Felsenstein’s “one-allele model” of speciation: The role of philopatry in the initial stages of host plant mediated reproductive isolation in *Enchenopa binotata*

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**Abstract** The study of speciation genetics is primarily concerned with identifying the genetic traits that allow divergent selection to overcome the homogenizing effects of gene flow. Felsenstein reviewed this race between gene flow and selection, concluding that speciation with gene flow was unlikely under a “two-allele model” (where two traits were necessary for reproductive isolation) but that divergence could occur quite easily under a “one-allele model.” Despite this finding, much of the sympatric speciation research involving phytophagous insects has relied on a two-trait model, where insects evolve both preferences for and increased performance on novel host plants. Philopatry (a tendency to remain where one was born) is known to occur in phytophagous insects and is a single trait isolation mechanism. However, it is traditionally invoked as simply augmenting reproductive isolation. Species in the *Enchenopa binotata* complex are believed to have speciated in sympatry. They exhibit host plant preferences, host specific performance advantages and strong philopatry. We experimentally shifted *E. binotata* to evolutionarily novel host plants. Previous research has demonstrated that the experimental population of insects possesses genetic variation in preference and performance to the novel host. The degree of philopatry at mating and egg-laying was assayed for the first four years under full choice conditions. Host plant preference and performance was assayed after eight years. Philopatry was an immediate and strong isolating mechanism, while preference for and performance on the novel host lagged. We therefore suggest that philopatry may be a more important mechanism in the early stages of a host shift than previously believed [Current Zoology 59 (5): 658–666, 2013].

**Keywords** Philopatry, Enchenopa, Ecological Speciation, Sympatric Speciation

All biodiversity can be explained in reduced terms by lineage splitting in the form of speciation events. Elucidating the mechanisms of speciation will allow biologists to explain patterns of diversity and potentially to predict the progress of evolution (Howard, 1998). In sexually reproducing species, these speciation mechanisms are believed to be ones that restrict the swamping effects of gene flow enough to allow divergent natural selection (or drift) to drive populations apart (Coyne and Orr, 2004). This process may be particularly difficult when gene flow is not prevented by physical or geographic barriers (Berlocher and Feder, 2002; Bolnick and Fitzpatrick, 2007). For speciation with gene flow to occur, divergence must occur faster than genetic homogenization due to gene flow.

In a classic paper, Felsenstein (1981) reviewed the race between the swamping effects of gene flow and divergent natural selection. He found two general cases: the first in which the same allelic substitution in two incipient populations leads to reproductive isolation and a second in which at least two different allelic substitutions, one in each population are necessary. It is important to note that “one-allele” and “two-allele” traits may actually be involved in quantitative traits; the key is whether the same substitutions occur in both populations or different substitutions occur in each population. Felsenstein concluded that in the absence of extrinsic isolating mechanisms appreciable gene flow would always overcome divergence when two or more alleles are involved in isolation, as recombination quickly breaks up gene combinations that promote isolation. However, he proposed that divergence would be possible when only one allele led to local adaptation and restricted gene flow. Such substitutions are resistant to the
homogenizing effects of gene flow and in fact may be spread across incipient species populations thereby increasing the rate of divergence. It is believed that while a one-allele model is more likely to lead to speciation with gene flow the conditions are too restrictive to be common. Recent work has focused on “magic traits” where one allelic substitution can cause assortative mating and ecological adaptation (Gavrilets, 2004; Servedio et al., 2011).

Phytophagous insects systems have long served as models for sympatric or ecological speciation, gaining prominence in the 1960s (Bush, 1975; Berlocher and Feder, 2002). In these groups, isolation is usually initiated through shifts to novel host plants involving two traits: behavioral changes in host plant preference and physiological changes in the ability to survive and reproduce (or perform) on the novel host plant (Dethier, 1954; Maynard Smith, 1966; Hawthorne and Via, 2001; Grassman et al., 2006). A great deal of work has shown that such preference and performance differences do exist among closely related insects inhabiting different host plants (see e.g., Wood, 1980; Diehl and Bush, 1989; Abrahamson et al., 2001; Hawthorne and Via, 2001). However, Felsenstein suggested that preference and performance combinations specific to novel and ancestral host plants will be broken up rapidly by recombination leading to maladapted combinations (e.g. individuals that prefer a novel host but are unfit to survive there). These same complications led earlier authors to insist that geographic isolation is necessary to restrict gene flow during the initial stages of speciation (Mayr, 1947; Futuyama and Mayer, 1980).

When insects use plant hosts as both food and habitat, they may exhibit philopatry: the tendency for an individual to remain in the habitat where it was born (Shields, 1982). In the case of phytophagous insects that use their host plant as a location for mating and oviposition, this will provide a significant barrier to gene flow and isolate populations along host lines (Wood et al., 1999). When host plants are long lived, these insects are considered philopatric if they remain on the same individual plant where they were born. Philopatry has been used interchangeably with host fidelity (e.g. Wood et al., 1999), but when a distinction is made, host fidelity refers to preference for a host plant species while philopatry indicates a predilection for an individual host plant. Although philopatry has been studied in some phytophagous insect systems, it is generally regarded as a force augmenting isolation that occurs through shifts in preference and performance and not an initiating force (Feder et al., 1994). This is curious considering Felsenstein highlighted a study by Balkau and Feldman (1973) where philopatry worked as a potential single trait isolating mechanism in his 1981 paper. Balkau and Feldman (1973) modeled a situation where recombination was reduced by restricted migration, and Felsenstein stated that: “The key to Balkau and Feldman’s model is that speciation in their model proceeds by substituting the same allele in both populations” (Felsenstein, 1981 p.133). Although not formally considered a magic trait (Servedio et al., 2011), if simple philopatry is enough to reduce gene flow and allow divergence to take place, then it would involve the same allelic substitutions in both incipient populations, and it will skirt the issues raised by a two trait preference and performance shift and eliminate this particular criticism of speciation with gene flow.

The two-marked treehopper Enchenopa binotata Say species complex (Hemiptera: Membracidae) is hypothesized to have diverged by shifts to novel host plants through a combination of genetic and non-genetic mechanisms (Wood, 1980; Wood and Keese, 1990). Each of the species in this complex is found on a single species of woody host plant from at least nine plant species across six different plant families native to North America (Lin and Wood, 2002; Hamilton and Cocroft, 2009). Species in the E. binotata complex are univoltine. Eggs are laid in the host plant vascular tissue in the fall and hatch in the spring in response to the water phenology of their host (Wood and Keese, 1990; Wood et al., 1990). This leads to asynchronous life histories and shifts in mating windows resulting in some degree of allochronic isolation. Strong preferences for and increased performance on the host plant has been experimentally demonstrated (Wood, 1980; Wood and Guttman, 1982; Wood and Guttman, 1983). Strong philopatry also exists, with insects tending to remain on the individual plant where they were born, even when conspecific host plants are available (Wood et al., 1999). The protective egg froth females lay over their egg masses contains a chemical attractant that leads to egg mass clustering among conspecific females (Wood and Patton, 1971). Larger clusters encourage the presence of attendant ants that positively affect juvenile survivorship (Wood, 1982). Additionally, communication among mates occurs through vibrational signals that can only be sent and received by individuals on the same branch (Rodriguez et al., 2004). Thus philopatry is likely under
strong selection because of the role it plays in juvenile survivorship and mate recognition.

We used a long term experiment to determine the relative strength of both philopatry and host plant preference and performance during the initial stages of a host shift. Insects from one population on *Viburnum lentago* were collected and distributed into large experimental cages that were permanently located outdoors and exposed to the natural elements. Each cage contained two host plants in three different treatments: two ancestral host plants (the Nannyberry, *V. lentago*), two novel host plants, or one ancestral and one novel host plant. The novel host plant (the congeneric Wayfaring tree, *V. lantana*) is not native to North America and is therefore not believed to have been a host plant in the evolutionary history of *Enchenopa binotata*. Previous results from this project demonstrated genetic variation for preference and performance on the novel host plant; therefore natural selection should be able to act on these traits during a host shift (Tilmon et al., 1998). Additionally, a pilot study using the same host plants showed strong philopatry on both the ancestral *V. lentago* and the novel *V. lantana* (Wood et al., 1999). We assessed philopatry during the first four years of the experiment and host plant preference and performance after eight years (to allow time for host plant adaptation). *Enchenopa binotata* is univoltine, so each year is one insect generation.

### 1 Materials and Methods

In 1994 and 1995, six cages were constructed on the grounds of the University of Delaware. These cages were 2.4 meters tall, by 1.8 meters wide, by 9.1 meters long. They were screened to prevent gene flow in or out, as well as to protect *Enchenopa binotata* populations against predation. Two trees were planted in each cage, 7.3 meters apart, well within the dispersal ability of *E. binotata* (Wood and Guttman, 1981; Guttman et al., 1989). Two species of trees from the genus *Viburnum* were used. These congeneres were chosen so as to minimize asynchrony (as evidenced from an earlier study: Wood et al., 1999) and therefore only reflect the host fidelity aspects of the Host Water Phenology Hypothesis. Additionally, clones of each species were used to minimize genetic variation. One of the hosts (*V. lentago*) was native and known to support natural populations of *E. binotata*, and one host (*V. lantana*) was introduced from outside the range of *E. binotata*, precluding any possible ancestral association. The insects for the study came from *V. lentago*, so this was designated as the ancestral host. There were four replicates each of three treatments, as follows: *V. lentago-V. lentago, V. lentago-V. lantana, and V. lantana-V. lantana*. The cages were part of a larger study of sympatric speciation, and the hosts in each cage reflect part of that design. Cages with one of each host species (*V. lentago-V. lantana*) represent sympatric conditions (i.e., insects had access to both host plant species). Cages with two of the same host species (either *V. lentago-V. lentago* or *V. lantana-V. lantana*) represent allopatric isolated populations (i.e. access to alternate hosts was prevented by a barrier). These were set up in a block design. In the fall of 1995 (after mating had occurred), 12,000 females were collected from multiple trees in a stand of *V. lentago* in Winchester, Virginia. The females were then released in groups of 500 onto each host plant.

#### 1.1 Philopatry at mating and oviposition

Each year from 1995 to 1998 adults from one tree in each cage were marked on their pronota with white paint shortly after eclosion and prior to mating and oviposition. The host plant where insects were marked varied and was determined primarily by which host plant appeared to have the fewest individuals. These markings do not affect movement (Wood and Guttman, 1982; Tilmon, unpublished). Daily counts were made during the mating period and the oviposition period to determine location of mating and oviposition. We also recorded the number of mated pairs that were from the same host plant (either both originating on that host or the alternate host) or from different host plants (mixed matings). Individuals found mating or ovipositing on their host of origin were labeled as “residents” while those from the alternate host were called “non-residents.” Chi-square tests were used to determine if location of mating and oviposition of an individual deviated from the null expectation of 50% per host plant. Chi-square tests were also used to assess whether the proportion of mixed and within host mating pairs differed from the null (25% within mating from host 1, 25% within host mating from host 2, and 50% mixed matings).

#### 1.2 Preference and performance

To allow time for local adaptation, ovipositional preference for and performance ability on the novel host was assayed in 2003, eight years after the experiment began. Due to low population size at this stage of the experiment, we were unable to collect from all four cages per treatment, restricting sampling to two or three cages. In the fall, after mating, up to 28 females were collected from each tree across all three treatments. Fourteen of these females were isolated on individual *V.
lentago branches on additional host plants outside the cage, and 14 were isolated on individual V. lantana branches, also outside the cage. Sleeve cages were used to confine individual females to a single branch so that egg masses could be assigned to a given female. Each of these groups of 14 was split, with seven going to each of two plants per plant species (Fig. 1). This was used to account for individual host differences, although it should be noted that each plant is part of the same clone, minimizing genetic variation. These females remained isolated until late November when egg laying had been completed and the females had died. The sleeve cages were then removed (to avoid excessive damage to the branches under winter weather conditions) and egg masses counted to determine number of egg masses produced per female. The number of egg masses laid has been strongly correlated to number of hatched nymphs (TKW, unpublished). Females collected from the ancestral V. lentago and transferred to V. lentago served as a control to indicate the number of egg masses a female from the original population can lay. Deviations from this in the number of egg masses a female can lay are related to her physiological development on a host plant (performance) and her behavioral response to a host plant (preference). Although we used the number of egg masses to assay both preference and performance, different comparisons allowed us to disentangle these two effects. To assay preference we compared the ovipositional output of females who had developed on the same host plant species (the novel host V. lantana) but had been presented at oviposition with either the novel host plant or the ancestral V. lentago. This isolated the effect of behavior from development. To assay performance differences we compared the average number of egg masses females from the novel V. lantana laid on the novel host to the average number of egg masses females from the ancestral V. lentago laid on the novel host. In this comparison females developed on different host plant species but were presented with the same host plant species at oviposition in order to isolate the effects due to development from those of behavioral host plant acceptance.

Data from the main transfer treatments were analysed using the Kruskal-Wallis tests (Kruskal and Wallis, 1952). This non-parametric test was used because the data exhibited non-normal distributions resulting primarily from the large number of females that laid zero eggs. Individuals who lay zero eggs are biologically significant to the costs of a host shift, so our analyses needed to take these into account. In order to detect possible confounding aspects of the experimental design, Kruskal-Wallis tests were done between allopatric and sympatric conditions, the different cages from which the females were sampled, and the individual host to which they were transferred to see if they affected ovipositional performance. A Bonferroni correction was used for the multiple comparisons. No effects from either the original cage or the transfer host were expected, as all individual host trees used in the experiment are clones from one individual per species.

2 Results

2.1 Philopatry at mating and oviposition

Philopatry at oviposition was represented by the proportion of females found ovipositing on the host on which they were born and was determined for each cage across each of the four years assayed (Table 1) testing the proportion of residents against the null hypothesis of 50%. With few exceptions, females were much more likely to remain or return to their host plant during oviposition. Variation among cages and years precluded pooling of the data, but to provide a general summary the overall proportion of females ovipositing on their host of origin was 68% and of the cages where statistically significant philopatry was found the proportion ranged from 55% to 100%.

Philopatry at mating was represented two ways. The proportion of mated pairs where both male and female were residents (Table 2) was measured in order to re-
reflect the restriction of gene flow due to philopatry. We tested the proportion of resident-resident matings against the null hypothesis of 25%. As with philopatry at oviposition considerable variation precluded pooling of the data, so they are presented for each cage across all four years. Again, with few exceptions individuals were much more likely to be observed mating on their host plant of origin and with another individual from that same host plant. The overall proportion of mated pairs where both individuals were residents was 56%. When philopatry was detected, the proportions ranged from 60% to 91%.

Philopatry at mating was also represented as the number of individuals observed mating on their host plant of origin to reflect the innate philopatry of each individual during mating, regardless of the choice of mate (Table 3). While there were more instances here than in the data on mated pairs in which the proportion of residents that we observed mating did not differ from the null (50%), the number where nonresidents were more likely to be found did not change (two overall) and the majority of cages reflected philopatry at mating across all years. As before, variation precluded pooled data, but for illustrative purposes the overall proportion of individuals found mating on their host plant was 67%. When philopatry was detected, the proportions ranged from 60% to 91%.

2.2 Preference and performance

To detect possible confounding effects, Kruskal-Wallis tests were used to investigate differences within each transfer treatment among cage designs (allopatric vs. sympatric), among individual cages, and among the host plants were females were transferred. A Bonferroni correction was used to account for multiple comparisons (Appendix). As before, variation precluded pooled data, but for illustrative purposes the overall proportion of individuals that we observed mating did not differ from the null (50%). The number of residents observed mating on their host plant was 56%, and when the proportion of mated cages that reflected philopatry at mating across all years was 67%, and when the proportion of mated cages reflected philopatry at mating across all years was 67%. When philopatry was detected, the proportions ranged from 60% to 91%.

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Table 1 Philopatry at oviposition displayed as the percent of females that laid eggs on their host of origin

| Year | Viburnum lentago-Viburnum lentago | Viburnum lentago-Viburnum lantana | Viburnum lantana-Viburnum lantana |
|------|----------------------------------|----------------------------------|----------------------------------|
| 1995 | 90% (402) | 82% (669) | 85% (531) | 83% (466) | 82% (631) | 23% (771) | 75% (582) | 35% (801) | 95% (201) | 100% (302) | 86% (328) | 87% (238) |
| 1996 | 75% (436) | 66% (609) | 59% (451) | 52% (377) | 55% (448) | 64% (573) | 61% (480) | 68% (586) | 51% (364) | 52% (359) | 52% (386) | 50% (385) |
| 1997 | 70% (988) | 64% (692) | 62% (491) | 50% (164) | 55% (73) | 64% (915) | 61% (774) | 68% (950) | 83% (81) | 75% (206) | 63% (164) | 64% (167) |
| 1998 | 56% (164) | 67% (456) | 69% (543) | 61% (108) | 86% (72) | 85% (27) | 61% (322) | 63% (43) | 69% (26) | 79% (98) | 85% (118) | 71% (124) |

Sample sizes are presented in parentheses and P-values indicate the significance of departure from a null expectation of 50% (equal likelihood of female ovipositing on their birth host or the alternate host). Data are from the four cages in each of the three treatment types (two ancestral Viburnum lentago plants, one ancestral V. lentago plant and one novel Viburnum lantana plant, or two novel V. lantana plants) for each of the four years studied. Cages where the proportion of ovipositing females on the host of origin did not significantly differ from the null of 50% are highlighted in gray. Cages where the proportion of females ovipositing on the alternate host was higher than the proportion ovipositing on the host of origin are indicated in bold.
### Table 2  Philopatry at mating displayed as the percent of mating pairs where both male and female were residents

| Year | Viburnum dentatum-Viburnum dentatum | Viburnum dentatum-Viburnum latifolia | Viburnum latifolia-Viburnum latifolia |
|------|-------------------------------------|-------------------------------------|-------------------------------------|
|      | 15 | 42 | 54 | 61 | 15 | 42 | 54 | 61 | 15 | 42 | 54 | 61 |
| 1995 | 78% (27) | 71% (41) | 67% (27) | 65% (26) | NA | NA | 63% (49) | NA | 21% (19) | NA | P = 0.7891 |
| 1996 | 71% (48) | 75% (142) | 69% (55) | 70% (20) | 54% (144) | 8% (164) | 62% (69) | 7% (119) | 29% (14) | 81% (36) | 56% (43) | 83% (23) |
| 1997 | 71% (178) | 61% (175) | 53% (118) | 86% (22) | 42% (59) | 54% (347) | 45% (179) | 77% (275) | 88% (24) | 60% (65) | 60% (47) | 46% (33) |
| 1998 | 69% (62) | 53% (59) | 60% (58) | 50% (6) | 83% (18) | 44% (9) | 52% (124) | 46% (11) | 43% (14) | 38% (72) | 60% (50) | 70% (43) |

Sample sizes are presented in parentheses and P-values indicate the significance of departure from a null expectation of 25% (likelihood of both male and female in a pair from the host plant where they were found mating being due to chance). Data are from the four cages in each of the three treatment types (two ancestral Viburnum dentatum plants, one ancestral V. dentatum plant and one novel Viburnum latifolia plant, or two novel V. latifolia plants) for each of the four years studied. Gray highlighting is used to indicate cases where the proportion of mated pairs in which both male and female were residents did not significantly differ from the null of 25%. Bold font is used to indicate cases where the proportion of mated pairs in which both male and female were residents was less than the null of 25%.

### Table 3  Philopatry at mating displayed as the percent of individuals in a mating pair that were born on the plant where they were found mating

| Year | Viburnum dentatum-Viburnum dentatum | Viburnum dentatum-Viburnum latifolia | Viburnum latifolia-Viburnum latifolia |
|------|-------------------------------------|-------------------------------------|-------------------------------------|
|      | 15 | 42 | 54 | 61 | 15 | 42 | 54 | 61 | 15 | 42 | 54 | 61 |
| 1995 | 87% (54) | 83% (82) | 83% (54) | 81% (52) | NA | NA | 80% (98) | NA | NA | 45% (38) | NA | P = 0.5164 |
| 1996 | 83% (96) | 85% (284) | 80% (110) | 80% (40) | 68% (288) | 23% (328) | 74% (138) | 14% (238) | 46% (28) | 85% (72) | 74% (86) | 91% (46) |
| 1997 | 80% (356) | 74% (350) | 60% (236) | 91% (44) | 58% (118) | 67% (694) | 51% (358) | 78% (550) | 90% (48) | 74% (130) | 73% (94) | 56% (66) |
| 1998 | 75% (124) | 68% (118) | 71% (116) | 75% (12) | 86% (36) | 50% (18) | 67% (248) | 59% (22) | 61% (28) | 63% (144) | 71% (100) | 77% (86) |

Sample sizes are presented in parentheses and P-values indicate the significance of departure from a null expectation of 50% (equal likelihood of an individual mating on their birth host or the alternate host). Data are from the four cages in each of the three treatment types (two ancestral Viburnum dentatum plants, one ancestral V. dentatum plant and one novel Viburnum latifolia plant, or two novel V. latifolia plants) for each of the four years studied. Cages where the proportion of mating individuals on the host of origin did not significantly differ from the null of 50% are highlighted in gray. Cages where the proportion of mating individuals on the alternate host was higher than the proportion ovipositing on the host of origin are indicated in bold.
and development on the host plant. To assay the evolution of host plant preference after eight years, we compared individuals from the novel *V. lantana* on *V. lentago* to those on the ancestral *V. lentago* (Fig. 2). We found that these females laid more egg masses on average (*P* = 0.004) on the ancestral *V. lentago* ($\bar{x}$ = 5.41, *n* = 42) than on the novel *V. lantana* where they had been collected ($\bar{x}$ = 2.58, *n* = 45). To assay performance adaptation to the novel *V. lantana*, we compared the average number of egg masses laid by females from the novel *V. lantana* to the average number of egg masses laid by females from the ancestral *V. lentago* on the novel host (Fig. 2). It was found that when females are taken from the ancestral *V. lentago* and placed on the novel *V. lantana* there was no statistical difference in the number of egg masses they lay ($\bar{x}$ = 3.76, *n* = 70) as compared to the females that were sampled from the *V. lantana* populations and transferred to a *V. lantana* ($\bar{x}$ = 2.58, *n* = 45) (*P* = 0.128). It was also found that females from the novel *V. lantana* populations experienced an increase in the average number of egg masses produced when transferred to the ancestral *V. lentago* (*P* < 0.005), although they still produced fewer egg masses on the ancestral *V. lentago* as compared to those females from the *V. lentago* associated populations that were transferred to *V. lentago* (*P* < 0.05). We interpret this to mean that there are costs to developing on the novel *V. lantana*.

### 3 Discussion

Using experimental host shifts of *Enchenopa binotata* from a long term field experiment, we found innate philopatry to be a strong and immediate barrier to gene flow while the evolution of preferences for and performance on novel host plants lagged. Specifically, females still expressed a preference for the ancestral host, while performance of the females confined to the novel host was equal on both ancestral and novel host plants. Earlier research demonstrated heritable variation in these experimental populations for host plant preference and performance on the novel hosts (Tilmon et al., 1998). Considering this and noting that host plant specific preferences and performance exist in natural *E. binotata* populations, our experimental insect populations should be able to evolve increased preference for and performance on the novel host plants. Although the sympatric and allopatric cage designs were not a focus of this study, it is interesting to note that philopatry was maintained even when both trees in a cage were of the same species and that preference for and performance on the novel host did not increase in cases where the populations were confined to a cage with access only to the novel host. Given how quickly philopatry works to isolate insects along host plant lines, the evidence supports this as a more important isolating mechanism in the initial stages of a host shift.

The innate philopatry in the *Enchenopa binotata* species complex likely stems from two behavioral traits. First, the protective froth that females deposit over their egg masses contains attractive compounds that encourage egg mass clustering by multiple females (Wood and Patton, 1971). The juvenile *E. binotata* cannot hop or fly and are confined to the branch where they were born, forming large aggregations until maturity. It has been shown that larger aggregations of *E. binotata* juveniles are better at recruiting mutualistic ants than are smaller aggregations (Wood, 1982). Further, these attendant ants have a positive impact on juvenile survivorship. The result is that *E. binotata* populations are often found on one or a few individual host plants, even in a large stand of available conspecific hosts (TKW pers. obs.). Second, *E. binotata* males attract mates using su-

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**Fig. 2** Mean number of egg masses produced by females for the four different transfer treatments

Females collected from the ancestral *Viburnum lentago* and transferred to *V. lentago* served as the control. Host plant preference was assayed by comparing the mean number of egg masses laid by females collected from the novel *V. lantana* and transferred to the novel *V. lantana* to the mean number of egg masses laid by females collected from the novel *V. lantana* and transferred to the ancestral *V. lentago*. The mean number of eggs laid per female was higher on the ancestral *V. lentago* than on the novel *V. lantana* indicating they retained a preference for their ancestral host. Performance on the novel host plant was assayed by comparing the mean number of egg masses laid by females collected from the novel *V. lentago* and transferred to the novel *V. lantana* to the mean number of egg masses laid by females collected from the novel *V. lantana* and transferred to the novel *V. lantana*. The mean number of eggs laid per female was not statistically different between treatments indicating females had not adapted physiologically to the novel *V. lantana*. The number of females for each treatment is shown above the bar for that treatment. Females from the control treatment laid significantly more egg masses than any other treatment.
Philopatry is found across a wide range of organisms, including fish, birds, amphibians, mammals and other insects (Refsnider and Janzen, 2010). Although it is more likely to operate as an isolating mechanism in organisms like phytophagous insects on long lived hosts (where the host plant is both food and home), it can act to reduce gene flow in any group where the natal habitat is the site of mating and birth. The evolution of philopatry is a single trait mechanism and is therefore not disrupted by gene flow early on (Balkau and Feldman, 1973). More attention needs to be paid to philopatry in the context of speciation genetics.

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### Appendix

| Transfer Treatment | Geographic Cage Design | Individual Cage | Individual Transfer Host |
|--------------------|------------------------|-----------------|--------------------------|
| *V. lantana* to *V. lantana* | *P* = 0.30 | *P* = 0.33 | *P* = 0.09 |
| *V. lantana* to *V. lentago* | *P* = 0.09 | *P* = 0.14 | *P* = 0.27 |
| *V. lentago* to *V. lantana* | *P* = 0.22 | *P* = 0.66 | *P* = 0.20 |
| *V. lentago* to *V. lentago* | *P* = 0.71 | *P* = 0.03 | *P* = 0.07 |

Geographic Cage Design refers to the host plant species in a cage (either sympatric with one *Fiburnum lentago* and one *V. lantana* or allopatric with two of the same host species). The non-parametric Kruskal-Wallis test was used in all cases. Numbers presented are the resulting *P*-values. Due to multiple comparisons, a Bonferroni correction was used. After the correction α=0.05 becomes α=0.02. No factors were significant.