

## HABITAT AVOIDANCE: OVERLOOKING AN IMPORTANT ASPECT OF HOST-SPECIFIC MATING AND SYMPATRIC SPECIATION?

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**Abstract.**—Understanding speciation requires discerning how reproductive barriers to gene flow evolve between previously interbreeding populations. Models of sympatric speciation for phytophagous insects posit that reproductive isolation can evolve in the absence of geographic isolation as a consequence of an insect shifting and ecologically adapting to a new host plant. One important adaptation contributing to sympatric differentiation is host-specific mating. When organisms mate in preferred habitats, a system of positive assortative mating is established that facilitates sympatric divergence. Models of host fidelity generally assume that host choice is determined by the aggregate effect of alleles imparting positive preferences for different plant species. But negative effect genes for avoiding nonnatal plants may also influence host use. Previous studies have shown that apple and hawthorn-infesting races of *Rhagoletis pomonella* flies use volatile compounds emitted from the surface of fruit as key chemosensory cues to recognize and distinguish between their host plants. Here, we report results from field trials indicating that in addition to preferring the odor of their natal fruit, apple and hawthorn flies, and their undescribed sister species infesting flowering dogwood (*Cornus florida*), also avoid the odors of nonnatal fruit. We discuss the implications of nonnatal fruit avoidance for the evolutionary dynamics and genetics of sympatric speciation. Our findings reveal an underappreciated role for habitat avoidance as a potential postmating, as well as prezygotic, barrier to gene flow.

**Key words.**—Apple maggot fly, fruit volatiles, habitat avoidance, host-specific mating, reproductive isolation.

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Speciation in sexually reproducing organisms is predicated on the evolution of reproductive barriers to gene flow between populations. These barriers can take the form of inherent differences reducing the propensity of individuals from different populations to mate, to form zygotes following coupling [after mating], or to produce zygotes that develop into viable and fertile (reproductive) progeny (Dobzhansky 1937, 1951). Understanding speciation therefore requires discerning the historical and biogeographic context of traits that reproductively isolate populations during the formative stages of divergence (Coyne and Orr 2004).

Apple and hawthorn-infesting populations of the tephritid fruit fly *Rhagoletis pomonella* are a model system for sympatric host race formation and speciation in real time (Bush 1994; Berlocher and Feder 2002; Jiggins and Bridle 2004). As early as the mid-1800's, Walsh (1864) proposed that certain host-specific phytophagous insects could speciate in the absence of geographic isolation in the process of shifting and adapting to new host plants. In particular, Walsh (1867) cited the recent shift of *R. pomonella* from its native host hawthorn (*Crataegus* spp.) to introduced, domesticated apple (*Malus pumila*) in the 1800's as a potential example of sympatric divergence in progress. Bush (1966, 1969a,b) subsequently argued that the entire complex of six or more taxa comprising the *R. pomonella* sibling species complex, to which apple and hawthorn flies belong, possessed several attributes consistent with the complex having radiated sympatrically via host-plant shifts.

Sympatric speciation via host shifting is a form of ecological divergence that occurs without geographic isolation and in the face of gene flow (Schluter 2001; Via 2001). For *R. pomonella*, the ecological selection pressures driving sympatric divergence emanate from colonizing and specializing

on novel host plants (Bush 1969a, b). One important adaptation contributing to sympatric differentiation is host-specific mating. When organisms mate in preferred environments or habitats, a system of positive assortative mating is established that can facilitate sympatric divergence (Maynard Smith 1966, Diehl and Bush 1989, Rice and Hostert 1993, Kawecki 1996, Johnson and Gullberg 1998; Kondrashov et al. 1998, Fry 2003). For *R. pomonella*, as is true for many phytophagous insects, habitat specific mating is manifested in the form of host fidelity; adult flies tend to mate on or near the same species of host fruit that they infested as larvae (Feder et al. 1994). Factors that influence which host plant a fly chooses to reside on therefore determine its potential mates, and populations of flies preferring different host species will be reproductively isolated from one another by a premating barrier to gene flow. Mark-recapture studies have estimated that host fidelity can reduce interhost migration and mating between the apple and hawthorn-infesting races of *R. pomonella* to 4–6% per generation (Feder et al. 1994, 1998), and has the potential to generate near complete isolation between *R. pomonella* and related sibling species, such as the blueberry maggot, *R. mendax* (Feder and Bush 1989). Discerning how specialist insects such as *Rhagoletis* discriminate among potential host plants is therefore a central element for understanding sympatric race formation and speciation because these are key traits responsible for driving habitat-specific mating, generating prezygotic reproductive isolation, and initiating population divergence.

Proper host plant identification for phytophagous insects involves specific visual, chemical, tactile, and gustatory cues (Bernays 2001). Studies of host recognition for the apple-infesting race of *R. pomonella* have implied that apple fruit odor is the most important long-to-intermediate range cue

used by apple flies to locate apple trees (Prokopy and Roitberg 1984). Apple flies rely on a combination of visual and chemosensory cues at distances of less than a meter to locate fruit in the tree canopy (Prokopy and Roitberg 1984), and on tactile, visual, and chemical cues once they have alighted on fruit. Recent work has shown that apple and hawthorn flies also use fruit volatiles to discriminate between apple and hawthorn trees (Linn et al. 2003). Synthetic blends of apple and hawthorn fruit volatiles have been identified that induce the same behavioral response in apple and hawthorn origin flies in flight tunnel assays as whole fruit extracts (Zhang et al. 1999; Nojima et al. 2003a). In no choice tests, apple and hawthorn flies preferentially flew upwind in the tunnel to reach source spheres containing their natal compared to nonnatal fruit blend (Linn et al. 2003). Apple and hawthorn flies were also disproportionately trapped in the field on spheres baited with their natal versus nonnatal fruit volatiles in choice trials (Linn et al. 2003). These data imply that the apple race has, within the past 150 years, evolved an increased preference for (attraction to) a unique mix of apple fruit volatiles, whereas hawthorn fly populations have retained their ancestral preference for the odor of hawthorn fruit.

Evidence suggests that *R. pomonella* flies may also actively avoid nonhost fruit volatiles. (We use the term "avoid" here to refer to the tendency of a fly to stay away from a particular alternative host-fruit odor, without implying that this response is dictated by any immediate harm to the individual, like the toxic effect of a chemical or its association with predators). First, addition of nonnatal volatiles to the apple and hawthorn fruit odor blends antagonized the upwind flight behavior of apple and hawthorn flies in wind tunnel assays (Linn et al. 2005a). The results imply, but do not prove, avoidance behavior because the reduced response of flies to mixed blends may be due to disruption of the positive signal of the natal blend rather than a deterrence effect of the nonnatal volatiles per se. Second,  $F_1$  hybrids between the apple and hawthorn host races of *R. pomonella*, as well as *R. pomonella* and its hypothesized sister species infesting flowering dogwood (*Cornus florida*) (Berlocher 1999, 2000), all failed to respond to apple, hawthorn, or dogwood fruit odor blends at 200  $\mu\text{g}$  doses that elicit maximal upwind flight behavior in parent taxa (Linn et al. 2004). A portion of hybrid flies (30–50%) did respond in 2000  $\mu\text{g}$  dose tests. Such high concentrations of natal fruit volatiles in the odor plume deter parental taxa from reaching source spheres (Linn et al. 2004). Moreover, hybrids responding in the high dose trials predominately oriented to a 1:1 combination of their parent's blends, a mixture that generally has antagonistic effects on apple, hawthorn, and dogwood flies (Linn et al. 2005a).  $F_1$  hybrids therefore display response patterns consistent with them possessing avoidance alleles for alternative parental fruit volatiles.

Demonstrating fruit-odor avoidance is not a straightforward process, however. Previous no choice tests of fly behavior to fruit blends in the flight tunnel were predicated on control experiments in which flies did not respond to odorless red spheres positioned 1 m upwind (Linn et al. 2003). Consequently, any fly exhibiting directed flight to reach an odor-baited, red sphere could be considered to show a positive

response to the tested fruit blend. However, flies not responding to nonnatal volatiles are not necessarily avoiding the blend, because they may merely not recognize the odor. As discussed above, adding nonnatal volatiles to the natal blend is also not a direct test for avoidance behavior. Demonstrating fruit odor avoidance requires that flies first be attracted to a host cue having a different sensory modality than olfaction (e.g., a visual fruit stimulus). If addition of a nonnatal fruit blend to an attractive fruit model can be shown to diminish orientation to the model, then chemosensory avoidance can be inferred. Even so, this "avoidance behavior" is probably best viewed as the nonnatal volatiles having an antagonistic effect on visual cue acceptance.

Here, we test for avoidance behavior in *R. pomonella* through a series of two-way field choice trials between red sticky sphere traps baited with apple, hawthorn, or a recently identified flowering dogwood fruit blend (Nojima et al. 2003b) versus blank, odorless control spheres. We also investigate the effects of individual chemical components of natal and nonnatal blends, as well as 1:1 combinations of natal plus nonnatal fruit odors, on fly capture to determine (1) whether single compounds alone were capable of inducing fly behavior or if whole blends were required, and (2) whether combined blends caused similar levels of avoidance as nonnatal volatiles alone. The field trials were performed on resident, wild fly populations infesting an abandoned apple orchard, a hawthorn copse, and two flowering dogwood tree (*Cornus florida*) stands. We report significantly reduced rates of fly capture on nonnatal blends compared to blank spheres, supporting the avoidance hypothesis.

## MATERIALS AND METHODS

### Overview of Study

The basic experimental design involved a series of replicated two-way choice experiments in the field between a red sticky sphere baited with a given fruit odor treatment tested against a blank, odorless control sphere (Fig. 1). Red sphere traps were used because they have been shown to be visually attractive to *R. pomonella*, mimicking ripe host fruit. Therefore, our rationale was that comparisons of capture rates between fruit odor versus blank spheres would indicate the relative preference, avoidance or nonresponse of resident *R. pomonella* flies for a particular chemical or blend of volatiles. Field trials were conducted from July to October 2003 at four study sites in the midwestern United States: a mixed variety apple orchard near Fennville, Michigan; a copse of hawthorn trees located 1 km from the apple orchard at Fennville; and two stands of flowering dogwood trees in Granger, Indiana, and Cassopolis, Michigan. Allozyme surveys have indicated that the apple and hawthorn populations at the Fennville site are genetically differentiated from each other, displaying significant allele frequency differences at several loci (Feder and Bush 1989). Previous flight tunnel tests have also shown that flies from each of the four study sites preferentially orient to the volatile blend of their natal host fruit (Linn et al. 2003, Nojima 2003a,b).



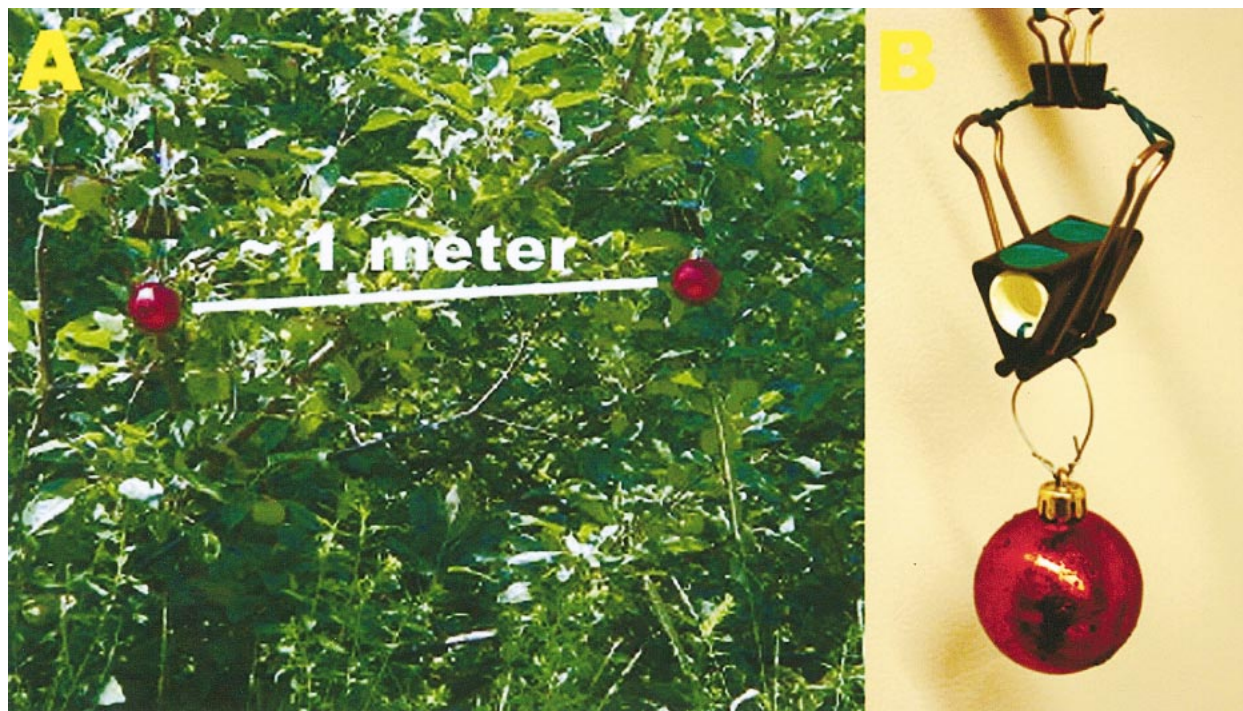


FIG. 1. (A) Paired two-way choice trial of a fruit odor blend tested against a blank odorless control sphere spaced 1 m apart in an apple orchard; (B) Red sphere trap with fruit odor lures housed in opposite sides of a binder clip.

#### *Fruit Odor Lures and Traps*

The fruit odor lures used in the study were prepared by Sutterra LLC (Bend, OR). The lures consisted of a white scintillation vial cap (1.4 cm dia.) filled to half volume with a 1:3 mixture of a particular fruit volatile odor and wax. (The composition of the wax odor delivery system is a proprietary blend of Sutterra LLC). Odor release rates from the wax lures have been estimated to be about 35  $\mu\text{g/hr}$ . (J. Fisher, pers. obs.), equivalent to about 50 ripe red delicious apples (Prokopy et al. 1987). Apple and hawthorn flies can be deterred by high doses (2000  $\mu\text{g}$ ) of their natal fruit blend in flight tunnel assays (Linn et al. 2004). Thus, it was important for release rates from lures to be in accord with levels naturally found within host trees (apple trees typically contain hundreds of ripe fruit, and hawthorn and dogwood trees thousands of fruit), as was attempted in the current study. Lures have been found to retain potency for up to one month in the field (J. Fisher, pers. obs.), and were changed on a bi-weekly basis in the study. A number of different odor treatments were tested in the apple orchard, hawthorn copse, and dogwood tree stands (see Tables 1, 2, 3 for lists of treatments). Synthetic apple, hawthorn, and dogwood blends were prepared according to Zhang et al. (1999) and Nojima et al. (2003a,b). Lures containing either individual compounds or subcomponent mixtures of the blends were made such that the concentrations of chemicals in the lures were the same as when they were part of the entire fruit blend. For 1:1 combinations of fruit blends, lures contained one dose equivalent of each of the two blends. Two lures were placed inside the opposite ends of a black binder clip that was attached to a tree branch by twist tie wire (Fig. 1B). Red, plastic Christ-

mas tree bulbs (5 cm dia.) coated with Tanglefoot insect spray (Tanglefoot Co., Grand Rapids, MI) were hung from the bottom of the clips to trap flies.

#### *Experimental Design*

A given two-way trial consisted of a red sphere trap baited with a particular fruit odor tested against a blank, odorless control sphere positioned one meter away in the tree canopy. Each odor treatment was replicated six times at a study site, with each paired trial of spheres nested within a block of all of the odor treatments tested at the site. In the apple orchard, each block of treatments was performed in a separate row of apple trees, with the two-way trials positioned on the south facing sides of alternate trees. At the hawthorn and dogwood fly sites, replicate blocks were arrayed within a set of three trees, with each two-way trial separated by a minimum of six meters in the tree canopy. Traps were monitored for flies every third day over a total of either eight (hawthorn copse) or six (apple orchard and dogwood stands) different time periods at the study sites. Due to differences in fly population density within and between trees, as well as variation in the attractiveness of individual traps related to their specific positions in the tree canopy, we did not randomize the locations of two-way trials within blocks after each three-day trial period. Instead, the positions of the odor baited sphere and blank sphere constituting a given two-way replicate trial were exchanged every three days, such that the pair of spheres resided in each of the two test positions in a tree for an equal amount of time during the field season. The positions of treatments within blocks and the initial assignment of treatments for

TABLE 1. Results for two-way fruit odor field trials conducted at an apple orchard in Fennville, Michigan, in 2003. Given are the total number of flies captured on each fruit odor treatment tested, the number trapped on odor baited (scented) and control (blank) spheres, and the percentages of the total capture on baited traps through the course of the field season. Natal fruit blend and component compounds are in bold.

Apple flies						
Odor treatment	Total capture	Scented ( <i>n</i> )	Blank ( <i>n</i> )	% on scent	$\chi^2$	<i>P</i> -value
<b>Apple</b>	690	417	273	60.4%	30.05	****
Haw	452	155	297	34.3%	44.61	****
Dogwood	691	229	462	33.1%	78.57	****
<b>butyl hexanoate</b>	437	324	113	74.1%	101.88	****
3-methylbutan-1-ol	338	138	200	40.8%	11.37	***
1-octen 3-ol	501	149	352	29.7%	82.25	****
Met + Oct	434	171	263	39.4%	19.50	****
<b>Apple</b> + Haw	632	236	396	37.3%	40.51	****
<b>Apple</b> + Dog	737	254	483	34.5%	71.15	****
<b>Apple</b> + Met	615	213	402	34.6%	58.09	****
<b>Apple</b> + Oct	572	236	336	41.3%	17.48	****
<b>Apple</b> + Met + Oct	741	298	443	40.2%	28.37	****

\*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ , as determined by chi-squared tests to an expected 50:50 capture ratio.

spheres in a given two-way trial within trees were determined by random draw.

## RESULTS

A significantly greater number of males than females were trapped at all four study sites regardless of fruit odor treatment (overall apple orchard sex ratio of males (m) to females (f) trapped = 8.5 : 1,  $\chi^2 = 156.6$ ,  $n = 7,111$  flies,  $P < 0.0001$ ; hawthorn copse sex ratio m : f = 3 : 1,  $\chi^2 = 513.7$ ,  $n = 1962$ ,  $P < 0.0001$ ; combined dogwood stand sex ratio m : f = 1.5 : 1,  $\chi^2 = 40.6$ ,  $n = 637$ ,  $P < 0.0001$ ). However, in all cases the proportion of males to females did not differ significantly between fruit odor versus blank control spheres (data not shown). Consequently, captures of male and female flies were pooled for all further data analysis. In addition, because of position effects associated with the locations of traps within and among trees, statistical tests were performed on the pooled totals for all six replicates of a given fruit odor treatment within the three day test periods. Moreover, because trap ratios did not differ significantly between the two dog-

wood fly sites (data not shown), we pooled the Cassopolis, Michigan and Granger, Indiana data for statistical analysis.

Resident apple, hawthorn and dogwood flies were all trapped significantly more often on red, tanglefoot-coated spheres baited with their respective natal fruit volatile blend than on blank, odorless control traps (Tables 1–3). For all three different host taxa tested, a little over 60% of flies were captured on their natal scent. Preference for the natal host odor was consistent across time. Greater proportions of resident flies were trapped on their natal blend versus blank spheres within each of the three-day trial periods in the apple orchard and hawthorn copse (apple orchard:  $Z = 2.21$ ,  $P < 0.05$ ,  $n = 6$  trials; hawthorn copse:  $Z = 2.53$ ,  $P < 0.01$ ,  $n = 8$ , as determined by one-tailed Wilcoxon signed-rank test). The only exception was the dogwood stands where during one test period equal numbers of flies were caught on dogwood baited versus blank spheres ( $Z = 2.21$ ,  $P < 0.05$ , one-tailed Wilcoxon signed-rank test across test periods favoring dogwood odor versus blank traps,  $n = 6$ ).

In contrast to the results for the natal fruit blends, signif-

TABLE 2. Results for two-way fruit odor field trials conducted in a hawthorn copse in Fennville, Michigan, in 2003. Given are the total number of flies captured on each fruit odor treatment tested, the number trapped on odor baited (scented) and control (blank) spheres, and the percentages of the total capture on baited traps through the course of the field season. Natal fruit blend and component compounds are in bold.

Hawthorn flies						
Odor treatment	Total capture	Scented ( <i>n</i> )	Blank ( <i>n</i> )	% on scent	$\chi^2$	<i>P</i> -value
Apple	153	36	117	23.5%	42.88	****
<b>Haw</b>	191	116	75	60.7%	12.17	***
Dogwood	111	41	70	36.9%	7.58	**
butyl hexanoate	170	85	85	50.0%	0.00	NS
<b>3-methylbutan-1-ol</b>	141	71	70	50.4%	0.01	NS
1-octen 3-ol	183	65	118	35.5%	15.35	****
Met + Oct	219	80	139	36.5%	15.89	****
<b>Haw</b> + Apple	174	80	94	46.0%	1.13	NS
<b>Haw</b> + Dog	73	24	49	32.9%	8.56	**
<b>Haw</b> + BH	256	122	134	47.7%	0.56	NS
<b>Haw</b> + Met + Oct	156	50	106	32.1%	20.10	****
<b>Haw</b> + Oct	135	59	76	43.7%	2.14	NS

\*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ , as determined by chi-squared tests to an expected 50:50 capture ratio.

TABLE 3. Results for two-way fruit odor field trials conducted in two dogwood stands in Granger, Indiana, and Cassopolis, Michigan, in 2003. Given are the total number of flies captured on each fruit odor treatment tested, the number trapped on odor baited (scented) and control (blank) spheres, and the percentages of the total capture on baited traps through the course of the field season. Values are combined totals pooled from the two sites. Natal fruit blend and component compounds are in bold.

Dogwood flies						
Odor treatment	Total capture	Scented (n)	Blank (n)	% on scent	$\chi^2$	P-value
Apple	97	34	63	35.1%	8.67	**
Haw	83	32	51	38.6%	4.35	*
<b>Dogwood</b>	94	58	36	61.7%	5.15	*
butyl hexanoate	74	28	46	37.8%	4.38	*
<b>3-methylbutan-1-ol</b>	71	47	24	66.2%	7.45	**
<b>1-octen 3-ol</b>	67	37	30	55.2%	0.73	NS
<b>Dog + Apple</b>	71	31	40	43.7%	1.14	NS
<b>Dog + Haw</b>	72	27	45	37.5%	4.50	*
<b>Dog + BH</b>	8	0	8	0.0%	8.00	**

\*  $P < 0.05$ , \*\*  $P < 0.01$ , as determined by chi-squared tests to an expected 50:50 capture ratio.

icantly fewer apple, hawthorn and dogwood flies were captured on spheres baited with their nonnatal blends compared to blank spheres (Tables 1–3). In general, less than 38% of resident flies were trapped on nonnatal blend spheres. Once again, nonnatal fruit odor avoidance showed a consistent pattern across time (apple fly preference for blank over hawthorn baited spheres across time periods:  $Z = 2.02$ ,  $P < 0.05$ ,  $n = 6$  trials; apple fly for blank over dogwood odor sphere,  $Z = 1.86$ ,  $P < 0.05$ ,  $n = 6$ ; hawthorn fly for blank over apple odor sphere:  $Z = 2.53$ ,  $P < 0.01$ ,  $n = 8$ ; hawthorn fly for blank over dogwood odor sphere  $Z = 1.69$ ,  $P < 0.05$ ,  $n = 8$ ; dogwood fly for blank over hawthorn odor sphere:  $Z = 2.03$ ,  $P < 0.05$ ,  $n = 6$ ; dogwood fly for blank over apple odor sphere:  $Z = 1.63$ , borderline significance  $P = 0.052$ ,  $n = 6$ ). However, in the apple orchard and hawthorn copse, the same or slightly greater numbers of resident flies were caught on the dogwood baited than blank spheres for the first two trapping periods. But relatively few flies were captured in the dogwood volatile tests during the first two periods, and the pattern dramatically reversed itself as fly captures increased in the test periods that followed. In the dogwood stand for one of the six test periods, two more flies were captured on the apple baited than blank spheres (5 vs. 3). But again, fly numbers were low at this time on Sept. 19.

Preference and avoidance trap-capture patterns observed for the apple, hawthorn, and dogwood fruit blends also were observed for certain natal and nonnatal volatile compounds constituting the blends. For example, traps baited with butyl hexanoate, a major component of the apple blend, caught almost three times more apple flies compared to odorless control traps (Table 1). In contrast, fewer apple flies were caught on traps baited with 3-methylbutan-1-ol, 1-octen-3-ol, or a mixture of these two chemicals, than on blank spheres (Table 1). Both 3-methylbutan-1-ol and 1-octen-3-ol are major components of the dogwood blend. In contrast, fewer dogwood flies were trapped on spheres baited with butyl hexanoate, than on blanks, but more were captured on traps baited with 3-methylbutan-1-ol (Table 3).

However, not every natal or nonnatal compound affected fly capture. For example, hawthorn flies were not differentially trapped on butyl hexanoate or 3-methylbutan-1-ol baited spheres versus blank controls (Table 2), the former being a minor component of the hawthorn blend and the latter an

essential volatile of the blend needed for maximal hawthorn fly response (Nojima et al. 2003a). Hawthorn and apple flies were both captured significantly less often on spheres baited with the dogwood specific volatile 1-octen-3-ol than on odorless traps (Tables 1, 2). However, it was not apparent whether dogwood flies were preferentially attracted to 1-octen-3-ol. More resident dogwood flies were captured on 1-octen-3-ol baited spheres than blank controls (55% vs. 45%), but the total capture was not great ( $n = 67$  flies) and the difference was not statistically significant (Table 3). Thus, *R. pomonella* flies were not summarily captured to a greater or lesser extent on traps baited with compounds solely on the basis of whether the volatiles formed part of their natal blend.

In general, 1:1 mixtures of natal plus nonnatal fruit blends caused flies to be captured less frequently on spheres (Tables 1–3) than on duplicate spheres with no odor. For the majority of trials, the proportion of resident flies captured on spheres baited with 1:1 combinations of natal plus nonnatal blends was statistically as low as that observed for the nonnatal blend tested alone (hawthorn flies captured on combined haw plus dogwood blend vs. blank = 32.9%, hawthorn flies capture on dogwood blend vs. blank = 36.9%,  $P = 0.63$  for significant difference, Fisher's exact test; apple flies captured on combined apple plus haw blend = 37.3%, apple flies captured on haw blend = 34.3%,  $P = 0.30$ ; apple flies captured on combined apple plus dogwood blend = 34.5%, apple flies captured on dogwood blend = 33.1%,  $P = 0.61$ ; dogwood flies captured on combined dogwood plus haw blend = 37.5%, dogwood flies captured on haw blend = 38.6%,  $P = 1.0$ ; dogwood flies captured on combined dogwood plus apple blend = 43.7%, dogwood flies captured on apple blend = 35.1%,  $P = 0.27$ ). The exceptions was the haw plus apple blend in the hawthorn copse, where ratios for the combined blend were intermediate between the natal and nonnatal blends tested alone (combined blend capture vs. blank = 46.0%, apple blend capture = 23.5%, haw blend capture = 60.7%,  $P = 0.003$ ,  $P = 0.13$  for difference between combined capture and haw and apple blend captures, respectively).

## DISCUSSION

Our field studies provide empirical support for the avoidance hypothesis; populations of apple, hawthorn, and dog-



wood flies tend to prefer the fruit volatile blend of their natal host plant and are deterred by nonnatal volatiles. Natal blends were associated with capture rates 34 to 38% higher than background captures on blank spheres. In contrast, nonnatal blends caught 37 to 69% fewer flies than blank spheres. Moreover, these values likely underestimate the degree of fruit odor discrimination in the field. Many of the flies captured on control spheres in natal blend tests were undoubtedly drawn into the general vicinity of the two-way trial by the natal fruit volatiles emitted from the source sphere. Within a range of 1 m, precise localization of an odor source assumes lesser relative importance to *Rhagoletis* flies than alighting on a fruit of acceptable size and color on which to mate and oviposit (Prokopy and Roitberg 1984). As a result, the ratio of natal blend versus control captures will tend to underestimate the attraction of flies to their natal fruit volatiles. In contrast, nonnatal fruit volatiles likely deterred many flies from the vicinity of both odor and blank spheres in the avoidance trails. Consequently, a fair proportion of flies captured on nonnatal spheres may represent a subset of the population that are either indifferent to or even attracted to nonnatal fruit volatiles, thereby reducing our estimates of fruit odor avoidance. In this regard, flight tunnel tests have revealed variation (polymorphism) in natal and nonnatal fruit odor specificity behaviors among individual apple, hawthorn, and dogwood flies (Linn et al. 2005b). From 5 to 25% of flies from a given host population will respond to both their natal and nonnatal blends. Recently, Linn et al. (2005a) have also devised a flight tunnel assay to test for avoidance behavior in flies. Experiments conducted on a line of apple flies established from Geneva, New York produced similar results as our field trials (Linn et al. 2005a), supporting a general tendency for the majority of individuals in the apple race to avoid hawthorn fruit volatiles. Further field and laboratory testing involving additional sites would confirm the universality of habitat avoidance in *Rhagoletis*, although in the current study, flies at both dogwood sites displayed similar avoidance behavior to nonnatal volatiles.

Our field studies also show that preference and avoidance behaviors for *R. pomonella* flies extend to certain individual volatile compounds, as well as to entire fruit blends. However, in the field trials, not every natal or nonnatal compound affected fly capture. For example, hawthorn flies did not appear to prefer or avoid butyl hexanoate, a minor component in the hawthorn blend and a major attractant to apple flies. Moreover, hawthorn flies were not preferentially trapped on 3-methylbutan-1-ol baited spheres, an essential component for upwind flight enhancing fly response in the flight tunnel tests when included in the hawthorn blend (Nojima 2003a).

It is tempting to speculate that the lack of response of hawthorn flies to certain key ingredients of the apple and dogwood blends may have predisposed *R. pomonella* to shift to these host fruits. Menken and Roessingh (1998) have suggested that such a mechanism may exist for host shifts wherein the presence of low levels of a particular compound in the natal host may facilitate a shift to a novel host that has the same component chemical. However, in the case of butyl hexanoate, it is not clear whether the lack of response is the ancestral condition for hawthorn flies or is due to ongoing gene flow from sympatric apple fly populations. Also, it is

not certain whether hawthorn flies shifted to flowering dogwood or vice versa. In addition, although hawthorn flies may not avoid certain individual compounds in the apple and dogwood blends, in our field tests they did tend to avoid the apple and dogwood blends in their entirety. Thus, the relevancy of the hawthorn race's indifference to butyl hexanoate and 3-methylbutan-1-ol for facilitating host shifts remains to be determined. Further testing of individual compounds and subcomponent mixes, as well as determination of the physiological and genetic bases for fruit odor preference and avoidance, are needed to address this issue. In this regard, tests of hawthorn fly populations from the southern United States, outside of the range of overlap with apple flies, may help to resolve whether the indifference of hawthorn flies to butyl hexanoate observed in the Fennville, Michigan, field trials is consistent across the range of the hawthorn race and is the ancestral state for *R. pomonella*.

Combined mixtures of natal plus nonnatal blends also resulted in significantly reduced captures of flies. This finding reiterates that apple, hawthorn, and dogwood flies do not merely fail to recognize the odor of alternative host fruit, but also generally tend to avoid them. Moreover, avoidance of nonnatal blends appears to be stronger than the inherent attraction invoked by components of the natal blend. In five of six instances, the relative proportions of flies caught on the combined blend were similar to the reductions seen when nonnatal blends were tested alone. For the one exception (haw plus apple blend tested in the hawthorn copse), the proportion captured on the combined blend (compared to blank spheres) was intermediate between the apple and haw blends tested alone, but still much lower than that observed for the natal haw blend. Similar responses to a mix of natal and nonnatal blends have been found in flight tunnel assays (Linn et al. 2005a). The results for the combined blend tests in the field and laboratory underscore that chemosensory changes accompanying *R. pomonella* host shifts involve not just the derivation of a new preference for a novel fruit that overcomes an ancestral aversion behavior, but also the rapid evolution of avoidance for ancestral fruit volatiles.

The existence of habitat avoidance behavior in *R. pomonella* has important theoretical implications for sympatric host race formation and speciation. First, negative avoidance alleles provide a possible explanation for why apple  $\times$  hawthorn, apple  $\times$  dogwood and hawthorn  $\times$  dogwood fly  $F_1$  hybrids do not respond to normal doses of host fruit volatiles in flight tunnel tests (Linn et al. 2004). These flies possess alleles for avoiding both of their parental fruit blends, and so respond to neither. If the impaired chemosensory response of hybrids affects their ability to locate host fruit—a hypothesis that can be directly tested by mark-recapture field studies—then habitat specific mating would be an important postmating, as well as prezygotic, barrier to gene flow during sympatric host race formation. Like sexual selection, hybrids would have difficulty securing mates because they possess abnormal phenotypes that preclude them from fully participating in the “mating leks” formed on alternative host trees. Moreover, postzygotic isolation would further cast host-specific mating as a single-variant sympatric speciation model. Selection for nonnatal host avoidance would not only generate positive assortative mating, but also directly reduce

the inclusive fitness of disassortatively mating individuals. For example, individuals migrating to nonnatal host plants and mating with flies of the opposite host race would tend to produce progeny that were subsequently less able to find either parental host and mate. When hybrids are unfit, reinforcement is also possible (Coyne and Orr 2004), providing additional avenues for sympatric divergence to proceed toward completion through selection for increased mate discrimination based on traits not affecting habitat performance.

Second, the existence of avoidance behavior implies that epistatic interactions between host fruit odor recognition and avoidance genes may play a role in habitat specific mating. In the absence of such interactions, host shifts could be genetically constrained if flies heterozygous for alternative avoidance alleles have reduced fitness in all genetic backgrounds. This would make it difficult for a new avoidance mutation for hawthorn to spread in the derived apple race because individuals possessing the mutation would initially all be unfit heterozygotes. A potential solution to this dilemma is that in certain genetic backgrounds (e.g., in apple flies homozygous for apple preference alleles) new avoidance genes for the ancestral host (hawthorn) are dominant, creating a ridge in the phenotypic fitness surface allowing avoidance behavior for hawthorn to evolve in the apple fly population without reducing intrahost fitness, generating both pre- and postzygotic isolation from hawthorn flies in the process.

Third, the ancestral state for hawthorn flies (and probably for most phytophagous insects) is likely to be avoidance of novel plants. This implies that host shifts involve not only changes resulting in the evolution of new preferences for novel host, but that these changes overcome previous avoidance behaviors to the derived host, and are accompanied by the rapid evolution of new avoidance behavior to the ancestral host. Although it is conceivable that the ancestral state in the hawthorn race was nonavoidance of apple fruit odor (i.e., that avoidance to apple in the ancestral hawthorn race evolved only after the formation of the apple race and in response to reduced fitness of hybrids), we consider this scenario less likely for several reasons. First, nonhost compounds often act as feeding deterrents to phytophagous insects (Bernays and Chapman 1987; Bernays et al. 2000; Bernays 2001). Second, *R. pomonella* survivorship in native crab apples is extremely low (Pree 1977). Thus, prior to the introduction of domesticated apple into North America about 400 years ago, avoidance to crab apple may have been favored. To the extent that crab apple and domesticated apple fruit share similar volatile profiles, hawthorn flies would have been predisposed to avoiding domesticated apple. The question of whether insects are generally predisposed to avoiding nonhost plants, even before they are first encountered in nature, is a fascinating one and may be addressed in *R. pomonella* through analysis of hawthorn-infesting populations in the South outside the range of overlap with the apple race.

In conclusion, our field studies empirically demonstrate an important and previously overlooked aspect of habitat specific mating bearing directly on sympatric host race formation and speciation for phytophagous insects; both preference for novel hosts and avoidance responses to ancestral hosts evolve quickly during host shifts. However, many questions remain to be answered concerning the genetics of host avoidance

behavior in *Rhagoletis* (e.g., genetic crosses imply that preference and avoidance behaviors are not just pleiotropic consequences of genetic changes to the same set of loci; J. Feder, H. Dambroski, C. Linn, S. Berlocher, A. Forbes, and W. Roelofs, unpubl. ms), as well as the generality of rapid habitat avoidance evolution in other host-specific phytophagous insect systems following host shifts, especially those hypothesized to have arisen sympatrically. Regardless, what seemed to be a relatively straightforward understanding of the basis for habitat choice (preference) and host-specific mating may oversimplify the biological complexity of the process. The result may have been an under appreciation of the potential for sympatric differentiation for phytophagous insects, as well as ecological speciation in general, regardless of geographic context.

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