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Are the apple maggot, *Rhagoletis pomonella*, and blueberry maggot, *R. mendax*, distinct species? Implications for sympatric speciation

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Abstract

Rhagoletis pomonella (Walsh) and *R. mendax* (Curran) (Diptera : Tephritidae) are major economic pests of apple and blueberry fruits, respectively, in eastern North America. The taxonomic status of these flies as distinct species has been in dispute because of their close morphological similarity, broadly overlapping geographic distributions and inter-fertility in laboratory crosses. Starch gel electrophoresis of soluble proteins was performed to establish the extent of genetic differentiation and levels of gene flow between blueberry infesting populations of *R. mendax* and apple and hawthorn infesting populations of *R. pomonella*. *R. mendax* and *R. pomonella* were found to be genetically distinct sibling species as eleven out of total of twenty-nine allozymes surveyed possessed species specific alleles. Data from three sympatric apple and blueberry fly populations in Michigan indicated that these flies do not hybridize in nature and gave no evidence for nuclear gene introgression. Differences in host plant recognition were implicated as important pre-mating barriers to gene flow between *R. pomonella* and *R. mendax*; a result supporting a sympatric mode of divergence for these flies.

Introduction

The taxonomic distinction between the apple maggot fly, *Rhagoletis pomonella* (Walsh), and the blueberry maggot fly, *R. mendax* (Curran), has been argued throughout this century. Confusion stems from the fact that these flies are morphologically very similar, have broadly sympatric geographic distributions and produce viable and fertile progeny in laboratory crosses (Bush, 1966; J. Frey, pers. comm.). Morphological differences in ovipositor length, femur coloration and wing band ratios led Bush (1966) to conclude that *R. pomonella* and *R. mendax* were distinct species. However, others consider *R. pomonella* and *R. mendax* to be potentially interbreeding host races rather than species (Diehl & Prokopy, 1986).

Determination of the level of gene flow and genetic divergence between apple and blueberry flies has important implications to models of sympatric speciation proposed for certain *Rhagoletis* species groups (Bush, 1969a, 1975). Because adult *Rhagoletis* flies mate almost exclu-

sively on or near the fruits of their host plant (Bush, 1969b, Prokopy *et al.*, 1971, 1972), mate choice is directly coupled to host selection. Variation for behaviors involved in host plant recognition may, therefore, produce pre-mating barriers to gene flow and result in the sympatric divergence of fly populations adapted to different hosts. Although host related phenotypic differences do not exist between *R. pomonella* and *R. mendax*, these flies still overlap in their host acceptance behaviors, host associated larval survivorship and adult emergence times (Diehl & Prokopy, 1986; Bierbaum & Bush, 1988 and submitted). Movement of *R. pomonella* and *R. mendax* between hosts with subsequent 'hybridization' is therefore possible under circumstances where blueberries (*Vaccinium* spp.) and huckleberries (*Galyussacia* spp.), the two primary Ericaceous hosts for *R. mendax*, are present in close proximity to domestic apples (*Malus pumila*) and hawthorns (*Crataegus* spp.), the primary Rosaceous hosts for *R. pomonella*. If substantial gene flow occurs between sympatric apple and blueberry maggot populations then *R. pomonella* and *R. mendax* should be recognized as 'host races' or 'biotypes' and some form of geographic isolation would probably be necessary for these flies to speciate.

Previous allozyme work revealed that although *R. mendax* and *R. pomonella* are very closely related, they do differ at the *fumarase* locus for which the common allele in *R. mendax* is absent from *R. pomonella* populations (Berlocher, 1980; Berlocher & Bush, 1982). This result suggests that either (1) gene flow is restricted at least unidirectionally from *R. mendax* to *R. pomonella* or (2) selection against *R. mendax* larvae in apples is intense and correlated with the *fumarase* locus. Larval survivorship studies, however, have shown only moderate selective differences associated with the host fruit environment (Bierbaum & Bush, submitted). Larval selection, therefore, cannot account for the absence of the *R. mendax fumarase* allele in *R. pomonella* populations. Nevertheless, concern was expressed that apple and blueberry flies used in the allozyme study originated from populations located hundreds of kilometers apart and that few populations and individual flies were analyzed (Diehl & Prokopy, 1986).

The current electrophoretic study examines the extent of hybridization and genetic divergence between *R. pomonella* and *R. mendax*. The experiment addresses problems with the earlier work by genetically analyzing several sympatric fly populations in western Michigan, U.S.A., as well as geographically distant populations from Nova Scotia, Canada, and Door County, Wisconsin, U.S.A. The results indicate that *R. pomonella* and *R. mendax* are genetically distinct and give no evidence that nuclear gene flow is occurring between these two sibling species in nature.

Materials and methods

Geographic and host distributions of flies. The geographic distribution of *R. mendax* is entirely contained within that of the more widespread *R. pomonella* in eastern North America (Bush, 1966). The blueberry maggot can be found from Nova Scotia, Canada, to Florida, U.S.A., and as far west as Michigan, U.S.A. *R. mendax* prefers Ericaceous host plants in the genera *Vaccinium* (blueberries) and *Gaylussacia* (huckleberries) whose fruits are similar in many respects (Bush, 1966). *R. mendax* larvae have also been reported infesting wintergreen (*Gaultheria procumbens*) and cranberries (*Vaccinium oxycoccoides*) but these two plants do not seem to be suitable for supporting large populations of the fly (Bush, 1966). In the northeastern United States and eastern Canada, *R. mendax* primarily attacks native low bush blueberries and huckleberries. From Michigan and New Jersey south to Florida, the fly can also be reared from domesticated high bush blueberries. A suggestion has been made that the high and low bush infesting forms of the blueberry maggot represent partially reproductively isolated host races (Diehl & Prokopy, 1986).

The distribution of the apple maggot differs from that of the blueberry maggot in that it occurs continuously as far west as Minnesota, U.S.A.

and in an isolated population in the highlands of Mexico. The apple maggot has also recently been introduced into several western states in the U.S.A. *R. pomonella* parasitizes a wide variety of Rosaceous hosts including apples, cherries, hawthorns, and occasionally plums, pears and rose hips (Herrick, 1920; Bush, 1966; Shervis *et al.*, 1970; Prokopy & Bush, 1972; Prokopy & Berlocher, 1980). However, apple and hawthorn infesting populations of *R. pomonella* predominate across the range of the fly.

Sampling scheme. Apple and blueberry infesting flies were collected from both western and northeastern regions of *R. mendax*'s range in Michigan and Nova Scotia (Fig. 1). All flies analyzed in the study were collected as larvae from infested fruit and were reared to adulthood in the laboratory. The Michigan collection consisted of three pairs of sympatric apple and the high bush blueberry infesting populations (Fig. 1, Table 1). Site 3 (MacMartin house) represents a location in Chickaming, Michigan, where host plants were in physical contact. At the other two sympatric Michigan sites, apple orchards and blueberry patches were separated by a distance of 1 km, which is still well within the cruising range of *Rhagoletis* adults (Phipps & Dirks, 1933; Bourne *et al.*, 1934; Maxwell, 1968; Neilson, 1971). Apple and blueberry flies were collected at the Michigan sites in both 1984 and 1985, with the exception that a blueberry crop failure prevented collecting at site 1 in 1985. *R. pomonella* flies were also sampled from hawthorns near Grant, Michigan (site 4) in 1985 and from hawthorns and apples outside of the range of *R. mendax* in Door Co., Wisconsin (site 5) in 1985.

Table 1. Collecting sites for *R. pomonella* and *R. mendax*. Numerical designations are given for sites along with the year and host fruit sampled. A = domestic apple, B(H) = high bush blueberry, B(L) = low bush blueberry, H = hawthorn

Site #	Location	Fruit collected	Year(s) collected
1.	Maple Island Rd, near Sullivan, Mich., U.S.A.	A, B(H)	84, 85
2.	Highway 46, near Mooreland, Mich., U.S.A.	A, B(H)	84, 85
3.	MacMartin home, Chickaming, Mich., U.S.A.	A, B(H)	84, 85
4.	112th Street, Grant, Mich., U.S.A.	H	85
5.	Ephraim, Door Co., Wisc., U.S.A.	H, A	85
6.	Research Station, Kentville, Nova Scotia, Canada	A	85
7.	Parrsboro, Nova Scotia, Canada	B(L)	85

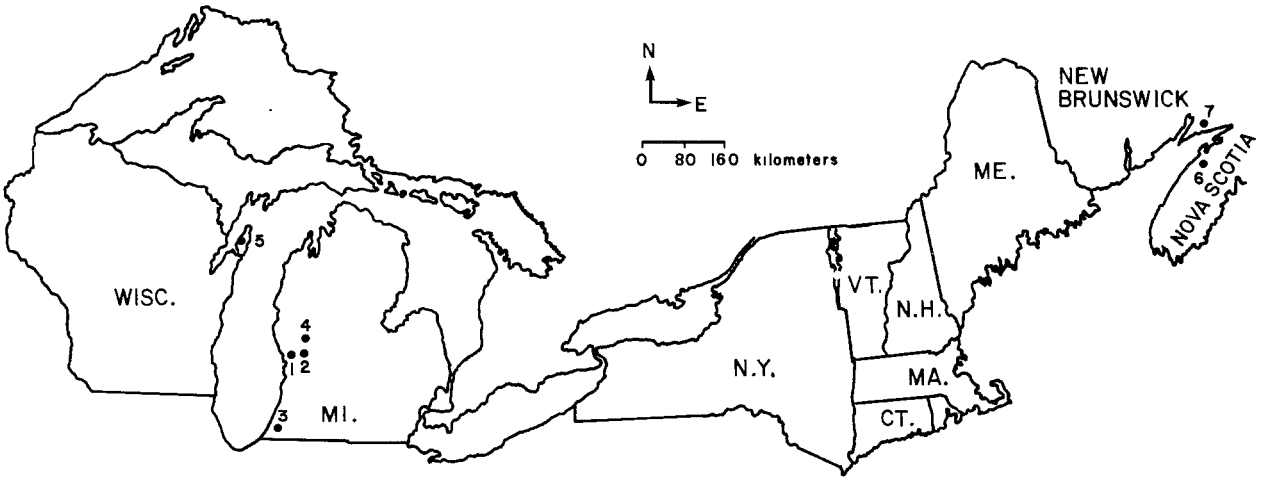

Fig. 1. Collecting sites for *R. mendax* and *R. pomonella* across the eastern United States and Canada. Site designations and descriptions are given in Table 1.

Flies from Nova Scotia were kindly supplied by Dr. Willis Neilson. *R. pomonella* were collected from apples in 1985 on the grounds of the agricultural research station in Kentville, Nova Scotia (site 6). *R. mendax* were sampled from endemic low bush blueberries in 1985 near Parrsboro, Nova Scotia (site 7), which is located approximately 100 km northwest of Kentville.

Electrophoresis. Standard starch gel electrophoretic techniques were used and are described fully elsewhere (Berlocher & Bush, 1982; Feder *et al.*, in press). Only adult flies were analyzed electrophoretically. Electromorphs were numerically designated according to their relative anodal mobilities with the most common allele at a locus for *R. pomonella* assigned a mobility of 100 and used as a standard. Isozymes that migrated the nearest to the cathode were designated system 1, the second nearest system 2, etc. A genetic basis has been established for all of the polymorphic loci scored in this study except for *NADH-diaphorase-3* (Berlocher & Smith, 1983; Feder *et al.*, in press; S. Berlocher, pers. comm.).

Data analysis. Significant deviations from Hardy-Weinberg genotypic expectations were determined by *G*-test. Alleles were pooled, as required, so that the genotype numbers of all classes were ≥ 1. The Levene correction (Spiess, 1977) was applied for sample sizes of < 100 or if genotypic classes had expected numbers < 1. *G*-contingency statistics were used to test for allele frequency heterogeneity. *G*-tests were performed on allele numbers rather than on frequencies to give a weighted *G* value for each test. Alleles for a locus were pooled, when necessary, to ensure that each cell in the *G*-test had an observed number ≥ 5. F-statistics were calculated by the method of Weir and Cockerham (1984) with variances estimated by jackknifing over populations or loci. Linkage disequilibrium was determined between non-allelic genes using the method of Burrows (Cockerham & Weir, 1977). Burrows disequilibrium values (Δ) were tested for significance by single degree of freedom Chi-square tests (Cockerham & Weir, 1977). Genetic distance measures were derived by the formula of Nei (1972) with variances estimated by jackknifing over loci.

Results

Twenty-nine allozyme loci were resolved electrophoretically (see Table 2 for a list of these enzymes along with their abbreviations). Fourteen loci were either monomorphic or showed limited

Table 2. Allozymes resolved for *R. pomonella* and *R. mendax*. Enzyme abbreviations are given in parentheses

Monomorphic or essentially fixed enzymes		Polymorphic enzymes	
Napthyl-acid phosphatase	(*Acph*)	Aminoacylase	(*Acy*)
Aconitase-1	(*Acon-1*)	Aconitase-2	(*Acon-2*)
Alcohol Dehydrogenase-1 & 2	(*Adh-1 & 2*)*	Adenylate kinase	(*Ak*)
Aldolase	(*Aldo*)	Aspartate amino transferase 1 & 2	(*Aat-1 & 2*)
NADH-diaphorase-1	(*Dia-1*)	NADH-diaphorase-2 & 3	(*Dia-2 & 3*)
Hexokinase	(*Hk*)	Fumarase	(*Fum*)
Malate dehydrogenase-1 & 2	(*Mdh-1 & 2*)*	β-Hydroxyacid dehydrogenase	(*Had*)
Peptidase-1 & 3	(*Pep 1 & 3*)	Isocitrate dehydrogenase	(*Idh*)
Superoxide dismutase	(*Sod*)	Malic enzyme	(*Me*)
Trehalase	(*Tre*)	Mannose phosphate isomerase	(*Mpi*)
Triose phosphate isomerase	(*Tpi*)	Peptidase-2	(*Pep-2*)
		Phosphoglucose isomerase	(*Pgi*)
		Phosphoglucomutase	(*Pgm*)

* Indicates an essentially fixed locus with the frequency of the common allele > 0.95.

variability (frequency of the common allele greater than 95%) for the same allele in both blueberry and apple populations (Table 2). The remaining 15 loci were polymorphic in either *R. mendax* or *R. pomonella* (Table 2; Appendices 1 and 2). Most polymorphic loci, excluding *Aat-1* which is sex-linked (Feder *et al.*, in press), were in Hardy-Weinberg equilibrium (data not shown). The 17 significant deviations observed in 298 tests did not differ appreciably from the number expected due to random type I error. The same was also true for the sex-linked allozyme *Aat-1* for females in that no significant deviation from Hardy-Weinberg equilibrium was observed for the locus out of 13 tests (females are the homogametic sex in Diptera).

R. mendax and *R. pomonella* were found to be genetically distinct sibling species. Although none of the 15 polymorphic loci resolved in the study was diagnostically fixed for alternative alleles in *R. mendax* and *R. pomonella* populations, 'species-specific' alleles were found for 11 different allozymes (*Aat-1 & 2*, *Dia-2 & 3*, *Acon-2*, *Acy*, *Pep-2*, *Fum*, *Pgi*, *Ak* and *Had*; see Appendices 1 and 2). In addition, *Me* showed highly significant gene frequency differences (0.40 or more) between sympatric apple and blueberry populations in Michigan (Appendix 1).

Species-specific alleles were not always present in high frequencies in *R. pomonella* or *R. mendax* populations. For instance, Ak^{111} and Had^{97} generally had frequencies of 5% or less for *R. pomonella* and *R. mendax*, respectively (Appendix 2). However, the common alleles for the allozymes *Aat-1* and *Dia-2* in *R. pomonella* were not present in any of the Michigan or Nova Scotia populations of *R. mendax* (Appendices 1 and 2). Conversely, Fum^{158} and $Dia\text{-}3^{91}$, the common alleles in *R. mendax* populations, were virtually absent from *R. pomonella* populations. One Fum^{158} and two $Dia\text{-}3^{91}$ alleles were scored for one apple infesting fly at site 3 (Chickaming, Michigan) in 1985 (Appendix 2). However, no other fly reared from apples possessed either of these two alleles.

The genotype of the aberrant apple infesting fly from Chickaming, Michigan, implies that it is *R. mendax* and not either *R. pomonella* or a F1 hybrid. *Dia-3* gene frequencies for *R. mendax* and *R. pomonella* at site 3 (Appendix 2) indicate that a F1 hybrid could be either heterozygous for $Dia\text{-}3^{91}/Dia\text{-}3^{100}$ or homozygous for $Dia\text{-}3^{100}$ but not homozygous for $Dia\text{-}3^{91}$ as the aberrant fly is. Also, 18% of the blueberry flies analyzed at site 3 have the same two locus genotype for *Fum* and *Dia-3* as the fly in question while no other apple fly does. Furthermore, if *Dia-3* and *Fum* are excluded from consideration, the probability of possessing the same genotype as the aberrant fly for the remaining 13 polymorphic loci is approximately 1×10^{-12} for an apple fly. In comparison, a blueberry fly has a more reasonable probability of 2×10^{-4} of being genetically identical.

F-statistics and genetic distance measures were calculated to quantify the amount of genetic divergence between *R. mendax* and *R. pomonella*. F-statistics derived by the method of Weir and Cockerham (1984) based on all 15 polymorphic loci revealed an overall F_{ST} value of 0.3106 ± 0.0600 (jackknife estimate of standard deviation calculated over loci) among apple and blueberry fly populations in 1985. This level of genetic differentiation is not atypical of values for other insect species and is at least an order of magnitude greater than reported intraspecific F_{ST} values (McCauley, 1987). Apple and blueberry flies were separated by an overall Nei genetic distance of 0.230 ± 0.078 (jackknife estimate of standard deviation calculated over loci) based on all 29 loci resolved in the study. The amount of genetic differentiation between *R. mendax* and *R. pomonella* was suprising because previous work (Berlocher & Bush, 1982) suggested that the sibling species were much more closely related being separated by a Nei distance of only 0.0750. It should be noted, however, that the Nei distance derived in the earlier work was based on a set of allozyme loci that only partially overlapped with that of the current study. For example, staining methods for *Aat-1*, *Dia-2* and *Dia-3* (three loci which have species specific alleles in high frequencies) were not perfected in *Rhagoletis* at the time of Berlocher and Bush's (1982) original survey of the genus.

Genetic analysis of hawthorn flies from Michigan and Wisconsin showed that they are very closely related to apple flies sharing all electromorphs in common (Appendices 1 and 2). Nei distances of only 0.0080 ± 0.0039 and 0.0112 ± 0.0062 were calculated between hawthorn and apple flies from Michigan and Wisconsin, respectively. These Nei distances fall within the range of values normally found for intra-specific comparisons between insect populations (see Figure 2 of Menken & Ulenberg, 1987). In addition, none of the unique *R. mendax* alleles was observed in any other hawthorn population of *R. pomonella*. This result suggests that reproductive isolation between hawthorn and blueberry flies is as complete as that between apple and blueberry flies. Sympatric hawthorn and blueberry sites are needed, however, to entirely rule out the possibility of introgression between these flies.

The electrophoretic data also lend little support for the suggestion of Diehl and Prokopy (1986) that low and high bush blueberry maggot populations represent partially reproductively isolated host races, as the overall Nei genetic distance between low and high bush forms of *R. mendax* (0.0111 ± 0.0047) was not significantly different from the value between apple populations of *R. pomonella* from Michigan and Nova Scotia (0.0073 ± 0.0031). Observed frequency differences between blueberry fly populations are therefore most likely due to geographic differentiation. Verification of this point, however, requires analysis of sympatric high and low bush blueberry populations.

Hybridization is rare, if it occurs at all, between sympatric blueberry populations of *R. mendax* and apple infesting *R. pomonella*. The diagnostic alleles for *Fum*, *Dia-2*, and *Dia-3* can be used to calculate a statistical confidence level for hybridization between blueberry and apple flies. Based on genotypic frequencies at the three sympatric Michigan sites, whenever *R. pomonella* and *R. mendax* mate approximately 43% of the resulting F1 hybrid offspring should be triple locus heterozygotes for *Fum*, *Dia-2*, and *Dia-3*. No fly was heterozygous for all three loci, however, out of a total of 654 flies analyzed; a result significant at the $P \leq 0.05$ level assuming a Poisson distribution with hybridization occurring at a frequency of 1.06% or greater.

The preceeding analysis assumes that *Fum*, *Dia-2*, and *Dia-3* are in linkage equilibrium and that viabilities are the same for hybrid larvae as they are for conspecific crosses. To determine the extent of genetic co-variation among *Fum*, *Dia-2*, and *Dia-3*, correlation coefficients based on Burrows disequilibrium values (Cockerham & Weir, 1977) were calculated between pairs of non-allelic genes. Significant disequilibrium was not observed for any test involving *Fum*, *Dia-2*, and *Dia-3* (data not shown) and, therefore, gene frequencies at these three loci appear to be evolving independently. The assumption of normal survivorship for F1 hybrid progeny may not be completely valid, however. Fecundities for interspecific matings, as measured by survivorship of F1 progeny to adulthood, can vary widely from cross to cross and may be reduced by as much as 50% compared with conspecific matings (W. Neilson, pers. comm.). However, unless tri-locus heterozygotes for *Fum*, *Dia-2*, and *Dia-3* have disproportionately lower viabilities (which is doubtful), *R. mendax* × *R. pomonella* crosses would still produce genotypically distinguishable hybrids. The frequency of detecting F1 hybrids, should, therefore, be reduced only in relation to the general decrease in hybrid survivorship.

Intraspecific allele frequencies were reasonably consistent across years at the Michigan sites. Six out of a total of 75 *G*-contingency tests indicated significant gene frequency differences across years at a given location (Table 3). Although the number of significant tests is higher than that expected by random type I error, the most pronounced frequency difference observed between 1984 and 1985 was 0.170 for $Pep\text{-}2^{110}$ for apple flies at site 1 (Appendix 1). Nei genetic distances based on only the 15 polymorphic loci were just 0.0030 in comparisons of pooled *R. mendax* populations between 1984 and 1985 and 0.0047 for *R. pomonella* populations.

Geographic variation was apparent across both *R. mendax* and *R. pomonella* populations.

Table 3. *G*-contingency tests for allele frequency heterogeneity across years and sites for *R. mendax* and *R. pomonella* populations (degrees of freedom for tests are given in parentheses). Allele frequencies for loci not showing significant variation within Michigan were pooled across populations and tested against the corresponding host population in Nova Scotia (N.S.) or Wisconsin. *Acon*-2, *Fum* and *Pgm* for *R. mendax* were tested on a site by site basis between Michigan and N.S.

Test	*R. mendax*	*R. pomonella*
Between years ('84-'85) at a given site	*Acon-2*/Site 3(3)*	*Aat-2*/[Site 1 (3)*, Site 2(3)*] *Acon-2*/[Site 1 (4)*, Site 2(4)*] *Pep-2*/[Site 1 (2)*]
Among Michigan sites ('84)	*Pgm* (2)***	*Acon-2* (8)***, *Aat-2* (6)* *Me* (2)*, *Mpi* (2)*, *Pgm* (2)**
Among Michigan sites ('85)	*Acon-2* (3)**, *Pgm* (1)*** *Fum* (1)**	
Between pooled Michigan sites and Nova Scotia (N.S.) ('85)	*Had* (1)*, *Me* (1)** *Pgi* (1)**	*Acon-2* (4)***, *Aat-2* (3)** *Aat-1* (1)*, *Me* (1)** *Pep-2* (2)*, *Pgi* (1)**
Between site 2/N.S. ('85) (Tests for Acon-2, Fum, Pgm)	*Acon-2* (3)***, *Fum* (1)*** *Pgm* (1)**	-
Between site 3/N.S. ('85) (Tests for Acon-2, Fum, Pgm)	*Acon-2* (3)***, *Pgm* (1)*	-
Between pooled Michigan apple sites and Wisconsin apple sites ('85)	-	*Had* (1)**, *Acon-2* (2)***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Overall F_{ST} values were significantly greater than zero in 1985 across both *R. mendax* and *R. pomonella* populations (0.0399 ± 0.0126 and 0.0157 ± 0.0070, respectively). Nine out of a total of 60 *G*-contingency tests indicated significant intraspecific allele frequency differences among Michigan populations in 1984 and 1985 (Table 3). Allele frequencies for loci not showing significant variation among Michigan populations in 1985 were pooled and tested for heterogeneity against populations from the same host in Nova Scotia and Wisconsin. Six out of a total of 15 loci tested showed significant allele frequency differences between apple maggot flies from Michigan and Nova Scotia while 2 of 15 tests were significant between Michigan and Wisconsin (Table 3). $Acon^{75}$ and Pgm^{111}, found in Michigan *R. pomonella* populations at average frequencies of 0.193 and 0.055, respectively, were not observed at the Kentville, Nova Scotia site (Appendix 1). All but the rarest alleles were shared in common, however, between Wisconsin and Michigan populations of *R. pomonella*. Six loci also displayed significant frequency differences between blueberry flies from Nova Scotia and at least one of the Michigan sites (Table 3). $Acon^{95}$, present in Michigan *R. mendax* populations at an average frequency of 0.196, was absent from the Parrsborro, Nova Scotia population (Appendix 1).

Discussion

The allozyme results verify that *R. pomonella* and *R. mendax* are genetically distinct species that hybridize rarely, if at all, in nature. Laboratory and field experiments indicate, however, that *R. mendax* and *R. pomonella* have the potential to hybridize whenever they come in contact. Furthermore, hybrid F1, F2 and backcross larvae produced from laboratory matings are viable and fertile and can be reared to adulthood in either apple or blueberry fruits (J. Frey, pers. comm.).

Premating isolating barriers must, therefore, restrict hybridization between blueberry and apple flies.

What premating barrier(s) restricts hybridization between *R. mendax* and *R. pomonella*? Evidence exists to rule out a number of possibilities. For instance, long range pheromones are not important sexual attractants in the *R. pomonella* group (Boller & Prokopy, 1976). Body coloration and wing pattern are believed to be species recognition cues during courtship within certain *Rhagoletis* species groups (Bush, 1966). However, the morphological similarity of *R. mendax* and *R. pomonella* and the ease with which these flies mate in the laboratory argue against strong ethological isolation. Blueberry flies eclose as adults approximately one week earlier than apple flies. However, sexually mature *R. mendax* and *R. pomonella* adults co-occur for over a month at all three sympatric sites in Michigan (J. Feder, pers. obs.). Allochronic isolation, therefore, does not appear to play a major role in maintaining species differences between blueberry and apple flies.

Differential host recognition is the most likely factor limiting contact between apple and blueberry flies. Significant differences have been found in the behavioral and chemosensory responses of *R. mendax* and *R. pomonella* to apples, hawthorns and blueberries (Diehl & Prokopy, 1986: Bierbaum & Bush, 1988; J. Frey, pers. comm.). Because *R. mendax* and *R. pomonella* mate almost exclusively on or near the fruits of their respective host plants (Prokopy *et al.*, 1971, 1972), species specific differences in host identification could produce strong premating barriers to gene flow. The unique alleles possessed by *R. mendax* and *R. pomonella* make it possible to test host plant fidelity through a genetic analysis of adults captured directly from interdigitated blueberry and apple plants. Until this test is completed, indirect evidence related to the blueberry crop failure at site 1 (Sullivan, Michigan) in 1985 suggests that host recognition is accurate and species specific. *R. mendax* did not attack apples or show any sign of introgression with *R. pomonella* at Sullivan despite the fact that blueberries were absent from the site in 1985 and apples were the only readily available host fruit.

Host plant recognition, although accurate, may not be absolutely perfect for *Rhagoletis* flies. One fly reared from apples in this study did possess a genotype characteristic of *R. mendax*. One possible explanation is that *R. mendax* females occasionally lay their eggs into apples. If true, the results of this study suggest that such host identification mistakes are rare and have little effect on promoting nuclear gene flow between the two species. Plasticity in host recognition may, however, be important in an evolutionary sense in allowing *R. pomonella* group flies to colonize and establish permanent populations on new host plants. A second explanation is that the *R. mendax* fly in question became accidently mixed with *R. pomonella* flies during rearing. Indirect support for the second possibility comes from the fact that *R. mendax* females have difficulty puncturing the skin of certain domestic apple varieties with their ovipositors (Diehl & Prokopy, 1986; Bierbaum & Bush, 1988). These mechanical difficulties reduce the likelihood that a *R. mendax* female laid her eggs into apples at the MacMartin site.

The inability of *R. mendax* females to lay their eggs into several apple varieties normally infested by *R. pomonella* could be an important element isolating the two species. However, the difficulties *R. mendax* has ovipositing into apples do not prevent *R. mendax* females from mating with *R. pomonella* males if blueberry fly females alighted on apples or apple fly males landed on blueberries. Mated *R. mendax* females could subsequently deposit their eggs into blueberries thereby promoting gene flow between *R. pomonella* and *R. mendax*. Thus, contact between *R. mendax* and *R. pomonella* can result in hybridization and potential introgression in spite of differences in ovipositional preference. Furthermore, although *R. mendax* females have difficulty ovipositing into certain apple varieties, they have little trouble penetrating hawthorns (Diehl & Prokopy, 1986), which are the native host of *R. pomonella* (Bush, 1966). However, genetic analysis of hawthorn flies suggests that they are as

genetically distinct from *R. mendax* as apple flies are.

Post mating reproductive isolation may also limit introgression between *R. pomonella* and *R. mendax*. Although apple and blueberry flies can be easily crossed, viability to adulthood may be reduced by as much as 50% for hybrid progeny (W. Neilson, pers. comm.). Although F1 hybrids are fertile (J. Frey, pers. comm.), adequate studies have not been done to determine the extent to which hybrid breakdown occurs in F2 and backcross progeny. However, even if postmating isolation exists between *R. pomonella* and *R. mendax* as a result of hybrid breakdown, it would not stop the formation of F1 progeny. Because F1 hybrids were not observed in the field, differences in host recognition must limit hybridization between *R. mendax* and *R. pomonella*.

Assortative mating due to differences in host recognition behaviors is an important component of sympatric speciation models. The direct connection in *Rhagoletis* between host recognition and mate selection could result in the sympatric divergence of host associated populations in the absence of geographic barriers to gene flow. Unfortunately, we may never know the exact chronological order or geographic context of hosts shifts which occurred during the evolution of the *R. pomonella* group. Therefore, it is difficult to directly assess the involvement of host plant fidelity in the speciation process. Nevertheless, host preference differences certainly help maintain divergence between *R. mendax* and *R. pomonella*. In the absence of these differences it is conceivable that populations of blueberry and apple flies would fuse. More will be known about this possibility after studies on hybrid sterility and breakdown are completed. Such studies will also be useful in allowing us to fine tune our estimated confidence level of hybridization between *R. mendax* and *R. pomonella*. If, however, F2 and backcross progeny prove to be reasonably viable and fertile then it is difficult to see how host specificity could not have played an important role in speciation. Of course, whether host recognition differences arose in sympatry or allopatry

Appendix 1. Allele frequencies for *Acon-2*, *Acy*, *Pep-2*, *Pgi*, *Me*, *Dia-2* and *Pgm* for *R. mendax* and *R. pomonella* populations across the eastern United States and Canada. Site designations are given in Table 1. Allele frequencies are only given for those electromorphs displaying the greatest amount of interspecific differentiation. A complete list of allele frequencies is available from the first author on request

Species	Site	Year	Host	Acon-2		Acy		Pep-2		Pgi		Me		Dia-2		Pgm	
				N	75	N	89	N	110	N	145	N	80	N	100	N	111
R. mendax	1	84	B	87	0.000	–	–	13	0.000	79	0.000	39	0.051	62	0.000	78	0.051
	2	84	B	79	0.000	–	–	52	0.000	79	0.000	66	0.015	52	0.000	79	0.019
	2	85	B	41	0.000	40	0.000	41	0.000	41	0.000	40	0.088	41	0.000	41	0.000
	3	84	B	72	0.000	–	–	29	0.000	72	0.000	72	0.076	72	0.000	72	0.028
	3	85	B	55	0.000	14	0.000	55	0.000	55	0.000	53	0.066	55	0.000	55	0.027
	7	85	B	39	0.000	–	–	13	0.000	39	0.000	39	0.000	39	0.000	39	0.000
R. pomonella	1	84	A	88	0.091	–	–	24	0.062	79	0.126	39	0.451	54	0.657	79	0.038
	1	85	A	41	0.171	41	0.158	41	0.232	43	0.139	42	0.512	42	0.560	43	0.047
	2	84	A	79	0.101	–	–	13	0.118	79	0.063	64	0.508	52	0.557	79	0.032
	2	85	A	38	0.263	39	0.154	40	0.175	38	0.079	39	0.615	38	0.632	39	0.026
	3	84	A	71	0.303	–	–	–	–	72	0.049	45	0.644	46	0.717	70	0.093
	3	85	A	44	0.227	44	0.125	44	0.239	45	0.100	45	0.622	45	0.656	45	0.089
	4	85	H	169	0.068	166	0.208	169	0.172	157	0.080	156	0.256	169	0.843	156	0.010
	5	85	H	51	0.000	51	0.167	51	0.216	51	0.079	51	0.196	51	0.765	51	0.039
	5	85	A	49	0.010	50	0.130	50	0.150	51	0.069	52	0.539	50	0.700	51	0.030
	6	85	A	39	0.000	–	–	13	0.077	39	0.025	39	0.398	39	0.731	39	0.000

Appendix 2. Allele frequencies for *Fum*, *Dia-3*, *Aat-1*, *Aat-2*, *Ak*, and *Had* for *R. mendax* and *R. pomonella* populations across the eastern United States and Canada. Site designations are given in Table 1. Allele frequencies are only given for those electromorphs displaying the greatest amount of interspecific differentiation

Species	Site	Year	Host	Fum		Dia-3		Aat-1		Aat-2		Ak		Had		
				N	158	N	89	N	−100	N	100	N	111	N	97	122
R. mendax	1	84	B	89	0.775	89	0.848	44	0.000	86	0.000	59	0.000	89	0.011	0.888
	2	84	B	79	0.798	79	0.892	66	0.000	79	0.000	75	0.000	79	0.000	0.785
	2	85	B	41	0.829	41	0.927	41	0.000	41	0.000	41	0.000	41	0.000	0.793
	3	84	B	72	0.701	72	0.917	72	0.000	72	0.000	72	0.000	72	0.014	0.771
	3	85	B	54	0.630	55	0.936	55	0.000	54	0.000	55	0.000	55	0.054	0.736
	7	85	B	39	0.910	39	0.744	39	0.000	39	0.000	39	0.000	39	0.000	0.885
R. pomonella	1	84	A	89	0.000	89	0.000	48	0.844	85	0.247	58	0.000	89	0.000	0.258
	1	85	A	42	0.000	31	0.000	42	0.809	42	0.214	42	0.000	48	0.000	0.274
	2	84	A	79	0.000	79	0.000	66	0.803	78	0.263	78	0.000	79	0.000	0.278
	2	85	A	39	0.000	39	0.000	39	0.769	39	0.295	39	0.013	40	0.000	0.263
	3	84	A	72	0.000	72	0.000	50	0.880	72	0.208	71	0.007	71	0.000	0.232
	3	85	A	45	0.022	42	0.024	44	0.784	45	0.233	44	0.046	45	0.000	0.311
	4	85	H	169	0.000	169	0.000	155	0.774	154	0.399	157	0.003	170	0.000	0.121
	5	85	H	51	0.000	51	0.000	50	0.900	51	0.441	50	0.000	51	0.000	0.176
	5	85	A	50	0.000	50	0.000	50	0.700	50	0.300	50	0.020	50	0.000	0.150
	6	85	A	39	0.000	39	0.000	39	0.885	39	0.244	39	0.000	39	0.000	0.205

would still be a matter of conjecture. However, genetic differences have been found between sympatric hawthorn and apple populations of *R. pomonella* (Feder *et al.*, 1988; McPheron *et al.*, 1988). The sympatric shift of *R. pomonella* from hawthorns to domesticated apples, which occurred within the last 150 years (Walsh, 1867), excludes the possibility of prior periods of geographic isolation. If differences in host recognition or some other inherent biological characteristics of apple and hawthorn flies can be related to the observed genetic differentiation, then taxa in the *R. pomonella* group are likely to diverge sympatrically by shifting and adapting to new host plants.

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Résumé

Est-ce que Rhagoletis pomonella *et* R. mendax *constituent des espèces distinctes? Implications pour la spéciation sympatrique*

R. pomonella Walsh and *R. mendax* Curran sont respectivement deux mouches très nuisibles aux pommes et aux myrtilles du N E des USA. La position taxonomique de ces mouches comme espèces distinctes a été longtemps mise en doute par suite de leur grande ressemblance morphologique, de l'important chevauchement de leurs répartitions et de leur interfécondité au laboratoire. L'électophorèse sur gel d'amidon de protéines solubles a été utilisé pour établir l'importance de la différenciation génétique et du flux génique entre *R. mendax* contaminant des myrtilles et *R. pomonella* contaminant des pommiers et des

aubépines. *R. mendax* et *R. pomonella* se sont révélées des espèces jumelles car, à l'exception de 11 alolozymes sur 29, chaque espèce possédait des allèles spécifiques. Les données concernant 3 populations sympatriques de mouches des myrtilles et des pommes du Michigan ont montré que des mouches ne s'hybrident pas dans la nature et n'ont fourni aucune indication sur une introgression de gènes nucléaires. Des différences concernant la découverte de hôtes sont impliquées comme obstacles prézygotiques importants au flux génique entre *R. pomonella* et *R. mendax*; ce résultat conforte l'hypothèse d'une divergence sympatrique de ces mouches.

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