1981

Insect Life History Patterns: Habitat and Geographic Variation

Edited by
Robert F. Denno and Hugh Dingle

With 62 Figures

Springer-Verlag
New York Heidelberg Berlin

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Chapter 3

The Role of Host Plants in the Speciation of Treehoppers: An example from the *Enchenopa binotata* Complex

THOMAS K. WOOD and SHELDON I. GUTTMAN

1 Introduction

Recent studies on the origin of insect races and speciation in certain phytophagous insect complexes have forced systematists to revise their ideas concerning modes of animal speciation. Rapid establishment of new races by insects on introduced plants has led some biologists to suggest that races and species may arise sympatrically (Bush 1969, 1975). Since much of the evidence is indirect, however, other workers still regard geographical isolation as a prerequisite for speciation in most groups of sexually reproducing animals (Mayr 1970).

Phytophagous and parasitic insects are particularly suited to sympatric divergence because of specialized feeding habits (Bush 1975). Maynard Smith (1966) showed that in theory disruptive selection operating on genotypes differing in habitat or host selection could lead to sympatric speciation. Allochronic life histories (Alexander and Bigelow 1960, Alexander 1968) or adoption of new host plants (Bush 1969, Huettel and Bush 1972, Knerer and Atwood 1973) have been implicated in the formation of insect races and reproductive isolation. Edmunds and Alstad (1978; Chapter 2) have demonstrated that sessile scale insects adapt to the defense system of individual pine trees. When dispersal abilities are limited and extinction rates high, newly colonized hosts may act as islands, with the insect population on one host plant partially isolated from demes on other adjacent trees (Simberloff 1976). Recently, Tauber and Tauber (1977a, 1977b) and Tauber et al. (1977) suggested a basis for sympatric divergence in a non-host-specific insect through selection for genes controlling diapause. These studies argue that sympatric speciation may have occurred, but, as Bush (1969) suggests, there is still a general paucity of detailed studies.

The Membracid, *Enchenopa binotata* (Fig. 3-1), has been historically considered as a single species. It occurs on a number of different host plants that occur throughout a broad geographical area from eastern North America to as far south as Panama (Metcalf and Wade 1965). Although very little is known about its tropical biology, host plant records indicate that it may be polyphagous in Central America. In Costa Rica, the species has been recorded on 13 host plants belonging to 12 genera in 6 families

(Ballou 1936). The distribution of *E. binotata* in eastern North America closely coincides with that of its 7 primary host plants: *Ptelea trifoliata* L. (hoptree); *Celastrus scandens* L. (bittersweet); *Robinia pseudoacacia* L. (black locust); *Cercis canadensis* L. (redbud); *Juglans nigra* L. (black walnut); *J. cinerea* L. (butternut); and *Viburnum* sp. Although these plants are sympatric over a wide geographic area, they seldom all occur in the same habitat, except where planted together as ornamentals.

Here we review studies showing that *E. binotata* in North America is a complex of reproductively isolated taxa which have diverged along host lines. Our objective in these studies has been to determine how host plants shape the life histories and promote speciation in the *E. binotata* complex. Wood (1980) postulated: (1) that the *E. binotata* progenitor was tropical, multivoltine and polyphagous, (2) that successful colonization of deciduous north temperate hosts resulted in coordination of egg-hatch with host phenology, (3) that the coordination of egg-hatch to host phenology on diverse hosts resulted in allochronic life histories and assortative mating, (4) that assortative mating was facilitated further by low vagility, female monogamy, habitat heterogeneity and ant mutualism, and (5) that the interaction of these factors promoted genetic polymorphism and reproductive isolation.

2 Study Area and Methods

All the studies reported here (electrophoretic studies excepted) were done with *Enchenopa* from Clinton County, Ohio. Our main field study area consisted of a 300 X 180 m site in the city of Wilmington, where treehopper populations on all hosts (except *C. scandens*) have coexisted for at least 10 years. In this area there were 156 individual host trees with branches from several tree species often interdigitating. Tree

Figure 3-1. Female of *Enchenopa binotata* on *Cercis canadensis* covering her egg mass with froth. Eggs are inserted into twigs.

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3 Life History Differe

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heights varied from under 7 m to 24 m depending on the species. Field studies on associated ants and the phenology and vagility of treehoppers were done at this site. Samples for electrophoresis were collected from this area, nearby areas in Clinton County, and also from Wooster and Oxford, Ohio.

Differences among the life histories, host plant selection and mate selection of *Enchenopa* on each host species were studied in a small area (3.6 × 4.8 m) less than 1 km from the main study area. Each host plant species was caged and stocked with field-collected female treehoppers from original host plants. There they were allowed to oviposit. The phenology of egg-hatch on each host was determined the following spring by daily examination. The day eggs first hatched (day 1) was used as a reference for a continuous time scale to compare life history events of *Enchenopa* on the various host plants. Treehopper maturation, adult survival, and the phenology of dispersal, precopulation and copulation were measured by making daily observations over a 102-day period with each day divided into 14 observational periods beginning at 7:30 a.m. and ending at 8:30 p.m. Individual males and females were identified by marking with enamel paint. Analysis of Variance and Duncan's Multiple Range Test were used to compare means.

3 Life History Differences of *Enchenopa* on Host Plants

3.1 Treehopper Coloration and Feeding Site

Coloration of *Enchenopa* adults and nymphs varies with host species. Adult females and males on *R. pseudoacacia* and *Viburnum* are light to dark brown, while on other hosts they are black. Color variation as well as differences in feeding sites among *Enchenopa* on different hosts are most striking in third to fifth instars. Nymphs are black and white on the two *Juglans* species and contrast strikingly with the leaf petiole on which they feed. On *R. pseudoacacia*, nymphs also feed on the petiole but are green. On *Viburnum*, *C. canadensis* and *C. scandens* nymphs are brown to gray, and blend with the twigs of their hosts. Nymphs on *P. trifoliata* feed on twigs, and are a grayish-brown with a yellow stripe on the dorsum (Wood 1980).

3.2 Oviposition Characteristics and Behavior

Females insert their egg masses into the bark of the various host plants. Egg masses contain a mean of 14.5 eggs on *R. pseudoacacia* and 7.0-8.6 eggs on the other hosts (Wood 1980). Following oviposition, egg froth, a white secretion from the ovipositor, is deposited over the mass of eggs (Fig. 3-1). The major component of egg froth is an ether-extractable lipid that comprises from 77.7 to 87.1% of the froth mass. The remainder of the egg froth mass is protein (9.0-21.8%) and carbohydrate (1.5-3.6%). The proportion of these froth components varies and is dependent on the host plant (Wood 1980). In a series of experiments we found that the ether-extractable lipid in the egg froth contained an ovipositional attractant. Once an egg mass was deposited on a branch and covered with egg froth the ovipositional attractant drew other females to the same branch to deposit eggs. As the number of egg masses increased, more and

more females were attracted to the branch. Consequently, egg masses are highly clumped and only a small proportion of the branches on an individual tree will have egg masses, but the ones that do can possess over 100 masses (Wood and Seilkop, unpubl.).

Egg froth deposited by females on different hosts has a distinctive color, shape and amount. When on the two *Juglans* species, the white egg froth shrinks and discolors shortly after deposition, becoming nearly indistinguishable from the host plant. Froth on the remaining host plants retains its color and shape.

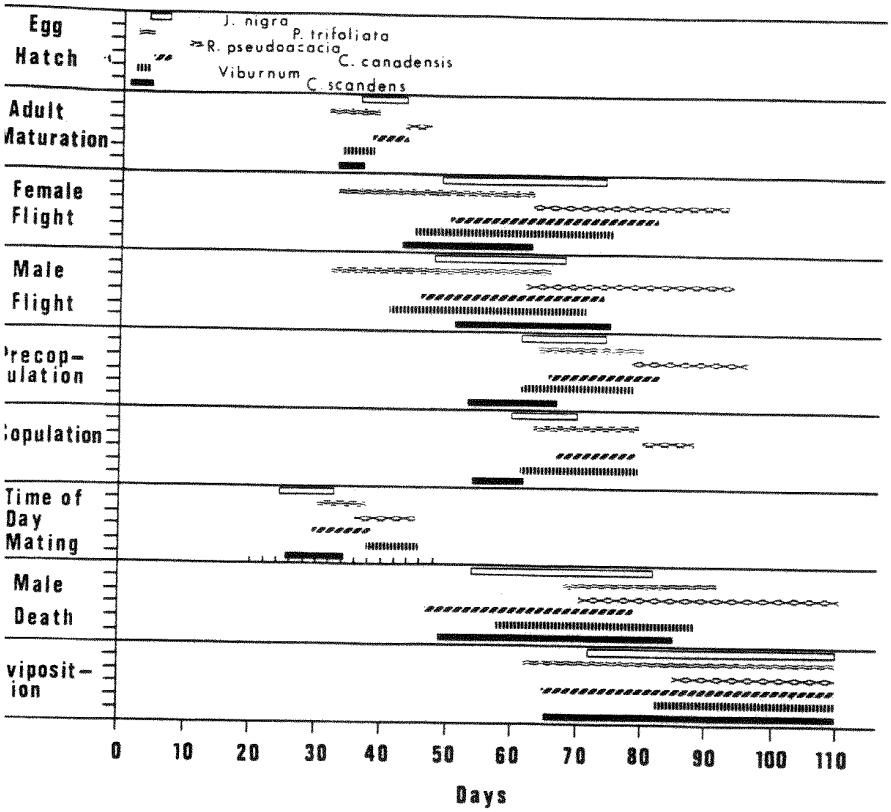
Females on *J. nigra* and *R. pseudoacacia* deposit their eggs in new branches from the current growing season. Females on *P. trifoliata* and *Viburnum* deposit eggs in second-year growth, while those on *C. scandens* oviposit in 1-3-year-old twigs. The most dramatic difference occurs on *C. canadensis*, where females deposit eggs in 2-4-year-old growth. Branch diameter appears to be a critical factor influencing the site of oviposition (distance from terminal bud) as well as egg mass density. For instance, branches chosen for oviposition on *C. canadensis* have significantly greater diameters than those chosen on other hosts. Although egg masses can be found on first-year twigs with diameters similar to those of other host species, small branches suffer high overwinter die back in southern Ohio. The young twig mortality associated with *C. canadensis* has proably selected for oviposition on larger twigs.

Observations of treehoppers in the field and in cages showed that there are seasonal differences in oviposition patterns among females on different hosts (Fig. 3-2). Also, the time of day females deposit eggs varies with host. For instance, females on *P. trifoliata*, *C. canadensis*, *V. opulus* and *C. scandens* deposit the majority of their eggs in the morning, while females on *J. nigra* deposit eggs in the afternoon, and those on *R. pseudoacacia* deposit eggs in the evening (Wood 1980).

3.3 Egg-Hatch and Adult Maturation

The hatching phenology of eggs differed among hosts (Fig. 3-2). Eggs in *P. trifoliata*, *V. opulus* and *C. scandens* always began to hatch before those on *J. nigra*, *C. canadensis* and *R. pseudoacacia*. In some years eggs on *R. pseudoacacia* hatched after egg-hatch was nearly completed on the other host species. Also, the sequencing of egg-hatch on hosts remained consistent over 4 years of observation (Wood and Guttman 1981). Except for *C. canadensis*, eggs hatched on host plants when they were flowering. On *C. canadensis*, eggs hatched after flowering when leaves were fully formed.

In addition to disparate oviposition and hatching phenology, nymphs raised on different hosts molted to adults at different times (Fig. 3-2). Generally, nymphs molted to adults 30 days after egg-hatch and the sequence of adult maturation on host plants paralleled that of egg-hatch. Thus, differences in adult maturation time were largely attributable to staggered egg-hatch rather than inequitable nymphal development rate. Nymphs on *P. trifoliata*, *V. opulus* and *C. scandens* molted to adults first, followed by those on *J. nigra* and *C. canadensis*, and finally by those on *R. pseudoacacia*. Initial sex ratios following molting were 1:1 on all hosts. Thereafter, male survival fell consistently before mating and throughout the mating period. Following mating, only 18% of the initial cohort of males was alive on all hosts. Also, there were temporal differences



gure 3-2. Phenological differences in egg-hatch, adult maturation, female and male spersal (flight), precopulation, copulation, male mortality and oviposition among the ost races of *Enchenopa*. Time scale is referenced to date of first egg-hatch. The time day mating occurs is on a different scale. The day is divided into one-hourly inter- ls beginning at 7:30 a.m. and ending at 9:30 p.m.

male treehopper mortality on the various host plants (Fig. 3-2). On all hosts the mber of females decreased before and during mating, but mortality was not as great that for males. By the end of the mating period, 76% of the female cohort was alive. llowing mating, females lived from 30 to 60 days (Wood and Guttman 1981). mber of females decreased before and during mating, but mortality was not as great that for males. By the end of the mating period, 76% of the female cohort was alive. llowing mating, females lived from 30 to 60 days (Wood and Guttman 1981).

Adult Dispersal

used the number of males and females gathered on the sides of the screen cages as index of dispersal from host plants. Based on this index, adults dispersed from hosts different times (Fig. 3-2), increasing the probability of meeting mates having de-

veloped on the same host. For example, females on all hosts but *J. nigra* and *V. opulus* differed in their times of dispersal. Similarly, males on *R. pseudoacacia* and *P. trifoliata* differed from each other and all other hosts in their dispersal behavior. Males on other hosts were not as clearly separated. Furthermore, treehoppers on different host plants displace one another by dispersing at different times of the day or at different temperatures (see Wood and Guttman 1981).

The majority of all *Enchenopa* were observed on the screens of cages before mating commenced. Three days after mating began, 63.2% of females and 52.2% of the males were recovered on the screens. Thus, a substantial amount of dispersal occurs prior to mating. For example, mating first occurred on *P. trifoliata* on day 55, and at that time, 72.2% of males and 84.5% of females had dispersed to the screen. Following mating and during oviposition, very few males or females dispersed (Wood and Guttman 1981). This experiment fails to differentiate between intra- and interhost dispersal but, as will be pointed out in the section that follows, the majority of dispersal is probably among conspecific hosts.

3.5 Vagility of Treehoppers

We hypothesize that low vagility acts in concert with low levels of intrahost dispersal to minimize contact among populations of *Enchenopa* on different hosts. A mark-recapture study was designed to estimate vagility. Adult *Enchenopa* were collected when the majority of nymphs on a given host species had molted to adults and before much flight activity had taken place. These were color-coded with enamel paint and reestablished on their original host. Counts of marked treehoppers were made daily on the release host and nearby plants for the first 4-5 days. Thereafter, counts were made every 2-3 days through mid-September (Wood and Guttman 1981).

The percentage of males and females recaptured at their site of release decreased rapidly during the first 17 days. During this period 7-63% of 217 released males were recovered on the release hosts and only 1-2% on nonhost trees. This time interval corresponded to the period of high mortality incurred during flight and before mating in the cage experiment. In the field, only a few marked males were observed on *Enchenopa* hosts other than their developmental ones. These males were observed 18.2 m from their release site and the remainder were observed within 4.5 m. By the time mating commenced in the cages, 6% of the released males were recaptured on their original host. After mating was completed on conspecific hosts in cages, very few marked males were observed in the field (Wood and Guttman 1981).

The decline in the number of marked females at release sites in the field also corresponded to the pattern of early mortality and flight activity observed in cages. The number of marked females dropped to zero 31-56 days after release on *J. nigra, C. scandens, C. canadensis* and *R. pseudoacacia.* This was not surprising considering the number of individual conspecific hosts and female mortality. However, observations of marked females on *P. trifoliata* and *V. prunifolium* demonstrated the tendency of females to remain at or near the release site. Between 10 and 13% of 88 marked females were observed on these hosts 43-64 days after release, respectively. If these data are adjusted using mortality figures from cage studies, they suggest that during

s period 35.0-41.4% of the females reared on a given tree remain to deposit eggs. ıen male recaptures are adjusted for mortality the data suggest that 5-20% of the les on a given tree remain to the end of the mating period. These adjusted values conservative, since mortality in the field is probably much greater than in the es. Throughout this study marked *Enchenopa* were observed forming precopulatory i mating pairs on the release tree, and females were also observed depositing eggs. r data suggests that populations of *Enchenopa* exhibit low vagility, and that the dis- sal which does occur is largely intrahost rather than interhost (also see Wood and ttman 1981).

Mating Activity

recognize precopulatory behavior and copulation as two separate activities. Pre- ıulatory behavior consisted of males sitting on females and lasted from a few onds up to two hours. Precopulatory behavior may result in copulation, but males) abandon females and move to others. Precopulatory pair formation provided a asure of when males on different hosts became sexually active.

Precopulatory pairs began to form first during the season on *C. scandens*, followed uccession by those on *P. trifoliata, J. nigra, V. opulus, C. canadensis* and *R. pseudo- cia* (Fig. 3-2). The mean day precopulatory pairs formed on all host plant species ered significantly except for those on *V. opulus* and *J. nigra*. Also, the time of day t pairs formed on *C. scandens* and *J. nigra* was the same as it was for *Enchenopa* on *rifoliata, C. canadensis* and *J. nigra*. However, *Enchenopa* on *V. opulus* and *R. udoacacia* differed from each other and from those on all other hosts in the time lay they formed precopulatory pairs (Wood and Guttman 1981).

Treehoppers on different hosts displaced one another by copulating at different es in the season (Fig. 3-2). The mean day *Enchenopa* copulations took place on *C. ıdens, J. nigra* and *R. pseudoacacia* differed significantly from each other and all er hosts. Mean day of mating for treehoppers on *V. opulus, P. trifoliata* and *C. adensis* did not differ from each other. The time of day copulations occurred her divided *Enchenopa* on some host plants (Fig. 3-2). Treehoppers on *V. opulus R. pseudoacacia* mated in the evening and differed from those on other hosts. ıs, *Enchenopa* on all hosts except *P. trifoliata* and *C. canadensis* mated at either erent times of the season or day (see Wood and Guttman 1981).

Competition for Mates

ıales outnumbered males on all hosts at the end of the mating period. However, ales generally mated only once, while males secured up to four copulations. ıs, as the number of receptive females decreases, competition for mates should ease. A consequence of this might be the dispersal of males from their host in ch of females. However, we did not notice an increase in male flight activity as number of virgins decreased. The lack of male dispersal may be explained by two s: First, male mortality was high at the end of the mating period, reducing the pool

of competing males. Second, males failed to differentiate between virgins and previously mated females. Apparently, male fitness is increased by remaining on hosts where females are already present. Also, temporal differences in male mortality (see Fig. 3-2) may reduce the probability for interhost matings, but these are probably the result of differential selective pressures associated with hosts that place constraints on male development and/or longevity.

4 Host Plant and Mate Selection

4.1 Host Plant Selection

In addition to the disparate life histories that *Enchenopa* obtains on its host plants, features of host plant and mate selection behavior may further promote reproductive isolation among host plant populations. Field observations suggest that females select and oviposit in the host species on which they develop. To test this hypothesis, *Enchenopa* were confined in large cages containing all 7 host plant species. *Enchenopa* from one host were placed on that host in the center of the cage with free access to the other 6 surrounding host plants. *Enchenopa* from all 7 host plants were tested in a similar manner. Males and females dispersed throughout the cages before mating, and considerable interhost movement was observed before oviposition. When females deposited egg masses, they selected their native host. There were very few ovipositional "mistakes" and most of these occurred between the two *Juglans* species. Despite small cages and exaggerated sympatry, it appears that females were able to select and oviposit on the host plant on which they developed (see Wood 1980).

4.2 Mate Selection

Using an experimental design similar to that above, we tested whether *Enchenopa*, when given free access to mates from all host plants, selected mates according to their particular host plant origin. Six of the 7 host species were covered with a single cage. Adult *Enchenopa* from each host plant were field-collected prior to mating and color-coded with enamel paint to indicate host origin. Hourly observations were made from 8:00 a.m. to 8:30 p.m. throughout the mating period. As in the previous experiment, males and females dispersed throughout the cage with considerable interhost movement before mating (Wood 1980, Wood and Guttman 1981).

Before mating occurred in *Enchenopa* there was a period of precopulatory pair formation during which males and females sat next to each other up to two hours. Precopulatory pair formation among males and females from different host plants was common (Wood 1980). In one year, 166 of 288 precopulatory pairs involved partners from the same host plant (Wood and Guttman 1981). These data suggest that males cannot distinguish receptive females on the basis of their host origin. However, of the 128 observed copulations, only 7 involved males and females of mixed plant origin. Several mixed copulations were of shorter duration than normal (< 142 min), suggesting that sperm may not have been transferred (Wood 1980). We conclude that females select males of the same host plant origin with which to mate.

5 Genetic Differentiation among Host Populations of Treehoppers

Determination of intra- and interhost differentiation was done by examining 15 presumptive loci with horizontal starch gel electrophoresis. Of these 15 loci, 7 were polymorphic and used for comparisons. Allele frequencies among *Enchenopa* indicate that all host plants of a given species support populations with identical predominant alleles at a given locus. However, the results of chi-square homogeneity tests demonstrate that significant differences in allele frequencies exist within most *Enchenopa* species. This genetic differentiation occurs at the microgeographic (individual tree) and macrogeographic levels as well as between developmental stages. The degree of differentiation varies depending upon the host population of *Enchenopa* being considered. For example, significant differences in allele frequency existed for only one locus in insects from individual *C. canadensis* in the main study area, while significant differentiation was present at 4 of the 6 variable loci tested in nymphs from *C. scandens* (Guttman et al. 1981).

The magnitude of genetic differentiation among *Enchenopa* host populations (Table 3-1) is much greater than within a host population (Guttman et al. 1981). Treehoppers on *J. nigra* are readily distinguished from the other host populations by the near fixation of a glutamate oxaloacetate transaminase allele. This allele is absent from most treehopper populations on other host plants but, if found, is present in low frequency (0.17 or less). Similarly, those *Enchenopa* populations on *P. trifoliata* are fixed, or nearly so, for a peptidase allele. Others which have this allele are populations on *J. nigra* and *C. canadensis*, where its frequency is 0.16 or less. A catalase allele is fixed in *Enchenopa* on *P. trifoliata*, while it occurs in low frequency in *J. nigra* and *C. canadensis* populations. Treehoppers on *R. pseudoacacia* possess a particular phosphoglucomutase allele in frequencies ranging from 0.62 to 0.75. This allele is absent in most other populations, but when it does occur its frequency does not exceed 0.11 (Guttman et al. 1981).

The statistic $\bar{D}$, as defined by Nei (1972) and modified by Nei (pers. comm.), was used to estimate the genetic distance within and among host populations of treehoppers. If it is assumed that the substitution of electromorphs occurs as a Poisson process, then $\bar{D}$ is the average number of substitutions per locus in the separate evolution of the two populations being compared. A total of 41 populations were analyzed. The mean genetic distance among the combined *Enchenopa* host populations is low ($\bar{D} = 0.11 \pm 0.06$) but is about three times the average distance of *Enchenopa* populations from only *J. nigra* ($\bar{D} = 0.04$), the most variable host population. The $\bar{D}$ between *Enchenopa* from *J. nigra* and the remaining host entities is 0.17 ± 0.05. The value for those from *P. trifoliata* is 0.13 ± 0.04, and the value for those on *R. pseudoacacia* is 0.13 ± 0.07. Treehoppers on *C. canadensis* show smaller distances than those from the previous three hosts. They maintain a $\bar{D}$ of 0.10 ± 0.04 relative to the other entities, and the smallest $\bar{D}$ between *C. canadensis* treehoppers and any other host population is 0.05, suggesting a large degree of genetic distinctness. Biochemically, *Enchenopa* on *Viburnum* and *C. scandens* ($\bar{D} = 0.01$) are very similar, suggesting that differentiation has been minimal (Guttman et al. 1981).

A dendrogram summarizing genetic distances and the history of divergence in the *E. binotata* complex was obtained following cluster analysis of $\bar{D}$ values by the un-

Table 3-1. Mean allele frequencies at 6 gene loci coding for enzymes in fifth-instar nymphs of *Enchenopa* on 6 host plants. All treehoppers were collected within a 10-km area in Ohio

Locus	Allele	*Juglans* (5) N = 150	*Ptelea* (4) N = 158	*Cercis* (4) N = 130	*Viburnum* (4) N = 140	*Robinia* (1) N = 26	*Celastrus* (5) N = 158
Est-1	*a*				0.01		
	b	0.08	0.05	0.05	0.19		0.15
	c	0.67	0.58	0.07	0.29		0.41
	d	0.25	0.18	0.80	0.39	1.0	0.29
	e		0.19	0.08	0.10		0.10
	f				0.02		0.05
Pep-2	*a*	0.15	0.99				
	b	0.53	0.01	0.34			0.34
	c	0.30		0.64	0.98	1.0	0.66
	d	0.01		0.02	0.02		
Pgi-2	*a*	0.20	0.14		0.01		
	b	0.77	0.86	0.98	0.93	0.92	0.98
	c	0.01		0.02	0.06	0.08	0.01
	d	0.01					0.01
Pgm-2	*a*	0.01	0.14	0.02	0.02		0.01
	b				0.01		
	c	0.95	0.86	0.94	0.96	0.35	0.92
	d	0.03		0.02		0.04	0.03
	e	0.01		0.02		0.62	0.04
Cat-1	*a*	0.45		0.02	0.03	0.04	0.03
	b			0.34			
	c	0.46		0.62	0.22		0.25
	d	0.09	1.0	0.02	0.73	0.96	0.70
	e				0.02		0.01
Got-1	*a*						0.02
	b	0.03	0.08	0.08	0.03		0.19
	c		0.83	0.92	0.97	1.0	0.78
	d	0.97	0.09				0.01

In parentheses are the numbers of trees from which samples were taken.
N = number of genomes sampled.
Code for alleles: Est, esterase; Pep, peptidase; Pgi, phosphoglucose isomerase; Pgm, phosphoglucomutase; Cat, catalase; Got, glutamate oxaloacetate transaminase.

weighted pair group method (Tateno, pers. comm.) (Fig. 3-3). The use of such a phenogram in making phylogenic inferences relies upon the assumption that there is a constant rate of gene substitution per unit length of time in all evolutionary branches. The dendrogram indicates that *Enchenopa* on *J. nigra* diverged first, followed by those on

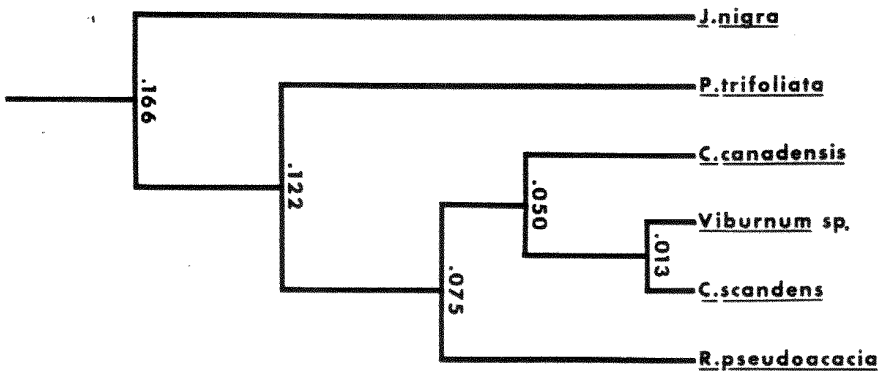


Figure 3-3. Dendrogram showing the relationships among the 6 host races of *Enchenopa* based on genetic distances.

P. trifoliata, R. pseudoacacia and *C. canadensis.* Treehoppers in the *Viburnum-C. scandens* group diverged most recently (Guttman et al. 1981). Finally, we feel that the genetic distances observed suggest that gene flow has been minimal among *Enchenopa* on some hosts and that reproductive isolation has occurred.

Finally, we will speculate on the time of divergence of these *Enchenopa* entities. Nei (1972) states that, if most of the biochemical changes detected are neutral or relatively neutral, then the rates of protein evolution would be stochastically constant, and protein differences would reflect not only phylogeny but could serve as an evolutionary clock indicating actual times the various cladogenetic events occurred. One need not be a strict "neutralist" to make use of Nei's model. Over all loci under selection, some will be evolving more conservatively, and others more rapidly, than the overall mean. If we examine a diversity of loci with differing evolutionary rates (Sarich 1977), it is to be hoped that we average the discrepancy in rates among individual loci. Genetic distance has been used as an estimate of divergence time in a number of studies, and times obtained appear to correlate well with estimates obtained from other evidence, such as geological history (Gorman et al. 1971, 1975, Nevo et al. 1974, Yang et al. 1974, Kim et al. 1976). However, several different methods have been used to calibrate the evolutionary clock. Sarich (1977) proposes using the correlation between albumin immunological distance (AID), obtained by micro-complement fixation, and Nei's genetic distance. In diverse groups, an AID of 35 = 1.0 $\overline{D}$ units if 3/4 of the loci investigated are slowly evolving and the remainder rapidly evolving (Sarich 1977). Albumin data in many taxa (Sarich 1969, Gorman et al. 1971, Wallace et al. 1973) suggest that 10 AID units correspond to a divergence time of 6 million years and, thus, a genetic distance of 1.0 = 21 million years. Time estimates calculated by this method for divergence of *Enchenopa* host races are presented in Table 3-2. Divergence times range from approximately 3.5 myr for the *Juglans nigra* split from the ancestral stock to 0.25 myr for the most recent split between *C. scandens* and *Viburnum.* Nei (1972) advocates the relationship expressed by the formula $t = \overline{D}/(2cn_T\lambda a)$, where t is the period of time since a pair of populations or species become isolated, $\overline{D}$ the genetic distance (expected number of amino

acid differences per protein), c the proportion of amino acid substitutions detected by electrophoresis, λa the rate of amino acid substitutions per polypeptide per site per year, and n_T the total number of codons (= number of amino acids) involved in the synthesis of a protein. Using the logic of Nevo et al. (1974), the above formula reduces to $t = 1.54 \times 10^6 \bar{D}$. This yields times of divergence that are exactly 13.63 times less than those calculated by the former method; these are presented in Table 3-2. While each method has certain advantages, we believe that it would be tenuous to attempt to choose between them. Therefore, they are included as upper and lower estimates of time of divergence for each *Enchenopa* host race. Both methods suggest that divergence has been a recent event and may be even more recent than the most conservative estimates. Regardless of these estimates, it is clear that genetic divergence of the *Enchenopa binotata* complex has occurred on these 6 broadly sympatric host plants in eastern North America.

6 The Role of Ants in the Life History and Dispersion of Treehoppers

We hypothesize that predaceous ants play a major role in affecting the survival and dispersion of treehoppers. Ants are attracted to and feed on the anal secretions (honeydew) of many Homoptera (Leston 1973a, 1973b, 1978). Similarly, we observed that aggregations of *Enchenopa* nymphs can be attended by ants. An examination of 150 branches with nymphs showed that 60 of these aggregations were attended by ants. The number of nymphs in attended aggregations averaged 3 times the number in non-ant-attended ones. These observations suggest that treehopper survival is greater where attending ants are present because they reduce predation from other invertebrates (Wood 1977, McEvoy 1979). Also, because ants are foraging for honeydew, large aggregations of treehoppers should be more attractive to ants than small ones.

To test this hypothesis 15 branches on each of 16 trees containing treehopper egg masses were tagged. When eggs hatched, counts of nymphs and ants were made until nymphs molted to adult (approx. 30 days later). As expected the mean number of nymphs/branch and the mean number of egg masses/branch were positively related. The mean number of nymphs on the 16 trees was positively related to the number of

Table 3-2. Time estimates of divergence for host races of the *Enchenopa binotata* complex. See text for additional explanation

Host race	Minimum estimate[a] (years B.P.)	Maximum estimate[b] (years B.P.)
J. nigra	256,000	3,486,000
P. trifoliata	188,000	2,562,000
R. pseudoacacia	116,000	1,575,000
C. canadensis	77,000	1,050,000
C. scandens	20,000	273,000

[a] Using the method of Nei (1972).
[b] Using the method of Sarich (1977).

aggregations on the tree which were attended by ants. Thus, the number of egg masses within a tree, the number of nymphs, and the number of tending ants were positively related. Nymphal survival on individual trees was related to the mean initial number of nymphs per branch and to the number of branches with nymphs that were ant-attended (Wood and Seilkop, unpubl.). When individual branches were considered independent of trees we found that branches with 11 or more nymphs were more consistently attended by ants and had a higher nymphal survival than those with few nymphs (Wood and Seilkop, unpubl.). Furthermore, McEvoy (1979) showed that treehopper survival is positively related to their proximity to ant nests.

We feel that ovipositional attractants in the egg froth, the clumped distribution of egg masses, and aggregation behavior in nymphs are all adaptations that concentrate the honeydew resource and encourage attendence by ants. Thus, as a consequence of ants, populations of *Enchenopa* tend to be scattered and disjunct.

Reproductive Isolation and the Evolution of Host Races

We propose that divergence of *Enchenopa* along host plant lines broadly fits a sympatric model for speciation. Based on our present knowledge of treehoppers, we postulate that the progenitor of North American *Enchenopa* was Neotropical and that females deposited eggs in masses covered with froth. *Enchenopa* occurs in the Neotropics on several different hosts (Ballou 1936), and Funkhouser (1951) proposes the Neotropics as the origin for treehoppers in general with subsequent radiations into north temperate regions. Egg froth in the tropics may serve as a protection from predators or parasites as well as a mechanism to ensure large aggregations of nymphs that concentrate honeydew and attract a defensive force of ants that are so abundant in the lowland tropics. Selection favoring females that were attracted to existing egg masses for oviposition should have been intense, given the fact that in our experiments nymphs on branches containing few egg masses attracted few ants and incurred severe mortality.

Enchenopa colonizing North America may have been polyphagous, or monophagous feeding on a single host with subsequent transfers to other hosts after its arrival. Regardless, inclement winters and synchronization of life history with the unique phenology of each host were problems to be incurred. Coordination of egg-hatch and nymphal development with optimal host nutrition (apparently when hosts are in flower) and avoidance of plant defenses were undoubtedly favored. As eggs hatched and nymphs developed in response to the phenology of their host plants, conditions which promoted host specialization and divergence were established. We see evidence for this today in the chronological differences in life histories of *Enchenopa* that occur on the various host plants. For instance, *Enchenopa* on *C. scandens, J. nigra* and *R. pseudoacacia* are effectively separated from each other by mating at different times during the season. On *Viburnum,* treehoppers mate in the evening, while those on *P. foliata* and *C. canadensis* are diurnal maters. *Enchenopa* on these latter two hosts disperse at different times during the season. Such differences should in part promote reproductive isolation among the host races.

Furthermore, tending ants appear to be a major factor in North America as well as the Neotropics, fashioning the life history of *Enchenopa* and dictating their highly clumped distribution. Successful colonization of new hosts should be a rare event considering the high mortality incurred by small populations of treehopper nymphs that fail to attract protective ants. Under these circumstances, dispersal should be as rare as we show, density-dependent, and occur only at high densities (see Wood and Guttman 1981). Consequently, as Janzen (1968) and Opler (1974) suggest, and Edmunds and Alstad show (1978; Chapter 2), host plants or individual trees may function as evolutionary islands with their isolated populations of coevolving herbivores.

Assortative mating within host plant species is almost assured considering disparate life histories, the mating system, and the insular nature of treehopper populations. We predict that the majority of females mate and deposit eggs either on the tree where they developed or on nearby conspecifics. The allelic heterogeneity we found among *Enchenopa* populations from adjacent conspecific host plants confirms this hypothesis (see also Guttman et al. 1981). This should promote genetic divergence of *Enchenopa* on different host plant species as well as among individual trees.

Selection of host-specific genotypes and assortative mating could lead to reproductive isolation among host races. The fact that females choose their developmental hosts for oviposition and mate mostly with males having developed on conspecific hosts suggests that significant divergence has occurred. The genetic differences we present among entities in the *Enchenopa* complex indicate that sufficient time has elapsed for the development of post-mating isolation mechanisms. Treehoppers on *J. nigra, P. trifoliata, C. canadensis* and *R. pseudoacacia* are electrophoretically the most distinct. Offspring from mixed matings among these *Enchenopa* or between these and treehoppers on *Viburnum* or *C. scandens* may have reduced fitness. Although *Enchenopa* on the latter two hosts are closest genetically, they are ecologically well-separated by differences in mating phenology. In this proposed model we feel that slight life history shifts of *Enchenopa* in response to host plants combined with behavioral and ecological factors are all that are needed to produce reproductively isolated species.

Further tests of our model proposed by Wood (1980) depend on:

(1) Detailed studies of tropical *Enchenopa*, since evidence for a tropical progenitor is tenuous.
(2) Understanding the mechanisms that coordinate treehopper life histories with host phenology.
(3) Determining the relative contributions of genetics and environment (host plants) to the variation in *Enchenopa* life histories.

Summary. Given the life history, host-plant interactions, and behavior, we feel that *Enchenopa* has diverged along host plant lines into a complex of reproductively isolated populations. The mechanism suggested for this divergence broadly fits into a sympatric model.

Acknowledgments. We appreciate the efforts of both the editors of this volume and an anonymous reviewer for their help in clarifying the manuscript. Portions of this work were supported by National Science Foundation Grants BMS 74-19764 A01, and a Miami University Faculty Research Grant.

Published with the approval of the Director of the Delaware Agricultural Experiment Station as Miscellaneous Paper No. 918, Contribution No. 495, of the Department of Entomology and Applied Ecology, University of Delaware, Newark, Delaware 19711, U.S.A.

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