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A phylogenetic analysis of the *Enchenopa binotata* species complex (Homoptera: Membracidae) using nymphal characters

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Abstract. Nymphal characters which distinguish the nine species of the *Enchenopa binotata* complex (key included) are delineated for future formal species descriptions. A phylogenetic tree using these characters is presented and compared to a tree derived from allozyme data. Three pairs of sister species are defined, two of these pairs meeting strict phylogenetic and biological criteria which suggest sympatric speciation through host shifts.

Introduction

The *Enchenopa binotata* (Say) complex of univoltine North American treehoppers consists of an array of nine 'biological species' associated with eight genera of deciduous host plants. These nine species presently are all considered *E.binotata* (Metcalf & Wade, 1965). Although there are two other available names which have been synonymized under *Enchenopa binotata*, *E.brevis* (Walker) and *E. porrecta* Buckton, because of the lack of host plant data and description of the associated nymphs in the original descriptions, and the lack of discrete adult characters, none of these names can at present be assigned to any of the nine species (Say, 1824; Walker, 1851; Buckton, 1903).

Allozyme analyses support the 'biological species' status of these nine species (Wood & Guttman, 1981, 1982, 1983, 1985; Guttman *et al.*, 1981, 1989; Guttman & Weight, 1989; Wood, 1992; Pratt *et al.*, 1992). Reproductive isolation of this complex is related to seasonal and diurnal differences in mating initiated by host plant phenological differences that influence the timing of egg hatch in the spring (Wood, 1980; Wood & Guttman, 1982, 1983; Wood & Keese, 1990; Wood *et al.*, 1990). The intimate relationship between host plant phenology and life history timing results in assortative mating of *Enchenopa* on different host plant species, which has been suggested as a mechanism that could have facilitated speciation in sympatry (Wood *et al.*, 1990; Wood & Keese, 1990). However, the lack of a phylogenetic hypothesis precludes a historical perspective as to the mode of speciation.

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The general lack of discrete characters for the species of the *Enchenopa binotata* complex have precluded traditional phylogenetic analyses and inhibited formal species descriptions. Male and female genitalia exhibit as many differences within species as between species (Pratt & Wood, in prep.), allozyme variation consists of frequency differences (Pratt *et al.*, 1992) and adult pronotal shape variation can only be measured by a suite of continuous measurements (Wood *et al.*, in prep.).

Although an allozyme derived phylogeny gives some insight into the mode of speciation in *Enchenopa* (Pratt *et al.*, 1992), there is considerable disagreement in the systematic community as to whether phylogenetic analyses based on allozyme data are reflective of evolutionary processes (Swofford & Olsen, 1990). Most of the phylogenies from allozyme data have been constructed from matrices of various genetic distances, using either cluster of additive tree methods. Unfortunately the different choices of genetic distances can give rather divergent phylogenies.

Mickevich & Johnson (1976) argued that the loss or gain of alleles are more important in the historical processes of evolution than are the frequencies of alleles, from which genetic distances are derived. They argued that the frequencies of alleles can change easily by drift and/or selection. On the other hand, allozyme analyses are also subject to sample size effects (Swofford & Berlocher, 1987), and unless sample sizes are exceedingly large, there is a high probability of coding a taxon as 'fixed' if a second allele is rare. It is therefore desirable to perform a second independent analysis which should support the phylogeny constructed from genetic distance data to determine if

the genetic distance phylogeny represents the historical evolution of the complex.

Although the differences in adult morphology between the different species of the *E.binotata* complex are difficult to code by traditonal methods for identification and phylogenetic analyses, last instar nymphs differ in coloration (Wood, 1980), which suggested that there may be other nymphal characteristics that are diagnostic of the species of the *Enchenopa binotata* complex. In this study, we ask whether nymphs of the nine *Enchenopa* species have characters, for future formal species descriptions, which permit (1) identification and (2) construction of a phylogeny.

Methods

First (three or more) and fifth (five or more) instar *Enchenopa* nymphs from each of the following host plants were examined: *Ptelea trifoliata* (L.), Wooster, Wayne Co., Ohio; *Cercis canadensis* (L.), Wooster, Wayne Co., Ohio; *Juglans nigra* (L.), Newark, Newcastle Co., Delaware; and Ithaca, Tompkins Co., New York; *Juglans cinerea* (L.), Ithaca, Tompkins Co., New York; *Robinia pseudoacacia* L., Harrisburg, Dauphin Co., Pennsylvania; *Celastrus scandens* (L.), Fair Hill, Cecil Co., Maryland; *Viburnum lentago* L., Winchester, Frederick Co., Virginia; *Liriodendron tulipifera* (L.), Newark, Newcastle Co., Delaware; and *Carya ovalis* (Wang); Sarg., Newark, Newcastle Co., Delaware. The *Enchenopa* species which use *Viburnum* and *Carya* as hosts have been found on other plant species of their respective genera, whereas the others appear to be monophagous. As an outgroup we used first and fifth instar nymphs of *Campylenchia latipes* (Say), the only closely related North American genus to *Enchenopa* (Metcalf & Wade, 1965), from Little Orleans, Allegany Co., Maryland (*Solidago*, Asteraceae as a host).

After examination of first (Fig. 1) and fifth instar (Fig. 2) nymphs of each species, thirty-one characters were coded (listed in Table 1). The discrete characters were coded as either present or absent, or either like a character or not. The continuous measurements were measured under a binocular dissecting microscope with an ocular micrometer that was calibrated with a ruler. These measurements and ratios were gap-coded and assigned codes as in Table 1. Each character was coded in a multistate condition and analysed using PAUP, version 2.4 (Swofford, 1985). The MULPARS option with both local and global branch swapping were used in separate analyses. Since all of the characters which had more than three states were continuous the WEIGHTS SCALE option was chosen. Four characters (indicated in Table 1) could not be ordered and were treated as unordered.

Results

All nine species of the *Enchenopa binotata* complex can be distinguished by fifth instar (see key) and to some extent first instar nymphal characters (Tables 1 and 2).

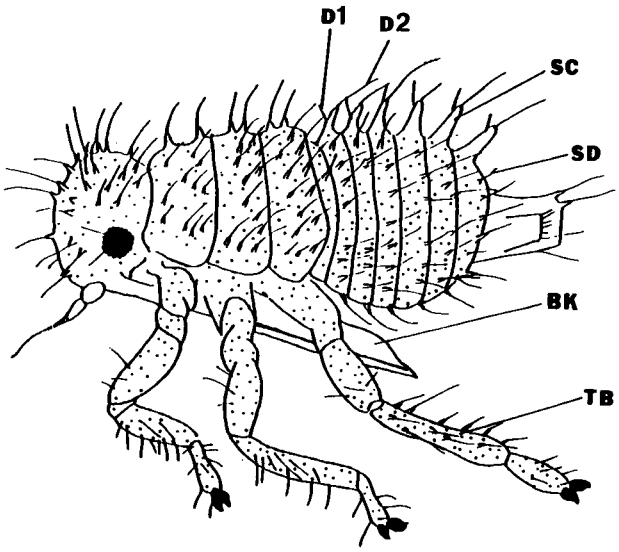

Fig. 1. An illustration of a first instar nymph of the *Enchenopa binotata* complex (host *Viburnum lentago*) showing the first instar characters used in the phylogenetic analysis. These characters are as follows: D1 = the D1 seta on the dorsal scolus of A3 (third abdominal segment); D2 = the D2 seta; SC = the dorsal scolus on A7; SD = the subdorsal seta on A8; BK = the beak; and TB = the tibia of T3.

The characters which separate the *Enchenopa* species on the different hosts do not involve major morphological changes, such as the presence or absence of morphological structures as seen between the outgroup *Campylenchia latipes* and the *E.binotata* complex, but rather continuous characters as seen between very closely related taxa: differences in colour, colour pattern, lengths of structures, etc. Although these characters are continuous, it suggests that they could be used for a phylogenetic analysis of the species complex.

From an analysis of nymphal characters using PAUP only one phylogenetic tree (Fig. 3A) was produced using both local and global branch swapping. The consistency index of that phylogenetic tree was 0.574. The phylogenetic analysis suggests that the species on *Robinia pseudoacacia* is the most plesiomorphic and from a closely related ancestor there followed two major branches in the evolution of the *E.binotata* complex. The members of the first branch have closely related hosts in the Juglandaceae, whereas the members of the second branch have hosts in five different plant families (as well as five different orders): *Cercis*–Caesalpiniaceae (Rosales), *Viburnum*–Caprifoliaceae (Dipsacales), *Ptelea*–Rutaceae (Sapindales), *Celastrus*–Celastraceae (Celastrales) and *Liriodendron*–Magnoliaceae (Ranales) (Cronquist, 1981).

This evolutionary arrangement of the species is supported by the characteristics of the egg froth placed over the egg masses (an adult character not included in the analysis). The members of the complex which use *Liriodendron*, *Viburnum*, *Cercis*, *Ptelea* and *Celastrus* (a monophyletic group according to the phylogenetic tree) all produce a

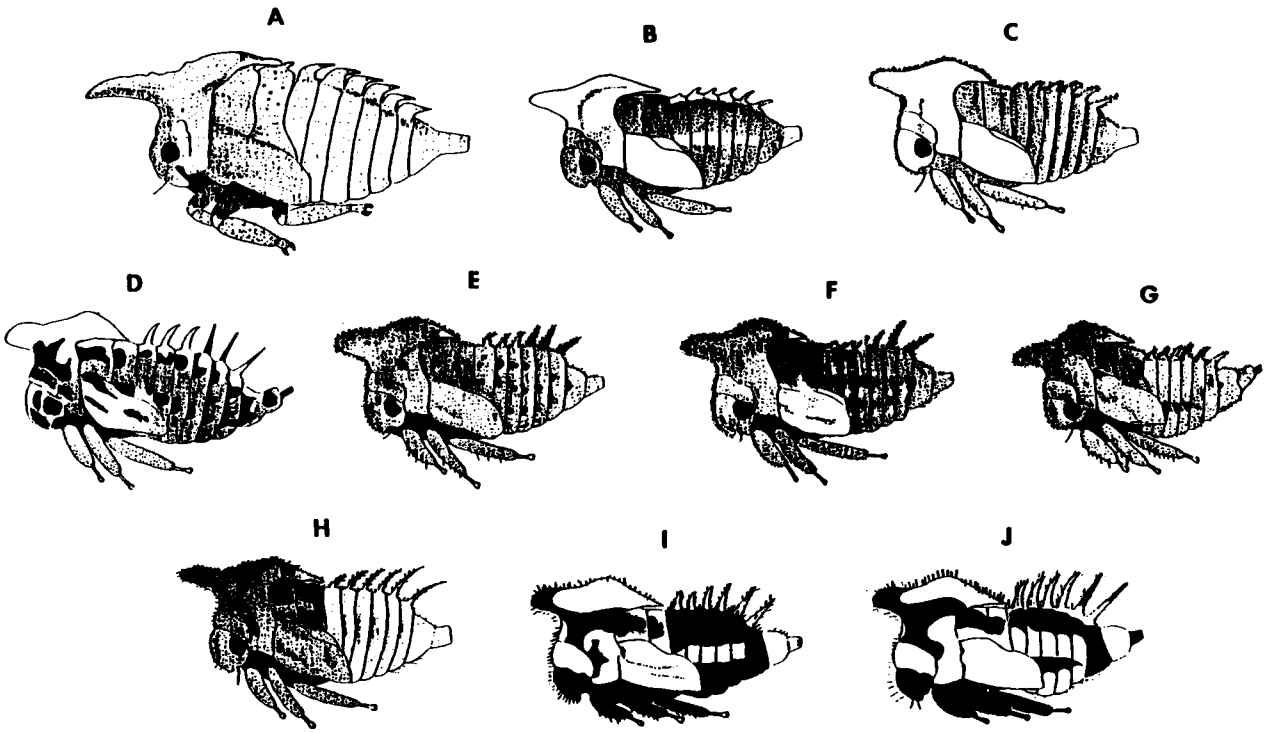

Fig. 2. The fifth instar nymphs are as follows: A = *Campylenchia latipes*, B = host *Robinia pseudoacacia*, C = host *Liriodendron tulipifera*, D = host *Ptelea trifoliata*, E = host *Celastrus scandens*, F = host *Viburnum lentago*, G = host *Cercis canadensis*, H = host *Carya ovalis*, I = host *Juglans cinerea*, and J = host *Juglans nigra*.

round-shaped egg froth, whereas the three which use species of Juglandaceae (which form another monophyletic group) all produce an elongate oval-shaped egg froth, and the species on *Robinia* produces a narrow elongate shaped egg froth, somewhat like the outgroup, *Campylenchia latipes* (Fig. 3A).

Discussion

The characters, such as colour, lengths of structures, etc., (Table 1) which discriminate the sympatric *Enchenopa* species are similar to those used to separate geographic races of other insects (Arnold, 1985). The resulting phylogenetic tree using these characters (Fig. 3A) is similar to a Distance Wagner Tree (Rogers Distances) based on allozymes (Fig. 3B) (Wood, 1992; Pratt *et al.*, 1992). The major similarities between the trees are: (1) the three members of the *Enchenopa binotata* complex on *Robinia pseudoacacia*, *Liriodendron tulipifera* and *Carya ovalis* are the most primitive; (2) the four on *Cercis canadensis*, *Viburnum lentago*, *Celastrus scandens* and *Ptelea trifoliata* cluster together; and (3) the two species which use *Juglans* sp. cluster together.

The differences between the two phylogenies are in the exact placements of the species which use *Carya ovalis*, *Liriodendron tulipifera* and *Cercis canadensis*. These differences are not inconsistent with the Rogers and Nei distance matrices. In the nymphal phylogeny (Fig. 3A) the *Enchenopa* species which uses hickory (*Carya*) as a host appears to be the sister group of the two species which use *Juglans* as hosts, whereas in the allozyme phylogeny (Fig. 3B) it does not appear to be more closely related to that group than to any other species of the *E. binotata* complex. According to both Rogers and Nei genetic distances, the closest species to the sister species which use *Juglans* as a host (other than each other) is the species on *Carya* (Pratt *et al.*, 1992). This relationship is also supported by their similarities in egg froth shape (an adult character) (Fig. 3A).

The species which uses *Liriodendron* as a host, in the nymphal phylogeny (Fig. 3A), because of topology and branch lengths, appears to be ancestral to the other species which produce a round-shaped egg froth over their egg masses. This ancestral distinction is not made in the allozyme analysis (Fig. 3B), yet the pair which use *Viburnum* and *Liriodendron* as hosts have the smallest Nei and second smallest Rogers distances of the paired comparisons among the nine species. Also the allozyme phylogeny does not show the species which use *Cercis* and *Viburnum* as hosts as sister species as does the nymphal phylogeny. Of the eight genetic distances between *Cercis* and other species of the complex, the species that uses *Viburnum* as a host is closest in Rogers distance and second closest in Nei distance.

Table 1. List of nymphal characters used for the phylogenetic analysis.

First instar characters	
(1)	A3 scolus length, 1 = 0.05, 2 = 0.06, 3 = 0.07, 4 = 0.08
(2)	A3 D2 seta length, 1 = 0.05, 2 = 0.10, 3 = 0.15 4 = 0.20–0.22, 5 = 0.27
(3)	A3 D1 seta, absent = 0, present = 1
(4)	A8 scolus length, 1 = 0.05, 2 = 0.08, 3 = 0.09, 4 = 0.10, 5 = 0.12, 6 = 0.15
(5)	A8 subdorsal setae length, 1 = 0.03, 2 = 0.08, 3 = 0.13, 4 = 0.15, 5 = 0.17, 6 = 0.18, 7 = 0.20
(6)	T3 tibia length, 1 = 0.30, 2 = 0.35–0.36, 3 = 0.40, 4 = 0.45
(7)	Beak length, 1 = 0.18, 2 = 0.22–0.23
Fifth instar characters	
(8)	Abdomen, concolorous green = 1 or not concolorous green = 2
(9)	White midline stripe, absent = 0, present = 1
(10)	Dorsal and ventral band, absent = 0, present = 1
(11)	No. rows discontinuous black blotches, 0 = 0, 1 = 1, 1, 2 = 2, 3 = 3
(12)	Bands, distinct = 1, faint = 2, absent = 3
(13)	Last 2 scoli shape, clawlike = 1, not clawlike = 2
*(14)	Wing pad coloration, brown = 1, white = 2, black and white = 3
(15)	A3 scolus, length/width, 1 = 0.5, 2 = 1, 3 = 2, 4 = 3, 5 = 4
(16)	A4 scolus, length/width, 1 = 1.5, 2 = 3, 3 = 4.5–5, 4 = 7
(17)	A5 scolus, length/width, 1 = 1, 2 = 2, 3 = 4–4.5, 4 = 8
(18)	A6 scolus, length/width, 1 = 1, 2 = 2, 3 = 3, 4 = 4, 5 = 5
(19)	A7 scolus, length/width, 1 = 2–3, 2 = 4.5–5, 3 = 6–6.7, 4 = 8
(20)	A8 scolus, length/width, 1 = 3–3.3, 2 = 4–4.5, 3 = 5–5.2, 4 = 7.5–8
*(21)	Head pigmentation, green = 1, brown = 2, black blotches = 3, black = 4
*(22)	Dorsal horn colour, brown–grey = 1, white–cream = 2, white–black = 3
*(23)	Lateral concolour prothorax, brown = 1, white–green = 2, white = 3, black–green = 4, black–white = 5
(24)	Scolus A7 length, 1 = 0.20, 2 = 0.30–0.38, 3 = 0.45–0.50, 4 = 0.55–0.65, 5 = 0.70–0.80
(25)	Scolus A8 length, 1 = 0.25, 2 = 0.30–0.40, 3 = 0.50–0.60, 4 = 0.65–0.75
(26)	T2 scolus absent = 0 or present = 1
(27)	A6 scolus, dark = 1, light = 0
(28)	Setae, reclinate = 1, not reclinate = 0
(29)	Setae, curly = 1, not curly = 0
(30)	Dorsal setae length, 1 = >0.03, 2 = 0.05, 3 = 0.10, 4 = 0.15–0.20
(31)	Horn width, 1 = 1.29–1.30, 2 = 1.40–1.58, 3 = 1.82–3.0

* Unordered characters.
The units for all continuous characters, except for ratios, are in mm.

Table 2. Table of character scores.

	Character no.*																														
Genus/host	First instar							Fifth instar																								
Enchenopa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Robinia	1	4	1	2	6	2	1	1	1	0	0	3	2	2	1	1	1	1	2	3	1	2	2	1	1	0	2	2	1	2	2
Cercis	3	5	1	4	3	2	1	2	0	0	1	2	2	1	3	2	2	3	2	2	2	1	1	2	2	0	3	2	1	4	3
J.nigra	4	4	1	6	7	1	2	2	1	1	0	3	2	2	5	3	5	6	5	6	4	3	5	5	4	0	1	1	2	4	2
Ptelea	1	2	1	3	2	1	2	2	0	0	2	1	2	3	4	4	4	4	5	5	3	2	4	3	3	0	3	2	2	4	2
Viburnum	2	4	1	3	5	1	1	2	0	0	2	2	2	1	1	1	1	3	4	4	2	1	1	2	2	0	3	2	2	4	2
Celastrus	1	4	1	4	7	2	2	2	0	0	3	2	2	1	4	3	3	4	3	3	2	1	1	2	2	0	3	2	2	4	1
J.cinerea	3	3	1	5	5	3	2	2	1	1	0	3	2	2	3	2	4	5	3	4	4	3	5	4	3	0	1	1	1	4	1
Liriodendron	2	3	1	3	4	4	2	1	0	0	0	3	2	2	2	2	2	3	3	4	1	2	3	3	3	0	2	2	2	4	2
Carya	4	5	1	5	7	2	2	1	0	0	0	3	2	1	6	5	6	5	6	7	2	1	1	4	3	0	2	2	1	3	3
Campylenchia																															
Solidago	1	1	0	1	1	1	1	1	0	0	1	2	1	1	3	1	2	2	1	1	2	1	1	4	3	1	2	2	1	1	3

* Characters are listed in Table 1.

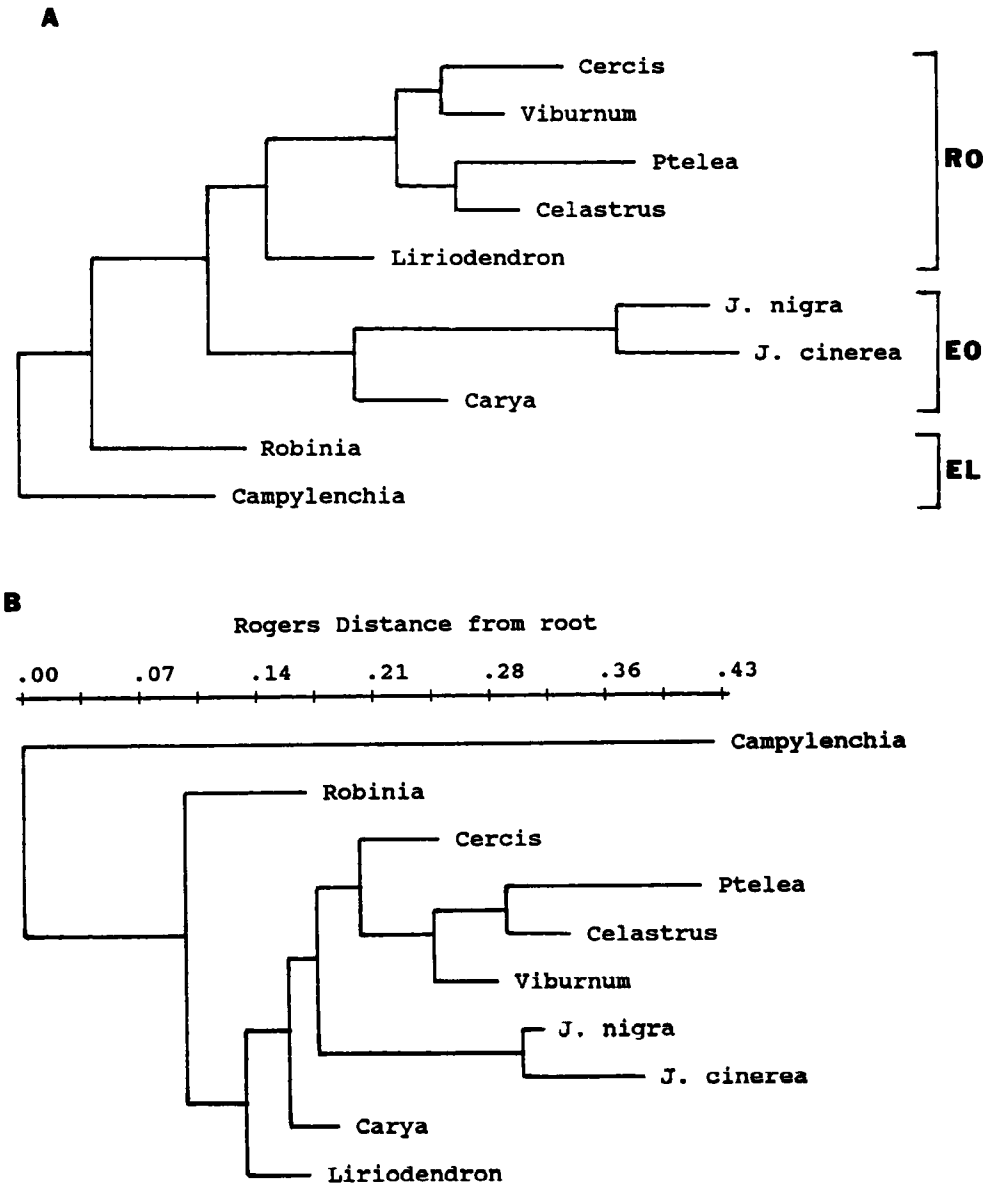

Fig. 3. (A) Phylogenetic tree using nymphal characters of the species of the *Enchenopa binotata* complex, labelled by their host plants, using *Campylenchia latipes* as an outgroup. The characters are shown in Table 1 and scored as in Table 2. The species form three groups according to the shape of the egg froth overlaying the egg mass. These groups are as follows: RO = round shaped egg froth; EO = elongate oval shaped egg froth; and EL = elongate shaped egg froth. (B) A distance Wagner tree with Rogers distances (from allozyme data) of the species of the *Enchenopa binotata* complex, using *Campylenchia latipes* as an outgroup (from Wood, 1992, and Pratt *et al.*, 1992).

The phylogenetic tree suggests there are three sister pairs in the *Enchenopa binotata* complex: the species on *Viburnum* and *Cercis*; on *Celastrus* and *Ptelea*; and on *Juglans nigra* and *J.cinerea* (Fig. 3A). If the species have historically speciated in sympatry, then those sister species should broadly overlap in geographic range. From present distribution maps, the geographic range overlaps (using Sorenson's Coefficient) for these three pairs are 75.6, 54.8 and 1.5, respectively (Wood, 1992; Pratt *et al.*, 1992). Although the *Enchenopa* on *Juglans nigra* and *J.cinerea* do not exhibit broad overlap in distributions, the *Enchenopa* on *J.cinerea* has a limited distribution which is entirely nested within the range of its sister species.

If sympatric speciation of these sister pairs were due to the host plant phenology hypothesis, as suggested previously, then these pairs of sister species should also differ either in their chronological time to mating or exhibit diurnal differences in mating, which would reproductively isolate them in sympatry. The two pairs on which biological information is available, *Viburnum* and *Cercis*, and *Celastrus* and *Ptelea* (Wood, 1992), fit Wiley's (1981) strict evidence for sympatric speciation. Of these two pairs, the

first differs in the time of day of mating and the second differs in the chronological time to mating (due to the accumulation of seasonal differences in egg hatch and development time to mating). Therefore these two pairs of sister species exhibit biological differences which would reproductively isolate them in sympatry.

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Appendix: fifth instar key

1 Scoli present on mesothorax and metathorax; abdominal scoli only 2 times as long as wide; setae very short and sparse; thorax grey and brown and abdomen green ..... *Campylenchia latipes*

1′ Scoli absent on the mesothorax and metathorax; last abdominal scolus generally 4 times as long as wide; setae variable and not sparse; colour variable .... *Enchenopa* (2)

2 Abdomen with green coloration 3

2′ Abdomen without green; either black, brown, and or grey 6

3 Abdomen mottled with green and black; abdominal scoli long and of subequal lengths (Fig. 2D); setae short less than 0.05 mm long; dorsal horn white and remaining part of pronotum darkly coloured .... on *Ptelea*

3′ Abdomen not mottled with black; abdominal scoli variable generally not of subequal lengths; setae longer than 0.05 mm; when dorsal horn white remaining pronotum also white 4

4 Abdominal scoli greatly reduced as in Fig. 2B; thorax green and white; setae short less than 0.07 mm long; abdomen often with a white stripe ..... on *Robinia*

4′ Abdominal scoli not so reduced as in either Figs 2C or 2H; or setae not so short greater than 0.14 mm long; thorax green or brown; no white stripe present on abdomen 5

5 Thorax brown; wing pad brown; scoli long and narrow as in Fig. 2H ..... on *Carya*

5′ Thorax white or sometimes with green, not brown; scoli generally short and broad as in Fig. 2C; dorsal horn white. ..... on *Liriodendron*

5′′ Dorsal horn not white; body evenly coloured with green, with red markings sometimes apparent ..... on *Viburnum*

6 Abdomen striped with black and white, or brown and white as in Figs 2I and 2J; setae long and straight; scoli long particularly first scolus on A3; dorsal horn white dorso-posteriorly 7

6′ Abdomen not striped with white; colour generally grey or brown not black; setae long and curled; scoli not so long particularly first scolus on A3 (Figs 2E–G); dorsal horn generally not white, but brown, brown-grey, or grey 8

7 First abdominal scolus less than one third the length of the last scolus ..... on *Juglans cinerea*

7′ First abdominal scolus greater than one third the length of the last scolus ..... on *Juglans nigra*

8 Abdomen with a weakly defined dark stripe as in Fig. 2G, not mottled ..... on *Cercis*

8′ Abdomen often mottled with white and brown, no darker stripe visible (Figs 2E and 2F) 9

9 First abdominal scolus highly reduced less than half the length of the last scolus as in Fig. 2F; colour pattern variable, sometimes mottled or sometimes evenly coloured ..... on *Viburnum*

9′ First abdominal scolus generally not so reduced as in Fig. 2E; colour pattern mottled grey-brown ..... on *Celastrus*