A combination of divergent natural and sexual selection is a powerful cause of speciation. This conjunction of evolutionary forces may often occur when divergence is initiated by ecological differences between populations because local adaptation to new resources can lead to changes in sexual selection. The hypothesis that differences in resource use contribute to the evolution of reproductive isolation by altering the nature of sexual selection predicts that: (1) differences in sexual traits, such as signals and preferences, are an important source of reproductive isolation between species using different resources; (2) there are identifiable sources of selection on sexual traits that differ between species using different resources; and (3) signals vary between populations using different resources to a larger extent than between populations using the same resource at different localities. Testing these predictions requires a group of closely-related species or populations that specialize on different resources and for which the traits involved in mate choice are known. The *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae) are host plant specialists in which speciation is associated with shifts to novel host plants. Mating in this complex is preceded by an exchange of vibrational signals transmitted through host plant stems, and the signal traits important for mate choice have been identified. In the *E. binotata* complex, previous work has supported the first two predictions: (1) signal differences between species are important in mate recognition and (2) host shifts can alter both the trait values favoured by sexual selection and the evolutionary response to that selection. In the present study, we tested the last prediction by conducting a large-scale study of mating signal variation within and between the 11 species in the complex. We find that differences in host use are strongly associated with differences in signal traits important for mate recognition. This result supports the hypothesis that host shifts have led to speciation in this group in part through their influence on divergence in mate communication systems. © 2010 The Linnean Society of London, * Biological Journal of the Linnean Society*, 2010, 99, 60–72.

**ADDITIONAL KEYWORDS:** phytophagous insects – specialization – speciation – vibrational communication.

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**INTRODUCTION**

Ecological speciation occurs when populations become reproductively isolated as they adapt to different environments (Rice, 1987; Schluter, 2001; Rundle & Nosil, 2005). When the traits influenced by local adaptation include mating signals and preferences, the combination of ecological differences and divergent sexual selection is an efficient cause of speciation (West-Eberhard, 1983; Kirkpatrick & Ravigné, 2002; Ritchie, 2007). There are many ways in which differences in resource use can lead to a change in mate communication systems (Cocroft, Rodriguez & Hunt, 2008). These include developmental plasticity (Landolt & Phillips, 1997; Rodríguez & Greenfield, 2003; West-Eberhard, 2003; Grace & Shaw, 2004; Schlichting, 2004; Etges *et al.*, 2007, Beckers & Schul, 2008; Rodriguez *et al.*, 2008);
selection for tuning of preferences and/or signals to environmental conditions (Endler, 1992; Fleishman, 1992; Boughman, 2002; Seehausen et al., 2008); differences in suites of predators (Abrahamson et al., 2001), some of which may eavesdrop on signals (Zuk & Kolluru, 1998); demographic changes that alter mating systems (Shuster & Wade, 2003); and ecological adaptation that incidentally changes signal production or perception (Miyatake & Shimizu, 1999; Podos, 2001; Vines & Schluter, 2006; Nosil et al., 2007). In addition, colonization of a new resource by a small number of individuals could lead to changes in sexual communication as a result of drift or through selection for less choosy females (Kaneshiro & Giddings, 1987). Each of these mechanisms has the potential to alter the relationship between variation in signals and in mating success (i.e. to change the nature of sexual selection).

The hypothesis that adaptation to the use of different resources leads to reproductive isolation and divergence through changes in sexual selection makes one main prediction: (1) differences in sexual traits, such as signals and preferences, should be an important source of reproductive isolation between species using different resources. Understanding the link between changes in resource use and divergence in sexual traits requires testing two further predictions: (2) there should be identifiable sources of selection (whether direct or indirect) on sexual traits that differ among populations using different resources. (3) Sexual traits should vary between populations using different resources to a larger extent than between populations on the same resource at different localities, thus distinguishing the effect of differences in resource use per se from the reproductive isolation that is expected to arise merely because populations on different resources are also separated in space. There has been mixed support for these predictions, with the most support accruing to the first (Rundle & Nosil, 2005; Funk, Nosil & Etges, 2006; Funk & Nosil, 2008). Most studies have tested only one or two predictions, however (Table 1), and the relationship between changes in resource use and divergence in sexual traits remains poorly understood. This is in part because strong tests of predictions (2) and (3) ideally require a group of several closely-related

<table>
<thead>
<tr>
<th>Prediction</th>
<th>Sticklebacks</th>
<th>Mosquitofish</th>
<th>Darwin’s finches</th>
<th>Fruit flies</th>
<th>Leaf beetles</th>
<th>Walking sticks</th>
<th>Brown planthoppers</th>
<th>Green lacewings</th>
<th>Enchenopa treehoppers</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>(2)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>(3)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Predictions: (1) Differences in signals/preferences are an important source of reproductive isolation; (2) Direct or indirect selection on signals/preferences differs among populations using different resources (3) Resource use is the main predictor of variation in signals/preferences.


Boughman, Rundle & Schluter (2005); McKinnon et al. (2004).

Rundle et al. (2000).


Huber et al. (2007).

Podos (2001).

Rice & Hostert (1993); Rundle et al. (2005).

Greenberg et al. (2003), although the authors’ findings could not he replicated independently (Coyne & Elwyn, 2006)).


Nosil et al. (2002, 2007); Sandoval & Nosil (2005); Arbuthnott & Crespi (2009).

Nosil et al. (2007).

Claridge et al. (1985); Butlin (1993, 1996).

Claridge (1985, 1990); Claridge et al. (1988).


Henry & Wells (2004).

Rodríguez et al. (2004).

Rodríguez et al. (2006); McNett & Cocroft (2008).

Present study.
species or populations that specialize on different resources and for which the traits involved in mate choice have been identified (Etges, 2002).

Study of the relationship between host shifts and divergence in sexual communication in the *Enchenopa binotata* Say species complex of tree-hoppers provides an ideal opportunity to test all of the above predictions (Cocroft et al., 2008). The *E. binotata* complex is a model for studies of speciation through host plant shifts (Wood, 1993; Coyne & Orr, 2004). Host shifts have occurred between distantly-related plants that constitute different selective environments, as demonstrated by reduced juvenile survivorship after reciprocal transplants (Wood, 1993). Host shifts also lead to ecological isolation. Because the insects' life history is tightly coordinated with the phenology of their host plant, shifts to hosts with differing phenologies lead to differences in the timing of egg hatch, adult eclosion, and mating (Wood & Keese, 1990; Wood, Olmstead & Guttmann, 1990). The high host fidelity of these insects further reduces encounters between individuals on different hosts (Wood, 1980; Tilmont, Wood & Pesek, 1998).

Reproductive isolation resulting from differences in sexual selection may be even stronger than ecological isolation in the *E. binotata* complex. When multiple species were experimentally placed in close proximity, interspecific courtship was frequent but rarely led to mating (Wood, 1980). The traits underlying this assortative mating were revealed by Hunt (1994), who showed that male *E. binotata* court females using substrate-borne vibrational advertisement signals. Subsequently, we have tested prediction (1), that differences in sexual communication systems between host-associated species are a source of reproductive isolation. For one population of *E. binotata* on *Viburnum*, females strongly discriminated against the signals of males from all but one of the other host-associated species tested (Rodríguez, Sullivan & Cocroft, 2004). Furthermore, female preferences for several male signal traits (studied in four sympatric species) have diverged between species, and male signal variation appears to have been strongly shaped by sexual selection exerted by female choice (Rodríguez, Ramaswamy & Cocroft, 2006). Differences in signals between species represent evolutionary divergence rather than phenotypic plasticity because most aspects of signals remain unchanged when individuals develop (Rodríguez et al., 2008) or signal (Sattman & Cocroft, 2003) on a nonhost plant.

We have also tested prediction (2), that there are causal links between host shifts and the evolution of communication. Sensory drive (Endler, 1992; Boughman, 2002) is one possibility (e.g., divergent selection arising from differences in the vibration-transmitting properties of the host plants). Signal transmission characteristics have been examined for hosts of two species in the *E. binotata* complex [those using *Cercis canadensis* (Fabaceae) and *Ptelea trifoliata* (Rutaceae)]. Comparison of male signals and plant transmission properties suggests that signal frequency has evolved in response to divergent selection for efficient transmission through the communication channels provided by their respective hosts (McNett & Cocroft, 2008). If this signal–environment match turns out to be a general pattern, it would have important consequences for reproductive isolation because frequency is the most important signal trait for mate recognition (Rodríguez et al., 2006). In addition, divergence of female preferences across host plants, including preferences for signal frequency (Rodríguez et al., 2006), contributes to the differences in selection acting on signals. An additional factor, wind-generated noise, does not appear to have a role in signal divergence. Wind is a major source of abiotic noise for vibrationally-communicating insects on plants, although the spectral properties of wind-induced vibrations do not differ between four host plant species in which they were measured (McNett, 2007; G. D. McNett & R. B. Cocroft, unpubl. data).

Even where selection on signals does not change after a host shift, the evolutionary response to that selection may change. Host species constitute different environments for development, and experimental host shifts reveal genotype × environment interactions (G × E) in signals and changes in genetic correlations among signal traits (Rodríguez et al., 2008). Thus, the genetic backgrounds of attractive phenotypes may vary across host plant species, and colonizing a new host could alter the dynamics of sexual selection (Rodríguez et al., 2008). G × E in male attractiveness also occurs as a consequence of host shifts in *Drosophila mojavensis* (Etges et al., 2007, 2009).

Having shown that differences in sexual communication cause reproductive isolation, and that use of different host species can favour such differences, we now test prediction (3), that signals in the *E. binotata* complex vary between populations using different resources to a larger extent than between populations using the same resource at different localities. The *E. binotata* complex offers a powerful test of this prediction because it affords a categorical measure of variation in ecological factors (host species) that varies independently of geographical distance. If factors associated with host shifts are the main cause of signal diversification in *Enchenopa*, then differences in host plant use should explain a greater proportion of signal variation than differences in locality for populations using the same host plant. If this diversification is important in speciation, it should occur in the signal traits that most contribute to behavioural...
isolation. If this prediction is not met, factors other than developing, signalling and selecting a mate on a new host may be responsible for signal diversification.

MATERIAL AND METHODS

SAMPLING

The *E. binotata* complex occurs throughout eastern North America (Lin & Wood, 2002). The present study aimed to record three populations spanning the distribution of each species, although, for rarer species, this was not possible (Fig. 1; for locality and sample size information, see Appendix). We collected nymphs and/or teneral adults in the field and reared them on potted host plants in the greenhouse [at both University of Missouri (MU) and Indiana University Southeast (IUS)]. This procedure ensured a reliable association between individuals and host plants because the presence of immatures indicates that reproduction has occurred on that plant, while adults can sometimes be encountered on nonhosts. Furthermore, species in the *E. binotata* complex are similar as adults, but distinctive in morphology and coloration during the nymphal stage (Pratt & Wood, 1992). The species in this complex have not yet been formally described, and we refer to them using their host plant genus.

SIGNAL MEASUREMENTS

Male signals in the *E. binotata* complex are produced in bouts in which the first few signals are lower in amplitude (Fig. 2A). Each signal, which is produced by abdominal tremulation, consists of a frequency-modulated whine followed by one or more pulses (Fig. 2B, C, D). Both whine and pulses are dominated by a single frequency (Fig. 2C, D, E, F), which is sometimes accompanied by lower-amplitude harmonics (Fig. 2C). At high densities, males may form stationary choruses (R. B. Cocroft, R. L. Rodriguez & R. E. Hunt, pers. observ.). We recorded individual males producing a single bout, as in Figure 2A. Comparisons among species are facilitated by the structural similarity of their signals (Fig. 3).

For each male, we measured the number of signals per bout, signal rate within a bout, whine length, frequency at the end of the whine, number of pulses, and pulse rate. To characterize signal frequency, we used time-domain rather than frequency-domain analysis. This was possible because of the relatively pure-tone nature of *Enchenopa* male signals (Fig. 2E). The frequency falls over the course of the whine, with most of the frequency change occurring during the initial low-amplitude portion (Fig. 2C). Although the frequency change is usually slight during the latter high-amplitude portion of the whine, measurements

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Localities at which collections were made for recording male signals. Names given are the host plant genus from which *Enchenopa binotata* were collected. The localities are (with numbers corresponding to numbers on map): *Carya* (2,9); *Celastrus* (5, 7, 12); *Cercis* (5, 7, 10); *Dirca* (7, 8, 14); *Juglans cinerea* (13); *Juglans nigra* (5, 7, 10); *Liriodendron* (11); *Ptelea* (5, 7); *Robinia* (5, 7, 12); *Viburnum* (5, 6, 10); *Sideroxylon* (1, 3, 4). For locality and sample size information, see Appendix.

made from amplitude spectra (as in Fig. 2F) would likely be influenced to some extent by host plant filtering properties. By measuring frequency from ten cycles at a consistent ‘landmark’, comprising the end of the whine where amplitude reaches a peak (Fig. 2B), we avoid the influence of plant filtering on our measurements (Cocroft et al., 2006). The frequency during the highest-amplitude portion of the signal is likely the most relevant for female choice: it has more energy than other frequencies in the signal, and synthetic signals containing only this frequency were approximately as attractive to females as natural signals in four species tested (R. L. Rodriguez & R. B. Cocroft, unpubl. data).

Recordings were made from 2001–06, both at IUS and at MU. The same protocols were used at each institution. Males were recorded 2–4 weeks after adult eclosion; a study of age-related variation in one species in the complex revealed no effect of age on signal variation over this time scale (Sattman & Cocroft, 2003).

MU: Each male was placed on the stem of a potted host plant, located on a vibration isolation table (Vibraplane, Kinetic Systems). Recordings were made by focusing the beam of a laser vibrometer (Polytec CLV 1000 with a CLV M030 decoder module; Polytec Inc.) on the host plant stem within 5 cm of the male. A small piece of reflective tape was attached to the stem to increase laser reflectance. The output was high-pass filtered at 60 Hz using a Krohn–Hite 3202 filter (Krohn-Hite Corporation) and sent to a Macintosh G4 computer through an Edirol UA–5 USB interface (Roland Corporation) and recorded with SoundEdit 16 Version 2 (Macromedia, Inc.) at 44.1-kHz sampling rate. We monitored male signals with a Radio Shack MPA-45 amplifier connected to an RCA.
loudspeaker and a Hameg HM 203–7 20 MHz oscilloscope (Hameg Instruments). For one population (E. binotata 'Dirca' from Ontario), signals were recorded in the field using a phono cartridge, but measurements from these recordings are directly comparable with the other measurements in the study.

IUS: Except for the following differences in equipment, procedures were the same as those followed at MU. The vibration isolation table used was a TMC Model 63–541. Recordings were made using a Polytec OFV 353 laser vibrometer and OFV 2602 decoder module. The output was sent to a Macintosh G4 with an Audiomedia III (Digidesign) digital interface and recorded with PEAK, version 3.0 (BIAS). Male signals were monitored using a TASCAM DA-3-MK II digital tape recorder connected to headphones.

The recording room at each location was maintained at approximately 24 °C. Because there was some temperature variation within the recording rooms, all signal variables were examined for temperature-related variation within each population; in most cases there was no influence of temperature, although, for four populations, it was necessary to adjust pulse rate and frequency to a common temperature of 24 °C using the slope of the regression of the signal trait on temperature.

When characterizing variation in vibrational signals it is important to minimize variation as a result of substrate effects. This effort is critical when the goal is to evaluate differences among species that signal on different substrates. We conducted two studies evaluating the influence of differences among plant individuals and species on variation in substrate-borne signals. For E. binotata (Sattman & Cocroft, 2003), none of the features measured here were affected by differences among individual plants of the same species. Although basic signal structure also did not change when the same individual male signalled on a nonhost plant, males produced fewer, shorter signals on a nonhost (Sattman & Cocroft, 2003; Rodríguez et al., 2008). Substrate-related variation can be minimized by making recordings close to the signaler on its own host species, as was performed in the present study, but it is not necessary to record all males of a given species on the same individual host plant (Cocroft et al., 2006).

STATISTICAL ANALYSIS
For the variance component analysis, measurements for the signals within a bout were first averaged for each individual. We conducted a nested analysis of variance using PROC GLM in SAS, version 6.12 (SAS Institute). This analysis requires at least two populations per host, so we excluded two rare species (E. binotata 'Juglans cinerea' and E. binotata 'Liriodendron') for which we obtained recordings for only one population. We used a model that corrects for the effects of the unbalanced design on the denominator degrees of freedom and mean square, with the effect of host species tested over the population-within-host species term, and the effect of population tested over error (Steel et al., 1997). To test the prediction that host plant species should more strongly influence signal differences than geographic differences, we compared the relative magnitude of the variance components. We used PROC VARCOMP to partition variation in signal traits among host plant species, populations within host species, and individuals within populations (sensu Steel et al., 1997).

RESULTS
There were significant differences among host-associated species in all signal traits (Table 2). There were also differences among populations within species (Table 2). We illustrate these findings with the two signal traits, namely frequency and whine length (Fig. 4), for which females in four species studied show the strongest preferences. Frequency and whine length preferences are closed (i.e. intermediate values are preferred) and the preferred values differ among species (Rodríguez et al., 2006). For the other signal traits, females showed weak open preferences or no preference (Rodríguez et al., 2006), so variation in these traits is unlikely to cause reproductive isolation. For frequency and whine length, there was a graded spectrum of geographic variation within species. In most species, there was little or moderate variation among populations (Fig. 4). However, for one species (E. binotata 'Viburnum'), variation among populations was comparable to that among species.

To assess the relative contribution of host shifts and geographical distance to signal divergence, we compared the magnitude of the variance components of these two factors. In all cases, the variance component as a result of species differences was substantially larger than the variance component as a result of population differences (Fig. 5). The variance component as a result of individual differences within populations was substantial for all parameters except signal frequency.

DISCUSSION
We tested a critical prediction of the hypothesis that host shifts have led to divergence in mate communication systems. Supporting this hypothesis, differences in male advertisement signals of Enchenopod treehoppers covary with differences in resource use. For most traits, geographical separation between populations using the same host plant species also
Table 2. Tests of hypotheses from mixed model analysis of variance, assessing variation due to use of different host plants (HOST), and to populations at different localities (LOC) using the same host

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency HOST</td>
<td>6</td>
<td>414986.75</td>
<td>32.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>14.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOC(HOST)</td>
<td>15</td>
<td>9471.75</td>
<td>58.00</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whine length HOST</td>
<td>6</td>
<td>1044027.84</td>
<td>8.53</td>
<td>0.0005</td>
</tr>
<tr>
<td>Error</td>
<td>14.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOC(HOST)</td>
<td>15</td>
<td>93966.00</td>
<td>8.21</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signals/bout HOST</td>
<td>6</td>
<td>232.94</td>
<td>18.67</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>11.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOC(HOST)</td>
<td>15</td>
<td>10.75</td>
<td>1.83</td>
<td>0.0292</td>
</tr>
<tr>
<td>Error</td>
<td>363</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signal rate HOST</td>
<td>6</td>
<td>11752600.55</td>
<td>7.23</td>
<td>0.0012</td>
</tr>
<tr>
<td>Error</td>
<td>13.74</td>
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</tr>
<tr>
<td>LOC(HOST)</td>
<td>15</td>
<td>1273446.33</td>
<td>5.84</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>353</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pulses HOST</td>
<td>6</td>
<td>61.50</td>
<td>25.56</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>11.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOC(HOST)</td>
<td>15</td>
<td>2.05</td>
<td>1.98</td>
<td>0.0155</td>
</tr>
<tr>
<td>Error</td>
<td>360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse rate HOST</td>
<td>6</td>
<td>143.44</td>
<td>9.19</td>
<td>0.0003</td>
</tr>
<tr>
<td>Error</td>
<td>14.16</td>
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<td>LOC(HOST)</td>
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<td>11.98</td>
<td>8.91</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>356</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note that model corrects for effects of unbalanced design on denominator d.f. and MS (see text).

Figure 4. Variation in frequency and whine length in the Enchenopa binotata complex. Shown are mean ± 95% confidence interval for each population, indicated by the name of their host plant; dotted lines surround populations of a given species, labelled according to host plant, except for the species on Viburnum, which is indicated by green (grey in print) symbols.

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has promoted changes in signals. The findings obtained in the present study thus provide support both for host shifts and host-independent effects as mechanisms of signal divergence. However, host shifts have a much stronger role, especially for signal frequency, the most important trait for mate recognition (Rodríguez et al., 2006). Once differences in signal traits have appeared (especially in signal frequency and whine length, for which females show strong closed preferences), female mate choice is likely to reduce gene flow and promote further divergence. In the *E. binotata* complex, differences in mating signals and preferences are an important source of reproductive isolation (Rodríguez et al., 2004, 2006), and there are causal links between host shifts and signal divergence (McNitt & Cocroft, 2008; Rodriguez et al., 2008). Together with the finding from the present study that signal variation is linked with resource use, these studies of sexual communication in *E. binotata* strongly support the hypothesis that host shifts contribute to speciation partly through their influence on the evolution of sexual communication.

Ecological and behavioural isolation are both important in the current coexistence of species in the *E. binotata* complex, and both may have been important during their initial divergence. Host shifts lead immediately to ecological isolation because sympatric individuals developing on different hosts eclose and mate at different times (Wood & Guttman, 1982; Wood & Keese, 1990; Wood et al., 1990) and because these relatively sedentary insects show high host fidelity in mating and oviposition (Wood, 1980). Host shifts also impose divergent natural selection, leading to specialization on the new host (Wood & Guttman, 1983; Tilmon et al., 1998). This combination of assortative mating and divergent selection may be sufficient to initiate speciation (Wood, 1993). However, we suggest that divergence in communication systems acts in synergy with ecological isolation and may have been important for completing the speciation process. Ecological isolation among *E. binotata* populations on different hosts is leaky: there is considerable overlap of mating periods between individuals on some hosts (Wood & Guttman, 1982), and mate-searching males can sometimes be found on hosts of other species in the complex during the mating period (R. B. Cocroft, unpubl. data). The importance of behavioural isolation was made clear when breeding adults from multiple host-associated species, maintained on potted host plants, were placed in close proximity: mixed-species courtship was frequent but heterospecific matings were rare (Wood, 1980). Divergence in signals and preferences (Rodríguez et al., 2004, 2006) contributes to this behavioural isolation.

The signals of male *Enchenopa* differ not only between populations using different hosts, but also to some extent among populations using the same host. This geographic variation could have its roots in interactions among *E. binotata* species because mate-searching males can sometimes be found on the ‘wrong’ host during the breeding season (R. B. Cocroft, unpubl. data). Host plants of the 11 species in the complex co-occur in a mosaic in which the set of common species differs among localities (Lin & Wood, 2002). As a consequence, processes such as gene flow among species on different hosts, or reinforcement in response to the cost of mismating, could promote different patterns of signal divergence at different localities. Signal variation could also be related to geographic variation in host plant characteristics, such as those that affect signal transmission, or to random factors such as genetic drift. Finally, in one case (*E. binotata* ‘Viburnum’), recent work suggests the presence of two genetically distinct lineages with different signal frequencies (Snyder, 2008).

**Figure 5.** Relative magnitude of variance components in signal measurements from male *Enchenopa binotata* as a result of variation among host-associated species, among populations using the same host, and among individuals within populations.
Viburnum-associated *E. binotata* populations use four closely-related hosts (*Viburnum cassinoides*, *V. lentago*, *Viburnum prunifolium*, and *Viburnum rufidulum*), but both lineages occur on each host species.

Although we examined host-associated variation only in male signals, female preferences can also change after a host shift. Such changes can occur as a byproduct of adaptation to different environments (Rundle *et al.*, 2005); as a consequence of selection favouring the avoidance of mating with individuals adapted to different hosts (Nosil *et al.*, 2003); because of selection favouring sensitivity at the frequencies that best transmit through the environment (Boughman, 2002; McNett & Cocroft, 2008); through Fisherian dynamics (Kokko *et al.*, 2002; Mead & Arnold, 2004; Rodríguez *et al.*, 2008); through developmental effects on preferences (Rodriguez & Greenfield, 2003); or because different signal traits or values are associated with male viability or condition on different host plants (Proulx, 1999; Lorch *et al.*, 2003). Given that any or all of these factors may lead to changes in mating preferences, and that, once differences in preferences appear, the strength of sexual selection would facilitate reproductive isolation and divergence (West-Eberhard, 1983), it is perhaps surprising that there are so few examples of closely-related, host-specific insects for which signals and preferences have been investigated for their role in ecological speciation (Claridge, 1985; Claridge *et al.*, 1988; Percy, Taylor & Kennedy, 2006; Etges *et al.*, 2007; Nosil *et al.*, 2007).

How general are the patterns seen in the *E. binotata* complex? Evidence that differences in host use promote differences in sexual communication also comes from the study of chemical signals in populations of *Timema* walkingsticks (Nosil *et al.*, 2007). Pairs of allopatric populations are more likely to be sexually isolated if they use different hosts than if they use the same host, as demonstrated by behavioural responses of males, and differences in olfactory cues likely explain these responses (Nosil *et al.*, 2007).

Another host-specific phytophagous insect in which the hypothesis that host shifts contribute to divergence through their effects on sexual selection has been examined in detail is the brown planthopper, *Nilaparvata lugens*. This species is a pest of rice (*Oryza*) and may be the result of two independent host shifts from *Leersia* plants (Jones *et al.*, 1996). The rice and *Leersia* forms occur sympatrically over a broad geographic range in India, south-east Asia and Australia, and Claridge *et al.* (1985, 1988) examined geographic variation in mating signals over much of that range. The first prediction, that there is assortative mating based on differences in sexual communication, is met: variation in pulse repetition rate in male signals influences mate choice and contributes to assortative mating between the two forms (Claridge *et al.*, 1985, 1988; Butlin, 1993, 1996). Although prediction (2) has not been tested, Claridge *et al.* (1988) suggested that plant signal transmission characteristics were unlikely to account for the signal differences contributing to reproductive isolation. Butlin & Tregenza (1998) suggested that changes in signals were largely a result of drift, although a quantitative genetic analysis revealed a signature consistent with directional selection (Butlin, 1996). The third prediction, that signal variation is more strongly correlated with host use than with geography, is not met: pulse repetition rates of the *Leersia* form are faster than those of the rice form in some areas, but slower in others (i.e. geography appears to explain more variation in pulse rate than does host use) (Claridge *et al.*, 1988). Whatever the causes of rapid signal evolution in *Nilaparvata*, the evolution of behavioural isolation is important for species coexistence (Claridge *et al.*, 1988) and may have been important during speciation.

Another group providing an instructive contrast with the *E. binotata* complex is the *Chrysoperla carnea* species complex of lacewings. In both cases, closely-related species differ more in signals than in morphology, especially in the *C. carnea* complex where some species have been recognized solely on the basis of song differences (Henry, 1985). Lacewings are predators that use plant stems to transmit their substrate-borne signals, but are not host plant specialists like *Enchenopa*. Some lacewing species, however, are restricted to broad classes of substrates, such as woody versus herbaceous plants. Henry & Wells (2004) tested the hypothesis that the signals of conifer-dwelling and grass/herbaceous plant-dwelling lacewings are adapted to these substrates. This hypothesis makes the untested though reasonable assumption that variation in signal-filtering properties within these broad classes of plants is less than that between them, although Cokl *et al.* (2007) showed that structurally similar plant species can differ in vibration-transmitting properties. There did not appear to be a strong effect of substrate type on signal transmission, measured either as propagation fidelity or as preferences for signals propagated through native versus foreign substrates. On the basis of those results, and the pattern that some closely-related species in the *C. carnea* complex differ in song but not ecology, it was suggested that environment-independent mechanisms explain signal differences.

Host-specific phytophagous insects constitute a large fraction of terrestrial diversity, and provide many likely examples of ecological speciation (Tauber...
& Tauber, 1989; Mallet, 2001; Price, 2002; Bush & Butlin, 2004). In these insects, diversification often occurs in concert with shifts to new host plants (Berlocher & Feder, 2002). After a host shift, ecological factors promote reproductive isolation between populations on ancestral and novel hosts (Tauber & Tauber, 1989; Wood, 1993; Berlocher & Feder, 2002; Funk et al., 2002). However, ecological isolation is often partial, and speciation may require additional sources of reproductive isolation (Claridge et al., 1988). A recent review of divergence in host-associated insects (Dres & Mallet, 2003) showed that gene flow was more restricted between closely-related taxa that experienced both ecological and behavioural isolation than between those that experienced ecological isolation alone. The latter were considered to be 'host races'; if these represent an intermediate stage of speciation, as was suggested by Dres & Mallet (2003), then the evolution of behavioural isolation may often be important during speciation in phytophagous insects. Host specialist insects provide an especially useful model for investigating the relationship between communication and speciation. They exhibit a great diversity of communication signals (Cocroft & Rodriguez, 2005), and the intimate relationship between insect and plant facilitates experimental study of the processes leading to divergence in mating signals and preferences. In this regard, the *E. binotata* complex (with its many members specializing on a diverse set of hosts, substantial signal divergence, and strong mate preferences) showcases an important and widespread process: the interaction between local adaptation and the evolution of reproductive isolation.

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APPENDIX

List of populations by host plant, number on map in Fig. 1, and locality (by state abbreviation in the USA), with number of males recorded from that host at that locality (Dirca palustris: ONT = Ontario, Canada)

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Map number</th>
<th>Locality</th>
<th>Number of males recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carya spp.</td>
<td>2</td>
<td>LA: Winn Co.</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>MD: Cecil Co.</td>
<td>2</td>
</tr>
<tr>
<td>Celastrus scandens</td>
<td>5</td>
<td>MO: Boone Co.</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IN: New Albany</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>NY: Ithaca</td>
<td>13</td>
</tr>
<tr>
<td>Cercis canadensis</td>
<td>5</td>
<td>MO: Columbia</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IN: New Albany</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>MD: Green Ridge</td>
<td>12</td>
</tr>
<tr>
<td>Dirca palustris</td>
<td>14</td>
<td>ONT: White Lake</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>IN: New Harmony</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IN: Harrison Co.</td>
<td>4</td>
</tr>
<tr>
<td>Juglans cinerea</td>
<td>13</td>
<td>NY: Oswego</td>
<td>3</td>
</tr>
<tr>
<td>Juglans nigra</td>
<td>5</td>
<td>MO: Columbia</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IN: Greenville</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>MD: Little Orleans</td>
<td>8</td>
</tr>
<tr>
<td>Liriodendron</td>
<td>11</td>
<td>OH: Wooster</td>
<td>1</td>
</tr>
<tr>
<td>Ptelea trifoliata</td>
<td>5</td>
<td>MO: Columbia</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IN: Crawford Co.</td>
<td>25</td>
</tr>
<tr>
<td>Robinia pseudoacacia</td>
<td>5</td>
<td>MO: Columbia</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>IN: New Albany</td>
<td>20</td>
</tr>
<tr>
<td>Sideroxylon Lycioides</td>
<td>3</td>
<td>AL: Moulton</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>MO: Van Buren</td>
<td>6</td>
</tr>
<tr>
<td>S. lanuginosum</td>
<td>1</td>
<td>TX: Austin</td>
<td>4</td>
</tr>
<tr>
<td>Viburnum lentago</td>
<td>6</td>
<td>KY: Bernheim Forest</td>
<td>24</td>
</tr>
<tr>
<td>V. prunifolium</td>
<td>5</td>
<td>MO: Columbia</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>MD: Little Orleans</td>
<td>6</td>
</tr>
</tbody>
</table>