HOST PLANT SHIFTS AND SPECIATION IN THE ENCHENOPA BINOTATA SAY COMPLEX

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ABSTRACT

In this review of the Enchenopa binotata Say complex evidence is presented that speciation has occurred through shifts in host plant utilization. All members of this complex are generally sympatric throughout their range in eastern North America on six genera of deciduous trees. Enchenopa is univoltine with a life history extending from May to October. Females which mate once, when given a choice assortatively mate and oviposit along host plant lines. Eggs hatch synchronously on each host but asynchronously among host species. Since males and females from a given host plant species are uniform in their age distribution this promotes assortative mating along host plant lines. Evidence is reviewed that suggests that Enchenopa allochronic life histories are mediated by host plant water relations which in the absence of other isolating mechanisms promotes assortative mating. In general the evidence to date suggests that a sympatric speciation model of host shifts is a plausible explanation for the divergence of this complex.

INTRODUCTION

The debate over whether shifts to novel host plants promotes speciation in phytophagous insects and whether these shifts can occur in sympatry is still very much alive. Proponents (Bush 1975) and opponents (Futuyma and Mayer 1980) agree that host shifts are a plausible speciation mechanisms but disagree on whether the empirical evidence supports a sympatric over one of several allopatric models. Empirically it is difficult, if not impossible, to demonstrate that a particular speciation event occurred in sympatry, only that it is the most parsimonious explanation relative to alternative models (Futuyma and Mayer 1980). The issue is ..hether mechanisms exist which would provide a barrier to gene flow between the parent and daughter sympatric populations. Most sympatric models invoke genes associated with mate and host selection as well as survival to initiate divergence (Bush 1975). Critics (Futuyma and Mayer 1980) argue that none of these could provide an initial barrier to restrict gene flow. These critics imply that some host characteristic must be present which is sufficiently disruptive to act as an extrinsic barrier to impede gene flow.

Here I would like to review work on the Enchenopa binotata Say complex (Homoptera: Membracidae) which I believe demonstrates that shifts in host plants have promoted speciation within this complex. I will also review mechanisms that suggest these shifts in host plants and the subsequent speciation could have occurred in sympatry.

Geographic Distribution

Historically, Enchenopa binotata Say was considered a single species with an extremely broad geographic range extending from Panama in Central America northward into southeastern Canada (Metcalf and Wade 1965). Within eastern North America Enchenopa binotata is found on 7 genera of deciduous trees: Ptelea trifoliata L. (Rutaceae), Viburnum sp. (Caprifoliaceae), Juglans nigra L. and J. cinerea L. (Juglandaceae), Celastrus scandens L. (Celastraceae), Robinia pseudoacacia L. and

Cercis canadensis L. (Fabaceae), and Lirodendron tulipifera L. (Magnoliaceae) (Wood 1980, Wood and Guttman 1985). The distribution (Little 1971) of these host plants show considerable geographic overlap. Both Cercis canadensis and Ptelea trifoliata have ranges that extend to 17° latitude in eastern Mexico. The southern most records of Juglans nigra are in southeastern Texas and extend northward to approximately 43° latitude. Juglans cinerea overlaps J. nigra around 35° latitude but extends northward to 49° latitude in northeast North America. Robinia pseudoacacia first appears at approximately 33° latitude and originally extended to 41° but because of its commercial use is now distributed as far north as southern Canada. Unlike the other hosts the original distribution of R. pseudoacacia was disjunct with one population in the Arkansas-Missouri region separated roughly by the Mississippi River Basin with the second population extending from northeastern Georgia through eastern Tennessee into southwestern Pennsylvania. This second population only extended as far east as the Appalachian Mountains. Enchenopa is found on three species of Viburnum (V. lentago L., V. prunifolium L. and V. rufidulum Raf.), the combined ranges of these hosts form an overlapping continuum from 30° latitude to approximately 52° in mid-central Canada. Lirodendron tulipifera is primarily eastern, reaching its southwestern limit at approximately 31° latitude and 93° longitude and extending in a northeasterly direction to 43° latitude.

Field sampling over eastern North America in addition to museum and literature records suggests there is considerable geographic overlap in Enchenopa host utilization. Enchenopa acquire "new hosts" in a northward direction from east Texas. The southern most records of Enchenopa are on Cercis 29° Lat. 99° Long.), followed in sequential order by Juglans (30° Lat. 93° Long.), Viburnum (33° Lat. 95° Long.), Ptelea (33° Lat. 95° Long.), Robinia (34° Lat. 97° Long.), Celastrus (36° Lat. 87° Long.) and on Lirodendron (39° Lat. 83° Long.). Geographically, the most southern area where Enchenopa occur on all hosts is in the Arkansas-Missouri region in the Mississippi River Basin (Wood and Guttman in prep.). Thus in the absence of any other evidence the distribution of the host plants and that of Enchenopa binotata suggests that it is a single polyphagous species in eastern North America.

Morphological and Life History Variation
        Enchenopa binotata is univoltine throughout eastern North America. Females begin oviposition in late July and continue throughout the fall until October. Eggs are inserted under the bark of the host plant and then covered with a white waxy secretion. Eggs are deposited in clusters or egg masses and often large numbers of egg masses occur on a single branch (Wood and Patton 1971). In the spring, eggs hatch synchronously on a given host species in April and May depending on latitude and year. Nymphs aggregate and are located by ants which collect honeydew and provide protection (Wood 1982).

        When I first began to work with this treehopper I noticed that there was some variation in the coloration and shape of the pronotum in both sexes. It wasn't until I began to see nymphs on the different host plants in the field that I suspected E. binotata to be anything but a variable species. For example, on Juglans late instars are black and white while those at the same stage on Robinia are green. Nymphs on other hosts also vary in coloration (Wood 1980).

        In a series of studies I satisfied myself that indeed Enchenopa on each host plant were different. Nymphs not only differ in color but also in aggregating habits and where they feed. Females select different oviposition sites, begin oviposition at different times during the season, oviposit at different times of the day and vary the number of eggs placed in each egg mass. The general physical appearance of the egg froth and its general composition are quite different on each host species. The above suggested to me that Enchenopa binotata may be a complex of races associated with each of the host genera (Wood 1980).

Host and Mate Selection

The above host plant related variation lead to asking whether females discriminate among hosts during oviposition. When females are confined to a field cage containing the entire range of possible host plants they disperse to hosts other than their natal host. However, when oviposition begins females move to their natal host with the result that few or no eggs are deposited on non-natal hosts. In the seven different combinations tested only Enchenopa from J. nigra and J. cinerea failed to clearly discriminate their natal host. Thus females are discriminating under these extreme experimental conditions (Wood 1980).

If females discriminate host plants then do males and females mate along host plant lines if given a choice? To test this possibility males and females from each of six hosts were collected, color coded and released on their natal host in a common cage containing all six host genera. Males and females from a given host were thus given free access to males and females from all other hosts. The results were very striking. Males and females dispersed throughout the cage to other hosts before the first mating was observed. Once mating began males and females formed precopulatory pairs in which over 50% of the pairs were of mixed host plant origin. However, only seven of 128 matings observed were of mixed host plant origin. Normal mating duration of Enchenopa ranges from one to one and a half hours and the seven mixed matings were of a shorter duration suggesting they were not successful. When I compared mating of males and females from a given host to that on other hosts, there appeared to be relative temporal differences. Furthermore, the majority of matings occurred on the females native host (Wood 1980).

It seems clear that under extreme experimental conditions Enchenopa from all hosts do not constitute a panmictic population. Enchenopa females mate on and with males from their natal host but also oviposit there, which indicates Enchenopa on each host constitutes a race or a reproductively isolated species. This type of host and mate discrimination provides a basis for assortative mating which could lead to genetic divergence and certainly could maintain reproductive isolation.

Electrophoretic Analysis

To substantiate that Enchenopa represent a complex of species an electrophoretic analysis of insects from each host was done. Enchenopa nymphs were collected from a number of sites and hosts in southern Ohio. In one site (300 x 180m area) Enchenopa on five of the six host species had co-existed for at least ten years. Here two different host species with Enchenopa often had interdigitating branches or were within close proximity of each other. If Enchenopa from each host were intermating in the field it should be evident at this site and they should be similar in their electrophoretic patterns (Guttman et. al.).

Of the 15 presumptive loci examined eight were monomorphic. Examination of the seven variable loci showed that Enchenopa from some hosts were distinct and could be characterized by either the fixation of alleles or by frequency differences. Differences in Enchenopa were consistent within a host even when collected from different localities or when collected from two host species with interdigitating branches. Within a site Enchenopa on Ptelea showed differences among individual trees suggesting that at least on this host, vagility is very low (Guttman et al. 1981). Mark recapture work confirms this finding (Guttman and Wood, in prep.).

The electrophoretic evidence supports the hypothesis that Enchenopa binotata is a complex of reproductively isolated species which diverged along host plant lines. The order of divergence based on this data is the following: Juglans, Ptelea, Robinia, Cercis, Celastrus, and Viburnum. Thus Enchenopa on Juglans nigra are the most divergent while those on Celastrus and Viburnum the most similar. The neutralist Nei (1972) model which assumes no selection estimates the time of

divergence for Enchenopa on Juglans at 256,000 yr. B.P. and the most recent
Celastrus-Viburnum divergence at 20,000 yr. B.P. (Wood and Guttman 1981).

Temporal Life History Variation

In southern Ohio, any one site potentially may have Enchenopa on all seven
host genera. This suggested that reproductive isolation may be enhanced by subtle
differences in Enchenopa life history timing on each of the host genera. Trees from
each of the six host genera were planted next to each other and each covered with a
cage. Females were then collected in the fall and released on their natal host to
oviposit. The following spring a continuous time scale was established the day the
first egg hatched in May and ending in October. Observations were made daily and
hourly (7:30 am to 8:30 pm from June through the end of August). Events that
occurred on a given host species were expressed in terms of when they occurred on
the time scale and could be compared in a relative sense to the same events on other
hosts (Wood and Guttman 1982).

Eggs hatch on each host at slightly different times, with eggs on Celastrus,
Viburnum, and Ptelea hatching first and generally at the same time. After egg hatch
is completed on these hosts, eggs on Cercis, Juglans, and Robinia hatch. Within a
host plant species, eggs hatch synchronously within a period of six to seven days.
Regardless of the group, eggs hatch at or near the time of flowering for a given
host, with the exception of Cercis. On Cercis egg hatch occurs during its
vegetative flush which is after flowering. Patterns of adult maturation generally
follow that of egg hatch although on Robinia maturation is later than on Juglans and
Cercis (Wood and Guttman 1982).

The number of males and females which flew to the screen of the cage on a
given day or time of day was used as an indication of their tendency to disperse
from their host. On any given host most of this dispersal occurred before the first
mating was observed. For example, approximately 80% of the insects on the screen of
the cage containing Ptelea were observed before the first mating. Once mating and
oviposition began very few males or females were observed on the screen. Enchenopa
from the different hosts differed in the temporal sequence when they flew to the
cage. For example, the majority of Enchenopa on Ptelea were observed on the cage
screen on the average 18 days before those from Cercis and 30 days before those from
Robinia (Wood and Guttman 1982).

When the temporal sequence and the time of day that precopulatory pairs are
formed on a host are compared to other hosts, it is clear that Enchenopa are
allochronic relative to each other. Only Enchenopa from Juglans and Viburnum formed
precopulatory pairs during the same temporal sequence but differed diurnally.
Enchenopa formed pairs on Juglans in the morning while on Viburnum in early evening.
The temporal sequence of mating was related to the formation of precopulatory pairs
although statistically those from Cercis, Ptelea, and Viburnum did not differ.
However, mating on Viburnum occurs in the early evening while on the other two hosts
it occurs in the morning. Only Enchenopa on Cercis and Ptelea share the same
temporal sequence or time of day they mate. Enchenopa on these two hosts are
separated in the temporal sequence of their flight from their hosts. On the
average, the differences of 14 days for males and 18 for females reduces the
potential for interhost mating (Wood and Guttman 1982). Indeed the low vagility for
Enchenopa on Ptelea (Guttman and Wood in prep.) suggests that most of the flight
observed may be within a tree rather than between trees.

Females mate once and during a limited seasonal time span so that as mating
proceeds on a given host the number of virgins declines. The decline in virgins
could promote competition among males and dispersal to natal or non-natal hosts to
find mates. However, no evidence of increased male flight activity toward the end
of mating was found. The lack of male dispersal can be accounted for in part by

differential mortality between males and females from a given host. Males have a
much shorter life span than females which results in lower numbers of males relative
to females as mating proceeds on a given host. The frequency of males forming
precopulatory pairs with previously mated females increases as the number of virgins
declines. This suggests that males treat every female encountered as a potential
mate. The duration of precopulatory pairing is often quite long and males can spend
considerable time courting non-receptive females. Since mating only occurs at
certain times of day this limits how many females can be approached. Male mortality
among Enchenopa from each of the six host genera is also allochronic which further
reduces the probability of gene flow through male dispersal (Wood and Guttman 1982).

        The above suggested that reproductive isolation is maintained in part by
subtle temporal differences in life history initiated by differences in plant
phenology. However, this still leaves open the question of whether gene flow among
Enchenopa associated with each host could be maintained through the movement of
males or mated females. Females from a given host were collected after mating was
completed, confined to their natal host and each of the other five hosts. In all,
36 combinations were tested. Mortality on some non-natal hosts was high indicating
a significant cost if the natal host is not selected. Females from some host plants
did not deposit eggs on non-natal hosts, or if they did, the number of egg masses
was lower than on natal hosts. Few eggs hatched on non-natal hosts compared to
those on natal hosts indicating a high cost to ovipositing on inappropriate hosts.
Perhaps the most interesting result was that eggs deposited on non-natal hosts
hatched at or near the time of normal egg hatch on that host and not at the time
eggs hatched on their natal host. This strongly suggested that some feature of the
host plant was mediating when eggs hatch rather than genetically determined (Wood
and Guttman 1983).

        To determine if gene flow could be maintained through the movement of males, a
total of 5,400 virgins were collected from all hosts and confined to their natal
host. Males were then released into cages containing conspecific females and those
with females from the other five host genera (36 combinations were tested). A
separate control of Enchenopa virgins from each host was established. Although the
analysis is not yet complete it is clear that male mortality on some non-natal hosts
such as Ptelea is almost complete within two weeks after their introduction and
before the onset of mating. Thus the inability of males to survive on some non-
natal hosts almost ensures no mating. Over the course of the two years during which
this experiment was replicated 315 matings were observed and only 31.43% were of
mixed host plant origin. In most combinations there were few egg masses deposited
and relatively few eggs hatched the following spring (Wood and Guttman in prep.).

Control of Egg Hatch
        The model (Wood 1980) proposed for the speciation of the Enchenopa binotata
complex hypothesized that the adoption of novel host plants with differing
phenologies initiated allochronic egg hatch and mating which promoted assortative
mating along host plant lines.

        The key to this model is whether the host plant rather than temperature or
photoperiod mediate when eggs hatch. In a series of recently completed experiments
I think there is conclusive evidence that host plant water relations mediate egg
hatch. Enchenopa eggs do not hatch if the plant is experimentally manipulated to
avoid dormancy. The majority of egg masses on non-dormant Celastrus are completely
overgrown by the bark of the plant. Eggs in branches collected in the early spring
will only hatch if liquid water is supplied to the branch. Eggs in branches
maintained on moist paper towels do not hatch suggesting that eggs must imbibe water
from the plant to develop. The most compelling evidence of water mediated control
is when branches collected at the same time are maintained under identical
photoperiod and temperature conditions and only the addition of liquid water to the
branch is manipulated. When water is added to branches at different times, egg

hatch is allochronic even though they were maintained under identical photoperiod and temperature conditions. The question is then, whether host plants take up water at different times or rates in the spring. In the spring of 1985 branches from each of the six hosts were collected at intervals and their water content determined. The increase in water content of branches throughout the spring from each of the six hosts differed from each other suggesting that they are taking up water at different times. Thus it would appear that different host plant water relations initiate allochronic Enchenopa life histories (Wood, Olmstead, Guttman, in prep.).

Assortative Mating

Since egg hatch is mediated by the host plant is there evidence to support the hypothesis that temporal differences in egg hatch leads to assortative mating along host plant lines? Because Enchenopa from each of the six host genera are reproductively isolated there may be behavioral differences which could account for the observed assortative mating along host plant lines. The model (Wood 1980) proposed suggests that allochronic life histories promotes assortative mating which is equivalent to saying that males and females assortatively mate by age class.

To test this aspect of the model, egg hatch was manipulated to provide six age classes of Enchenopa from Celastrus. The range within each age class was six days which is approximately the range of egg hatch on a host and mimics patterns observed in the field. Each age class was color coded and released into a common cage in an environmental chamber (28°C and 12:12 LD). Every hour (8:00 am to 5:00 pm) observations were made of precopulatory and mating pairs. Survival of males and females was determined at intervals. To determine if mating was equally likely among age classes the expected number of precopulatory and mating pairs among each age class based on their initial frequencies was compared with that observed. Both precopulatory and mating pairs were heavily biased toward individuals within the same age or an adjacent age class. Even though the Enchenopa in this experiment were from the same host species and indeed the same population they did not mate equally among the age classes. The mechanism producing this assortative mating lies in allochronic male mortality. Males of one age class simply died at different times relative to other age classes so that few males survive at the time adjacent or successive age class females are ready to mate. Since females from a given age class have only a certain span of time to mate, mating occurs among individuals that are very similar in age. If the data for precopulatory and mating pairs are adjusted to account for male mortality then mating ($P=.052$) occurs equally among age classes.

Speciation Model

Funkhouser (1951) considered the center of treehopper distribution in the western hemisphere to be the tropics with subsequent northern migrations. Seasonal and host plant records (Ballou 1936) suggest that in the tropics E. binotata may be moltivoltine and polyphagous on leguminous trees. If E. binotata moved north as implied by Funkhouser (1951) then its ability to extend its range may have been related to generalized host preferences. The habit of inserting eggs into plant tissue and covering them with wax would enhance their ability to overwinter but more importantly permit tracking some aspect of host phenology. Moving north into areas where plant growth is seasonally limited would restrict the number of generations and place intense selection on eggs which hatch in synchrony with the availability of plant nutrients. In more northern climates such selection pressures would be intensified resulting in a univoltine life history with eggs hatching relative to some aspect of plant phenology (Wood 1980).

The initial polyphagous habit would permit exploitation of large abundant hosts but low vagility would encourage moderate to high densities on individual trees or stands. Such a pattern combined with patchy distributions of hosts would further fractionate Enchenopa populations. Differences in ovipositional sites on

each host exposed eggs to varying plant physiological conditions which could produce asynchronous egg hatch among hosts on a microgeographic scale (Wood 1980).

The hosts of Enchenopa are evolutionally divergent, differences in nutrient quality could affect the timing of nymphal and sexual maturation of adults. Differences in nutrient quality could magnify the affect of allochronic egg hatch and promote assortative mating along host plant lines. Gene flow could occur initially on locally sympatric hosts but would be limited due to intense selection for the coordination of egg hatch to host phenology and insect genotypes adaptive to specific hosts (Wood 1980).

Since this hypothesis was proposed substantial experimental work has been done that supports it. At this point, I would suggest that Enchenopa moved northward on a leguminous tree. The divergence of Enchenopa to Juglans could have occurred in southeastern United States while the distributional and electrophoretic data suggest the remainder diverged in the upper Mississippi-Ohio River Basin. Although Enchenopa on C. canadensis have the southern most records, the electrophoretic data suggests a recent divergence. If this is true, Enchenopa could have moved north on this host with successive divergences. I think however, that it is more likely that there have been multiple shifts among hosts with completely different phenologies. A test of this hypothesis will come after a cladistic analysis is completed. If the shifts occurred in sympatry then closely related species should be on hosts with completely different phenologies (water relations) and very similar geographic ranges. Such shifts would produce sufficient differences in life histories to encourage assortative mating by age class and provide a major disruptive barrier to gene flow. Experimentally it seems clear that allochronic life histories mediated by the plant could initiate divergence in the absence of selection. However, it seems unlikely that there were not intense selection pressures associated with host shifts. High male and female mortality on non-natal hosts in our experiments and work with nymphs (Kiss 1983) suggests that either nutritional quality or secondary plant compounds are sufficiently different to produce intense selection for genotypes adaptive to a particular host. Intense selection combined with plant mediated life histories could effect rather rapid divergence. Development of even limited host and mate discrimination abilities would further accelerate the process, particularly if there was a cost to poor discrimination. The experiments discussed in this paper suggest the costs are extremely low egg hatch and nymphal survival.

In summary, although it is experimentally impossible to say that Enchenopa speciated in sympatry. I think there are mechanisms which support the model. The demonstration that differing plant water relations can act as an extrinsic disruptive force leading to assortative mating suggests to me that a sympatric model is as plausible as invoking an array of geographic barriers.

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