Western Washington University

Western CEDAR

WWU Graduate School Collection

WWU Graduate and Undergraduate Scholarship

2013

Short-range host preference in snowberry maggot flies depends on life history stage

John L. (John Lawton) Huddleston
Western Washington University

Follow this and additional works at: https://cedar.wwu.edu/wwuet

Part of the Biology Commons

SHORT-RANGE HOST PREFERENCE IN SNOWBERRY MAGGOT FLIES DEPENDS ON LIFE HISTORY STAGE

By

John Huddleston

Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Dietmar Schwarz

Dr. Merrill Peterson

Dr. Ben Miner

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a masters degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and noncommercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Signature: John Huddleston

Date: July 24, 2013

SHORT-RANGE HOST PREFERENCE IN SNOWBERRY MAGGOT FLIES DEPENDS ON LIFE HISTORY STAGE

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
John Huddleston
July 2013

ABSTRACT

Closely related animal species are often incompletely isolated reproductively and occasionally hybridize. Many host-specific insects are primarily isolated by the reproductive barrier of host choice and provide model systems to study gene flow in sympatry. Understanding conditions that alter barriers like host choice can help us understand reproductive isolation between these species. Hybridization occurs between microsympatric populations of apple and snowberry maggots in Bellingham, Washington which are also not isolated by mating season or assortative mating. I exposed apple and snowberry maggots to their natal fruits in two-way choice experiments to measure their short-range host preferences. I tested snowberry flies at different life stages to determine whether host preference is constant throughout their lives. Virgin flies show no preference for their natal hosts and rarely oviposit. After mating, female snowberry flies strongly prefer their natal host and oviposit solely in snowberries. Young synovigenic females may balance the costs of exploring nearby novel hosts and occasionally mating with heterospecific males with the benefits of finding nutrients to develop their eggs and mates to fertilize them. Mated females will spend most of their time on their natal host where males will follow them. As a result, hybridization between apple and snowberry maggot populations is most likely in the early season before females have mated and started ovipositing in their natal hosts. The fate of evolutionary interactions between species depends on the life history dynamics of the reproductive barriers that isolate them. Future studies should consider strength of reproductive isolation in this context.

ACKNOWLEDGMENTS

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Species are the fundamental units of biological diversity and are commonly defined by the biological species concept as reproductively isolated populations (Mayr 1963). In the absence of geographic separation, closely related sympatric species tend to be strongly isolated by prezygotic ecological and behavioral barriers such as temporal isolation or behavioral isolation by habitat or mate choice (Coyne and Orr 2004). Changes to ecological conditions or organismal behavior can weaken reproductive barriers and enable gene flow between closely related sympatric populations. Gene flow is expected to continue between sibling species after secondary contact and throughout their divergence (McPheron 1990, Mallet 2008). In extreme cases, this gene flow can lead to stable hybrid zones, fusion or elimination of parental populations, or hybrid speciation (Huxel 1999, Schwarz et al. 2007). As the consequences of gene flow between sympatric species may redefine those species, it is important to understand the ecological and behavioral barriers that prevent or enable hybridization. With this understanding we can better predict the evolutionary interactions between populations at intermediate stages on the continuum of speciation.

Host-specific insects are model organisms for the study of divergence with gene flow as a result of their species richness and ability to form new species in sympatry through host shifts and host-related assortative mating (Berlocher and Feder 2002). Host choice is a strong isolating barrier driven by the behavior of insects and the ecology and physiology of their host plants (Drès and Mallet 2002). Host-specific insects are under strong selective pressure to match their life cycles to the phenologies of their host plants, detect and orient toward host plant signals, and adapt to the larval environment of hosts (Berlocher and Feder 2002). Thus sympatric populations can be isolated through their host choice by non-overlapping mating periods, differential responses to host signals, or differential survival of larvae in host plants.

Host choice is a variable characteristic of host-specific phytophagous insects dependent on interactions between the physiological states of insects and their hosts. Prior to reproductive maturity, insects have dispersal patterns indicative of foraging for food while reproductively mature insects also search for mates and oviposition sites (Harrison 1980, Opp and Prokopy 1987). Correspondingly, the natal host preferences of virgin females are often much weaker than those of mated or older females (Anton et al. 2007). While physiological states can affect host choice, host choice can change physiology by increasing female oogenesis, egg-laying rates, and host use (Papaj 2000). These patterns indicate that host-specificity is correlated with the requirements of specific life stages.

Variable host choice should have important consequences for the stability of species in sympatry, especially if host choice is the primary reproductive barrier between those species. Gene flow is most likely to occur between sympatric populations whose life cycles include a stage during which they simultaneously lack host-specific behaviors and are reproductively mature. Early in the season before host-specific behavior increases, heterospecific encounters on non-natal hosts should be more likely. Connections between changing host preference and physiological state have been studied in Lepidoptera (Hern and Dorn 1999, Rojas 1999, Masante-Roca et al. 2007), Hemiptera (Wenninger et al. 2009), and Diptera (Petersson and Sivinski 2003). However, ontogenetic changes in host preferences have not been studied in the context of gene flow between diverging species.

The *Rhagoletis pomonella* species group is a model system for investigating the effects of host choice on gene flow between sympatric populations (Drès and Mallet 2002). The group consists of *R. pomonella* host races and latitudinal types, *R. zephyria*, *R. mendax*, the undescribed flowering dogwood fly, and *R. cornivora* (Smith and Bush 1997). These species and host races are under strong selective pressure to adapt to the conditions of their hosts (Michel et al. 2010) such as host phenology (Dambroski and Feder 2007) and larval fruit environment (Bierbaum and Bush 1990, Schwarz personal communication).

Since host plants are almost exclusively the site of assembly for mating pairs, host choice also tends to determine mate choice (Prokopy et al. 1971, Smith and Prokopy 1980). When the barrier of host choice is incomplete, mating isolation between members of the species group is also incomplete. For example, in the lab, *R. zephyria* will mate with both *R. pomonella* (Yee and Goughnour 2011) and *R. mendax* (Schwarz and McPheron 2007). *R. pomonella* host races from apple and hawthorn will also mate in the absence of host cues (Feder et al. 1994).

Host choice is primarily determined by the way *Rhagoletis* flies respond to the hierarchical combination of olfactory, visual, and tactile signals provided by their hosts. At long range (∼12 m), flies identify hosts by volatile chemicals emitted from ripening fruit (Linn et al. 2003), moving toward fruits from their natal host and away from other fruits (Linn et al. 2005*b*). At short range (<1 m), flies identify hosts visually based on the appearance of habitat structure and fruit clusters (Linn et al. 2003). At this scale, female *Rhagoletis* flies also use their ovipositors to perform tactile and chemical tests on potential host fruit. Probing allows females to evaluate the quality of the fruit and detect an oviposition-deterring pheromone left by other females after ovipositing (Prokopy 1972). When flies are exposed to blends of different host odors at close range, they appear to rely on the color and shape of fruit for host choice instead of the potentially confusing olfactory signals they receive (Forbes and Feder 2006). Mistakes in host detection and acceptance at this short range will manifest as variable host preference and potentially as shifts in natal hosts.

Rhagoletis females accept hosts for oviposition based on a combination of factors including density dependence (Roitberg et al. 1990), previous experience with other hosts (Prokopy et al. 1982, Papaj and Prokopy 1988), increased egg load (vanRanden and Roitberg 1996), age (Prokopy et al. 1988), and mating status (Opp and Prokopy 1986). Variation in these factors throughout the lives of plant parasites could lead to variation in

host choice. For closely related sympatric species, even temporary changes in host choice may be sufficient to allow interactions between species. When mating isolation is incomplete, as it is between many *Rhagoletis* species (Feder et al. 1994, Schwarz and McPheron 2007, Yee and Goughnour 2011), these interactions could result in hybridization and gene flow between populations and potentially prevent speciation.

Each member of the *R. pomonella* species group has emerged in the last 1.5 Myr through an initial host shift to a novel host and subsequent reduction of gene flow between populations (Smith and Bush 1997, Xie et al. 2007). Apple and hawthorn maggots formed as incipient host races through the introduction of non-native apples to the northeastern United States in the 1800s (Bush 1969, Feder et al. 1988). In that same region and time period, individuals of *R. zephyria* and *R. mendax* mated on the introduced honeysuckle (*Lonicera*), creating a hybrid population that maintained its preference for its new host (Schwarz et al. 2005). The sibling species of *R. pomonella* have arisen through host shifts from hawthorn to novel hosts such as snowberry, blueberry, and flowering dogwood (Xie et al. 2008). These resulting host races and species have so few fixed substitutions amongst them that the proper order of divergence within the group cannot be resolved with mitochondrial or nuclear markers (Berlocher 2000, Xie et al. 2008). Indeed, low levels of hybridization have been reported between sibling species in secondary contact even after extended isolation by geographic barriers (McPheron 1990, Feder et al. 1999). These results suggest that host shifts within the group have occurred recently, rapidly, and with continued gene flow between shifting populations.

Since host shifts have been so rapid and recent in the *R. pomonella* species group, the barriers between existing species and new hosts are most likely incomplete. Previous tests based on visual, olfactory, and ovipositional responses by species to natal and non-natal hosts suggest that host preference is incomplete within the group. Approximately 10-30% of *R. pomonella* flies from apple, hawthorn, and flowering dogwood respond to non-natal

host odors as strongly as natal odors (Linn et al. 2005a). *R. pomonella* host races from apple and hawthorn both experience reduced natal host preference when presented with a mix of natal and non-natal odors and visual signals (Forbes and Feder 2006). Flies from both host races will also oviposit into each other's respective hosts (Prokopy et al. 1988). In oviposition acceptance experiments with no-choice conditions, *R. zephyria* oviposited in honeysuckle and snowberry while *R. mendax* oviposited in blueberry, honeysuckle, and hawthorn (Schwarz et al. 2007).

For sympatric populations of *Rhagoletis* species, such incomplete host preference provides opportunities for individuals to meet and hybridize. Hybrids may experience extrinsic inviability through intermediate diapause timing (Dambroski and Feder 2007), reduced fecundity (Xie et al. 2008), or reduced response to parental host fruits (Linn et al. 2005a) and still remain intrinsically viable adults (Yee and Goughnour 2011). When these extrinsic barriers break down, the resulting viable hybrids can potentially form stable hybrid populations, as in the case of the *Lonicera* fly (Schwarz et al. 2005), or mediate gene flow between parental populations through backcrossing.

The combination of variable host preference and incomplete mating isolation could facilitate host shifts or gene flow between sympatric populations, particularly those coming into secondary contact. A natural experiment of this kind has been taking place in the last 30 plus years since the invasive apple maggot, *R. pomonella*, arrived in Washington State and came into secondary contact with the native snowberry maggot, *R. zephyria* (AliNiazee and Penrose 1981). The two species are incompletely isolated by mate choice (Yee and Goughnour 2011) and previous research suggests that significant introgression has occurred between populations in Washington (McPheron 1990, Green et al. in press). The geographic distributions of apples and snowberries overlap significantly and both hosts bear fruit during the same time of the year. Thus, unlike host races of *R. pomonella* on apple and hawthorn, sympatric populations of *R. zephyria* and *R. pomonella* are not

temporally isolated. The lack of geographic or temporal isolation makes host choice an important prezygotic barrier between these sympatric populations. Apple and snowberry plants in this region frequently grow adjacent to each other with interdigitating branches. Such spatial structure provides an ideal configuration to investigate potential gene flow between sympatric sibling species as a result of very short range host detection and acceptance. In Bellingham, Washington, fly trap catches have confirmed the presence of *R. pomonella* and *R. zephyria* at sympatric sites where both apples and snowberries occur (Gough personal communication). Genotypes of flies at these sites also reveal asymmetrical introgression between the populations, with all introgressed individuals found in apples (Green et al. in press). Preliminary transplant experiments with pure parentals have found fewer survivors among *R. pomonella* in snowberries than *R. zephyria* in apples, which suggests the asymmetry of introgression could be the result of extrinsic inviability of hybrids in snowberries (Schwarz personal communication).

In this study, I investigate the close-range host preferences of virgin, naïve snowberry and apple maggots, as well as the host preferences of virgin, experienced and mated snowberry maggots. Based on previous research, I expect snowberry and apple maggot flies to prefer their natal hosts and for that natal host preference to remain constant for snowberry maggot flies at each life stage. I tested these hypotheses in a laboratory with two-way choice host acceptance experiments. In the first set of experiments, virgin, naïve flies of each species were exposed to apple and snowberry fruit. Snowberry maggot flies from these experiments were then tested with hawthorn and snowberry under the assumption that no significant learning had occurred during the initial tests. Mated female snowberry maggot flies were exposed to apple and snowberry to compare the effects of mating status on host preference. Finally, I observed the frequency of mating between virgin flies in arenas with only apples and only snowberries to determine whether the presence of non-natal host fruit deters mating.

METHODS

Fly populations and rearing

The pupae from these experiments were collected in the fall of 2011 from infested apple and snowberry fruit in Bellingham, Washington. *R. pomonella* (apple maggot flies) pupae emerged from apples and *R. zephyria* (snowberry maggot flies) from snowberries. I placed pupae into petri dishes based on the fruit from which they emerged and initiated diapause by placing the dishes in a dark incubator at 4 °C for four months. Forty days before experiments were to begin, the pupae were moved into a climate chamber set at 22 °C to initiate eclosion. This configuration is equivalent to 650 degree days, which had been the mean time to eclosion in previous years (Schwarz personal communication).

Every day, I sorted emerging flies into cages, labelled by species and sex, that were equipped with a food strip (4:1 brown sugar to water and Tegosept anti-fungal agent) and water. I stored males and females in separate climate chambers (22 °C and a 16 h/8 h light/dark photoperiod) to prevent any potential habituation to mating cues (Schwarz and McPheron 2007). Eclosed flies were allowed to mature for one week before use in experiments. I controlled for the age of flies across six weeks of experiments by assigning to the same cohort any flies that emerged during the same week. Flies from separate weekly cohorts were kept in separate cages.

Two-way choice host acceptance

Experimental design

I performed three separate two-way choice experiments to measure host preference. In the first experiment, I measured the host preference of virgin, naïve snowberry and apple maggot flies by exposing individual flies simultaneously to apples and snowberries. In

the second experiment, I measured the host preference of virgin snowberry maggot flies from the first experiment by exposing them to hawthorns and snowberries to confirm the consistency of their initial host preference. In the third experiment, I measured the host preference of mated snowberry females, all of which had been tested originally as virgins. I used the same experimental arenas and behavioral protocols for all three experiments.

Experiments took place in the lab at two side-by-side workstations, each equipped with a 250 Watt mercury vapor light elevated on laboratory stands 39 cm above a platform for the experimental arena (Figure 1). The lights produced an illuminance of 35,000 lux and contributed to an average platform temperature of 28 °C. A small desk fan was set to low speed and angled to one side of the platforms to reduce the ambient temperature without directly blowing air on flies during experiments. I prepared four experimental arenas (two per sex) with square 20.32 cm Pyrex glass containers whose plastic snap-on lids were modified to consist primarily of a square of window screen with a single 2 cm hole for aspirating flies, and a foam plug for the hole (Figure 2a).

I collected branches with fresh apple and snowberry fruit into separate buckets each morning at sites near the lab where infested fruit had been observed in previous seasons. In the lab, the collected branches were placed in water-filled vases. In each arena, I placed a single apple with one or two leaves positioned to provide shade above both apple and snowberry fruit. The remainder of the arena was filled with enough snowberry branches, leaves, and fruit to roughly match the surface area of the apple fruit and leaves (10-20 berries, 4-5 branches with leaves) and provide a realistic environment for flies to explore. I replaced fruit when it was marked or probed by a female. The large surface area of both hosts' fruits and leaves minimized the likelihood that any other signals left by flies from earlier trials would affect the behavior of flies in later trials. Separate arenas were assigned to both sexes to prevent interactions between pheromones left by males or females that might alter behavior in the opposite sex.

Behavioral protocol

For each trial, I randomly selected the sex and species of the next fly to test and aspirated a matching fly into the least-recently-used arena for the chosen sex. The fly was allowed to adjust to its environment for 10 minutes. If the fly had not touched either host during that time, it was removed and placed in a separate cage. A subset of these uncooperative flies were reused to compensate for lack of numbers in the same host preference experiment or in the mating environment experiment. As these flies were in close proximity to both hosts in the apple/snowberry preference experiment, I did not expect any behavioral bias due to previous host experience. A post-hoc test excluding previously uncooperative flies from the final statistical analysis did not alter the significance of the result (generalized linear model with binomial error, $p < 0.001$). Uncooperative flies that never cooperated in the host preference experiments were omitted from statistical analysis.

From the time a fly first discovered a host, I used my custom observation software to track the total time the fly performed the specific actions of resting on the wall, searching snowberries or apples, and resting or grooming on snowberries or apples. The software allowed me to log a fly's current action and associate a timestamp with the moment the action was observed. I calculated differences between timestamps in the output log file to determine the amount of time each fly spent performing each action. Because I used the proportion of time each fly spent on a given host as its likelihood of interacting with other flies, I did not distinguish between time spent on branches, leaves, or fruit for the timing of search or rest behaviors. In addition to timing specific actions, I made qualitative notes about each fly's general behavior including whether the fly actively searched the surface of apple or snowberry fruit and whether the fly appeared to feed off the surface of the apple. Trials with cooperative flies were ended 10 minutes after the first host contact.

The same host preference protocol was repeated for mated *R. zephyria* females fol-

lowing the mating environment experiment. Trials with mated females took place after the initial six weeks of experiments with virgin flies. Thus, in addition to their mated status, females in these trials differed from virgin flies by age and experience. Mated females were only tested with apple and snowberry fruit, collected in the same manner described above.

Statistical analysis

Host preference was measured by the proportion of time spent on either host over the total time spent on both hosts. The time flies spent on the wall was analogous to time not spent grazing in two-way choice feeding experiments and was thus omitted from the preference calculation (Roa 1992). I did, however, confirm that flies generally spent more time on both hosts than they did on the wall. Virgin, naïve *R. zephyria* flies spent 2.6 times longer on hosts than walls and *R. pomonella* flies spent 4.6 longer on hosts than walls. To assess the significance of host preference for each pair of hosts tested, I used a generalized linear model (GLM) with a binomial error correction for an improvement of power over the recommendation by Lockwood (1998) for testing proportions in a two-choice case. Although sex is an independent factor in this design, I tested it along with host as a factor in the GLM. I tested the significance of my GLM with an ANOVA and a Chi-square test (R Core Team 2013). This approach allowed me to use the same proportions to test for an interaction between host and sex in addition to testing the effects of both factors individually.

As another measure of host preference, I calculated the difference in oviposition events per host for all experiments. Without any previous studies to indicate oviposition preference of *R. zephyria* females, I assumed there was no preference and expected to see an equal number of oviposition attempts in both hosts. Due to my small sample sizes, I used the exact goodness of fit test to determine whether the actual number of oviposition

events per host deviated from the expectation of no preference.

In the case where preferences differed between hosts or sexes, I used the host fidelity measures of host visit frequency and median duration to better understand the nature of the perceived preference. As these values are not independent but are also not bounded in the same way as the proportions of host preference, I applied Quade's test as suggested by Roa (1992) to compare median duration of visits between hosts. To compare the number of visits per host per flies, I used another GLM with a binomial error model. I used Mann-Whitney's U-test to compare those same values between sexes.

Mating environment

Experimental design

I sought to determine whether *R. zephyria* is more likely to mate in the presence of its native fruit, snowberry, or the introduced fruit, apple. I introduced an equal number of *R. zephyria* flies of each sex (four to seven) to arenas filled with either snowberries or apples. Arenas for mating were modified to include three holes in the lid for easier aspiration of flies (Figure 2b). Initially, I used virgin flies that had also been previously tested in host acceptance experiments. Toward the end of the summer I had to reuse previously mated males to keep the male/female ratios balanced. Experimental trials ran for four hours each day simultaneously with, and physically adjacent to, the host preference experiments where all flies would be exposed to the same environmental conditions.

Fresh snowberries and apples were picked each morning along with the host acceptance fruit. In one arena I placed two apples and at least three apple leaves for shade. In the other arena, I created a complex structure of snowberries similar to that found in the field, with interdigitating branches, leaves, and fruit.

11

Behavioral protocol

Females were introduced to the arenas before males and allowed to adjust to their new environment for ten minutes (Schwarz and McPheron 2007). The observation period began when the last males were added to the arenas. Mating pairs typically stay joined for 20 minutes or more, so mating trials were run simultaneously with host preference trials, with spot checks for mating pairs every 5-10 minutes. For each pair, I noted the time, fruit, and location of the pair (wall, fruit, or leaf/branch) before removing them into a separate cage for mated pairs. To maintain the ratio of males to females, I added a new male and female to the arena for each mated pair I removed.

Statistical analysis

I counted the total number of mating pairs found for each host and tested the significance of these values from the expectation of equal mating pairs per host using an exact goodness of fit test. Trials were run simultaneously, for the same period of time, and with the same number of flies.

RESULTS

Two-way choice host acceptance

Virgin, naïve flies on apple and snowberry

Virgin, naïve flies of both *R. zephyria* and *R. pomonella* slightly preferred their respective non-natal hosts over their natal hosts during two-way choice experiments (Figure 3). *R. zephyria* flies preferred apples over snowberries (mean ± SE; apple = 56% ± 5; p < 0.001, n=67) while *R. pomonella* flies preferred snowberries over apples (snowberry = 61% ± 12; p < 0.001, n=12). To test for any effect of flies that were originally uncooperative and then used again in a later trial, I reanalyzed host preferences without these flies. The same preferences were still detected for both species even after excluding uncooperative flies. I did not detect any difference in host preference between sexes. Not enough males survived diapause to allow comparison between the sexes of *R. pomonella*.

The two species had different behavior with respect to number of and duration of visits to the two hosts. *R. zephyria* flies visited both hosts an equal number of times with an average of three visits per host per trial and spent roughly the same amount of time per visit on each host (Table 1). In contrast, *R. pomonella* flies visited snowberries three times for every one time they visited apples (p < 0.001, n=12). However, the duration of these visits was not significantly different between hosts.

Neither species showed a preference for either host based on oviposition behavior. Two out of 37 virgin, naïve *R. zephyria* females (5%) probed snowberries extensively although there was no indication of marking behavior (Table 2). None of the females probed or marked apples. None of the 10 total *R. pomonella* females attempted to oviposit in either host.

Mated females on apple and snowberry

Mated *R. zephyria* females exhibited the opposite host preference of virgin, naïve females by spending more time on their natal host of snowberries (mean ± SE; snowberry = 67% ± 8, apple = 33% ± 8; p < 0.001, n=16, Figure 3). Similarly to virgin, naïve flies, mated females spent 2.7 times longer on host plants than on the wall with 709 seconds on hosts and 260 seconds on the wall. The same pattern of host visit frequency for virgin, naïve flies was also detected for mated *R. zephyria* females, who visited both apples and snowberries an average of three times per trial. Mated females differed from virgin, naïve flies in their host visit duration by spending 4.6 times longer on snowberries than apples (p < 0.001, n=16; Table 1). As with host proportions, mated females significantly preferred snowberries over apples for oviposition (Table 2). Eleven of the 16 mated females (69%) oviposited in snowberry and none in apple (p < 0.001, exact test for goodness of fit).

Virgin flies on hawthorn and snowberry

Virgin *R. zephyria* flies preferred the non-natal, native host of hawthorn over their natal host snowberry (mean ± SE; hawthorn = 63% ± 8, snowberry = 37% ± 8; p < 0.001, n=20). Although females appeared to spend more time on hawthorn than males I did not detect a significant difference between the sexes (mean ± SE; females on hawthorn = 68% ± 13, n=10; males on hawthorn = 58% ± 10, n=10). In contrast to the other host acceptance experiments, flies in this experiment spent a similar amount of time on hosts (411 seconds) as they did on the wall (463 seconds). Virgin *R. zephyria* flies visited hawthorns more frequently than snowberries, with 5 visits to hawthorn for every 3 visits to snowberry (p < 0.01, n=20; Table 1). The duration of these visits did not differ between hosts.

There were not enough oviposition events on either host to indicate a host preference between hawthorn and snowberry for virgin *R. zephyria* females. No females oviposited in snowberries (Table 2). One *R. zephyria* female probed hawthorn and marked the fruit indicating oviposition. No larva emerged from the probed and marked fruit, suggesting that either no egg was deposited or the egg did not develop.

Mating environment

I observed *R. zephyria* pairs in separate apple and snowberry arenas for a total of 1,544 minutes during which I counted 13 mating pairs in the apple arena and 8 pairs in the snowberry arena. These counts did not differ significantly from my expectation of equal numbers of mating pairs per arena (p=0.38, exact goodness-of-fit test).

DISCUSSION

Incomplete host preference

Host preferences for *R. zephyria* females changed with age and mating status. Virgin females exhibited a minor preference for non-natal hosts, while mated females strongly preferred their natal host. I observed incomplete host preference in all experiments with *R. zephyria* and *R. pomonella*, including those with mated *R. zephyria* females. In these latter experiments, *R. zephyria* females did occasionally explore non-natal hosts, but spent more time on their natal host, likely due to ovipositional activities rather than avoidance of the non-natal host. Thus, based on my definition of preference as proportion of time spent on a given host, I reject my hypotheses that *Rhagoletis* flies always prefer their natal hosts and that their host preferences are constant throughout their life history. I reconcile these results with previous research in the context of life history events such as aging and mating. Additionally, I consider possible explanations for incomplete host preference and use these results to describe a potential model for hybridization between these two species in sympatry.

Although incomplete natal host preferences have been previously reported in the *R. pomonella* species group (Prokopy and Bush 1973, Linn et al. 2005*a*, Forbes and Feder 2006, Schwarz et al. 2007), the flies in these earlier experiments were always mated and always preferred their natal hosts. Apple, hawthorn, and flowering dogwood flies each prefer their natal host when they are sequentially exposed to volatiles from natal and non-natal hosts in a wind tunnel. However, 10-30% of these flies exhibited incomplete host preference by responding equally strongly to both natal and non-natal volatiles (Linn et al. 2005*a*). In two-way host choice tests with visual and olfactory cues, apple and hawthorn flies generally prefer their natal host. An exception to this rule occurred

in cases where olfactory cues from natal and non-natal hosts were present on the same fruit and hawthorn flies could not discriminate between hosts (Forbes and Feder 2006). In multi-choice host choice experiments with snowberry, blueberry, honeysuckle, and hawthorn, *R. zephyria* and *R. mendax* both preferred their natal hosts but also failed to discriminate against honeysuckle (Schwarz et al. 2007). Early field studies of apple fly mating behavior reported that 1/6th of male/female encounters occurred on nearby sour cherry trees instead of apples (Prokopy and Bush 1973). The diversity of hosts parasitized by members of the *R. pomonella* species group (Smith and Bush 1997) and their recent divergence from a common ancestor (Xie et al. 2008) suggest that host preference has been sufficiently incomplete throughout the evolutionary history of this group to allow host shifts when coupled with rare mistakes in host acceptance by females. In contrast to the partial preference for natal hosts seen in these previous studies, virgin, naïve flies in our two-way choice experiments showed no preference for their natal hosts. Instead, both species behaved as host generalists, actively exploring the leaves, branches, and fruit of both hosts. I expect therefore that the lack of host preference is a characteristic of virgin flies that could not be detected by previous studies, due to their use of mated females and lack of short-range host choice conditions. Host generalism at this stage in their lives creates opportunities for sympatric populations of flies to mate and produce hybrid offspring in the early season.

Change of host preference with life stage

I hypothesized that *R. zephyria* flies would express a consistent preference for snowberries throughout their life history. I found that mated *R. zephyria* females significantly preferred their natal hosts over non-natal hosts, in stark contrast to virgin, inexperienced females. Additionally, mated females only accepted their natal fruit for oviposition. As a result, I reject my second hypothesis.

17

The observed shift for female *R. zephyria* flies from no host preference by virgins to a strong natal preference by mated females may be understood in the context of their synovigeny. Like most members of the order Diptera, *Rhagoletis* females require additional proteins and sugars to develop their eggs after emerging from diapause (Wheeler 1996). Males and females both require these nutrients for basic survival and obtain them primarily from aphid honeydew (Neilson and Wood 1966). However, females require more of these nutrients as they continue to develop eggs throughout adulthood (Webster 1979). As a result, *Rhagoletis* females spend the first weeks of their lives foraging for food without any particular preference for their natal host. During this period, flies will also start mating and continue to do so for the remainder of their lives (Prokopy and Bush 1973). Females begin to oviposit during their third week as adults (Webster 1979) after they have mated and acquired sufficient nutrients to develop their eggs. It is also likely that physical contact by females with their natal host is an important trigger for oviposition (Alonso-Pimentel et al. 1998). As oviposition becomes more important than foraging for food, females spend more time searching for and exploring their natal host fruit.

Although I found that both species spend more time on each other's natal hosts, the effect size of these preferences was not large. The average duration and number of visits by virgin, naïve *R. zephyria* flies to apples and snowberries were identical. This behavior shows that flies will explore novel host environments even in the presence of nearby natal hosts. Visits by *R. pomonella* to snowberries and virgin *R. zephyria* to hawthorns were shorter and more frequent than visits by these flies to their natal hosts. While this behavior may indicate rapid rejection of non-natal fruit by both species, the rapid movement of these flies between hosts is likely to attract the attention of potential heterospecific and conspecific mates (Prokopy and Bush 1973) increasing the likelihood of hybridization. The duration and frequency of these visits to non-natal hosts lends

additional support to the conclusion that these flies do not strongly discriminate between hosts at this stage of their lives.

The effect of life history events on behavior is not unprecedented for the *R. pomonella* species group. *R. pomonella* females do not exhibit a strong natal host preference in early season observations. Prior to reproductive maturity, flies will disperse away from their natal host for one or two weeks before returning to mate and oviposit (Opp and Prokopy 1987). Females found on their natal hosts in the early season occur primarily on leaves and branches instead of fruit (Smith and Prokopy 1980). Similar behavior was noted for *R. mendax* in the early season with a distinct transition from leaves to fruit after the first several weeks of the season (Smith and Prokopy 1982).

Potential causes of incomplete host preference

For sympatric *Rhagoletis* populations such as those in Bellingham, where natal and non-natal hosts overlap spatially, incomplete host preference could occur as a result of reduced foraging costs. *Rhagoletis* flies are reluctant to venture far from their natal hosts (Roitberg et al. 1982) and will try to minimize the distance they travel to find food. If non-natal hosts occur within a fly's normal foraging radius, there should be little to no cost to explore the nearby host. Flies may even benefit from exploring non-natal hosts if those hosts could provide an additional source of food, mates, or refuge from predators. In contrast, the cost of ovipositing in a non-natal host should be much higher for populations whose larvae have adapted to specific host fruit conditions. The expectation that different costs apply to host preference and host acceptance is consistent with my experiments, in which there was extreme spatial overlap between hosts. In these conditions, it was less costly for virgin flies to explore non-natal hosts and they showed no host preference. For mated *R. zephyria* females, however, the cost of ovipositing in the wrong host would be high and I saw a corresponding shift in host preference toward

19

their natal host.

Food sources within close range of natal hosts will satisfy the nutritional requirements of early life and minimize the cost of travelling outside the distribution of natal hosts. Females can then remain near natal host fruit where they will spend the majority of their middle and late life (Roitberg et al. 1982). Apples and snowberries regularly grow in the same plots throughout the Pacific Northwest. The proximity of apples to the natal host of *R. zephyria* provides these flies with a potential source of food. Like many other fruits, the surfaces of apples are often rich with many species of yeast and other fungi that can provide a source of nitrogen to flies (Chand-Goyal and Spotts 1996, Vadkertiova et al. 2012). Indeed, I observed over a dozen flies (16%) of both species and sexes feeding on the surface of apples for long, continuous periods. In the case of sympatric *Rhagoletis* populations in Bellingham, apples provide an accessible nitrogen source at short range from snowberries and could easily provide mating assembly sites for conspecifics and heterospecifics alike.

Rhagoletis flies could benefit from their incomplete host preference as it increases their chances of finding conspecific mates while foraging for food. Flies in the *R. pomonella* species group are polygamous and mate at least once a day, with reproductive success positively correlated to number of matings (Opp and Prokopy 2000). Females experience increased fecundity simply from the physiological process of mating and multiple matings increase their fertility and the longevity of their egg-laying (Opp and Prokopy 1986). Because offspring are almost always the result of fertilization by the female's last mate, males may also benefit from multiple matings by increasing their chances to be the last partner (Opp et al. 1990). Although it is unclear whether *Rhagoletis* flies have conspecific sperm precedence mechanisms like those found in *Drosophila* (Price et al. 2000), the long-term benefits of multiple mating behavior to microsympatric snowberry and apple maggot populations still likely exceed the costs of heterospecific matings in the early season. As

long as the last partner prior to oviposition is conspecific, both sexes of both species benefit from multiple matings. By switching to a more host-specific behavior after their initial mating, females increase the likelihood that the majority of their offspring will be from conspecific matings.

The inability of *Rhagoletis* flies to discriminate between natal and non-natal hosts could also lead to the incomplete host preference I observed. The olfactory signals flies typically rely on to detect their natal hosts at long range (Linn et al. 2003) are more likely to confuse flies at short range when volatiles from both hosts are present in similar concentrations (Dambroski et al. 2005, Forbes and Feder 2006). These sensory conflicts will affect populations whose hosts physically overlap such as those in Bellingham where apple and snowberry plants grow within less than a meter of each other. Flies emerging at these sites will rely primarily on visual and tactile feedback to identify hosts, making them prone to more accidental visits to non-natal hosts while foraging (Roitberg et al. 1982). *Rhagoletis* flies may also lack the ability to discriminate against non-natal hosts that they have not previously evolved to recognize. Apples are still a relatively recent introduction to the Pacific Northwest and *R. pomonella* is a recent invader to Washington (AliNiazee and Penrose 1981). As a result, neither *R. zephyria* nor *R. pomonella* populations in this region may be familiar with each other's natal hosts despite the historical overlap between populations in the Midwestern United States (Feder et al. 1999). If host avoidance requires the maintenance of specific genes (Forbes et al. 2005), populations in secondary contact after a long period of isolation may have lost this functionality through drift or selection.

It is still an open question whether the abilities to explore and occasionally accept non-natal hosts could be adaptive traits for *Rhagoletis* flies under adverse or unusual natal host conditions. Certainly, variation in host availability could increase selective pressure on fly populations. For example, changes in climate or host phenology (Dambroski and

Feder 2007) or anthropogenic influence (Teixeira and Polavarapu 2003) could all result in patchy geographic distribution of viable host plants. The reduction of host plants within the cruising range of flies increases intraspecific competition for a scarce resource (Davis et al. 2011). Additionally, *Rhagoletis* flies have to contend with other plant parasites that share their natal host (Roitberg et al. 1990) and predators (Mather and Roitberg 1987). *R. pomonella* flies that infest the relatively recent host of apples are less likely to be parasitized by braconid wasps than flies that infest hawthorns (Feder 1995). Escaping predation and parasitization has thus been suggested as a factor in host shifts. Finally, the distribution of natal host plants is not consistent for fly populations undergoing range expansions. Geographically expanding populations will likely experience population fragmentation or differential selection as they move through regions less well-populated with their natal hosts (Michel et al. 2007). Given the current continental distributions of both *R. zephyria* and *R. pomonella*, these species have almost certainly faced similar problems.

Implications of incomplete host preference for speciation

In light of my findings that *R. zephyria* and *R. pomonella* express a reciprocal preference for each other's natal hosts prior to mating, hybridization between these two species is more likely than previously expected (Feder et al. 1999). During this early stage in their lives, flies are equally likely to explore apples and snowberries thereby increasing the likelihood of heterospecific encounters and potential matings. Mating isolation between these species is weak in the absence of host cues (Yee and Goughnour 2011) and in this study I find that the presence of natal or non-natal host fruit does not reduce the number of mating events between conspecific *R. zephyria* flies. Based on this mating behavior and the preference of both species for non-natal hosts, I predict not only that flies of either species could meet on the same host, but also that conspecifics are likely to meet on the same "wrong" hosts and mate there. However, since mating for both species is

not completely assortative, is it unlikely that matings in these conditions would be solely between young conspecifics.

After mating, female host preference dramatically shifts toward natal hosts, reducing the probability that mated females will be present on non-natal hosts and mate with heterospecific males there. Although I did not test host preference for mated males, previous research suggests that males are not as dramatically affected as females by mating status (Anton et al. 2007). I do, however, expect males to learn which hosts on which they most successfully discover conspecific females and follow females to their natal hosts (Prokopy et al. 1989). Feeding and mating remain the fundamental priorities of males throughout their lives, while females obtain the additional goal of oviposition (Webster 1979). Low levels of hybridization could still occur between males exploring non-natal hosts and females ovipositing in natal hosts, although selection may favor males that mate with conspecifics (Smith and Prokopy 1980). Therefore, I expect the probability of heterospecific matings to be highest during the early season when host preference is incomplete for both sexes of both species. In the mid- to late-season, when the majority of females have mated, the probability of heterospecific matings will drop significantly and depend primarily on the preference of males for non-natal hosts and heterospecific mates. The proportion of virgin females in the population after the early season will also contribute to the likelihood of heterospecific matings.

Despite the increased likelihood of heterospecific matings in the early season, several factors could reduce the effective gene flow between sympatric populations. Mating occurs frequently throughout the season (Prokopy and Bush 1973). During the early season, mating events are not necessarily followed immediately by oviposition. The last partner a female has will fertilize 79-93% of her eggs due to sperm competition (Opp et al. 1990). Females that mate with a conspecific male after a heterospecific male are therefore less likely to produce hybrid offspring. Those eggs that are fertilized as a result

of heterospecific matings are more likely to be oviposited into the less viable substrate of unripe, early-season fruit (Papaj 2000). However, the larvae that emerge from eggs that are oviposited into ripe, early-season fruit will have an advantage over mid- and late-season larvae as they will have more time to consume food before diapause (Ragland et al. 2012).

In this study, I found that virgin *R. pomonella* and *R. zephyria* flies of both sexes exhibit no host preference and that host preference remains partially incomplete for *R. zephyria* females even after mating. Host acceptance, however, remains nearly complete for females regardless of mated status. Thus for each heterospecific pair, I expect hybrid eggs to be oviposited in the natal host of the female. Hybrids between male *R. zephyria* and female *R. pomonella* in snowberries or male *R. pomonella* and female *R. zephyria* in apples should be rare if they exist at all. If any aspects of host survival are dependent on traits inherited from the female (e.g., diapause timing or host-specific enzymes), this pattern of hybridization would reduce the intrinsic inviability of hybrids at sympatric sites and promote gene flow via females that oviposit in their natal host (Fox et al. 1995, Dambroski et al. 2005).

The pattern of introgression between populations of *R. zephyria* and *R. pomonella* in Bellingham can be understood in the context of our expectation that hybrid eggs will be oviposited in the natal host of the female partner. Multiple lines of evidence suggest that introgression between *Rhagoletis* populations in Bellingham only occurs in individuals emerging from apples. These introgressed flies must be the result of initial matings between *R. zephyria* males and *R. pomonella* females and oviposition of eggs into apples. This same hypothesis was first suggested by McPheron (1990) and has since been supported by mating experiments between virgin flies of both species, in which heterospecific pairs of *R. zephyria* males and *R. pomonella* females were more common than those of *R. pomonella* males and *R. zephyria* females (Yee and Goughnour

2011). Evidence that *R. zephyria* males possess more generalist mate preferences was discovered in mating trials between *R. zephyria* and *R. mendax*, in which mating pairs of *R. zephyria* males and *R. mendax* females were more common than the alternate pairing (Schwarz and McPheron 2007). Tests of mitochondrial DNA from introgressed flies in Bellingham could confirm that their female parents are indeed *R. pomonella* flies. The combined effects of complete natal host acceptance by females and asymmetrical mating preferences between the species may be enough to account for the asymmetrical hybridization found in Bellingham without invoking asymmetry in postzygotic isolating barriers such as intrinsic inviability of hybrids. However, asymmetrical population sizes for these two species may still be a contributing factor to the observed asymmetrical introgression, as might asymmetrical postmating, prezygotic barriers.

Conclusion

The emergence and maintenance of species in sympatry not only depends on ecological conditions that facilitate gene flow but also the life history states of the interacting populations themselves. The system of sympatric *R. zephyria* and *R. pomonella* populations in Bellingham provides a natural demonstration of these conditions. The incomplete host and mating preferences of both species and proximity of their natal hosts enables gene flow in the early stages of their lives. Gene flow during the middle and late season will likely be reduced when host preferences become stronger for females after mating. If the pattern of host acceptance I see for *R. zephyria* applies to *R. pomonella* as well, the nature of gene flow between these populations will be limited by oviposition of hybrid eggs only in the natal hosts of females. From this demonstration, I find that reproductive isolation in sympatry and ecological preferences exist on a continuum and that discrete life history events can reconfigure the ranges of these continua.

LITERATURE CITED

AliNiazee, M., and R. Penrose. 1981. Apple maggot in Oregon: A possible threat to the Northwest apple industry. Bulletin of the Entomological Society of America **27**:245–246.

Alonso-Pimentel, H., J. B. Korer, C. Nufio, and D. R. Papaj. 1998. Role of colour and shape stimuli in host-enhanced oogenesis in the walnut fly, *Rhagoletis juglandis*. Physiological Entomology **23**:97–104.

Anton, S., M. Dufour, and C. Gadenne. 2007. Plasticity of olfactory-guided behaviour and its neurobiological basis: lessons from moths and locusts. Entomologia Experimentalis et Applicata **123**:1–11.

Berlocher, S. 2000. Radiation and divergence in the *Rhagoletis pomonella* species group: inferences from allozymes. Evolution **54**:543–557.

Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annual Review of Entomology **47**:773–815.

Bierbaum, T., and G. Bush. 1990. Genetic differentiation in the viability of sibling species of *Rhagoletis* fruit flies on host plants, and the influence of reduced hybrid viability on reproductive isolation. Entomologia Experimentalis et Applicata **55**:105–118.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution **23**:237–251.

Chand-Goyal, T., and R. Spotts. 1996. Enumeration of bacterial and yeast colonists of apple fruits and identification of epiphytic yeasts on pear fruits in the Pacific Northwest United States. Microbiological Research **151**:427–432.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Dambroski, H., C. Linn, and S. Berlocher. 2005. The genetic basis for fruit odor discrimination in *Rhagoletis* flies and its significance for sympatric host shifts. Evolution **59**:1953–1964.

Dambroski, H. R., and J. L. Feder. 2007. Host plant and latitude-related diapause variation in *Rhagoletis pomonella*: a test for multifaceted life history adaptation on different stages of diapause development. Journal of Evolutionary Biology **20**:2101–12.

Davis, J. M., C. R. Nufio, and D. R. Papaj. 2011. Resource quality or competition: why increase resource acceptance in the presence of conspecifics? Behavioral Ecology **22**:730–737.

Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences **357**:471–92.

Feder, J. 1995. The effects of parasitoids on sympatric host races of *Rhagoletis pomonella* (Diptera: Tephritidae). Ecology **76**:801–813.

Feder, J., C. Chilcote, and G. Bush. 1988. Genetic differentiation between sympatric host races of the apple maggot fly *Rhagoletis pomonella*. Nature **336**:61–64.

Feder, J., S. Williams, S. H. Berlocher, B. A. McPheron, and G. L. Bush. 1999. The population genetics of the apple maggot fly, *Rhagoletis pomonella* and the snowberry maggot, *R. zephyria*: implications for models of sympatric speciation. Entomologia Experimentalis et Applicata **90**:9–24.

Feder, J. L., S. B. Opp, B. Wlazlo, K. Reynolds, W. Go, and S. Spisak. 1994. Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. Proceedings of the National Academy of Sciences of the United States of America **91**:7990–4.

Forbes, A. A., and J. L. Feder. 2006. Divergent preferences of *Rhagoletis pomonella* host races for olfactory and visual fruit cues. Entomologia Experimentalis et Applicata **119**:121–127.

Forbes, A. A., J. Fisher, and J. L. Feder. 2005. Habitat avoidance: overlooking an important aspect of host-specific mating and sympatric speciation? Evolution **59**:1552–9.

Fox, C., K. Waddell, and T. Mousseau. 1995. Parental host plant affects offspring life histories in a seed beetle. Ecology **76**:402–411.

Gough, H., personal communication. Unpublished raw data.

Green, E., K. Almskaar, S. Sims, T. Arcella, W. Yee, J. Feder, and D. Schwarz. in press. Rapid molecular species identification of morphologically cryptic apple and snowberry maggots in Western Washington (Tephritidae: *Rhagoletis*). Environmental Entomology .

Harrison, R. 1980. Dispersal polymorphisms in insects. Annual Review of Ecology and Systematics **11**:95–118.

Hern, A., and S. Dorn. 1999. Sexual dimorphism in the olfactory orientation of adult *Cydia pomonella* in response to alpha-farnesene. Entomologia Experimentalis et Applicata **92**:63–72.

Huxel, G. 1999. Rapid displacement of native species by invasive species: effects of hybridization. Biological Conservation **89**:143–152.

Linn, C., H. Dambroski, S. Nojima, J. L. Feder, S. H. Berlocher, and W. L. Roelofs. 2005a. Variability in response specificity of apple, hawthorn, and flowering dogwood-infesting *Rhagoletis* flies to host fruit volatile blends: implications for sympatric host shifts. Entomologia Experimentalis et Applicata **116**:55–64.

Linn, C., J. L. Feder, S. Nojima, H. R. Dambroski, S. H. Berlocher, and W. Roelofs. 2003. Fruit odor discrimination and sympatric host race formation in *Rhagoletis*. Proceedings of the National Academy of Sciences USA **100**:11490–3.

Linn, C., S. Nojima, and W. Roelofs. 2005b. Antagonist effects of non-host fruit volatiles on discrimination of host fruit by *Rhagoletis* flies infesting apple (*Malus pumila*), hawthorn (*Crataegus* spp.), and flowering dogwood (*Cornus florida*). Entomologia Experimentalis et Applicata **114**:97–105.

Lockwood, J. R., III. 1998. On the statistical analysis of multiple-choice feeding preference experiments. Oecologia **116**:475.

Mallet, J. 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences **363**:2971–86.

Masante-Roca, I., S. Anton, L. Delbac, M.-C. Dufour, and C. Gadenne. 2007. Attraction of the grapevine moth to host and non-host plant parts in the wind tunnel: effects of plant phenology, sex, and mating status. Entomologia Experimentalis et Applicata **122**:239–245.

Mather, M., and B. Roitberg. 1987. A sheep in wolf's clothing: tephritid flies mimic spider predators. Science **236**:308.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge.

McPheron, B. 1990. Genetic structure of apple maggot fly (Diptera: Tephritidae) populations. Annals of the Entomological Society of America **83**:568–577.

Michel, A. P., J. Rull, M. Aluja, and J. L. Feder. 2007. The genetic structure of hawthorn-infesting *Rhagoletis pomonella* populations in Mexico: implications for sympatric host race formation. Molecular Ecology **16**:2867–78.

Michel, A. P., S. Sim, T. H. Q. Powell, M. S. Taylor, P. Nosil, and J. L. Feder. 2010. Widespread genomic divergence during sympatric speciation. Proceedings of the National Academy of Sciences USA **107**:9724–9.

Neilson, W. T. A., and F. A. Wood. 1966. Natural source of food of the apple maggot. Journal of Economic Entomology **59**:997–998.

Opp, S., and R. Prokopy. 1986. Variation in laboratory oviposition by *Rhagoletis pomonella* (Diptera: Tephritidae) in relation to mating status. Annals of the Entomological Society of America **79**:705–710.

Opp, S., and R. Prokopy. 1987. Seasonal changes in resightings of marked, wild *Rhagoletis pomonella* (Diptera: Tephritidae) flies in nature. Florida Entomologist **70**:449–457.

Opp, S., and R. Prokopy. 2000. Multiple mating and reproductive success of male and female apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephritidae). Journal of Insect Behavior **13**:901–914.

Opp, S. B., J. A. Y. Ziegner, N. G. A. Bui, and R. J. Prokopy. 1990. Factors influencing estimates of sperm competition in *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). Annals of the Entomological Society of America **83**:521–526.

Papaj, D. 2000. Ovarian dynamics and host use. Annual Review of Entomology **45**:423–448.

Papaj, D., and R. Prokopy. 1988. The effect of prior adult experience on components of habitat preference in the apple maggot fly (*Rhagoletis pomonella*). Oecologia **76**:538–543.

Petersson, E., and J. Sivinski. 2003. Mating status and choice of group size in the phoretic fly *Norrbomia frigipennis* (Spuler) (Diptera: Sphaeroceridae). Journal of Insect Behavior **16**:411–423.

Price, C., C. Kim, J. Posluszny, and J. Coyne. 2000. Mechanisms of conspecific sperm precedence in *Drosophila*. Evolution **54**:2028–2037.

Prokopy, R., E. Bennett, and G. Bush. 1971. Mating behavior in *Rhagoletis pomonella* (Diptera: Tephritidae) I. Site of assembly. The Canadian Entomologist **103**:1405–1409.

Prokopy, R., and G. Bush. 1973. Mating behavior of *Rhagoletis pomonella* (Diptera: Tephritidae): IV. Courtship. The Canadian Entomologist **105**:873–891.

Prokopy, R., S. Cooley, and S. Opp. 1989. Prior experience influences the fruit residence of male apple maggot flies, *Rhagoletis pomonella*. Journal of Insect Behavior **2**:39–48.

Prokopy, R., S. Diehl, and S. Cooley. 1988. Behavioral evidence for host races in *Rhagoletis pomonella* flies. Oecologia **76**:138–147.

Prokopy, R. J. 1972. Evidence for a marking pheromone deterring repeated oviposition in apple maggot flies. Environmental Entomology **1**:328–332.

Prokopy, R. J., A. L. Averill, S. S. Cooley, and C. A. Roitberg. 1982. Associative learning in egglaying site selection by apple maggot flies. Science **218**:76–7.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ragland, G. J., S. B. Sim, S. Goudarzi, J. L. Feder, and D. A. Hahn. 2012. Environmental interactions during host race formation: host fruit environment moderates a seasonal shift in phenology in host races of *Rhagoletis pomonella*. Functional Ecology **26**:921–931.

Roa, R. 1992. Design and analysis of multiple-choice feeding-preference experiments. Oecologia **89**:509–515.

Roitberg, B., M. Mangel, and G. Tourigny. 1990. The density dependence of parasitism by tephritid fruit flies. Ecology **71**:1871–1885.

Roitberg, B. D., J. C. V. Lenteren, J. J. M. V. Alphen, F. Galis, and R. J. Prokopy. 1982. Foraging behaviour of *Rhagoletis pomonella*, a parasite of hawthorn (*Crataegus viridis*), in nature. The Journal of Animal Ecology **51**:307.

Rojas, J. 1999. Influence of age, sex and mating status, egg load, prior exposure to mates, and time of day on host-finding behavior of *Mamestra brassicae* (Lepidoptera: Noctuidae). Environmental Entomology **28**:155–162.

Schwarz, D., personal communication.

Schwarz, D., B. M. Matta, N. L. Shakir-Botteri, and B. A. McPheron. 2005. Host shift to an invasive plant triggers rapid animal hybrid speciation. Nature **436**:546–9.

Schwarz, D., and B. A. McPheron. 2007. When ecological isolation breaks down: sexual isolation is an incomplete barrier to hybridization between *Rhagoletis* species. Evolutionary Ecology **9**:829–841.

Schwarz, D., K. D. Shoemaker, N. L. Botteri, and B. A. McPheron. 2007. A novel preference for an invasive plant as a mechanism for animal hybrid speciation. Evolution **61**:245–56.

Smith, D., and R. Prokopy. 1980. Mating behavior of *Rhagoletis pomonella* (Diptera: Tephritidae) VI. Site of early-season encounters. The Canadian Entomologist **112**:585–590.

Smith, D., and R. Prokopy. 1982. Mating behavior of *Rhagoletis mendax* (Diptera: Tephritidae) flies in nature. Annals of the Entomological Society of America **75**:388–392.

Smith, J. J., and G. L. Bush. 1997. Phylogeny of the genus *Rhagoletis* (Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase II. Molecular Phylogenetics and Evolution **7**:33–43.

Teixeira, L., and S. Polavarapu. 2003. Evolution of phenologically distinct populations of *Rhagoletis mendax* (Diptera: Tephritidae) in highbush blueberry fields. Annals of the Entomological Society of America **96**:818–827.

Vadkertiova, R., J. Molnarova, D. Vranova, and E. Slavikova. 2012. Yeasts and yeast-like organisms associated with fruits and blossoms of different fruit trees. Canadian Journal of Microbiology **58**:1344–1352.

vanRanden, E., and B. Roitberg. 1996. The effect of egg load on superparasitism by the snowberry fly. Entomologia Experimentalis et Applicata **79**:241–245.

Webster, R. 1979. Long-term intake of protein and sucrose in relation to reproductive behavior of wild and laboratory cultured *Rhagoletis pomonella*. Annals of the Entomological Society of America **72**:41–46.

Wenninger, E. J., L. L. Stelinski, and D. G. Hall. 2009. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. Environmental Entomology **38**:225–34.

Wheeler, D. 1996. The role of nourishment in oogenesis. Annual Review of Entomology **41**:407–431.

Xie, X., A. P. Michel, D. Schwarz, J. Rull, S. Velez, A. A. Forbes, M. Aluja, and J. L. Feder. 2008. Radiation and divergence in the *Rhagoletis pomonella* species complex: inferences from DNA sequence data. Journal of Evolutionary Biology **21**:900–13.

Xie, X., J. Rull, A. P. Michel, S. Velez, A. A. Forbes, N. F. Lobo, M. Aluja, and J. L. Feder. 2007. Hawthorn-infesting populations of *Rhagoletis pomonella* in Mexico and speciation mode plurality. Evolution **61**:1091–105.

Yee, W. L., and R. B. Goughnour. 2011. Mating frequencies and production of hybrids by *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae) in the laboratory. The Canadian Entomologist **143**:82–90.
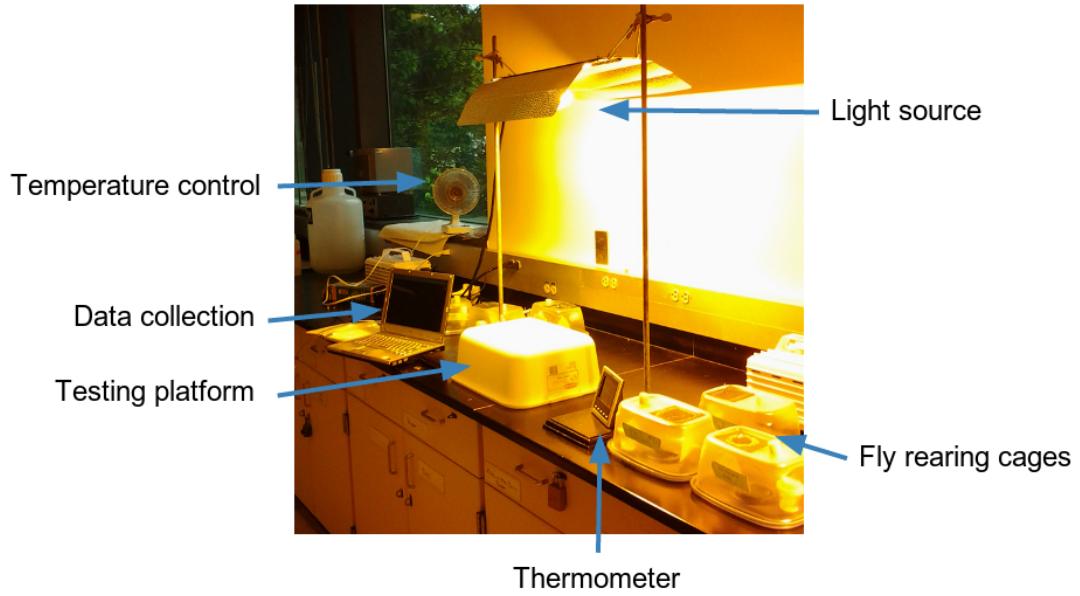
Figure 1. Configuration for host preference experiments in the lab showing one of two side-by-side experimental stations.

(a) Host preference enclosure

(b) Mating environment enclosures

Figure 2. Configuration of host preference and mating environment experiments.
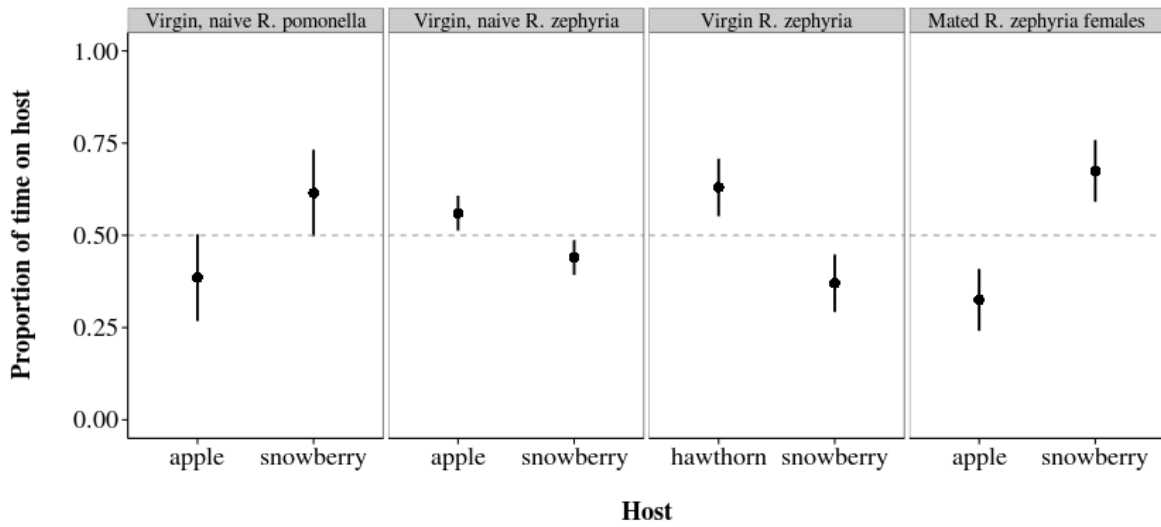
Figure 3. Host preference measured by proportion of time spent on hosts (mean ± SE) for *R. pomonella* and three life stages of *R. zephyria* flies (virgin and naïve, virgin and experienced, and mated). Difference in host preference was significant in all experiments (p < 0.001).

Table 1. Host-specific visits per trial and median duration per visit (mean $\pm$ SE) for each species group.

Species group	Host	Visits/trial	Median duration/visit (s)
Virgin *R. pomonella*	snowberry	3.4 $\pm$ 0.8 ***	128 $\pm$ 59
	apple	1.1 $\pm$ 0.4 ***	149 $\pm$ 62
Virgin, naïve *R. zephyria*	snowberry	3.6 $\pm$ 0.4	92 $\pm$ 22
	apple	3.8 $\pm$ 0.4	88 $\pm$ 20
Virgin *R. zephyria*	snowberry	3.4 $\pm$ 0.7 **	61 $\pm$ 32
	hawthorn	4.9 $\pm$ 0.7 **	44 $\pm$ 14
Mated *R. zephyria*	snowberry	2.9 $\pm$ 0.6	251 $\pm$ 68 ***
	apple	3.1 $\pm$ 0.7	54 $\pm$ 18 ***

* p < 0.05, ** p < 0.01, *** p < 0.001

Table 2. Probing events by host and life stage of *R. zephyria* females.

Category	Snowberry	Apple	Hawthorn	Total Females
Virgin, naïve	2	0	-	37
Virgin, experienced	0	-	1	10
Mated, experienced	11 ***	0 ***	-	16

* p < 0.05, ** p < 0.01, *** p < 0.001