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The role of host-plant fidelity in initiating insect race formation

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ABSTRACT

Advocates of sympatric speciation postulate that habitat-/host-associated mating and reproduction are critical conditions to reduce genetic constraints (recombination and migration) that otherwise impede divergence. To determine whether host-plant fidelity of insects could establish these conditions, we experimentally created sympatric host shifts. We shifted first instars of the treehopper *Enchenopa* (Homoptera: Membracidae) from their host-plant *Viburnum lentago* to three novel *Viburnum* species (*V. lantana*, *V. utile* and *V. prunifolium*). After eclosion, adults were marked and allowed to move freely between original and novel hosts. Hourly observations were made throughout each of the 33 days of the mating period to record host origin and location of individuals in mating pairs. At the onset of oviposition, daily observations were made to record the position within cages of marked ovipositing females and observations were made every 3 days to determine the location of every female within a cage. Under the conditions of this experiment, when dispersal distances between original and novel hosts were small, male and female host fidelity contributed to non-random mating and oviposition. Although some adults dispersed back to the original host, most did not. In the control treatment, containing two presumably equally 'acceptable' *V. lentago* plants, fidelity to the plant on which adults were raised resulted in non-random mating and oviposition. Our results suggest that host fidelity in the initial stages of a host shift could be a facilitating factor that allows divergent selection on host-associated performance traits and perhaps eventually host preference.

Keywords: *Enchenopa*, gene flow, insects, plants, speciation, sympatric.

INTRODUCTION

For some phytophagous insects, changes or shifts in host-plant usage are hypothesized to initiate disruptive selection leading to specialization and divergence (Bush, 1975, 1994; Wood, 1993). There is little doubt that many phytophagous insects are host-plant specialists in that they are restricted to a single species or a limited array of species (Strong *et al*., 1984). Most documented cases of host shifts involve the introduction of insect phytophages

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to novel habitats or situations where localized habitat destruction has forced changes in host-plant use (Strong *et al.*, 1984). For example, *Rhagoletis* (Bush, 1994), *Papilio* (Thompson, 1993) and *Euphydryas* (Singer *et al.*, 1993) have shifted to novel host-plants within recent history suggesting host specialization can occur rapidly. In many examples, complete or partial spatial isolation was involved for some period of time in the process of specialization.

What is controversial is whether specialization can occur when both original and novel hosts are within close proximity and equally abundant, allowing reciprocal movement of insects between hosts for protracted periods of ecological or evolutionary time. Divergence-with-gene-flow models (Templeton, 1989) are often invoked to explain sympatric host race formation and speciation in insects (Tauber and Tauber, 1989). The issue with these hypotheses is whether there are factors that override the major genetic constraints of recombination and migration (Felsenstein, 1981; Rice and Hostert, 1993) that otherwise impede divergence. How can a population respond to 'specializing' (directional) selection when it faces potential genetic influx from populations exposed to different selective regimes, or when it has the potential to disperse in favour of another habitat?

Simulation models and laboratory experiments suggest genetic constraints can be overcome when mating and reproduction take place within a habitat or host (Maynard Smith, 1966; Diehl and Bush, 1989; Kondrashov and Mina, 1986; Rice and Hostert, 1993). There are two explanations how the essential elements of host-associated mating and reproduction may be initially achieved. One hypothesis suggests that, when dispersers are presented with a choice of host-plants, pre-existing genetically determined variation in preference allows colonization of novel hosts. Thus, it is host preference that initially establishes host-plant-associated mating and reproduction (Diehl and Bush, 1989). Alternatively, we suggest that, in some cases, all that is required for colonization is that the novel host be within the range of 'acceptability' for oviposition and survival of offspring. Persistent colonization could be achieved if fidelity to that host was maintained by adult offspring in succeeding generations. Under both hypotheses, once novel hosts are colonized and mating/reproduction occurs on the host, then selection on genetically variable performance traits could lead to directional, divergent host specialization.

Host-plant discrimination, or preference, is sometimes invoked as a promoter of, and sometimes as an impediment to, host shifts. Some argue that pre-existing variation in host preference can lead to spontaneous novel host shifts (Diehl and Bush, 1989; Bush, 1994), whereas others argue that host preference hierarchies are conservative (Thompson, 1993), acting like other performance traits as constraints making host shifts unlikely in a sympatric host-plant setting (Futuyma *et al.*, 1993). Whether host preference precedes or is the result of a host shift is open to question and may depend on the types of insects studied (Via, 1990).

It is important to note that many host specialization studies often involve highly mobile insects (e.g. Lepidoptera) whose reproductive biology, hosts (annuals or herbaceous perennials) and heterogeneous habitats dictate that individuals must make frequent host selection decisions based on preference (Thompson, 1988; Singer *et al.*, 1989; Thompson and Pellmyr, 1991). A distinction needs to be drawn, however, between host preference and host fidelity (philopatry). For various biological reasons, not all insects are highly mobile and individuals are not constantly forced to choose a particular host species. For example, heteropterous insects that utilize large, long-lived hosts such as trees and shrubs can sustain large populations on individual plants for relatively long periods of ecological time

(Edmunds and Alstad, 1978; Southwood, 1978). It is these kinds of insects where host fidelity could play a role in the initial stages of a host shift.

Habitat fidelity may be a critical initial stage in host specialization when the hosts involved are long-lived trees. If the offspring of colonizing individuals find themselves on a novel plant that is not 'unacceptable', and a large proportion remain to mate and reproduce, then selection imposed by the plant on performance traits could promote divergence. In this sense, both host preference and host fidelity bring about the same essential initial conditions of host-associated mating and reproduction hypothesized as requirements for sympatric divergence (Via, 1990). An additional requirement would be the existence in the colonizing population of genetic variation in critical performance traits that would allow adaptation to a new host species. This genetic variation should manifest itself in a genotype by host interaction where some genotypes perform better on one host than another. Such interactions would allow selection for genotypes adapted to particular host species (Tilmon, 1995). Divergence, however, could not occur if there was substantial reciprocal migration and very moderate selection. Philopatry or host fidelity during mating and reproduction could reduce the effect of reciprocal migration in the early stages of a host shift. Host fidelity of a colonizing population may be essential to allow selection for host or habitat preference, which has been shown experimentally to evolve rapidly (Rice and Salt, 1990).

Discerning the factors that promote divergence is an inferential process for extant species such as the *Enchenopa* species complex (Homoptera: Membracidae). Observation and experimental manipulation of this extant species complex suggest that speciation occurred through host shifts in sympatry (Wood, 1993). But those characteristics which delineate closely related species in sympatry, including host preferences and reproductive isolation mechanisms, can either be interpreted as the cause or the result of the speciation event. Phylogenetic reconstruction can lend support for a postulated mechanism of speciation if relationships are consistent with predictions of the speciation model. For the *Enchenopa* species complex, phylogenetic evidence supports a model of sympatric divergence through host shifts. But no matter how supportive experimental and phylogenetic inferences are, in a strict sense they do not prove the speciation hypothesis.

In this paper, we investigate the role of philopatry in the initial stages of an experimentally created host shift. We ask whether philopatry could be a contributing factor to initially disrupt reproductive cohesion between insects on original and novel hosts in the absence of spatial considerations. This study was designed to assess the feasibility of establishing a long-term experimental field test to determine if sympatric host shifts can lead to specialization and ultimately speciation.

Life history of the *Enchenopa* species complex

The life histories of members of the *Enchenopa binotata* species complex (in the process of being formally named) are well known (Wood, 1980, 1993; Guttman *et al.*, 1981, 1989; Wood and Guttman, 1981, 1982, 1983; Guttman and Weigt, 1989; Wood and Keese, 1990; Wood *et al.*, 1990; Pratt and Wood, 1992, 1993; Hunt, 1994). For present purposes, it is important to know that the life history of the univoltine species associated with *Viburnum lentago* begins with synchronous egg hatch in May (within a 12 day period). The timing of egg hatch is an effect of host-plant phenology, although there is a genetic component to egg development time (T.K. Wood, unpublished data). In general, nymphal development

takes over a month. The time interval between eclosion and the onset of mating is roughly 3 weeks. Mating occurs over a 4 week period where females mate once but males can mate several times. When the mating period for the population is completed, there are very few living males. Mating is followed by approximately 4 months of oviposition. Throughout oviposition, females may deposit 20 or more egg masses containing 6–10 eggs each.

METHODS

To directly determine the role of host fidelity in the initial stages of a host shift, we performed experimental host shifts using an *Enchenopa* species which occurs on *Viburnum lentago* (Table 1). In the field, we established three pairs of sympatric hosts, each consisting of a *V. lentago* (original host) plus one of three novel hosts in the genus *Viburnum*. We also established a *V. lentago–V. lentago* control. All the individual *V. lentago* plants were clones. The four treatments, each enclosed within a 1.8 m^3 cage, were as follows: a *V. lentago* plant with a *V. lentago* plant; *V. lentago* with *V. lantana*; *V. lentago* with *V. prunifolium*; *V. lentago* with *V. utile*. The critical condition of the experiment was to maximize the potential for insect movement between trees to promote random mating and oviposition. Thus each pair of trees was separated by only 75–125 cm and paired trees were approximately the same size. Such minimal distances are well within the normal dispersal range of individual *Enchenopa*, while eliminating confounding factors such as the transmission of substrate-borne vibrational mating signals (Hunt, 1994). There were sufficient resources for one replicate of each treatment. To standardize the sympatric treatments, the *V. lentago* plants were planted in the same polar position (south) in each of the three original–acquired host combinations.

Individual females were isolated in 1993 on *V. lentago*. When egg hatch occurred in the early spring of 1994, their offspring were transferred by camel hair brushes to both *V. lentago* and to one of the novel *Viburnum* species – *V. lantana*, *V. utile* or *V. prunifolium* – and to the *V. lentago–V. lentago* control. Nymphs were confined to individual plants or to branches covered by sleeve cages. Although we attempted at egg hatch to maintain an even split family design between *V. lentago* and particular novel *Viburnum*, by the time adult eclosion occurred, some families had experienced extinction on one or the other host. The spirit of the split family approach was used to minimize potential genetic differences in development time and mate recognition signals between insects on original and novel host-plants.

Shortly after eclosion, adults were marked with enamel paint on their pronota to indicate host origin. Marked adults were temporarily confined to sleeve cages on each host-plant. On the day before mating was anticipated to begin (14 July 1994), the sleeve cages were removed from the branches to permit free movement of insects within and between trees in the cages throughout the 33 day mating period. To observe matings, we made observations every daylight hour each day for 33 consecutive days to record the host origin of individuals in mating pairs and the host where mating occurred. To obtain another measure of host fidelity, daily records were made to determine the position and origin of each adult within a cage.

We calculated the expected mating frequencies of the eight possible mating combinations if mating was random, and used chi-square to determine whether expected and observed differed. In the *V. lentago–V. utile* combination, the low number of matings resulted in

expected frequencies of less than 5, invalidating the chi-square test. In that combination, the classes were collapsed to four to test whether females were randomly mating on the respective hosts.

Since we knew the daily location of adults, we determined the proportion of the individuals that remained on their release host. We summed over 3 day intervals the number of males or females remaining on their release host and divided that figure by the sum of those observed within the cage during the same interval to determine the proportion of individuals that remained as residents or dispersed to the other host. These proportions were arc-sine transformed. The slopes (Table 2) were compared using an autoregression model with an interaction between a tree indicator ('dummy') variable and time (Neter and Wasserman, 1974). Because performing the Durbin-Watson test indicated serial autocorrelation, the first-order autoregressive model was calculated using the AUTOREG procedure of SAS.

Oviposition is a prolonged process necessitating a slightly different protocol to observe females depositing egg masses and to determine patterns of dispersal. Females on *Viburnum* begin to oviposit in the morning when temperatures reach approximately 15°C. When temperatures reached this level, daily observations of each plant were made twice within a 2 h period to record the host and origin of females depositing egg masses. Every 3 days we recorded the host position and origin of each female in the cages. Daily oviposition records and 3 day records of location were analysed in a similar way to the data collected during mating.

RESULTS

We initially ask some general questions (Table 1) regarding experimental design, sexual differences and original–novel host factors which could affect the proportions of residents and dispersers observed in mating pairs (Table 2). We define residents as individuals released and observed on a plant and dispersers as those observed on the opposite plant. The nature of the experimental design where *V. lentago* plants were placed on the south and novel hosts on the north could result in directional dispersal. Using a chi-square test of independence (2×2 tables), we found no evidence that (in the absence of treatment factors) males or females dispersed more in one polar direction than the other. We also found that males and females did not differ in their tendency to be residents or dispersers (Table 1).

When we ask whether males released on *V. lentago* differed in their tendency to be residents or dispersers compared to those on novel hosts, we found they were homogeneous in their proportions. However, in a test for homogeneity between females raised on *V. lentago* and those on novel hosts, we found they were not. To locate the source of heterogeneity, we tested for homogeneity among the novel hosts in the proportion of resident and dispersing females. As expected, females on the three novel hosts were not homogeneous with respect to proportion of residents and dispersers. Examination of the data suggested that the source of heterogeneity was females from *V. prunifolium*. When *V. prunifolium* females were removed from the analysis, females on *V. lantana* and *V. utile* were homogeneous in their tendency to disperse. In fact, females from these hosts were homogeneous in their tendency to disperse when compared to those on *V. lentago*. Thus, the only evidence that insects on novel hosts behave differently from those on the original *V. lentago* in terms of their tendency to disperse was females from *V. prunifolium*. Our data

Table 1. Chi-square tests for independence for experimental factors that could affect the proportion of residents and dispersers observed in mating pairs (Table 2)[a]

Position effect

	Males		Females	
	Residents	Dispersers	Residents	Dispersers
South	117	34	128	32
North	97	50	99	71
	Adjusted $\chi^2 = 0.95$		Adjusted $\chi^2 = 0.49$	

Comparison by sex

	Residents	Dispersers
Males	214	84
Females	227	71
	Adjusted $\chi^2 = 0.26$	

Novel host–*V. lentago* comparison

	Males		Females	
	Residents	Dispersers	Residents	Dispersers
All *V. lentago*	141	51	158	38
All novel	73	33	69	33
	Adjusted $\chi^2 = 0.31$		Adjusted $\chi^2 = 4.87$ *	

Comparison of females on novel hosts

	Residents	Dispersers	Residents	Dispersers
V. lantana	31	9	31	9
V. utile	15	2	15	2
V. prunifolium	23	22		
	$\chi^2 = 10.66$ ** (d.f. = 2)		Adjusted $\chi^2 = 0.01$	

Females from *V. lentago* with those from *V. lantana* and *V. utile*

	Residents	Dispersers
V. lantana	31	9
V. utile	15	2
V. lentago	158	38
	$\chi^2 = 1.145$ (d.f. = 2)	

[a] Residents are individuals that mated on the host they were released onto and dispersers are individuals that moved to the other host. * $P \leq 0.05$; ** $P \leq 0.005$.

do not allow us to distinguish between what could be perceived as female preference for the original *V. lentago* and other factors such as female crowding, plant health or plant architecture.

Table 2. A test of the role of host plant fidelity in promoting non-random mating among treehoppers (*Enchenopa*) in a first-generation sympatric host shift[a]

	Plant polar position								
	South				North				
Male/female pairs:	SS	SN	NS	NN	NN	NS	SN	SS	χ^2
V. lentago × *V. lentago*									
Observed matings	21	3	14	3	23	1	7	0	63.2**
Gene flow	31/144 = 21.53%								
Gene flow from opposite plant	23/82 = 28.1%				8/62 = 12.9%				4.8*
V. lentago × *V. lantana*									
Observed matings	22	8	7	1	25	14	6	6	51.1**
Gene flow	49/178 = 27.53%								
Gene flow from opposite plant	17/76 = 22.4%				32/102 = 31.4%				1.77
V. lentago × *V. prunifolium*									
Observed matings	29	11	14	11	19	3	4	2	49.5**
Gene flow	58/186 = 31.18%								
Gene flow from opposite plant	47/130 = 36.2%				11/56 = 19.6%				5.0*
V. lentago × *V. utile*									
Observed matings	21	2	0	0	10	2	5	4	27.6**
Gene flow	17/88 = 19.32%								
Gene flow from opposite plant	2/46 = 4.3%				15/42 = 35.7%				13.9**

[a] Categorization of individuals observed in mating pairs are by plant position and release origin. Column headings of south and north indicate the position/host species where matings took place. Subcolumn headings of S or N are used to indicate first the origin of the male and then the female (first row in each treatment). Gene flow indicated in the second row for each treatment is simply the number of individuals which mated off their release host divided by the total number of individuals in mating pairs. The third row in each treatment indicates the number of individuals that dispersed and mated divided by the total number of individuals mating on that plant. Chi-square values in this row indicate a test of equality of proportions. * $P \leq 0.05$; ** $P \leq 0.005$.

Mating

The insects in the three host shift treatments and the controls (*V. lentago–V. lentago*) exhibited non-random mating with respect to mating site, and male and female host origin (Table 2). Since females mate only once, whereas males can mate more often [but most often don't (Wood and Guttman, 1982)], a conservative estimate of gene flow during the mating period can be obtained by calculating the proportion of individuals that mated off their release host on the opposite tree. As directly measured by observed matings, there was only 22% gene flow between insects on the two plants in the control (Table 2). In the host shift treatments, there was gene flow (Table 2) between the two insect populations on original and novel hosts of 19.32% (*V. lentago–V. utile*), 27.53% (*V. lentago–V. lantana*) and 31.18%

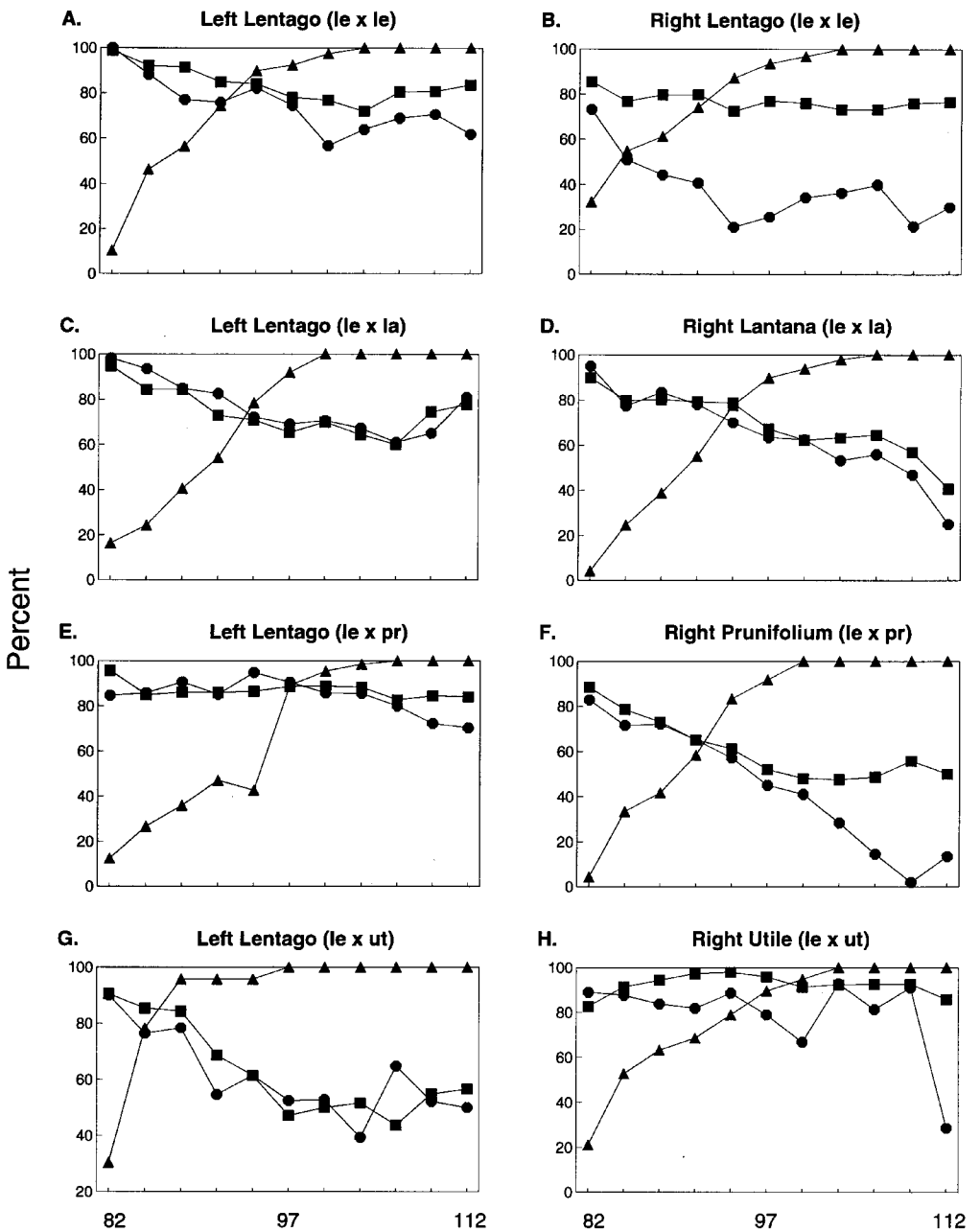

Fig. 1. Host-plant fidelity of males and females during mating. Untransformed percentages of males (circles) and females (squares) are plotted to show temporal changes in the proportion of individuals within the cage remaining on their release host. Triangles indicate cumulative percent of matings over time (days) that occurred on that plant. Day 82 was 16 July 1994 and day 112 was 15 August 1994.

Table 3. Temporal changes in survival and host fidelity throughout mating as indicated by slopes derived from autoregression analysis[a]

	Plant polar position			
	South		North	
	Survival	Host fidelity	Survival	Host fidelity
V. lentago × *V. lentago*				
Males	–0.78	–0.87	–0.78	–0.62
Females	–0.57[B]	–0.54[B]	–0.74	–0.16[B]*
V. lentago × *V. lantana*				
Males	–0.84	–0.75	–0.37*	–1.09
Females	–0.78	–0.49[B]	–0.47*	–0.84[B]
V. lentago × *V. prunifolium*				
Males	–0.60	–0.37	–0.89*	–1.60*
Females	–0.34	–0.19[A]	–0.56[B]*	–0.77[B]*
V. lentago × *V. utile*				
Males	–0.70	–0.85	–0.57*	–0.54[A]
Females	–0.92	–0.90	–0.62*	–0.03[A]*

[a] Slopes presented for host fidelity are untransformed, while statistical analysis was done on arcsine transformed data. Slopes for survival were calculated using the number of males or females recorded in daily counts that were originally released on that plant. Significance levels are $P \leq 0.05$. [A] Slope does not differ from zero; [B] slopes of males and females on that plant differ; * slopes of that sex differ between plants.

(*V. lentago–V. prunifolium*). None of the sympatric treatments differed from the control (χ^2 test of homogeneity). This value of gene flow is misleading, since it does not account for the asymmetrical gene flow that may take place in the initial stages of a host shift. Such asymmetrical gene flow is characteristic of two of the three experimental sympatric treatments and the control (Table 2). If host preference was highly developed, movement from the novel host back to the original host would be predictable, resulting in asymmetrical gene flow during the approximate 33 day mating period. This was the case for only one of the three sympatric treatments, *V. lentago–V. prunifolium*. Asymmetrical gene flow was achieved in the *V. lentago–V. utile* treatment by movement of insects from the original to the novel host (Table 2). Asymmetrical gene flow did not occur in the *V. lentago–V. lantana* treatment.

The means by which non-random mating and asymmetrical gene flow were achieved are evident when temporal patterns of host fidelity are overlaid with mating activity (Fig. 1). In the *V. lentago–V. lentago* control, female host fidelity, throughout the 33 day observation period, remained relatively constant at around 80% (Fig. 1A,B) but declined differentially over time as indicated by differences in the slopes (Table 3). On one (south) *V. lentago* plant, male host fidelity closely paralleled that of females. On the opposite (north) *V. lentago* plant, male host fidelity declined during the first 10 days of mating. This decline in male host fidelity contributed to the 28% gene flow to the population on the opposite (south) plant. Since approximately 80% of the matings on both plants occurred during this 10 day period, the result was that 87% of the individuals in mating pairs on the north plant were those released on that plant. Thus high male and female host fidelity during the peak of

mating on the south plant interacting with high female but declining male fidelity on the north plant resulted in the observed asymmetric gene flow between the two populations when the trees were only 105 cm apart. This control shows that, when host-plants are equally 'acceptable' and insects are developmentally synchronized, high male–female host fidelity can make a significant contribution to non-random mating and asymmetric gene flow.

Perhaps the most difficult thing for many critics of sympatric speciation to accept is fidelity of insects to a novel host in the initial stages of a host shift. In the *V. lentago–V. lantana* sympatric treatment, the two trees were separated by 125 cm. On both hosts, male and female host fidelity closely paralleled each other (Fig. 1C,D) and began to decline simultaneously. The coincident timing of declining insect host fidelity resulted in symmetrical gene flow between the two hosts (Table 2). The *V. lentago–V. prunifolium* treatment, where trees were separated by 107 cm, perhaps most clearly demonstrates the effect of asymmetric host fidelity on gene flow (Fig. 1E,F). Male and female host fidelity was consistently high on *V. lentago*, with the result that relatively few individuals dispersed to *V. prunifolium*. However, this was not true for *V. prunifolium*, in that both male and female host fidelity declined during the period when only 46–58% of the matings were completed on the respective hosts. It was this asymmetric influx of adults from *V. prunifolium* to *V. lentago* that produced the 36% gene flow in that direction but only a 19% gene flow in the opposite direction to *V. prunifolium*. In the *V. lentago–V. utile* sympatric treatment where trees were separated by 75 cm, mating occurred very quickly on *V. lentago* when male and female host fidelity was high (Fig. 1G,H). Once mating was almost completed, host fidelity declined. On *V. utile*, male and female host fidelity was consistently the highest of all the treatments. The more prolonged mating period on *V. utile* and dispersal from *V. lentago* resulted in a 20% gene flow to the population on *V. utile*. The high level of insect host fidelity on *V. utile* resulted in only a 4% gene flow back to the population on *V. lentago*.

As planned in the experimental design, there were no developmental differences in seasonal mating time (*t*-test) which could obscure the role of host fidelity in promoting non-random mating in either the control or any of the sympatric treatments. Since we are interested in the initial role of host fidelity, differences in mating time would be a confounding variable. Other experiments have shown that differences in life-history timing can also promote or maximize non-random mating (Wood and Keese, 1990).

Differential host-related survival could also promote non-random mating through asymmetry in the availability of mates. To test for this possibility, we used autoregression of daily adult counts against days to calculate and compare slopes to determine whether males or females released on particular trees declined at different rates throughout the mating period. In the control (*V. lentago–V. lentago*), males and females released on either plant did not decline differentially (Table 3). In contrast, in the three sympatric treatments of original and novel hosts, there were differences in male and female estimated survival (Table 3). However, such differential host effects on survival were most evident 14 days after the onset of mating, when 77% of all matings in all treatments were completed, and probably contributed little to the observed pattern of non-random mating.

Oviposition

At the end of November, we had observed 1980 egg masses being deposited. We categorized these data by treatment and by origin of ovipositing females (resident or disperser)

Table 4. Egg masses deposited by females remaining on the release host and those dispersing to the other plant within each of the treatment cages[a]

Treatment	Residents	Dispersers	% Dispersers
V. lentago × *V. lentago*	410	165	28.7
V. lentago × *V. lantana*	180	113	38.6
V. lentago × *V. prunifolium*	378	168	30.7
V. lentago × *V. utile*	212	86	28.6

[a] Percentage of egg masses deposited by dispersers is their contribution to the cage total. The proportions among the four treatments are not homogeneous ($\chi^2 = 9.90$, $P = 0.019$).

Table 5. Distribution of egg masses by plant within each of the four treatments[a]

Treatment	Residents	Dispersers	% Dispersers	χ^2
V. lentago × *V. lentago*				
south	220	91	29.26	0.106
north	190	74	28.03	
V. lentago × *V. lantana*				
lentago (S)	150	101	40.24	2.067
lantana (N)	30	12	28.57	
V. lentago × *V. prunifolium*				
lentago (S)	148	95	39.09	14.248 *
prunifolium (N)	230	73	24.09	
V. lentago × *V. utile*				
lentago (S)	113	65	36.52	12.626 *
utile (N)	99	21	17.50	

[a] Residents are those females originally released on the plant and dispersers are those from the opposite plant. Chi-square tests for each treatment are for equality of proportions. * $P \leq 0.005$.

(Table 4). A chi-square test of homogeneity ($\chi^2 = 9.90$, $P = 0.019$) showed that the four treatments were not homogeneous. The lack of homogeneity was due to the *V. lentago*–*V. lantana* treatment.

A more detailed categorization (Table 5) showed that the movement of females between trees was not symmetrical in all treatments (chi-square test for equality of proportions). In the control (*V. lentago*–*V. lentago*), females that dispersed reciprocally to the opposite plant contributed equal proportions of egg masses. This symmetrical gene flow was expected, since *V. lentago* was the host of origin of the insects in this experiment and both trees were clones and presumably equally 'acceptable' in terms of quality. It should be borne in mind that on both trees over 70% of the egg masses deposited were by females marked and released on that tree approximately 5 months prior to the completion of oviposition.

In the *V. lentago*–*V. lantana* treatment, there was no difference between the respective trees in the proportion of egg masses deposited by female residents and those that dispersed to that plant (Table 5). Although it is tempting to suggest that both host-plants might appear to be equally 'acceptable', the lack of statistical differences is probably due to overall low numbers of egg masses on *V. lantana* owing to female mortality.

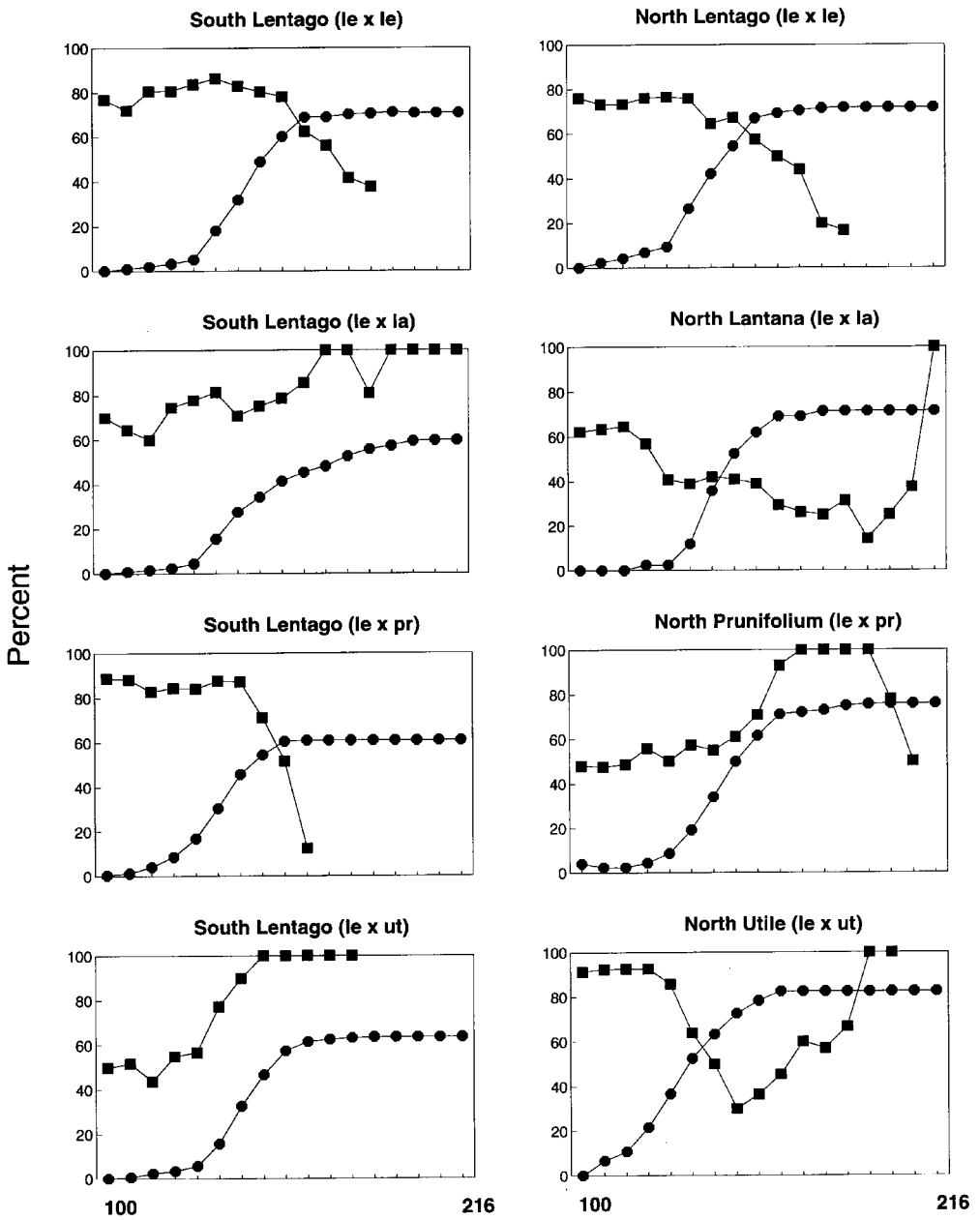

Fig. 2. Female host fidelity during oviposition. Squares represent the percentage of the females within the cage remaining on the plant they were released onto. Circles represent the cumulative percent of eggs deposited by females released on that plant.

The test for equality of proportions of oviposition between residents and dispersers showed they were not equal in the *V. lentago–V. prunifolium* and the *V. lentago–V. utile* treatments. In both these treatments, a higher proportion of egg masses deposited on

V. lentago were from females that dispersed from the opposite novel host (*V. prunifolium* or *V. utile*). In the reciprocal direction, there was a significantly lower proportion of egg masses deposited on the novel host from females that dispersed from *V. lentago*. When these two treatments were individually compared to the control (*V. lentago–V. lentago*) using a chi-square test of homogeneity, they were not homogeneous. The asymmetry shown in the two sympatric treatments where some females moved from the novel hosts back to the original host could indicate some preference for that host. Although some females may have found the novel host 'unacceptable', many did not, because 75.9 and 82.5% of the egg masses deposited on the novel hosts were by females that were raised as nymphs on that host and remained for the 5 month duration of oviposition.

At any time during oviposition, females could potentially switch plants. Our 3 day records of the origin and position of each female within a cage suggest they did not. In the control (*V. lentago–V. lentago*), female fidelity to the tree of release was consistently high during the period when most of the oviposition occurred (Fig. 2). The decline in the proportion of resident females after most of the egg masses were deposited is a reflection of death due to age. The control suggests that, if dispersal occurs, it occurs during the later part of the mating period (Fig. 1) and that there is little movement during oviposition (Fig. 2).

The three sympatric treatments lend support to the above conclusions, in that female host fidelity on the novel hosts declined during the later days of the mating period (Fig. 1). However, during the period when most of the oviposition occurred on these hosts, the proportion of resident females remained at a plateau with only minor fluctuations, probably due to variation in counting. Near the completion of oviposition, what appeared to be major increases in the proportion of resident females on a tree was the result of a decline in numbers within the cage owing to mortality, thus distorting the proportions. In only two of the treatments did the apparent increase in resident females during oviposition appear to be real. In the *V. lentago–V. lantana* treatment, female fidelity to *V. lentago* declined after mating (Fig. 1) and these females appeared to disperse back to *V. lentago* during the early part of oviposition (Fig. 2). The same can be said for *V. lentago* in the *V. lentago–V. utile* treatment (Figs 1, 2).

DISCUSSION

Host shifts probably occur in a localized region where both original and novel hosts co-occur within the dispersal range of the insect. Females may deposit eggs and offspring may survive on novel hosts; however, the host shift may fail if adult offspring disperse *en masse* back to the original host during mating and oviposition (T.K. Wood, unpublished data). The intention of this experiment was to maximize the potential of random mating and oviposition by using (1) small distances between trees, (2) small trees to encourage inter-host movement, (3) developmentally similar adults and (4) split families to minimize any potential genetic variation in mate recognition signals. Thus, the non-random mating and oviposition demonstrated here when dispersal distances were unrealistically small suggests that, once colonization of a novel host occurs, initial host fidelity may be a critical step in initiating divergence. It is host fidelity which in some extant *Enchenopa* species probably contributes to genetic variation of insects associated with trees of the same species within and between localized habitats (Guttman *et al*., 1981, 1989; Guttman and Weigt, 1989).

The demonstration that host fidelity on novel host-plants can promote an asymmetrical

reduction in gene flow in the first generation of a host shift is critical to the development of field-testable speciation models of divergence with gene flow. In this experiment, restricted gene flow between paired *Enchenopa* populations due to host fidelity was higher but approximated the 6–20% estimated gene flow (Barton *et al.*, 1988; Feder *et al.*, 1988, 1994) between apple and hawthorn races of *Rhagoletis pomonella* in abandoned orchards where dispersal distances were several orders of magnitude greater. Neither observed levels of gene flow between races of these flies, nor levels of gene flow in our experimental *Enchenopa*, can result in speciation unless there are high levels of selection (Barton *et al.*, 1988) for host-adapted performance traits or additional mechanisms such as life-history asynchrony, or differences in mate recognition systems which augment isolation. For our experimental host-shifted *Enchenopa*, plant-induced life-history asynchrony could augment host fidelity to allow selection on host associated differences in performance traits (Tilmon, 1995) and ultimately alter patterns of variation in mate recognition signals that may have existed in the original population prior to the host shift.

Other work on this *Enchenopa* experimental system has shown that each of the three novel host-plants used in this experiment have differential effects on important genetic performance traits, such as longevity, fecundity and nymphal viability. These traits show a significant genotype by host interaction, which is necessary for divergence on novel hosts, and a selection experiment showed that progress towards host race formation can be achieved in three generations of isolation (Tilmon, 1995). Although host effects on some performance traits are quite strong, if gene flow is extensive, divergence cannot occur. Host fidelity apparently is an intrinsic trait of these *Enchenopa* treehoppers; it was quite evident in the control as well as two of the host-shift treatments. Such host fidelity, if maintained over time, can serve as an important disruptive force to augment host-plant-induced differences in life-history timing which promote asynchronous mating (Wood and Guttman, 1981, 1982, 1983; Wood and Keese, 1990; Wood *et al.*, 1990). There is a genetic component to egg development time and other traits, but recent work has demonstrated that these novel *Viburnum* affect the timing of *Enchenopa* life history (due to differences in flowering phenology), resulting in asynchronous mating between insects on original and novel hosts in the first generation of a host shift (T.K. Wood, unpublished data). We believe that host fidelity during mating and oviposition, interacting with host-plant-induced asynchronous mating, will provide the degree of prezygotic isolation that permits host-associated divergence in critical genetic performance traits. When this process is overlaid on spatial scales that are ecologically realistic, we hypothesize that the essential requirements for host shifts that could result in speciation are in place. As pointed out by Carson (1989), whether sympatric race formation occurs or not has important implications for insect pest management as well as evolutionary genetics. Like many important conceptual issues in evolutionary biology, race formation in the face of gene flow is amenable to experimental field tests. The results presented here and elsewhere support the feasibility of long-term direct field experiments to address sympatric models of speciation.

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