former will be associated with lower signal variability (Gerhardt 1994; Shaw & Herlihy 2000; Gerhardt & Huber 2002); and (v) for open preferences that differ in strength or sign, signal traits should be shifted towards the preferred values (Houde & Endler 1990; Endler & Houde 1995; Simmons *et al.* 2001).

We evaluated the importance of female mate choice in signal evolution by testing these predictions across four species in the *Enchenopa binotata* species complex of treehoppers. This complex consists of 11 or more species that occur in close sympatry across eastern North America, and differ in male mating signals. In the mating system of these insects, male–female duetting facilitates pair formation, so female response signals provide an assay of mate preference (Rodríguez *et al.* 2004). We found that preferences differ among species in the *E. binotata* complex, and that signals match the values preferred by the females to a degree that is determined by preference strength and shape.

2. MATERIAL AND METHODS

(a) *The Enchenopa binotata species complex* (Hemiptera: Membracidae)

Each species in this clade specializes on a different species of woody plant in the forest understory, edge and canopy. The *E. binotata* complex is a case study of sympatric speciation involving shifts to novel host plants (Wood & Guttman 1983; Wood & Keese 1990; Wood *et al.* 1990; Lin & Wood 2002;

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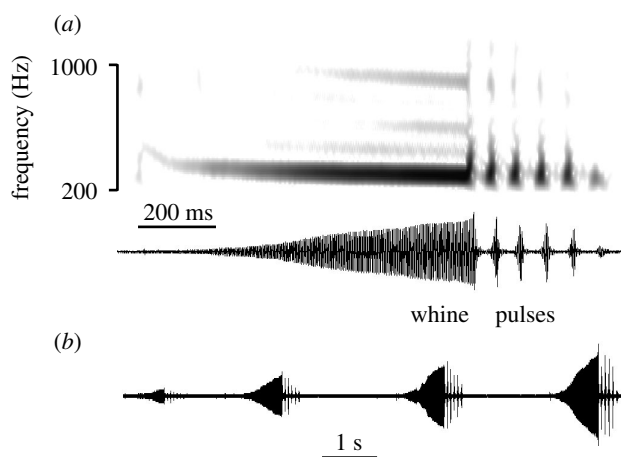


Figure 1. (a) Spectrogram and waveform of a recording of a signal produced by a male *E. binotata* 'Viburnum'. (b) Bout consisting of four signals that increase in amplitude.

Cocroft *et al.* in press). Host shifts cause assortative mating, because the insects' life-history timing is regulated by host plant phenology, so insects on plants with different phenologies acquire allochronic life cycles. Host shifts also result in divergent ecological selection promoting host specialization and host fidelity.

Sexual communication is important for assortative mating in this complex. As in many herbivorous insects (Claridge 1985; Henry 1994; Cokl & Virant-Doberlet 2003; Cocroft & Rodríguez 2005), pair formation in *E. binotata* is facilitated by exchanges of plant-borne vibrational signals between males and females (Hunt 1994; Sattman & Cocroft 2003; Rodríguez *et al.* 2004). Mate-searching males produce signals, and female responses elicit localized searching by the males. Signal structure is conserved across the complex, and consists of a tone with harmonics and a frequency sweep, the whine, followed by a series of pulses (figure 1a). In spite of overall similarity in signal structure, there is quantitative signal variation among species and individuals (R. B. Cocroft 2006, unpublished data). Females choose among males by responding to signals on the basis of this variation, influencing the likelihood of being located by males (Rodríguez *et al.* 2004).

(b) General

Experiments were performed during April–August 2003–2005. To ensure that females were sexually receptive and responsive to signals, we tested virgin females reared from nymphs collected in Boone County, MO. We reared nymphs on their host plants in an outdoor facility in the University of Missouri–Columbia campus. We tested females 4–6 weeks after their adult moult, at the peak of their receptivity (Wood 1993). We tested females from the *E. binotata* species that specialize on *Celastrus scandens*, *Cercis canadensis*, *Ptelea trifoliata* and *Viburnum rufidulum* host plants. The male signals of these species offer a representative example of variation in the complex, including its extremes in frequency and length, and two intermediate species. Species in this complex await formal description, and we refer to them by the name of their host plant, thus *E. binotata* 'Celastrus', etc.

(c) Stimulus design

We tested the effect of variation in six signal traits that differ among species in the *E. binotata* complex (Rodríguez *et al.* 2004; R. B. Cocroft 2006, unpublished data). These traits

included dominant frequency at the end of the whine, whine length, number of signals per bout, length of the interval between signals, pulse number and pulse rate (figure 1). We varied one trait at a time while keeping the other traits set to the mean of the local population from which females were drawn. The values assayed for each trait span the range of variation in the complex. For signal frequency, this range (100–500 Hz) did not reach frequencies high enough to describe the full shape of the *E. binotata* 'Celastrus' preference, so we extended the range of frequencies to 720 Hz for two additional females.

Rather than relying on natural variation in mating signals, we synthesized signals with a custom-written program in MATLAB v. 5.2.1 (Mathworks Inc., Natick, MA). Preliminary experiments showed that these signals are as effective in eliciting female responses as playbacks of recorded male signals (R. L. Rodríguez 2006, unpublished data). The artificially generated signals use a constant frequency rather than a frequency sweep; this greatly streamlines the playback procedure, as they do not require compensation for differential frequency filtering during propagation along plant substrates (Cocroft & Rodríguez 2005).

Stimuli had the typical *E. binotata* signal bout structure of gradually increasing amplitude (figure 1b). We used a structure of four signals that increased in amplitude in the pattern: 25, 75, 100 and 100% of maximum amplitude. We varied this pattern only for the experiment testing the signal number, where the one-signal stimulus had 100% amplitude and the two-signal stimulus had a pattern of 75 and 100% amplitude.

(d) Stimulation and recording of female responses

We played back stimuli to females through the stems of potted plants (*ca* 50 cm tall) of their host species. To introduce stimuli to a plant stem, we attached a magnet to the stem with wax (Endevco, San Juan Capistrano, CA) and placed an electromagnet *ca* 2 mm from it. The electromagnet received signals from a Macintosh G4 computer, amplified with an Optimus MPA-40 amplifier. Stimulus presentation was controlled with a custom-written program in MATLAB. The plant was placed on a vibration isolation table (Vibraplane, Kinetic Systems, Boston, MA) to minimize the noise generated by building vibrations. We isolated the plant from the table with shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY), further to reduce building vibrations and isolate the plant from table resonance.

For testing, we placed females on the prepared stems, *ca* 5 cm from the magnet. We recorded the playbacks and the elicited female responses by focusing the beam of a laser vibrometer (Polytec CLV 1000 with a CLV M030 decoder module; Polytec Inc., Auburn, MA) on a small (*ca* 2 mm²) piece of reflective tape affixed to the stem. The laser beam was approximately perpendicular to the stem, and the laser source was *ca* 50 cm from the plant. The laser signal was high-pass filtered at 60 Hz (Krohn-Hite 3202; Krohn-Hite Corporation, Brockton, MA). The output of the filter was sent to a Macintosh G4 computer via an Edirol UA-5 USB interface (Roland Corporation, Japan), and recorded with SOUNDEDIT 16 v. 2 (Macromedia Inc., San Francisco, CA) at a sampling rate of 44.1 kHz. We monitored the playbacks and female responses using a Radio Shack MPA-45 amplifier connected to an RCA loudspeaker and a Hameg

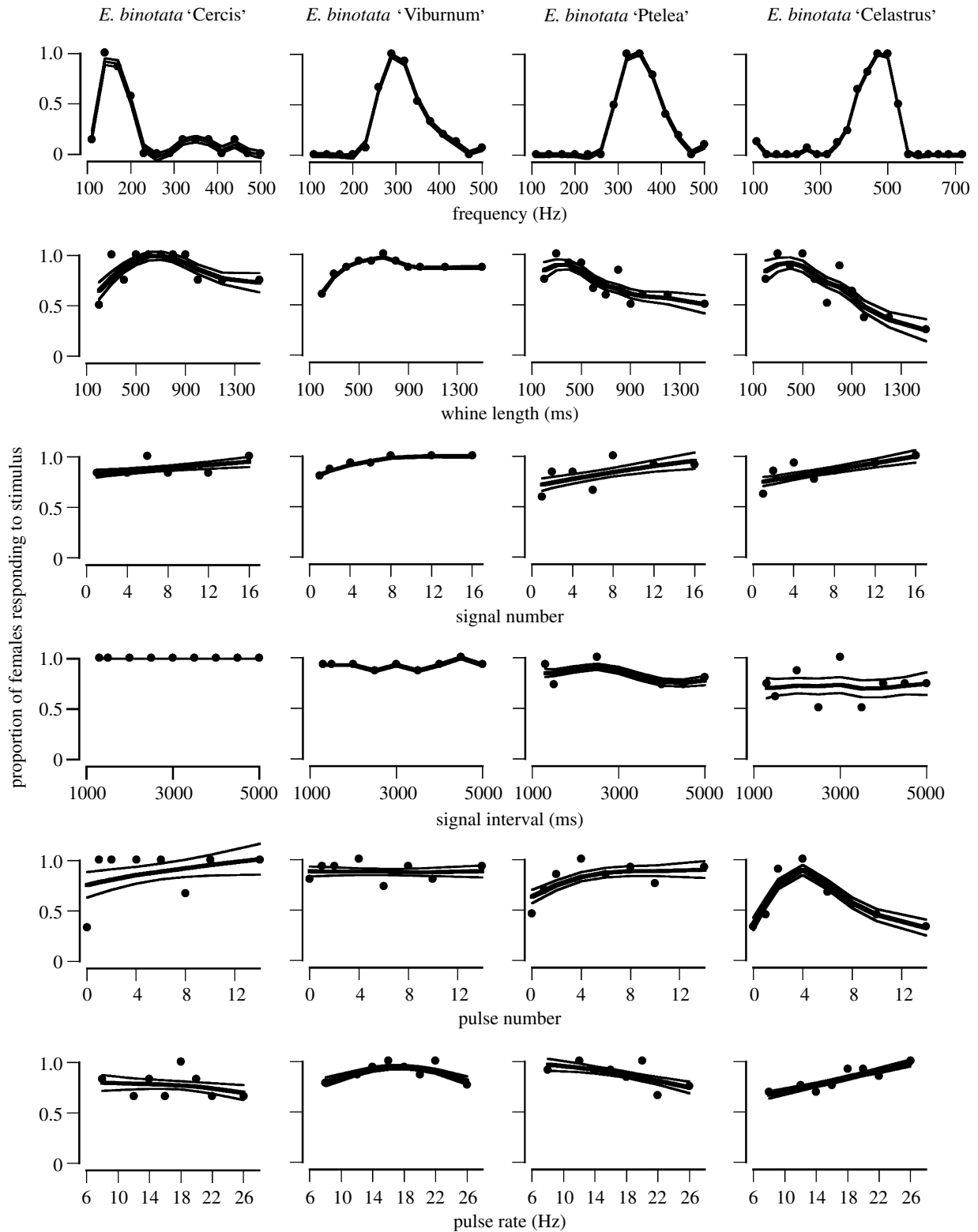


Figure 2. Proportion of females responding to stimuli varying in frequency, whine length, signal number, signal interval, pulse number and pulse rate. Each column corresponds to one species. Dots indicate the proportion of females that responded; lines indicate the spline regressions of those proportions ± 1 s.e. The *x*-axes show the range of variation tested for each signal trait, which corresponds to the range in the *E. binotata* species complex.

HM 203-7 20 MHz oscilloscope (Hameg Instruments, Mainhausen, Germany). Air temperature was kept at 24–25 °C.

We adjusted stimulus peak amplitude at the point where the females were placed on the plant stem (*ca* 5 cm from the magnet). We used an amplitude (0.3 mm s^{-1}) corresponding to the average amplitude of a male signalling

ca 5 cm away, on the basis of measurements of over 10 males from three species in the *E. binotata* complex (from *Cercis*, *Ptelea* and *Viburnum*; R. L. Rodríguez and G. McNett 2006, personal observation). Females usually walked a short distance after being placed on the stem, so they received stimulation within a few centimetres from the point of amplitude calibration.

Table 1. Analysis of the responses of *E. binotata* females, testing the effect of variation in stimulus traits, species, their interaction and female individual identity.

	factor	d.f.	χ^2	<i>p</i>
frequency	species	3	0	1.0
	linear	1	41.23	<0.0001
	species \times linear	3	176.28	<0.0001
	quadratic	1	3.77	0.05
	species \times quadratic	3	154.18	<0.0001
whine length	individual	48	123.67	<0.0001
	species	3	0	1
	linear	1	0.22	0.64
	species \times linear	3	31.47	<0.0001
	quadratic	1	15.1	0.0001
signal number	species \times quadratic	3	10.74	0.013
	individual	44	255.22	<0.0001
	species	3	0.0001	1
	linear	1	12.64	0.0004
	species \times linear	3	5.41	0.14
signal interval	quadratic	1	0.003	0.96
	species \times quadratic	3	0.18	0.98
	individual	49	184.43	<0.0001
	species	3	-0.00003	—
	linear	1	0.0000003	1
pulse number	species \times linear	3	2.71	0.44
	quadratic	1	0.000003	1
	species \times quadratic	3	2.89	0.089
	individual	40	174.68	<0.0001
	species	3	0	1.0
pulse rate	linear	1	4.99	0.025
	species \times linear	3	9.65	0.022
	quadratic	1	2.67	0.10
	species \times quadratic	3	3.97	0.26
	individual	41	145.29	<0.0001
pulse rate	species	3	0.0002	1
	linear	1	0.13	0.72
	species \times linear	3	15.49	0.0014
	quadratic	1	2.96	0.085
	species \times quadratic	3	3.83	0.28
	individual	47	239.75	<0.0001

(e) Female preference analysis

Our experimental unit is an individual female; the data contributed by each female are her responses to variation in a signal trait. In each of the experiments testing the effect of variation in a signal trait, we presented each female with a random sequence of all the stimuli representing variation in that trait. The time between each stimulus was 15 s, and the time between experiments testing the effect of variation in different signal traits was 2 min. The number of females tested in each experiment was 13–15 for each of the three species, but for *E. binotata* ‘Cercis’ $n=7$ for frequency, signal number and pulse rate; $n=4$ for whine length; $n=3$ for pulse number; and $n=1$ for signal interval (the small sample for this species was owing to low survivorship among the nymphs we collected).

We observed whether females responded to one or more signals in a stimulus bout, analysing the recordings with SOUND EDIT. We used logistic regressions (Hosmer & Lemeshow 2000) to analyse variation in female responses according to stimulus variable (tested as linear and quadratic terms), species and female individual identity (as a random term; see Rodríguez *et al.* 2004). We report the likelihood ratio χ^2 -tests for these terms. The main purpose of this analysis was to identify species differences in preferences,

using the interaction between stimulus and species as an indicator (see Olvido & Wagner 2004). Lack of interaction indicates preferences that are similar among species.

Because linear and quadratic terms can yield a simplistic view of the actual shape of preferences (Brodie *et al.* 1995; Ritchie 1996; Blows *et al.* 2003; Brooks *et al.* 2005), we used cubic splines to characterize preferences without making assumptions about their shape (Schluter 1988). To this end, we scaled to 1 the proportion of females responding to the stimuli, dividing by the maximum proportion of females that responded in each experiment to each species. We used these data to calculate splines ± 1 s.e. based on 100 bootstraps with the program created by D. Schluter (www.zoology.ubc.ca/~schluter/lab.html). This program calculates a running regression with a window size optimizing the prediction of deleted observations. This window determines the curve stiffness, with larger windows giving smoother functions. We used the squared standard deviation of the values calculated by the spline regressions as an index of preference strength, as in Schluter (1988).

(f) Preference–signal correspondence

To compare preferences with the distribution of signal traits, we obtained the distribution of male signal traits from

Table 2. Relationship between the preference strength and the distribution of signal traits in *E. binotata*. (The effect of preference strength on the signal–preference mismatch was tested only for closed preferences. The effect of preference strength on the CV of signal traits was tested separately for closed and open preferences.)

	closed preferences				open preferences			
	factor	d.f.	<i>F</i>	<i>p</i>	factor	d.f.	<i>F</i>	<i>p</i>
preference–signal mismatch	strength	1, 2	14.21	0.064				
	species	3, 2	3.19	0.25				
	interaction	3, 2	0.80	0.60				
male signal trait CV	strength	1, 2	7.8	0.11	strength	1, 6	0.38	0.56
	species	3, 2	0.93	0.56	species	3, 6	0.23	0.87
	interaction	3, 2	0.22	0.88	interaction	3, 6	0.20	0.89

a library of recordings of the Missouri populations from which the females were drawn (R. B. Cocroft 2006, unpublished data). Signals were recorded using laser vibrometry (as described previously), with the laser beam focused on a small piece of reflective tape within a few centimetres of a male on the stem of a potted host plant. Differences between the mean temperatures at which females were tested and males recorded were less than 1 °C.

We tested the relationship between preferences and signals using the between–male coefficient of variation (CV), and the preference–signal mismatch using the difference between the peak of the preference and the mean of the signal trait (where 0 is perfect correspondence). We estimated peaks (points of maximum response) only for closed preferences. If there was more than one point at the peak, we took the mean of those points. We then obtained the absolute value of the difference between the preference peak and the mean of the male signal trait, and standardized for among-species and among-trait comparisons by dividing it by the male mean. We used least-squares regressions, including preference strength, species and their interaction in the model. Ongoing studies of variation in mitochondrial and nuclear genes within and among species in the *E. binotata* complex (R. L. Snyder 2006, unpublished data) reveal that they are very similar for the genetic markers used (perhaps reflecting recent divergence), and their relationships are not yet resolved; accordingly, here we treat their relationships as a ‘star’ phylogeny, i.e. four closely related but independent lineages.

3. RESULTS

(a) Female preferences

Female responses were influenced by variation in most of the signal traits tested; only signal interval had no influence (figure 2; table 1). (For signal interval, the χ^2 was negative, probably owing to a rounding error; the very low magnitude of the term indicates no effect.) Note that for *E. binotata* ‘Cercis’, we were able to test only one female for signal interval; as with the other species, there was no indication of a preference (figure 2). The first step in comparing preferences among species was the test of the stimulus \times species interaction. For most preferences, this interaction was significant, either for the linear or quadratic terms, or both (table 1). Only for the signal number was there no interaction (table 1), indicating that preferences for signal number were similar among species (figures 2 and 3). We then focused on the shape of the preference functions to elucidate differences among species.

Preferences for signal frequency and whine length were closed (favouring intermediate values), and differences

among species lay in the preferred values (figures 2 and 3). The standard error curves (figure 3) show that preferred frequencies differed for all four species. Preferred whine lengths were similar for *E. binotata* ‘Cercis’ and *E. binotata* ‘Viburnum’, and for *E. binotata* ‘Ptelea’ and *E. binotata* ‘Celastrus’, with females from the first two species preferring longer whines than females from the last two species (figure 3). Preferences for signal number were open (favouring more signals in a bout) and similar among species (figure 2). Preferences relating to signal pulses differed among species in shape: preferences for pulse number were either open (favouring more pulses), flat (no preference) or closed (figure 2), while preferences for pulse rate were either open (favouring slower rates in two species and faster rates in one species) or closed (figure 2).

Because we presented each female with a random sequence of all stimuli for any one signal trait, we were able to test the effect of individual variation on how females responded to the stimuli. We found a significant individual component in all preferences (table 1), which is consistent with individual variation in preferences.

(b) Preference–signal correspondence

We compared preferences with the distribution of signal traits among species (figure 3) to test the predictions of the hypothesis that female preferences have been important in shaping male signal evolution. There were two signal traits for which females from all four species had closed preferences—frequency and whine length (figure 3)—so we could test prediction (i), i.e. for closed preferences mean signal values will correspond to the preferred values. There was a very close match between preferences and signals, with the match being closer for frequency than for whine length (figure 4a(i), (ii)).

To test prediction (ii), i.e. preference strength will influence the correspondence between preferred values and signal traits, we used all closed preferences: frequency and whine length in the four species, pulse number in *E. binotata* ‘Celastrus’, and pulse rate in *E. binotata* ‘Viburnum’ (figure 3). We found a marginally significant relationship between preference strength and the preference–signal mismatch (table 2), but the magnitude of the *F*-ratio (equal to 14.21) shows that this is owing to low statistical power rather than to a weak effect. Since the preference strength \times species interaction was non-significant and had a small *F*-ratio (table 2), we removed it from the model to increase power. This test revealed a significant relationship with preference strength

($F_{1,5}=16.44$, $p=0.0098$): stronger preferences were associated with smaller mismatches (figure 4a(iii)).

Prediction (iii) states that preference strength will influence signal trait variability. We tested this prediction separately for closed and open preferences, because the former were stronger (figure 4b; table 3). For closed preferences, the relationship between preference strength and signal trait CVs was non-significant (table 2), but the magnitude of the F -ratio (equal to 7.8) shows that this is owing to low statistical power rather than a weak effect. Since there was no preference strength \times species interaction (table 2), we removed the interaction term from the model to increase power. This test revealed a significant relationship between preference strength and signal trait CVs ($F_{1,5}=15.15$, $p=0.012$): stronger preferences were associated with lower CVs (figure 4b). By contrast, there was no such relationship for open preferences (table 2; figure 4b), probably because they were much weaker (figure 4b(iii); table 3), while the signal traits showed much more variation in their CVs (compare the axes of figure 4b(i) and (ii)).

Prediction (iv) states that closed preferences will be associated with lower signal variability than open preferences. We found a significant relationship between preference shape and signal trait CVs that met this prediction (figure 4c; table 3).

Prediction (v) states that, for open preferences, signal traits should be shifted towards the values preferred by the females. We could apply this prediction only to the preference for pulse rate, where females from three species had open preferences differing in sign. Females from *E. binotata* 'Cercis' and *E. binotata* 'Ptelea' preferred slower pulse rates, while females from *E. binotata* 'Celastrus' preferred faster pulse rates (figure 2). Male pulse rates differed accordingly (figure 3). The other species, *E. binotata* 'Viburnum', had a closed preference for pulse rate, with the preferred value matching that of the males (figure 3).

4. DISCUSSION

Females showed preferences for most of the signal traits that vary among species in the *E. binotata* complex. Most preferences differed among species, with females preferring different signal values and attending in varying ways and degrees to different signal traits. These species differences in female preferences suggest that there may have been a pattern of divergent selection on male signals in the *E. binotata* complex. We tested five predictions of this hypothesis among four closely related species, finding support for all of them: (i) for closed preferences, the mean values of male signal traits corresponded to the values preferred by females; (ii) preference strength influenced the degree of this correspondence; (iii) preference strength influenced the variability of signal traits; (iv) closed preferences were associated with lower variability in signal traits; and (v) open preferences differing in sign were associated with the expected differences in signal traits. Previous tests of the role of mate choice in signal evolution have lent support to the first (Ritchie 1996; Shaw 2000; Mendelson & Shaw 2002; Gerhardt 2005a,b), second (Butlin 1993), fourth (Gerhardt 1994; Shaw & Herlihy 2000; Gerhardt & Huber 2002) and fifth (Houde & Endler 1990; Endler & Houde 1995) predictions. This is the first study to test all these predictions comprehensively, and

yields strong evidence that female choice has been an important agent of signal evolution.

Members of the *E. binotata* complex specialize on different host plants, so one important issue is the extent to which species differences in preferences are owing to their development on different host plants, i.e. to phenotypic plasticity and host plant effects. We have not evaluated plasticity in *E. binotata* female preferences, but three lines of evidence suggest a minor role for plasticity in preference divergence. First, plasticity has little influence on the other half of the signal-receiver system: inducing male *E. binotata* to signal on foreign host plants causes little or no change in most signal traits—except that, being host-specific, males produce fewer and shorter signals (Sattman & Coccoft 2003). Rearing males on different host plants reveals substantial plasticity and genotype \times environment interactions, but plasticity does not exceed the range of variation of the species (R. L. Rodríguez 2006, unpublished data). Second, studies of female preferences in other species reveal plasticity and genotype \times environment interactions, but again these do not exceed the range of variation of the species (Rodríguez & Greenfield 2003). Third, the host-specificity of the members of the *E. binotata* complex (Wood & Guttman 1983) suggests that reaction norms are unlikely to have been shaped by selection on performance on multiple host plants, but instead by selection on single hosts. Thus, the observed preference–signal correspondence would be unlikely to arise as a consequence of unselected variation in reaction norms in novel environments. We suggest that *E. binotata* communication systems have probably been shaped by selection on each species' host, with differences in preferences arising from divergent selection among species.

Several factors may influence selection on female preferences. Members of the *E. binotata* complex are highly host-specific and show reduced fitness on the host plants of other species in the complex (Wood & Guttman 1983). Although partial reproductive isolation arises from host fidelity and allochronic life histories owing to differences in host plant phenology (Wood & Keese 1990; Wood *et al.* 1990), mating seasons may overlap, and mate-searching males occasionally disperse across host plants (R. B. Coccoft 2006, personal observation). Thus, mating with heterospecifics may influence offspring fitness, and mate recognition may be important. Female choice may also influence offspring fitness in other ways. There is substantial repeatability (Sattman & Coccoft 2003) and genetic variation in several male signal traits (R. L. Rodríguez 2006, unpublished data); further, individual variation in female responses to playbacks (Rodríguez *et al.* 2004; this study) is consistent with individual differences and genetic variation in preferences. Genetic variation in preferences and signals will result in Fisherian coevolution, facilitating divergence (West-Eberhard 1983; Kokko *et al.* 2002; Mead & Arnold 2004). In addition, variation in male signals may be condition-dependent (Jennions *et al.* 2001; Brandt & Greenfield 2004; Hunt *et al.* 2004), and if correlations between signal traits and condition vary among host plants, there may be divergent selection on preferences (Coccoft *et al.* in press). Another factor that may influence preference divergence involves the interaction between sexual selection and adaptation to novel environments, which is of particular relevance in the context of speciation and host shifts in

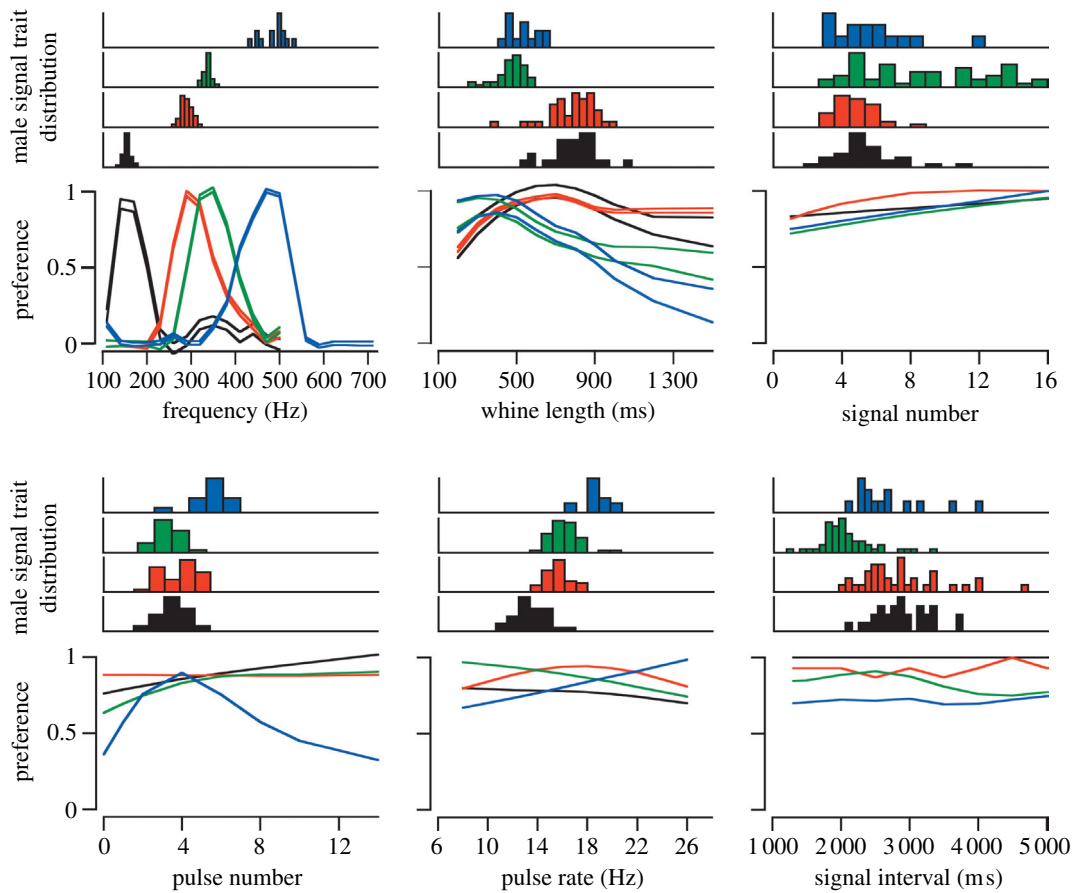


Figure 3. Comparison of preferences and signals. Curves indicate spline regressions as in figure 2, shown are the ± 1 s.e. curves for frequency and white length to emphasize species differences in preference peaks. For other preferences, differences lay in the shape rather than in the peaks, so we only show the regression curves for simplicity. Histograms show the distribution of male traits. Species are indicated by colours: black, *E. binotata* ‘Cercis’; red, *E. binotata* ‘Viburnum’; green, *E. binotata* ‘Ptelea’; blue, *E. binotata* ‘Celastrus’.

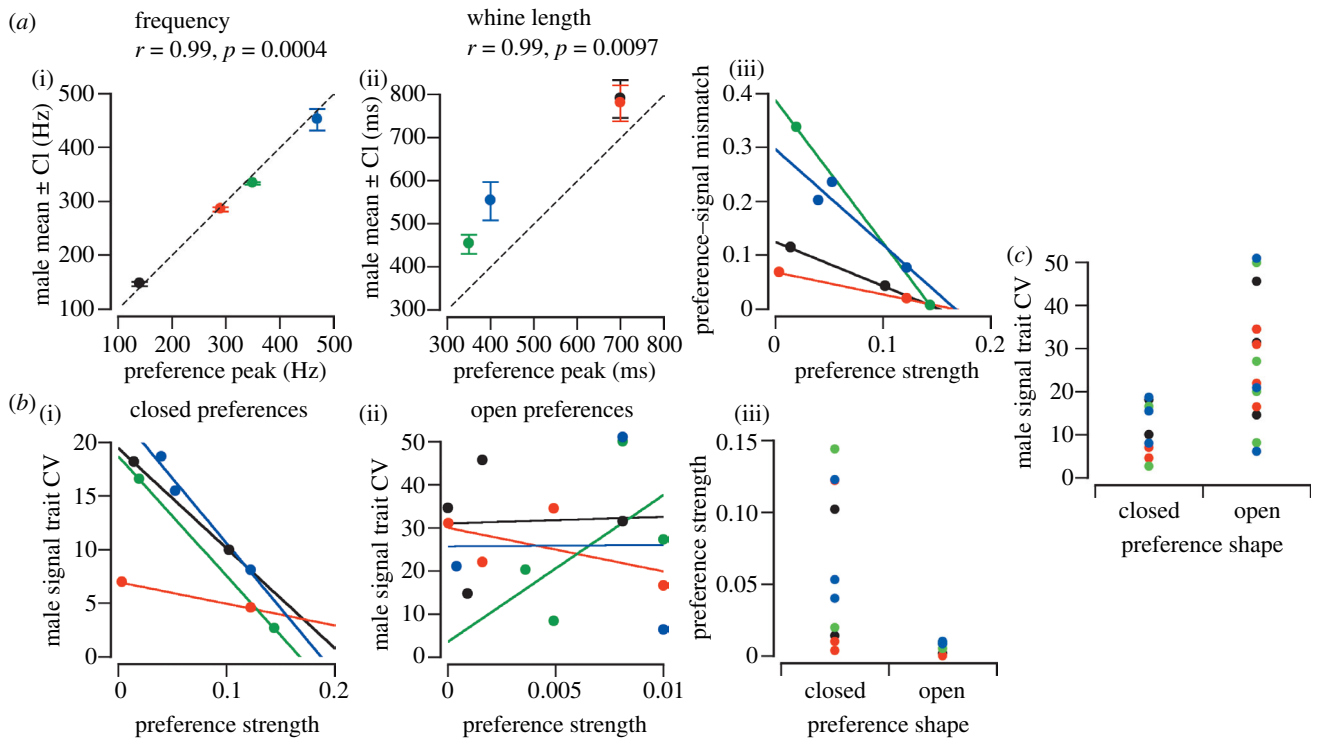


Figure 4. Preference-signal relationships. Species are indicated by colours as in figure 3. (a(i), (ii)) For frequency and white length there was a strong correlation between preference peaks and mean signal values; dashed lines indicate a 1:1 relationship. (a(iii)) Stronger closed preferences were associated with smaller preference-signal mismatches. (b(i), (ii)) Preference, strength influenced signal trait CVs for closed but not for open preferences. (b(iii)) Closed preferences were stronger than open preferences. (c) Closed preferences were associated with lower signal trait CVs than open preferences.

Table 3. Relationship between the preference shape (closed versus open) and the coefficient of variation of male signal traits, and female preference strength in *E. binotata*.

factor	relationship between preference shape and male signal trait CV			relationship between preference shape and strength		
	d.f.	<i>F</i>	<i>p</i>	d.f.	<i>F</i>	<i>p</i>
shape	1, 11	6.10	0.031	1, 11	6.30	0.029
species	3, 11	0.42	0.99	3, 11	0.14	0.93
interaction	3, 11	0.08	0.97	3, 11	0.09	0.97

plant-feeding insects (Cocroft *et al.* in press). Condition-related mate choice may facilitate adaptation to novel environments (Lorch *et al.* 2003) and, conversely, adaptation to novel host plants may result in divergence in preferences (Rundle & Nosil 2005). Finally, our finding of strong preference–signal correspondence suggests that models of sexual conflict (Tregenza *et al.* 2006) are not important in *E. binotata* mating systems. This idea is in accordance with the active role that duetting females play in pair formation, suggesting that they are not exposed to male harassment (Alexander *et al.* 1997).

In conclusion, the *E. binotata* species complex offers a strong example of female preferences having a key role in signal evolution, where following a host shift sexual communication may act in synergy with host fidelity, plasticity in life-history timing and ecological selection (Wood & Guttman 1983; Wood 1993; Cocroft *et al.* in press) in the process of speciation.

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