

Genetic Variation in Performance Traits and the Potential for Host Shifts in *Enchenopa* Treehoppers (Homoptera: Membracidae)

K. J. TILMON,¹ T. K. WOOD, AND J. D. PESEK²

Department of Entomology and Applied Ecology, University of Delaware, Newark, DE 19717

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ABSTRACT Host plant shifts by phytophagous insects are often hypothesized as means through which new species could genetically differentiate. For genetic divergence to occur between populations on original and novel hosts, the colonizing population must possess genetic variation in performance traits allowing use of and selection by the new host. To test for the existence of genetic variation in *Enchenopa* treehoppers for the use of novel host plants in the genus *Viburnum*, we performed a 4-way full sib analysis. Related insects were exposed to 4 different plant species in greenhouse and field settings: the original host plant and 3 novel host species. We estimated genetic variation in performance traits of male and female longevity, and female fecundity and calculated fitness values, nymphal survival, and male and female developmental time to adult eclosion. We found significant genetic variation in traits influencing both survival and reproduction. There were significant interactions between genotype and host, indicating some genotypes are more adept than others at using new hosts. We conclude that sufficient genetic variation exists in this population to facilitate successful shifts to these new hosts.

KEY WORDS *Enchenopa*, Membracidae, genetic variation, host shifts, speciation

SHIFTS BY PHYTOPHAGOUS insects to novel host plants (either in sympatry or allopatry) are powerful means through which insects might genetically differentiate. If the novel host poses selective hurdles that drive divergence along host plant lines (Strong et al. 1984) the result may be the formation of new races or species. For genetic divergence between host-associated insect populations to be initiated, the colonizing population must initially possess genetic variation in performance traits, permitting some individuals and their progeny survival on, selection by, and adaptation to the new host (Etges 1993). If populations lack the genetic variation to permit a host shift then constraints are imposed, limiting the direction of evolution (Futuyma et al. 1993). However many systems show more genetic flexibility than constraints, allowing adaptation to surprisingly novel conditions (Barker and Thomas 1987). The possibility of host-driven divergence and speciation events in phytophagous insects, and also insect population responses to exotic or genetically engineered plants, depends in part on existing performance-associated genetic variability in a colonizing population.

Evidence that populations exhibit genetic variation in performance on different host plants comes from systems where different host associations are already established. In such systems, populations have devel-

oped host-associated polymorphisms (Via 1991), have achieved host race status (Bush 1992), or have undergone speciation (Wood 1980, 1993; Wood and Guttman 1982, 1983; Guttman et al. 1981). Data on the initial genetic variation that precedes and permits a host shift, the pre-existing genetic potentials of a population before a host shift, are lacking in these systems.

Divergence and speciation of *Enchenopa* treehoppers have been hypothesized to be the result of host shifts to phenologically different plants (Wood and Keese 1990, Wood et al. 1990). Host shifts are hypothesized to initiate a process that promotes assortative mating, restricted gene flow, and plant specialization in the absence of geographic isolation (Wood and Keese 1990; Wood et al. 1990, 1998). In the extant univoltine *Enchenopa binotata* Say species complex (species yet to be named), the initiation of egg development is mediated by plant phenology (Wood 1980, Wood and Guttman 1982, Wood et al. 1990). An important consequence to *Enchenopa* of using phenologically different host species is asynchronous life histories, assortative mating, and subsequent genetic divergence (Wood 1980, Wood and Guttman 1982). Host plant specialization and speciation in the *E. binotata* species complex has occurred across 6 plant families (Leguminosae, Juglandaceae, Caprifoliaceae, Celastraceae, Magnoliaceae, Rutaceae), between genera within a family (Leguminosae: *Cercis*, *Robinia*; Juglandaceae: *Carya*, *Juglans*) and even within a genus (*Juglans nigra* L.; *J. cinerea* L.). *Enchenopa* on *Carya* and *Viburnum* are found on more than 1 species in their respective host genera. However, the other

¹Department of Entomology, Cornell University, Ithaca, NY 14853-0901.

²Department of Food and Resource Economics, University of Delaware, Newark, DE 19717.

members of the *E. binotata* species complex are very host specific, restricted to 1 plant species (Wood 1993). Given this high degree of host specificity, the likelihood of successful host plant shifts by *Enchenopa*, with subsequent speciation, depends on the existence of performance trait variation relative to new hosts, permitting host shifts.

Host specialization and speciation has occurred in the extant *E. binotata* species complex on 2 species within a plant genus (*Juglans*). This suggests that experimental shifts to novel host plants even within a plant genus could provide a model to examine pre-existing genetic variation in host use relative to speciation. Differential plant species effects on life history timing is a central component of the *E. binotata* sympatric speciation hypothesis. Experimental novel host shifts to congeneric plant species with different plant phenologies and effects on performance traits would enhance the credibility of the *Enchenopa* speciation hypothesis.

Sib analysis permits determination of the relative importance of environmental versus genetic influence on the expression of fitness traits (Falconer 1989). We report the results of a 4-way full sib analysis of *Enchenopa* treehoppers. This experiment was conducted in the greenhouse and in the field to test for genetic variation in performance of an *Enchenopa* species on 3 novel hosts in the genus *Viburnum* (Caprifoliaceae). The cosmopolitan genus *Viburnum* is very diverse; species differ widely in flowering phenologies and chemical makeup, and range from deciduous to evergreen.

Preliminary data suggests some *Viburnum* species vary in the ability of *Enchenopa* to colonize them. We tested for genetic variation in *Enchenopa* in response to these plants for traits of longevity, fecundity, nymphal survival, and developmental time.

Materials and Methods

We tested for variation in 1 species in the *E. binotata* complex in the ability to use novel host plants within the genus *Viburnum*. The *Enchenopa* population used in this experiment was originally from *V. lentago* L., which served as the control host. The 3 novel *Viburnum* species were *V. prunifolium* L., *V. lantana* L. and *V. utile* Hemsley (a semievergreen), *V. lantana* and *V. utile* are introduced to the United States, so the possibility of past contact between *Enchenopa* (a New World insect) and these plants can be excluded. *V. prunifolium* is an *Enchenopa* host in some regions. It is native to North America and has little geographic range or habitat overlap with *V. lentago*; seldom do the 2 species co-occur except in cultivation. Other experiments suggest that experimental shifts of *Enchenopa* to these phenologically different *Viburnum* species have an effect on their life history, altering the timing of egg hatch, adult eclosion, and mating (Wood 1993).

Members of the *E. binotata* species complex are univoltine, with their entire life cycle spent in or on the host plant. Little nymphal or adult movement from the natal plant occurs, even when the plant species is

inappropriate (Wood et al. 1998). Egg hatch occurs in spring, adult maturation in early summer, mating in midsummer, and oviposition into plant vascular tissues throughout the fall, into November (Wood and Guttman 1982, Wood 1993).

Experiment 1. In the summer of 1992, in preparation for a full sib study in 1993, we established family lines of *Enchenopa* from *V. lentago*. The *Enchenopa* colony from which family lines were isolated was established 2 generations earlier in 1990 with insects taken from an isolated stand of *V. lentago* near Winchester, VA, and maintained on cloned *V. lentago* plants in a greenhouse. All *Viburnum* spp. plants in this study were clones propagated from 1 representative of their respective species.

Families for the study were established by observing the colony for copulations, then isolating 14 mated females on individual *V. lentago* branches across 9 shrubs with fine-mesh polyester sleeve cages. The egg masses deposited in any given branch represented a family of unique parentage: females mate only once and though males can mate more than once it is unlikely that in this large colony any of the 14 families shared a sire. In the fall of 1992, we moved plants containing egg masses to an outside common garden for overwintering. In spring of 1993, just prior to egg hatch, plants were brought back into the greenhouse. Each family was maintained as a unit through egg hatch and adult eclosion in the spring and early summer.

After adult eclosion was completed but prior to mating, each of the 14 families from *V. lentago* was divided equally into 4 groups. One-fourth of each family was then transferred to each of the following hosts: *V. lentago*, *V. prunifolium*, *V. lantana*, and *V. utile*. A total of 612 individuals (261 males, 351 females) was used. Quarter-family transfers to a given host species were all kept on different branches of a common host tree, either a large *V. lentago*, *V. lantana*, *V. prunifolium*, or *V. utile* to minimize any individual tree effects during the mating period. Matings took place in the greenhouse within polyester sleeve cages on plant branches. Sisters were mated with brothers, minimizing sire effects. A full sib rather than a half sib analysis was used because males transfer fewer sperm on the 3rd and subsequent matings (Greene 1997), and half sib designs require a male to fertilize more than 2 females. When matings were observed, copulating pairs were gently coaxed into vials to complete mating. After mating was completed and pairs had separated, females were individually confined in sleeve cages on branches of their transfer host species in the field. Eighty-three females were distributed across 11 cloned *V. lentago*, 96 females across 9 *V. lantana*, 87 females across 10 *V. prunifolium*, and 85 females across 15 *V. utile*. Plants ranged from 2 to 8 yr old and were located on or near the University of Delaware arboretum. We checked individual females each week over a 5-mo period (mid-July through December), counting egg masses and determining mortality dates, to estimate fecundity and longevity on the original and novel hosts.

Experiment 2. The following experiment was performed an independent study similar in design to that performed in Experiment 1 to examine variation in *Enchenopa* development time on novel host plants. Mated female *Enchenopa* from the same colony were used to establish families. They were distributed across 15 plants containing eggs of these families in a greenhouse just prior to the start of the experiment in 1994. As the eggs of families from *V. lentago* branches during the experiment were transferred 1st instars daily to small, individually potted plants in the greenhouse. One-quarter of the plants were transferred to a *V. lentago*, *V. prunifolium*, *V. lantana*, or *V. utile*. Thus, each of the 4 families was distributed equally across 4 individual plants of the 4 *Viburnum* species. All *Viburnum* species were used as host plants.

We transferred a total of 100 nymphs every 5 d during nymphal development into a 1-wk period, into early June. The number of nymphs still alive in each plant. Upon adult eclosion, we recorded the sexed the number of insects in each sub-group. These data were used to estimate survival and development time.

Traits Tested. The traits measured were male and female longevity, fecundity and calculated female fitness (Experiment 1), nymphal survival and female development time (Experiment 2). Longevity was measured as the difference, in days, between the date of male or female emergence (to the nearest week). Male and female emergence were used as the starting point for each family because we had no data on their emergence. Both male and female emergence were used for each family because males emerge earlier than females. Fecundity was measured as the number of egg masses female produced. By employing concepts of life tables, longevity (age at death), fecundity can both be taken as measures of an individual female fitness (lifetime fitness increase) representing her contribution to the next generation. Complete life tables were calculated for each individual female. Mean values are detailed by Lentgen et al. (1991) by Via (1991). This variable was used as a measure of lifetime fitness (Lentgen et al. 1991).

To assess nymphal survival, we constructed a mean dummy variable for nymphal survival. To calculate nymphal survival in each family, we used nymphal development period as a proxy for age classes, which we counted survival of nymphs served living to 1 age class.

Experiment 2. The following year, in spring 1994, we performed an independent full sib experiment similar in design to that performed in 1993 to determine variation in *Enchenopa* nymphal survival and development time on novel hosts. In summer 1993, 18 mated female *Enchenopa* from *V. lentago* were isolated to establish families. They were isolated on *V. lentago* branches across 15 plants in the field. Branches containing eggs of these family lines were brought into the greenhouse just prior to egg hatch in the spring of 1994. As the eggs of families hatched from the *V. lentago* branches during the first 2 wk of May, we transferred 1st instars daily with a camel's-hair brush to small, individually potted host plants maintained in the greenhouse. One-quarter of each family was transferred to a *V. lentago*, *V. prunifolium*, *V. lantana*, or a *V. utile*. Thus, each of the 18 families was distributed equally across 4 individual plants representing each of the 4 *Viburnum* species. Eighteen plants of each *Viburnum* species were used, for a total of 72 plants. We transferred a total of 1,357 nymphs.

Every 5 d during nymphal development (about a 5 wk period, into early June), we counted the number of nymphs still alive in each family sub-group on their plant. Upon adult eclosion, each day we counted and sexed the number of insects maturing in each family sub-group. These data were used to calculate nymphal survival and development time to adult eclosion.

Traits Tested. The traits tested for main effects were male and female longevity (1993), female fecundity and calculated female fitness values (1993) (Experiment 1), nymphal survival (1994) and male and female development time to adult eclosion (1994) (Experiment 2). Longevity was calculated as the difference, in days, between the average eclosion day for males or females in a family and individual death day (to the nearest week). Mean family eclosion day was used as the starting point to estimate longevity for each family because we lacked egg hatch dates for them. Both male and female mean eclosion dates were used for each family because on average males mature earlier than females. Fecundity was assessed as the number of egg masses females laid before they died. By employing concepts of population demography (life tables), longevity (age-specific survivorship) and fecundity can both be taken into account to generate an individual female fitness value (individual rate of increase) representing her contribution to the next generation. Complete life tables were generated for each individual female. Methods for calculating fitness values are detailed by Lenski and Service (1982) and by Via (1991). This variable is useful as a demographic measure of lifetime fitness (Via 1991, Lenski and Service 1982).

To assess nymphal survival to adulthood, we constructed a mean dummy variable, hereafter referred to as nymphal survival. To calculate mean nymphal survival in each family sub-group, we divided the nymphal development period into increments of 5 d representing age classes, concomitant with the days on which we counted survivors. Those individuals observed living to 1 age class but not to the subsequent

age class were given survival values equal to the number of days leading up to their last survived age class. These survival values for all individuals within family sub-groups could then be used in standard analysis of variance (ANOVA). Individual developmental time to adult maturation was calculated as the day upon which an individual matured minus the mean day of egg hatch for its family. Individual developmental times were used to calculate mean developmental time for males or females within a family sub-group on a given host plant.

Statistical Analyses. Unless otherwise stated, we used ANOVA to test for effects of family (genotype), host plant (environment), and family by host (genotype by environment) interactions. We have consistently found individual tree effects on measured insect traits to be inconsequential (perhaps because we used cloned *Viburnum*), and therefore have excluded them from analysis. The family effect is treated as random and the host effect is treated as fixed because individual tree effects were not included. The family by host interaction is tested on the error term, but the host effect that in a balanced design would have been tested using the family by host effect as an error term has no test when the design is not balanced. We rectified this by using approximate *F*-tests (Satterthwaite 1946). This standard approach creates a synthetic error term that is a combination of family by host and error, with appropriately adjusted degrees of freedom.

Whether reproductive performance is measured by total egg masses or by fitness value a certain fraction of the data is zero, because some females died without laying eggs. Although use of zero values violates both the normal and homoskedastic assumptions of ANOVA, removing zero values is unsatisfactory: females with zero fecundity are part of the cost of a host shift, and their lack of fecundity is valid data that should not be ignored. To test fitness values for effects of family, host, and family by host interaction by using an analysis not sensitive to zero values in the data, we employed the tobit procedure (Tobin 1958) conducted using the GLIMMOD and LIFEREG procedures in SAS (Sas Institute 1989, 1992). This is a latent variable approach. It is assumed that there is an unobservable quantity for each individual (representing perhaps nutrition) that is negative or zero when no egg masses are produced but equal to reproductive value when egg masses are produced. The tobit model is:

$$F_{ijk}^* = \mu + \text{family}_i + \text{host}_j + \text{family}^* \text{host}_{ij} + \epsilon_{ijk}$$

$$F_{ijk} = 0 \quad \text{if } F_{ijk}^* \leq 0$$

$$F_{ijk} = F_{ijk}^* \quad \text{if } F_{ijk}^* > 0 \quad [1]$$

Testing of effects in the model is done using likelihood ratio tests. Under the null hypothesis the test statistic is distributed as chi-square. The degrees of freedom are the same as the effect tested.

To examine cross-host family correlations in performance measures, we calculated Pearson correlation coefficients for each of the traits measured in experiment 1 and 2 (SAS Institute 1989).

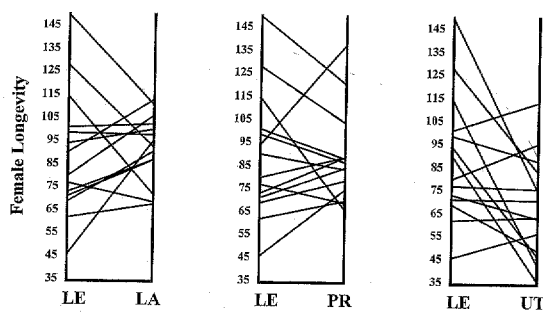


Fig. 1. Family means of *Enchenopa* female longevity in days on *V. lentago* (LE), *V. lantana* (LA), *V. prunifolium* (PR), and *V. utile* (UT). Points on either axis connected by a line represent 2 portions of the same family and their mean longevity on the original (*V. lentago*) and novel host plant (indicated beneath the axes). For a given family, an ascending line indicates greater longevity on the novel host; a descending line indicates shorter longevity on the novel host. Crossing is indicative of a family by host interaction (Table 1), in which families react differently to different plants.

Results and Discussion

The goal of the experiments presented in this paper was to assess the genetic basis for performance traits on novel host plants in the context of experimental host shifts. By placing genetically related individuals in different environments, sib analysis provides a means of assessing genetic variability. Because male *Enchenopa* reproductive biology prevents half sib analysis, it was not possible to evaluate maternal effects, that potentially confound full sib analysis (Falconer 1989).

In the experiments described above, family effects are indicative of genetic variation between family groups for some trait. Host effects indicate that the environment has a significant effect on a given trait. The family by host interaction is especially important because it indicates that some genotypes are more adept than others in using particular new hosts, and those particular hosts can select for host-adapted genotypes. Every stage of the *Enchenopa* univoltine life cycle occurs on or in its host plant. For herbivores with such an intimate host association, the host plant surely serves as a powerful selective force. The family by host (genotype by environment) interaction is indicative of the type of genetic variation that could allow populations of phytophagous insects, under the right circumstances, to diverge on different host plants in response to plant selective pressures.

Female longevity is an important fitness component in the *Viburnum* *Enchenopa* species because an individual female can oviposit continually for up to 5 mo, and longevity has a tight positive correlation with fecundity (T.K.W., unpublished data). Female longevity showed significant family and host effects ($P \leq 0.0098$ and $P \leq 0.0053$, respectively), and a significant family by host interaction ($P \leq 0.0452$) (Table 1; Fig. 1). The crossing patterns apparent in Figs. 1-4 are indicative of a family by host interaction.

Enchenopa males die long before females, shortly after the narrow window of the mating period; thus,

extended longevity appears to be less important for males than females. For male longevity, there was a significant family effect ($P \leq 0.0405$), but neither a host effect ($P \leq 0.0957$) nor family by host interaction ($P \leq 0.0569$) (Table 1). Because in experiment 1 males were only on novel hosts during the mating period (and not as nymphs prior to mating), we interpret the lack of significant host effect and family by host interaction in longevity as the host having little time to assert an effect.

Female fecundity is an especially important fitness trait because it represents an individual's genetic contribution to the next generation. Here, fecundity was measured as the number of egg masses a female deposited. For *Enchenopa* on the *Viburnum* species used in this study, number of egg masses is a good indicator not only of actual egg number (concealed under the protective egg mass wax layer) but also of number of hatching nymphs in the spring (host does not significantly effect percentage of egg hatch) (T.K.W., unpublished data). Female fecundity showed significant family and host effects ($P \leq 0.0290$ and $P \leq 0.0462$) (Table 1), and family by host interaction ($P \leq 0.0396$) (Table 1; Fig. 2).

We consider female fecundity values of zero to be valid data. However, because our use of zeros violates ANOVA assumptions, we also calculated female fitness values from longevity and fecundity data, and employed a tobit analysis (not sensitive to zeros) to verify our ANOVA conclusions. Results of the tobit analysis of fitness values concur with the ANOVA conclusions for female longevity and fecundity. There were significant family and host effects ($P \leq 0.0001$ and $P \leq 0.0001$), and a significant family by host interaction ($P \leq 0.0001$) (Table 2; Fig. 3).

Ability to survive on a host through the vulnerable immature phase is crucial for any population undergoing the initial stages of a host shift. For nymphal survival, measured in the 1994 experiment using different families than those in the 1993 experiment, there was a significant host effect and family by host interaction ($P \leq 0.0001$ and $P \leq 0.0001$) (Table 1; Fig. 4). There was no significant family effect ($P \leq 0.4486$).

Developmental time to pupation or adult eclosion is often cited as an index of fitness, more rapid development correlating with greater fecundity (Via 1984, Etges 1993). However for these *Enchenopa* there is no such correlation (K.J.T., unpublished data), and so in the context of this study we consider it a less important trait. Male developmental time to adult eclosion showed a significant host effect ($P \leq 0.0001$) and family by host interaction ($P \leq 0.0001$), but no family effect ($P \leq 0.2819$) (Table 1). Female developmental time to adult eclosion showed significant family and host effects ($P \leq 0.0033$ and $P \leq 0.0001$), and a significant family by host interaction ($P \leq 0.0299$) (Table 1). There was no significant correlation between female developmental time and ultimate fecundity in these females (K.J.T., unpublished data).

We consider the significant family by host interactions (present in all measured traits except male longevity) the most critical results. Although it is tempt-

Table 1. Analysis of variance

Trait	Source
Male ^a Longevity	Family
	Host
	Family*Host
	Error
Female ^b Longevity	Family
	Host
	Family*Host
	Error
Female ^c Fecundity	Family
	Host
	Family*Host
	Error
Nymphal ^d Survival	Family
	Host
	Family*Host
	Error
Male ^e Development	Family
	Host
	Family*Host
	Error
Female ^e Development	Family
	Host
	Family*Host
	Error

^a The family*host effect is tested.

^b Days between family male or

^c Number of egg masses.

^d Individuals were assigned sur

^e Days between mean family ha

ing to also consider whether across novel hosts relative must always be careful in the presence of interaction (1993). However, because so about the overall difference performance traits between host means and standard errors

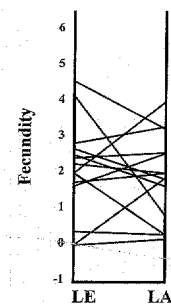


Fig. 2. Family means of *Enchenopa* fecundity (number of egg masses) on either axis connected by a line represent 2 portions of the same family and their mean fecundity on the original (*V. lentago*) and novel host plant (*V. lantana*). For a given family, an ascending line indicates greater fecundity on the novel host; a descending line indicates shorter fecundity on the novel host. Crossing is indicative of a family by host interaction (Table 1), in which families react differently to different plants.

Table 1. Analysis of variance for performance traits of *Enchenopa* on original and novel host plants

Trait	Source	df	Type III sum of squares	Mean square	F-value ^a	p-value
Male ^a Longevity	Family	13	15,264.11	1,174.16	2.04	0.0405
	Host	3	3,687.57	1,229.57	2.23	0.0957
	Family*host	38	22,369.30	588.67	1.44	0.0569
	Error	206	84,079.58	408.15		
Female ^b Longevity	Family	14	71,546.91	5,110.49	2.53	0.0098
	Host	3	28,468.29	9,489.43	4.79	0.0053
	Family*host	41	83,916.32	2,046.74	1.44	0.0452
	Error	307	435,118.64	1,417.32		
Female ^c Fecundity	Family	13	285.69	21.98	2.19	0.0290
	Host	3	85.4	28.47	2.89	0.0462
	Family*host	39	393.94	10.10	1.48	0.0396
	Error	295	2,018.66	6.84		
Nymphal ^d Survival	Family	17	14,331.23	843.01	1.03	0.4486
	Host	3	13,521.54	4,507.18	9.62	0.0001
	Family*host	50	41,816.50	836.33	4.68	0.0001
	Error	1,286	229,867.89	178.75		
Male ^e Development	Family	16	260.04	16.25	1.23	0.2819
	Host	3	321.79	107.26	8.68	0.0001
	Family*host	38	591.04	15.55	2.46	0.0001
	Error	222	1,406.09	6.33		
Female ^e Development	Family	16	393.54	24.6	2.76	0.0033
	Host	3	400.00	133.33	15.74	0.0001
	Family*host	37	353.62	9.56	1.55	0.0299
	Error	199	1,223.88	6.15		

^a The family*host effect is tested on the error term. Family and host effects use approximate F-tests with a synthetic error term (see text).
^b Days between family male or female mean eclosion and individual death day to the nearest week.
^c Number of egg masses.
^d Individuals were assigned survival values equal to number of days survived before death, to the nearest 5 d.
^e Days between mean family hatch and individual adult eclosion.

ing to also consider whether performance is uniform across novel hosts relative to the original host, one must always be careful in interpreting main effects in the presence of interactions (Freund and Wilson 1993). However, because some readers may be curious about the overall differences in *Enchenopa* performance traits between hosts, we include pooled trait means and standard errors in Table 3.

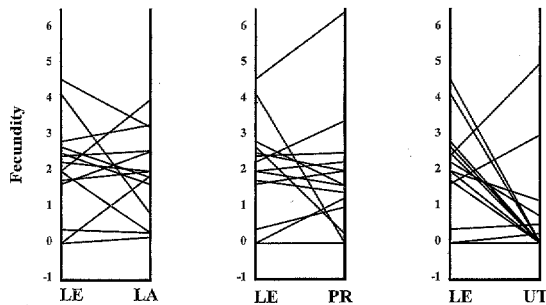


Fig. 2. Family means of *Enchenopa* female fecundity estimated by number of egg masses on *V. lentago* (LE), *V. lentana* (LA), *V. prunifolium* (PR), and *V. utile* (UT). Points on either axis connected by a line represent 2 portions of the same family and their mean fecundity on the original (*V. lentago*) and novel host plant (indicated beneath the axes). For a given family, an ascending line indicates greater fecundity on the novel host; a descending line indicates lower fecundity on the novel host. Crossing is indicative of a family by host interaction (Table 1), in which families react differently to different plants.

Pearson correlation coefficients were calculated for male and female longevity, female fecundity, nymphal survival, and male and female developmental time. No patterns of cross-host family correlations appear, except the significant correlations between families on *V. lentana* and *V. prunifolium* for traits of female longevity ($r = 0.59, P \leq 0.032$), nymphal survival ($r = 0.51, P \leq 0.030$), and female developmental time ($r = 0.52, P \leq 0.047$); this indicates that families performing well for these traits on *V. lentana* also tend to do so on *V. prunifolium*. Other significant correlations exist positively between families on *V. prunifolium* and *V. utile* for male longevity ($r = 0.62, P \leq 0.022$), and negatively between families *V. lentago* and *V. utile* for nymphal survival ($r = -0.51, P \leq 0.037$).

Perhaps the most important traits to consider in our study are female longevity, fecundity, fitness values, and nymphal survival. These traits are relevant indicators of performance because they directly impact the gene pools of subsequent, and potentially adapting generations. Significant family by host interactions

Table 2. Tobit analysis of *Enchenopa* female fitness values on original and novel host plants

Source	df	Chi-square	P
Family	13	26.9	0.0001
Host	3	102.16	0.0001
Family*host	39	55.20	0.0001

Female fitness values are from life tables generated for each individual female. As noted in the text, the tobit analysis is a maximum likelihood model and tests performed are likelihood ratio tests.

Table 3. Pooled performance trait means of *Enchenopa* on original and novel host plants

Performance trait	Host plant			
	<i>V. lentago</i>	<i>V. lantana</i>	<i>V. prunifolium</i>	<i>V. utile</i>
Male ^a longevity	52.87 ± 2.51 (71)	63.73 ± 3.33 (58)	51.30 ± 1.74 (66)	54.94 ± 3.21 (66)
Female ^a longevity	90.50 ± 4.88 (78)	94.60 ± 4.02 (92)	89.99 ± 4.81 (83)	68.32 ± 4.13 (82)
Female ^b fecundity	2.20 ± 0.33 (83)	1.93 ± 0.30 (96)	1.92 ± 0.31 (87)	0.92 ± 0.24 (85)
Nymphal ^c survival	27.27 ± 0.78 (343)	23.29 ± 0.83 (329)	22.85 ± 0.85 (336)	13.86 ± 0.69 (349)
Male ^d development	38.63 ± 0.27 (100)	42.22 ± 0.35 (71)	41.44 ± 0.40 (74)	40.48 ± 0.43 (35)
Female ^d development	40.81 ± 0.33 (88)	44.42 ± 0.28 (70)	44.34 ± 0.41 (62)	42.16 ± 0.43 (36)

Pooled mean performance measures ± SE, with number of measured individuals in parentheses. *V. lentago* is the host of origin. Means are not compared due to the confounding presence of interactions (Table 1) on the interpretation of main effects.

^a Days between male or female family mean eclosion and individual death day to the nearest week. Females whose death dates were not determined were removed from this analysis.

^b Number of egg masses.

^c Individuals were assigned survival values equal to number of days survived before, to the nearest 5 d.

^d Days between mean family hatch and individual adult eclosion.

existed for these (and other) traits. This indicates that some genotypes are more adept than others in using new hosts.

These results experimentally document that a population of a species in the *E. binotata* complex contains genetic variation in fitness-related traits that could allow shifts to novel hosts. This genetic variation is the necessary raw material from which natural selection could produce adaptations to novel hosts. Selection experiments with *Enchenopa* maintained on *V. lantana*, *V. prunifolium*, and *V. utile* indicate that observable adaptation to these novel hosts can occur in 4 generations (Tilmon 1995). In the presence of other factors such as differences in life history timing (Wood 1993) and host-associated mating and oviposition (Wood et al. 1998) this genetic variation is essential to

permit host-imposed selection to promote genetic divergence, host race formation, and ultimately, perhaps speciation.

The novel host plants to which these *Enchenopa* were exposed were chosen because of their diverse flowering phenologies and because preliminary data suggested that the insects would survive on them, making the experiments possible. However, there was no a priori reason to expect a genetically diverse response to them, in the form of a family by host (genotype by environment) interaction. Because *V. lantana* and *V. utile* are both introduced into the United States, *Enchenopa* could not have had any previous host association with these species that would have influenced the results reported here. However, it cannot be ruled out that the ancestors of the *V. lentago*

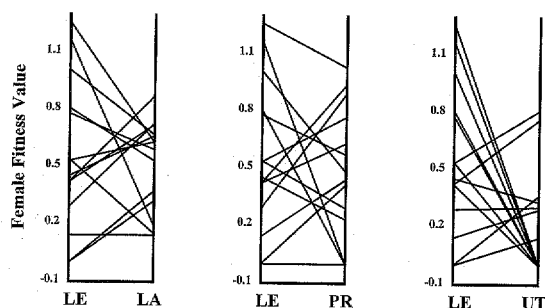


Fig. 3. Family means of *Enchenopa* female fitness values (calculated in life tables) on *V. lentago* (LE), *V. lantana* (LA), *V. prunifolium* (PR), and *V. utile* (UT). Points on either axis connected by a line represent 2 portions of the same family and their mean fitness value on the original (*V. lentago*) and novel host plant (indicated beneath the axes). For a given family, an ascending line indicates greater fitness value on the novel host; a descending line indicates lower fitness value on the novel host. Crossing is indicative of a family by host interaction (Table 1), in which families react differently to different plants.

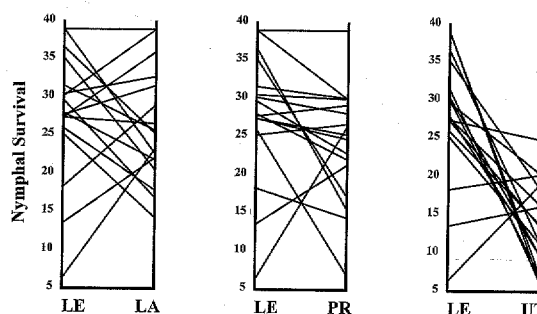


Fig. 4. Family means of *Enchenopa* nymphal survival values on *V. lentago* (LE), *V. lantana* (LA), *V. prunifolium* (PR), and *V. utile* (UT). Points on either axis connected by a line represent two portions of the same family and their mean nymphal survival value on the original (*V. lentago*) and novel host plant (indicated beneath the axes). For a given family, an ascending line indicates greater nymphal survival value on the novel host; a descending line indicates lower survival value on the novel host. Crossing is indicative of a family by host interaction (Table 1), in which families react differently to different plants.

Enchenopa used in this study on *V. prunifolium* in the evolution of

One could argue that such changes in host plant use are expected, and that the differences are not be different enough to provide a strong selection pressure. However, one must keep in mind that plant taxonomic changes can naturally predict their impact on insect life history timing. Two *Enchenopa* species are host specific on 2 different host plants, the genus *Juglans*. Furthermore, host transfer experiments reported for *Enchenopa* do not support the idea that *Enchenopa* species are selectively neutral in their ability exhibited for these hosts. This may explain why *Enchenopa* species in different geographic regions on different hosts are congeners does not support *Enchenopa* divergence.

The results presented here suggest that host-associated genetic variation could exist within an *Enchenopa* population. This variation could play an important role in host divergence and speciation.

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<i>V. lentago</i>	<i>V. utile</i>
0 ± 1.74 (66)	54.94 ± 3.21 (66)
9 ± 4.61 (83)	68.32 ± 4.13 (82)
2 ± 0.31 (87)	0.92 ± 0.24 (85)
5 ± 0.85 (336)	13.86 ± 0.69 (349)
4 ± 0.40 (74)	40.48 ± 0.43 (35)
4 ± 0.41 (62)	42.16 ± 0.43 (36)

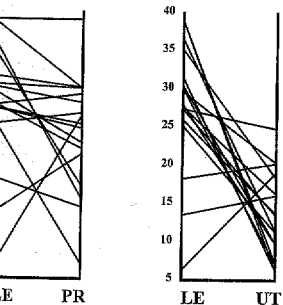
V. lentago is the host of origin. Means are in effects.

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Enchenopa nymphal survival val- *tana* (LA), *V. prunifolium* (PR), either axis connected by a line e same family and their mean e original (*V. lentago*) and novel h the axes). For a given family, eater nymphal survival value on g line indicates lower survival issing is indicative of a family by which families react differently

Enchenopa used in this study had contact with *V. prunifolium* in the evolutionary past.

One could argue that successful shifts within a plant genus are expected, and that congeneric plants might not be different enough to provide notable differential selection pressure. However, it should be borne in mind that plant taxonomic groupings do not necessarily predict their impact on herbivore fitness or life history timing. Two *Enchenopa* species in this complex are host specific on 2 different plant species in the genus *Juglans*. Furthermore, levels of mortality in the transfer experiments reported here indicate that *V. lentago* *Enchenopa* do not react to other *Viburnum* species as selectively neutral hosts. The genetic variability exhibited for these novel *Viburnum* species in different geographic regions; that these hosts are congeners does not eliminate the potential for *Enchenopa* divergence on them.

The results presented here suggest that genetically based, host-associated performance differentials do exist within an *Enchenopa* population. This type of variation could play an important role in host-driven divergence and speciation events in *Enchenopa*.

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