Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers

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For specialized herbivorous insects, shifts to novel host plants can have dramatic evolutionary consequences. If mating traits diverge, assortative mating can develop between ancestral and novel host populations and facilitate speciation. Mating signals may diverge under a variety of scenarios. Signal differences may be a consequence of divergence in correlated traits, such as body size. If local communication environments differ, mating signals may also diverge through selection for enhanced transmission. We tested these hypotheses using 2 closely related species in the *Enchenopa binotata* complex of treehoppers. Each member of this complex specializes on a different host plant species. Their communication modality may make signal divergence likely after a host shift: like many plant-dwelling insects, *Enchenopa* communicate using substrate-borne vibrations for which the plant itself is the transmission channel. Each species' mating signal is a relatively pure tone, and differences between species in signal frequency are critical for mate recognition. Whereas no support was found for a correlated selection hypothesis, we found support for a signal transmission hypothesis: both species use a signal frequency that transmits well in their contrasting communication environments, suggesting that host shifts may favor signal divergence and ultimately behavioral isolation. *Key words:* host specialist, phytophagous, sensory drive, signal transmission, speciation. *[Behav Ecol 19:650–656 (2008)]*

Plant-feeding insects constitute 40% of all animal species (Strong et al. 1984; Bush and Butlin 2004) and are thought to have diversified through shifts to novel host plants (Feder et al. 1988; Funk et al. 2002). Host shifts lead to divergent selection, assortative mating, and potentially to reproductive isolation between populations on the ancestral and novel hosts. This can happen when plant phenology differs between hosts, leading to an allochronic shift in life-history timing (Wood and Keese 1990). Host fidelity may also contribute to reproductive isolation (Wood 1980; Jaenike 1990; Feder et al. 1994; Bernays 1998; Via 1999). However, these reproductive barriers may not completely eliminate interactions between host-associated populations (Drès and Mallet 2002). Additional isolation may come from traits associated with mating, such as mate attraction signals. Divergence in such traits favors assortative mating and may lead to reproductive isolation (West-Eberhard 1983; Schluter and Price 1993; Boughman 2002; Coyne and Orr 2004). Only a few studies have addressed plant-related changes in insect mating signals (e.g., Landolt and Phillips 1997; Etges and Ahrens 2001), but if host shifts alter the nature of selection on mating signals, this could increase the likelihood that host shifts result in speciation.

Here we address hypotheses to explain mating signal evolution related to host shifts. We use 2 closely related species in the *Enchenopa binotata* species complex (Hemiptera: Membracidae), which is a clade of host-specialist insects that occur sympatrically throughout much of the eastern United States (Wood 1993; Cocroft et al. 2008). Species in the *E. binotata* complex communicate using substrate-borne vibrational signals transmitted through the leaves and stems of their host plant, as do many other plant-feeding insects (Virant-Doberlet

and Cokl 2004; Cocroft and Rodríguez 2005). Male mating signals in the *E. binotata* complex all consist of a pure-tone "whine" followed by a series of pulses, but vary in several traits, particularly frequency (Cocroft et al. 2008). Frequency also is the single most important signal trait for female mate recognition because signals of different species differ more in frequency than in other signal traits (Cocroft et al. 2008), and females strongly prefer the signal frequencies of conspecific males (Rodríguez et al. 2006). We investigate the ultimate causes of frequency differences in 2 species within the complex: the species that uses eastern redbud (Fabaceae: *Cercis canadensis*) and the species that uses wafer ash (Rutaceae: *Ptelea trifoliata*). The species on *C. canadensis* communicates using a lower signal frequency than the species on *P. trifoliata* (Figure 1). Species in the *E. binotata* complex are awaiting description and will be referred to here using their host plant affiliation (i.e., *E. binotata* "Cercis" and *E. binotata* "Ptelea").

For host-specialist insects like *E. binotata*, the host plant plays a central role in nearly every aspect of the life cycle, including communication and mate-searching behavior. This intimate relationship suggests at least 4 hypotheses that could account for mating signal variation following a host shift. First, signal variation could be an immediate consequence of signaling on a new substrate. Previous research has shown this not to be the case in *E. binotata*: when males are moved between different host plant species, signal frequency remains unchanged—that is, frequency in these tonal signals is a property of the signaller, not the substrate (Sattman and Cocroft 2003; Cocroft et al. 2006). Second, signal variation could be an immediate consequence of developing on a novel host plant. Previous research has also shown this not to be the case for *E. binotata*: reciprocal transplant experiments that reared *E. binotata* Ptelea on 2 different hosts showed little or no influence on signal frequency (Rodríguez et al. 2007).

Here we test 2 additional hypotheses for how host shifts could lead to signal frequency differences between *E. binotata* Ptelea and *E. binotata* Cercis. First, we test the hypothesis that frequency differences are a consequence of changes in body size. Among vibrationally communicating insects,
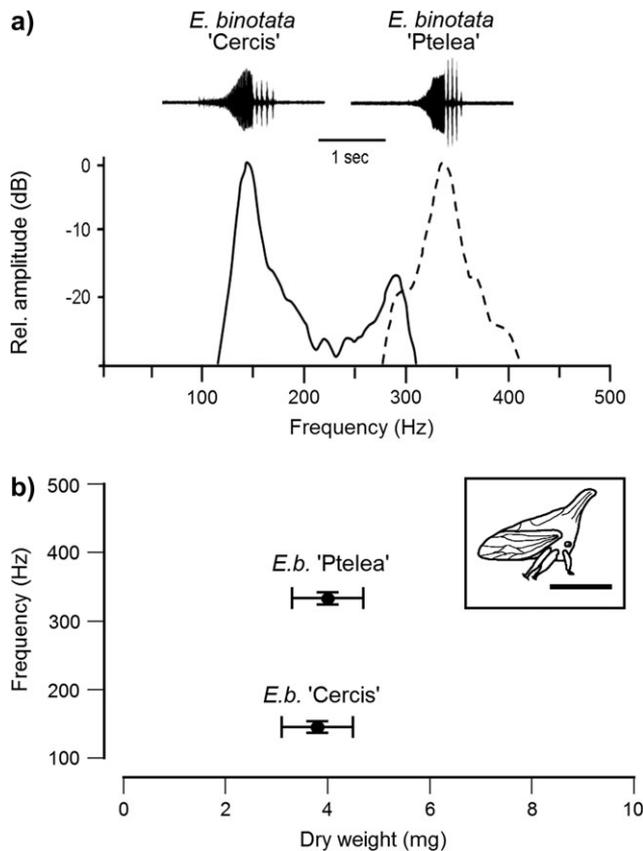
Figure 1
Variation in male mating signal frequency and dry weight for *Enchenopa binotata* 'Cercis' and *E. binotata* 'Ptelea.' (a) Waveforms of each species' signal with the corresponding amplitude spectra showing the frequency difference between species. (b) Variation in dry weight (milligrams ± SD) and frequency (Hertz ± SD) within each species and a drawing of a representative male (inset; males of both species similar; scale bar = 3 mm). Dry weight was not significantly different between species ($t_{113} = 1.83$, $P = 0.07$; mean dry weight ± SD: *E. binotata* Cercis = 3.8 ± 0.70 mg, $n = 56$; *E. binotata* Ptelea = 4.0 ± 0.70 mg, $n = 59$).

erties makes 2 predictions. The first is that the hosts of species with different signal frequencies must differ in their signal transmission properties. Transmission properties can be represented by plotting attenuation as a function of frequency (see below). These transmission curves are analogous to adaptive landscapes (Simpson 1944), where a peak in the attenuation curve represents a frequency range of optimal signal transmission. Transmission curves are likely to differ between hosts because of variation in physical structure and plant mechanical properties (Michelsen et al. 1982; Read and Sanson 2003). However, if transmission curves do not differ between the host plants of 2 species with different signal frequencies, then the hypothesis is rejected.

The second prediction is that, given differences in transmission properties of the host plants, male signal frequency should match the peak of optimal transmission, thereby maximizing long-range transmission and signal detection (Endler 1992; Schluter and Price 1993). Studies of the green stink bug have shown that the frequency of its vibrational signals matches the transmission curves of some of its common host plants (Miklas et al. 2001; Cokl et al. 2005), whereas a study of 2 lacewing species, one of which signals on conifers and the other on herbaceous plants and grasses, found no match (Henry and Wells 2004). However, no study has yet investigated the role of sensory drive in signal divergence where it would be most expected: closely related species, each restricted to a single host plant. If there is a signal–environment match, then signals should transmit better through the substrate where they are typically used than through other substrates, such as plant parts where the insects do not occur, or the substrate used by closely related species. In contrast, if signals do not match the optimum frequency more closely in the environment in which they are used, then adaptation to different host plants cannot explain the divergence in frequency.

MATERIALS AND METHODS

Study system

Members of the *E. binotata* species complex are a widely cited example of sympatric speciation through shifts to novel host plants (Wood and Guttman 1983; Wood 1993; Coyne and Orr 2004). These species occur sympatrically throughout much of the eastern United States on host plants that are evolutionarily diverse, being represented in several different plant families (Rutaceae, Celastraceae, Fabaceae, Juglandaceae, Adoxaceae, Thymelaeaceae, Rhamnaceae, and Magnoliaceae). Pair formation in *E. binotata* is mediated by their plant-borne vibrational signals (Hunt 1994; Cocroft et al. 2008). Males use a "call-fly" strategy while searching for mates, producing advertisement signals to which receptive females respond. Female response then stimulates males into a localized search. Results of the most recent phylogeny of the *E. binotata* complex (Lin and Wood 2002) show that the 2 species used in this study are closely related, but their precise relationship is unresolved.

Body size measurements

To test the hypothesis that the difference in frequency between *E. binotata* Cercis and *E. binotata* Ptelea is due to a difference in body size, we compared dry body weight between 56 males of *E. binotata* Cercis (1–3 males from 39 different host plants) and 59 males of *E. binotata* Ptelea (1–3 males from 31 different host plants). Males were collected in and around Columbia, Boone County, Missouri, then dry frozen, thawed, and air dried before being weighed on a Mettler AB54S electronic balance to the nearest 0.1 mg.

larger species have lower frequency signals, although there is considerable scatter around the best-fit line (Cocroft and De Luca 2006). Body size can change as a result of host shifts (Messina 2004), and if the larger of the 2 *Enchenopa* species has a lower frequency, this would be consistent with the hypothesis that the difference in frequency is a by-product of a change in body size. In contrast, if the 2 species do not differ in size or if the species with the lower frequency is smaller, this hypothesis is rejected. Second, we test the hypothesis that signal frequencies have diverged as a result of adaptation to host plants with different signal transmission properties. For the many plant-feeding insects that communicate with vibrations, the stems and leaves of host plants represent the transmission environment. Plant tissues act as frequency filters that attenuate signals and limit the long-range transfer of information (Bell 1980; Michelsen et al. 1982; Bradbury and Vehrencamp 1998). If there is selection on males to use a signal frequency that propagates with little loss through the substrate and/or on females to favor frequencies that allow males to be detected and assessed from a greater distance, then populations on hosts with different physical properties might be under selection for use of different optimal signal frequencies.

The hypothesis that frequency differences are a consequence of adaptation to host plants with different transmission prop-

Signaling sites

Before testing the hypothesis that the frequency difference between *E. binotata* Cercis and *E. binotata* Ptelea reflects adaptation to different host plant transmission properties, it was crucial to determine where on the host plant communication takes place. Behavioral observations were made in 2003 and 2004 throughout the breeding seasons on host plants found within a 20-km^2 area in Boone County, MO, in local community parks and natural recreation areas. To identify specific plant stems and branches used for transmitting signals, we clipped onto host plant stems a Signal Flex SF30 Universal Tuner Pickup and monitored signaling behavior using a battery-powered Johnson JA-004 Mini-amp/speaker. For some stems and branches, communication was inferred if multiple individuals were observed on the stem 3 or more times throughout the breeding season. The rationale for this inference is that adult treehoppers spend most of their time feeding, and there is no spatial separation between feeding and signaling sites (Shugart 2004). Consequently, if males and females are present on a stem during the mating season, signaling is almost certainly occurring on that stem.

Frequency attenuation curves of the host plants

We tested the predictions of the signal transmission hypothesis by comparing plant transmission properties between host plant species. Each *E. binotata* species was found almost exclusively on the stems and leaf petioles of its host plant, although the species differed with respect to the plant part most frequently used (see Results). Stems and leaf petioles thus represent the environments to which signal adaptation would be expected. We measured the filtering properties of both plant parts for both host plant species. One branch of approximately 1 m in length (measurement includes both stems and leaves) was removed from 20 different plants for each host plant species. The branches removed were those known or inferred to have been used by signaling *Enchenopa*. Branches were cut, capped with a water vial, brought into the laboratory, and clamped at the base in the same spatial orientation as in the field. To ensure that laboratory-based measurements reflected those expected under natural conditions, we conducted preliminary tests in spring/summer of 2003 on potted host plants within the size range of those used by the insects. We tested for changes in transmission properties due to cutting a stem and applying a clamp to its base for mounting it in the laboratory. Attenuation curves for distal stem portions were robust to this procedure (McNett GD, unpublished data). Additionally, because conducting transmission measurements required 3–3.5 h per stem, we tested for drift in transmission properties over time; this was negligible for a 4-h period. For each branch, we measured the transmission properties for 1 leaf petiole (*C. canadensis*, mean length = 3.3 cm, *n* = 20; *P. trifoliata*, mean length = 5.7 cm, *n* = 20) and 1 woody stem (both species, mean length = 20 cm, *n* = 20). For both species, the average stem distance is approximately 20 cm (*C. canadensis*: 19.2 ± 3.0 standard deviation [SD]; *P. trifoliata*: 20.2 ± 4.0 SD) between the point on a stem with the largest diameter used by the insects and the point with the smallest diameter used by the insects. We thus report transmission functions on the stem as the relative attenuation occurring over 20 cm.

To measure attenuation, we used a 5-s band-limited noise stimulus (100–1000 Hz) imparted into the stem with an ET-132-203 Electrodynamic Shaker (Labworks, Costa Mesa, CA) that was placed in contact with the base of the stem. The noise stimulus was played from a Macintosh G3 computer using SoundEdit software (v. 16), high-pass filtered at 60 Hz

(Krohn-Hite 3202 filter, −24 dB per octave, Krohn-Hite Corporation, Brockton, MA), and amplified (Pioneer A-305 stereo amplifier). Amplitude compensation was made at the base of the stem to ensure that each frequency had equal energy once imparted (Cocroft 1996). The transmitted noise stimulus was recorded on a separate Macintosh G3 (4 4100 Hz sampling rate, 16 bit resolution) using a National Instruments data acquisition board and a custom-written program using Labview (v. 6.0; National Instruments, Austin, TX). Recordings were analyzed using a custom-written program in Matlab (v. 6.5; Mathworks, Natick, MA).

Measurements of the transmitted stimulus were made using a method detailed elsewhere (McNett et al. 2006); only the essential points are given here. This method involves processing the signals of 2 orthogonally aligned transducers. Plant stems vibrate in 2 dimensions as signals propagate, but transducers are maximally sensitive along 1 axis. Common methods that use a single transducer, therefore, will often underestimate the actual vibrational amplitude because it is unlikely that the transducer will be aligned with the major axis of vibration. Two orthogonally aligned transducers establish a 2-dimensional plane and allow accurate measurements of vibration amplitude for a given frequency. We arranged the laser beams of 2 laser vibrometers (Polytec CLV 1000 with a CLV M030 decoder module; Polytec Inc., Auburn, MA) orthogonally to each other and to the long axis of the stem or leaf petiole being measured. To obtain an attenuation curve, we took a ratio of amplitude values (i.e., transmission function) across a range of frequencies (100–1000 Hz) spanning the frequencies used by the *E. binotata* complex (∼140 to 500 Hz). The amplitude values used in the transmission function were derived from the 2 orthogonally aligned transducers. The transmission functions we obtained represent the filtering properties between 2 points separated by 20 cm length of woody stem or 2 points along the entire length of a leaf petiole (petiole length varies between host plant species, see above). One transmission function was thus obtained for 1 stem and 1 petiole per tree, for 20 trees of each host plant species. The peak of the transmission function obtained for each woody stem and petiole per branch was set to 0 dB relative to the frequency that transmitted best through that plant part.

Attenuation by environment

We assessed adaptation to the local environment in male signals of both *E. binotata* species by comparing signal attenuation across transmission environments. We used the measured transmission curves to estimate the attenuation that a sample of individual male signals (*n* = 20 for each species) would experience relative to the best frequency in a given plant part. We used the peak frequency of each male signal to obtain an attenuation estimate. All signals used were obtained from males collected from populations near Columbia, Boone County, Missouri.

RESULTS

Body size measurements

Males of *E. binotata* Cercis and *E. binotata* Ptelea did not differ significantly in dry body weight (t_{113} = 1.83, P = 0.07; mean dry weight ± SD: *E. binotata* Cercis = 3.8 ± 0.70 mg, *n* = 56; *E. binotata* Ptelea = 4.0 ± 0.70 mg, *n* = 59; Figure 1b). Instead, there was a slight trend in the opposite direction than that predicted by a negative size–frequency relationship: the lower frequency *E. binotata* Cercis was slightly smaller than higher frequency *E. binotata* Ptelea (Figure 1b). There is thus no
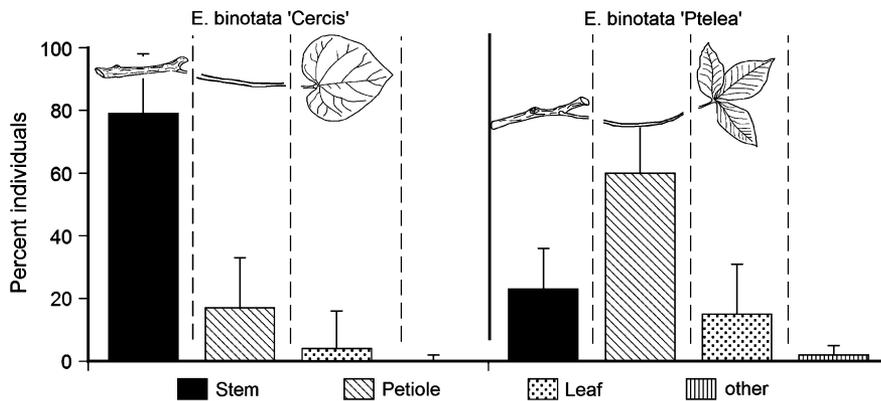
Figure 2
Signaling environments of *Enchenopa binotata* adults within each host plant. *Enchenopa binotata* 'Cercis' (*n* = 33 host plants) primarily communicates through the stems of its host plant, whereas *E. binotata* 'Ptelea' (*n* = 20 host plants) primarily communicates through leaf petioles.

support for the hypothesis that size differences are responsible for the difference in frequency in these 2 species.

Signaling sites

Both species preferred the distal portions of branches, as do other membracids (Price and Carr 2000), instead of basal positions nearer the trunk. Within these distal branch portions, however, individuals preferentially occupied different plant parts (Figure 2). *Enchenopa binotata* Cercis was found more often on the stems of its host plant, whereas *E. binotata* Ptelea was found more often on leaf petioles. Furthermore, use of a particular plant part was more consistent in *E. binotata* Cercis: the percentage of individuals found in the preferred environment was greater in *E. binotata* Cercis than in *E. binotata* Ptelea. The average stem diameter used by *E. binotata* Ptelea = 2.8 ± 0.9 mm SD (range 1.5–5.4 mm, *n* = 86), and the average petiole diameter = 1.2 ± 0.4 mm SD. The average stem diameter for *E. binotata* Cercis = 2.7 ± 0.9 mm SD, and the average petiole diameter = 1.2 ± 0.4 mm SD (range 1.4–5.4 mm, *n* = 169).

Frequency attenuation curves of the host plants

The shape of the transmission curves differed between plant parts and plant species. For *C. canadensis*, stems transmitted low frequencies with the least attenuation (Figure 3), whereas leaf petioles transmitted higher frequencies with the least attenuation. For *P. trifoliata*, stems also transmitted lower frequencies with the least attenuation (Figure 3), whereas leaf petioles transmitted midrange frequencies with the least attenuation. Because the 2 species use not only different host plants but also different plant parts, they encounter very different communication environments. The first prediction of the hypothesis that signal divergence is due to adaptation to different host plant properties—that the transmission environments of the 2 treehopper species differ—is thus supported.

Attenuation by environment

Attenuation estimates confirmed the qualitative match between signal frequency and the transmission curve of a given plant part, evident in Figure 3. The signals of each species experienced the least attenuation in the plant part predominantly used on that species' own host plant (*E. binotata* Cercis: $F_{2,57}$ = 2021.4, $P < 0.0001$; *E. binotata* Ptelea: $F_{2,57}$ = 357.8, $P < 0.0001$; Figure 4). The signals of *E. binotata* Cercis performed dramatically better when transmitted through the woody stems that this species commonly uses than in the leaf petioles it rarely uses. Signals of *E. binotata* Ptelea also performed best on the petioles where communication more often takes place, although differences in attenuation are less pronounced (Figure 4), in part because the average transmission curves are flatter (Figure 3). The signals of each species also performed significantly better in their own transmission environment than that normally used by the other species (Figure 4), supporting the second prediction of the hypothesis that signal divergence is a result of adaptation to different host plant properties.

DISCUSSION

We found support for both predictions of the hypothesis that signal differences between these 2 closely related species are due to adaptation to the signal transmission properties of their respective hosts. First, transmission properties differ between plant environments, in this case between plant parts as well as host plant species. Second, the frequency of each species transmits with the least attenuation in its own plant environment. We found no support for the hypothesis that the differences in signal frequency are a by-product of size differences because body size did not differ between the 2 species. Two other hypotheses, that frequency differences are a result of signaling or developing on a different host plant, have been rejected in previous studies. Of the 4 hypotheses emphasized here to explain frequency differences between *Enchenopa* species, only the signal transmission hypothesis is supported. In addition to selection from female mate choice (Rodríguez et al. 2006), host plant transmission properties are a possible agent of signal evolution, suggesting that shifts to novel host plants can favor divergence in vibrational mating signals. The use by males of frequencies that transmit well through host plant tissues could benefit both males and females. Females might benefit from the ability to detect males from greater distances, thereby gaining more information about the availability of mates. Males would gain from obtaining a larger signal active space.

An alternative hypothesis for a signal–environment match is that it results from behavioral feedback—that is, that individuals choose an environment that transmits their signal with little attenuation. In the absence of other causes of frequency change, we would expect signal frequency to remain the same after a host shift because the insects would choose an environment within the new host that has the same signal-transmitting properties as the old host. Any changes in signal frequency after a host shift would have to arise from other causes such as developmental plasticity; however, experiments with *E. binotata* Ptelea revealed that developing on a different host does not alter signal frequency (Rodriguez et al. 2007). Although individuals are likely to choose favorable sites for signal
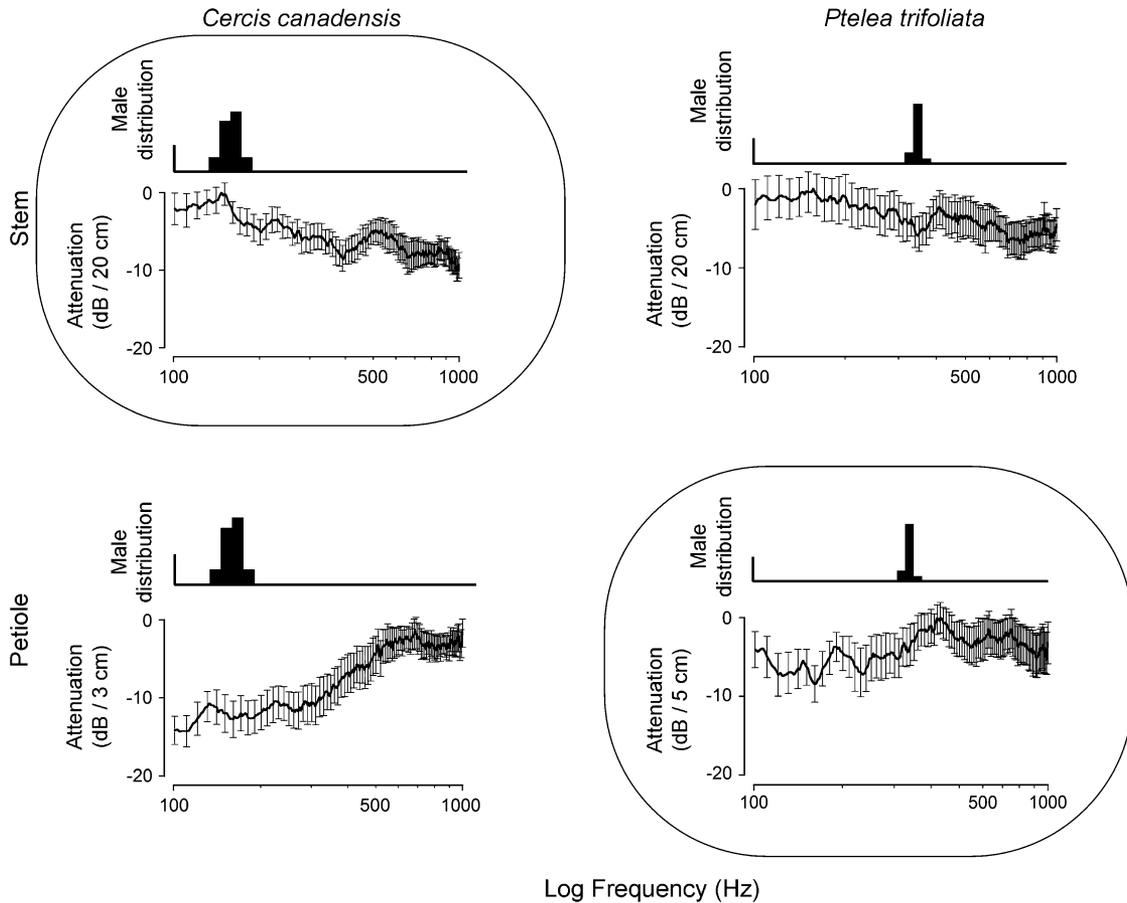
Figure 3
Transmission function curves (attenuation per distance ± standard error) showing differential filtering in stems and petioles of *Cercis canadensis* and *Ptelea trifoliata* (*n* = 20 stems, 1 from each of 20 plants). Transmission curves are relative to the frequency transmitting with the least attenuation (0 dB) in that module. Histograms above each curve represent the distribution of male signal frequency. The boxed curve for each species represents the transmission properties of the plant module predominantly used for communication.

transmission, where available (e.g., Bennet-Clark 1987; Heindl and Winkler 2003; Elias et al. 2004), males will be constrained in their choice of signaling sites by the distribution of females. Females, in turn, are likely to be under fecundity selection,

maximizing access to nutritional resources (Roff 1992) rather than to male signals. Accordingly, given that 1) we have found no evidence for immediate changes in signal frequency after a host shift (Sattman and Cocroft 2003); 2) within the
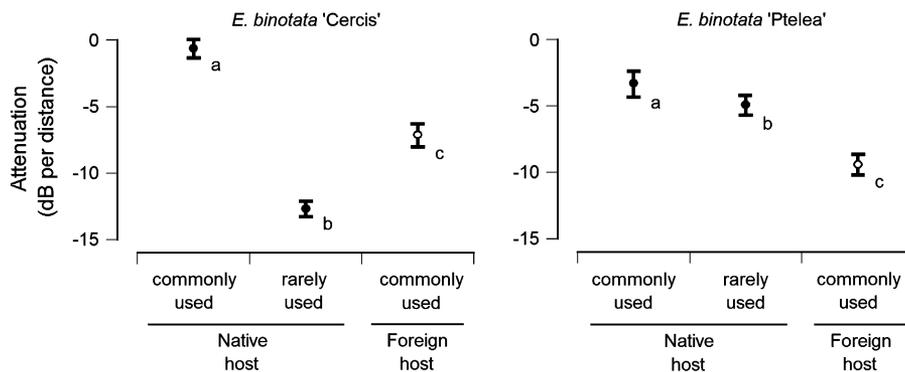
Figure 4
Signals of each species transmit best in the host plant part typically used for communication. Relative attenuation (decibels ± SD) of the mating signals of *Enchenopa binotata* 'Cercis' (*n* = 20 males) and *E. binotata* 'Ptelea' (*n* = 20 males). For each species, the average attenuation for 3 plant parts is given. Filled circles represent signal attenuation on the native host plant, in the plant part commonly used (e.g., woody stems for *E. binotata* Cercis) and the plant part rarely used (e.g., leaf petioles for *E. binotata* Cercis). Open circles represent signal attenuation on the other host plant, in the plant part commonly used by the other *Enchenopa* species. Attenuation differed significantly across plant parts for both species (*E. binotata* Cercis: $F_{2,57}$ = 2021.4, $P < 0.0001$; *E. binotata* Ptelea: $F_{2,57}$ = 357.8, $P < 0.0001$; symbols with unique subscripts are significantly different).

E. binotata complex, frequency differences are closely associated with changes in host use (Cocroft et al. 2008); and 3) male choice of signaling sites is likely to be constrained by the distribution of females, we feel that the most likely explanation for the signal–environment match in the 2 species examined here is that it is due to selection on signal frequency arising from host plant transmission properties.

Whether plant environments can favor vibrational signal divergence has been an open question (Cocroft and Rodríguez 2005). Widely held beliefs about vibrational signals predict convergence in signal design, rather than divergence. For example, it has been assumed that plant-dwelling insect species should use broadband signals for efficient transmission (Michelsen et al. 1982; Bradbury and Vehrencamp 1998). This idea is based on the expectation that the filtering properties of plants are unpredictable: a wide bandwidth will allow at least some frequencies to reach the intended receiver. Also, studies that have tested the predictions leading to signal divergence have produced mixed results. Henry and Wells (2004) found no support for signal–environment matching or differential transmission in 2 green lacewing species (*Chrysoperla* spp.) that use very different assemblages of plant substrates. In contrast, studies of the cosmopolitan green stinkbug (*Nezara viridula*) have shown that it uses a frequency range that transmits well on some of its common host plants (Miklas et al. 2001; Cokl et al. 2005).

The present study is the first to address the signal transmission hypothesis using large sample sizes, host specialists, methods for accurately measuring signal amplitude, and clearly defined communication environments. The lack of consensus from previous studies may result from the absence of one or more of these features. First, most studies have reported estimates of plant transmission properties based on very small sample sizes, which may not accurately characterize the signal environments encountered by a population of insects. Second, all previous studies have involved taxa that use multiple plant species, which complicates predictions about signal adaptation. Third, although plant stems vibrate in 2 dimensions during vibration propagation, previous studies have used approaches that measure stem vibration in only 1 dimension. Such an approach is likely to underestimate signal amplitude and reduce the accuracy of measured transmission curves (McNett et al. 2006, see Materials and Methods). Finally, behavioral observations in the field have not usually been available to precisely identify the signaling environment to which adaptation should be expected.

Substrate-borne vibrational communication is widespread in plant-feeding insects (Claridge 1985; Henry 1994; Virant-Doberlet and Cokl 2004; Cocroft and Rodríguez 2005). This is the first study to support the hypothesis that changes in host plant use can impose natural selection on insect vibrational signals, and it may be significant that support was found in strict host specialists (this study) but not in a pair of species that use a range of different plants (Henry and Wells 2004). Generalist species may face a very different selective environment, especially in the variability of signaling substrates, and a hypothesized adaptation to such unpredictability is to use signals containing a broad band of frequencies, which may fare better in the "frequency lottery" than signals that use a narrow band of frequencies (Michelsen et al. 1982). However, at least 1 host generalist (the green stinkbug, *N. viridula*) uses narrowband signals. Its signal frequency is well matched to the transmission properties of some of the common hosts in its introduced range, and the use of a narrowband signal is hypothesized to be an adaptation to a frequency "window" around 100 Hz that occurs in a number of plant substrates used by this species (Miklas et al. 2001; Cokl et al. 2005). To understand the nature of selection imposed by host plants on signal traits in generalist species, then, it may be important to measure multiple hosts. Much more work will be needed, however, before generalizations can be made about how vibrational communication systems evolve in response to the environments created by the tissues of living plants.

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