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Sympatric speciation by allochrony?

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Sympatric speciation was once thought most improbable, but careful study of some systems, particularly the apple maggot (*Rhagoletis pomonella*) and related *Rhagoletis* species, has led to its reinstatement as a likely mode of speciation in some cases. Different species and host races in this clade of flies often have highly specialized host preference, and along with frequent evolutionary shifts to different fruit species between sister taxa, there is a likely effect of the timing of adult emergence that follows host fruiting phenology. This is known as "allochronic" isolation (from the Greek, meaning "different timing"). This overview covers recent discoveries by Inskeep et al. (2021) showing how allochrony is a major factor in preventing gene flow between a pair of sister species of *Rhagoletis* on different host fruits. Although the authors do not claim to prove sympatric speciation, it does seem very likely, and the work clearly underscores how readily host shifts via allochrony can aid sympatric speciation.

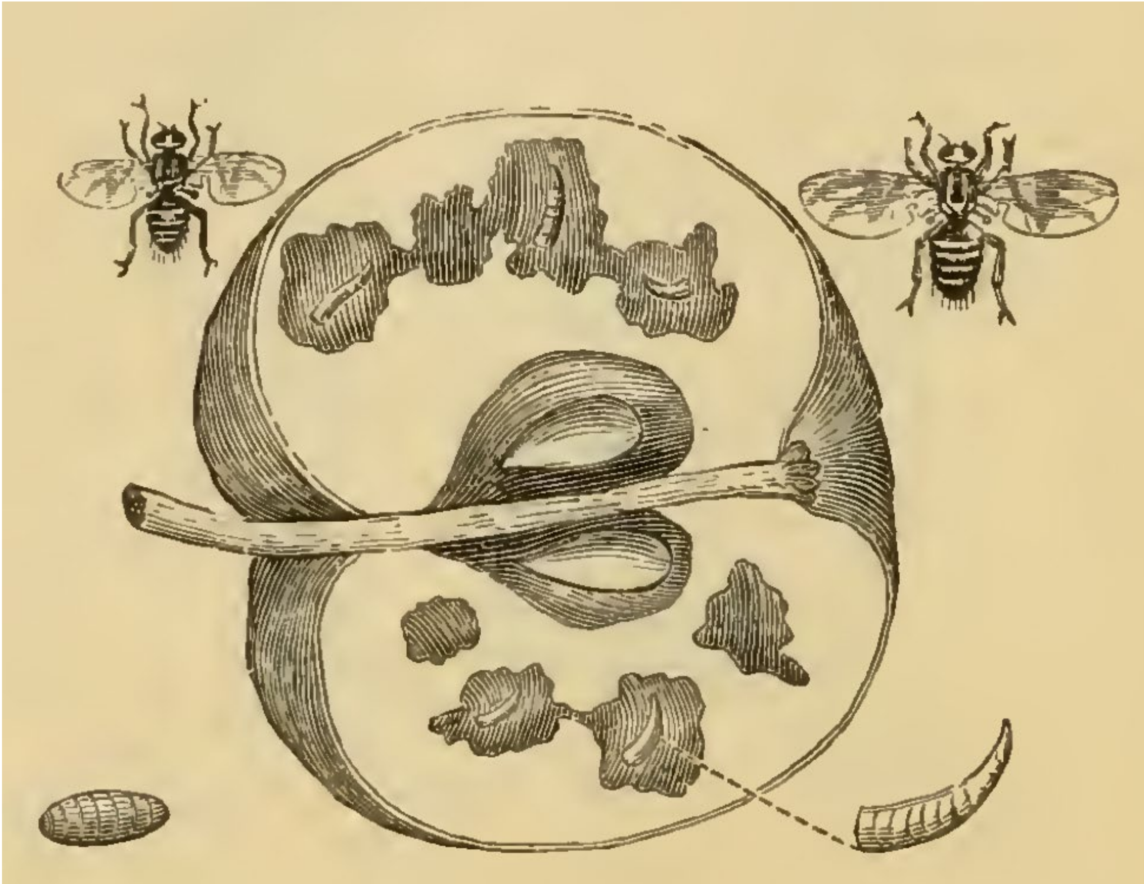

Fig. 1. The Apple Maggot (*Rhagoletis pomonella*) was described as a species when it began to infest apples in the mid-19th Century. From B.D. Walsh (1867).

It all seemed clear by the 1960s. The origin of new species, or speciation, was believed to require complete geographic isolation, or "allopatry." Gene flow was thought likely to swamp genetic divergence and prevent the evolution of reproductive isolation. Ernst Mayr in his monumental book (Mayr 1963), sought to explain away supposed cases of "sympatric" speciation (i.e. speciation in the face of gene flow) and sympatric ecological "races" either as cases of secondary contact after allopatric divergence, or merely due to phenotypic plasticity with no genetic divergence component. The paper we discuss here (Inskeep et al. 2021) provides a particularly clear example of a potential sympatric speciation event in the "true fruit fly" group, *Rhagoletis* (Tephritidae). First, however, it is worth briefly describing around 60 years of research into the genus to show why *Rhagoletis* has become a model for sympatric speciation.

In graduate school at Harvard in the early 1960s, the young Guy Bush took a course taught by Ernst Mayr and George Gaylord Simpson. For this course, he wrote a term paper on sympatric speciation, and following the doctrine of his day, argued it couldn't happen. However, while writing this essay he learned that the *Rhagoletis pomonella* group of true fruit flies had been implicated in sympatric speciation, and thus began the research that led to a large series of studies on *R. pomonella* group species and "host races", leading up to the paper reviewed here (Inskeep et al. 2021). Ernst Mayr became a member of Bush's PhD committee, and strongly encouraged him in his goal of carrying out a study of *Rhagoletis* systematics, because he thought that Bush would "put that unresolved example [of sympatric speciation] to rest once and for all" (Bush 1998).

After studying their behaviour in the field, Bush realized that *Rhagoletis* and other tephritid fruit flies mated on their host plant fruits, which come from diverse families of flowering plants. Males patrol territories on fruits of their host trees and wait for females to arrive. Bush intuited that a shift in host preference could thereby result in a mating bias among males and females with similar genetic preferences for new host fruit species, and so reproductive isolation could result as a by-product of a shift host plant choice. This form of pleiotropy is today argued, somewhat inappropriately, perhaps, to be a "magic trait" (Gavrilets 2004): if selection favours divergence in ecological preference, this leads "magically" to reproductive isolation due to mating between partners that have similar genetically based ecological preferences, because both males and females of the divergent form are liable to exhibit the same new ecological preference.

The Apple Maggot *Rhagoletis pomonella* is a North American fly species whose larvae natively feed on hawthorn fruit. It was first described as a species soon after it suddenly became a pest of apples in the mid 19th Century (Walsh 1867); see Fig. 1. Apples had been introduced to North America some 300 years earlier, but in the 19th Century, a wave of advance of this new pest of apples Westwards from the East coast was documented. Walsh was a correspondent of Darwin and was the first to propose that natural selection between "phytophagic varieties" might lead to the origin of new species. Bush realized that sympatric speciation was after all a likely hypothesis for this host shift. He was understandably nervous about advocating for such a "crackpot idea" in his dissertation, especially with Ernst Mayr on his committee. Luckily, he managed to procrastinate finishing his 1964 dissertation and submitted it when Mayr was out of town, so the defence of his PhD went unopposed. Later Bush went to the Evolution meetings to give a presentation on his work with *Rhagoletis* only to find that Theodosius Dobzhansky, another opponent of sympatric speciation, was chairing the session in which he was speaking. After the talk, nobody made any comments, except for Dobzhansky who told him: "Sympatric speciation is like the measles; everyone gets it, and we all get over it!" (Bush 1998).

The history of the local host switch to apple and wave of advance showed it clearly happened in sympatry, but there was still little evidence for the switch to apple being genetic rather than merely phenotypic. Bush, followed by his students and ex-students (led particularly by Jeff Feder and Stewart Berlocher), then their students and postdocs, have since studied this system further. At the University of Texas, Bush collaborated with a chemist, Barrie Kitto, to look for differences in allozymes between the apple and hawthorn host races, but most of the enzymes were polymorphic in both, and so the effort seemed a failure. Bush actually exhorted Feder not to use allozymes (Bush 1998), but Feder disobeyed his supervisor. By increasing sample sizes he showed clear allele frequency differences between the host races. Bruce McPheron, D.C. Smith, and Berlocher, who was by then a professor at University of Illinois, made similar findings at the same time, and three papers were then published back to back in Nature proving genetic differences (Feder et al. 1988; McPheron et al. 1988; Smith 1988). After this breakthrough many other papers were published documenting in great detail the hawthorn/apple switch. In particular, it became obvious that as well as showing genetic differences in host preference, the two host races also were isolated in time, with adults of the apple race having a genetic predisposition to emerge a few weeks earlier than the hawthorn race, in accordance with the different times of fruiting of their hosts. Thus, the magic trait of host switch also involved some allochronic isolation between the host races.

However, even by the time of Bush's original thesis work, it was known that many other sibling species or host races existed in the *R. pomonella* group feeding on a number of other native fruit species (Bush 1966). Most of these species are at least partially sympatric in Eastern forests of North America, suggesting that sympatric host switches similar to that in the hawthorn/apple switch likely occurred multiple times to trigger the rapid radiation of these natural host races and species (Berlocher 1998; Powell et al. 2013). The current paper is on a host switch leading to one such example involving the "sparkleberry fly," which feeds on *Vaccinium arboreum*. This represents one of the first papers published on *Rhagoletis* that uses DNA data resequenced from across the genome, in this case reduced representation RADSeq data (Inskeep et al. 2021).

In the published version of his PhD thesis, Bush noted some slight morphological differences, but had included the sparkleberry fly within the species *Rhagoletis mendax* which normally feeds on various species of blueberry and deerberry (like the sparkleberry they are also members of the genus *Vaccinium*). *Rhagoletis mendax* (the Latin name means mendacious, which presumably refers to its cryptic morphological status) is itself closely related to *R. pomonella* but just distinct enough to have been recognized as a separate species (Bush 1966). A sister relationship of sparkleberry flies with blueberry/deerberry-feeding *R. mendax* is confirmed here using current genomic evidence based both on concatenated data and coalescent-based species tree phylogenetic reconstructions. Genetic differences between the two forms are conserved in sympatry as well as in allopatric populations (Inskeep et al. 2021). Whereas sparkleberry is found only in the Southern states, where it occurs in sympatry with blueberry- and deerberry-feeding *R. mendax*, blueberries and *R. mendax* are also found Northwards all over Eastern North America and into Canada.

Was this an example of allochronic isolation? Yes, almost certainly. The sparkleberry fruits much later in the fall and winter than the highbush blueberries, which are late summer and early fall fruiters. Infested fruits were collected from the field from across the ranges of these species, and controlled experiments reported here investigated the emergence of flies the following year. The sparkleberry flies track their host phenology by emerging around two months later than blueberry flies, even from sites in sympatry. Since there is no overlap of emergence dates there is therefore no opportunity for gene flow. This finding demonstrating a genetic predisposition to different phenology parallels an earlier study documenting a three-month difference in eclosion times in field data for the same species (Payne and Berlocher 1995). In contrast, the three-week allochrony between apple and hawthorn host races of *R. pomonella*, and the thirty day difference in emergence between the hawthorn race and flowering dogwood fly are weaker barriers, and there is concomitantly more evidence for gene flow: 4-6% and ~1% per generation, respectively.

Does the allochronic isolation found here lead to an absence of gene flow? Were there any hybrids between the two? Initially, data suggested some genetic intermediates with *R. mendax* on sparkleberry hosts. However, after careful reanalysis of these putative intermediates, together with sequence data from a third species, the putative intermediates were found most likely to consist of two F1 hybrids between the sparkleberry fly and a more distant species, the "flowering dogwood fly," rather than with *R. mendax*. In addition, two individuals of apparently nearly pure flowering dogwood flies were found feeding on sparkleberry. Unlike blueberry flies, flowering dogwood flies emerge late in the fall and overlap broadly in areas of sympatry with the emergence times of sparkleberry flies (in this study, the sympatric site collected for emergence was Kentucky). The flowering dogwood fly is sister to the apple and hawthorn flies in the species *R. pomonella*, and is thus more distantly related to *R. mendax* than the sparkleberry fly. It is unclear if the hybrids between dogwood and sparkleberry flies ever lead to longer term introgression, but other hybrids or apparent intermediates in Arkansas and Alabama suggest gene flow would be possible. Nonetheless, the two species are undoubtedly stable to this potential for gene flow, with phylogenetic and genetic evidence for genetic differences being maintained across their combined range.

Following earlier papers by some of these authors, Inskeep et al. summarize their paper with a likely history for this radiation. The most genetically divergent flies treated here are hawthorn feeders from the Eastern and Western sides of the Sierra Madre mountains in Mexico. Earlier genetic evidence based on rather few sequenced loci suggested that large inversions on several chromosomes originated allopatrically in these Mexican populations, and that these inversions may harbour differences that led to differences in eclosion time due to different responses to daylength (Feder et al. 2003; Xie et al. 2008). These forms, it was proposed, then spread Northwards into the United States during the Pleistocene, where they hybridized before radiating onto different fruit species in part via allochronic evolution. The hybridization could have led to the inversion polymorphisms present in the North American *R. pomonella* flies and provided greater ecological flexibility, allowing shifts in host choice and emergence timing. Hybridization of ancestral species has been observed to be associated with rapid adaptive radiations in other systems, for instance cichlid fish in Lake Victoria (Meier et al. 2017). The switches from hawthorn to snowberry (*Rhagoletis zephyria*), blueberry and dogwood, and from blueberry to sparkleberry would, like the hawthorn to apple shift, represent examples of these adaptive shifts.

However, the authors are rightly somewhat hesitant about this history, which depends on inferring ancestral host plants and areas of species origin from current host plants, geographic distribution, and phylogeny. Inferring ancestral character states is a notoriously difficult problem in comparative analysis, especially when sister species rarely share the same states (Schluter et al. 1997), as is true here for host plants. This problem is greatly exacerbated when there is gene flow among the taxa (Mallet et al. 2016), which again is likely here. It is not even clear to us that hawthorn was the ancestral host. The North American species *Rhagoletis cornivora* is even more distantly related to the *pomonella* clade studied here than the Mexican "*R. pomonella*" assumed to be ancestral to this ingroup (Xie et al. 2008), and as its name implies, feeds on dogwoods (*Cornus* spp.). Thus it is possible that the original hosts of the common ancestor were dogwoods, not hawthorns, with the *R. pomonella*-like flowering dogwood fly expressing an ancestral, rather than a derived host plant preference. Similarly, *R. mendax* may have evolved to feed on blueberries from a sparkleberry feeding ancestor, rather than vice versa.

Nonetheless, it is clear that *R. mendax* and the sparkleberry fly are sisters and that the larval host shift required a switch in timing that must have greatly reduced gene flow in sympatry. Of course, these changes may have involved a period of allopatry and the authors did not claim to prove sympatric speciation. Yet because allochrony alone can provide such a powerful source of genetic isolation, there doesn't seem a much greater likelihood of speciation in allopatry than in sympatry. Given the full current sympatry of the sparkleberry fly with *R. mendax*, prior allopatry seems an unnecessary hypothesis. The work also highlights an aspect of rapid evolutionary radiations that is repeatedly observed in a number of different systems: adaptive divergence is often followed by convergent evolution leading to renewed hybridization among related species, which may itself further fuel more adaptive radiation by continuing to provide recombinant variation (Gillespie et al. 2020).

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