The Sympatric Speciation of *Rhagoletis pomonella*

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Rhagoletis pomonella, also called the "railroad worm," is the apple maggot fly native to North America that originally fed on the fruit of the wild hawthorn (*Crataegus spp*). Over the past 130 years, however, *R. pomonella* has evolved to feed on a variety of different fruits, including those that are found on the domestic apple tree (*Malus pumila*). Not native to North America, the wild apple which was introduced to Europe by merchants, was brought to America by colonists in the form of seeds. The temperate zones of North America, like those of Asia and Europe, proved to be an ideal place for growing apples. These seeds were then planted throughout the United States because of their ability to bare fruit. More specifically, John Chapman born in 1775, better known as Johnny Appleseed, spent his life planting apple seeds on his 100,000 square mile trek throughout the Midwest (http://www.americaslibrary.gov/cgi-bin/page.cgi/jb/revolut/apple_1). Additionally, he sold trees from his nursery that were planted elsewhere, thereby being one of the strongest contributors to the spread of the apple tree. Chapman therefore probably established many of the orchards that served as factors in the speciation process.

The apple maggot fly is an example of a phytophagous insect that practices host fidelity, meaning it mates on or near the fruit of its host plant. Now, because of the preference for two different host plants and realizing the dependent nature of the fly to the host plant, these host races could be representing speciation in action. By understanding the influence that host fidelity and other factors have on reproductive isolation, a model for ongoing speciation can be constructed. Taking into account the rapid establishment of new host races in lieu of a geographic barrier, host fidelity is thought to act as a premating barrier in sympatric speciation. This, along with other biological attributes, has led researchers to hypothesize that *Rhagoletis pomonella* is a current example of sympatric speciation in action.

One of the first scientists to explore this topic was Guy L. Bush, and his preliminary research began back in the 1960s. Before being able to explore sympatric speciation, Bush had to understand the biological characteristics of the fly. First, he looked at courtship behavior. *Rhagoletis* bodies, like most Tephritidae, are decorated with brightly contrasting patterns along with wings that usually possess intricate, species specific, and often sexually dimorphic patterns. Furthermore, Bush showed that the distinct body and wing patterns act as visual releasers in courtship (Bush, 1969). These visual releasers and displays are noted in Tauber and Toschi's study as being wing patterns and various courtship actions performed by both male and female flies (Tauber and Toschi, 1964). The study consisted of monitoring courtship and documenting similarities in actions of each sex. Although important, Bush also found that these visual releasers are only effective at close range, at a point where the flies have already congregated on the host plants. So, here it is understood that the host fruit serves as the rendezvous point, on which the courtship, mating, and later oviposition take place (Bush, 1969). Taken together, the host and mate selection are directly related, consequently serving as an important feature for sympatric speciation. Each host race of fly prefers and attacks a different host plant, and because the visual releasers are only effective in close proximity, this strongly serves as a factor in reproductive isolation between the two races.

Understanding that courtship only took place after congregation on a host plant, Bush looked at the process of host selection. Despite not being monophagous, *Rhagoletis* display a fairly narrow host preference and restrict choice to plant species of a single genus or related genera. At this point, Bush did not understand the factors for host selection, but this did set up the area for future research. Bush did, however, understand the diapause and emergence of the *Rhagoletis*. The life cycle of *Rhagoletis pomonella* begins when the adult fly ecloses from the ground in spring and seeks out host fruit on which to mate and ovipost. Once courting and mating has occurred, the eggs are deposited in the fruit of the host plant. When the eggs hatch, they feed within the fruit until fall when the fruit abscises from the tree. Upon hitting the ground, the larvae leave the fruit and borrow 2 to 5 inches below surface where they pupate or undergo the transformation from larval to adult structures. Once spring arrives, the *Rhagoletis* adults typically eclose over a 2 to 4 week span corresponding to the maximum availability of host fruits, thus continuing the life cycle (Bush, 1968). The diapause is usually broken by a period of low temperature or by winter. Upon elcosion, *R. pomonella* typically survive for between 20-30 days in field conditions (Porter, 1928). Bush found the most interesting feature of *Rhagoletis pomonella* to be in its ecological diversity because speciation has occurred with a shift to a new host family in every case (Bush, 1969). This ecological diversity is unlike the walnut-infesting *Rhagoletis suavis* group that only infests a single genus of host plants, therefore making *R. pomonella* quite unique.

In particular, the first reports of *R. pomonella* using apple trees came over one hundred years ago from the Hudson River Valley and soon spread to Massachusetts and Connecticut, trees that were likely planted by of originally from Mr. Chapman. Today, some of these populations of apple and hawthorn infesting races display slight differences in body size, number of postorbital bristles, and ovipositor length (Bush, 1966). Bush also references the studies of Pickett and Neary from 1940, explaining that both races emerge from the pupal stage corresponding to when their prospective host plant fruits are at optimal time, or ripe, for oviposition (Bush 1969). At this point in the 1960s, Bush had studied and made observations about the speciation of *Rhagoletis pomonella* into apple and hawthorn host races that would later be explored in more depth. In 1969, Bush layed out a model for how evolution in *R. pomonella* could occur based on a single-locus Mendelian basis for host selection:

1) Diapause and emergence times are ultimately under genetic control.
2) Initial orientation to and selection of host plant is in response to a chemical cue.
3) Host selection has a genetic basis. In this case homozygous AA and heterozygous Aa individuals move preferentially to haws while homozygous aa flies move to apples.
4) A mutates to a locality where apples are available and a few homozygous individuals are eventually reproduced as a result of recombination.

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5) Host plant and mate selection are positively correlated. Individuals move preferentially to their respective host plants depending on their genotype, and mating occurs on host plant.

After looking at this model, it is clear that Bush thought about, touched upon, and set up the future research of speciation for *R. pomonella* in terms of host fidelity, allochronic isolation, fruit odor preferences, and hybrid limited fitness.

One of the most recognized scientists to follow in Bush's footsteps, and who actually worked along with Bush later on, is Jeffrey L. Feder. Feder is currently a professor at the University of Notre Dame who gained a Ph.D. from Michigan State University and performed postdoctoral work at Princeton University and University of Chicago. Still fascinated with understanding speciation, Feder continues his research on the speciation of *R. pomonella* (http://www.nd.edu/~biology/JeffreyFeder.shtml).

Specifically, Feder's research focuses on the role of host fidelity concerning *Rhagoletis pomonella*. In 1994, Feder conducted an experiment using three mark-and-recapture studies at a site near Grant, Michigan to investigate how host fidelity could restrict gene flow between the sympatric apple- and hawthorn-infesting races of *R. pomonella* (Feder et al. 1994).

The first study was entitled the "Field-Release Experiment" that released naïve apple-and hawthorn-origin flies in the middle of the study site under neither the apple and hawthorn trees (Feder et al. 1994). After the release, the flies were captured and the distributions of marked apple- and hawthorn-origin flies was tallied in order to examine the preference of flies based solely on genetic differences in host preference (Feder et al. 1994). In order to eliminate the effect of allochronic isolation, or differences in eclosing times, the team released large numbers of flies throughout the season. Allochronic isolation is isolation caused by difference in geologic timing, so by releasing the flies continuously throughout the season, this was eliminated. Also, the flies were released in the middle neutral territory, so the team eliminated the possible tie between eclosing and host preference. This allowed for the experiment to assess host preference strictly based on genetic preference wihtout being influenced by the time of season or under which tree the fly eclosed. Feder et al. reported that almost all of flies recaptured on apple trees were of apple-origin, but less than half of all flies recaptured on hawthorn trees were of hawthorn-origin (Feder et al. 1994). This result indicated a host-preference difference for the races. When Feder figured the average relative preference of the flies he found that a little over half of apple-origin flies had a preference for apple over hawthorn trees, whereas almost all of hawthorn-origin flies preferred hawthorn over apple trees (Feder et al. 1994). The apple-origin flies were found on both the apple and hawthorn trees, whereas almost all hawthorn-origin flies were found on hawthorn trees. Together these results indicate a clear difference between naïve apple- and hawthorn-origin flies in their propensities to accept apple and hawthorn trees. Because almost all of the flies captured on the apple trees were of apple-origin, and the average propensity for a apple-origin fly to choose apple over hawthorn was of only around half, the data suggests that the more recently derived (apple) flies have an only partially developed preference for apple, with significant propensity to go to haw still remaining. The hawthorn-origin flies were found almost completely on hawthorn trees, indicating that the ancestral species has a much lower propensity to attack the apple tree. These results support the idea that the *R. pomonella* are in the beginning stages of speciation.

The next experiment, the "Host-Switch Experiment," released adults of both races among both host plant species. One goal was to compare the numbers of flies recaptured on the host race under which they were released (Feder et al. 1994). These flies were released in the same timeline so as to eliminate allochronic biases. In the end, about three quarters of marked flies recaptured on apple-trees were apple-origin flies; however, apple- and hawthorn-origin flies were captured in roughly equal numbers on hawthorn release trees. The difference between this experiment and the previous was that the flies were released under both their own host plant and the other races'. In the end however, the two experiments yielded similar results, showing that apple-origin flies had a greater propensity to accept the ancestral native plant. Furthermore, this experiment indicated that a genetic preference trumped eclosion when considering host plant preference. Despite eclosing under the other host race's plant, both species strongly preferred their own host plant. Also, by taking these numbers Feder found the average host fidelity for apple-origin flies for apple trees was about 90.0 %, and roughly 80.0% for hawthorn-origin flies for hawthorn trees. For the apple fly, this number was determined by calculating the number of apple-origin flies released under apple trees that were found on apple trees in comparison to number of apple-origin flies found on either host plant. The same was done with the hawthorn flies. This experiment agrees with previous work, which strongly suggests a genetically based difference for host preference between the two races.

In the third and final experiment, "Net-Release Experiment," the goal was to determine levels of host fidelity taking into account all natural factors effecting the reproduction of the flies, including allochronic isolation. After two seasons of data were collected, hawthorn-origin flies had a 95.0% level of host fidelity and apple-origin flies had a 93.1% host fidelity level (Feder et al. 1994). Based upon the three different mark-and-recapture studies, Feder et al. concluded that genetically based differences in host selection, location of adult eclosion, and allochronic isolation all contribute to host fidelity and consequent limited gene flow. In the "field-release experiment" genetic preference was shown by the large number of both races being found on their respective host plants despite being released under a neutral tree. The "host-switch experiment" also showed a genetic preference for the races because even when released under the other races' host plant, the flies were still recaptured in large numbers under their origin plant. This experiment also showed that when released under their origin plant mimicking natural eclosion, the races were almost all captured on their origin plant. Finally, in the "net-release experiment," the flies were captured under the tree when they eclosed. Then, they were marked and released back where they were found in order to try and assess all factors that contribute to host fidelity (this is different from the other experiments because the flies were released multiple times over the season, and thus did not take into account natural eclosion times). This experiment showed that host fidelity, taking into account all factors including allochronic isolation, was fairly high in *R. pomonella.* However, because of the ~6% level of genetic exchange between the races the story is not complete. After establishing the means for host fidelity, Feder suggested that fruiting phenologies of apples and hawthorns may affect pupal development rates.

Despite overlapping in geographic distribution, apple and hawthorn trees mainly differ in their respective fruiting times (Bush, 1966). The apple trees' fruiting time occurs roughly three weeks before the hawthorn trees', therefore causing the apple-infesting *R. pomonella* flies to both enter diapause and eclose earlier than hawthorn-infesting. Because of the short life span, roughly 30 days, the insects' association and adaptation to the fruiting phenologies of their respective host plants could play an important role in sympatric speciation. Picking up where Feder left off, Dambroski and Feder set out to explore whether differences in host plant fruiting times leads to variations in pupal diapuase length, which could be a factor in reproductive isolation (Dambroski & Feder, 2007). In this study, Dambroski and Feder explored this topic by comparing the times at which apple- and hawthorn-infesting flies undergo diapause, as well as the seasonal time when *R. pomonella* break diapause and eclose in the spring. Dambroski and Feder hypothesized this mechanism for speciation knowing that in sympatry ecological barriers can be represented by allochronic prezygotic isolation caused by "life history adaptations." After calculating the statistics for multiple sympatric races from different sites, Dambroski and Feder found that the apple-infesting flies eclose earlier in the spring than hawthorn-infesting flies and also undergo a longer prewinter period in the soil. The findings of the Dambroski and Feder study suggest that diapause timing can rapidly evolve to coincide with host plant phenology. (Dambroski & Feder, 2007). The importance of this study is that it shows how the fruiting times of the host plants are directly related to both the diapause and eclosion of the flies, thereby serving as a legitimate premating barrier. Taken into consideration with the role of host fidelity, these two factors severely limit the actual possibility for sexual reproduction between the two races. Both of these factors therefore rely on host selection and preference and the means for this determination.

In 2003, Linn et al. studied the influence of fruit odor on host preference in *R. pomonella*. Up to this point, how *R. pomonella* distinguished among possible host plants was unkown. Using flight-tunnel assays and field tests, Linn et al. showed that apple- and ancestral hawthorn-infesting host races of *R. pomonella* use fruit odor as a key cue in distinguishing and selecting their respective host plants (Linn et al. 2003). Linn et al. created synthetic apple and hawthorn fruit volatile blends that contained the biologically active chemical components of the individual fruit odors. These volatiles were then placed on rubber septa and attached to a red ball at the upwind end of the constructed wind tunnel. Three different scenarios were conducted, a blank sphere, an apple sphere, and a hawthorn sphere. The blank served as a control, and neither race flew upwind to the sphere. In contrast, almost all of the apple-origin flies flew upwind to the sphere with apple fruit volatiles, and almost all of the hawthorn-origin responded similarly to their natal fruit volatile as well. Less than a quarter of the apple-origin flies, however, flew upwind to their nonnatal fruit volatile (hawthorn), and even fewer hawthorn-origin flies flew upwind to their nonnatal fruit volatiles (apple). These results coincide with Feder's in that the more recently derived species (apple) had a higher propensity to fly to its nonnatal fruit volatiles than the hawthorn-origin flies, possibly signifying the ancestral preference still found in some apple-origin individuals. Specifically, the studies showed that both apple- and hawthorn-infesting flies were captured using natal fruit volatiles of their host plants, indicating a direct preference and use of the host fruit in locating a host plant. (Linn et al. 2003).

Because the flies use fruit odor volatiles to find their host plants before mate selection and mating, host fruit volatiles play an integral role in premating reproductive isolation. To further develop this idea and expand on the importance of fruit volatiles, Andrew A. Forbes et al. conducted a study that focused on host plant avoidance. In his study, Forbes et al. found that not only do the flies prefer the odor of their natal fruit, they also avoid nonnatal fruit odors (Forbes et al. 2005). Despite the positive preference flies have for their natal fruit volatiles and the low preference for nonnatal volatiles, there is no gaurentee that this behavior is due to avoidance; instead, it could be due to non-recognition. Displaying avoidance, however, offers a more difficult task especially because avoidance behavior is thought to be demonstrated when nonnatal fruit volatiles have an antagonistic effect on a visual cue that is normally accepted and preferred (Forbes et al. 2005). The basic experimental consisted of a series of two-way choice field experiments between a sticky red ball covered in fruit odor and a blank, odorless control ball. The balls were painted red because they have been shown to attract all races of *R. pomonella*. Forbes et al. figured that by documenting flight patterns between the control and different fruit volatiles, preference, avoidance, or nonresponse could be displayed. Preference was based on the idea that when coated with the natal fruit volatile, the individual races would prefer the red sphere with odor over the blank odorless sphere. Avoidance, on the other hand, would occur when the flies would go to the odorless blank sphere instead of the red sphere coated in the nonnatal fruit volatiles. The logic here is that despite having a preference for the red odorless spheres over the blank spheres, the nonnatal volatiles, when applied to the red spheres, would cause the flies to avoid the red spheres all together. The results of the experiment showed that with both races capture rates were 35% higher with the natal blends over the odorless sphere. In contrast, the red spheres with nonnatal blends captured 37-69% fewer flies than the blank, odorless spheres (Forbes et al. 2005). Forbes et al.'s results support the hypothesis that nonnatal fruit volatiles cause flies to avoid other races' host fruit volatiles. These findings also suggest a possible link to explaining the postzygotic isolation of hybrid flies.

With all of these prezygotic isolating factors taken into account the question must be raised about the possibility of postzygotic isolating factors that might also contribute to the sympatric speciation of *R. pomonella*. Realizing the importance of fruit odor discrimination, Linn et al. wanted to study the response hybrids would have to host-fruit odors. In their study, they found that F_1 hybrids between apple and hawthorn races did not respond to either host fruit volatiles in wind-tunnel assays (Linn et al. 2004). These findings suggest that hybrids might actually suffer a fitness disadvantage when trying to locate fruit for reproduction. Fitness refers to the capability of an individual to reproduce and pass on his or her genes to the next generation, so without the ability to locate a host plant the hybrid would not be able to reproduce and therefore suffer a fitness disadvantage. Linn et al. suggested the hypothesis that inability to recognize host fruit volatiles may have been caused by conflicts in the hybrids' neural pathways that deal with preference and avoidance for a host plant (Linn et al. 2004). Taking into account the studies of preference and avoidance for natal and nonnatal fruit blends, Linn believed that the hybrids of the host races had conflicting neural recognition pathways. For example, a hybrid's pathway may have conflicts because genetically it both prefers and avoids the same fruit volatile, thereby leaving it with no preference at all. Taking on this idea, Olsson et al. performed a study analyzing olfactory receptor neuron (ORN) responses in F_1 hybrid *Rhagoletis*. In their study, electrophysiological analyses revealed considerable changes in hybrid ORN responses when compared to parent ORN responses (Olsson et al. 2006). This alteration in the receptor neuron could be a contributor to the reduced response of hybrids to host plant volatiles and thus be directly connected to both prezygotic and postzygotic isolation in connection with the sympatric speciation of *Rhagoletis pomonella.* It is interesting to note the evolution of the studies surrounding the sympatric speciation of *R. pomonella*, for what started off as a basic ecological study has since progressed into a neurobiological study concerning receptor neurons. There seems to be no end in sight for experiments and studies regarding this unique study system.

In conclusion, Guy Bush's observations and initial experiments on *Rhagoletis pomonella* lead to a vast array of future research. Various scientists studied host fidelity, the influence of host phenology, and fruit odor discrimination as possible prezygotic barriers that could serve as factors in sympatric speciation. Following these, Linn et al. and Olsonn et al. assessed fruit odor discrimination in F_1 hybrids in terms of a postzygotic barrier that could serve in reinforcing the sympatric speciation for *R. pomonella*. Looking back at Bush's model in reference to current data, it is clear that some of his ideas have been supported. For example, Dambroski and Feder's experiment on diapause variation supported Bush's idea that diapause and emergence times are ultimately under genetic control. Also, Linn et al.'s study on host fruit discrimination supported Bush's hypothesis that the initial orientation to a host plant is the result of a chemical clue. Furthermore, almost all of the studies discussed contribute to Bush's theory that host plant and mate selection are positively correlated in one way or another. Every study showed a direct tie and influence that selecting a host plant has on the life cycle of *R. pomonella.* Taken all together, *Rhagoletis pomonella* seems like a great example of a current species undergoing sympatric speciation.

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