The Genetics and Ecology of Sympatric Speciation: A Case Study

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Abstract. Mating occurs on the larval host plant in all Rhagoletis species (Diptera: Tephritidae). We show how this attribute, when coupled with certain differences in other biological traits, strongly influences the mode of speciation. In species of the suavis species group, host shifts have never occurred during speciation, and larvae feed on the husks of any walnut species (Juglans spp.), which are highly toxic. Taxa are allopatric or parapatric and exhibit deep phylogenetic nodes suggesting relatively ancient speciation events. Traits responsible for species and mate recognition, particularly in parapatric species, are morphologically distinct and strongly sexually dimorphic. All aspects of their biology, genetics, and distribution are consistent with a slow rate of allopatric speciation followed by morphological divergence in secondary contact. In contrast, speciation in the pomonella species group has always involved a shift to a new, usually unrelated, non-toxic host, and all taxa within these groups are sympatric, monophagous and morphologically indistinguishable from one another. Phylogenetic nodes are very shallow, indicating recent sympatric speciation. Sympatric divergence is promoted by genetic variation which allows a portion of the original species to shift to a new habitat or host. Evidence suggests that changes in a few key loci responsible for host selection and fitness on a new host may initiate host shifts. By exploiting different habitats, competition for resources between diverging populations is reduced or avoided. We provide evidence that in phytophagous and parasitic insects sufficient intrinsic barriers to gene flow can evolve between sister populations as they adapt to different habitats or hosts to allow each population to establish independent evolutionary lineages in sympatry.

Key words: allopatric speciation, host race, phylogeny, Rhagoletis, speciation rates, sympatric speciation.

Introduction

Ever since Charles Darwin (1859) proposed that new species could arise by ecological specialization in the absence of geographic barriers, the likelihood of sympatric or ecological speciation has been hotly debated. As the Modern Synthesis gained conceptual dominance during the mid-twentieth century, a consensus emerged among evolutionary biologists that sympatric speciation, at least in sexually reproducing animals, rarely, if ever, occurred. Prominent leaders in the field such as Ernst Mayr (1947, 1982) and Hampton Carson (1975, 1989) forcefully promoted the widely accepted view that reproductive isolation between populations can arise only during periods of geographic isolation through the accumulation of many mutations each of small effect. Studies over the past 20 years on natural populations, however, indicate that sympatric speciation may be far more common and widespread among both plants (Grant 1985; Davies 1993) and animals (Howard and Berlocher in press) than previously realized. Well-documented examples in animals include insects and other invertebrates (Tauber and Tauber 1989; Bush 1994), fish (Schliewen et al. 1994; Schluter 1996), and even birds (Smith et al. 1997). Recent theoretical treatments also indicate that biologically reasonable intensities of divergent selection and penetration can result in non-allopatric ecological speciation (for references see Rosenzweig 1995; Johnson et al. 1996).

Research on natural sister populations, either in the process of divergence or having recently completed ecological speciation, also do not support the view that speciation invariably requires the accumulation of many small genetic changes (Peder et al. 1994; Bradshaw et al. 1995; Buckley et al. 1997; Grant and Grant 1997; McMillan et al. 1999).
Recent findings (Dorweiler et al. 1993; Orr 1993; Bradshaw et al. 1995; Fry et al. 1996; Shoemaker and Ross 1996) support our long-held working hypothesis that genes at relatively few key loci affecting mate recognition, habitat choice and fitness in alternative habitats can be sufficient to initiate the process of ecological race formation and speciation (Bush 1969b, 1975, 1982; Bush and Smith 1997).

Several factors have contributed to our failure to appreciate the important role of such gene changes in the early stages of speciation. Until recently most research focused on the genetic basis of post-mating sterility and genome incompatibility (Wu and Palopoli 1994). These comparative studies generally dealt with taxa which had speciated so long in the past or which were not even sister species that it was impossible to identify which genes were involved in the speciation event and which had accumulated after speciation. Few studies dealt with taxa actually in the process of speciation. Such studies measure the genetics of species differences, but do not identify genes responsible for speciation (Templeton 1981). Because genetic differences between most species are large, these investigations left the impression that speciation required the accumulation of many gene changes.

Emphasis on the search for the genetic basis of post-mating isolation was also fostered by the assumption that any gene flow between populations, no matter how small, would inhibit divergence. As emphasized by Mayr (1963) traits responsible for pre-mating isolation are seldom perfect, particularly during the initial stages of divergence. Early investigators, therefore, assumed that the strength of divergent selection could not be sufficient to overcome gene flow in sympatry. Consequently, speciation must occur allopatrically. A general consensus emerged that temporal, ecological and ethological factors responsible for pre-mating isolation evolved simply as by-products of evolutionary events during periods of geographic isolation (Mayr 1988). Thus, a systematic search for genes involved in pre-zygotic reproductive isolation (e.g. mate recognition and habitat preference) has not been conducted.

Population genetic models such as those of Felekstein (1981) strengthened the view that gene flow between populations adapting to different habitats would inhibit divergence. These studies led to the conclusion that conditions for sympatric speciation were so restrictive that they were unlikely to be encountered in nature. Furthermore, related work indicated that reinforcement of character differences involved in premating isolation is not likely to occur in nature. Unfortunately, laboratory experiments such as the recent study of reinforcement by Hosten (1997), and theoretical treatments which find little evidence for reinforcement, do not factor in genetic variation in habitat preference and habitat associated mating. When such variation is considered, the conditions for reinforcement are reasonably met in nature (Schlüter 1994; Bull and Possingham 1995; Johnson et al. 1996). These studies indicate that divergent ecological selection pressures can be a primary cause of race formation and speciation in sympatry, and that ecological speciation in sympatry tends to occur much more rapidly than speciation due to drift and founder events in allopatric populations.

The species problem and host races

Contributing to the controversy surrounding the plausibility of sympatric speciation is the lack of agreement as to what constitutes a species. Proponents of the Modern Synthesis, particularly those dealing with animals, rely primarily on some form of the biological species concept (BSC) proposed by Mayr (1963). Two taxa are considered to be separate species if they are reproductively isolated from one another and incapable of exchanging genes. If gene flow occurs between populations they are generally not considered species. Although this definition appears to offer a clear cut dichotomy, it is biologically misleading and operationally impractical. The BSC cannot be logically applied to taxa that reproduce asexually or to allopatric populations. The existence of reproductive isolation can only be determined in nature when taxa are sympatric. Although artificial hybridization experiments which reveal sterility between allopatric taxa clearly indicate that they have reached species level, it is unclear how to treat taxa if little or no sterility exists. Finally, it is usually impractical to conduct such tests on most taxa, and one is left to speculate as to how a given pair of taxa would behave if contact was established naturally. This inability to treat allopatric populations objectively is a limitation inherent in all species concepts and should be acknowledged.

A more serious limitation of the BSC is that it cannot be applied rationally to sympatric taxa which maintain distinct phenotypic and genotypic differences despite a limited amount of gene flow through interbreeding. Recent molecular studies on closely related sympatric taxa recognized as distinct, closely related sister species (the products of a single speciation event) reveal that gene flow between them may still occur (Feder et al. 1995; Taylor et al. 1997). Such neospecies have acquired sufficient genetically based differences to maintain independent genotypes and distinct phenotypes, yet are still capable of sustaining some gene flow. These are the kind of taxa most likely to provide insight into the number and kind of genes involved in the speciation process rather than species which have long been isolated and show strong post-mating reproductive isolation.

The recently proposed species concept of Mallet (1995a) is less assumption laden and avoids the circularity, incon-
sistencies, untestability and essentialistic nature of the biological species concept and other elaborate, theory laden species concepts. Mallet has proposed that sister species be recognized only when they maintain distinctly different sympatric genotypic clusters, or in cladistic terms, sympatric clusters of genetic apomorphies (Dover 1995). Defining a species on the basis of genotypic clusters not only provides a useful operational species definition, but, as emphasized by Mallet (1995b), such clusters represent the level of differentiation most interesting to those studying speciation. However, as in the case of the BSC, without a "test of sympathy" this definition can only be applied subjectively to geographically isolated sister populations.

Generally sympatric sister taxa that maintain distinct genotypic clusters exploit different habitats or hosts. However, in several well documented examples where an invertebrate has recently established a sympatric population on a new host or habitat, the genetic differences maintained between sister taxa are small (Voutilhe et al. 1988; Abe 1991; Stanhope et al. 1992, 1993; Bush 1993b; Goldson and Mackenzie 1994; Mackenzie and Goldson 1994; Mackenzie 1996). Do such populations represent species or races? In many respects they behave like species: they mate assortatively; they utilize different habitats; and, more importantly, they maintain genetically distinct sympatric genotypes.

In the case of phytophagous insects such as Rhagoletis, the difference between a host race and a species appears to be in the degree of genetic divergence. Differences between taxa in the early stages of speciation are probably limited to relatively few key loci responsible for adaptation to alternative hosts, such as host and habitat preference, mate choice and fitness. Unfortunately, we have little information on the number or kinds of genetic changes occurring during the initial phase of speciation in the examples cited above. However, these sympatric populations adapting to different hosts or habitats do maintain significantly different gene frequencies at several loci in both immature and adult stages over generations. Such loci in Rhagoletis appear to be closely linked, or may directly be responsible for, adaptation to the host and host preference (Feder et al. 1994).

A case study

In the following section, we compare two Rhagoletis species groups. Members of the Rhagoletis pomonella species group are hypothesized to have diverged sympatrically in the course of ecological colonization and adaptation of new hosts. In contrast, speciation in the Rhagoletis suavis species group occurred allopatrically in the absence of a host shift. Molecular phylogenies are used to infer rates and patterns of species divergence in the two species groups. These rates and patterns are then compared to the context of observed biological differences between the pomonella and suavis species groups. Such comparisons provide insight into how the ability to shift to new hosts during the course of speciation affects the rate and mode of speciation in these flies.

Previous studies on phytophagous insects and habitat specialists indicate that animals which speciate sympatrically differ predictably in certain key attributes from those that speciate allopatrically (Bush and Howard 1986). Among these are differences in the taxonomic status of taxa within the species groups, the geographic distributions of taxa, the ability of taxa to exploit a novel host plant environment, and the rate of genetic divergence between taxa.

For example, in species groups in which non-allopatric (parapatric and sympatric) speciation has been the predominant mode, we expect to find: 1) sympatric or overlapping host or habitat races or closely related sister species; 2) pre-mating reproductive isolation between sympatric ecological races, with little or no post-mating incompatibility; 3) occupation of different habitats or hosts by sympatric ecological races and sister species; 4) little or no geographic variation in morphological characters separating closely related ecological races or sister species; 5) relatively rapid rates of ecological race formation and speciation; 6) parallel (multiple origin) ecological speciation, with pre-mating reproductive isolation evolving independently between host or habitat species as they adapt to similar, but ecologically equivalent areas (Scheffer 1996); and 7) a molecular signature where closely related sister species are more closely related to certain populations of the "parent species" than some populations of the parent species are to each other.

On the other hand, in species groups where allopatric speciation has been the predominant mode, we expect to find: 1) sister species with non-overlapping geographic ranges; 2) post-mating reproductive isolation between allopatric sister species, and reduced fertility between the geographic races of one species; 3) allopatric races and sister species that utilize the same host or habitat; 4) geographic variation in morphological characters separating closely related sister species; 5) relatively slow rates of geographic race formation and speciation; 6) no parallel speciation, with allopatric sister species showing no coherent pattern of divergence as described in 6 above; and 7) a molecular signature where all sister populations of a species form a single, phylogenetically distinct branch from all populations of the "parent species".

These predictions obviously do not pertain to instances of coevolution where a parasite speciates with its host, or speciation resulting from hybridization between previously isolated species. These have their own unique attributes
which will not be considered here.

The biology of Rhagoletis

The larvae of all Rhagoletis species infest the fleshy fruit of a wide range of monocots, dicots and gymnosperms. For example, the apple maggot fly, R. pomonella, infests apple (Malus pumila; Rosaceae), R. persimilis infests fairy bell fruit (Desporum trachycarpum; Liliaceae), R. juniperina infests juniper berries (Juniperus virginiana; Cupres-saceae) and R. striatella infests husk tomato (Physalis sp.) (Smith and Bush 1997). Most species are univoltine although some have a facultative diapause and may produce a small second generation under certain environmental conditions (Boller et al. 1976). In temperate North America, adult eclosion of each species is precisely timed to coincide with the period of host fruit maturation most suitable for oviposition. Closely related sympatric taxa are often allochronically isolated or nearly so. Oviposition generally occurs from five to twelve days after eclosion depending on the species and environmental conditions. A key feature of all Rhagoletis species is that mating occurs on or very near the host fruit which serves as a rendezvous for courtship and mating. Males will patrol a fruit for hours and court any female that arrives for mating or oviposition. Courtship involves the display of distinct wing patterns and use of short range or contact pheromones. Host and mate choice are therefore tightly linked, a common feature in the mating behavior of many parasites and other habitat specific organisms. Host selection is mediated by both visual and olfactory cues. Ultimately, selection of a host for mating and oviposition is determined by specific chemical cues emanating from the host plant and their fruits (Prokopy et al. 1987). Changes in host perception can have a direct effect on mate choice.

Distribution, morphological divergence and contrasting patterns of host relationships

The genus Rhagoletis is distributed throughout the Palaearctic, Nearctic and Neotropical regions which represent the three major centers of endemism in the genus (Bush 1966). Species groups within Rhagoletis exhibit two contrasting patterns of host plant utilization and geographic distribution (Bush 1969a). The first is represented by the R. pomonella species group. Each of the six well characterized sibling species, and several recently discovered taxa whose status is yet to be determined, infest different host plant species (Table 1). This includes the genetically distinct apple host race which established itself as a major pest of introduced apples (Malus pumila) under sympatric conditions from native flies infesting hawthorns (Crataegus) in eastern North America about 150 years ago (Feder et al. 1988; Bush et al. 1989; Bush 1993b). All sympatric pairs of sister taxa in this group infest the fruits of different host plants in different genera and sometimes hosts in different families. With minor exceptions, all are morphologically indistinguishable. All except two taxa are also broadly sympatric in eastern North America (Figs. 1 and 2). The exceptions are R. sepulchralis, which extends across North America (Fig. 1), and a little studied geographically isolated Mexican population infesting Crataegus mexicana (Fig. 1). This pattern of sympatry between morphologically similar sister species and host races is typical of several other Rhagoletis species groups in all three biogeographic regions where this genus is represented.

The second, less common pattern is displayed by the suavis group whose six species indiscriminately infest any species of walnut in the genus Juglans (Table 1; Fig. 3). Although flies occasionally infest apricots and peaches growing near heavily infested walnuts, these flies have never established permanent populations on such fruits. Unlike members of the pomonella group, all species in the suavis species group are morphologically very distinct and sexually dimorphic in wing patterns and body coloration used in species recognition and courtship displays. Species in the R. suavis group are either allopatric or, when parapatric, are allochronically isolated, are restricted to different altitudes and have very different body coloration. For example, in Arizona where they co-exist parapatrically, Rhagoletis juglandis is yellow and occurs mostly below 2,000 m while R. boycei is completely black and is found above 1,500 m.

Rates of speciation

The two contrasting patterns of host utilization and distribution appear to have had a profound effect on the rate at which species are generated in these two species groups. Even though the number of described species in the pomonella and suavis species groups is roughly the same, the time frame in which these species have arisen is radically different. This rate difference is readily seen by visually comparing the branching patterns within the pomonella and suavis species groups in the phylogenetic tree shown in Fig. 4.

Figure 4 shows the relationships of mitochondrial cytochrome oxidase II (COII) sequences from 5 of the 6 species in the suavis species group (Table 1). Using Brower's (1994) estimate of 2.3% sequence divergence per million years (1.1–1.2% per lineage) for silent sites in arthropod mtDNA, we estimate that these five suavis group species shared a common ancestor approximately 2 MYA (4.37% COII divergence for R. suavis and R.
### Table 1. Host plant use and geographic ranges of species in the *Rhagoletis pomonella* and *R. suavis* species groups.

<table>
<thead>
<tr>
<th>Species</th>
<th>Status</th>
<th>Host plant(s)</th>
<th>HP Family</th>
<th>Distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. pomonella</strong> Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pomonella</td>
<td>s</td>
<td><em>Crataegus brainerdii, C. brachycarpa,</em> <em>C. crus-gali,</em> <em>C. flabelata,</em> <em>C. flaevus; C. greggiana, C. holmesiana; C. lavina, C. macrosporum, C. marshallii, C. mollis, C. pedicellata, C. pruinosa, C. punctata,</em> <em>C. viridis, C. texana,</em> <em>(C. douglasi, C. monogyna)</em></td>
<td>Rosaceae</td>
<td>E NA</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>nr. pomonella</td>
<td>hr/ns?</td>
<td><em>Malus pumila</em></td>
<td>Rosaceae</td>
<td>(W NA)</td>
<td>4</td>
</tr>
<tr>
<td>nr. pomonella*</td>
<td>hr/ns?</td>
<td><em>Crateagus specios</em></td>
<td>Rosaceae</td>
<td>SC NA</td>
<td>5</td>
</tr>
<tr>
<td>nr. pomonella*</td>
<td>gr/ns?</td>
<td><em>Crateagus mexicana</em></td>
<td>Rosaceae</td>
<td>C MEX</td>
<td>2</td>
</tr>
<tr>
<td>nr. pomonella*</td>
<td>hr</td>
<td><em>Malus pumila</em></td>
<td>Rosaceae</td>
<td>C MEX</td>
<td>1</td>
</tr>
<tr>
<td>nr. pomonella*</td>
<td>hr</td>
<td><em>Prunus cerasus</em></td>
<td>Rosaceae</td>
<td>[NC, W NA]</td>
<td>6</td>
</tr>
<tr>
<td>nr. pomonella*</td>
<td>hr/ns?</td>
<td><em>Prunus englesi, P. umbilata</em></td>
<td>Rosaceae</td>
<td>SE NA</td>
<td>1</td>
</tr>
<tr>
<td>nr. pomonella*</td>
<td>hr</td>
<td><em>Rosa rugosa</em></td>
<td>Rosaceae</td>
<td>[NE NA]</td>
<td>7</td>
</tr>
<tr>
<td>zephyria</td>
<td>s</td>
<td><em>Symphoricarpos albus var. albus,</em> <em>Symphoricarpos albus var. laevisatus,</em> <em>Symphoricarpos occidentalis.</em></td>
<td>Rosaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mendax</td>
<td>s</td>
<td><em>Vaccinium ashei, V. angustifolium, V. corymbosum, V. stamineum,</em> <em>Gay schwarzi, G. froidoisa.</em></td>
<td>Ericaceae</td>
<td>E NA</td>
<td>9</td>
</tr>
<tr>
<td>nr. pomonella</td>
<td>ns</td>
<td><em>Vaccinium arboreum</em></td>
<td>Ericaceae</td>
<td>SE NA</td>
<td>10</td>
</tr>
<tr>
<td>carnivora</td>
<td>s</td>
<td><em>Cornus obliqua, C. stolonifera,</em></td>
<td>Cornaceae</td>
<td>NE NA</td>
<td>1</td>
</tr>
<tr>
<td>nr. mendax</td>
<td>ns</td>
<td><em>Cornus florida</em></td>
<td>Cornaceae</td>
<td>E NA</td>
<td>11</td>
</tr>
</tbody>
</table>

| **R. suavis** Group |        |                                                                                |             |              |           |
| suavis            | s      | *Juglans nigra, J. cinerea, [J. regia], J. sieboldiana*                        | Juglandaceae| E NA-C NA    | 1         |
| complete          | s      | *Juglans nigra, J. microcarpa,* *J. major, [J. regia],* *(J. caifornica, J. hindii, [J. regia]).* | Juglandaceae| C NA        | 12        |
| juglandes         | s      | *Juglans major, [J. regia].*                                                   | Juglandaceae| (W NA)      | 13        |
| boycei            | s      | *Juglans major, [J. regia].*                                                   | Juglandaceae| AR, NM-NC MEX| 12        |
| soqui             | s      | *Juglans mollis*                                                              | Juglandaceae| AR, NM-NC MEX| 12        |
| ramosae*          | s      | *J. major var. glabrata = J. mollis?*                                           | Juglandaceae| EC MEX      | 12        |

*Not included in Fig. 1.*

*gr = geographic race, hr = host race, ns = new species, s = species, | = plant introduced, ( ) = fly introduced.*


**boycei**). Including *R. ramosae* in the calculation, we estimate that six species have evolved in the *suavis* group over the last 2 MY, giving an approximate average speciation rate of one new species every 320,000 years.

Figure 4 also shows the COI relationships of 10 of the 14 taxa in the *pomonella* group (Table 1). Arguments similar to those used above allow us to estimate that the ten *pomonella* group taxa shown in Fig. 4 shared a
common ancestor approximately 2.6 MYA (6.1% divergence for *R. cornivora* and *R. pomonella* from *Crataegus viridis*). Thus, over the past approximately 2.6 MY, 10 of the 14 taxa in the *pomonella* group have evolved. Including the 4 *pomonella* group taxa not included in Fig. 4 in the calculation (these four taxa have almost certainly arisen since the divergence of *R. cornivora*), the overall average rate of speciation in the *pomonella* group, in the same time period considered for the *suavis* group species, is estimated to be approximately one new species every 180,000 years, or almost two times faster.

To make the comparisons more meaningful however, we need to consider rates of divergence in smaller time intervals, as opposed to examining only overall average rates of divergence. Eight of the ten *pomonella* group taxa in Fig. 4 form a tightly-knit cluster with a maximum sequence divergence (uncorrected) within the clade of 0.87%. We estimate the time to common ancestry of this clade as approximately 400,000 years. Thus, for the past 400,000 years, new taxa in the *pomonella* group have been generated at an average rate of 1 every 50,000 years. This is more than six times faster than the average rate observed in the *suavis* group.

We realize that the methods used in the above analysis provide at best a crude approximation of speciation rates. However, it was not our intent here to carry out a rigorous analysis of the differences in the patterns of diversity observed in the *pomonella* and *suavis* groups. This would require consideration of many factors, including extinction rates, and also taking into consideration the fact that
not all of the *pomonella* group taxa listed in Table 1 are described species (the species status of many of these taxa is not certain). We did, however, attempt to provide rough estimates that would allow us to contrast the topological differences in the phylogenies of these species groups that are evident from examination of Fig. 4.

**Host shifts and the mode of speciation**

Why are the rates of speciation so different between the *R. pomonella* and *R. suavis* groups? Two elements, the ability to shift hosts and differences in their modes of speciation, appear to account for the rate differences. In the *pomonella* species group speciation has always been accompanied by a shift to a new host, and there is abundant evidence that these shifts occurred sympatrically (Bush 1993b; Bush and Smith 1997). The colonization of apples (*Malus pumila*) by *Rhagoletis pomonella* from its native host *Craetaegus*, provides an example of how such sympatric shifts occur (Bush 1969b; Feder et al. 1988, 1989; Bush et al. 1989; Feder and Bush 1989).

Apples, which were introduced to New England from Europe in the early 17th century, remained free of attack by *Rhagoletis* until about 1860. In that year a farmer in the Hudson River Valley of New York reported his apple crop destroyed by an infestation of fly maggots. The fly was soon identified as *R. pomonella* and was also found infesting *Craetaegus* throughout eastern North America. Although exactly where and when *R. pomonella* first established the first population on apple is unknown, it is clear that the event occurred at a site where *Craetaegus* was
Fig. 3. The distribution of six *Rhagoletis suavis* group species including *R. boycei*, *R. completa*, *R. juglandis*, *R. ramosae*, *R. suavis*, and *R. zoqui*.

Growing within the flight range of apples.

Several observations support this conclusion (Bush et al. 1989). The Hudson River Valley and adjacent regions in New York, Pennsylvania, Massachusetts and Connecticut lie near the center rather than at the periphery of the abundance of both *Rhagoletis pomonella* and *Craeaeus* infested by these flies (Phipps 1983). Native crab apples are high in phenolic content and have never been found infested so did not serve as the original host for the apple infesting race. Because adult *Rhagoletis* can also travel several kilometers in a 24 hour period in search of food, oviposition sites and mates (Boller and Prokopy 1976), it is unlikely that an apple tree would remain sufficiently isolated for any evolutionarily significant length of time without a visit from this fly. Colonization is most likely to occur when apples and haws grow in close proximity. As we have shown elsewhere (Bush 1993a; Johnson et al. 1996), this would allow divergent selection to act on loci controlling traits important in adaptation to a new host such as host seeking and preference behaviors as well as those genes responsible for fitness.

The apple and haw races have already diverged significantly in these traits. Not only do they maintain genetically distinct populations (Feder et al. 1988; McPherson et al. 1988) but they also show habitat specific mating preferences (Feder et al. 1994), differences in eclosion times (Smith 1988b; Feder et al. 1994), and host-associated fitness trade-offs associated with developmental rates and larval survival (Feder 1998). These factors are important in coordinating life-history events with host phenology.
and other environmental conditions experienced by the two races. Such fine adjustments necessary for survival are difficult to identify and measure which may explain why they have been ignored in searches for host-associated trade-offs in other insects (Rauscher 1992).

One factor, phenological differences in fruit maturation, stands out as a major selective force promoting divergence between populations adapting to different hosts. Feder (1998) has shown that adapting to apple, which fruits earlier than hawthorn, required an acceleration in the developmental rate of the apple population in order to synchronize eclosion time with the maturation of its apple host fruit. Advancing the time of eclosion, which is under genetic control (Smith 1988b), was accompanied by rapid changes in a suite of other characters which allowed the apple fly to adapt to the higher temperatures experienced earlier in the summer (Feder 1998). Similar shifts in eclosion times by R. pomonella are found in other recently established host races. For instance, a highly localized cherry race which eclosed 2–3 weeks before apple flies, developed on Prunus cerasus (Table 1) in Door County, Wisconsin, probably in the 1960s (Bush 1974). In Utah about 1983, a second cherry race evolved independently on P. cerasus which emerges well before local hawthorn flies infesting native Crataegus douglasii (Jorgensen et al. 1986; Messina 1989). A similar case of allochronic host race formation is the host race of R. pomonella infesting Crataegus opaca in East Texas and Louisiana. This species fruits in April and May and is sympatric with other Crataegus species which normally fruit in September and October. We have yet to undertake long term detailed biological and genetic studies on these taxa. However, if they have responded to the colonization of a new host in a similar way to the pattern of divergence found in the apple race, many of these taxa are either species or well developed host races. Finally, the eclosion time of a recently discovered Rhagoletis species infesting the tree-like Vaccinium arborescens in the southeast U.S.A. does not overlap with its closely related sympatric sister species, R. mendax, from which it is genetically distinct (Fig. 4).

As emphasized earlier, the status of several pomonella group taxa listed in Table 1 is yet to be resolved. As for
the majority of other _pomonella_ group taxa listed in Table 1, evidence supports the view that they also have evolved in sympatry by colonizing a new host plant (Bush et al. 1989; Feder et al. 1995, 1998; Bush and Smith 1997). With the exception of Mexican _pomonella_, all are sympatric in eastern North America (Figs. 1 and 2) and all sister species and host races utilize different hosts. As indicated by their close genetic relationships, the majority have speciated or established host races very recently.

The case of the Mexican population of _R. pomonella_ which diverged in geographic isolation presents an interesting perspective on the species problem. Although this _Crataegus_ infesting population has diverged considerably at the genetic level from the population of _pomonella_ on _Crataegus_ in the northeast (Fig. 4), the two populations differ only slightly in a minor morphological trait. It is not clear if the Mexican population represents a distinct species or simply a geographic race. Like _R. pomonella_ in eastern North America, Mexican _pomonella_ infests hawthorn (_Crataegus mexicana_), uses this same host for mating, and has independently colonized apples introduced in the 16th century by the Spanish over 100 years before apples reached New England (Bush 1964). If the northeastern and Mexican populations establish contact by range expansion, would they fuse into one freely interbreeding species or, because they are close ecological equivalents, would they establish a narrow parapatric hybrid zone? Should Mexican _pomonella_ be recognized as a distinct species or simply a genetically distinct geographic race? As we have been unable to carry out hybridization experiments between these taxa, it is a subjective call. If these populations have developed sufficient post-mating incompatibility they may remain distinct where parapatric. If not, they may fuse as they both mate on and infest the fruits of _Crataegus._

While speciation is invariably accompanied by a shift to a new host in the _pomonella_ group, the pattern of speciation is dramatically different in the _suavis_ group which has remained anchored to _Juglans_ throughout its evolutionary history. Unlike the non-toxic fruits infested by members of the _pomonella_ group, walnut husks in which larvae feed gregariously contain juglone, an extremely toxic phenolic compound. This substance may impose a level of specialization as a dietary requirement, thus restricting the ability of _suavis_ group species to shift to non-walnut hosts. Specialization of this type has been proposed for _Drosophila paca_ which requires a sterol for normal larval development found in its semia cactus host plant (Heed and Kircher 1965). Possibly the shift to walnuts by the early progenitor of the _suavis_ group gained a means for coping with juglone and in the process acquired a dependency. Whatever the cause for the restriction of _suavis_ group species to _Juglans_, the allopatric or parapatric distribution of sister species and the fact that they all share the same hosts strongly supports an allopatric mode of speciation.

Conspicuation of the _suavis_ group species and their walnut hosts can also be ruled out. All extant North American _Juglans_ species evolved long before the _suavis_ group began to diversify. _Juglans_ spp. are widespread throughout the Holarctic and Neotropical regions and have an extensive fossil record. The genus originated in North America approximately 60 MYA during the Paleocene while present day species appeared over 40-45 MYA (Fjellstrom and Parfit 1995). The _suavis_ group is probably between 2-5 MYA, using the estimated age of the _suavis_ group calculated above as the minimum and 5 MYA as the maximum, which is when the floristic connection between California and Arizona was broken (Axelrod 1975). In support of this latter date, _J. hindsii_ and _J. californica_, both native to California, were free of _Rhaboletis_ until _R. completa_ was introduced in the early 1920s (Booey 1934). There was ample opportunity for populations of the early ancestors of the _suavis_ group to become geographically isolated on _Juglans_ for extended periods of time as the distribution of these plants shifted in response to climatic changes (Manchester 1987).

**Conclusion**

There is a growing body of evidence that post-mating reproductive isolation in plants (Davies 1993) as well as animals (Schlueter 1996 and references therein) is not a prerequisite for evolutionary divergence, and can occur in the face of considerable gene flow. Differentiation during adaptive radiation into new habitats, or in the case of _Rhagoletis_, to new hosts is probably the outcome of strong divergent natural selection (Bush 1994; Rosenzweig 1995; Johnson et al. 1996; Schluter 1996). To emphasize the vital contribution of resource environment, Schluter has referred to this form of sympatric divergence as "ecological speciation" which involves a process in which reproductive isolation evolves from divergent selection (Bush 1993a; Johnson et al. 1996; Schluter 1996). It is not surprising that a large degree of ecological differentiation is a consistent feature of sympatric species.

Phenotypic differences in behavior, ecological preferences and mate recognition may arise sympatrically in response to divergent selection pressures experienced by sister populations of a phytophagous insect while they adapt to different hosts or habitats. Gene flow during the process of divergence does not prevent host race formation and speciation. Results of hybridization experiments also confirm that hybrids between sister species in the _pomonella_ group are fertile and show little or no post-mating reproductive isolation (Bush 1993b). Hybrid swarms between such species are not present however,
suggesting that these cases did not originate as a result of secondary contact (Schluter 1996). Additionally, as pointed out by Feder (1998) and Feder et al. (1998), when fitness is strongly environment-specific intermediate phenotypes are at a pronounced disadvantage in exploiting either host resource.

The fact that mating occurs on or within a preferred host or habitat is probably the most important single factor promoting sympatric divergence in response to divergent ecological selection pressures in phytophagous parasites such as Rhagoletis. In other animals with strong habitat preferences mate choice may be based on morphological attributes which have evolved in concert with morphological differentiation that has occurred during the course of adaptation to alternative habitats (Schluter 1996). When genetic variation in host or habitat preference is coupled with variation in mate choice and fitness, host or habitat races and species are likely to evolve rapidly as long as there is a vacant host or habitat open for colonization (Johnson et al. 1996). The availability of a new host or habitat is obviously an important limitation to sympatric speciation. Ecological speciation via a host shift is probably a rare event under natural conditions as it must await the introduction of a new host plant free of major competitors. However, introductions by alterations in climate and ecological conditions or by active or passive long-distance seed dispersal are probably far more frequent than the opportunity for vicariant geographic isolation. The differences in the frequencies of these occurrences should manifest themselves as different topologies in phylogenetic trees of organisms influenced by these events.

Another aspect of ecological speciation that emerged from studies on host and habitat races is that pre-mating reproductive isolation may evolve in parallel in independent populations experiencing similar ecological selection pressures (Schluter 1996). This is exemplified in Rhagoletis by the independent evolution of apple host races in northeastern North America and in the central highlands of Mexico. It is quite possible that even within the northeastern population of R. pomonella, the apple race has arisen several times throughout the range of Cragaecus. As these populations expand they can fuse, possibly obliterating any trace of their independent origins.

Evidence is mounting from research on a variety of organisms that ecological selection pressures are the primary cause of rapid sympatric speciation in both plants and animals (Bush 1994; Schluter 1996 and chapters in Howard and Berlocher 1998). We are only just beginning to understand the processes involved in these examples of adaptive radiations in novel environments. Plants and animals with similar parasitic or habitat specific life styles are widespread and ecological speciation is likely to be far more common than we realize. We need many long term studies on a variety of such organisms. Only then can we hope to establish the biological and environmental factors which promote sympatric speciation in nature and understand the processes that give rise to the earth's biological diversity.

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