

**Speciation of the *Enchenopa binotata*  
complex (Insecta: Homoptera:  
Membracidae)**

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**Abstract**

The central question of this review is whether mechanisms which promote host-plant specialization initiate a process that ultimately results in genetic divergence of sympatric populations of phytophagous insects. The *Enchenopa binotata* species complex (Insecta: Homoptera: Membracidae) is used as a model to deduce, from extant plant-insect interactions, mechanisms which could have initiated sympatric divergence. Comparison of closely related species pairs, derived from an inferred phylogeny of extant *Enchenopa* species, suggests that asynchronous mating induced by differences in plant phenology may have initiated divergence. Although secondary contact cannot be excluded, geographic distributions of extant species are not allopatric, suggesting a possible sympatric origin of the species complex.

Experimental host-plant shifts demonstrate, in one generation, that phenologically novel host plants can result in asynchronous mating of *Enchenopa*. The existing data for the *Enchenopa* complex suggest that partial or complete mating asynchrony is achieved first through shifts to novel plants that disrupt extrinsically mating synchrony. Genetic divergence occurs secondarily through selection pressures imposed by novel host plants.

**INTRODUCTION**

The question of whether host-plant specialization of insects leads to speciation, in the context of sympatric models, has a long and controversial history in evolutionary biology (Mayr, 1947, 1963; Bush, 1975; Futuyma & Mayer, 1980; Wood, 1980; Jaenike, 1981; Templeton, 1981; Futuyma, 1986; Bush & Howard, 1986; Butlin, 1987; Barton, Jones & Mallet, 1988; Tauber & Tauber, 1989; Rice & Salt, 1990). Intrinsic (Bush, 1975) or gradual (Futuyma, 1986) sympatric models assume selection on insect genetic traits which differentially affect the fitness of individuals that colonize novel plant species (Rausher, 1984a,b). If a stable resource-associated genetic polymorphism is achieved, continued selection could lead to speciation (Maynard Smith, 1966). Bush (1975) and his colleagues (Feder, Chilcote & Bush, 1988; McPheron, Smith & Berlocher, 1988) have argued that intrinsic host recognition traits linked with traits influencing survival or the timing of the life history (Smith, 1988) are sufficient to promote divergence in the *Rbagoletis pomonella* complex (Feder *et al.*, 1988). However, experimental tests of some of these assumptions in other arthropods have generally not been supportive (Gould, 1979; Rausher, 1984a; Jaenike, 1989; Via, 1990; but see Diehl & Bush, 1989) but some models make sympatric speciation an attractive hypothesis while others do not (Maynard Smith, 1966; Pimm, 1979; Felsenstein, 1981; Rausher, 1984b; Diehl & Bush, 1989).

Most empirical research on insect sympatric speciation has concentrated on intrinsic genetic traits that influence diapause (Tauber & Tauber, 1977a,b; Tauber, Tauber & Masaki, 1986), ecological preference (Bush, 1975; Rice & Salt, 1988,

1990), or fitness (Rausher, 1984a) as mechanisms to disrupt a gene pool to allow genetic divergence. Invariably the insects chosen for study, such as the *Enchenopa* complex (Wood, 1980), are morphologically very similar and there is an a priori reason to suggest resource-based polymorphism. If the organisms in the complex are shown to be sibling or biological species, extant mechanisms that resolve the selection–recombination problem (Felsenstein, 1981) such as habitat preference (Diehl & Bush, 1989) or diapause (Tauber & Tauber, 1982), can be interpreted either as the cause (Tauber & Tauber, 1982), or the effect of speciation (Henry, 1982; Butlin, 1987). With the exception of the *Rbagoletis pomonella* complex, where host shifts are believed to have occurred during the last 200 years (Bush, Chapter 14) and reproductive isolation has not yet developed, there is little evidence that the postulated mechanisms effected divergence through sympatric host shifts.

Ideally, a case for sympatric speciation would involve mechanisms deduced from extant organisms, independent phylogenetic evidence, and an experimental test of the mechanism. Over the last 26 years, this has been the progression of my work on the eastern North American *Enchenopa binotata* (Fig. 1) species complex. In this review, I will describe evidence which suggests that divergence of this species complex could have occurred in sympatry.



**Figure 1** An *Enchenopa* female on *Cercis canadensis* placing egg froth over an egg mass. Eggs are inserted in a mass under the bark of the branch. The horn-like structure is the pronotum.

## THE *ENCHENOPA BINOTATA* SPECIES COMPLEX: BIOLOGY AND HOST-PLANT-RELATED VARIATION

### General life history

All members of the *Enchenopa binotata* species complex are univoltine. Although there are host-plant related differences in timing, the general life history described here for *Enchenopa* on *Celastrus scandens* (bittersweet) is representative of the complex. In Delaware, egg hatch begins in late April or early May over a 7–10-day period. Essentially synchronous egg hatch results in a uniformly aged cohort which matures to the adult stage approximately 30 days later in early June. Adult maturation occurs within a 10-day period, where males eclose on the average 1–2 days before females. Mating begins 3–4 weeks later, around the first day of July, and is completed within 10–15 days. Males appear to mature sexually before females, since there is a 5–10-day span between the first attempted mating and the first copulation. Females mate once while males may mate several times. Under semi-field conditions most male mortality occurs before early August while females live well into November (Wood & Guttman, 1982).

Oviposition begins in the middle of July and is continuous through early November. Females deposit eggs in clusters or egg masses containing on average seven eggs (Wood, 1980). Eggs are inserted into woody stems where they contact the vascular tissues of the plant. Once an egg mass is deposited, the ovipositional wound is covered with a lipid-based secretion known as egg froth (Wood & Patton, 1971; Wood, 1982). During the prolonged oviposition, an average female may deposit 20 or more egg masses. When oviposition is completed in November, most of the egg masses are deposited in a relatively small proportion of the host's branches (Wood, 1980, 1982; Wood & Guttman, 1982).

The following points of the life history are essential to understanding the mechanisms which may have promoted divergence of the *Enchenopa binotata* species complex: (1) synchronous egg hatch, (2) uniform age structure, (3) temporal delay between adult maturation and mating, (4) limited period of mating, (5) females mate once, (6) males die substantially earlier than females, (7) placement of eggs in contact with plant vascular tissue, (8) prolonged oviposition, and (9) clustering of egg masses on branches.

### Host-plant-associated variation

In eastern North America, *Enchenopa binotata* occurs on eight plant genera distributed among six different plant families (Table 1). With the exception of the *Enchenopa* on *Viburnum*, *Carya* and perhaps *Juglans*, the remaining *Enchenopa* are monophagous. Evidence that the six monophagous, two polyphagous and one possible oligophagous (on the two *Juglans* species) *Enchenopa* are biological species is presented later. Since the species in the *Enchenopa binotata* complex have not been formally named, they will be referred to by their associated host-plant genus or, in the case of the two on *Juglans*, by plant species.

The first suggestion that *Enchenopa binotata* was not a single polyphagous species was provided by host-associated variation in female pronotal colour, size and shape. This observation, combined with dramatic host-related differences in fifth instar nymphal colouration, aggregating habits, and feeding sites suggested considerable

**Table 1** Host plants of the *Enchenopa binotata* species complex.

| Genus               | Species                           | Family         |
|---------------------|-----------------------------------|----------------|
| <i>Ptelea</i>       | <i>trifoliata</i> (L.)            | Rutaceae       |
| <i>Juglans</i>      | <i>nigra</i> (L.)                 | Juglandaceae   |
| <i>Juglans</i>      | <i>cinerea</i> (L.)               | Juglandaceae   |
| <i>Carya</i>        | <i>illinoensis</i> (Wang) K. Koch | Juglandaceae   |
|                     | <i>ovalis</i> (Wang) Sarg.        |                |
|                     | <i>cordiformis</i> (Wang) K. Koch |                |
|                     | <i>laciniosa</i> (Michx.) Loud.   |                |
|                     | <i>ovata</i> (Mill.) K. Koch      |                |
| <i>Celastrus</i>    | <i>scandens</i> (L.)              | Celastraceae   |
| <i>Liriodendron</i> | <i>tulipifera</i> (L.)            | * Magnoliaceae |
| <i>Robinia</i>      | <i>pseudoacacia</i> (L.)          | Leguminosae    |
| <i>Cercis</i>       | <i>canadensis</i> (L.)            | Leguminosae    |
| <i>Viburnum</i>     | <i>cassinoides</i> L.             | Caprifoliaceae |
|                     | <i>rufidulum</i> Raf.             |                |
|                     | <i>lentago</i> L.                 |                |
|                     | <i>prunifolium</i> L.             |                |

intraspecific variation. Other characteristics such as diurnal differences in oviposition, seasonal timing of oviposition, oviposition sites, egg froth composition, and egg mass size which varied with host species also suggested distinct species or races in the *E. binotata* complex (Wood, 1980).

### Assortative mating and hybridization

To determine whether *E. binotata* is a complex of host races or cryptic biological species, males and females from six host species were placed in a common field cage where they were free to select mates and host plants. Although some females dispersed to inappropriate hosts and formed mixed precopulatory pairs, mating in general occurred on the female's natal host and only 6 of 103 matings were between individuals from different hosts. Although other pre-mating barriers could not be excluded, the observed assortative mating appeared to be the result of temporal mating asynchrony related to host-plant origin (Wood, 1980). These results supported the model of Maynard Smith (1966), in which a stable resource-associated polymorphism could develop through assortative mating, effected by host fidelity during mating.

Three attempts to hybridize six host-associated *Enchenopa* have been made by transferring males from one host and confining them with females on their host. Males from four hosts, when confined to *Ptelea* and *Juglans*, died before mating. In other transfers to inappropriate hosts, male mortality was high but mating did occur. The viability of eggs from these matings could not be established as they were accidentally destroyed the following year (Wood, unpublished).

Male and female genitalia of all *Enchenopa* in the *binotata* complex, regardless of host-plant origin, are remarkably similar and are of no taxonomic value in species recognition (Pratt & Wood, unpublished) and preliminary karyotype analysis failed to detect any differences in chromosome number (Greene & Wood, unpublished).

The lack of genitalic differences and similar chromosome numbers suggest no mechanical impediments to prohibit dispersing males from successful mating with females on other host species. However, high male mortality on inappropriate hosts and tight host-associated assortative mating suggests that little or no gene flow occurs between *Enchenopa* utilizing different hosts through male dispersal.

### **Host selection and the costs of inappropriate oviposition**

When females from a host species were given free access to oviposit on seven plant species in a cage, they made few or no mistakes in selecting the appropriate host species. None of six host-associated *Enchenopa* female cohorts were ambivalent in host selection for oviposition even though individuals were observed on inappropriate hosts prior to oviposition (Wood, 1980).

Although female host selection is tight, another experiment was done to test whether gene flow among *Enchenopa* on different hosts could occur through the dispersal of mated females. When females associated with each of six plant genera were forced to oviposit on inappropriate *Enchenopa* hosts, females either: (1) died rather than oviposit, (2) deposited few egg masses, (3) produced few hatching eggs, or (4) produced no adult offspring (Wood & Guttman, 1983).

These results suggest that extensive gene flow does not occur among *Enchenopa* on different host-plant species through the movement of mated females and pointed to considerable genetic divergence in host preference and the ability to survive on inappropriate host species. The coupling of host selection fidelity during oviposition, fitness costs of inappropriate oviposition, host-associated mating, and assortative mating suggest that resource-associated divergent selection could operate as described by Maynard-Smith (1966).

### **Allozyme variation**

Initial allozyme analysis of seven plant-associated *Enchenopa* demonstrated fixed or nearly fixed differences between four, while frequency differences separated the remaining three (Guttman, Wood & Karlin, 1981; Wood & Guttman, 1985). Although sample sizes and number of polymorphic loci were low, *Enchenopa* populations were from habitats where introgression was possible. Since the respective gene pools were distinguishable, *Enchenopa* on each host were considered to be either a race or biological species. Fixed allelic differences disappeared when six *Enchenopa* were collected over broader geographic ranges, but differences in allelic frequencies were maintained within and between geographic areas (Guttman & Weigt, 1989; Pratt, Wood & Datz, unpublished). Evidence that *Enchenopa* on *Carya* and *Juglans cinerea* are biological species is presented in Pratt *et al.* (unpublished).

### **Asynchronous life histories and mating**

Assortative mating of this univoltine complex of species was hypothesized to be the result of asynchronous mating associated with different host species (Wood, 1980). In common garden experiments, seven of the host-associated *Enchenopa* species confined to separate cages differed in the timing of egg hatch, seasonal time of mating, diurnal mating patterns, and periods of flight activity. For *Enchenopa* on an

array of host species, asynchrony of egg hatch interacting with apparent host effects on development time resulted in asynchronous restricted seasonal mating where females mate once. The longevity of males was substantially shorter than that of females, with most mortality occurring before and during the limited interval of female sexual receptivity. Because of host-associated differences in life-history timing, male mortality was also asynchronous (Wood, 1980, 1988; Wood & Guttman, 1982, 1985). The relationship between the timing of egg hatch and flowering phenology of the host plants suggests that the consequence of utilizing phenologically different host species was asynchronous mating (Wood, 1980; Wood & Guttman, 1982, 1985). Thus, host-associated differences in life-history timing and limited mating periods appear to augment genetic differences in host preference and survival to isolate extant *Enchenopa* species.

### Vagility

For *Enchenopa* there is only a relatively short period in the life history where dispersal is possible. Eggs are in plant tissue for as long as 10 months and nymphs cannot, under normal circumstances, leave the host. Nymphs in the *Enchenopa binotata* complex interact facultatively with ants which appears to favour low female vagility and persistent yearly colonization of trees. Attracting and maintaining ant attendance is a function of nymphal aggregation size on branches. Large nymphal aggregations are attended earlier by more ants than smaller aggregations, and as a result have higher survival. Nymphal survival on individual trees is related to the number of nymphs on the tree and whether nymphs are ant attended (Wood, 1982). Persistent colonization of individual trees appears to be related to the yearly predictability of ant mutualists. Since a female's fitness is directly related to offspring being tended by ant mutualists, this suggests female dispersal should only occur at high densities (Wood & Guttman, 1981) and that genetic traits associated with philopatry should be favoured.

Nymphal aggregations are established the previous year by ovipositing females and adult aggregations are loosely maintained until males become sexually active. Flight occurs before and during the pre-mating period but decreases as mating continues (Wood & Guttman, 1981). Once oviposition begins, the ovipositional attractant(s) in egg froth placed over egg masses (Fig. 1) attracts females to a limited number of branches within the host and there is a rapid decline in male and female flight. At the peak of oviposition, on some host species, aggregations of females are common and persistent for weeks unless disturbed. At the completion of oviposition on some host species, several hundred egg masses can be deposited in a branch 1–2 feet long (Wood, 1980, 1982).

When males and females were marked, dispersal between trees of the same species during the pre-mating stage was low and dispersal to different *Enchenopa* host species was not detected at all. Although marked insects declined on the original host during the pre-mating period (Wood & Guttman, 1981), the decline corresponded to high mortality in caged control populations (Wood & Guttman, 1982). At the end of mating, few marked males were recaptured on their original host which also coincided with high mortality of caged populations. Females were observed on their original host up to 2 months after being marked. Recaptures adjusted for non-predator related mortality indicated that 35–41% of the females remained on their original

host for their entire lifespan (Wood & Guttman, 1981). The few recaptured dispersed marked males were recovered on their appropriate hosts within 18 m of the release host (Wood & Guttman, 1981) while in another study all but two marked females were recaptured on adjacent touching trees of the same species (Guttman, Wilson & Weigt, 1989).

Low *Enchenopa* dispersal between trees and apparent philopatric tendencies suggest that gene flow within a microhabitat is limited. If true, then a reasonable prediction would be the existence of statistical differences in allozyme frequencies of *Enchenopa* among trees of the same species (Guttman *et al.*, 1981). Guttman *et al.* (1989) examined three *Enchenopa* species and found evidence of among-tree differences in allozyme frequency for *Enchenopa* on *Cercis*, *Juglans* and *Ptelea*. In summary, an important consequence of a female's gain in fitness from ant mutualists are genetically based ovipositional behaviours that increase the probability that offspring will be ant attended. These same behaviours reduce dispersal by mated females (Wood & Guttman, 1982).

### Summary of evidence for reproductive isolation

At present I consider the seven *Enchenopa* associated with seven plant genera and the two on different *Juglans* species as biological species. Nymphal morphology provides evidence that each of the nine *Enchenopa* are distinct species. Differences in colouration, setal morphology, shape of the pronotal horn, and the length and shape of the dorsal abdominal scoli of fifth instars can be used to distinguish the nine species (Pratt & Wood, in press). Adult female pronotal shape and size can also be used to distinguish the nine species but the differences are not discrete (Wood, Datz & Pratt, unpublished).

Asynchronous and host-associated assortative mating (Wood, 1980), host selection for oviposition (Wood, 1980), and the inability of females to produce viable offspring on inappropriate host species (Wood & Guttman, 1983) supports the species status of seven *Enchenopa*. Consistent allozyme frequency differences within and among geographic regions (and habitats) where introgression was possible indicate all nine host-associated *Enchenopa* are isolated divergent gene pools (Guttman *et al.*, 1981; Wood & Guttman, 1985; Guttman & Weigt, 1989; Pratt *et al.*, unpublished). The allozyme and morphological variation among species in the *E. binotata* complex are in the range typically used to distinguish geographic races of other insects. This suggests either a recent historical origin or that these characters have not been subject to divergent selection, or a combination of both.

### PLANT PHENOLOGY HYPOTHESIS

Recognition that *Enchenopa binotata* is a complex of biological species presents the dilemma of whether the asynchrony of life histories which facilitates reproductive isolation is the cause or the effect of speciation. Extant host-insect interactions suggest that shifts to phenologically novel hosts disrupted mating synchrony which permitted genetic divergence. If life-history timing is mediated extrinsically by the host plant rather than an intrinsic insect genetic characteristic, then recombination would have no effect on mating asynchrony. Rapid selection for host-plant adapted genotypes is



far more plausible if the nullifying effects of recombination do not affect the mechanism that maintains mating asynchrony. In this hypothesis, the phenology of the host plant operates in a manner analogous to an allopatric barrier.

### **Evidence for plant mediation of life-history timing**

In extant *Enchenopa* there is a relationship between the timing of egg hatch and the flowering phenology of the host plant (Wood, 1980; Wood & Guttman, 1982, 1985). The first direct evidence that the timing of egg hatch is mediated by the host plant rather than by an intrinsic genetic trait in the insect was the observation that eggs deposited on an inappropriate host hatched at a different time to those on appropriate host species (Wood & Guttman, 1983). Eggs are inserted under the bark in contact with the vascular tissue of the stem (Wood & Patton, 1974; Wood, 1980) and they require liquid water from the plant to begin development (Wood, Olmstead & Guttman, 1990). Asynchronous egg hatch can be induced experimentally by manipulating the time at which water is added to excised branches with eggs. Since six of the host species differ in branch water content during the late winter and early spring, temporal plant differences in the ascent of sap were hypothesized to be the factor initiating asynchronous egg hatch. Thus, a critical step in initiating asynchronous *Enchenopa* life histories appeared to be under extrinsic mediation (Wood *et al.*, 1990).

For host-induced differences in life-history timing to be the mechanism initiating divergence, there must be year to year consistency in patterns of egg hatch. Over 3 years of study, eggs on three hosts always hatched earlier than those on three others. Within each of these two groups, differences in development time to mating and diurnal mating patterns result in asynchronous mating (Wood & Guttman, 1982).

### **Experimental test of host-induced assortative mating**

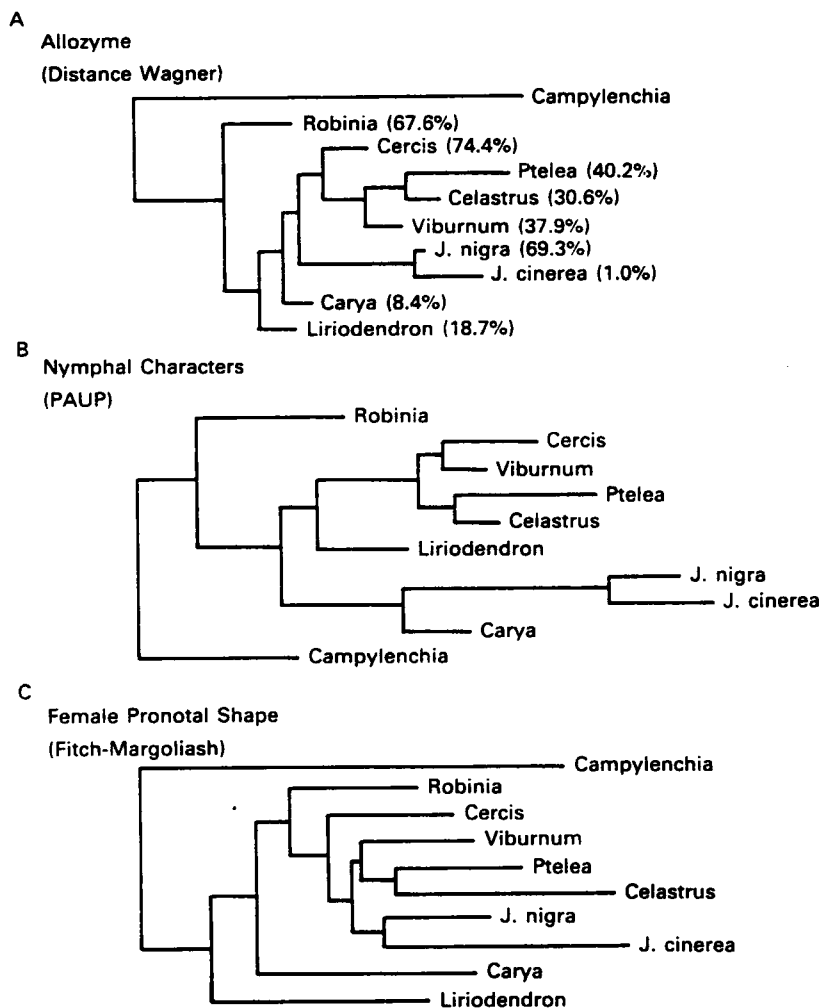
Although extant *Enchenopa* species appear to mate assortatively through asynchronous mating (Wood, 1980), this is not a direct test of whether host-induced asynchronous mating could initiate divergence. Assortative mating could be the result of other pre-mating barriers. However, experimental manipulation of one species (*Enchenopa* on *Celastrus*) demonstrated that plant-induced differences in life-history timing can result in asynchronous mating through uniform female maturation and asynchronous male mortality. Thus, assortative mating as a consequence of host-plant effects on the timing of egg hatch and development appear to be a mechanism (Wood & Keese, 1990) to minimize the potential effect of recombination.

## **PHYLOGENETIC RELATIONSHIPS**

Consistent yearly patterns of mating asynchrony interacting with host effects on fitness (Wood, 1980; Wood & Guttman, 1982, 1983) appear to be the requisites for divergence. If this hypothesis is correct then phylogenetic patterns should reflect these mechanisms. To attempt a historical reconstruction of divergence, phylogenies were inferred using three independent data sets and techniques. For each of the analyses, *Campylenchia*

*latipes*, the only North American species closely related to the *Enchenopa binotata* complex was used as an outgroup.

Sixteen polymorphic allozyme loci from 3725 *Enchenopa* and 77 *C. latipes* produced essentially the same tree regardless of whether the data were treated as distances (Biosys-1, Swofford & Selander, 1989) or characters (PAUP, Swofford, 1985). The distance Wagner tree (Fig. 2A) places *Enchenopa* from *Robinia*, *Liriodendron* and *Carya* at the base of the tree. *Enchenopa* on *J. nigra* and *J. cinerea* are more derived but placed together. Of these two, the *Enchenopa* on *J. cinerea* is the most derived. The remaining *Enchenopa* form a separate group, with those on *Cercis* and *Viburnum* being more plesiomorphic to those on *Ptelea* and *Celastrus*. Of this group, the *Enchenopa* on *Ptelea* is the most derived. The lack of fixed differences as well as



**Figure 2** Dendrograms suggesting relationships among nine host-plant associated *Enchenopa* species, using *Campylenchia latipes* as an outgroup. In part A the percentages indicate the proportion of the host plant's range occupied by *Enchenopa* on that host.

small genetic distances among the *Enchenopa* species indicates that divergence has been rather recent (Pratt *et al.*, unpublished).

A more traditional phylogenetic analysis (PAUP, Swofford, 1985) using 33 nymphal characters results in a tree (Fig. 2B) very similar to the allozyme derived distance Wagner tree (Fig. 2A). The few differences that do exist are compatible with the genetic distance data and the topology of the distance Wagner tree (Pratt & Wood, in press).

Pronotal colour, shape and size differs among the *Enchenopa* associated with different host plants. Pronotal shape (Fig. 1) can be measured and analysed using canonical discriminate analysis (Wood & Pesek, 1992). The pronota of more than 2400 females were traced with a digitizer. Digitized data ( $X - Y$  coordinates) were used to derive 300 variables that reflect pronotal shape of each individual. Canonical discriminate analysis was used to remove correlations among these variables and to generate a Mahanobis distance matrix for the *Enchenopa* species and *Campylenchia*. The dendrogram (Fig. 2C) from a Fitch-Margoliash analysis (Felsenstein, 1986; Phylip, ver. 2.7) using the female distance matrix, is in general agreement with phylogenies inferred from allozyme and nymphal characters.

Although there are differences in topology, all three trees (Fig. 2A-C) are in general agreement as to which *Enchenopa* are plesiomorphic and which are apomorphic. For the following discussion, the allozyme distance Wagner tree and genetic distances were used to define 12 closely related pairs of *Enchenopa* species (Table 2). Some pairs are not, in a strict cladistic sense (Wiley, 1981), sister species but will be referred to as such in the remainder of this paper.

If speciation of the *Enchenopa* complex was a result of sympatric shifts to phenologically novel plants that imposed differential selection pressures, then closely related *Enchenopa* should be geographically sympatric, differ in critical life-history

**Table 2** 'Sister' pairs of *Enchenopa* species associated with nine species of host plants. 'Sister' species were defined by genetic distance and topology of the Distance Wagner tree (Fig. 2A).

| <i>Enchenopa</i><br>'sister' host-<br>associated<br>species | %<br>Geographic<br>sympatry | Egg<br>hatch | Development<br>time to<br>mating | Asynchronous<br>mating | Diurnal<br>mating<br>preference |
|---|-----------------------------|--------------|----------------------------------|------------------------|---------------------------------|
| <i>Robinia/Liriodendron</i>                                 | 38.6                        | +            | +                                | +                      | ?                               |
| <i>Liriodendron/Carya</i>                                   | 92.4                        | +            | +                                | +                      | ?                               |
| <i>Carya/J. nigra</i>                                       | 29.6                        | +            | ?                                | ?                      | ?                               |
| <i>J. nigra/J. cinerea</i>                                  | 1.5                         | 0            | ?                                | ?                      | ?                               |
| <i>J. nigra/Cercis</i>                                      | 88.5                        | +            | +                                | +                      | +                               |
| <i>Cercis/Viburnum</i>                                      | 75.6                        | +            | 0                                | 0                      | +                               |
| <i>Viburnum/Celastrus</i>                                   | 72.9                        | +            | +                                | +                      | +                               |
| <i>Celastrus/Ptelea</i>                                     | 54.8                        | +            | +                                | +                      | 0                               |
| <i>Viburnum/Ptelea</i>                                      | 63.7                        | +            | +                                | 0                      | +                               |
| <i>Robinia/Cercis</i>                                       | 81.6                        | +            | +                                | +                      | +                               |
| <i>Cercis/Carya</i>   | 17.7                        | +            | ?                                | ?                      | ?                               |
| <i>Viburnum/Liriodendron</i>                                | 39.1                        | +            | -?                               | +                      | ?                               |

Data from Pratt *et al.*, unpublished; Wood & Guttman, 1982, 1985 were used to determine percentage geographic sympatry and indicate statistically significant differences in the timing of egg hatch, development time to mating, asynchronous mating and diurnal mating preference.

+ = significant difference; 0 = no difference; +? = inferred difference; and ? = no available data.

characteristics and in their ability to use the host plant of a 'sister' species. A consistent pattern would indicate that these factors contribute to present-day ecological isolation and could be those that initiated divergence and speciation.

### **Geographic correlates of phylogeny**

Since phytophagous insects are dependent on their host plant, one potential indirect measure of the historical length of the association could be the amount of the host geographic area that is occupied by the insect. A high degree of distributional similarity between both could indicate concomitant historical dispersal, especially into glaciated regions of eastern North America. With two under-sampled *Enchenopa* exceptions (on *Carya* and *Liriodendron*), there appears to be a correspondence between host area occupied and phylogenetic position (Fig. 2A). Generally, more plesiomorphic *Enchenopa* species occupy a larger proportion of their host plant's range than do apomorphic species, suggesting either a long host association or greater dispersal ability. Although this relationship must be considered tentative, pending more sampling, the extensive distribution of both host and its associated *Enchenopa* in glaciated regions indicates colonization during the last 20 000 years. The lack of fixed allelic differences among the *Enchenopa* complex and relatively low host geographic areas occupied by apomorphic species implies that speciation and dispersal could have been recent (Pratt *et al.*, unpublished).

Of the 12 potential 'sister' species pairs listed in Table 2, none presently show classic allopatric geographic distribution. Although secondary contact cannot be excluded, seven of the pairs share 54.8 – 92.4% of the geographic range and must be considered sympatric. Three pairs share 29.6 – 39.1% of their geographic range, however these 'sister' pairs involve *Enchenopa* on either *Carya* or *Liriodendron* where adequate distributional data are not available. At this point I would consider these pairs sympatric. Only two pairs suggest parapatric distributions in that they share less than 17.7% of the geographic area at the extreme of the 'sister' species distribution.

### **Life-history timing, reproductive isolating mechanisms and fitness correlates**

The plant phenology hypothesis suggests that divergence was initiated by host characteristics which disrupted mating synchrony. Phylogenetic support for the hypothesis would be life-history differences between sympatric 'sister' *Enchenopa* species. Of the 12 pairs where adequate experimental data are available, there is a remarkable congruence with this prediction (Table 2). Ten of the 12 pairs differ in the timing of egg hatch and 8 of 9 differ in the length of development to mating. Seven of 9 are asynchronous in mating and the two pairs that are not, differ in the time of day they mate (morning v. evening).

If there was selection for host-adapted genotypes and speciation was recent, then latent genetic variation in the ability of extant *Enchenopa* to utilize different host species could reflect the sequence of host shifts suggested by their phylogeny. When first instar nymphs from each of six *Enchenopa* species were transferred to non-natal host plants (Wood & Datz, unpublished), *Ptelea* and *Juglans* were completely unacceptable hosts (100% mortality) for all *Enchenopa* nymphs except those native to them. Although mortality varied, all *Enchenopa* nymphs survived on the remaining

four host species. *Robinia* is the most acceptable host plant for all *Enchenopa* nymphs regardless of their host origin, since they have the same or higher survival compared to that on their natal host. The remaining host species (*Celastrus*, *Viburnum* and *Cercis*) had more variable effects on nymphal survival of *Enchenopa* from different host-plant species. In general, nymphal survival was equal to or lower than that on the natal host species (Wood & Datz, unpublished).

Survival to reproduction and the ability to oviposit is the most critical step in promoting successful host-plant shifts. Of the 30 non-natal transfer combinations, only 8 had females that survived to oviposit (Table 3). Five of these combinations were *Enchenopa* transferred to *Robinia*. In all but one of the 8 combinations, the number of egg masses deposited per female was substantially lower than comparable females raised on their natal host. The lower number of egg masses represents an interaction between lower survival during oviposition and a reduction in fecundity. In two combinations, where sufficient egg masses were deposited to permit comparison, the number of eggs in each mass was substantially lower than those from females raised on their natal host (Wood & Datz, unpublished).

Of the six *Enchenopa* studied, non-natal host-plant effects on survival, reproduction and fecundity are reflected by their phylogenetic position. The host of the most plesiomorphic *Enchenopa* is *Robinia* where all *Enchenopa* survived to reproduce, although fecundity was lower than on their respective natal hosts. At the opposite extreme, the most apomorphic *Enchenopa* are on *Ptelea* and *J. nigra*. These hosts resulted in 100% nymphal mortality for all non-natal *Enchenopa*. *Enchenopa* from *Celastrus* lived to reproduce on *Viburnum* and *Cercis*, while those on *Ptelea* lived to reproduce only on *Cercis*. In both of these cases, reduced but successful reproduction occurred on hosts with more plesiomorphic *Enchenopa* (Wood & Datz, unpublished). The pattern described here is consistent with the Futuyama & McCafferty (1990) hypothesis which suggests host shifts should occur from chemically or nutritionally less difficult hosts to those that are more difficult.

### EXPERIMENTAL HOST-PLANT SHIFTS

Survival of extant *Enchenopa* species to reproduction on non-natal hosts, and the isolating mechanisms discussed above are compatible with the hypothesized phylogeny.

**Table 3** The number of egg masses per female for females that survived from the first instar to reproduction on their natal and non-natal hosts. Zeros indicate either no survival to reproduction or failure to deposit egg masses.

| Original host    | Transferred to: |               |                |                 |               |                  |
|------------------|-----------------|---------------|----------------|-----------------|---------------|------------------|
|                  | <i>Robinia</i>  | <i>Cercis</i> | <i>Juglans</i> | <i>Viburnum</i> | <i>Ptelea</i> | <i>Celastrus</i> |
| <i>Robinia</i>   | 4.9             | 0             | 0              | 0               | 0             | 0                |
| <i>Cercis</i>    | 0.8             | 5.9           | 0              | 0               | 0             | 0                |
| <i>J. nigra</i>  | 2.2             | 0             | 4.3            | 0               | 0             | 0                |
| <i>Viburnum</i>  | 0.4             | 0             | 0              | 4.5             | 0             | 0                |
| <i>Ptelea</i>    | 1.8             | 0.5           | 0              | 0               | 24.0          | 0                |
| <i>Celastrus</i> | 0.9             | 8.5           | 0              | 1.3             | 0             | 5.2              |

Overall, the pattern suggests that speciation in this complex of treehoppers was initiated through host shifts to phenologically different host plants that altered life-history timing and imposed selection favouring extant genotypes capable of surviving and reproducing on novel hosts. Although secondary contact cannot be excluded, the geographic distributions of 'sister' taxa provide no evidence to suggest classic allopatry, supporting the hypothesis that speciation could have occurred in sympatry or parapatry.

The plant phenology hypothesis is parsimonious, in that oviposition mistakes on phenologically novel host plants should sufficiently disrupt life-history timing to allow for assortative mating. Host effects on development time, survival and fecundity should favour host adaptive traits and host preference. Direct evidence for the hypothesis would be to induce experimentally sympatric shifts to novel host plants that, over time, resulted in life-history asynchrony and detectable genetic divergence in fitness traits. In the following two sections I provide the first experimental evidence for this hypothesis.

### **Oviposition 'mistakes'**

A host shift can only be made through the movement of mated females that make ovipositional mistakes. Such 'mistakes' could be the result of genetic variation in host preference (Bush, 1975), overcrowding or death of a host plant. Sympatric host shift models (Bush, 1975) predict that sufficient genetic variation exists within a population to permit successful colonization of new host species. A corollary is that some genetic traits permit successful colonization on some hosts but not on others. In the initial stage of a host shift, the models predict differential mortality on females during oviposition and their offspring. To test this hypothesis, females collected from a single *Viburnum lentago* population were forced to oviposit on an array of eight novel native and introduced *Viburnum* species and hybrids (Table 4). With the exception of one *Viburnum* species normally utilized in the eastern United States, female mortality was higher during a 7-day period and fecundity lower on novel *Viburnum* than on *V. lentago* (Wood, Pratt & Greene, unpublished).

### **Asynchronous egg hatch, adult maturation and mating**

To test the hypothesis that the phenology of the above eight novel hosts disrupts life-history synchrony of offspring, the timing of egg hatch, adult maturation and mating were determined on these plants grown on the same greenhouse bench. If egg hatch is correlated with plant flowering phenology (as on extant *Enchenopa* host plants), manipulated shifts to *Viburnum* that flower earlier should result in significantly earlier egg hatch. As predicted, the temporal sequence of egg hatch (Table 4) was earlier on the novel plant species than on the parent host (*V. lentago*) which is the last to flower in the spring (Wood *et al.*, unpublished). The chronology of adult maturation did not reflect the temporal pattern of egg hatch (Table 4). Adults on novel hosts matured earlier, at the same time, and later than on *V. lentago*. Thus, the interaction between host effects on the timing of egg hatch and nymphal development time produced novel *Enchenopa* populations that were not synchronized with those on the parent host.

To determine host effects on the chronology of mating, males and females were

**Table 4** Chronological patterns of egg hatch, adult maturation and mating of *Enchenopa* on nine species or hybrids of *Viburnum*.

| Host                        | Chronological pattern of |                                  |                                   |
|-----------------------------|--------------------------|----------------------------------|-----------------------------------|
|                             | Egg hatch<br>± S.E.      | Adult maturation<br>± S.E.       | Mating<br>± S.E.                  |
| <i>V. rhytidophyllum</i>    | 7.2 ± 0.2<br>(21)        |                                  |                                   |
| <i>V. opulus</i>            | 7.2 ± 0.1<br>(280)       | 57.0 ± 0.5<br>(51)               | 107.0 ± 0.0<br>(1)                |
| <i>V. dentatum</i>          | 7.3 ± 0.9<br>(58)        | 51.4 ± 0.4 <sup>a</sup><br>(66)  | 105.0 ± 5.9 <sup>a</sup><br>(10)  |
| <i>V. utile</i>             | 7.5 ± 0.1<br>(199)       | 48.9 ± 0.2<br>(252)              | 91.4 ± 1.2<br>(52)                |
| <i>V. burkwoodii</i>        | 7.5 ± 0.1<br>(260)       | 52.1 ± 0.3<br>(154)              | 95.3 ± 1.5<br>(44)                |
| <i>V. lantana</i>           | 7.5 ± 0.1<br>(693)       | 54.4 ± 0.1<br>(891)              | 109.7 ± 0.8<br>(245)              |
| <i>V. rhytidophylloides</i> | 8.0 ± 0.1<br>(260)       | 53.7 ± 0.5<br>(30)               | 99.7 ± 2.4 <sup>a</sup><br>(12)   |
| <i>V. prunifolium</i>       | 9.2 ± 0.0<br>(1179)      | 53.7 ± 0.1<br>(1835)             | 104.9 ± 0.6 <sup>a</sup><br>(512) |
| <i>V. lentago</i>           | 9.9 ± 0.1<br>(425)       | 51.2 ± 0.1 <sup>a</sup><br>(488) | 104.1 ± 1.2 <sup>a</sup><br>(100) |

A Kolmogorov-Smirnov two-sample test and a 't'-test were used to determine if the frequency distributions and the means on novel hosts differed from those on *V. lentago*. Both tests provided identical results and the same superscripts indicate no difference ( $P < 0.05$ ) from that on *V. lentago*. Numbers in parentheses indicate number of individuals. Low numbers of matings on *V. opulus* precluded comparison.

confined to cages on their new host. The chronology of mating generally reflected that of adult maturation. The mean day of mating (Table 4) on two novel hosts was earlier than on *V. lentago*. The 12.5- and 8.8-day differences between the mean day of mating on these hosts and *V. lentago* demonstrates that plant phenologies promoting early egg hatch combined with minimal host effects on development time do result in early asynchronous mating. The mean day of mating on three novel hosts did not differ from that on *V. lentago*. Although *Enchenopa* on these hosts hatched earlier, host-plant effects on development time brought them into mating synchrony. On one novel host the mean day of mating was later than *V. lentago*. The 5.6-day difference between the means was the result of increased nymphal maturation time. The 18.3-day difference between the mean day of mating on two novel hosts represented almost complete allochry in mating. The magnitude of this difference attests to the power of interacting effects from plant phenology and apparent nutritional quality, causing asynchrony in the life histories of these sap-feeding insects in only one generation (Wood *et al.*, unpublished).

When *Enchenopa* on all novel hosts were compared to those on *V. lentago* to determine if the proportion of observed matings differed, all differed. Although the occurrence of unobserved matings cannot be excluded, it is possible in the early

stage of a host shift that a significant proportion of females go unmated. Unmated females may be those that were not capable of obtaining sufficient nutrition to mature sexually. If confirmed, unmated females, in addition to mortality, represent a powerful selective pressure during the first generation of a host shift (Wood *et al.*, unpublished).

As expected, some novel hosts imposed high mortality on nymphs, with extinction occurring on one novel host 15 days after egg hatch. During the mating period, most novel hosts had no effect on mortality compared to that on *V. lentago*. On one novel host, male mortality was lower while on two it was higher. Female mortality on two hosts was higher than on *V. lentago*. Shortly after mating was completed an additional extinction occurred. The lifetime survival distributions of adult males differed from that on *V. lentago* on all but two novel hosts. On all but one novel host female lifetime survival distributions differed from *V. lentago* (Wood *et al.*, unpublished).

The number of eggs in each egg mass was greater on three novel hosts than on *V. lentago*. Only *Enchenopa* on one novel host did not differ from *V. lentago*. Although individual clutch size appears to be the same or higher on novel hosts, overall lifetime fecundity of these populations was lower than on *V. lentago*. On two novel hosts the number of egg masses deposited was extremely low (there were not enough for destructive sampling), suggesting only a small proportion of females on these hosts were capable of egg production (Wood *et al.*, unpublished).

This experiment demonstrates that the essential predictions of the intrinsic and plant phenological hypotheses of sympatric host shifts were met by the *Enchenopa* system. Forced oviposition 'mistakes' were costly in terms of initial female mortality and fecundity. The life-history synchrony of offspring was disrupted through the interaction of host effects on the timing of egg hatch and development time, resulting in asynchronous mating on some novel hosts relative to the parent population, and there were also fitness effects (Wood *et al.*, unpublished). Thus the original parent population of *Enchenopa* on *V. lentago* contained sufficient genetic variation to permit successful colonization of novel host species.

Although mating synchrony was disrupted, the question of how effective asynchronous mating is as a pre-mating barrier to dispersing males and females remains to be determined for these *Viburnum Enchenopa*. Other studies (Wood & Keese, 1990) suggest that, in the absence of spatial and genetic considerations, assortative mating should occur between some combinations of these experimentally created *Viburnum* asynchronous host 'races'. The ultimate rate of sympatric divergence of these 'races' should be a function of the degree of mating asynchrony, the intensity of selection, genetically based behavioural tendencies toward philopatry (Wood & Guttman, 1981; Guttman *et al.*, 1989; Wood, 1982) and microhabitat influences on gene flow between *Enchenopa* on parent and novel hosts.

## SUMMARY

Although far more and better evidence for sympatric speciation has been produced since the early critiques of Mayr (1947, 1963), an unequivocal case for sympatric speciation among extant species is difficult to make. In reality, as with most speciation models, nobody will know whether the extant *Enchenopa binotata* complex actually speciated in sympatry. The best that can be done is to demonstrate a plausible mechanism by which populations can differentiate in sympatry (Futuyma & Mayer,



1980; Butlin, 1987). In my estimation, the present data for the *Enchenopa* complex are more consistent with a sympatric model than any variant of allopatric models. My interpretation of the existing *Enchenopa* data is that, if sufficient genetic variation exists within a population to permit successful colonization of novel hosts, partial or complete mating asynchrony can be induced extrinsically through shifts to phenologically novel plants. Life-history asynchrony then permits genetic divergence to occur through selection pressures imposed by novel host-plant species. If novel hosts impose high fitness costs, as with extant *Enchenopa*, divergence will be rapid and selection for traits associated with host preference intense. Ultimately, only long-term experiments using the *Enchenopa* on novel *Viburnum* species can test stringently whether or not sympatric host shifts can lead to sufficient genetic differentiation for biological species formation. Only this kind of documentation will resolve the debate for the *Enchenopa* complex.

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