

## HOST PLANT RELATIONSHIPS AND SPECIATION IN LEAF-MINING AGROMYZID FLIES ON UMBELLIFERAE

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### 1. Introduction

Twenty years ago, Bush proposed how two distinctive features of the radiation of phytophagous insects - great species diversity and host specificity - could be causally related, in his model of speciation via formation of host plant races. Working on *Rhagoletis* (Tephritidae), he proposed a model of sympatric speciation with the following important elements: (1) host specificity; (2) mating occurring on the host plant, both sexes being attracted to the host; (3) divergence of populations associated with different hosts (a) initiated by assortative mating among individuals genetically predisposed to select a particular host, and (b) reinforced by directional selection, in which each host-associated population becomes increasingly adapted to its host, leading, for example, to allochronic isolation by synchronization with the host or other ecological factors.

As Bush has emphasized, however, the mode of speciation prevalent in a particular animal group largely depends on features of the biology of the group in question. Therefore, the biological diversity of phytophagous insects may be expected to lead to diversity in the processes of speciation. Zwölfer and Bush (1984) generalized this model to other groups of phytophagous insects and presented a framework for an analysis of speciation.

Leaf-mining Agromyzidae (Diptera) share many features of the biology of *Rhagoletis* that are implicated in speciation via host-race formation. We have initiated an analysis of speciation in this group of phytophagous insects on umbelliferous plants to investigate how applicable Bush's model may be to speciation in other groups of phytophagous insects.

### 2. Biology of leaf-mining agromyzidae

Leaf-mining agromyzids of the genus *Phytomyza* are speciose, highly host-specific endophytophagous flies. Closely related species often occur on different host plants, indicating that, as in *Rhagoletis*, speciation often is associated with a host plant shift. Mating occurs on the host. The different host plants differ strikingly in phytochemistry and ecology, which may lead to selection for divergence. Also as in *Rhagoletis*, *Phytomyza* spp. have a highly aggregated niche structure (Zwölfer & Bush, 1984). Most activities are tightly associated with the host, so that host plant choice strongly influences adult and larval habitat and food, as well as mating biology, pupation and hibernation sites.

Adults emerge in the spring after overwintering as pupae in the soil near the host. Adults of both sexes are attracted to the host plant. Females use their boring ovipositors to make large numbers of "feeding punctures" in the leaves of their host, and eat the cell sap that exudes. On this diet, these tiny flies can live for a month or more. Males come to the host plant to mate. Eggs are laid in leaves of the host. Development from egg to pupa takes about 9-12 days. The fully-grown larva cuts an exit slit and drops to the ground, pupating in the upper layers of the soil.

Leaf-mining *Phytomyza* spp., however, differ from *Rhagoletis* in ways that may be important in processes of speciation. First, in contrast to frugivorous *Rhagoletis*, leaf-mining *Phytomyza* feed on well-defended plant parts, characterized by strongly divergent secondary chemistry. This suggests that survival genes may be more important here than in *Rhagoletis* feeding on ripe pulpy fruits, which are among the least defended parts of plants. Second, leaf-mining *Phytomyza* feed on mature leaves available throughout the growing season, allowing more than one generation per year. This suggests less opportunity for allochronic isolation than in the largely univoltine *Rhagoletis* spp. associated with a seasonally more restricted food source.

### 3. Materials and methods

#### 3.1. Systems studied

Among the *Phytomyza* spp. associated with umbelliferous hosts in Central Europe, three systems common in our region and differing, according to the literature, in degree of divergence, were studied in detail:

- (1) *Phytomyza spondylii* - a species recognized in the most recent literature (Griffiths, 1973) as two distinct species, *P. spondylii* and *P. pastinacae*, each feeding on both *Heracleum sphondylium* and *Pastinaca sativa*;
- (2) *Phytomyza chaerophylli* - an oligophagous species feeding on different hosts of the tribe Scandiceae; its principal hosts in Central Europe are *Anthriscus silvestris* and *Chaerophyllum temulum*. Nowakowski (1962) suggested it may be a complex of monophagous species of host races; and
- (3) *Phytomyza angelicae* - feeding on *Angelica silvestris*, and recently lumped by Griffiths (1973) with *P. laserpitii* on *Laserpitium latifolium*.

#### 3.2. Field studies

Detailed field ecological studies, including population dynamics of the umbelliferous hosts as well as of the leaf-mining flies, were carried out during 1983-1986 in about 30 populations in the southwestern parts of the Swiss Jura and the Upper Rhine Valley. Populations were sampled regularly to provide material for the establishment of laboratory stocks, and for population-genetical investigations using enzyme electrophoresis.

#### 3.3. Laboratory studies

Laboratory studies included experiments on host plant choice, host suitability and mating behaviour, as well as phytochemical investigations (TLC) of the umbelliferous hosts and population-genetic studies of the insects using horizontal starch gel electrophoresis methods described by

Menken (1982). A total of up to 18 enzyme systems were investigated and analysed using Wright's (1951) F-statistics.

#### 4. Results and discussion

##### 4.1. Population structure

Our analysis of population structure, focusing on  $F(st)$ , shows that these flies occur in populations with a high degree of local genetic differentiation.  $F(st)$  is a measure of the degree of differentiation among local subdivisions of a population of a species - in this case, among local populations of the total species population. If  $F(st)$  is zero, then no local differentiation exists. Some very mobile butterflies like *Danaus plexippus*, *Pieris rapae*, and *Yponomeuta cagnagellus* approach this situation (Table 1). In contrast, in the *Phytomyza* species investigated, high  $F(st)$  values are found, comparable to those of sedentary species like *Euphydryas editha*, known to exist in highly local and well differentiated subpopulations. Populations of these leaf-mining flies thus possess the kind of population structure that has been postulated to lead to host-associated divergence in *Rhagoletis*. Further F-statistics,  $F(is)$  and  $F(it)$ , as well as results from our field studies, also indicate that local populations of these agromyzids are small and highly inbred (Table 2).

Table 1. Comparison of the standardized variation ( $F(st)$ ) in different insect taxa (See Latscha, 1986 for references).

Species	Family	$F(st)$
<i>Danaus plexippus</i>	Nymphalidae	.009
<i>Pieris rapae</i>	Pieridae	.014
<i>Yponomeuta cagnagellus</i>	Yponomeutidae	.027
<i>Brosophila pseudoobscura</i>	Drosophilidae	.030
<i>Drosophila melanogaster</i>	"	.044
<i>Drosophila robusta</i>	"	.055
<i>Phytomyza laserpitii</i>	Agromyzidae	.088
<i>Euphydryas chalcedona</i>	Nymphalidae	.090
<i>Euphydryas editha</i>	"	.118
<i>Phytomyza chaerophylli</i>	Agromyzidae	.120
<i>Phytomyza angelicae</i>	"	.148

In summary our results indicate that population structure is such as to allow local divergence of populations, including divergence associated with different host plants.

Table 2. F-statistics (Wright, 1951) for three *Phytomyza* species.

Species	$F(is)$	$F(it)$	$F(st)$
<i>P. chaerophylli</i>	.378	.442	.120
<i>P. angelicae</i>	.433	.513	.148
<i>P. laserpitii</i>	.779	.800	.088

#### 4.2. Host-associated divergence

Is there selection that would drive such divergence? In two of the three cases studied so far, at best very slight divergence caused by genetic drift in small, isolated populations and/or habitat-specific selection could be recognized among populations, suggesting that in these cases such mechanisms are unlikely to produce new species.

(1) *P. spondylii* turned out to be a single species, oligophagous on *H. sphondylium* and *P. sativa*. All females accept both hosts for oviposition and feeding, and no genetic differentiation could be detected so far, resulting in an overall genetic identity (I) between flies on different hosts of  $I = .895$  (Saner, 1986) comparable with values found for conspecific populations on other insect taxa.

(2) Similar results were obtained for the oligophagous *P. chaerophylli* on *A. silvestris* and *C. temulum*. Again, all females accept both hosts for oviposition and feeding, and almost no genetic divergence could be found (overall genetic identity  $I = .974$ ). However, slight but significant differentiation was detected at one locus (Pgm), in which the same alleles occurred at different frequencies in populations associated with different hosts in allopatric situations; where both hosts occurred sympatrically, electromorph frequencies were intermediate (Frey, 1986).

Our so far limited data on host plant secondary chemistry come from TLC of polyacetylenes, coumarins and terpenoids of leaf surface waxes. Though there are quantitative differences of composition, the hosts of *P. spondylii* (*Heracleum* and *Pastinaca*) and of *P. chaerophylli* (*Anthriscus* and *Chaerophyllum*) contain qualitatively similar mixtures of these compounds. Also, the alternative hosts of each of these two leaf-miners grow in very similar habitats and often occur together. Furthermore, the hosts of each species are characterized by nearly identical vegetative phenologies, and - both fly species having multiple overlapping generations - leave little opportunity for allochronic isolation (Fig. 1).

(3) Results for "*P. angelicae*", however, strongly indicate that host-associated divergence and speciation have occurred (Latscha, 1986). "*P. angelicae*" was revealed to consist of two closely related sibling species, *P. angelicae* monophagous on *A. silvestris* and *P. laserpitii* monophagous on *L. latifolium*, differing in four completely diagnostic loci (Idh, Est -1, Est -2, Lap). This indicates that there is effectively no gene flow between them, even where they occur sympatrically. Nei's genetic identity for the two species is  $I = .591$ , a value comparable to sibling species in other insect genera, e.g., *Ectoedemia* ( $I = .547$ ; Wilkinson et al., 1983), *Drosophila* ( $I = .563$ ; Ayala et al., 1974), and *Rhagoletis* ( $I = .741$ ; Morgante et al., 1980).

The host plants of these two very closely related *Phytomyza* spp. are much more divergent chemically and ecologically than in cases (1) and (2). While a similar pattern for polyacetylenes was found in both species, *Angelica* gives spots that match with linear and angular furanocoumarins, whereas in *Laserpitium* - at least in leaves of this one species - no furanocoumarins could be detected. Terpenoid patterns of the two plants are also quite different. Furthermore, the hosts of this sibling species pair tend to occur at different elevations (although there is broad overlap),

and show marked differences in habitat preference and phenology (Fig. 1).

Transplantation experiments showed that *P. angelicae* larvae can survive in *Laserpitium* but that *P. laserpitii* cannot in *Angelica*. This suggests that speciation in this sibling species pair may have been initiated by a shift from *Angelica* to *Laserpitium* requiring only evolution of the host-recognition mechanism followed by adaptation to the habitat and phenology of *Laserpitium* (Latscha, 1986).

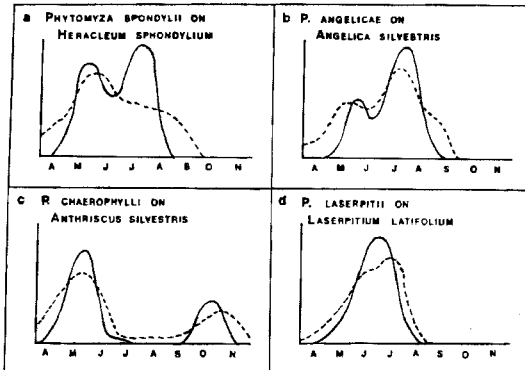


Figure 1. Phenology of host plants (---) and flies (—).

## 5. Conclusion

Our field and laboratory studies indicate that divergence in these flies is strongly influenced by characteristics of their biology, and that processes of speciation may be different among closely related agromyzids. In oligophagous species, like *P. chaerophylli* and *P. spondylIIi*, speciation in allopatry is most probable, while *P. angelicae* and *P. laserpitii* may have diverged in sympatry via formation of host plant races. Further studies will be conducted to unravel the mechanisms of speciation and adaptive radiation in these leaf-mining Agromyzidae.

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