A Field Test of Differential Host-Plant Usage between Two Sibling Species of Rhagoletis pomonella Fruit Flies (Diptera: Tephritidae) and its Consequences for Sympatric Models of Speciation

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A FIELD TEST OF DIFFERENTIAL HOST-PLANT USAGE BETWEEN TWO SIBLING SPECIES OF RHAGOLETIS POMONELLA FRUIT FLIES (DIPTERA: TEPHRITIDAE) AND ITS CONSEQUENCES FOR SYMPATRIC MODELS OF SPECIATION

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Speciation in the Rhagoletis pomonella sibling-species complex may be initiated in sympatry when these true fruit flies shift and adapt to new host plants (Bush, 1966, 1969a, 1969b, 1975). The four described species (R. pomonella, R. mendax, R. zephyria, and R. cornivora) and one undescribed taxon (“Cornus florida fly”) comprising the group overlap broadly in their geographic distributions across North America, with each species infesting a different set of host plants (Bush, 1966). In addition, larvae are obligate internal parasites in the fruits of their host plants, and adults mate almost exclusively on or near these fruits (Prokopy et al., 1971, 1972, 1974, 1975).
graphic distributions in eastern North America, and ical similarity, almost completely overlapping geo-
1814 through this century due to their close morpholog-
ical barriers to gene flow to result eventually in the com-
fect host plants. Third, we have to demonstrate that host specialization can, with time, evolve sufficiently to cause the complete reproductive isolation of populations.

Ecological, behavioral, and genetic studies on popula-
tions of R. pomonella infesting apple (Malus pa-
rnula) and hawthorn (Crataegus spp.) have suggested that host specialization can occur for conspecific populations in the absence of geographic barriers to gene flow (Feder et al., 1988, 1990a, 1990b; Feder and Bush, 1989; McPherson et al., 1988; Prokopy et al., 1988; Smith, 1988). Differences in host preference and developmental timing were implicated as key factors causing apple and hawthorn populations to be partially reproductively isolated. Hawthorn and apple “host races” of R. pomonella may therefore represent incipient species. However, it remains to be seen whether host-associated traits can evolve into effective enough barriers to gene flow to result eventually in the complete reproductive isolation of R. pomonella populations.

The objective of the current study is to establish the efficiency of differential host usage as a premating isolating mechanism between the blueberry maggot fly, R. mendax (Curran), and the apple maggot fly, R. pomonella (Walsh). The taxonomic distinction between R. mendax and R. pomonella has been in dispute throughout this century due to their close morphological similarity, almost completely overlapping geographic distributions in eastern North America, and ability to interbreed in laboratory crosses (Bush, 1966; Diehl and Prokopy, 1986). However, electrophoretic studies of larvae collected from infested blueberry, hawthorn, and apple fruits (Berlocher, 1976; Berlocher and Bush, 1982; Feder et al., 1989a) have shown that R. mendax and R. pomonella are genetically distinct taxa, possessing “species-specific” alleles for 11 different allozyme loci (R. mendax having unique alleles for two loci and R. pomonella having unique alleles for nine). The allozyme results gave no indication that R. mendax and R. pomonella hybridize in nature and suggested that differential host utilization is an important factor restricting gene flow between the two species.

The unique alleles possessed by R. pomonella and R. mendax permit a direct field test of the effectiveness of differential host usage as a premating reproductive isolating mechanism. Using the genotype of a fly for loci possessing species-specific alleles, it is possible to type most adults as either R. pomonella or R. mendax and, consequently, to infer whether an individual infested an apple/hawthorn or blueberry fruit as a larva (see Feder et al., 1989a). Genetic analysis of adults collected from sympatric host plants would therefore provide a measure of interhost migration (i.e., host fidelity prior to alighting) for R. pomonella and R. mendax between apple/hawthorn and blueberry plants under natural field conditions.

Note, however, that R. mendax and R. pomonella are not diagnostically fixed for alternative alleles for any allozyme locus. As a result, a small percentage of flies will not be genetically identifiable as either R. mendax or R. pomonella because they will not possess a species-specific allele characteristic of one or the other taxa.

The present study is not a direct test of whether any currently existing host-utilization differences between R. mendax and R. pomonella were responsible for initiating speciation for these taxa. Because we do not know the history of the R. pomonella/R. mendax split, we can only hypothesize on the geographic context and chronological order in which reproductive isolating mechanisms arose between these species. Consequently, differential utilization of blueberries, hawthorns, and especially apples by R. mendax and R. pomonella may have developed after populations of these flies were already partially reproductively isolated due to other factors, such as postmating sterility. However, as we mentioned above, differences in host preference have accompanied the recent sympatric formation of new host races in the species R. pomonella, suggesting that host specialization evolves quite early in the divergence process for these flies.

Regardless of when or how differential host plant usage evolved for R. mendax and R. pomonella, we can still test its effectiveness as a premating barrier to interspecific gene flow at the present time. The results from such a study should indicate whether traits responsible for host fidelity have the potential to cause the complete reproductive isolation of R. pomonella populations. If reproductively active R. pomonella and R. mendax adults frequently come into contact on host plants, then ethological premating or postmating re-
productive isolation must also be responsible for main-
taining the genetic integrity of these two species, a result inconsistent with predictions of sympatric-speciation models.

Life History and Biology of Rhagoletis Flies
Rhagoletis mendax and R. pomonella are, in general, univoltine across their respective ranges in eastern North America (Bush, 1966; Dean and Chapman, 1973). Females identify appropriate host-plant species by specific visual, tactile, and olfactory cues (Prokopy, 1968; Prokopy et al., 1973, 1987, 1988; Moericke et al., 1975; Fein et al., 1982; Owens and Prokopy, 1986; Papaj and Prokopy, 1986). Males are attracted by the same cues, and mating occurs almost exclusively on or near the fruits of the host plant (Prokopy et al., 1971, 1972). Multiple insemination is common for R. pomonella females, and sperm precedence has been documented in these flies (Opp, 1988). In fact, individuals of both sexes may mate as many as 30 times within a two-
week period (Opp, 1988), indicating that R. pomonella has an extremely polygamous mating system. Mark-
and-recapture studies have also shown that R. pomo-
nella are highly vagile, as adults have been observed to travel at least 1.6 km in search of host plants (Max-
well and Parsons, 1968), with only about 10% of marked flies recaptured on "release trees" (Maxwell, 1968; Reissig, 1977). Once a suitable host is found, adult females lay their eggs in the flesh of the plant's fruit while the fruit is still on the tree. Eggs hatch within two days, and immatures develop through three larval instars over a period of 3–5 weeks. A larva feeds and completes development only within the single host fruit in which it hatched. After the fruit abscises from the host plant and falls to the ground, late third-instar larvae leave the fruit and burrow a few inches into the soil, where they pupate after 3–4 days. Flies therefore overwinter as pupae in the soil beneath the tree they infested as larvae during the preceding summer. Most flies terminate diapause and eclose as adults the next spring, although some pupae require two winters of chilling in the field before completing development (Phipps and Dirks, 1933).

**Allozyme Studies of Field-Collected Adults and Larvae**

**Materials and Methods.**—Field experiments analyzing host fidelity for *R. mendax* and *R. pomonella* were conducted at a study site near the town of Chickaming, in the southwestern comer of Michigan. Previous genetic analysis of larvae from infested blueberry and apple fruits at this site in 1984 and 1985 gave no evidence of any hybridization or nuclear gene flow between the two species (Feder et al., 1989a). Adult flies were collected only from the apple tree and a row of blueberry plants that were in physical contact at the northern edge of a blueberry patch. Large numbers of flies were observed mating on the adults that we could genetically identify as *R. mendax* and *R. pomonella*. We therefore found no evidence for any interhost migration of *R. mendax* or *R. pomonella* between blueberry and apple plants. The alleles *Fum* and *Dia-3* were only found in adult flies collected from blueberry bushes (Table 1: allele frequencies were homogeneous across all three collecting dates for both blueberry and apple flies and so were pooled across dates). Conversely, *Dia-2*, *Aat-I*, *Aat-2*, *Acon-2*, *Dia-2*, *Dia-3*, and *Fum* suggest that these individuals were not hybrids of *R. pomonella* but were *R. mendax*. In fact, each of these three flies was at least 270,000 times more likely to be *R. mendax* than *R. pomonella* and from 270 to 4,200 times more likely to be *R. mendax* than an F1 hybrid. Irrespective of these three individuals, of the 114 adults that we could genetically identify as *R. mendax* and the 127 adults identified as *R. pomonella*, not a single fly was captured on the "wrong" host species. Consequently, reproductively active *R. mendax* and *R. pomonella* adults did not frequent each other's host plants, even for foraging purposes, despite the fact that the apple and blueberry plants surveyed were in physical contact with one another.

The electrophoretic results for field-dissected larvae were identical to those for adults (Table 1). All 85 larvae dissected from blueberry fruits in the field had at least one diagnostic allele for *R. mendax* (i.e., *Fum* or *Dia-3*), and none of these flies possessed a species-specific allele characteristic of *R. pomonella* (i.e., *Acon-2*, *Dia-2*, *Aat-I*, *Aat-2*, *Acon-2*, *Dia-2*, *Dia-3*, and *Fum*). Conversely, all 120 larvae infesting apples had at least one unique *R. pomonella* allele, and no individual carried a diagnostic *R. mendax* marker. We therefore found no evidence for any hybridization or nuclear-gene introgression between *R. mendax* and *R. pomonella*, as not a single larva possessed a genotype characteristic of either an F hybrid or F2 backcross at the Chickaming, Michigan, site, despite the fact that approximately 90% of all offspring resulting from hybrid matings would be expected to carry at least one *R. pomonella* allele and one *R. mendax* allele for *Aat-1*, *Aat-2*, *Acon-2*, *Dia-2*, *Dia-3*, and *Fum*.

The species-specific alleles for *Aat-1*, *Aat-2*, *Acon-2*, *Dia-2*, *Dia-3*, and *Fum* can be used to calculate a statistical confidence level of hybridization between *R. mendax* and *R. pomonella*. Combining results from the current and previous studies (Feder et al., 1989a), we have now electrophoretically analyzed a total of 421 larvae from blueberries and 486 larvae from apples at three different sympatric field sites in western Mich-
igan and have yet to score a single potential hybrid. If we assume that the probability of genetically detecting offspring from a \textit{R. mendax} × \textit{R. pomonella} mating is 90\% at all three of these sites, that interspecific crosses have the same fecundity as intraspecific matings, and that hybrid larvae have viabilities in host fruits equivalent to those of pure \textit{R. mendax} and \textit{R. pomonella}, then finding no \textit{F}_1 hybrid among these 907 larvae is significant at the \( P \leq 0.05 \) level, based on a Poisson distribution with hybridization occurring at a frequency of 0.37\% or greater.

Differential host utilization may not, however, be the only factor responsible for the lack of detectable \textit{F}_1 hybrids between apple and blueberry flies. Ethological premating isolation and postmating sterility could also be factors contributing to the absence of hybrids. To determine whether \textit{R. mendax} and \textit{R. pomonella} have the potential to mate and produce viable \textit{F}_1 larvae if they ever happen to come into contact on host plants, we conducted hybridization experiments in the field and laboratory.

\textbf{Field Hybridization Experiment}

\textit{Materials and Methods.} — A portion of the field-captured adults were used for a simple hybridization experiment. Male and female flies which we collected from different host plant species at the Chickaming, Michigan, site on July 16 were placed into 15 cm × 15 cm × 15 cm wire-mesh cages immediately following capture (total of four cages: two cages each containing four females collected from blueberry and four males from apple; two cages each containing four males from blueberry and four females from apple). Leaves of both host plants were added to the cages to simulate field conditions. We monitored the mating activities of these flies for ten minutes before freezing them in liquid nitrogen.

\textit{Results.} — \textit{R. mendax} and \textit{R. pomonella} readily mated in the field hybridization experiment. Three pairs of \textit{R. mendax} males and \textit{R. pomonella} females and four pairs of \textit{R. mendax} females and \textit{R. pomonella} males were observed in copula within two minutes of having been introduced into mating cages at the Chickaming site. However, the remainder of the flies in these cages did not mate during the ten-minute observation period. Also, there appeared to be some mechanical difficulty in two of the \textit{R. mendax} male × \textit{R. pomonella} female pairings. In these instances, males had difficulties mounting females and sustaining the correct mating position. \textit{R. mendax} has a smaller average body size than \textit{R. pomonella} and \textit{Rhagoletis} flies are sexually dimorphic, with females being larger than males (Bush, 1966). Although these allometric differences could account for the mechanical difficulties experienced by the two \textit{R. mendax} males in mating with \textit{R. pomonella} females, the field hybridization experiments suggest that ethological premating barriers are probably not strong between \textit{R. mendax} and \textit{R. pomonella}. The polygamous nature of the mating system and field observations of a considerable amount of male rape (Opp, 1988; Feder, pers. observ.) also imply that sexual selection is somewhat lax in these flies. All available evidence therefore suggests that blueberry and apple flies will mate when they come into contact on host plants.

\begin{table}
\centering
\begin{tabular}{llllllllllll}
\hline
\textbf{Host} & \textbf{Stage} & \textbf{Sex} & \textbf{Allele} & \textbf{N} & \textbf{F} & \textbf{L} & \textbf{F} & \textbf{L} & \textbf{F} & \textbf{L} & \textbf{F} & \textbf{L} & \textbf{F} & \textbf{L} & \textbf{F} & \textbf{L} & \textbf{F} & \textbf{L} \\
\hline
\end{tabular}
\caption{Allele frequencies for \textit{R. mendax} and \textit{R. pomonella} collected from interdigitated blueberry (B) and apple (A) host plants, respectively, at a field site near Chickaming, Michigan (see text for locus abbreviations). Allele frequencies are given only for species-specific alleles at each locus. Abbreviations: Ad = adults captured directly from host plants; L = larvae dissected from host fruits; F = female; M = male; \( N = \) sample size.}
\end{table}
Table 2. Classification of larvae from the laboratory hybridization experiment, based on their multilocus genotypes for Aat-1, Aat-2, Acon-2, Dia-2, Dia-3, and Fum. The larval sample was divided into four different time periods, as discussed in the text. Unidentified larvae were those that could not be genetically classified because they did not possess a diagnostic allele for any of the six allozymes listed above.

<table>
<thead>
<tr>
<th>Time period</th>
<th>R. mendax female × R. pomonella male</th>
<th>R. mendax male × R. pomonella female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hybrid</td>
<td>R. mendax</td>
</tr>
<tr>
<td>July 16–19</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>July 19–21</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>July 21–29</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>July 29–August 5</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Total for last three periods</td>
<td>48</td>
<td>10</td>
</tr>
</tbody>
</table>

Laboratory Hybridization Experiment

Materials and Methods.—A more detailed hybridization experiment was conducted in the laboratory to determine whether R. mendax × R. pomonella crosses are fertile and produce genetically distinguishable F1 hybrid progeny. On July 16, ten adult females captured on apples at the Chickaming, Michigan, site were placed into two wire mesh cages (five females per cage). Likewise, ten females collected from blueberries on the same date were put into two separate cages (total of four cages for the two species). Uninfested blueberry and apple fruits were added to the two R. mendax and two R. pomonella cages, respectively, to provide females with fruit for oviposition, and the flies were transported back to the laboratory. On July 19, the fruits were removed from each of the cages and placed into separate storage trays. Trays were individually covered with fine mosquito netting to prevent larval migration. Fresh fruit was then added to each cage along with five “heterospecific” males collected on July 16 from the opposite species of host plant. Fruit was subsequently replaced every other day, dated, and stored separately by date. After 18 days of storage, infested fruit was dissected, and larvae were frozen in liquid nitrogen. The crosses were terminated on August 5, and the nine surviving blueberry flies (five females and two males) and seven surviving apple flies (three females and six males) were dissected from host fruits dated July 19-21 (Table 2). In addition, pure R. mendax, R. pomonella females, and two R. mendax and two R. pomonella male crosses produced similar results. The alleles Aat-2<sup>100</sup>, Dia-2<sup>100</sup>, Acon-2<sup>273</sup>, and Aat-1<sup>100</sup> (normally found only in R. pomonella populations) were observed in progeny only after the addition of male apple flies to the two blueberry female cages. In all, approximately 79% of the 61 larvae analyzed from R. mendax female × R. pomonella males crosses and 27% of the 66 larvae scored from the reciprocal pairing could be unambiguously identified as hybrids because they possessed diagnostic alleles for both species (Table 2).

Females in the laboratory experiments hybridized with “heterospecific” males within two days of their introduction into the mating cages, as hybrid larvae were dissected from host fruits dated July 19–21 (Table 2). In addition, pure R. mendax and R. pomonella offspring were dissected from blueberry and apple fruits, respectively, placed into the mating cages on July 29 (Table 2). Both R. mendax and R. pomonella females therefore carried stored “heterospecific” sperm from the field for at least 13 days. These results also show that females did not begin mating with heterospecific males only after they had completely depleted stored supplies of homospecific sperm in their spermathecae.

The percentage of hybrid larvae from R. mendax female × R. pomonella male crosses rose to almost 100% after July 19 (Table 2), however, suggesting that the propensity for these flies to interbreed may have increased with time and decreasing sperm supply; but the proportion of hybrid larvae from the R. mendax male × R. pomonella female cages remained essentially the same over time (Table 2).

A single male and female mating pair did not produce all of the hybrids detected in either of the recip-
rocal interspecific laboratory crosses. The genotypes of hybrid larvae for Aat-2 and Acon-2 indicated that at least five different individuals (three of one species and two of the other) produced progeny from the R. mendax female × R. pomonella male cages and that at least two R. pomonella females and one R. mendax male gave rise to progeny in the reciprocal cross.

**Discussion**

Differential host usage is an important factor helping to maintain species differences between R. mendax and R. pomonella at the present time. Field-hybridization experiments demonstrate that R. mendax and R. pomonella have the potential to mate when they come into contact on host plants. Laboratory crosses show that hybrids can survive to late-instar stages in either apples or blueberries. (T. J. Bierbaum and G. L. Bush [unpubl.] have found that hybrids can survive from egg to adult stages in egg-transplant experiments conducted in the field.) Furthermore, the laboratory experiments indicate that hybrid larvae are genetically identifiable, based on their multilocus genotypes for Aat-1, Aat-2, Acon-2, Dia-2, Dia-3, and Fum. Consequently, the complete lack of detectable hybrid larvae from dissected field fruit cannot be the result of only ethological premating or postmating isolation. Rather, it is a reflection of the limited extent to which reproductively active R. mendax and R. pomonella adults meet, due to their preferences for blueberry and apple host plants, respectively. Without these differences in host preference, it is conceivable that populations of apple and blueberry maggot flies would interbreed freely and fuse. Therefore, in instances when host fidelity in the R. pomonella species group is not perfect and taxa occasionally come into contact on host plants, we may expect to find evidence for low levels of nuclear gene introgression. Such a situation could explain the presence of the common Had allele of R. zephyria (Had\textsuperscript{111}) in western populations of R. pomonella (McPherson, 1987, 1989).

Differences have previously been found in the behavioral and chemosensory responses of R. mendax and R. pomonella to apple, hawthorn, and blueberry fruits (Diehl and Prokopy, 1986; Bierbaum and Bush, 1988; J. Frey and G. L. Bush, unpubl.). However, these differences were not discrete in laboratory and field tests, and some overlap was observed in the host-acceptance behaviors of the two species (Diehl and Prokopy, 1986). The finding of what appears to be complete host fidelity for these flies under natural conditions suggests that previous laboratory and field cage experiments did not provide the correct combination or incorporate all of the specific cues responsible for proper host identification.

Further studies of mate choice, interspecific fertility, and hybrid viability and breakdown are still needed to quantify the contributing roles that these factors may play in restricting effective gene flow between R. mendax and R. pomonella and to fine-tune our confidence level of hybridization. Although F\textsubscript{1} hybrid progeny are fertile and produce viable F\textsubscript{2} and backcross offspring in laboratory crosses, preliminary results suggest that the fecundity of F\textsubscript{1} hybrids is reduced (perhaps by as much as 50%) compared to pure species (J. Frey, pers. comm.). Postmating isolation may, therefore, further reduce the likelihood of introgression between R. pomonella and R. mendax in those rare instances when these two species happen to hybridize in the field.

Assortative mating due to differences in host utilization is an important component of sympatric speciation models (Bush, 1969a, 1969b, 1975). The direct connection between host choice and mate selection in R. pomonella group flies makes it possible for variation in traits affecting host utilization to act as genetically based, premating isolating mechanisms. The results of the current study suggest that such host-preference traits have the potential to evolve into effective enough barriers to gene flow to result in complete reproductive isolation between R. pomonella populations. Other studies have shown that host-associated traits differ between recently formed and sympatric “host races” of the species R. pomonella (Feder et al., 1988, 1990a, 1990b; McPherson et al., 1988; Prokopy et al., 1988; Smith, 1988). It therefore appears likely that speciation in the R. pomonella group can be initiated in the absence of geographic isolation as a consequence of these flies shifting and adapting to previously unexploited host plants.

**Acknowledgments**

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NOTES AND COMMENTS


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