

VIBRATIONAL COMMUNICATION AND REPRODUCTIVE ISOLATION IN THE *ENCHENOPA BINOTATA* SPECIES COMPLEX OF TREEHOPPERS (HEMIPTERA: MEMBRACIDAE)

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Abstract.—Sexual communication can contribute to population divergence and speciation because of its effect on assortative mating. We examined the role of communication in assortative mating in the *Enchenopa binotata* species complex of treehoppers. These plant-feeding insects are a well studied case of sympatric speciation resulting from shifts to novel host-plant species. Shifting to hosts with different phenologies causes changes in life-history timing. In concert with high host fidelity, these changes reduce gene flow between populations on ancestral and novel hosts and facilitate a rapid response to divergent natural selection. However, some interbreeding can still occur because of partial overlap of mating periods. Additional behavioral mechanisms resulting in reproductive isolation may thus be important for divergence. In *E. binotata*, mating pairs form after an exchange of plant-borne vibrational signals. We used playback experiments to examine the relevance of inter- and intraspecific variation in male advertisement signals for female mate choice in a member of the *E. binotata* species complex. Female signals given in response to male signals provided a simple and reliable assay. Male species and male individual identity were important determinants of female responses. Females failed to respond to the signals of the two most closely related species in the complex, but they responded strongly to the signals of conspecific males, as well as to those of the most basal species in the complex. Communication systems in the *E. binotata* species complex can therefore play a role in reproductive isolation. Female responses were influenced by among-individual variation in male signals and females, suggesting the involvement of sexual selection in the evolution of these communication systems.

Key words.—Diversity, insect communication, mate choice, speciation, vibrational signals.

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The study of speciation is concerned with the causes of population divergence and evolutionary independence between lineages. The importance of ecology in initiating divergence is well recognized (Schluter 1998, 2001), but ecological differences alone may be insufficient because mating between individuals adapted to different resources can homogenize different populations and hinder divergence (Mayr 1963; Felsenstein 1981; Johnson and Gullberg 1998). Differentiation may thus require assortative mating as well as divergent selection (Kondrashov et al. 1998).

Differences in mating signals and other traits that mediate reproductive interactions can contribute to assortative mating and reproductive isolation (Darwin 1871; Maynard Smith 1966; West-Eberhard 1983; Eberhard 1985, 1994, 1996; Andersson 1994; Van Doorn et al. 1998; Higashi et al. 1999; Panhuis et al. 2001). Differences in mating signals between populations and species can arise through a variety of processes. There may be divergent selection on signals and preferences due to variation in habitat signal transmission properties (Endler 1993; Marchetti 1993; Endler and Basolo 1998; Boughman 2002), to signaling that indicates habitat-dependent individual condition (Andersson 1994; Rowe and Houle 1996; Jennions et al. 2001), or to divergent sexual selection (Darwin 1871; Fisher 1958; West-Eberhard 1983; Kelly and Noor 1996; Höbel and Gerhardt 2003). There may be selection favoring the avoidance of mismatching between different species or locally adapted populations (Dobzhansky 1940; Howard 1993). There may also be correlated responses to divergent selection on other traits (Emelianov et al. 2001; Hawthorne and Via 2001).

The role of mating signals in assortative mating and speciation is highlighted in hypotheses of divergence in sym-

patry, that is, in the absence of geographic barriers to gene flow. Although controversial (Mayr 1963; Futuyma and Mayer 1980), the hypothesis of sympatric speciation has received growing empirical and theoretical support, and it may be an important mechanism of speciation (Maynard Smith 1966; Dieckmann and Doebeli 1999; Higashi et al. 1999; Kondrashov and Kondrashov 1999; Tregenza and Butlin 1999; Hendry et al. 2000; Shaw et al. 2000; Wilson et al. 2000; Boughman 2001; Berlocher and Feder 2002). Sympatric speciation may be especially important in the diversification of plant-feeding insects (Wood and Guttman 1982, 1983; Bush et al. 1989; Tauber and Tauber 1989; Feder 1998; Berlocher and Feder 2002; Emelianov et al. 2003).

An important case study of sympatric speciation is the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae), a clade of nine species of host-specific, plant-feeding insects. It is hypothesized that shifts to novel host plants and a high degree of host fidelity led to allochronic life histories and disruptive selection favoring host specialization (Wood 1980, 1993; Wood and Guttman 1982, 1983; Wood and Keese 1990; Wood et al. 1990; Tilmon et al. 1998; Drès and Mallet 2002). In addition to divergence in life-history traits, sexual communication may be important for population isolation in *E. binotata*. There is evidence of behavioral mechanisms that favor mating between individuals from the same host-plant species. When *Enchenopa* adults of one species were given access to adults of five other species, mating occurred almost exclusively between conspecific pairs in spite of frequent mixed-species courtship (Wood 1980). The mechanism involved is likely to be vibrational communication (Michelsen et al. 1982; Gogala 1985), which in treehoppers mediates social interactions between parents and

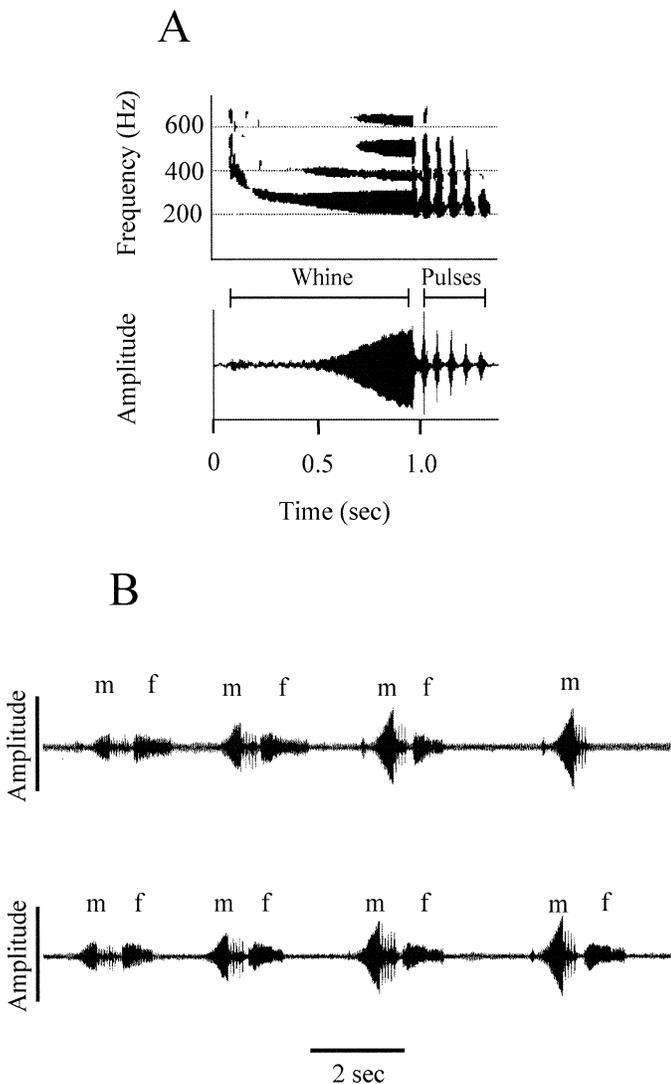


FIG. 1. (A) Sonogram (top) and waveform (bottom) of the signal of an *Enchenopa binotata* male from *Viburnum lentago*. (B) Recordings of two playbacks of bouts of four male signals (m) and the elicited female responses (f). In the top example, the female responded to three of the four male signals in the bout; in the bottom example, the female responded to all signals.

offspring, siblings, and mates (Hunt et al. 1992; Hunt 1993, 1994; Cocroft 1996, 1999a,b, 2001). Male *E. binotata* produce advertisement signals consisting of a tone (sometimes with harmonics) that slightly drops in frequency, followed by a series of pulses (Fig. 1A; Hunt 1994); signals are produced in bouts of several signals. Mating occurs after a duet between the male and the female, as in many vibrational communication systems (e.g., De Winter and Rollenhagen 1990; Heady and Denno 1991; Hunt et al. 1992; Wells and Henry 1992; Hunt 1993; Henry 1994; Wells and Henry 1994). Female *E. binotata* signal their receptivity by alternating their response signals (a tone with harmonics) with those of the male (Fig. 1B). Female response signals elicit local mate searching by males, and duets typically end in mating (R. L. Rodríguez, L. E. Sullivan, and R. B. Cocroft, unpubl. data). Female responses to male signals thus provide a reliable and

easily obtained assay of mate recognition and preference. Because most *E. binotata* females mate only once (Wood and Guttman 1982), the likelihood of mating encompasses a substantial portion of the variation in male reproductive success in these species.

Across the *E. binotata* species complex, male signals are similar in basic structure (Fig. 1A) but show quantitative variation among and within species in several signal characteristics (R. B. Cocroft, R. Hunt, and R. L. Rodríguez, unpubl. data). This pattern of a common theme with many fine variations among closely related species is widespread in the acoustic mating signals of frogs and insects (Cocroft and Ryan 1995; Harrison and Bogdanowicz 1995; Ryan and Rand 1995; Shaw 1999) and in many other sexual traits in many animal groups (Eberhard 1990). Signal variation in the *E. binotata* species complex suggests that there may be cues relevant to mate recognition and mate choice available for females. If so, the female responses that facilitate pair formation should vary according to male signal traits.

We presented females of one *E. binotata* species with the signals of males from six different species, including their conspecifics, and tested for variation in their responses according to the species and individual identity of the males. Species in the *E. binotata* complex await formal taxonomic description; we refer to them by the names of their host plants. We used *E. binotata* females from *Viburnum lentago*, and presented them with the signals of males from *V. lentago*, *Juglans nigra*, *Ptelea trifoliata*, *Celastrus scandens*, *Cercis canadensis*, and *Robinia pseudoacacia*. These trials encompassed six of the nine species of the *E. binotata* species complex. We did not include *E. binotata* from *Liriodendron*, *Carya*, and *Juglans cinerea* because we did not have sufficient recordings of the signals of their males.

MATERIALS AND METHODS

Experiments were performed during July–September 2002 at the University of Missouri-Columbia. *Enchenopa binotata* individuals were obtained from the seventh generation of a colony established in 1995 with over 6000 females collected on *Viburnum lentago* near Winchester, Virginia, and maintained in outdoor enclosures at the University of Delaware. This population is part of a host-shift experiment established by the late T. K. Wood in which the treehoppers have been allowed to adapt to three other *Viburnum* species in regimes of sympatry and allopatry. The females employed in our experiments came from three different treatments (hereafter, female provenance). A comparison of their behavior did not show a significant effect of provenance on their response to conspecific versus heterospecific signals (see Results).

Females used in our experiments were shipped as one- to two-week-old virgin adults from Delaware to Columbia, and they were tested when they were two to six weeks old, during their sexually receptive period. Female *E. binotata* become sexually receptive at an adult age of approximately three to four weeks, and may live for up to four months (Wood 1993).

Stimulus Generation and Playback

Our experimental design presented random sequences of single stimuli to individual females. We tested 24 females,

and each received three random sequences of male mating signals. We obtained the playback stimuli from a library of recordings of male advertisement signals (R. B. Cocroft, R. Hunt, and R. L. Rodríguez, unpubl. data). We randomly chose five male recordings for one population of each of the six species for which we had obtained recordings. Recordings of *V. lentago* males were obtained from the same experimental populations from which our females were derived; recordings of all other species were derived from a sympatric suite of Missouri populations. An ongoing assessment of signal variation suggests that differences between species are substantially greater than differences within species, and that the signals of males from Missouri *Viburnum* populations are similar to those of Virginia *Viburnum* males (R. B. Cocroft, R. Hunt, and R. L. Rodríguez, unpubl. data).

We arranged the chosen recordings in random playback sequences and presented each female with three different sequences (McGregor et al. 1992). A sequence consisted of the signals of six species presented in random order, each species represented by one male. Each female was thus exposed to three sets of six signal bouts for a total of 18 signal bouts. Male recordings for each set were chosen by sampling randomly (without replacement for each sequence, with replacement for each set) from the pool of five males of each species.

For playback, we affixed a magnet to the stem of a *V. lentago* plant with wax, and positioned an electromagnet 2 mm away from it. The electromagnet received a signal played from a Macintosh G4 computer and amplified with an Optimus MPA-40 amplifier. The plant was placed on top of a vibration isolation table (Vibraplane, Kinetic Systems, Boston, MA) to minimize noise generated by building vibrations. We isolated the plant from the table with acoustic foam to minimize any influence of table resonance.

We recorded our playbacks and the elicited female response signals by focusing the beam of a laser vibrometer (Polytec CLV 1000 with a CLV M030 decoder module; Polytec, Inc., Auburn, MA) on a small (~2 mm²) piece of reflective tape affixed to the plant stem. The beam of the laser was perpendicular to the vertical axis of the stem, and the laser was positioned ~15 cm away from the plant stem. The laser signal was high-pass filtered (Krohn-Hite 3202) at 60 Hz. The output of the filter was sent to a Macintosh G4 computer and recorded using SoundEdit (Macromedia, Inc., San Francisco, CA) with a sampling rate of 44.1 kHz. We monitored the playbacks and responses using a Radio Shack MPA-45 amplifier connected to an RCA loudspeaker and to a Hameg HM 203-7 20 MHz oscilloscope. Temperature was maintained at $23.2 \pm 0.3^\circ\text{C}$.

Before playback, we digitally filtered the recordings of male signals to compensate for the differential filtering of their frequency components due to propagation along the plant stem between the electromagnet and the point where experimental females were placed. We used custom-written software in Matlab (Mathworks, Inc., Natick, MA) to play back band-limited noise (50–5000 Hz) through the plant stem, record the noise, calculate the change in amplitude at each frequency, and generate a digital filter that compensated for it (see Cocroft 1996). We then applied the filter to the recordings of the male signals, and used these modified files

for presentation to the females. If the configuration of the plant changed (e.g., if it dropped a large leaf), we filtered the male signals again before proceeding with the playback trials.

The peak amplitude of the stimuli was adjusted at the point where experimental females were placed (5 cm from the playback magnet) to be equal to that of a male signaling 5 cm away, based on measurements of nine conspecific males (mode = 0.3 mm sec^{-1}). Females usually walked a short distance just after being placed on the stem, and thus playbacks were conducted with females within a few cm of the target point. We presented each female with the three playback sequences each separated by 1 min of silence to prevent habituation.

Playback Trials and Signal Analysis

Playbacks preserved the variation present among the signals of different males (see below). However, because females give more responses when males produce more signals per bout (R. L. Rodríguez and R. B. Cocroft, unpubl. data), we standardized the number of signals per bout to four. This value was the mean in a sample of 248 *E. binotata* males from *V. lentago* from the experimental populations from which our females were derived (R. Hunt, R. B. Cocroft, and T. K. Wood, unpubl. data). When a recording had more than four signals per bout, we eliminated the additional signals; if a recording had less than four signals, we repeated the last signal in the recording, taking care to preserve the individual male's pattern of intersignal intervals. This balance between the preservation of among-male variation and the standardization of the number of signals per bout allowed us to evaluate variation in female responses according to the species and individual characteristics of male signals.

We quantified female response in three variables: whether the female responded at all or not (presence or absence of response during a bout of four signals), the number of male signals in a bout that elicited a female response (0–4 responses), and response length (measured as the mean length of the responses in a given bout). We analyzed the recordings of female responses with SoundEdit, and measured response length to the nearest 3 msec.

Statistical Analysis

The data showed homogeneity of variance. We employed a multivariable model of binary logistic regression (Hosmer and Lemeshow 2000) to analyze the data for whether the females responded to the playbacks or not. We illustrate these results in Figure 2 with mean responses for each species, calculated by averaging responses for females across males, and for species across females; we refer to these averaged data as the probability of response.

For females that responded to male signals, there were two more variables to analyze: the number of responses elicited by a bout and their length. The relevance of these two variables for mate choice and the reproductive success of males and females is unclear. More and longer responses may improve the chance of a searching male locating the female, but whether that is the case remains to be tested. We therefore avoided attaching undue weight to either variable, and summarized

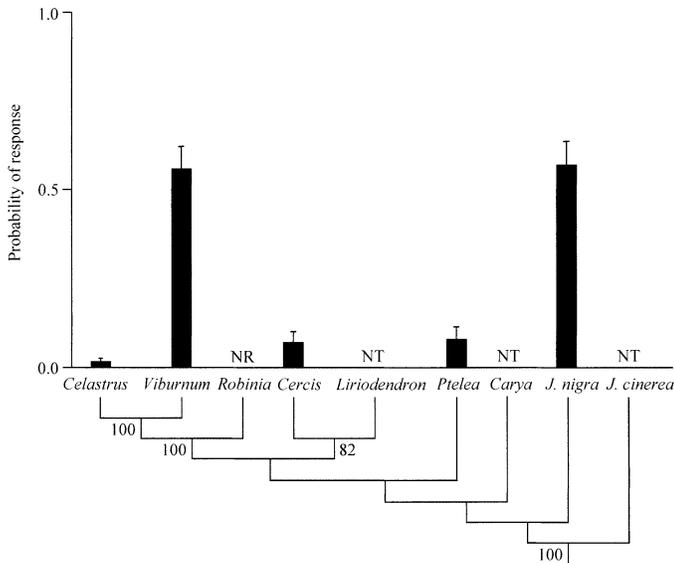


FIG. 2. Probability of a female response (mean \pm SE) being given in response to male advertisement signals of six species of the *Echenopa binotata* species complex. Species identity of the male signals is indicated by the host plant of the treehoppers. Phylogenetic tree drawn from Lin and Wood (2001), branch lengths not to scale, bootstrap values $<50\%$ not shown. This tree was obtained by means of maximum likelihood analysis of mitochondrial sequences (Lin and Wood 2001). NR, no female responded to these playbacks; NT, not tested due to insufficient male recordings.

our results in a single variable that describes the total response time elicited by each signaling bout (number of responses \times mean response length), given that there was a response. Females that did not respond at all to a given bout are missing data in this analysis, that is, they are not counted as having zero response time. We used a mixed-model, three-way ANOVA performed with the General Linear Model, which allowed for an unbalanced design.

To explore the influence of male signal variation on female responses, we performed a principal component analysis on five characteristics of the male signals: fundamental frequency, length of the whine section, intersignal interval, pulse rate, and number of pulses (see Fig. 1). Fundamental frequency was measured at the end of the whine section. Intersignal interval was the period between the beginning of one signal and the beginning of the next one, within a bout. This interval was measured between the penultimate and last signals of a bout. The number of pulses following the whine was counted; pulse rate was calculated by measuring the interval separating the first and last pulses. We evaluated the influence of these signal variables on the responses of females by testing the fit of linear, quadratic, and cubic models. Although the male signals presented to the females represent a random sampling of the populations from which they were drawn, we treated them as fixed effects because the presentation of their characteristics to the females was fixed. We present mean \pm 1 SE data.

RESULTS

Females responded preferentially to the signals of conspecifics, and discriminated against the signals of closely

TABLE 1. Logistic regression of the probability of a female response according to the species identity of the signaling male (relative to the response to signals of conspecific males), the individual identity of the male, and the individual identity and provenance of the responding female. Test that all slopes are zero: $G = 170.67$, $df = 8$, $P < 0.001$ ($n = 72$ trials with 24 females).

	Z	P
Species identity		
<i>E. binotata</i> from <i>Celastrus</i>	-4.30	<0.001
<i>E. binotata</i> from <i>Cercis</i>	-5.41	<0.001
<i>E. binotata</i> from <i>J. nigra</i>	0.10	0.92
<i>E. binotata</i> from <i>Ptelea</i>	-3.85	<0.001
Male individual identity	0.41	0.68
Female individual identity	0.30	0.76
Female provenance	-0.66	0.51

related species. The binary logistic regression revealed significant effects of species identity on the females' probability of response, and no significant effects of male identity, female identity, or female provenance (Table 1). The females never responded to the signals of males from *Robinia* in 72 trials with 24 females. We therefore excluded the trials with signals of *Robinia* males from the logistic regression analysis in order to prevent the problem of a zero cell count (Hosmer and Lemeshow 2000). Figure 2 shows the mean probability of response according to the species identity of the signaling male. Note that the signals of the most basal species in the clade were as acceptable as conspecific signals (Fig. 2; Table 1). The two most discriminated-against species where the most closely related: *E. binotata* from *Celastrus* and *Robinia*.

We then analyzed the data for the total response time of females, for those cases where females responded to male signals. We examined whether response time varied independently of the probability of response by testing for a correlation between these two variables. To perform this analysis we obtained mean values of the responses elicited by the signals of each species for each individual female, and used those values to calculate the mean responses shown in Figure 3: mean probability of response on the x-axis, and mean total response time on the y-axis. There was a strong positive correlation between the probability of response and the total response time (Pearson product-moment correlation: $r = 0.91$, $P = 0.03$, $n =$ mean responses to the six species that elicited female responses). That is, when male signals were likely to elicit female responses, they also elicited longer responses.

A three-way ANOVA revealed significant effects of species identity, male identity, and female identity on total response time (Table 2). It was not possible to test for the effect of female provenance with the General Linear Model because of collinearity with female individual identity. We therefore performed a nested ANOVA that included female individual identity within female provenance. This test showed no significant effect of provenance ($F_{2,19} = 2.77$, $P > 0.05$).

We analyzed variation in male signal characteristics with principal component analysis. The analysis of the male signals used in our playbacks yielded two principal components with eigenvalues higher than 1.0, which together explained 80% of the variation in the signals. The first principal component (PC1) mainly represented variation in signal fre-

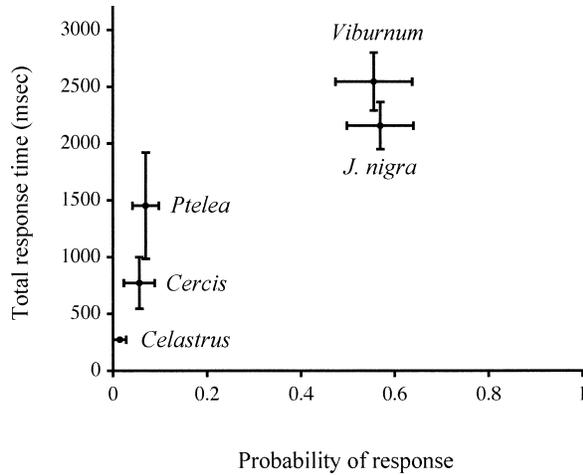


FIG. 3. Correlation between total response time and the probability of response (both shown mean \pm SE), in female *Enchenopa binotata* presented with male advertisement signals. Species identity of the male signals is indicated by the host plant of the treehoppers.

quency (loading = -0.60), whine length (loading = 0.55) and pulse rate (loading = -0.56 ; see Figure 1 and Materials and Methods for definition of these characteristics). The second principal component (PC2) mainly represented variation in intersignal interval (loading = 0.65) and pulse number (loading = 0.69). The signals of the different species in the *E. binotata* complex that we used in our playbacks were distinguished mainly by variation in the first principal component, that is, variation in frequency, whine length, and pulse rate, although variation in the second principal component (intersignal interval and pulse number) provided additional resolution (Fig. 4).

To analyze the influence of this variation in male signals on female responses, we averaged the responses of individual females to each male signal of each species. We thus obtained the mean probability of response for each male signal. These data varied continuously between 0–1; accordingly, we used arcsine-transformed data (Sokal and Rohlf 1995). We analyzed the total response time of the females averaged in the same way. Female responses varied non-linearly with some male signal characteristics. We therefore tested whether variation in female responses best fit linear, quadratic, or cubic models, plotting the probability of response according to each characteristic of the male signals (Fig. 5). These plots are suggestive of “closed” preference functions (see Gerhardt 1991; Gerhardt and Huber 2002) for frequency and pulse rate, “open-ended” preference functions for whine length and intersignal interval, and no preference for pulse number. Total response time showed a similar pattern, with one exception: females gave longer responses to signals with lower pulse rates ($F_{1,27} = 17.42$, $P < 0.001$).

DISCUSSION

Enchenopa binotata females from *Viburnum lentago* showed differential responses to male signals according to their species and individual identity. Conspecific signals were more likely to elicit a response than some heterospecific signals, and they also elicited longer responses. Responses were

TABLE 2. ANOVA of total female response time, according to the species identity of the signaling male, the individual identity of the male, and the individual identity of the responding female.

	df*	MS**	F	P
Species identity	4	7444627	12.05	<0.001
Male individual identity	10	2616583	4.24	<0.001
Female individual identity	21	3506575	5.68	<0.001
Error	55	617643		

least likely to be elicited by the closest relatives, but the signals of the more distantly related *E. binotata* from *Juglans nigra* were as effective as conspecific signals. The vibrational communication system that mediates pair formation in *E. binotata* can thus play a role in reproductive isolation. Females can identify males on the basis of their advertisement signals, and they may regulate the initiation of mating accordingly. This finding suggests an explanation for why mixed-species matings are rare, although mixed-species courtship can be frequent in experiments that bring together more than one species (Wood 1980). Furthermore, variation in female responses can influence the likelihood of mating because of the role of male-female duetting in pair formation: If a female does not respond to a male, he will not be prompted to search for her on the plant and may fly away. Therefore, the likelihood of female *E. binotata* from *V. lentago* mating with males from *Robinia*, should they encounter them, is extremely low because they never responded to their signals, as is the case for males from *Celastrus*, *Cercis*, and *Ptelea*, in decreasing order of improbability. The equal probability of female response for the signals of conspecific males and males from *J. nigra* indicates that different character combinations can achieve equal attractiveness, and that the re-

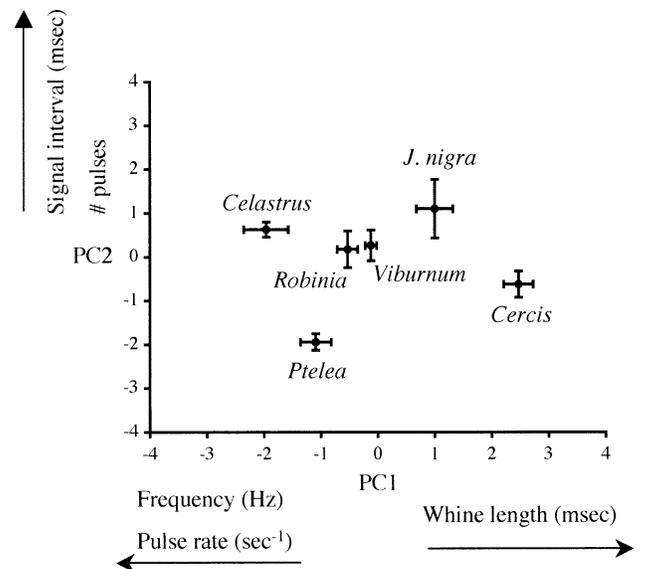
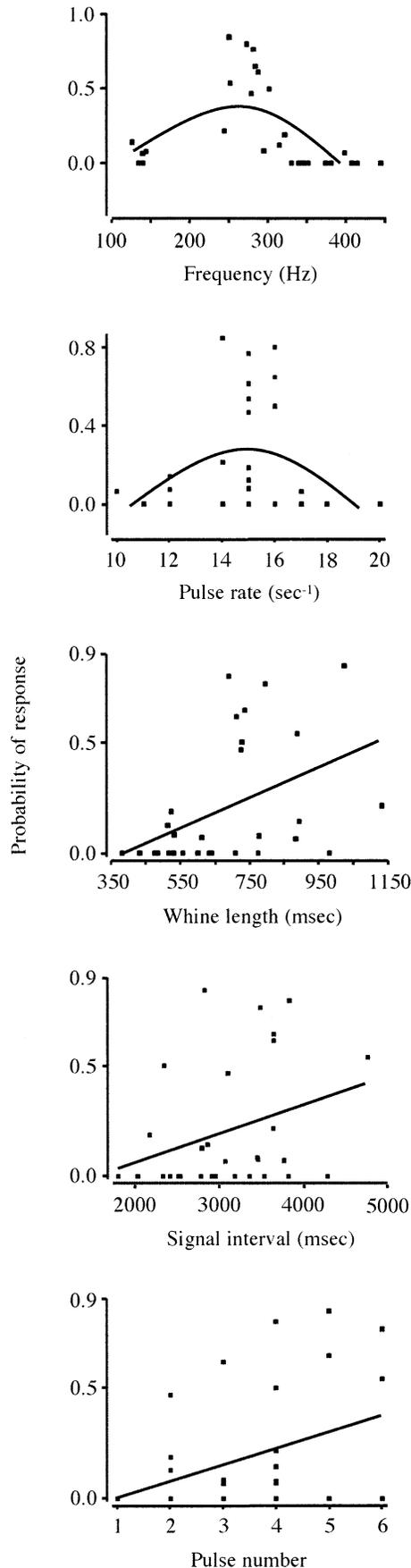


FIG. 4. Variation in the characteristics of male signals used in our playbacks. Principal component analysis of variation in the male signals. Mean \pm SE scores are shown for PC1 and PC2. On PC1, signal frequency and pulse rate increase towards the left, and whine length increases towards the right. On PC2, intersignal interval and pulse number increase towards the top.



productive isolation provided by the duetting system in *E. binotata* is only partial. It can nevertheless complement the reproductive isolation provided by host fidelity, allochrony and host specialization (Wood 1980, 1993; Wood and Guttman 1982, 1983; Wood and Keese 1990; Wood et al. 1990; Tilmon et al. 1998).

In addition to their current role in reproductive isolation, differential female responses to male signals may have contributed to the process of divergence in the *E. binotata* clade. The importance of this contribution depends on the timing of the appearance of signal variation and differential responses, relative to the process of divergence. Communication systems can evolve rapidly if they are influenced by few genes (see Ritchie and Phillips 1998), if selection is strong (e.g., as expected under sexual selection; West-Eberhard 1983), and if developmental plasticity generates diversity and facilitates the response to selection (West-Eberhard 2003). Several factors may have shaped communication systems in the *E. binotata* complex. Our results are consistent with at least two potential factors. One possible source of selection is the avoidance of mismating with heterospecifics (Dobzhansky 1940; Howard 1993; Coyne and Orr 1997). In accord with the lack of association between prezygotic isolation and genetic distance in sympatric *Drosophila* species (Coyne and Orr 1997), we found that discrimination of signals according to species identity was not positively associated with genetic distance, as discrimination was strongest against the closest relatives and weaker against the most distantly related species in the clade (Fig. 2). However, it is not clear that mating with closer relatives would be disfavored more strongly than mating with more distant members of the clade. We currently have no data on the relative likelihood of encounters between species in the *E. binotata* complex, or on the relative costs of mismating between different species.

Another factor that may have an important influence on communication systems is variation in the strength or direction of sexual selection (West-Eberhard 1983; Markow and Hocutt 1998). The finding of variation in *E. binotata* female responses according to male individual identity suggests intraspecific mate choice. Furthermore, the finding of among-female variation in response signals may reflect individual differences in sexual receptivity or in mate preferences. Future experiments will have to evaluate the importance of these factors before conclusions can be drawn about the evolution of mate discrimination in these insects. In particular, our experimental design cannot distinguish between the functions of mate recognition and mate choice. Nevertheless, the probability of female response was only influenced by male spe-

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FIG. 5. Variation in the probability of female response according to the characteristics of male signals used in our playbacks. From top to bottom: the probability of response to male signals according to frequency and pulse rate best fit quadratic models ($F = 15.54$, $P = 0.0005$; $F = 4.06$, $P = 0.05$, respectively); the probability of response best fit linear models for whine length and intersignal interval ($F = 8.55$, $P = 0.007$; $F = 3.30$, $P = 0.08$, respectively). There was no significant variation according to pulse number ($F = 2.58$, $P = 0.12$). The figure shows untransformed data; tests were performed on arcsine-transformed data.

cies identity, whereas total response time was also influenced by individual male and female identity. Thus, total response time may provide within-species mate discrimination. Testing this possibility will require evaluating the influence of variation in response time on the likelihood of pair formation.

The principal component analysis of male signal characteristics, and the corresponding variation in female responses, indicated that most of the signal characteristics measured may be important in influencing female responses and in distinguishing the signals of different species in the *E. binotata* complex. There are two reasons why our analysis does not describe independent female preference functions for different male signal traits. First, variation in each male signal characteristic was not independent of the other characteristics. Second, our experiments assayed a range of variation in male signals extending beyond the normal range of any one species, and the pattern of female preference according to intraspecific variation may differ. Consequently, the importance of between- and within-species variation will have to be evaluated. Nevertheless, our data suggest hypotheses about the form of selection on male signals due to female preferences: Different female preferences may be in operation in *E. binotata*, some closed and some open ended (Gerhardt 1991; Gerhardt and Huber 2002).

In conclusion, variation in female responses according to the species identity of signaling males suggests that communication systems may play a role in reproductive isolation in the *E. binotata* species complex. Individual variation in male advertisement signals and female responses suggests that sexual selection may play an important role in this system. These results provide a strong rationale for a detailed assessment of variation in male signals and female responses in the *E. binotata* species complex.

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LITERATURE CITED

- Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.
- Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? *Annu. Rev. Entomol.* 47:773–815.
- Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411:944–948.
- . 2002. How sensory drive can promote speciation. *Trends Ecol. Evol.* 17:571–577.
- Bush, G. L., J. L. Feder, S. H. Berlocher, B. A. McPherson, D. C. Smith, and C. A. Chilcote. 1989. Sympatric origins of *R. pomonella*. *Nature* 339:346.
- Cocroft, R. 1996. Insect vibrational defence signals. *Nature* 382: 679–680.
- . 1999a. Offspring-parent communication in a subsocial treehopper (Hemiptera: Membracidae: *Umberonia crassicornis*). *Behaviour* 136:1–21.
- . 1999b. Parent-offspring communication in response to predators in a subsocial treehopper (Hemiptera; Membracidae: *Umberonia crassicornis*). *Ethology* 105:553–568.
- . 2001. Vibrational communication and the ecology of group-living, herbivorous insects. *Am. Zool.* 41:1215–1221.
- Cocroft, R. B., and M. J. Ryan. 1995. Patterns of advertisement call evolution in toads and chorus frogs. *Anim. Behav.* 49: 283–303.
- Coyne, J. A., and H. A. Orr. 1997. “Patterns of speciation in *Drosophila*” revisited. *Evolution* 51:295–303.
- Darwin, C. 1871. *The descent of man and selection in relation to sex*. J. Murray, London.
- De Winter, A. J., and T. Rollenhagen. 1990. The importance of male and female behaviour for reproductive isolation in *Ribautodelphax* planthoppers (Homoptera: Delphacidae). *Biol. J. Linn. Soc.* 40:91–206.
- Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. *Nature* 400:354–357.
- Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. *Am. Nat.* 74:312–321.
- Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philos. Trans. R. Soc. Lond. B.* 357:471–492.
- Eberhard, W. G. 1985. *Animal genitalia and sexual selection*. Harvard Univ. Press, Cambridge, U.K.
- . 1990. Animal genitalia and female choice. *Am. Sci.* 78: 134–141.
- . 1994. Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution* 48:711–733.
- . 1996. *Female control: Sexual selection and cryptic female choice*. Princeton University Press, Princeton NJ.
- Emelianov, I., M. Drès, W. Baltensweiler, and J. Mallet. 2001. Host-induced assortative mating in host races of the larch budmoth. *Evolution* 55:2002–2010.
- Emelianov, I., F. Simpson, P. Narang, and J. Mallet. 2003. Host choice promotes reproductive isolation between host races of the larch budmoth *Zeiraphera diniana*. *J. Evol. Biol.* 16:208–218.
- Endler, J. A. 1993. Some general comments on the evolution and design of animal communication systems. *Philos. Trans. R. Soc. Lond. B* 340:215–225.
- Endler, J. A., and A. L. Basolo. 1998. Sensory ecology, receiver biases, and sexual selection. *Trends Ecol. Evol.* 13:415–420.
- Feder, J. L. 1998. The apple maggot fly, *Rhagoletis pomonella*. Pp. 130–144 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution* 35:124–135.
- Fisher, R. 1958. *The genetical theory of natural selection. A complete variorum edition*. Oxford Univ. Press, New York.
- Futuyma, D. J., and G. C. Mayer. 1980. Non-allopatric speciation in animals. *Syst. Zool.* 29:254–271.
- Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. *Anim. Behav.* 42:615–635.
- Gerhardt, H. C., and F. Huber. 2002. *Acoustic communication in insects and anurans*. University of Chicago Press, Chicago, IL.
- Gogala, M. 1985. Vibrational communication in insects (biophysical and behavioural aspects). Pp. 117–126 in K. Kalmring and N. Elsner, eds. *Acoustic and vibrational communication in insects*. Parey, Berlin.
- Harrison, R. G., and S. M. Bogdanowicz. 1995. Mitochondrial DNA phylogeny of North American field crickets: perspectives on the evolution of life cycles, songs, and habitat associations. *J. Evol. Biol.* 8:209–232.
- Hawthorne, D. J., and S. Via. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* 412:904–907.
- Heady, S. E., and R. G. Denno. 1991. Reproductive isolation in

- Prokelisia* planthoppers (Homoptera: Delphacidae): acoustic differentiation and hybridization failure. *J. Insect. Behav.* 4: 367–390.
- Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* 290:516–518.
- Henry, C. S. 1994. Singing and cryptic speciation in insects. *Trends Ecol. Evol.* 9:388–392.
- Higashi, M., G. Takimoto, and N. Yamamura. 1999. Sympatric speciation by sexual selection. *Nature* 402:523–526.
- Höbel, G., and H. C. Gerhardt. 2003. Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). *Evolution* 57:894–904.
- Hosmer, D. W., and S. Lemeshow. 2000. *Applied logistic regression*. 2nd ed. John Wiley & Sons, New York.
- Howard, D. S. 1993. Reinforcement: Origin, dynamics, and fate of an evolutionary hypothesis. Pp. 46–69 in R. G. Harrison, ed. *Hybrid zones and the evolutionary process*. Oxford Univ. Press, Oxford, U.K.
- Hunt, R. E. 1993. Role of vibrational signals in mating behavior of *Spissistilus festinus* (Homoptera: Membracidae). *Ann. Entomol. Soc. Am.* 86:356–361.
- . 1994. Vibrational signals associated with mating behavior in the treehopper, *Enchenopa binotata* Say (Hemiptera: Homoptera: Membracidae). *J. NY Entomol. Soc.* 102:266–270.
- Hunt, R. E., J. P. Fox, and K. F. Haynes. 1992. Behavioral response of *Graminella nigrifrons* (Homoptera: Cicadellidae) to experimentally manipulated vibrational signals. *J. Insect Behav.* 5: 1–13.
- Jennions, M. D., A. P. Møller, and M. Petrie. 2001. Sexually selected traits and adult survival: a meta-analysis. *Q. Rev. Biol.* 76:3–36.
- Johnson, P. A., and U. Gullberg. 1998. Theory and models of sympatric speciation. Pp. 79–89 in D. J. Howard and S. H. Berlocher, eds. *Endless forms. Species and speciation*. Oxford University Press, New York.
- Kelly, J. K., and M. Noor. 1996. Speciation by reinforcement: a model derived from studies of *Drosophila*. *Genetics* 143: 1485–1497.
- Kondrashov, A. S., and F. A. Kondrashov. 1999. Interactions among quantitative traits in the course of sympatric speciation. *Nature* 400:351–354.
- Kondrashov, A. S., L. Y. Yampolsky, and S. A. Shabalina. 1998. On the sympatric origin of species by means of natural selection. Pp. 90–98 in D. J. Howard and S. H. Berlocher, ed. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- Lin, C. P., and T. K. Wood. 2001. Molecular phylogeny of the North American *Enchenopa binotata* species complex (Homoptera: Membracidae). *Ann. Entomol. Soc. Am.* 95:162–171.
- Marchetti, K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362:149–152.
- Markow, T. A., and G. D. Hocutt. 1998. Reproductive isolation in Sonoran Desert *Drosophila*. Testing the limits of the rules. Pp. 234–244 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- Maynard Smith, J. 1966. Sympatric speciation. *Am. Nat.* 100: 637–650.
- Mayr, E. 1963. *Animal species and evolution*. Belknap Press, Cambridge, MA.
- McGregor, P. K., C. K. Catchpole, T. Dabelsteen, J. B. Falls, L. Fusani, H. C. Gerhardt, F. Gilbert, A. G. Horn, G. M. Klump, D. I. Kroodsma, M. M. Lambrechts, K. E. McComb, D. A. Nelson, I. M. Pepperberg, L. Ratcliffe, W. A. Searcy, and D. M. Weary. 1992. Design of playback experiments: the Thornridge Hall NATO ARW consensus. Pp. 1–9 in P. K. McGregor, ed. *Playback and studies of animal communication*. NATO ASI Series A: life sciences, Vol. 228. Plenum Press, New York.
- Michelsen, A., F. Fink, M. Gogala, and D. Traue. 1982. Plants as transmission channels for insect vibrational songs. *Behav. Ecol. Sociobiol.* 11:269–281.
- Panhuis, T. M., R. Butlin, M. Zuk, and T. Tregenza. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* 16:364–371.
- Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B.* 263:1415–1421.
- Ritchie, M. G., and S. D. F. Phillips. 1998. The genetics of sexual isolation. Pp. 291–308 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- Ryan, M. J., and A. S. Rand. 1995. Female responses to ancestral advertisement calls in tungara frogs. *Science* 269:390–392.
- Schluter, D. 1998. Ecological causes of speciation. Pp. 114–129 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- . 2001. Ecology and the origin of species. *Trends Ecol. Evol.* 16:372–380.
- Shaw, K. L. 1999. A nested analysis of song groups and species boundaries in the Hawaiian cricket genus *Laupala*. *Mol. Phylogenet. Evol.* 11:332–341.
- Shaw, P. W., G. F. Turner, M. R. Idid, R. L. Robinson, and G. R. Carvalho. 2000. Genetic population structure indicates sympatric speciation of Lake Malawi pelagic cichlids. *Proc. R. Soc. Lond. B* 267:2273–2280.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. Freeman, New York.
- Tauber, C. A., and M. J. Tauber. 1989. Sympatric speciation in insects: perception and perspective. Pp. 307–344 in D. Otte, and J. A. Endler, eds. *Speciation and its consequences*. Sinauer: Sunderland, MA.
- Tilmon, K. J., T. K. Wood, and J. D. Pesek. 1998. Genetic variation in performance traits and the potential for host shifts in *Enchenopa* treehoppers (Homoptera: Membracidae). *Ann. Entomol. Soc. Am.* 91:397–403.
- Tregenza, T., and R. K. Butlin. 1999. Speciation without isolation. *Nature* 400:311–312.
- Van Doorn, G. S., A. J. Noest, and P. Hogeweg. 1998. Sympatric speciation and extinction driven by environment dependent sexual selection. *Proc. R. Soc. Lond. B.* 265:1915–1919.
- Wells, M. M., and C. S. Henry. 1992. The role of courtship songs in reproductive isolation among populations of green lacewings of the genus *Chrysoperla* (Neuroptera: Chrysopidae). *Ann. Entomol. Soc. Amer.* 87:73–744.
- . 1994. Behavioral responses of hybrid lacewings (Neuroptera: Chrysopidae) to courtship songs. *J. Insect Behav.* 7: 649–662.
- West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. *Q. Rev. Biol.* 58:155–183.
- . 2003. *Developmental plasticity and evolution*. Oxford Univ. Press, New York.
- Wilson, A. B., K. Noack-Kunmann, and A. Meyer. 2000. Incipient speciation in sympatric Nicaraguan crater lake cichlid fishes: sexual selection versus ecological diversification. *Proc. R. Soc. Lond. B.* 267:2133–2141.
- Wood, T. K. 1980. Divergence in the *Enchenopa binotata* Say complex (Homoptera: Membracidae) effected by host plant adaptation. *Evolution* 34:147–160.
- . 1993. Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). Pp. 299–317 in D. R. Lees, and D. Edwards, eds. *Evolutionary patterns and processes*. Academic Press, New York.
- Wood, T. K., and S. I. Guttman. 1982. Ecological and behavioral basis for reproductive isolation in the sympatric *Enchenopa binotata* complex (Homoptera: Membracidae). *Evolution* 36: 233–242.
- . 1983. *Enchenopa binotata* complex: sympatric speciation? *Science* 220:310–312.
- Wood, T. K., and M. C. Keese. 1990. Host-plant-induced assortative mating in *Enchenopa* treehoppers. *Evolution* 44:619–628.
- Wood, T. K., K. L. Olmstead, and S. I. Guttman. 1990. Insect phenology mediated by host-plant water relations. *Evolution* 44: 629–636.