

The Apple Maggot Fly, *Rhagoletis pomonella*

Flies in the Face of Conventional Wisdom about Speciation?

Jeffrey L. Feder

It is often said that Darwin never really tackled the problem posed by the title of his thesis *On the Origin of Species*. Instead, he offered a proof of organismal evolution and one long argument that natural selection was a prime engine for evolution. But the full title of Darwin's work was *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*. Darwin therefore pondered how new species formed, and his answer was by natural selection, the same process responsible for change within populations.

Darwin could be rightly accused of being somewhat ambivalent as to the geographic context of speciation. But Benjamin Walsh was not. As early as 1864, Walsh proposed that certain host-specific phytophagous insects could speciate sympatrically (i.e., in the absence of complete geographic isolation) by shifting and adapting to new host plants. In particular, Walsh (1867) cited the shift of the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae, Walsh), from its native host hawthorn (*Crataegus* L. spp.) to introduced, domestic apple (*Malus pumila* L.), an event that occurred in the Hudson Valley Region of New York in the mid-1800s, as an example of an incipient sympatric speciation event (for further discussion of the natural history of this host shift, see Bush, 1966, 1992; Bush et al., 1989). Subsequently, the term *host race* has been used to describe this purported initial stage in sympatric divergence, host races being defined as partially reproductively isolated, conspecific populations that owe their isolation to host-associated adaptations (Diehl and Bush, 1984). Host races therefore represent a special class of ecological polymorphism, one in which the polymorphism pleiotropically results in reproductive isolation. This is in contrast to the situation where an ecological polymorphism does not affect the pattern of mating or gene flow within a species, instead being maintained by some form of balancing, frequency-

or density-dependent selection (Wilson, 1989). It is interesting to note that like *R. pomonella*, many reported cases of host races appear to involve introduced plants (Diehl and Bush, 1984), a situation where a new potential host interaction suddenly becomes available.

In this chapter, I examine the evidence for sympatric host race formation in *R. pomonella*. It seems most appropriate to concentrate on *R. pomonella* because it is the fly that prompted Walsh (1864, 1867) to propose, and later Guy Bush (1966, 1969a,b, 1975a,b) to refine, the concept of sympatric speciation via host race formation. Before delving into the data, however, I first outline Bush's (1966, 1969a,b, 1975a,b) general model for sympatric host race formation, placing his model in context with the known biology of *R. pomonella* and in juxtaposition with previous thinking about postzygotic reproductive isolation.

Bush's Model for Sympatric Host Race Formation

Bush's model for sympatric race formation and speciation rests on two main pillars:

Host (habitat) specific mating. Host-specific mating for *Rhagoletis* translates into adult flies mating on and ovipositing into the same species of host fruit that they fed within as larvae (figure 10.1). I shall refer to any such tendency as *host fidelity*. Host fidelity is important because it establishes a system of positive assortative mating that acts as a premating barrier to gene flow between demes specialized on alternative plants.

Host-associated fitness trade-offs. Fitness trade-offs are necessary to offset any "leakiness" that may exist in host fidelity (figure 10.1). As such, they act as post-

The Life Cycle of *Rhagoletis pomonella*

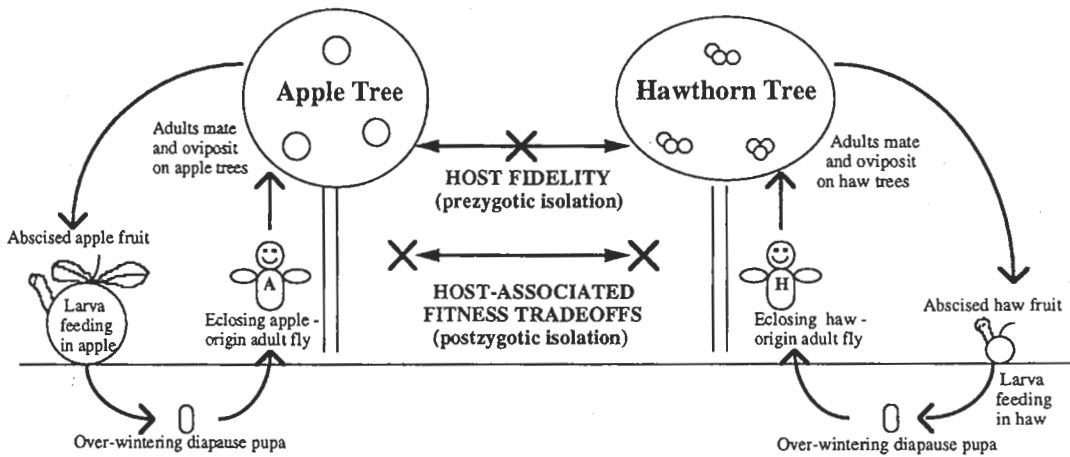


Figure 10.1. Summary of the life cycle of *R. pomonella* emphasizing the roles that host fidelity and fitness trade-offs play in isolating apple- and hawthorn-infesting races of the fly.

zygotic barriers to gene flow. Postzygotic isolation in animals has become synonymous with sterility or inviability problems in hybrids or backcrosses due to the detrimental mixing of incompatible genomes (Mayr, 1963). But this is not what Bush is arguing for. Rather, he is saying that in the earliest stages of divergence, postzygotic isolation is primarily the by-product of divergent selection pressures imposed by different habitats. Hybrids between very recently formed races or incipient species will therefore usually not die strictly because of inherently deleterious gene-gene interactions. Such hybrids will often thrive under laboratory conditions (see Schluter, this volume), as is the case for offspring between apple and hawthorn flies (Reissig and Smith, 1978; Smith, 1988; S. H. Berlocher and G. L. Bush, unpublished). Rather, hybrids will survive poorly because the genes they possess produce phenotypes that make them ill-suited for the niches occupied by their parents. Hybrid sterility and inviability problems that are intrinsic to the genetic environment will therefore tend to be secondary phenomena in speciation, completing a process already well under way. It is adaptation to variation in the external physical and biotic environment that provides the critical push that gets the process rolling. One need only look at the explosive nature of species genesis associated with adaptive radiations to find supporting evidence for this view (see chapters by Schluter and by McCune and Lovejoy, this volume).

To have a fitness trade-off in *Rhagoletis*, then, the same trait(s) or gene(s) that confer an advantage to a fly on one host plant must simultaneously incur a cost on alternative hosts (Dethier, 1954). Fitness trade-offs are equivalent to antagonistic pleiotropy. However, observing negative performance correlations in insects across

different plants does not guarantee the existence of antagonistic pleiotropy (Futuyma and Philippi, 1987; Via, 1991; Mackenzie, 1996). This is because linkage disequilibrium can also produce negative correlations. (I define linkage disequilibrium in this instance as repulsion phase gametic disequilibrium between derived genes [i.e., nonancestral alleles] fixed at different loci in host-associated populations.) Such linkage disequilibrium may often be the cause for negative performance correlations when comparisons involve different insect species or geographically separated demes specialized on different plants. But negative correlations due to linkage do not constitute fitness trade-offs in the strict sense. This is because recombination can theoretically generate a "jack of all trades" genotype that has high fitness on all host plants. The genetic architecture of host specialization is important because it is difficult for negative performance correlations to evolve *de novo* in sympatry via linkage. Very simply, an allele giving an insect an advantage on one plant, but having no detrimental consequence on other hosts, has the capacity to spread and rapidly fix through the metapopulation as a whole. This is true unless sympatric demes exchange few migrants and new mutations increasing performance arise almost simultaneously in different host demes at tightly linked loci. Consequently, to initiate sympatric speciation in *Rhagoletis*, a new mutation, preexisting allele and/or combination of genes, must in all likelihood result in antagonistic pleiotropy.

Few would argue that when host-specific mating and fitness trade-offs exist, substantial reproductive isolation can evolve in sympatry as a pleiotropic by-product of host-associated adaptation (Rice and Hostert, 1993). Consequently, the critical question is not whether sympatric

speciation is theoretically possible, because it is (see Kondrashov et al., and Johnson and Gullberg, this volume). Rather, it is whether sympatrically formed host races exist in nature. That is, do host-specific mating and fitness trade-offs ever evolve in tandem within geographically contiguous insect populations?

Genetic Evidence for *R. pomonella* Host Races

The first question to tackle is whether partially reproductively isolated and genetically differentiated host races of *R. pomonella* actually exist on apples and hawthorns. The answer to this question is yes. Sympatric pairs of apple- and hawthorn-infesting fly populations collected from field sites across eastern North America consistently displayed significant allele frequency differences at six allozyme loci: *Malic enzyme (Me)*, *Aconitase-2 (Acon-2)*, *Mannose phosphate isomerase (Mpi)*, *NADH-Diaphorase-2 (Dia-2)*, *Aspartate amino transferase-2 (Aat-2)*, and *Hydroxyacid dehydrogenase (Had)* (Feder et al., 1988, 1990; Feder and Bush, 1989; McPheron et al., 1988; Berlocher and McPheron, 1996). These six allozyme loci map to only three different regions of the genome (*Aat-2* and *Dia-2* map to linkage group I; *Me*, *Acon-2*, and *Mpi* are tightly linked on group II; and *Had* is on group III; Berlocher and Smith, 1983; Feder et al. 1989). Significant levels of linkage disequilibrium have been found between nonallelic genes within, but not between, each of these three genomic regions (Feder et al., 1988, 1990). Seven other polymorphic allozyme loci displayed little differentiation between the host races (Feder et al., 1990). Walsh and Bush therefore appear vindicated in their claim of host races in *R. pomonella*. Furthermore, the documented historical time frame of the shift from hawthorns to apples argues for a sympatric origin for the apple race (Walsh, 1867; Bush et al., 1989).

Host Fidelity in *R. pomonella*

The next issue is whether Bush's model for sympatric race formation is true. Regarding host fidelity, the first pillar of his model, the answer again is yes. Ron Prokopy and co-workers (Prokopy et al., 1971, 1972) showed in a series of field experiments that *R. pomonella* mate exclusively on or near the fruit of their host plants. Studies on the oviposition acceptance behaviors of naive apple- and hawthorn-origin flies have also suggested that genetically based differences in host preference exist between the races (Prokopy et al., 1972, 1988). Although females of both host races prefer to oviposit into hawthorns, hawthorn-origin females are much more averse to ovipositing into apples than are apple-origin flies (Prokopy et al., 1972, 1988). Mark-release-recapture studies conducted at a field site near the town of Grant, Michigan,

indicated that host fidelity limits interhost movement of adults between apple and hawthorn trees to ~6% per generation (Feder et al., 1994). A number of factors contributed to this host fidelity, including inherent differences in host preference, apple flies being averse to emigration when they eclose from beneath apple trees, and eclosion time differences between the races causing allochronic isolation (Feder et al., 1994; Smith, 1988). Data from the mark-release-recapture study also showed that the mating success of interhost migrants was not statistically different from that of resident flies, suggesting an absence of ethological isolation (Feder et al., 1994, 1998). In addition, the oviposition behavior of immigrant females was not significantly different from that of non-migrants (Feder et al., 1994, 1998). Finally, experimental crosses have given no indication of any sterility or inviability barriers between apple and hawthorn flies (Reissig and Smith 1978; Smith, 1988; Berlocher and Bush, unpublished). Consequently, estimates of interhost migration derived from the mark-release-recapture study accurately reflect levels of genetic exchange between the host races.

The Search for Host-Associated Fitness Trade-offs in *R. pomonella*

The mark-release-recapture study cannot be the complete story, however. Although the results confirm the existence of fairly strong host fidelity in *R. pomonella*, they also indicate that host fidelity is not absolute. If the effective level of gene flow between apple and hawthorn populations is actually 6% per generation (the level suggested by the mark-release-recapture study), then allozyme frequency differences between the races would quickly disappear. Some form of host-associated selection is therefore required to counteract this gene flow. But there is little evidence for host-related fitness trade-offs in phytophagous insects (Futuyma and Moreno, 1988; Jaenike, 1990; Via, 1990; Futuyma and Keese, 1992; but for possible exceptions, see Gould, 1979; Mitter et al., 1979; Fry, 1990; Karowe, 1990; Via, 1991; Mackenzie, 1996). This would seem to cut the foundation from beneath Bush's second pillar for sympatric divergence. Nevertheless, the allozyme data imply that divergent selection is acting on apple and haw races, as allele frequencies for *Me*, *Acon-2*, *Mpi*, *Aat-2*, *Dia-2*, and *Had* have remained consistently different between apple and hawthorn populations at the Grant, Michigan, site, despite gene flow, over at least an 11-year period since they were first monitored in 1984 (Feder et al., 1990, 1993, 1997a). What then is the source of the selection maintaining the genetic integrity of the host races for the three regions of the genome displaying host-associated differentiation?

One logical choice would be some sort of feeding adaptation affecting larval survivorship within host fruits (Bush, 1969a,b, 1975a,b). But reciprocal egg transplant experiments performed by Prokopy et al. (1988) gave no

evidence for any feeding specialization in larvae related to chemical or nutritional differences between apple and hawthorn fruits. Apple and hawthorn larvae survived equally well in hawthorn fruits. Both races fared equally poorly in apples. While these results accord with hawthorns being the ancestral host of *R. pomonella*, they are distressing regarding the issue of fitness trade-offs.

So, why do *R. pomonella* females continue to oviposit into apples when apples are nutritionally inferior to hawthorns for larval survival? Part of the reason is enemy-free space (Hairston et al., 1960; Bernays and Graham, 1988; Jaenike, 1990). Levels of braconid parasitism, interspecific competition (from a number of different moth species and plum curculio weevils), and intraspecific competition are much lower for flies infesting apples than for those infesting hawthorns (Feder, 1995; Feder et al., 1995). These factors were excluded from Prokopy et al.'s (1988) larval survivorship estimates and greatly compensate for the nutritional handicap of feeding within apples (Feder, 1995; Feder et al., 1995). But an escape from parasitoids and competitors does not constitute a fitness tradeoff in the strict sense for *R. pomonella*, for if a hawthorn-origin female were to oviposit into apples, then her offspring would receive the same beneficial escape from parasitoids that apple-origin larvae enjoy. In other words, aside from traits contributing to host fidelity, there is no genetic basis for the apple race's escape from natural enemies. Without a genetic basis for such a trait, there can be no genetic cost and hence no antagonistic pleiotropy or fitness trade-off. Some other factor besides enemy-free space must be responsible for maintaining genetic differentiation between the host races at the allozyme loci.

Life-History Traits, Host Plant Phenology, and Fitness Trade-offs in *R. pomonella*

Detoxification of plant secondary compounds and enemy-free space are but two potential avenues for host-associated adaptation. There are others. A number of observations suggest that *Me*, *Acon-2*, *Mpi*, *Dia-2*, *Aat-2*, and *Had* either encode or are linked to genes affecting development rates in *R. pomonella*. Flies possessing the alleles *Me 100*, *Acon-2 95*, *Mpi 37*, *Dia-2 100*, *Aat-2 +75*, and *Had 100* leave host fruits, pupate, and eclose earlier than other flies (Feder et al., 1993; Berlocher, unpublished). The allozymes present in higher frequencies in the hawthorn race at the Grant site therefore appear to be associated with faster rates of development in *R. pomonella*.

Patterns of geographic and temporal variation observed for the allozymes also implicate ambient temperature as a prime factor affecting the genetics of the host races. First, *Me*, *Acon-2*, *Mpi*, *Dia-2*, *Aat-2*, and *Had* all display latitudinal allele frequency clines in both apple and hawthorn races (Feder and Bush, 1989; Feder et al., 1990; Berlocher and McPherson, 1996). Second, allozyme

frequencies in the hawthorn race at the Grant site correlate with spring temperatures over an 11-year period beginning in 1984 (Feder et al., 1993, 1997a).

The action of ambient temperature on fly development is a prime suspect for selection acting on the allozymes or linked loci. But what mediates this selection such that it differentially affects the host races? A likely candidate is host plant phenology. An insect such as *R. pomonella* that is univoltine, overwinters in the soil in a facultative pupal diapause, and has a limited adult longevity (Boller and Prokopy, 1976) must be developmentally synchronous with host acceptability to maximize fitness. Asynchrony in any stage of the life cycle is disastrous for a fly, leading to either its immediate death or lower viability / fecundity because it is in the wrong developmental state for the season. This could be due, for example, to the larva or pupa not entering diapause before the onset of winter or to an adult being active before or after host fruits are present.

Apples and hawthorns represent different temporal resources. Fruits on apple varieties favored by *R. pomonella* peak ~3 weeks earlier in the season than hawthorns (Feder et al., 1993). This difference has many important consequences to the developmental profiles of apple and hawthorn flies. One consequence that I will initially focus on is that larvae leave abscised apple fruits and pupate in the soil an average of over 16 days earlier in the season than they do from hawthorns (Feder et al., 1994). Because *R. pomonella* are facultative diapausers, apple-origin pupae that develop too rapidly in the summer run the risk of breaking diapause prematurely and directly developing into adults. Almost all *R. pomonella* larvae do, in fact, fail to diapause when held at temperatures above 28° C (Prokopy, 1968), and small second generations of apple race flies have been reported in the field (Caesar and Ross, 1919; Porter, 1928; Phipps and Dirks, 1933; Dean and Chapman, 1973). Nondiapausing flies are inevitably doomed; either they eclose at times when suitable host fruits are no longer available or they commit to, but do not complete, adult development before the onset of winter and subsequently freeze to death. The greater exposure of apple-pupae to warm weather preceding winter may therefore favor flies with deeper diapauses (or lower basal metabolic/development rates) in the apple than the hawthorn race. Selective pressures are likely to be different for hawthorn flies. The relatively late phenology of hawthorns means that slow-developing hawthorn flies may not enter pupal diapause quickly enough before the onset of winter and freeze to death. I shall henceforth refer to this idea as the diapause trade-off hypothesis.

The Prewinter Experiment—An Empirical Test of the Diapause Trade-off Hypothesis

In order to test the diapause trade-off hypothesis, co-workers and I (Feder et al., 1997a) experimentally ma-

nipulated environmental rearing conditions for hawthorn flies to determine whether we could induce a genetic response at the six allozymes displaying host-associated differentiation. In particular, we systematically altered the time period preceding winter for different subsamples of hawthorn-origin pupae collected from the Grant site (figure 10.2). Our rationale was that lengthening the pre-wintering period would expose hawthorn pupae to extended periods of warm weather, conditions they would face if they were infesting a host plant with an earlier fruiting phenology like apples. Our expectation was that such treatments would selectively eliminate pupae in shallow diapauses or with high metabolic/development rates from the surviving hawthorn-fly population. Conversely, brief prewinter treatments (e.g., 2 days), would favor rapidly developing pupae that quickly enter diapause.

What pattern of genetic response does the diapause trade-off hypothesis predict for the prewinter selection experiment? Actually, there are two predictions. First, that populations of diapausing hawthorn flies that survive in-

creasing periods of prewinter heating and the ensuing chilling period should become increasing more similar to populations of apple flies in their genetic constitution. After all, we are exposing hawthorn pupae to environmental conditions that we believe mimic those faced by apple flies. If these conditions are affecting the allozymes then we should see a response in the direction of the apple race. Second, nondiapausing flies that eclose prior to winter should display high frequencies of electromorphs more common to the hawthorn race at the Grant site (i.e., *Me 100*, *Acon-2 95*, *Mpi 37*, *Dia-2 100*, *Aat-2 +75*, and *Had 100*), as these alleles have been previously correlated with fast development rates (Feder et al., 1993, unpublished).

Analysis of the prewinter experiment supported the diapause trade-off hypothesis (figure 10.3; Feder et al., 1997a). The predicted genetic response was observed for *Me*, *Acon-2*, *Mpi*, *Dia-2*, and *Aat-2*, as allele frequencies in surviving flies became more "applelike" with longer prewinter heat treatments (see figure 10.3 for results for *Me 100*). In fact, we came very close to genetically trans-

Experimental Design for Pre-Winter Experiment

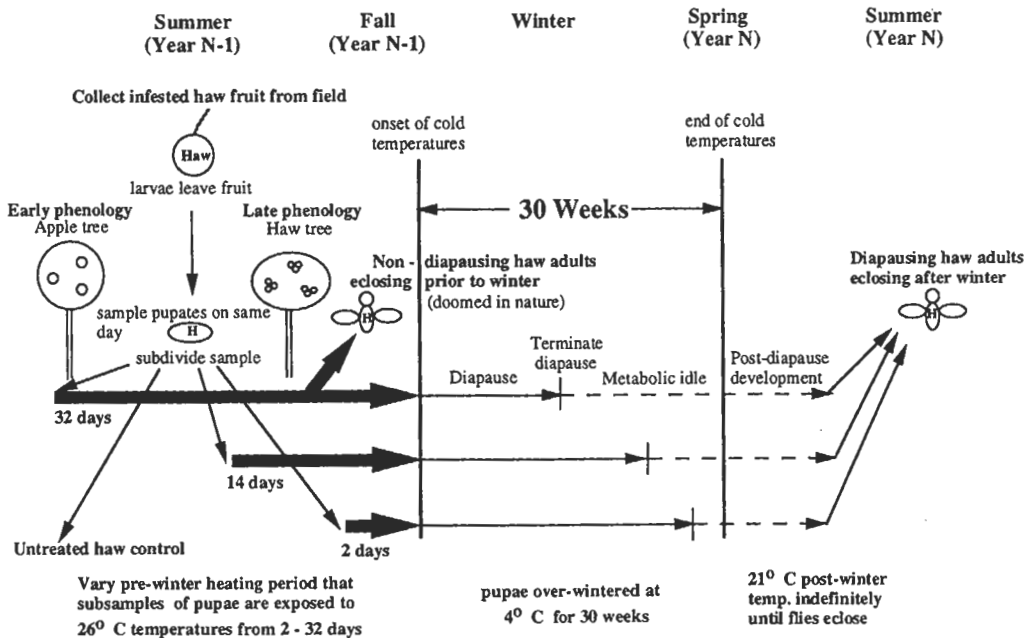


Figure 10.2. Overview of the experimental design for the prewinter study (for a detailed description of methods, see Feder et al., 1997a). Infested fruits were collected from beneath a hawthorn tree at the Grant, Michigan, study site on September 15, 1989, and placed on wire screens above plastic trays in the laboratory. Puparia were collected from the plastic trays on a daily basis and divided into subsamples. One daily subsample was immediately frozen to serve as an untreated genetic control. The remaining subsamples were held at 26°C in a constant temperature room for 2, 7, 14, 21, 28, or 32 days, after which time they were placed in cold storage for 30 weeks at 4°C to simulate winter. Adult flies were collected as they eclosed during the periods preceding (nondiapausing flies) and following chilling (diapausing flies). Flies were also sampled from an apple tree at the Grant site on August 15, 1989, to provide a baseline control for the apple race. Flies were genetically scored for *Me*, *Acon-2*, *Mpi*, *Dia-2*, *Aat-2*, and *Had* using standard starch gel electrophoretic techniques.

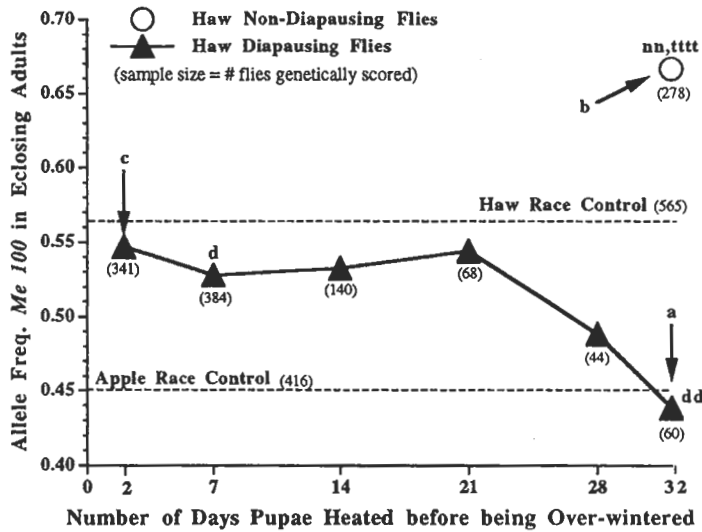
Me 100 results for Hawthorn Race

Figure 10.3. Allele frequencies for *Me 100* in diapausing (triangles) and nondiapausing (circle) hawthorn fly adults for the 2–32-day treatments in the prewinter experiment. The *Me 100* frequencies in the untreated apple and hawthorn fly control samples are designated by the dashed lines. d = significant allele frequency difference between diapause flies and hawthorn control sample as determined by one-tailed, randomized Fisher exact tests. n = significant difference between non-diapause flies and hawthorn control sample. t = significant difference between diapause and non-diapause flies for the same heat treatment. Number of letters designates significance level (one letter = $P < 0.05$, two = $P < 0.01$, four = $P < 0.0001$). Arrow “a” indicates how similar the allele frequency for *Me 100* for diapausing hawthorn flies in the 32-day treatment is to the untreated apple fly control. Arrow “b” points to *Me 100* frequency for nondiapausing hawthorn flies in the 32-day treatment being much greater than that for either diapausing flies or the untreated hawthorn fly control. Arrow “c” shows that the frequency of *Me 100* in the 2-day treatment was slightly lower than that of the untreated hawthorn fly control.

forming the hawthorn race into the apple race after only a single generation of mass selection by the 32-day treatment (see arrow “a” in figure 10.3). A significant proportion of pupae also eclosed as nondiapausing adults in the 32-day treatment. As expected, these nondiapausing flies had very high frequencies of *Me 100*, *Acon-2 95*, *Mpi 37*, *Dia-2 100*, *Aat-2 +75*, and *Had 100* (see arrow “b” in figure 10.3).

The prewinter experiment answers many questions. Most importantly, it establishes a direct, empirical link among ambient temperature, host phenology, fly development, and the six allozyme loci. The experiment also implies that the apple race was derived from standing genetic variation in the hawthorn race and, in particular, from pupae comprising the slowest developing, tail end of the hawthorn fly distribution.

Issues Raised by the Prewinter Experiment

The results from the prewinter experiment also raise several issues, however. I will attempt to answer what I

feel are a number of the most pertinent and thought-provoking questions below. The reader is referred to Feder et al. (1997a) for further discussion of the data.

Does the selection that occurs in the hawthorn race also occur in the apple race? If not, then extrapolating the results for the hawthorn race to the apple race would be like comparing oranges (or haws) to apples. Could it be that the remainder of the genome of apple flies is sufficiently different from that of hawthorn flies to cause relative fitnesses at the allozyme loci to differ between the races? If true, then hybrids between the races might have similar fitnesses to apple flies if they were to infest apples.

The preponderance of evidence argues against the above scenario, at least as far as the allozymes are concerned. Recall that only the three genomic regions containing *Me*, *Acon-2*, *Mpi*, *Dia-2*, *Aat-2*, and *Had* show host-related differentiation. Other polymorphic allozyme loci display little host-associated or geographic variation (Feder et al., 1990). This suggests that gene flow between the races is sufficient to homogenize allele frequencies at loci not directly under selection or linked to such genes.

Alternatively, balancing selection could also account for the similarity of allozyme frequencies at these other loci in the races, but this seems a less likely explanation given the number of loci involved and the known 6% level of genetic exchange between apple and hawthorn populations each generation. Either way, genetic differences between the races do not appear to be evenly dispersed throughout the genome but clumped in specific regions. Consequently, polymorphism in the remainder of the genome does not appear to be acted upon in a substantively different way between, as opposed to within, the races. Clinal patterns of variation for *Me*, *Acon-2*, *Mpi*, *Dia-2*, *Aat-2*, and *Had* in the apple race underscore this point by indicating that the apple race is responding to the same selection pressures in a similar manner as the hawthorn race (see next question). Finally, the apple race at the Grant site showed a qualitatively similar genetic response to selection as the hawthorn race in a just completed, parallel prewinter experiment (Feder et al., unpublished).

Does the prewinter experiment accurately reflect the situation in nature? After all, the experiment was conducted under artificial laboratory conditions. There is no guarantee that selection detected under such a situation also operates in nature. In this regard, an important implication of the diapause trade-off hypothesis (DTH) is that the same selective factors differentiating the host races should also operate within the races. The results from the prewinter experiment predict that allozyme frequencies should track local ambient temperature conditions, since temperature affects both the diapause status of flies and the fruiting times of host trees (Phipps and Dirks, 1933; Lathrop and Dirks, 1945; Glass, 1960; Oatman, 1964; Reissig et al., 1979).

Earlier, I presented two lines of evidence supporting a relationship between ambient temperature and the allozymes. The first was that *Me*, *Acon-2*, *Mpi*, *Dia-2*, *Aat-2*, and *Had* display latitudinal allele frequency clines among both apple- and hawthorn-fly populations in the United States (Feder et al., 1990; Feder and Bush, 1989; Berlocher and McPheron, 1996). It so happens that populations from northern (i.e., colder) latitudes possess higher frequencies of *Me 100*, *Acon-2 95*, *Mpi 37*, *Dia-2 100*, *Aat-2 +75*, and *Had 100*, the alleles associated with faster development rates. In addition, the clines show several perturbations in allele frequencies that coincide with differences in ambient temperature conditions among local collecting sites (Feder and Bush, 1989, 1991). Second, allozyme frequencies in the haw race at the Grant site have tracked spring temperature conditions since 1984 (Feder et al., 1993, 1997a). What I did not mention earlier, however, is that they track temperatures in the spring of the preceding year. Because most pupae break diapause after their first winter, flies sampled in year N are representative of pupae that survived the previous season (year N - 1). High spring temperatures in year N - 1 meant an

early field season and correlated with increased frequencies in year N of alleles common to the apple race (or southern populations). Conversely, cold springs selected for alleles more common in northern populations.

Is the selection generated in the prewinter experiment strong enough to account for the continued differentiation of the host races in the face of gene flow? The answer is yes, at least with respect to gene flow from the hawthorn into the apple race. The apple race pupates about 16 days on average earlier than the hawthorn race. We could therefore get a relative gauge of the force of selection by comparing treatments in the prewinter experiment that differ by approximately 2 weeks; the 26°C, 15:9-hour light:dark conditions used to rear pupae in the experiment being close to those experienced by apple flies at the height of the field season in late July to early August. Based on this criterion, selection coefficients (s values) against *Me 100*, *Acon-2 95*, *Aat-2 +75*, and *Had 100* homozygotes (or homozygotes at closely linked genes) calculated for hawthorn flies from a comparison of the untreated control and the 14-day treatment under an additive fitness model are 0.264, 0.254, 0.083, and 0.552, respectively. These coefficients are greater than those needed to counteract the estimated 6% gene flow from the hawthorn into the apple race each generation for the three genomic regions displaying differentiation (*Me* = 0.063, *Acon* = 0.136, *Aat* = 0.074, *Had* = 0.041; these values were calculated assuming that apple and hawthorn control samples represent allele frequencies in the races after interbreeding but prior to selection). The apple race therefore continues to persist as a diverged genetic entity from the hawthorn race due, in part, to developmental adaptations in pupae stemming from differences in the fruiting phenologies of apples and hawthorns.

What is maintaining the genetic integrity of the hawthorn race? The prewinter experiment really only explains the source of selection impeding introgression of certain regions of the genome from the hawthorn into the apple race. For the sympatric host race formation model to work, selection must also be operating in the opposite direction or the metapopulation as a whole would simply become more "applelike." And, the metapopulation is not becoming more "applelike," as allele frequencies for the six allozyme loci have remained consistently different between the host races at the Grant, Michigan, site since 1984 (Feder et al., 1993, 1997a). Earlier, I stated that the late phenology of hawthorns should select for faster rates of development in hawthorn pupae in order for them to achieve diapause before the first frost. This should favor individuals possessing the alleles for faster development rates (i.e., *Me 100*, *Acon-2 95*, *Mpi 37*, *Dia-2 100*, *Aat-2 +75*, and *Had 100*). However, the shortest heat treatment in the prewinter experiment (2 days) did not result in any marked increase in the frequencies of "hawthorn race" alleles in surviving

adults. In fact, the frequency of *Me 100* in the 2-day treatment was actually slightly lower than that of the untreated hawthorn race control (see arrow "c" in figure 10.3). This appears to contradict an important component of the diapause trade-off hypothesis. If it is true that short prewintering periods do not select for fast fly development, then what is limiting effective gene flow from the apple into the hawthorn race?

First, the overwintering conditions that were used in the prewinter experiment (30 weeks of chilling at 4°C) turn out to constitute what would be a very long winter for hawthorn pupae at the Grant site (Feder et al., 1997b). Experiments in which we have varied the length of the overwintering period for pupae indicate that 30 weeks of chilling at 4°C select against *Me 100*, *Acon-2 95*, *Mpi 37*, *Dia-2 100*, *Aat-2 +75*, and *Had 100* (Feder et al., 1997b). These data show that we actually did select for fast fly development in the 2-day prewinter treatment, but the long overwintering period used in the prewinter experiment counteracted the effects of this selection.

Second, the need to synchronize adult eclosion with host fruit availability also limits gene flow. Apple flies eclose an average of ~10 days earlier than hawthorn flies at the Grant site (Feder, 1995), a difference coinciding with the earlier phenology of apples. So, the races are being pulled apart allochronically as a consequence of selection for different mean eclosion times. Due to a quirk in the ecology and genetics of eclosion, this selection is likely to act against "hybrids" (offspring from apple × hawthorn fly matings) at the Grant site. The allozymes *Me 100*, *Acon-2 95*, *Mpi 37*, *Dia-2 100*, *Aat-2 +75*, and *Had 100* are all found in higher frequencies in the first individuals to eclose in both host races (Feder et al., 1993), and the apple race possesses lower frequencies of these "fast development" alleles than the hawthorn race at the Grant site. Taken together, this suggests that apple flies are actually genetically predisposed to eclose later, not earlier, than hawthorn flies if the two races were to be reared under identical environmental conditions. And, in fact, the period between pupation and eclosion is ~1 week longer for apple flies at the Grant site; they have a head start in that they pupate an average of 16 days earlier than hawthorn flies in the fall, but eclose only 10 days sooner the following summer. The reason that apple flies eclose earlier in nature is therefore environmental. The inherently slower development rate of apple flies is offset by the higher temperatures and longer photoperiods that these flies experience as larvae and pupae. Hybrids between the races should therefore have genotypes that translate into intermediate eclosion times (development rates) when reared under controlled conditions. But in nature, of course, this would not be the case, as apples and hawthorns are seasonally asynchronous. Consequently, hybrids should eclose earlier than apple flies if they were to infest apples and later than hawthorn flies if they were to infest hawthorns. The repercussions of this are straightforward. Hybrids would eclose too early in the

season to optimally utilize apples and too late to attack hawthorns. They would not eclose at the happy median that would let them use both hosts. This is another example of how gene × environmental interactions, rather than deleterious gene × gene effects, are responsible for isolating the races.

A third piece of the puzzle is that selection also appears to be acting on larval development rates. The alleles *Me 100*, *Acon-2 95* and *Mpi 37* not only correlate with adult eclosion time, but also with when larvae complete feeding, exit host fruits, and pupate (Filchak and Feder, unpublished data). We have found that the quality of apple and hawthorn fruits kept outdoors under natural, field conditions deteriorates faster than that for fruits kept in a protective, open-air garage (Filchak and Feder, unpublished data). Consequently, larval mortality was significantly greater in the field compared to garage, suggesting that field conditions favor rapidly developing larvae that quickly leave host fruits (Filchak and Feder, unpublished data). Consistent with this hypothesis, allele frequencies for *Me 100*, *Acon-2 95*, and *Mpi 37* were also significantly higher in surviving field- than garage-reared larvae for both host races (Filchak and Feder, unpublished data). These results imply that genotype specific mortality is, after all, occurring during the larval life-history stage of *R. pomonella*. The caveat is that the selection is not happening in the manner originally envisioned by Bush (1969a,b; 1975a,b), who thought feeding adaptations of *R. pomonella* to chemical and/or nutritional differences between apple and hawthorn fruits would be the key to larval survivorship. Rather, the prime factor appears to be how fast fruits necrose. Also, the selection is directional, favoring the alleles *Me 100*, *Acon-2 95*, and *Mpi 37* (and/or linked genes) in both host races. As we discussed, these same alleles (or linked blocks of genes) can be discovered in pupae, especially in the apple-fly race, as they correlate with premature diapause termination. Host-dependent fitness trade-offs in *Rhagoletis* may therefore be due as much to differences in the relative strengths of directional selection pressures acting on different life-history stages, as disruptive selection affecting any one particular stage (i.e., divergent selection results from the summation of directional selection vectors across the life-histories of the races being of different sign). The necessity to consider details of the entire life-cycle highlights one of the difficult challenges posed to documenting fitness trade-offs for phytophagous insects.

What is becoming increasingly clear is that the host races represent semiautonomous populations residing in a balanced state on alternative adaptive peaks. Gene flow between the populations is not sufficient to counteract selection and perturb the races from their respective peaks. The interaction of ambient temperature conditions, host-plant phenology, fruit decay, and fly development is a major factor shaping the adaptive landscape. The valley between the peaks is not the result of any inherently negative genetic interaction between the genomes

of the host races but stems from divergent selection pressures exerted externally by the environment. Finally, spatial and temporal variation in ambient temperature conditions provide an explanation for the existence of standing developmental variation within the hawthorn race, sufficient genetic variation to allow a portion of the hawthorn fly population to shift and adapt to the earlier phenology of apples.

Why can't flies evolve a compromise combination of developmental characters that permit them to effectively use both apples and hawthorns? Why isn't the range of developmental responses plastic and fine-tuned to prevailing environmental conditions? Why can't modifier genes evolve and expunge any negative pleiotropic consequences of the allozymes? For instance, why can't a modifier gauge and counteract any detrimental consequences that fast larval development has on individuals that subsequently experience warm prewinter temperatures as pupae? Why do the six allozymes have such similar effects across so many different life-history stages of *R. pomonella*?

As for the last question, the answer is probably that the allozymes encode or are linked to genes whose products have fundamental consequences to basal metabolic levels in *R. pomonella*. (I use the word *linked* here because there is no proof that selection is working directly on the allozymes. Also recall that the six allozymes map to three different regions of the genome and that linkage disequilibrium has been observed in each of these regions [Feder et al., 1988, 1990], thereby increasing the chances that selection may actually be acting on a gene[s] linked to the allozymes.) As to why development is not more plastic, one must always remember that not everything is possible. Sometimes developmental constraints really do exist in nature. After all, individuals can have only a single ontogeny. Nevertheless, sensory inputs from the environment could still be used to modify the unfolding of this ontogeny. But such information must be both reliable (an accurate predictor of things to come) and consistent (provide the same message about what to do for the future) for insects infesting different host plants if plasticity rather than specialization is to evolve. For example, think about a modifier gene that slows down pupal development during warm weather periods at the Grant site. Such a modifier locus could be beneficial for apple flies, as they develop relatively early in the season when one hot day is likely to be followed by others. But such a gene could be detrimental to hawthorn flies that develop later in the season when weather is more erratic and when maximal utilization of every warm day is more critical to ensure the completion of prediapause development.

The above scenario emphasizes that it may be much easier for nonspecific modifiers to differentially evolve in each of the races than for a specific modifier conferring developmental plasticity to arise and spread through

both races. (By a nonspecific modifier I mean an allele whose phenotypic effect is expressed regardless of the genetic background. A specific modifier refers to a gene that affects a trait only in the presence of certain other nonallelic genes.) After all, the races are already semi-autonomous entities. The reduction in gene flow could only facilitate this process and further hasten the continued divergence of the races at auxiliary loci.

If nonspecific modifiers can fix independently in the host races, then why hasn't the apple race evolved an obligate diapause to counteract its longer prewinter exposure to warm weather and elevated day lengths? The reason is probably one of balance and timing, specifically, a balance between the depth of pupal diapause and the timing of adult eclosion. Although pupae in deep diapauses avoid the risk of premature eclosion in the fall, they also emerge significantly later than other flies during the following summer (Feder et al., unpublished). There seems to be no mechanism to entirely decouple these two aspects of development in *R. pomonella*. They are correlated characters. Consequently, I suspect that obligately diapausing apple flies would eclose too late in the field season to effectively utilize apples as a host resource.

*Does the general paucity of trade-offs found for phytophagous insects argue against a high incidence for sympatric divergence? Could *R. pomonella* be the exception rather than the rule?* Yes, if the paucity were real. But Rausher (1992) has commented that most tests for trade-offs have concentrated on metabolic costs associated with detoxification of host secondary compounds, while neglecting costs "associated with coordinating life-history events with host plant phenology, with tolerance of microclimatic conditions and with escape from predators via crypsis" (p. 70). In addition, experimental designs have often been restricted so that only one or a few factors influencing fitness were actually measured. The results from *R. pomonella* certainly point to the relationship between host phenology and insect development as an overlooked source of trade-offs. They also point to the need to take a "holistic" approach to the study of fitness trade-offs examining an insect's entire life cycle, not just feeding stages. A plethora of fitness trade-offs may therefore await discovery in phytophagous insects, sending a cautionary note about overinterpreting the current shortfall of examples as an indicator of the unlikelihood of nonallopatric speciation (Butlin, 1987).

But why should developmental synchrony be a rich source for fitness trade-offs when plant secondary compounds appear not? I have argued that traits involved in sympatric race formation must be "nonlabile," that is, inflexible traits that at some point become incapable of compensatory adjustments to changes in the environment (Futuyma and Moreno, 1988). If they were not, then phenotypic plasticity (a phytophagous generalist) would be a common evolutionary solution to host plant hetero-

geneity. For reasons discussed above, developmental specialization may often be rigid and host specific. For instance, once a *Rhagoletis* pupa breaks diapause, there is no turning back—the fly is committed to adult development. This being the case, life-history traits adapting flies to the phenology of one host plant will limit their opportunity to utilize alternative hosts with different phenologies. In contrast, traits associated with metabolic detoxification may be more labile. For instance, it may be possible to induce detoxification pathways only when they are needed or to ameliorate their costs when they are not needed. Also, a wider repertoire of behaviors may be available in the arsenal of insects to combat plant defenses than there are to modify development. The end result of this being that life-history traits are better candidates for fitness tradeoffs than are traits involved in the detoxification of plant compounds.

How much of the genome must be impervious to gene flow for two populations to be considered species? The host races differ in at least three regions of the genome that impart partial postmating reproductive isolation by developmentally adapting the races to their respective host plants. We have yet to study the genetics of host preference, so there may well be more than three regions. Whatever the final number, however, a substantial part of the *R. pomonella* genome would seem to be “open” to interracial gene flow. We also do not know how many regions differ between recognized sibling species in the *R. pomonella* group. But again, the answer is not the entire genome. Taxa in the *R. pomonella* complex are not fixed for alternative alleles at allozyme loci, the only exception being the distantly related *R. cornivora* (Berlocher and Bush, 1982; Berlocher et al., 1993). Instead, they share most allozymes in common, differing in allele frequencies at certain loci (Berlocher and Bush, 1982; Berlocher et al., 1993; Berlocher and Feder, unpublished). This pattern could be explained by shared ancestral polymorphism. But it could also be due to a low level of gene flow among taxa. If the latter, then most *R. pomonella* species are not quantitatively different from host races, especially the more recently derived taxa. In both cases selection counteracts gene flow to varying degrees across the genome. The main difference is that at the species level gene flow is lower and/or the intensity of selection is greater. *R. pomonella* group species do not appear to be unique in this regard. Numerous examples of the introgression of genomic regions between species can be found in the literature (Harrison, 1990), suggesting that “the field of genetic recombination is broader than the taxonomic species and groups that are behaving as evolutionarily independent entities” (Templeton, 1989, p. 10). The possession of a completely autonomous, self-cohesive gene pool is therefore not a required calling card for a “good species.” This is not to say that genetic incompatibilities and negative epistatic interactions will not be found extending across large

portions of the genome between certain species pairs (see chapters by Naviera and Maside, and Wu and Hollocher, this volume), just that such extensive closure of two genomes may often accumulate after speciation.

A New or Old Species Definition?

The realization that the entire genome need not crystallize or be completely impervious to gene flow when new species form and that disruptive selection can be a prime impetus for divergence necessitates a rethinking of species concepts. One recent suggestion is that species are genotypic clusters that can overlap spatially without fusing (Mallet, 1995). In essence, this represents a genetic updating of Darwin's original species definition. While this definition may not be an end-all, it does free us from the self-referential problem of species as reproductively isolated entities (Wallace, 1865; Mallet, 1995), a concept that confuses cause and effect (Paterson, 1985; Mallet, 1995; see Templeton, this volume).

Application of the cluster definition is not without its difficulties, however. Exactly how different do two genetic clusters have to be to be considered different species? How many genes and how much differentiation at these loci? For successful speciation, a minimum of two complementary genes are needed according to Dobzhansky (1937) and Mayr (1963). Could our criterion therefore be differentiation for at least two unlinked loci (J. Mallet, personal communication)? But if just a couple of unlinked genes are required for species designation, then shouldn't the *R. pomonella* host races be considered different species? This seems a bit premature to me. But where to draw the line on a seeming continuum in levels of divergence is not a problem unique to the genotypic cluster definition. Rather, the problem is an inherent result of the speciation process itself.

One possible solution would be to calculate likelihood scores that individuals with a particular genotype belong to one taxon as opposed to another based on a representative sample of loci spaced throughout the organism's genome (a variant of this idea was first suggested to me by J. Mallet). If (1) a sample of individuals can be sorted into two distinguishable genotypic clusters such that there is less than a 5% chance in misassigning a randomly chosen individual from one cluster into the opposing cluster and (2) the population of individuals comprising each of the clusters is in Hardy-Weinberg equilibrium for a majority of loci, then the clusters could be considered species. A corollary of finding distinguishable sympatric clusters will frequently be that geographically distant populations of the same species exchange more genes, and hence are more genetically similar to each other, than sympatric populations of different species. In other words, populations of the same species will eventually form what appears to be a monophyletic clade, even if the populations had multiple origins. This corollary pro-

vides one basis for distinguishing host races from species (see Berlocher, ch. 8 this volume); enough gene flow occurs between local host race populations that although the races maintain their genetic differences in sympatry, local host race populations are genetically more similar to one another than each is to other populations infesting the same host plant in other areas of the species range. (Note: genetic similarity refers to a representative sample of loci evenly spaced throughout the genome. It is possible that if only those loci under selection were considered, then populations infesting the same host plant would be most similar across the entire species range.)

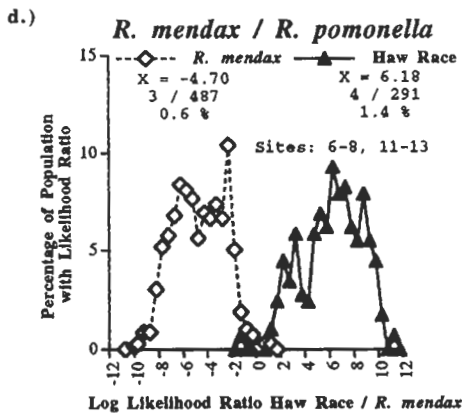
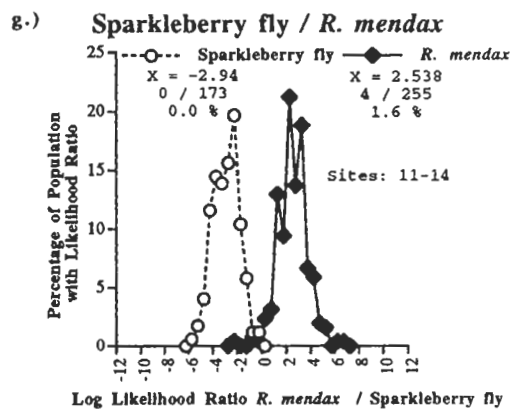
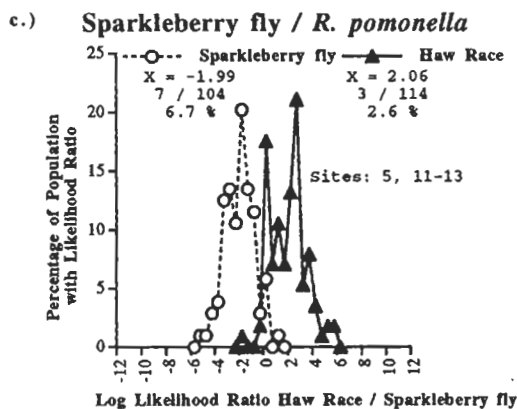
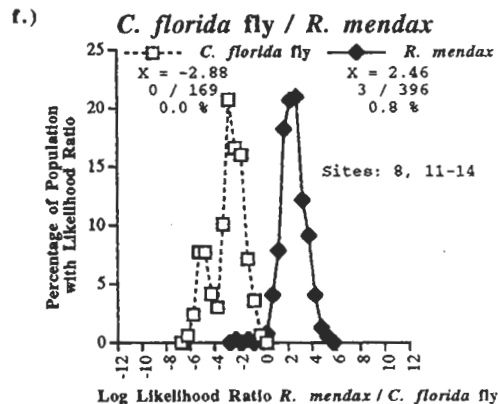
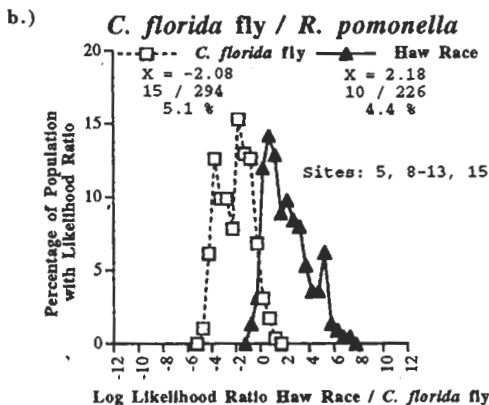
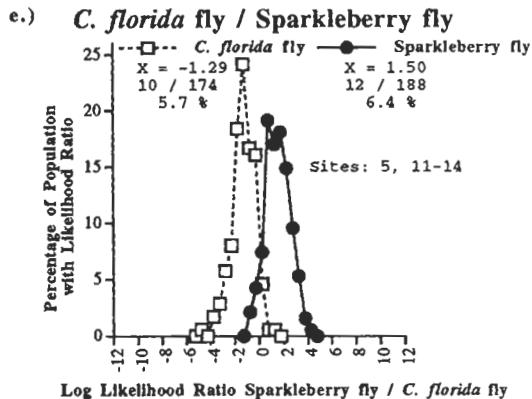
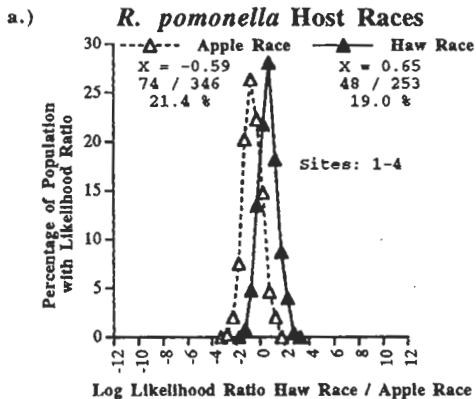
An example of a likelihood analysis is shown in figure 10.4 for the *R. pomonella* complex using a 17-locus allozyme data set. Graphs of the distributions of lod scores are given for pairwise comparisons among the apple- and hawthorn-infesting populations of *R. pomonella*, the undescribed *Cornus florida* fly (collected from flowering dogwood), the undescribed sparkleberry fly (collected from *Vaccinium aboreum*), and *R. mendax* (collected from high bush blueberries, *V. corymbosum*, and deerberries, *V. stamineum*; see Berlocher, ch. 8 this volume, for further discussion of the biology of these flies). (Lod score = \log_{10} of the likelihood ratio that a fly comes from one taxa versus another given the fly's genotype, i.e., the likelihood that a fly has a given genotype given that the fly belongs to taxon 1 divided by the likelihood that the fly has this genotype and came from taxon 2). Such an analysis is well suited for *R. pomonella* group flies because their host plant affiliations provide an a priori basis for assigning individuals to different taxa (e.g., the genotypes of flies collected from apples are tested against flies collected from hawthorns at sympatric sites). The results indicate that apple and hawthorn populations of *R. pomonella* constitute host races and not sibling species. Although apple and hawthorn flies form discernible genetic clusters in pairwise tests of sympatric populations collected from across the Northeast (figure 10.4a), the clusters overlap to such an

extent that ~20% of the flies sampled from one host plant would be misclassified as belonging to the other race based on their genotypes, far above the 5% threshold set for species status. In addition, local populations of apple and hawthorn flies are sometimes more genetically similar to one another than to other geographically distant populations of flies infesting either apples or hawthorns, respectively (Berlocher, unpublished; Feder, unpublished; McPheron, unpublished). The likelihood analysis does suggest that several closely related taxa to apple and hawthorn flies in the *R. pomonella* group should be classified as species (figure 10.4b-g), although the *Cornus florida* fly and sparkleberry fly appear to be just at the 5% cutoff point for species status. Finally, there were few instances of significant single locus deviations from Hardy-Weinberg equilibrium for any of the five taxa included in the analysis (total of 19 significant tests out of 393 conducted, none significant on a tablewise basis using the sequential Bonferroni method; Rice, 1989).

Figure 10.4a-d (first column of graphs in the figure) highlights the continuum in the levels of genetic differentiation existing between *R. pomonella* host races, and sibling species. This observation lends credence to Bush's (1966, 1969a,b, 1975a,b, 1992) argument for the fluid nature of sympatric race and species formation in the *R. pomonella* group, that host races represent the initial, formative stage in a gradual, sequential process triggered by ecological adaptation that results in sympatric speciation. Further work is still needed, however, on the transition period from host races to sibling species to confirm the rate and chronology of sympatric speciation in the *R. pomonella* group (see Berlocher, ch. 8 this volume).

The genotypic cluster method is not foolproof, however. Further work is needed to establish guidelines for sorting individuals into clusters when populations do not differ by some a priori criterion, such as their host affiliation. An exhaustive genetic data set is also required to perform the analysis that may be prohibitive for certain

Figure 10.4. Genotypic cluster analysis for *R. pomonella* group flies based on a 17-locus allozyme data set (see h for a list of the 17 loci). Flies included in the study were the apple- and hawthorn-infesting populations of *R. pomonella*, the undescribed *Cornus florida* fly (collected from flowering dogwood), the undescribed sparkleberry fly (collected from *Vaccinium aboreum*) and *R. mendax* (collected from high bush blueberries [*V. corymbosum*] and deerberries [*V. stamineum*]). Lod scores (= \log_{10} of the likelihood ratio that a fly comes from one taxon versus another given the fly's genotype) were calculated for individual flies collected from pairs of different host plants at sympatric sites or sites in close geographic proximity (see h for a list of study sites and collecting years). The results for a given pair of taxa were then combined across sites to generate the composite distributions of lod scores shown in a-g. To calculate lod scores, genotype frequencies were first determined at each of the 17 allozyme loci in the two populations (p1, p2) infesting alternative host plants (h1, h2, respectively) at a site. For a given fly collected from either h1 or h2, the frequency of the genotype that the fly possessed at locus 1 in p1 was divided by the frequency of that genotype in p2. Next, the product of these individual locus ratios was taken across all 17 loci. The logarithm base 10 of this product represents the \log_{10} likelihood ratio (or lod score) that a fly of a given genotype infested h1 versus h2. Plots of the distributions of these ratio scores over 0.5 unit intervals appear in a-g. Also given are the mean lod scores averaged across sites for each taxa in the various pairwise comparisons (designated by X), the number of individuals collected from a given host plant that would be mistyped as belonging to the alternative taxa over the total number of individuals sampled, the percentage of misclassifications, and the sites included in the analysis.



- h.)
- | | |
|---------------------------|------------------------------|
| Collecting Sites: | Allozymes (17 Loci): |
| 1. Grant, MI. 1984 | Aconitase-1&2 |
| 2. Ephriam, WI. 1984 | Adenylate kinase-2 |
| 3. Gas City, IN. 1987 | Alcohol dehydrogenase-1 |
| 4. Urbana, IL. 1986 | Aldolase |
| 5. Fairfield, IL. 1981 | Aspartate aminotransferase-2 |
| 6. Sawyer, MI. 1985 | NADH-Diaphorase-1&2 |
| 7. Amherst, MA. 1986 | Fumarase |
| 8. Princeton, N.J. 1988 | Glucosephosphate isomerase |
| 9. Beltsville, MD. 1989 | Hydroxy acid dehydrogenase |
| 10. Bowling Gr., VA. 1989 | Isocitrate dehydrogenase |
| 11. Clark Hill, S.C. 1989 | Malate dehydrogenase-1&2 |
| 12. Fort Valley, GA. 1989 | Mannose phosphate isomerase |
| 13. Tuskegee, AL. 1989 | Phosphoglucomutase |
| 14. Gainesville, FL. 1989 | Triosephosphate isomerase |
| 15. Nacogdoches, TX. 1989 | |

taxa. Along these lines, additional thought is required to control for the number and type of genes included in an analysis. Finally, the method has its own unique peculiarities. For example, it is possible for two subpopulations that recently became completely reproductively isolated to still be considered the same species because they have yet to accumulate sufficient genetic divergence to sort themselves into discernible clusters. But the genetic cluster definition does provide a criterion for species demarcation that at least in principle could be uniformly applied across taxa. The definition does not constrain species to monophyletic or bifurcate origins, nor does it bias our thinking as to how clusters (species) form (Mallet, 1995). And it has the added bonus of potentially working for both sexual and asexual taxa.

An Open Mind toward Speciation

In conclusion, *Rhagoletis pomonella* may not be that unconventional after all, at least not if you were to have asked Charles Darwin. Darwin said from the beginning that speciation was the natural outcome of populations adapting to different environments. This certainly appears to be the case in *Rhagoletis*. Also, the apparently fluid nature of races and species in the *R. pomonella* group (see Berlocher, ch. 8 this volume) is what he said would be expected from an active process like speciation. Benjamin Walsh certainly would not disagree and would add the caveat that complete geographic isolation is not always required to initiate speciation.

So why in these current times do so many consider *R. pomonella* so unconventional and sympatric speciation so controversial? The following passage from C. I. Wu (1996) denotes a commonly held belief that

among all differences between species, the traits contributing to reproductive isolation are the most intriguing. Although in itself the phenomenon seems to make no sense (what good does it do to produce sterile progeny?), reproductive isolation is crucial for nascent species to continue their divergence without their unique innovations being lost by blending through gene migration. (p. 105)

Instead of dwelling on the history of this view, I think it more insightful to say that it is "the production of nascent species, rather than the acquisition of hybrid inviability or sterility, that seems the most intriguing event in speciation. To achieve this, presumably adaptive innovations must be protected from gene flow by selection" (J. Mallet, personal communication). *Rhagoletis* flies are controversial because they argue that speciation can be triggered by natural selection. This is not to say that reproductive isolation itself is directly selected for, but that isolation evolves as an inadvertent by-product of adaptation to the environment (Rice and Hostert, 1993). We therefore should not think of reproductive isolation in

purely genetic terms divorced from external ecological pressures. The fitness of a phenotype is, after all, a product of the interaction of genes with the environment as mediated through development. Consequently, the ruckus over sympatry is somewhat tangential to the central issue of disruptive selection. For given that new innovations can be sheltered from gene flow by selection, the complete allopatric separation of populations no longer becomes an absolute prerequisite for divergence. This does not mean that geographic considerations do not and have not often facilitated the adaptive divergence of populations. Allopatry certainly relaxes the initial restriction of antagonistic pleiotropy associated with sympatry, making it easier for divergence to occur via linkage (i.e., the sequential fixation of alternative sets of alleles at linked genes that each confer a selective advantage in one habitat but that have no or even detrimental effects on fitness in alternative environments relative to the ancestral allelic state). The possibility of reproductive isolation caused by negative epistasis and drift is also enhanced. But sometimes divergent selection pressures may be strong enough to pull populations apart in sympatry. Is it any wonder that Darwin was ambivalent when it came to geography?

The reascension of Darwin's view for a direct role of the environment and natural selection in speciation may be Guy Bush's and *Rhagoletis*'s most long-lasting legacy. As Guy is wont to say, "to understand the critical early stages of speciation, one must fully understand the biology of one's study organism(s). This is how one can identify the key traits under selection that initiate population divergence. Each case is likely to be different and have its own particular set of circumstances and rules" (Bush, personal communication).

If nothing else, this volume testifies to Guy's view that a great diversity of patterns and processes underlies speciation, a view that was fostered and nurtured by Bush through his constant reminders to us to keep an open mind concerning speciation.

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