GENETICS, EXPERIENCE, AND HOST-PLANT PREFERENCE IN *EUROSTA SOLIDAGINIS*: IMPLICATIONS FOR HOST SHIFTS AND SPECIATION

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Abstract.—Host-associated mating is crucial in maintaining the partial reproductive isolation between the host races of *Eurosta solidaginis* (Diptera: Tephritidae), a fly that forms galls on *Solidago altissima* and *S. gigantea*. (We refer to flies reared from *S. gigantea* as gigantea flies and those reared from *S. altissima* as altissima flies.) We measured the host preference of males and females of both host races, F_1 hybrids between the host races, F_2, and backcrosses to both host races. Male and female altissima flies and female gigantea flies had high host fidelity, whereas male gigantea flies had low host fidelity. This result suggests that there may be gene flow between the host races due to nonassortative mating that occurs when male gigantea mate with altissima females on *S. altissima*. This indicates assortative-mating mechanisms in addition to host-associated mating are required to produce the partial reproductive isolation between the host races that has been observed. Nongenetic factors had no influence on host preference. Larval conditioning did not influence host preference: reciprocal F_1 hybrids reared in *S. altissima* and *S. gigantea* both preferred *S. gigantea*. Adult experience had no impact on host preference: females preferred their natal host plant regardless of which host they encountered first as an adult. The hypothesis that maternal effects influence preferences was rejected because male and female flies did not show a consistent preference for the host plant of their mother. We also found no evidence that preference was a sex-linked trait because F_1 and backcrosses to the host races with different combinations of X chromosomes from the two host races preferred *S. gigantea*. Our results indicate that host preference is not determined by a large number of genes because preference of hybrids did not correspond to the proportion of the genome derived from each host race. The strength of the ovipuncture preference for *S. gigantea* by gigantea females, the females of both reciprocal F_1 hybrids, the backcross to gigantea, and F_2s indicates that preference is inherited nonadditively at a limited number of loci. The F_1 female hybrids, however, had a weaker host preference for *S. gigantea* than the pure gigantea host race, indicating that there may be incomplete dominance or modifier loci. Males had different host preference patterns than females, with individual male gigantea and male F_1 hybrids usually exhibiting preference exclusively for *S. gigantea* or *S. altissima*. One hypothesis explaining the difference in host preference between males and females is that the same gene influences both female and male host preference, but it is a sex-influenced gene. Thus, males carrying the gene for *S. gigantea* preference have an intermediate host preference, whereas females have a strong host preference to *S. gigantea*. In summary, we found that the host preference that produces host-associated mating is inherited nonadditively at a relatively small number of loci on autosomal genes. This mode of inheritance meets the assumptions of models of sympatric speciation, indicating that the host races could have evolved in sympatry.

Key words.—Assortative mating, *Eurosta solidaginis,* gall, host preference, host race, hybrid crosses, *Solidago,* sympatric speciation.

In herbivorous insects, changes in host-plant preference for mating and oviposition sites are crucial in determining whether host shifts, host expansion, or host specialization occur. Changes in host preference can also be critical in the formation of new species (Bush 1975; Tauber and Tauber 1989). Whether host shifts can result in sympatric speciation is intensely controversial (for a review, see Bush 1994), although there is increasing empirical evidence that it does occur (Feder 1998; Feder et al. 1998). Recent models have shown that sympatric speciation can take place under a variety of ecologically plausible conditions (Johnson et al. 1996; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999). Sympatric speciation in herbivorous insects due to a host shift requires genetically based changes in host preference (Bush 1975; Futuyma and Mayer 1980). Computer models show that sympatric speciation can occur even when fitness in two habitats (such as two different host plants) and mate choice are dependent on different, independently in-

herited quantitative traits if there is disruptive selection (Johnson et al. 1996). Kondrashov and Kondrashov (1999) found that the likelihood of sympatric speciation increases as the number of loci influencing fitness increases and as the number of loci influencing mate choice decreases. Sympatric speciation is facilitated if mate choice is coupled with host-plant preference (Bush 1975; Rice 1984, 1987; Diehl and Bush 1989). In this case, the same genes determine host selection and assortative mating, so that assortative mating is a correlated character of host preference, eliminating the need for linkage between these loci (Rice 1984, 1987; Diehl and Bush 1989; Johnson et al. 1996).

The assumptions of sympatric speciation models (Kondrashov and Mina 1986; Bush 1992) can be tested in populations that are in intermediate stages of the speciation process by studying the factors that impede or facilitate gene flow. By examining populations that are partially reproductively isolated, it is possible to determine the factors that contributed to the initiation of speciation. After speciation is completed, many characters that were not responsible for speciation may contribute to reproductive isolation. Host races are populations that are partially reproductively isolated

from each other due to their association with different host plants (Diehl and Bush 1984) and thus are ideal for studying the speciation process.

Many factors can influence host-plant preference, including insect genetics, experience, and the physiological state of the insect (Miller and Strickler 1984; Courtney et al. 1989). Sympatric speciation models include assumptions about the inheritance of host preference, but how host preference is inherited is relatively poorly understood (Thompson 1988). We are aware of only one study that has examined the inheritance of oviposition preference between host races (Sezer and Butlin 1998). Sezer and Butlin found that host preference is determined by a small number of loci, with dominance for the preference for one host plant. Studies of other levels of reproductive isolation also indicate that a relatively small number of loci may control host preference. Huettel and Bush (1972) conducted hybridization studies on two species of tephritid, gall-inducing flies and concluded that oviposition preference was strongly influenced by one locus with a modifier locus. Carson and Ohta (1981) conducted hybridization studies between two allopatric populations of *Drosophila grimshawi.* They found that preference was determined by a single-locus, two-allele system, indicating that simple genetic determination of host preference is possible.

Adult or larval experience can also potentially influence host preference and the course of speciation. Several studies have demonstrated that adult experience can influence host preference (Wood 1980; Jaenike 1982, 1990; Prokopy et al. 1982; Wood and Guttman 1983). It has also been proposed (Craighead 1921), but never clearly established (Mitter and Futuyma 1983), that larval experience can influence adult host preference. Huettel and Bush (1972) concluded that larval conditioning had an important impact on adult host preference in hybridization studies of two tephritid gall-inducers. Jaenike (1988) developed a model that showed that experience combined with genetic differences in host preference could lead to more rapid differentiation of populations than genetic factors alone. Although conditioning may facilitate initial host shifts, it cannot lead to sympatric speciation, which is defined as the genetic differentiation of populations (Kondrashov and Mina 1986; Johnson et al. 1996.)

In this study, we assessed the impact of adult experience, larval experience, and genetics on host preference in *Eurosta solidaginis,* a tephritid fly that induces galls on two species of goldenrod, *Solidago altissima* and *S. gigantea.* We will refer to flies that originate on *S. altissima* as altissima flies and those that originate on *S. gigantea* as gigantea flies. The fly populations on these two plant species have been hypothesized to be host races that originated in sympatry (Waring et al. 1990; Craig et al. 1993; Brown et al. 1996; Itami et al. 1998). Data support the hypothesis that the population on *S. gigantea* originated via a host shift by flies from *S. altissima* (Waring et al. 1990; Brown et al. 1996; Itami et al. 1998). The host races are genetically differentiated, having small but significant differences in allozyme frequencies (Waring et al. 1990; Itami et al. 1998). Characteristics of the host races meet critical assumptions underlying sympatric speciation models. First, there is strong assortative mating due to host-plant preference (Craig et al. 1993). Second, there is strong disruptive selection for mating-site and oviposition-site selection (Craig et al. 1997).

In this study we asked three questions: (1) What is the host fidelity of males and females of the two host races? Habitat fidelity can be defined as the probability that an individual will be found in the habitat where it is most fit (Johnson et al. 1996). *Eurosta solidaginis* mates on the host plant and individuals that mate on the alternative host plant produce F_1 hybrids that have low fitness (Craig et al. 1997). Thus, the tendency to remain on and mate on the natal host plant is a measure of habitat fidelity that we will term host fidelity. The strength of host fidelity will influence the strength of reproductive isolation. By measuring the strength of host fidelity of each host race and of hybrids between them, we can assess the conditions that either facilitate or impede gene flow between the host races. (2) Is host preference a genetically determined trait or does larval or adult experience influence adult host preference? Sympatric speciation models require genetic determination of host preference. Host preference is nonrandom oviposition on plant resources that are offered simultaneously or sequentially; a preferred oviposition site is one that is attacked at a higher rate than if attack were random (Craig et al. 1989). Host preference may or may not correlate with host fidelity. (3) What is the mode of inheritance of host preference? Once we determined that host preference was a genetically determined trait, we examined its mode of inheritance, which will also help elucidate the conditions under which gene flow is likely to occur. Whether alleles for host preference are dominant or sex linked will influence the direction of gene flow. Determining the number of genes involved will reveal how well the characteristics of these host races fit the assumptions of sympatric speciation models.

Tests of Hypotheses on the Determinants of Host Preference

To answer the three questions listed above, we tested the specific hypotheses listed below by examining the preferences of both host races, F_1 hybrids between the host races, and backcrosses. The degree of assortative mating due to host-plant preference depends on the behavior of both males and females. However, male behavior has been almost completely ignored in previous studies. Therefore, we tested all of these hypotheses (except the adult experience hypothesis) on both males and females.

Hypotheses on genetic factors influencing host preference

The autosomal gene hypothesis.—This hypothesis proposes that host preference depends on the autosomal genotype of the fly. It predicts that the males and females resulting from reciprocal F_1 crosses will have the same host preference regardless of the host plant that they had been reared in or the direction of the cross. Two variants of this hypothesis are given below.

The autosomal gene with dominance hypothesis predicts that preference is determined by autosomal genes with one allele for host preference exhibiting dominance. It predicts that both reciprocal F_1 hybrids will have a preference for the same host species. If preference is determined at a single locus with dominance, then we would expect the following

three results, and this would allow us to reject the polygenic hypothesis: (1) complete preference in the backcross to the host race with the allele for dominant preference; (2) a 1:1 ratio in host preference in the backcross to the host race with the allele for recessive preference; and (3) a 3:1 ratio in host preference in the F_2s.

The polygenic autosomal gene hypothesis is that multiple autosomal loci influence host preference. Polygenic inheritance of preference could produce complex patterns of preference among different crosses depending on how many loci were involved and the dominance relationships among alleles. Alternatively, if there were completely additive genetic variance, then it would be predicted that flies would have intermediate preferences relative to the proportion of their genome derived from each host race.

The sex-linked trait hypothesis.—Under this hypothesis, the genes that influence host preference are located on the sex chromosomes. It predicts that the reciprocal male F_1 crosses will prefer the host plant of their mother because genes for preference are carried on the X chromosome. The hypothesis also predicts that female backcrosses that are homozygous for X chromosomes from the host races would differ in host preference from those heterozygous for the X chromosomes.

The maternal effect hypothesis.—This hypothesis proposes that offspring will prefer their mother's host plant due to the inheritance of extra-nuclear factors from the mother. The hypothesis predicts that reciprocal F_1 crosses between the host races will have different host preferences: each will prefer the host plant of the mother.

Hypotheses on nongenetic factors influencing host preference

The adult experience hypothesis.—Regardless of their genotype, offspring will prefer the resources that they first experienced as adults.

The larval experience hypothesis.—Regardless of their genotype, adults prefer their larval host plant. This hypothesis predicts that F_1 hybrids between the host races reared on different hosts should have opposite preferences: each should prefer the host they were reared on.

Natural History of Eurosta solidaginis

Uhler (1951) described the life history of *E. solidaginis,* and Abrahamson and Weis (1997) provided an extensive review of the evolution and ecology of this species. Ming (1989) recognized two subspecies of *E. solidaginis* on the basis of wing morphology. All flies in this study are of the eastern subspecies, *E. solidaginis solidaginis. Eurosta solidaginis* induces stem galls on two goldenrod species, *S. altissima* and *S. gigantea.* Genetic individuals of these two goldenrod species can form extensive clones through lateral spread of rhizomes. *Eurosta solidaginis* is univoltine and, in Minnesota, adults emerge in mid to late May. The mean emergence time of gigantea flies is 2–21 days earlier than that of the altissima flies (Craig et al. 1993; Itami et al. 1998). Adult *E. solidaginis* live for approximately 10 days. Each host race mates on the bud of their host plant (Craig et al. 1993). Males sit at the top of a bud and wait for females to alight for mating or watch for females on nearby buds (T.

Craig, J. Itami, and J. Horner, pers. obs.). After mating, females inject an egg into the unexpanded leaves of a host plant's terminal bud. Insertion of the ovipositor leaves a visible wound that we term an ovipuncture. An egg is not inserted with every ovipuncture. After hatching, the larva burrows down the bud several millimeters before settling just below the apical meristem. Galls become apparent 21 days after oviposition. The larvae reach maximum size by the end of the growing season and overwinter within the gall. Most stems are host to a maximum of one fly.

METHODS

All preference tests were conducted in outdoor cages at Cedar Creek Natural History Area (CCNHA), 50 km north of Minneapolis, Minnesota in Bethel, Minnesota from 1993 to 1996. Except for those in the adult-experience experiment, all flies experienced a common environment prior to the initiation of preference testing. In the first 3 years, flies were reared on host plants grown in a common garden that is described in Craig et al. (1997). In the common garden, replicates of the two host species were grown in 1-m² plots. The two plant species were distributed in a checkerboard pattern in the garden. Mating combinations were randomly assigned to plots within the garden. In 1996, the flies were reared from plants grown in 20-L pots. Oviposition occurred in 10 large cages, as described in Craig et al. (1999). After oviposition, the plants were randomly mixed together. The P_1 flies used in the experiments consisted of a mixture of flies reared in the common garden and from galls collected in the field the previous fall from both host species in a 50-km radius of Minneapolis, Minnesota. We used wild flies to run tests of P_1 flies simultaneously with all tests on the hybrids. The relatively small number of flies from the garden did not always provide a sufficient number of P_1 flies for comparison with the hybrids.

Female flies were mated before being preference tested because unmated females will not oviposit (Uhler 1951; T. Craig, J. Itami, and J. Horner, unpubl. data). Females were then preference tested in the absence of males because we wanted to measure host preference in the absence of nonhost-associated mating. The presence of mates would have introduced a confounding variable into the measurement of host preference. Females will mate more than once (T. Craig, J. Horner, and J. Itami, unpubl. data), and both host races have a strong preference for mating on their host plant (Craig et al. 1993), so measuring host preference after the initial mating still gives an indication of mating-site preference. Male preference was tested using unmated males. Unmated males assume positions on the bud of host plants whether in the presence or absence of females (Craig et al. 1993). Mating- and oviposition-site preferences were highly correlated in previous studies (Craig et al. 1993).

Except for those flies in the adult-experience experiment, flies were not exposed to plants prior to their preference test. All flies were reared from galls stored in screen bags mixed together for overwintering in outdoor cages. Flies were mated in 0.125-m³ screen cages without host plants. Mating usually occurs the morning after emergence and females immediately begin to oviposit. Flies that mated soon after conditions be-

came favorable were immediately placed in the preference cages. Any flies that had not completed mating before 1100 h were held in cool, moist conditions until the following morning for preference testing. If a fly was mated one day but had not been preference tested, then it was placed in a preference cage early the next morning before temperature and light conditions reached the thresholds where flies became active. This avoided the confounding variable of testing preference under different environmental conditions.

Host preference was measured in choice arenas, which were 1-m^3 cages containing 10 host plants growing in 3.8-L pots, five with a single ramet of *S. altissima* and five with a single ramet of *S. gigantea.* Ramets were grown from rhizomes potted the previous year that were collected from a wide range of sites within 20 km of CCNHA. We drew numbers to randomly assigned flies to a choice arena. Flies were released on a neutral surface in the center of the arena and allowed to select from among the potential hosts. Twenty choice arenas were available to be used simultaneously, although usually fewer replicates were running at any one time. After each preference test, the plants in each cage were replaced with new potted plants, randomly selected from a common pool of plants that had not been ovipunctured, before the next replicate was run.

Except for flies in the adult-experience experiment, we measured host preference for males and females as the proportion of time spent on each host plant. Males occupy positions at the top of a bud and wait for females to alight for mating. Thus, the amount of time spent on the bud indicates mating-site preference and the potential for mating with the other host race. Females mate either when they initially alight on a bud occupied by a male or when males approach them while they are ovipositing on a bud. Female preference was also measured by counting the number of ovipunctures on each host plant because the number of ovipunctures is strongly correlated with the number of eggs oviposited (Craig et al. 1997). In addition, oviposition-site preference is highly correlated with mating-site preference (Craig et al. 1993).

Larval Experience, Maternal Effects, and the Mode of Inheritance of Host-Plant Preference Experiments

To test the *larval experience, maternal effects, autosomal genotype, dominance, sex-linked gene*, and *polygenic* hypotheses, we measured host preference of P_1, reciprocal F_1 crosses, F_2, and backcross generations in the choice arenas. The F_1 flies were produced by mating female gigantea and male altissima (the GA cross) and by mating female altissima with male gigantea (the AG cross). The females then oviposited into their natal host plants: gigantea females into *S. gigantea* and altissima females into *S. altissima.* The F_1 flies on each host species thus had the same larval experience and maternal influences but a different genetic composition from the P_1s on that host. If the F_1 and P_1 flies reared from the same host species differed in preference, we could conclude that neither larval experience nor maternal effects had an influence on preference. However, if the F_1 and P_1 offspring reared on the same host had the same host preferences, it would be impossible to know whether larval experience, maternal effects, or both had influenced host preference. We did not attempt

to have the flies oviposit into the alternative host plant because the extremely strong host preference of both host races (Craig et al. 1993) makes it very difficult to obtain an adequate number of ovipositions on the alternative host plant. In addition, attempts to transfer *E. solidaginis* eggs dissected from one host plant to the alternate host plant failed.

Backcross flies were predominantly produced by mating F_1 females with the males of each host race. A few backcross hybrid offspring were also produced by crossing F_1 males with females of each host race. The hybrid flies were produced from crosses that were reared in an experimental garden described in Craig et al. (1997) and from large population cages.

All mating combinations were preference tested simultaneously each year with two exceptions. The F_2 flies were preference tested only in 1993. In 1996, only P_1s and F_1 hybrids were tested, because backcross offspring did not survive. Each day, all available mating combinations of both sexes were started simultaneously. The number of replicates of each mating combination that were run each day varied due to the yearly variation in survival among crosses (see Craig et al. 1997) and due to daily variation in the emergence of the hybrid flies. For example, because of the small number of F_2 flies available, there were many days when this replicate was not run. Preference tests of P_1 flies (of the same sex as the hybrid combinations being tested) were run each day at the same time as the hybrid combinations. The emergence rates of the two host races normally peaks at slightly different times (Craig et al. 1993; Itami et al. 1998). Therefore, gall-rearing temperature was regulated on a large sample of both host races to ensure the continuous emergence of P_1 flies from both host races throughout the experimental period.

The host preference of each fly was measured during a 2-h period of activity in the choice arenas. In this experiment, plants were distributed in the choice arenas in such a way as to simulate the clonal growth pattern of goldenrod in the field. To accomplish this, plants were arranged in a circle that consisted of two semicircles of each host species. The semicircles of ramets were oriented in one of the four cardinal directions at random among trials. Flies were placed on a wooden pot stake in the center of the experiment to start the trial. The 2-h period was initiated when the fly flew from the stake. Every 10 min the position of the fly was recorded. If the fly was on a plant, the species was noted. If a fly was found on the cage, it was replaced on the stake. If a fly became inactive, those periods were not counted as part of the 2-h period. Inactivity was defined as a total lack of movement over a 10-min period. Inactivity was usually due to weather conditions. Cloudy weather and temperatures that are too warm or cold will quickly cause *E. solidaginis* to become inactive (Uhler 1951; Abrahamson and Weis 1997). If a female fly did not exhibit oviposition behavior or if a male fly did not land on a bud during the 2-h period, the trial was not included in the analyses. If possible, the fly was placed in another trial on a subsequent day. Oviposition behavior was defined as searching the bud and pausing to probe it with the ovipositor. Not all females that searched buds oviposited. Flies were cryptic and the trial was not included in the analysis of host preference based on the time spent on a plant if the fly could not be located in two consecutive observation

periods. Ovipunctures were used as a measure of oviposition preference. Female flies were included in the analysis of oviposition preference based on ovipuncture number even if they were not observed in every time period. They were included if they were observed at least in the first and last periods. This ensured that all oviposition had occurred in a 2-h period. We did not determine egg number because this requires destructive sampling, and we wanted to rear the flies for use in further experiments. There is a highly significant correlation between number of ovipunctures and the number of eggs oviposited in both host races (Craig et al. 1997).

Adult Experience Experiment

To test the adult experience hypothesis, flies of both host races were reared from puparia that were dissected from galls to preclude exposure to potential cues in the gall during emergence as adults. Once adults had emerged, females were mated with males of the same host race in the absence of any host plants. Mated females (40 of each host race) were randomly assigned to one of two treatments: initial exposure to *S. altissima* or initial exposure to *S. gigantea.* Four cages were used for the initial exposure. Each initial-exposure cage was constructed of nylon mesh and measured 1.5 m high × 1 m length × 1 m width. Two cages contained four 20-L pots of *S. altissima,* and the other two cages contained four 20-L pots of *S. gigantea.* We set a period of 2 h of continuous, active searching of the host plant as the minimum initial-experience period. We surveyed cages every 20 min for activity. When the weather is cool or cloudy, flies are inactive and do not search the host plant (Uhler 1951; T. Craig, J. Itami, and J. Horner, unpubl. data). The summer of 1993 was exceptionally cool and cloudy, so acquiring a 2-h period of activity sometimes took up to 72 h. After an initial exposure lasting at least 2 h (and as long as 72 h, depending on weather conditions), females were placed individually into choice arenas. The choice arenas were 1-m³ cages constructed of nylon mesh. Each choice arena contained five 3.8-L pots of each of the two host species (10 pots total). Each pot contained a single ramet, and the pots were randomly assigned to a position in a circular array. Females were placed onto a neutral surface (a wire flag) in the center of the choice arena. They were left in the choice arena for 1 h or until at least three plants were ovipunctured, whichever came first. The plant species ovipunctured were then recorded.

Statistical Analysis

Host preference was analyzed using one-way analysis of variance (ANOVA) on the proportion of total ovipunctures and the proportion of total time spent on *S. gigantea.* Periods spent on neutral surfaces, such as the cage, were not used in calculating the time ratios. Treatments were not included in the analysis if we had five or fewer replicates. All proportions were arcsine-square-root transformed prior to analysis to normalize the variance. To examine which treatments differed significantly, we used Tukey's multiple range tests.

Results
Inheritance of Host Preference Experiments

We had nearly 100% mating success for all hybrid males and females, which indicates that these flies had normal vigor.
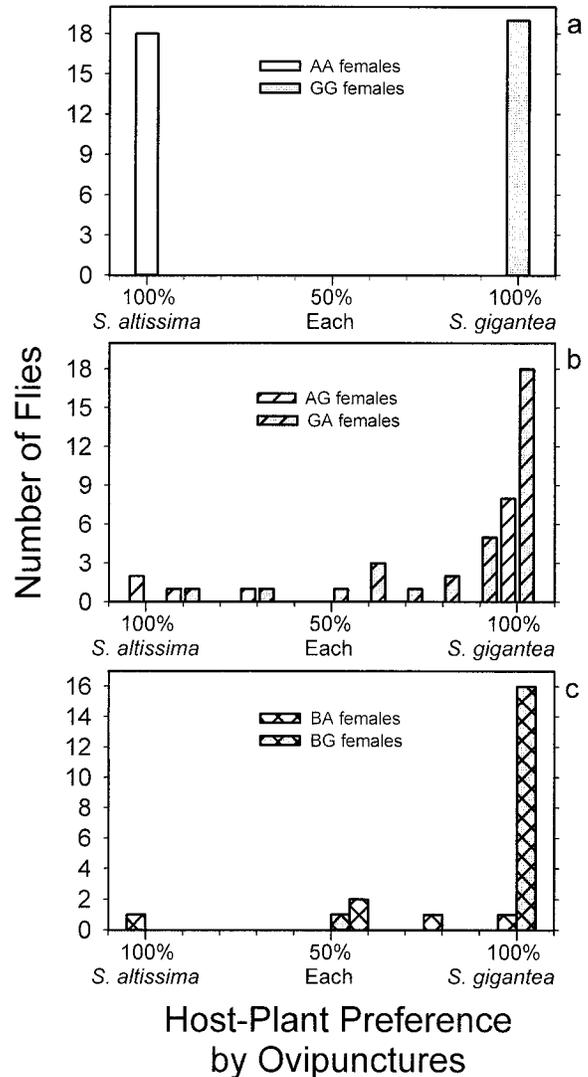

FIG. 1. In the inheritance of preference experiment, the individual ovipuncture preferences of: (a) the P_1s, AA (altissima female × altissima male) and GG (gigantea female × gigantea male); (b) the F_1 hybrids between the host races, AG (altissima female × gigantea male) and GA (gigantea female × altissima male); and (c) the backcrosses of F_1s with the pure host races, BG (F_1 × gigantea male or female) and BA (F_1 × altissima male or female).

However, we cannot make a meaningful statistical comparison of mating frequencies between hybrid and pure host race flies because we made an extra effort to obtain the maximum number of hybrid flies for preference testing by carefully controlling the light, humidity, and temperature conditions in the hybrid-mating cages. Normal vigor of females of different crosses is also indicated by ovipuncture rate: females from different crosses did not differ in the mean number of ovipunctures made in a 2-h period (mean ± SD = 21.93 ± 20.46, $F_{6,106} = 2.01$, $P > 0.05$).

There were significant differences in ovipuncture preference among female flies of different crosses (Fig. 1, one-way ANOVA $F_{4,97} = 78.37$, $P < 0.0001$). Both the AA and GG females (the pure host races) exhibited strong host fidelity, ovipuncturing only their natal host plant. Dominance of *S.*

gigantea preference was indicated by the ovipuncture preference for *S. gigantea* by the AG, GA, and BG crosses.

A Tukey's test divided the ovipuncture preferences of the crosses into three mutually exclusive groups. The first group consisted only of altissima females (AA) that oviposited exclusively on *S. altissima.*

The second group identified by the Tukey's analysis included gigantea (GG) flies that oviposited solely on *S. gigantea* and the BG cross that ovipunctured over 97% of the time on *S. gigantea* (Fig. 1c). Only one of the 17 BG females ovipunctured both host species. The sex-linked hypothesis was not supported because these BG females had different combinations of X chromosomes from the two host races, but they had the same host preference. Fifteen of the BG females were produced by mating AG or GA females, which had one X chromosome from the altissima host race and one X chromosome from the gigantea host race, with gigantea (GG) males. The expected ratio of offspring from this cross would have would have been 50% homozygous for gigantea and 50% heterozygous for the X chromosomes from the two host races. Mating gigantea females in a cage with F_1 males from both reciprocal crosses produced the remaining two BG females. Because the host race origin of the X chromosome from the F_1 male could not be determined, these females could again have been heterozygous or homozygous for their X chromosomes.

The third homogenous group as determined by the Tukey's analysis consisted of the AG and GA crosses, which predominantly ovipunctured *S. gigantea* (mean percent of ovipunctures on *S. gigantea* $\pm$ SD; GA = 83.0 $\pm$ 28.0%; and AG = 78.0 $\pm$ 36.0%) but which also occasionally oviposited on *S. altissima.* The results do not support the prediction of the larval experience hypothesis that the GA cross would prefer *S. gigantea* where it developed, and that the AG cross would prefer *S. altissima* where it matured. Individual females had a range of ovipuncture preferences (Fig. 1b) with 50% of the GA cross and 33% of the AG cross making at least one ovipuncture on *S. altissima.*

Due to small sample size, the BA and F_2 flies were not included in the statistical analyses. Of the five backcross altissima females, three had strong ovipuncture preferences for *S. altissima* and two had a strong preference for *S. gigantea* (Fig. 1c.). All four F_2 females oviposited exclusively on *S. gigantea.*

Females of different crosses differed in the proportion of time they spent on each host plant (Fig. 2, $F_{4,91} = 14.78$, $P < 0.0001$). Host fidelity of the pure host races, as measured by the amount of time spent on the host plant, was weaker than host fidelity as indicated by the proportion of ovipunctures on the natal host plant. A Tukey's analysis showed that crosses fell into two homogeneous groups. The first group contained only AA females, which spent a large majority of their time on *S. altissima.* The second group consisted of the GG, AG, GA, and BG crosses that all tended to prefer *S. gigantea.* This similarity in host preference of the hybrids to the pure gigantea host race again support the dominance hypothesis. Due to small sample size, the BA and F_2 flies were not included in the statistical analyses.

Males from different crosses differed in their host preferences (Fig. 3, $F_{4,86} = 14.78$, $P < 0.0001$) and males of the
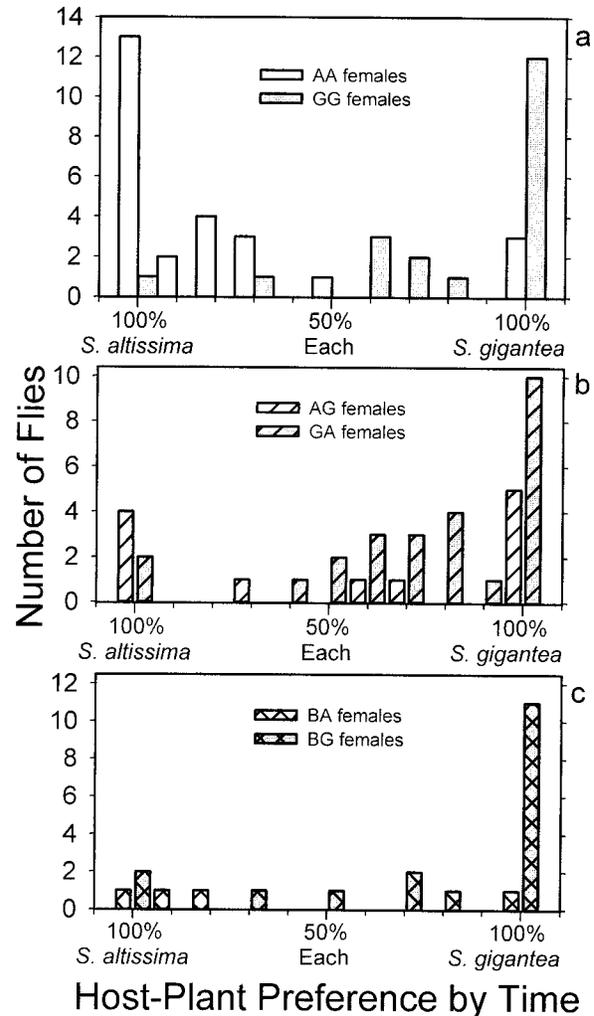

FIG. 2. In the inheritance of preference experiment, the distribution of time spent on the two host plants by females of: (a) the P_1s, AA (altissima female $\times$ altissima male) and GG (gigantea female $\times$ gigantea male); (b) the F_1 hybrids between the host races, AG (altissima female $\times$ gigantea male) and GA (gigantea female $\times$ altissima male); and (c) the backcrosses of F_1s with the pure host races, BG (F_1 $\times$ gigantea male or female) and BA (F_1 $\times$ altissima male or female).

pure host races differed in their degree of host fidelity. A Tukey's test divided the crosses into three groups. The first group consisted of AA males that had a strong preference for their own host plant; only four of 37 AA males were recorded on a *S. gigantea* bud (Fig. 3a). The second group consisted of the GG, GA, and AG crosses that did not have consistent host preferences. Individual males in these crosses frequently showed an exclusive preference for either *S. altissima* or *S. gigantea* (Fig. 3a, b). The similarity of host preferences between the pure gigantea (GG) and the F_1 crosses (mean percent of time on *S. gigantea* $\pm$ SD; GG = 56.25 $\pm$ 47.58%; and AG = 56.25 $\pm$ 45.00%, GA = 42.01 $\pm$ 48.48%) supports the dominance hypothesis. The prediction of the sex-linked hypothesis that each cross should prefer the host of the mother was not supported. The GA and AG crosses had X chromosomes from different host races, but they had
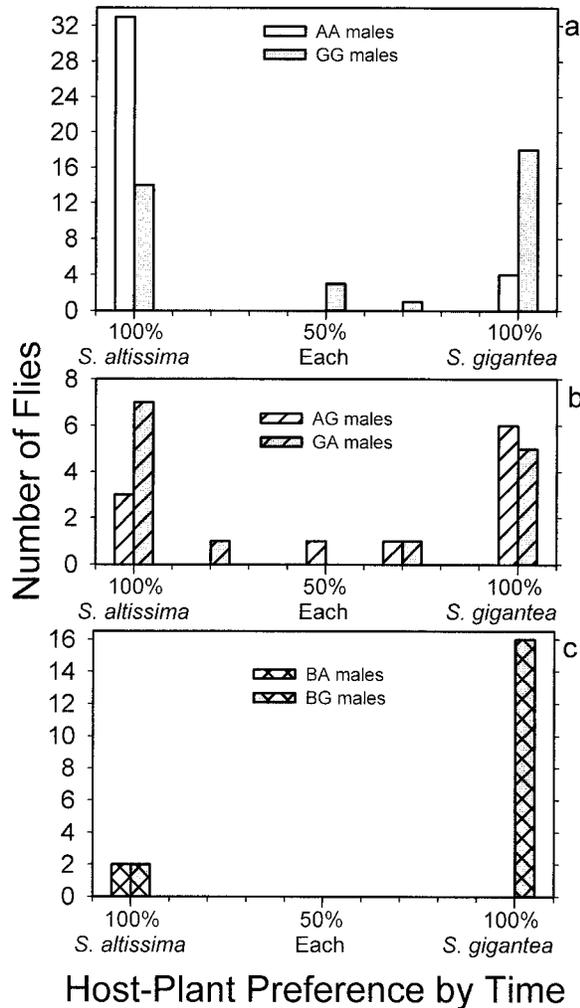
Host-Plant Preference by Time

FIG. 3. In the inheritance of preference experiment, the distribution of time spent on the two host plants by males of: (a) the P₁s, AA (altissima female × altissima male) and GG (gigantea female × gigantea male); (b) the F₁ hybrids between the host races, AG (altissima female × gigantea male) and GA (gigantea female × altissima male); and (c) the backcrosses of F₁s with the pure host races, BG (F₁ × gigantea male or female) and BA (F₁ × altissima male or female).

similar host preferences. Note also that AA males and AG males, both of which had an X chromosome from the altissima race, had different host preferences. The third group consisted of the BG cross that had a stronger preference for *S. gigantea* (Fig. 3c) than the GG flies. Due to small sample size, the BA and F₂ flies were not included in the statistical analyses. The three F₂ male flies all had a preference for *S. gigantea*.

Adult Experience Experiment

The adult experience hypothesis was not supported because, with only one exception, females ovipunctured only their natal host species, regardless of initial experience (Table 1). The bud from the one exception was dissected and we found that an egg had been oviposited.

TABLE 1. Host preference in the adult experience experiment. The number of mated female flies choosing each host plant (*Solidago*) species. The results are given for the first three host plants ovipunctured by each fly.

Fly host race	Initial experience	Acceptance	
		S. gigantea	*S. altissima*
gigantea	*S. gigantea*	20	0
	S. altissima	20	0
altissima	*S. altissima*	0	20
	S. gigantea	1[1]	19

[1] Only one host plant was oviposited by this female.

DISCUSSION

Our results indicate that host preference in *E. solidaginis* is inherited nonadditively at a limited number of autosomal loci. No evidence of nongenetic influences on preference was detected: preference was not significantly influenced by larval or adult experience. Autosomal inheritance is indicated because the predictions of the sex-linked and maternal-effects hypotheses were not supported. Contrary to the prediction of the sex-linked trait hypothesis, male F₁ progeny from the two reciprocal crosses, with X chromosomes from different host races, had the same host preferences. This hypothesis also predicted that host preference of BG females homozygous for their sex chromosomes would differ from that of BG females heterozygous for their sex chromosomes in their host preference, but they did not. Contrary to the prediction of the maternal effects hypothesis that flies should prefer the host plant of their mother, both F₁ crosses preferred *S. gigantea*.

The strong ovipuncture preferences for *S. gigantea* of the AG, GA, and BG females (i.e., similar to that of the pure gigantea [GG] females) support the dominance hypothesis. An intermediate ovipuncture preference, however, was found in some AG and GA hybrids, which is very rare in the pure host races (this study; Craig et al. 1993; Itami et al. 1998). Intermediate preference could be due to incomplete penetrance of the dominant allele or alleles for *S. gigantea* preference. It may also indicate that other modifer genes influence the expression of host preference.

The similarity of host preferences of GA and AG males to that of the pure gigantea host race males (GG) also supported the dominance hypothesis. Most individuals from these populations spent their time exclusively on one or the other host species. This could have resulted from an absolute host preference for one host species by each individual. Alternatively, it could result from an intermediate host preference. In studies where males were continuously observed, they rarely move once they have landed on a host plant (T. Craig, J. Itami, and J. Horner, unpubl. data). Thus, males may have stayed on the first plant that they encountered by chance, even if they would accept both host species.

Male GG, AG, and GA had intermediate host preferences, whereas the females from these crosses had a strong preference for *S. gigantea*. One hypothesis explaining these sexual differences in host preference is that host preference is a dominant, sex-influenced trait. As a result, the gene that produces strong *S. gigantea* preference in females could pro-

duce an intermediate host preference in males. Pure altissima males that were homozygous for the altissima preference gene had high host fidelity. Males that were homozygous or heterozygous for gigantea genes had an intermediate host preference. The extremely strong host preference of the BG males for *S. gigantea* is enigmatic, and it is not explained by this hypothesis. An alternative hypothesis is that host preference may be determined by different loci in males and females.

The prediction of the polygenic hypothesis that a hybrid (F_1, F_2, and backcrosses) host preference would correspond with the proportion of the genome derived from each host race was not supported. The preference pattern suggests that a single gene of large effect strongly influences oviposition preference. Larger sample sizes for the behavior of backcross and F_2 flies are needed so that their preference ratios could be statistically tested against the expected Mendelian ratios if preference was determined by different numbers of genes. It has been difficult to obtain more backcrosses to the altissima host race to test this hypothesis. F_1 hybrids have a low survival rate on most genotypes of both hosts, making it difficult to obtain the large numbers of F_1 flies needed to form backcrosses. The backcrosses to altissima also have had extremely low survival. In two of the four years, we did not obtain any backcross altissima survivors (T. Craig et al. 1997; T. Craig, J. Itami, and J. Horner, unpubl. data). This indicates that there may be postreproductive barriers to gene flow. Craig et al. (1997) hypothesized that this postreproductive isolation is due to adaptation to host plants by the host races and not genetic incompatibilities of the populations. F_1 hybrids can survive on particularly benign genotypes of host plants of both species (Itami et al. 1998; T. Craig, J. Itami, and J. Horner, unpubl. data), providing support for this hypothesis.

Implications for Gene Flow between the Host Races

Mate preference is strongly influenced by host-plant preference in *E. solidaginis* (Craig et al. 1993; Itami et al. 1998), so weak host fidelity creates the potential for gene flow. Patterns of host preference and survival indicate that gene flow would be primarily unidirectional from the altissima to the gigantea host race. The low host fidelity of gigantea (GG) males creates the greatest potential for gene flow resulting from mating between gigantea males and altissima females on *S. altissima* plants. The resulting F_1 females (AG) would have a preference for *S. gigantea,* so they would be most likely to mate with gigantea males. Male F_1 flies have intermediate preferences, so some flies would also backcross to the gigantea host race. These backcrosses to gigantea have a high survival rate (Craig et al. 1997), indicating that introgression of altissima alleles into the gigantea host race is possible. Both sexes of these gigantea backcrosses also have a strong preference for *S. gigantea,* promoting introgression into the gigantea host race. Introgression from the gigantea to the altissima host race would be limited. Male F_1 flies had intermediate host preferences, so some flies could also backcross to the altissima host race. However, gene flow in this direction would be minimized because of the low survival rate of altissima backcrosses (Craig et al. 1997; T. Craig, J. Itami, and J. Horner, unpubl. data).

The low host fidelity of gigantea males creates the potential for extensive gene flow that could obliterate host-race differences, but several other factors may limit nonassortative mating. First, there is weak assortative mating in the absence of the host plant (Craig et al. 1993). Second, allochronic isolation limits opportunities for interhost race matings: gigantea males have the earliest emergence times and altissima females the latest (Craig et al. 1993; Itami et al. 1998). Third, spatial isolation between the host species in some areas may limit encounters between the host races. Finally, host fidelity of males may increase in the presence of females: there was nearly complete assortative mating on the host plants when both host races and host species were present in experimental cages (Craig et al. 1993).

Low gigantea host fidelity may be expressed under some conditions but not others, creating spatial and temporal variation in gene flow. Relatively strong genetic differentiation was indicated in the eastern United States (Waring et al. 1990; Brown et al. 1996), but low levels of differentiation were found in Minnesota, where this study was conducted (Waring et al. 1990; Itami et al. 1998). Experimental studies have shown that when *S. gigantea* is absent or when the host species are mixed together, gigantea males are likely to move to *S. altissima* and mate there, whereas gigantea females and altissima males and females will not shift host species (Itami et al. 1998). Therefore local variation in the spatial distribution and emergence times of the host races may determine the rate of gene flow due to gigantea male host infidelity in different locations (Itami et al. 1998).

Implications for Speciation

The *E. solidaginis* host races meet critical assumptions of sympatric speciation models. They have genetically based host preferences determined by a limited number of loci, which satisfies a critical assumption of sympatric speciation models for herbivorous insects (Bush 1975; Futuyma and Mayer 1980; Kondrashov and Mina 1986; Johnson et al. 1996; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999). Host preference creates reproductive isolation (Craig et al. 1993), so the host races satisfy the requirement that sympatric speciation is possible when more than one (but a small number of) loci determine reproductive isolation (Johnson et al. 1996; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999). Kondrashov and Kondrashov (1999) also found that the probability of sympatric speciation increases as the number of loci influencing fitness in two different habitats increases, and Craig et al. (1997) concluded from a study of *E. solidaginis* that the adaptations required for survival on the two host species were probably influenced by a large number of loci.

The host races of *E. solidaginis* have diverged so that there is an association between preference for a host plant and fitness on that host plant (Craig et al. 1993, 1997; Itami et al. 1998). The sympatric speciation model of Johnson et al. (1996) indicates that this stage could have been reached as the result of a shift in host preference that created partial reproductive isolation followed by the genes for fitness in the two habitats coming into linkage disequilibrium with genes for habitat preference. The Johnson et al. (1996) model

also indicates that following this initial divergence, the evolution of disequilibrium among genes for habitat preference, those for habitat fitness, and those for nonhabitat assortative mating can lead to the rapid evolution of complete reproductive isolation. Nonhabitat assortative mating in *E. solidaginis* results from the allochronic isolation of the host races and weak assortative mating in the absence of host plants (Craig et al. 1993). Experimental studies have indicated that the allochronic isolation may have evolved as a reproductive isolating mechanism (Horner et al. 1999). Strengthening of these traits could lead to the evolution of complete reproductive isolation and speciation.

LITERATURE CITED

Abrahamson, W. G., and A. E. Weis. 1997. Evolutionary ecology across three trophic levels: goldenrods, gallmakers and natural enemies. Princeton Univ. Press, Princeton, NJ.

Brown, J. M., W. G. Abrahamson, and P. A. Way. 1996. Mitochondrial DNA phylogeography of host races of the goldenrod ball gallmaker, *Eurosta solidaginis* (Diptera: Tephritidae). Evolution 50:777–786.

Bush, G. L. 1975. Sympatric speciation in phytophagous parasitic insects. Pp. 187–206 *in* P. W. Price, ed. Evolutionary strategies of parasitic insects and mites. Plenum Press, New York.

———. 1992. Host race formation and sympatric speciation in *Rhagoletis* fruit flies (Diptera: Tephritidae). Psyche 99:335–355.

———. 1994. Sympatric speciation in animals: new wine in old bottles. Trends Ecol. Evol. 9:285–288.

Carson, H. L., and A. T. Ohta. 1981. Origin of the genetic basis of colonizing ability. Pp. 365–370 *in* G. G. E. Scudder and J. L. Reveal, eds. Evolution today: proceedings of the second international congress of systematic and evolutionary biology. Hunt Institute, Carnegie Mellon Univ., Pittsburg, PA.

Courtney, S. P., G. K. Chen, and A. Gardner. 1989. A general model for individual host selection. Oikos 55:55–65.

Craig, T. P., J. K. Itami, and P. W. Price. 1989. A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. Ecology 70:1691–1699.

Craig, T. P., J. K. Itami, W. G. Abrahamson, and J. D. Horner. 1993. Behavioral evidence for host-race formation in *Eurosta solidaginis*. Evolution 47:1696–1710.

Craig, T. P., J. D. Horner, and J. K. Itami. 1997. Hybridization studies on the host races of *Eurosta solidaginis*: implications for sympatric speciation. Evolution 51:1552–1560.

Craig, T. P., J. K. Itami, C. Shantz, W. G. Abrahamson, J. D. Horner, and J. V. Craig. 1999. The influence of host plant variation and intraspecific competition on oviposition preference and offspring performance in the host races of *Eurosta solidaginis*. Ecol. Entomol. 25:1–12.

Craighead, F. C. 1921. Hopkin's host-selection principle as related to certain cerambycid beetles. J. Agric. Res. 22:189–220.

Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. Nature 400:354–357.

Diehl, S. R., and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Annu. Rev. Entomol. 29:471–504.

———. 1989. The role of habitat preference in adaptation and speciation. Pp. 345–365 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Feder, J. L. 1998. The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation. Pp. 130–144 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, New York.

Feder, J. L., S. H. Berlocher, and S. B. Opp. 1998. Sympatric host race formation and speciation in *Rhagoletis* (Diptera: Tephritidae): a tale of two species for Charles D. Pp 408–441 *in* S. Mopper and S. Y. Strauss, eds. Genetic structure and local adaptation in natural insect populations: effects of ecology, life history, and behavior. Chapman and Hall, New York.

Futuyma, D. J., and G. C. Mayer. 1980. Non-allopatric speciation in animals. Syst. Zool. 29:254–271.

Huettel, M. D., and G. L. Bush. 1972. The genetics of host selection and its bearing on sympatric speciation in *Procecidochares* (Diptera: Tephritidae). Ent. Exp. Appl. 15:465–480.

Horner, J. D., T. P. Craig, and J. K. Itami. 1999. The influence of oviposition phenology on survival in the host races of *Eurosta solidaginis*. Ent. Exp. Appl. 93:121–129.

Itami, J. K., T. P. Craig, and J. D. Horner. 1998. Factors affecting gene flow between the host races of *Eurosta solidaginis*. Pp. 375–404 *in* S. Mopper and S. Y. Strauss, eds. Genetic structure and local adaptation in natural insect populations: effects of ecology, life history, and behavior. Chapman and Hall, New York.

Jaenike, J. 1982. Environmental modification of oviposition behavior in *Drosophila*. Am. Nat. 119:784–802.

———. 1988. Effects of early adult experience on host selection in insects: some experimental and theoretical results. J. Insect Behav. 1:3–15.

———. 1990. Host specialization in phytophagous insects. Annu. Rev. Ecol. Syst. 21:243–247.

Johnson, P. A., F. C. Hoppensteadt, J. J. Smith, and G. L. Bush. 1996. Conditions for sympatric speciation: a diploid model incorporating habitat fidelity and non-habitat assortative mating. Evol. Ecol. 10:187–205.

Kondrashov, A. S., and F. A. Kondrashov. 1999. Interactions among quantitative traits in the course of sympatric speciation. Nature 400:351–354.

Kondrashov, A. S., and M. V. Mina. 1986. Sympatric speciation: when is it possible? Biol. J. Linn. Soc. 27:201–223.

Miller, J. R., and K. L. Strickler. 1984. Finding and accepting host plants. Pp. 127–150 *in* W. J. Bell and R. T. Cardé, eds. Chemical ecology of insects. Sinauer Associates, Sunderland, MA.

Ming, Y. 1989. A revision of the genus *Eurosta* Loew, with a scanning electron microscopic study of taxonomic characters (Diptera: Tephritidae). M.S. thesis, Washington State University, Pullman, WA.

Mitter, C., and D. J. Futuyma. 1983. An evolutionary-genetic view of host-plant utilization by insects. Pp. 427–459 *in* R. F. Denno and M. S. McClure, eds. Variable plants and herbivores in natural and managed systems. Academic Press, New York.

Prokopy, R. J., A. L. Averill, S. S. Cooley, and C. A. Roitberg. 1982. Associative learning in egg laying site selection by apple maggot flies. Science 218:76–77.

Rice, W. R. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. Evolution 38:1251–1260.

———. 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. Evol. Ecol. 1:301–314.

Sezer, M., and R. K. Butlin. 1998. The genetic basis of oviposition preference differences between sympatric host races of the

brown planthopper (*Nilaparvata lugens*). Proc. R. Soc. Lond. B 265:2399–2405.

Tauber, C. A., and M. J. Tauber. 1989. Sympatric speciation in insects: perception and perspective. Pp. 307–344 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer Associates, Sunderland, MA.

Thompson, J. N. 1988. Evolutionary genetics of oviposition preference in swallowtail butterflies. Evolution 42:1223–1234.

Uhler, L. D. 1951. Biology and ecology of the goldenrod gall fly, *Eurosta solidaginis* (Fitch). Cornell Univ. Agricultural Experimental Station, Memoir no. 300. Ithaca, NY.

Waring, G. L., W. G. Abrahamson, and D. Howard. 1990. Genetic differentation among host-associated populations of the gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). Evolution 44: 1648–1655.

Wood, T. K. 1980. Divergence in the *Enchenopa binotata* Say complex (Homoptera: Membracidae) effected by host plant adaptation. Evolution 34:147–160.

Wood, T. K., and S. I. Guttman. 1983. *Enchenopa binotata* complex: sympatric speciation? Science 220:310–312.