CHAPTER 4

Divergence in Key Host Examining and Acceptance Behaviors of the Sibling Species Rhagoletis mendax and R. pomonella (Diptera: Tephritidae)

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Host-associated races and species of phytophagous insects provide an opportunity to address fundamental behavioral and evolutionary problems concerning natural selection and the evolution of reproductive isolation. Several plant protection programs have also focused on insect biotypes during their efforts to increase insect resistance in major crop plants (e.g., Gallun 1977; Gallun and Khush 1980; Sogawa 1982). Unfortunately, behavioral and life-history traits causing differences in the host plant specialization of insect species are poorly understood at both the genetic and phenotypic levels. Through studying these traits, we hope to gain insights into the biological mechanisms governing the evolution of host-associated races and species. In this paper we focus on a field study of the host examining and acceptance behaviors (sensu Miller and Strickler 1984) of two sibling species of Rhagoletis fruit flies.

One of the species, R. mendax, occurs in the eastern United States and Canada infesting the fruits of high and low bush blueberries (Vaccinium corymbosum and V. angustifolium), and a few representatives of the genus Gaylussacia (Ericaceae). The second species, R. pomonella, infests apple (Malus sp.), hawthorne (Crataegus sp.), rose hips (Rosa rugosa), sour cherries (Prunus cerasus L.), and occasionally other plants distributed among six genera of the Rosaceae in the United States, Canada, and Mexico (Bush 1966; Shervis et al. 1970; Prokopy and Bush 1972; Prokopy and Berlocher 1980). Independent lines of evidence based on chromosomal, morphological, and electrophoretic data (Bush 1966; Berlocher and Bush 1982) indicate mendax and pomonella are two of the most closely related species within the pomonella group. Although there is a high level of genetic similarity between the species, they have consistent differences in morphological (Curran 1932; Bush 1966), electrophoretic (Berlocher 1980; Berlocher and Bush 1982), life-history, and behavioral traits (Bierbaum and Bush 1988). The genetic similarity between these species has allowed us to hybridize them in the laboratory, and rear interspecific hybrid larvae in apples and blueberries under natural conditions in the field (Bierbaum and Bush 1988). This enables a genetic analysis of life-history and behavioral traits differentiating these closely related species.
From a theoretical standpoint, the formation of host races appears to be an important step in the development of new species of phytophagous insects (Bush 1974; 1975a; Bush and Diehl 1982). This topic has been of long-standing interest to evolutionary biologists and has been extensively debated (Mayr 1963; Bush 1969; 1975b; Bush and Howard 1986; Futuyma and Mayer 1980; Futuyma 1983; Jaenike 1981; Felsenstein 1981; Rice 1984). Theoretical analyses have shown that the formation of host races can be facilitated when mating occurs on the host plant (Bush 1975a; Rice 1984); under these conditions mate and host selection are closely linked in the behavioral repertoire and host selection can serve as a major mechanism to restrict gene flow between populations. Theories have also postulated that the evolution of a new host-associated species often involves genetic changes in at least two major components of the insect’s genome. First, the adults need to acquire the ability to preferentially find and accept a new host for oviposition and in some cases mating, and second, the larvae must be able to survive on the new host (Bush 1975a; Bush and Diehl 1982). Population genetic models (Felsenstein 1981; Rice 1984) have explored various mechanisms of host race formation and speciation, and have shown how fitness differences in alternate habitats and the level of habitat-based assortative mating can influence the evolution of reproductive isolation. Their results indicate the importance of data on host selection and survival abilities in understanding the formation of host-associated species.

Although a few empirical studies have ascertained genetic differences in the viabilities and host acceptance behaviors of closely related races and species of phytophagous insects (e.g., Hatchett and Gallun 1970; Huettel and Bush 1972; Knerer and Atwood 1972; 1973; Phillips and Barnes 1975; Carson and Ohta 1981; Bierbaum and Bush 1983), there is relatively little information on the phenotypic and genetic bases of these important traits (for reviews see Diehl and Bush 1984; Futuyma and Peterson 1985). In field experiments we have tested for differences in the host acceptance behaviors and larval-to-adult survival rates of mendax and pomonella flies on apple and blueberry plants. In this paper we focus on the results of behavioral experiments designed to test if the species differ in: i) the percentage of females ovipositing in apple and blueberry fruits, ii) the total number of eggs laid in each host fruit, and iii) number of occurrences, sequence, and duration of the host examining and acceptance behaviors preceding and following oviposition. We have identified key behavioral differences between mendax and pomonella that will serve as a basis for further genetic analyses.

MATERIALS AND METHODS

To record the number of occurrences, sequence, and duration of 14 host examining and acceptance behaviors displayed by mated
females, we developed a BASIC language Behavioral Sequence Recording Program for a TRS-80, model 100 portable microcomputer (Tandy Corporation, Fort Worth, Texas). The duration of the behavioral events was recorded with an accuracy of 1.0 seconds. Each plant was subdivided into bottom, middle, and top regions of the fruits, leaves, and branches in order to record the position of the released insect. This resulted in 126 behavior and plant location combinations that could be recorded by pressing single keys on the computer keyboard. A behavioral display was counted as a new occurrence if it was preceded by a different behavior or it occurred in a new plant position (e.g., bottom, middle, or top regions of the fruit). During releases of several mated females to blueberry and apple plants, we made videotape records of their behavior using a GP-5A Hitachi color video camera with a Schneider-Kreuznach macro-telephoto lens. These video records helped to detail several behaviors that were incorporated into the computer program for collecting the quantitative field observations.

We worked with mature (18 to 25 day-old) mated pomonella females obtained near Hart, MI, and mendax from Sawyer, MI. The flies were reared from pupae collected the previous summer and chilled at 4°C for six months, then incubated at 25°C with a 15L:9D photoperiod. Adults were held in 15 cm x 15 cm x 15 cm wire screen and plexiglass cages at a density of 10 females and 5 males, and were reared to maturity on a standard yeast hydrolysate and brown sugar diet (Prokopy and Boller 1970). The females were naive in the sense of not being exposed to either blueberry or apple plants prior to their testing in the field.

On the first day of each observation, a single female was released to the fruit of one of the two host plants and her behavior was recorded. She was then taken to the lab and held in a constant temperature chamber at 25°C for one day; on the third day, she was brought back to the field and released to the alternative host plant. One half of the test group was released to blueberries first, and the second half was released first to apples. The behavior of each fly was recorded until: i) it flew off the plant to the ceiling or sides of the field cage or ii) 20 minutes had elapsed. We completed a total of 166 female releases to blueberries and apples; 82 of these observations were pomonella flies and 84 were mendax flies.

Plants for each observation were kept inside a 3m x 2m x 2m clear saran plastic mesh cage in an abandoned field adjacent to a wooded nature preserve on the MSU campus. We used a potted 2.4m high apple tree and a 1.2m blueberry bush with fruits wired to their branches immediately prior to the release of a female. The fruits were free of pesticides and were harvested one day before their use; they were protected in the field from insect infestation by nylon mesh bags covering the branches of plants. Prior to the observations on each female, five McIntosh apples were
wired to the apple tree and a set of five clusters of Bluehaven blueberry fruits (with three ripe fruits per cluster) were attached to the blueberry bush. The fruits were placed in the same marked locations on the host plants for each release.

Data files were stored in the laboratory on computer disks at the end of each day of observations. Records in the files consisted of numerical and alphabetical codes specifying a behavior, location on the plant, and starting and finishing times of the behavioral display. The number of occurrences and duration time of behaviors displayed by an individual female at different plant locations, and the total time (residence time) on the fruits, leaves, and branches were calculated from the records. The following results focus on five behaviors that were closely linked with the act of oviposition into a fruit. The results for additional behaviors and plant locations will be reported elsewhere.

RESULTS

Residence Times on Fruits, Leaves, and Branches

Figure 1A gives the distributions of the total time spent by individual mendax and pomonella females on apple fruits. The time spent by pomonella females on apples was almost three times the mean residence time for mendax flies. The mendax distribution was skewed in the direction of individuals that spent short periods of time (less than 200 seconds) on apple fruits. R. pomonella females showed a broader range of residence times on apples than mendax. The distributions for most of the behaviors we analyzed do not match a normal probability function, and therefore we used nonparametric statistics to analyze our data. Using the Mann-Whitney U-test (Sokal and Rohlf 1981), the distributions of the time spent by mendax and pomonella females on apples are significantly different (U(s)=1454.0, P<0.01).

A large number of mendax females did not alight on the leaves of the apple plant (see zero histogram bar for the mendax distribution in Figure 1B), but over half the pomonella flies spent greater than 75 seconds on apple leaves. This resulted in a four-fold longer residence time for pomonella compared to mendax (Figure 1B, U(s)=1295.0, P<0.01).

There was a different pattern to the time spent by the same females on blueberries. Although there was no significant difference between mendax and pomonella in the length of time spent on blueberry leaves (Figure 2B, U(s)=909.5, P>0.2), differences occurred for the total time spent on blueberry fruits (Figure 2A, U(s)=1069.5, P<0.05). The longer mean residence time of mendax females on these fruits was due to a larger fraction of females that engaged in previpositional, ovipositional, and postovipositional behaviors.
TOTAL RESIDENCE TIME ON APPLE FRUITS

R. mendax

\[ \bar{x} = 200.8 \]
\[ n = 42 \]

R. pomonella

\[ \bar{x} = 588.4 \]
\[ n = 41 \]

Figure 1A. Total time spent by R. mendax and R. pomonella females on apple fruits during 20-minute observation periods.
TOTAL RESIDENCE TIME ON APPLE LEAVES

R. mendax

\[ \bar{x} = 75.7 \]

\[ n = 42 \]

R. pomonella

\[ \bar{x} = 312.6 \]

\[ n = 41 \]

Figure 1B. Total time spent by R. mendax and R. pomonella females on apple leaves during 20-minute observation periods.
Figure 2A. Total time spent by *R. mendax* and *R. pomonella* females on blueberry fruits during 20-minute observation periods.
TOTAL RESIDENCE TIME ON BLUEBERRY LEAVES

R. mendax

$\bar{x} = 197.7$

$n = 42$

R. pomonella

$\bar{x} = 200.1$

$n = 41$

Figure 2B. Total time spent by R. mendax and R. pomonella females on blueberry leaves during 20-minute observation periods.
The total residence time on branches of both host plants was small compared to the residence times on either fruits or leaves, and comparisons between the species did not show differences in the amount of time they spent on either blueberry (U(s)=905.5, P>0.1) or apple (U(s)=911.5, P>0.1) branches.

**Host Examining and Acceptance Behaviors Displayed on Fruits**

The following focuses on several host acceptance behaviors strongly associated with ovipositing into blueberry and apple fruits. Prior to bending her abdomen for oviposition, a female walked with her head tilted towards the fruit with the labellum of her mouthparts periodically touching the surface (lapping) (i.e., she touched the surface of the fruit, then raised her mouthparts, and repeated the behavior). On a few occasions, a female also touched the surface of the fruit with the tip of her third antennal segment (antennating). Following either lapping or antennating, she turned approximately 180 degrees, bent her abdomen and probed the fruit with her ovipositor. Probing the fruit is often (but not always) followed by the insertion of an egg into the puncture. After an egg was laid, the female began dragging her ovipositor over the surface of the fruit. During this behavior, she emitted a marking pheromone that partially deterred other females from laying an egg in the same fruit. (Prokopy 1972; Boller 1981; Boller and Hurter 1985).

The species showed differences in the number of occurrences of the lapping behavior on apples. *R. mendax* females displayed this behavior less often than *pomonella* females (Figure 3A, U(s)=1149.5, P<0.01; 55 percent of the *mendax* females did not display the behavior compared to 27 percent of the *pomonella* flies). In contrast, *mendax* females touched the surface of blueberry fruits with their labellum a greater number of times than *pomonella* females (Figure 3B, U(s)=1207.0, P<0.01).

Antennating was a relatively rare behavior, but differences existed between the species in the number of antennal contacts with both blueberry and apple fruits. On apples, the mean number of antennal contacts per *pomonella* female was 35 times the value for *mendax* (Figure 4A, U(s)=1032.0, P<0.01). On blueberries, the reverse occurred with *mendax* females displaying 14-fold more antennating than *pomonella* (Figure 4B, U(s)=967.0, P<0.05).

*R. mendax* and *R. pomonella* showed pronounced differences in the number of ovipositor probes into apple fruits (Figure 5A, U(s)=1155, P<0.01). None of the 42 *mendax* females probed apples with their ovipositor, whereas *pomonella* females probed apples from 0 to 9 times, with a mean of 1.2. *R. pomonella* females often probed an apple fruit for several brief periods prior to oviposition. This exploratory probing and the previously described lapping and antennating behaviors probably provided essential sensory information stimulating a female to either
**Behavior: Mouthparts Touching the Surface of an Apple Fruit**

- **R. mendax**
  - $\bar{x} = 1.3$
  - $n = 42$

- **R. pomonella**
  - $\bar{x} = 3.4$
  - $n = 41$

*Figure 3A. Number of occurrences of the lapping behavior by R. mendax and R. pomonella females on apple fruits.*
Figure 3B. Number of occurrences of the lapping behavior by R. mendax and R. pomonella females on blueberry fruits.
Figure 4A. Number of occurrences of the antennating behavior by *R. mendax* and *R. pomonella* females on apple fruits.
Figure 4B. Number of occurrences of the antennating behavior by R. mendax and R. pomonella females on blueberry fruits.
Figure 5A. Number of occurrences of the probing behavior by *R. mendax* and *R. pomonella* females on apple fruits.
oviposit or depart from the fruit.

A different pattern of ovipositional probes was observed on blueberry fruits; several mendax females rejected apple fruits but accepted blueberries. This alteration of behavior resulted in a four-fold greater mean number of probes in blueberries by mendax females compared to pomonella females (Figure 5B, U(s)=1209.5, P<0.01).

The differences in the preovipositional behaviors of mendax and pomonella were correlated with differences in the percentage of females that laid at least one egg into a fruit. Individuals initiating more preovipositional behaviors had a higher chance of laying an egg in the fruit. We refer to a female that lays one or more eggs per 20-minute observation period as an acceptor. On blueberries, the percentage of mendax acceptors was significantly higher than the percentage of pomonella acceptors, and the reverse pattern was observed on apples. Unexpectedly, the percentage of pomonella females ovipositing in blueberries did not differ significantly from the fraction ovipositing in apples (Table 1).

The two species also differed in the number of eggs laid per female in apples and blueberries. When averaged over accepting and nonaccepting females, mendax flies laid an average of 1.7 eggs in blueberries compared to 0.4 eggs per pomonella female (F=15.7, P<0.01). No mendax females accepted apples, and pomonella flies had significantly higher rates of oviposition (0.3 eggs per female) into this fruit (F=13.7, P<0.01).

The differences in rates of egg laying were linked with differences in the occurrence of ovipositor dragging on each host fruit. The pomonella females initiated a greater number of drags on the surface of apple fruits (Figure 6A, U(s)=990.5, P<0.01) compared to mendax females. The opposite relationship held on blueberries where mendax females invested all their eggs (Figure 6B, U(s)=1202.5, P<0.01).

Lastly, the two species differed in the amount of time spent in specific grooming behaviors on each host fruit. After dragging, both species preened the ovipositor with hind tarsi. This behavior was displayed primarily on fruits, and rarely occurred on either branches or leaves. On blueberries, mendax females spent a greater period of time preening their ovipositor compared to pomonella females (Figure 7A, U(s)=1247.5, P<0.01), and the differences between the species were again reversed on apples (Figure 7B, U(s)=1206.0, P<0.01).

DISCUSSION

R. mendax and R. pomonella have diverged in several key host examining and acceptance behaviors. The lack of probing and
Table 1. The percentage of *R. mendax* and *R. pomonella* females ovipositing in McIntosh apples and Bluehaven blueberries during field release experiments.

<table>
<thead>
<tr>
<th>Host plant</th>
<th>McIntosh apple</th>
<th>Bluehaven blueberries</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species tested</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. pomonella</em></td>
<td>11/41=0.27 (a,d)</td>
<td>10/41=0.24 (b,e)</td>
</tr>
<tr>
<td><em>R. mendax</em></td>
<td>0/42=0 (a,c,e)</td>
<td>25/42=0.60 (b,c,d)</td>
</tr>
</tbody>
</table>

Values followed by the same letter (a,b,c,d, or e) are significantly different from each other at the P=0.01 level using the G-test of independence.

Oviposition in apples by *mendax* females is consistent with electrophoretic data (Berlocher 1980; Berlocher and Bush 1982; Feder et al., unpublished) that found no evidence of *mendax* or *mendax x pomonella* hybrids in adults reared from apples. The low larval-to-adult viability of transplanted *mendax* larvae in apple fruits (14 percent compared to 39 percent in blueberry fruits; Bierbaum and Bush 1988), the results of electrophoretic studies, and data on ovipositional preferences indicate *mendax* females do not utilize apple as a host plant.

Surprisingly, *pomonella* females laid roughly equal numbers of eggs in apples and blueberries under field cage conditions. This was unexpected since prior studies indicated *pomonella* flies did not infest blueberries in nature (Bush 1966; Berlocher 1980; Prokopy and Berlocher 1980; Berlocher and Bush 1982). *R. pomonella* females, however, have laid viable eggs in blueberry
BEHAVIOR: PROBE A BLUEBERRY FRUIT WITH THE OVIPOSITOR

R. mendax

$\bar{x} = 2.1$

$n = 42$

R. pomonella

$\bar{x} = 0.5$

$n = 41$

Figure 5B. Number of occurrences of the probing behavior by R. mendax and R. pomonella females on blueberry fruits.
BEHAVIOR: DRAG OVIPOSITOR ON AN APPLE FRUIT

R. mendax

\[ \bar{X} = 0.1 \]
\[ n = 42 \]

R. pomonella

\[ \bar{X} = 1.0 \]
\[ n = 41 \]

Figure 6A. Number of occurrences of the dragging behavior by R. mendax and R. pomonella females on apple fruits.
Figure 6B. Number of occurrences of the dragging behavior by *R. mendax* and *R. pomonella* females on blueberry fruits.
Figure 7A. Total time spent by R. mendax and R. pomonella females in the ovipositor preening behavior on blueberry fruits.
BEHAVIOR: PREENING THE OVIPOSITOR WITH THE TARSI
LOCATION: APPLE FRUIT

R. mendax

\[ \bar{x} = 1.7 \]
\[ n = 42 \]

R. pomonella

\[ \bar{x} = 15.5 \]
\[ n = 41 \]

Figure 7B. Total time spent by R. mendax and R. pomonella females in the ovipositor preening behavior on apple fruits.
fruits when caged for several days on the branches of blueberry plants (Pickett 1937; Pickett and Neary 1940). Diehl and Prokopy (1986 and pers. comm.) are performing host selection experiments on mendax and pomonella flies that address a subset of topics covered in our own studies. In the laboratory 30 percent of their pomonella females oviposited in Red Delicious apples and high bush blueberries, but in field experiments using the same host fruits none of the pomonella flies oviposited in blueberries. The reason for this difference between their field and laboratory results is not discussed in their paper, but their field results do not agree with our results and the experiments of Pickett and Neary (1940) that showed pomonella females readily oviposited in blueberry fruits under field cage conditions.

Diehl and Prokopy (1986) also asserted that data did not support the designation of mendax and pomonella as distinct species. This conclusion is not consistent with the empirical evidence, and we do not agree with their assessment. Electrophoretic studies (Berlocher 1980; Berlocher and Bush 1982; Feder et al. unpublished) have determined that the species maintain unique alleles at four enzyme loci in both sympatric and allopatric populations. Consistent morphological differences between the species have been established for ovipositor length, wing band ratios (Bush 1966), male genitalia (Curran 1932; Bush 1966), and pupal dimensions (Bierbaum and Bush, manuscript). Interspecific mating trials and no-choice ovipositional experiments (McAlister and Anderson 1935; Pickett 1937; Pickett and Neary 1940) have indicated that these behavioral traits are involved in the reproductive isolation between the species. No F1 pupae were obtained from crosses between mendax females and pomonella males caged on apple and blueberry plants, whereas the reciprocal cross produced F1 pupae on both fruits. Hybrid adults were also produced from the latter cross, but the larval-to-adult survivorship was lower for hybrids compared to progeny from within species crosses (Bierbaum and Bush 1988). Therefore, the formation of interspecific hybrids in nature is unlikely. If this event occurs, it would most likely result from pomonella females mating mendax males and ovipositing in blueberry fruits. Thus, any occasional gene flow between the species should be primarily unidirectional from pomonella to mendax. Additional traits that could serve as mechanisms of reproductive isolation have not been thoroughly studied. These include differences in the prelighting host finding behaviors of mendax and pomonella males and females and the mating ability, ovipositional behavior, and viability of hybrid progeny.

In addition to differences in their ovipositional behaviors, mendax and pomonella females showed large differences in: i) residence times on leaves and fruits, and ii) examining and acceptance behaviors tightly linked with oviposition. The examining behaviors included touching the surface of the fruit with the mouthparts (lapping), antennating, and probing the fruit
with the ovipositor; behaviors following oviposition included dragging the ovipositor on the fruit surface and preening it with the hind tarsi.

Compared to mendax females, pomonella females spent more time on both the fruits and leaves of apple plants, and the reverse relationship held for the mean residence times of the two species on blueberries. A large fraction of the differences in residence times on fruits was due to differences in examining, probing, and dragging behaviors. R. mendax flies displayed these behaviors more often than pomonella on blueberries, and the reverse pattern was observed on apples. The species investing the most eggs in a fruit also completed more ovipositor draggings on the fruit surface. This is consistent with prior experimental evidence indicating that the dragging behavior is tightly associated with oviposition into a fruit (Prokopy 1972).

The lapping, antennating, and probing behaviors probably provide essential chemosensory information, stimulating a female to oviposit into or depart from the fruit. Recent studies that have characterized chemosensilla on the ovipositor, tarsi, and antennae of pomonella flies have identified two longitudinal groves at the apical portion of the ovipositor contain chemosensilla that respond to NaCl solutions (Liscia et al. 1982). Contact chemosensory sensilla have been identified on the tarsi of pomonella females by Bowdan (1984), who found that different cells within a sensillum showed distinct electrophysiological responses to ovipositional-deterrent pheromone, sucrose, and salt solutions. Fein et al. (1982) used apple volatiles to study electroantennogram responses of pomonella males and females, and found a variety of GLC-fractioned apple extracts stimulated antennal sensilla. With the exception of the study by Fein et al. (1982), few experiments have tested the responses of mendax or pomonella chemosensilla to naturally occurring fruit compounds, and there is a need for more electrophysiological work with such compounds.

A few studies by other researchers have tested for evolutionary divergences in the adult host acceptance behaviors of closely related populations and species of insects infesting different host plants. One experiment established clear differences in the preferences of sympatric host races. In an interesting set of greenhouse experiments, Phillips and Barnes (1975) observed differences in ovipositional preferences of races of codling moths infesting apples, walnuts, and plums. They found the apple race strongly preferred apple plants, and the walnut and plum races preferred walnut trees over both apples and plums. The ovipositional preferences of walnut x apple and plum x apple F1 hybrids were intermediate to the wild parental populations, indicating a genetic basis for differences in preference.

A high degree of specificity in ovipositional preference has
also been established for host races of sawflies infesting balsam fir, black spruce, and white spruce (Knerer and Atwood 1972; 1973), and closely related species of gall-forming tephritid flies (Huettel and Bush 1972). In both studies, laboratory choice experiments revealed a virtually complete ovipositional preference for the host plant infested by each race or species in nature. Huettel and Bush (1972) studied the genetic basis of host selection using F1, F2 and backcross hybrids, and found that genetic variation at a single locus could explain the differences in preference between the species.

Several other studies have found allopatric populations within a species that have diverged in host preferences or larval viabilities in response to variation in the abundance of alternate plants in different geographic regions (e.g., Singer 1971, 1983; Hsiao 1978; Carson and Ohta 1981; Rausher 1982; Tabashnik 1983; Papaj 1986). In several of these examples, evolutionary changes in host preference have occurred during a host range expansion in which one or more locally abundant host plants were added to the total number used by the species. Although these studies have helped clarify the nature of evolutionary differentiation between populations of phytophagous insects, an improved understanding of the phenotypic and genetic bases of behaviors controlling differential host plant use by closely related insect species is essential. By continuing our analysis of host examining and acceptance behaviors of *mendax*, *pomonella*, and their hybrid progeny, we hope to understand the genetic bases of these behavioral traits. The methods we have developed permit detailed step-by-step analyses of differences in key host acceptance behaviors displayed by each species on blueberry and apple plants. Through focusing on the inheritance of these traits, we hope to gain an improved understanding of characters thought to be important in the formation of host races and species of *Rhagoletis* fruit flies. We believe this approach can also provide insights into behavioral mechanisms controlling colonization of plants by insect herbivores.

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SUMMARY

Our study focuses on the host examining and acceptance behaviors of two sibling species of *Rhagoletis* fruit flies infesting different rosaceous and ericaceous host plants. Field cage experiments tested for differences in the post-alighting behaviors of mated *R. mendax* and *R. pomonella* females on apple and blueberry plants. These experiments utilized a portable microcomputer to provide a quantitative, sequential record of the host examining and acceptance behaviors displayed on both host plants and pinpointed differences between the species in key post-alighting behaviors. This quantitative behavioral approach determined significant behavioral differences amenable to further genetic analysis, and provided essential information for understanding the evolutionary processes involved in host race and species formation.

There were several differences in the examining and acceptance behaviors of the species on apple and blueberry fruits. These included: i) touching surface of the fruit with the labellum of the mouthparts (lapping), ii) touching the fruit surface with the tip of the third antennal segment (antennating), iii) bending the abdomen and probing the fruit with the ovipositor for egg laying, iv) dragging the ovipositor on the fruit to deposit an oviposition-deterrent pheromone, and v) preening the ovipositor with the tarsi. The differences between the species in their preovipositional behaviors were correlated with differences in the percentage of females laying at least one egg in a fruit. The percentage of *mendax* females ovipositing in Bluehaven blueberries was significantly higher than the percentage of *pomonella* females; the opposite pattern was observed on the fruits of McIntosh apple plants. In addition, the numbers of eggs laid per *mendax* female in blueberries was higher than the number of eggs per *pomonella* female, and the reverse pattern was found in apple fruits. *R. pomonella* females resided for longer periods of time on apple fruits and leaves than *mendax* flies, and the reverse relationship held for the time spent by each species on blueberry fruits.

Our continuing analysis of host examining and acceptance behaviors displayed by *R. mendax*, *R. pomonella*, and their hybrid progeny should help ascertain the phenotypic and genetic bases of behaviors controlling differences in the host plant use of these closely related species. By focusing on these behaviors, we hope to gain an increased understanding of characters thought to be important in the formation of host races and species of *Rhagoletis* fruit flies.
REFERENCES


