

ABSTRACT

Title of Document: PATTERNS OF DIVERSIFICATION IN
PHYTOPHAGOUS INSECTS: PHYLOGENY AND
EVOLUTION OF *PHYTOMYZA* LEAF-MINING
FLIES (DIPTERA: AGROMYZIDAE)

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Plant-feeding insects account for about one fourth of macroscopic biodiversity. This study aims to document factors contributing to this diversity by investigating phylogenetic relationships within a large radiation of herbivorous insects, *Phytomyza* leaf-mining flies (Diptera: Agromyzidae).

After a brief introduction (Chapter 1), a general overview of phylogenetic patterns in phytophagous insects is presented, based on over 200 phytophagous insect phylogenies from the recent literature (Chapter 2). A few salient results include 1) host use conservatism at the family level predominates, with shifts occurring at about 5% of speciation events; 2) host shifts are a major contributor to speciation, occurring in about half of 145 speciation events tabulated; 3) insect-host associations mostly reflect colonization of already diversified host plant clades; and 4) variation in diversification rates is not yet well-documented for phytophagous insects, except at the broadest scale.

Chapter 3 is a phylogenetic study of the genus *Phytomyza sensu lato*, using over 3,000 nucleotides of DNA sequence data from three genes. Results indicate that the genus *Chromatomyia*, considered by some as synonymous with *Phytomyza*, is in fact polyphyletic and nested within *Phytomyza*. Possible parallelism in a biological trait (internal pupation in leaf tissue) which is one of the defining traits of species in the former *Chromatomyia* is discussed. In addition, the internal classification of *Phytomyza* is assessed and revised insofar as the data permit.

Divergence times for the Agromyzidae, and also for *Phytomyza* and related genera, were estimated using a molecular phylogeny calibrated by three agromyzid fossils (Chapter 4). Results suggest that the temperate *Phytomyza* group of genera originated in the relatively warm Eocene epoch. Ranunculaceae, a primitive plant family, is inferred as the ancestral host for a clade including most *Phytomyza* species, but is probably secondary to feeding on more derived plant families (“asterid clade”). Ten clades were identified for comparison of diversification rates between Ranunculaceae- and asterid-feeding lineages, which showed that asterid-feeding clades exhibit higher rates of diversification. *Phytomyza* originated approximately at the early Oligocene global cooling event, but contrary to expectations, diversification significantly slowed during the Oligocene cool period, when suitable habitats for *Phytomyza* were presumably widespread.

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(DIPTERA: AGROMYZIDAE)

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Foreword

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Chapter 1: Introduction

With nearly 400,000 species known and many more awaiting discovery and description, plant-feeding insects comprise approximately one fourth of macroscopic biodiversity (Strong et al. 1984). This diversity represents one of the dominant ecological and evolutionary forces which has shaped life on earth, and its explanation is the major theme of this dissertation. Evolution of phytophagy (plant-feeding) has probably occurred more than 50 times in insects, and is often accompanied by a significant increase in rates of species accumulation (Mitter et al. 1988). Much of this diversity is thought to have resulted from evolutionary interactions with the highly diverse flowering plants (Ehrlich and Raven 1964, Farrell 1998, Mayhew 2007). Phytophagous insects are also notable for their high degree of host-plant specialization; probably over 75% of species feed only on members of one plant family (Bernays and Chapman 1984), and many insect species feed only on a single plant species (e.g. Scheffer and Wiegmann 2000). Elevated rates of speciation are thought to be correlated with host specialization, but the mechanisms that drive this linkage are poorly understood.

The prevailing theme in the macroevolution of insect-plant interactions is the tension between host use conservatism and colonization of novel hosts. In one sense, this represents a paradox: associations of insect groups with specific plant groups can be extraordinarily stable, persisting tens of millions of years, yet some degree of lability in host use is necessary to explain the diversity of phytophagous insects observed today.

Not only are host shifts (usually to related plants) probably an important driver of speciation for plant-feeding insects (Berlocher and Feder 2002), but even rare colonizations of unrelated plants may serve to open up new “adaptive zones” in which adaptive radiation can occur.

Because many host associations are historically stable, phylogenies are especially important in documenting and explaining patterns of host use in phytophagous insects (Mitter and Farrell 1991, Farrell et al. 1992). Much of the literature in this area has centered around the influential idea of coevolution (Ehrlich and Raven 1964), which, as originally formulated, postulates that insects and plants have been locked in an ancient, ongoing evolutionary struggle, each adapting and diversifying in response to the other. When such ancient plant/insect associations persist, coevolution may result in a pattern in which insects which are “primitive” within a certain lineage may also be associated with “primitive” plants (Farrell 1998, Ward et al. 2003). However, other kinds of historical signatures may also be important in phytophagous insect evolution. For example, one type of pattern has been noted (e.g. Farrell et al. 1992, Wiens and Donoghue 2004) which could be called “biome tracking”. Because certain biomes (especially tropical forests) have historically occupied much greater area in past epochs, many insect groups may have originated in such biomes and later adapted to other climates and habitats (e.g. temperate forests or grasslands). This kind of evolutionary trend may also result in patterns detectable in relationships between modern species (i.e. phylogeny) and their ecological characteristics.

Determining the historical timing of evolutionary events can be essential in understanding the evolutionary effect of past host plant associations and climates. However, this is often difficult because the fossil record is sparse for many kinds of insects. Combining phylogenetic information, especially that derived from DNA sequence data, with available fossil data is an especially powerful approach that has recently been used for many different organisms (Welch and Bromham 2005). These new dating methods have also provoked a renewed interest in the study of variation in evolutionary rates of diversification (e.g. Davies et al. 2004, Ree 2005, McKenna and Farrell 2006, Moreau et al. 2006). However, methods for estimating divergence times and diversification rate variation are still rapidly developing, and much work remains to be done.

Leaf-mining flies (Agromyzidae) are a promising system for phylogenetic studies of host use evolution and diversification. The phylogeny of the family Agromyzidae has recently been investigated by Dempewolf (2001) and Scheffer et al. (2007), providing a firm footing for more detailed studies of individual clades. Leaf-mining flies exemplify many of the characters of phytophagous insects in general, including high diversity (>2,800 species) and an unusually high degree of specialization (99% of species restricted to hosts in a single family; Spencer, 1990). All species are internal plant feeders, a trait that has been linked to a higher degree of specialization and host fidelity (Mitter and Farrell 1991). As the common name suggests, most feed in leaf tissue, forming an externally visible trace, or mine, but a significant number of species feed in stems, seeds, or other tissues. Hosts for the Agromyzidae are relatively well-

documented, since larvae in leaf mines are often easily located and reared. Over 140 plant families are attacked, including most major plant groups (Spencer 1990, Benavent-Corai et al. 2005), but hosts are primarily those with herbaceous growth form. Some agromyzids are important pests of agricultural and ornamental plants (Spencer 1973). Unlike many insects, Agromyzids are more diverse in temperate than tropical regions; this is especially true of the largest genus, *Phytomyza*, which includes over 630 described species, almost entirely in the temperate northern hemisphere. Spencer (1990) noted that *Phytomyza* species exhibit a strong association with the “primitive” plant family Ranunculaceae (buttercup and columbine family), and hypothesized that the ancestral *Phytomyza* species was associated with this plant family. Later shifts to more derived, diverse herbaceous plant families such as Asteraceae (daisy family) may have further accelerated species diversification.

This dissertation begins with a general overview of phylogenetic patterns found in recent literature on phytophagous insects (Chapter 2). Next, this study aims to use DNA sequence data to estimate phylogenetic relationships within *Phytomyza*, and then use the results to update the classification of the genus and comment on the evolution of certain life history traits (Chapter 3). The phylogeny will then be used to study patterns of host shift between plant families in *Phytomyza* and to test Spencer’s hypothesis of an ancestral association with the Ranunculaceae (Chapter 4). Using fossils to calibrate divergence times on the molecular phylogeny, events in the evolution of *Phytomyza* will finally be

compared to the history of the host plant groups and the biomes they inhabit. One major goal is to determine which factors have influenced changes in diversification rate in this group.

Chapter 2: The Phylogenetic Dimension of Insect-Plant Interactions: A Review of Recent Evidence

The dramatic expansion of research on insect/plant interactions prompted by Ehrlich and Raven's (1964) essay on coevolution focused at first mainly on the proximate mechanisms of those interactions, especially the role of plant secondary chemistry, and their ecological consequences. Subsequently, in parallel with the resurgence of phylogenetics beginning in the 1970s and 80s, there arose increasing interest in the long-term evolutionary process envisioned by Ehrlich and Raven (e.g., Benson et al. 1975, Zwölfer 1978, Berenbaum 1983, Mitter and Brooks 1983, Miller 1987). Since the early 1990s, spurred in part by the increasing accessibility of molecular systematics, there has been a happy profusion of phylogenetic studies of interacting insect and plant lineages. The results so far have reinforced skepticism about the ubiquity of the particular macro-evolutionary scenario envisioned by Ehrlich and Raven, now commonly termed "escape and radiation" coevolution (Thompson 1988). However, this model continues to inspire and organize research on the evolution of insect/plant assemblages because it embodies several themes of Neo-Darwinism, each of interest in its own right, which have been taken up anew in the modern re-embrace of evolutionary history. In this chapter we attempt to catalog some of the postulates about phylogenetic history derivable from Ehrlich and Raven's essay, and evaluate their utility for explaining the structure of contemporary insect/plant interactions.

The “escape and radiation” model (review in Berenbaum 1983) tacitly assumes, first, that the traits governing species’ interactions, such as insect host plant preference, are phylogenetically conserved due to constraints such as limited availability of genetic variation. Such constraints create time lags between successive insect and plant counter-adaptations, allowing the lineage bearing the most recent innovation to increase its rate of diversification. A related general implication is that, because of genetic or other constraints on evolutionary response to new biotic surroundings, the structure of present-day insect/plant interactions (e.g., who eats whom) will be governed more by long-term evolutionary history than by recent local adaptation. This postulate parallels a broader recent shift in thinking about community assembly, from a focus on equilibrium processes to a greater appreciation of the role of historical contingency (Webb et al. 2002, Cattin et al. 2004, DiMichele et al. 2004). Third, the “radiation” component of “escape and radiation” perfectly encapsulates the New Synthesis view, lately enjoying a revival (Schluter 2000), that diversification is driven primarily by ecological interactions. Insect/plant interactions have figured prominently in the modern re-examination of all three of these broad postulates.

This chapter attempts a survey the recent evidence on the phylogeny of insect-plant interactions, focusing chiefly on among-species differences in larval host plant use by herbivorous insect lineages (largely neglecting pollinators, which are treated elsewhere), and organized around the themes sketched above. We draw mostly on literature of the past dozen years, i.e., subsequent to early attempts at a similar survey (e.g., Mitter and Farrell 1991, Farrell and Mitter 1993). Given the great diversity of

phytophage life histories and feeding modes, full characterization of host use evolution will require, in addition to hypothesis tests in particular groups, the estimation of relative frequencies of alternative evolutionary patterns across a broad sampling of lineages. Our emphasis here is on the latter approach. A complete catalog is no longer feasible, but we have made a concerted and continuing effort to compile as many phylogenetic studies of phytophagous insect groups as possible. These are entered into a database which at this writing contained over 1000 entries, many of which were obtained from the Zoological Record database. Our analyses and conclusions are based chiefly on approximately 200 of these reports which contain both a phylogenetic tree and information on host plant use. Many of the phylogenies are based on DNA sequences, while for others the chief evidence is morphology. This data base, intended as a community resource to promote further synthesis, is available at www.chemlife.umd.edu/entm/mitterlab, as are the data compilations and other supplementary materials mentioned in the text. Our nomenclature follows Angiosperm Phylogeny Group (2003; hereafter APGII) for angiosperm families and higher groups, and Smith et al. (2006) for ferns.

Conservatism of Host-Plant Use

Full understanding of the influence of evolutionary history on insect/plant associations will require a broad accounting of the degree to which the different dimensions of the feeding niches of phytophagous insects are phylogenetically conserved. Much evidence on some aspects of this question has accumulated in the past decade.

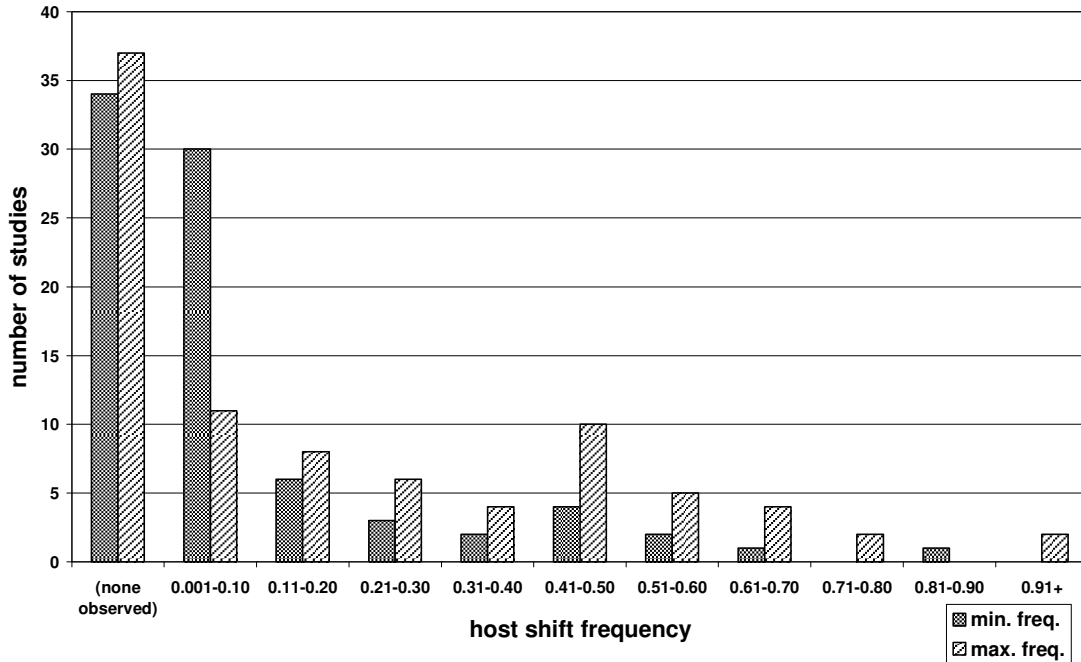
Conservation of Host-Taxon Associations

The strongest generalization that can be made about the evolution of host plant use is that related insect species most often use related hosts. This long-standing conclusion is now supported by numerous studies in which the history of host taxon use has been reconstructed, most often under the parsimony criterion, on an insect phylogeny inferred from other characters. An early compilation (Mitter and Farrell 1991) of the few phylogenetic studies then available (~25) suggested that on average, less than 20% of speciation events were accompanied by a shift to a different plant family; strictly speaking, the compilation was of the fraction of branches subtended by the same node on the phylogeny which have diverged in host family use, as inferred under the parsimony criterion. We have now repeated that calculation using essentially all applicable phylogenies we could find, totaling 93 (27 Coleoptera, 28 Hemiptera, 19 Lepidoptera, 12 Diptera, 5 Hymenoptera, and 1 each of Thysanoptera and of Acari [honorary insects for the purposes of this chapter]). Some of the uncertainty in host shift estimates comes from incomplete sampling of species. In the earlier compilation, host shift frequency was calculated as the total number of host family shifts inferred under the parsimony criterion, divided by one less than the number of sampled species with known hosts. This should be an unbiased estimate of the actual frequency of host shifts, if the included species are a random subset of the clade sampled. However, sampling in phylogenetic studies is often deliberately over-dispersed across subclades (e.g., genera within a tribe), which should tend to inflate the average evolutionary distance among sampled species and hence the apparent frequency of host shifts. To evaluate the importance of this effect, we also calculated a corrected frequency estimate, dividing the number of shifts detected on the

phylogeny by the total number of species with known hosts, including ones not included in the phylogenetic study. We will refer to these two estimates, in the order here described, as maximum versus minimum. In further contrast to the earlier tabulation, this one excluded the relatively few polyphagous species (defined here as those using more than two plant families); several phylogenies including a high proportion of polyphagous species were excluded, as well. A detailed tabulation of the phylogenies is given in Supplementary Table S2 (www.chemlife.umd.edu/entm/mitterlab), while the results are summarized in Fig. 2.1.

The histogram of Fig. 2.1 shows a result very similar to that of the earlier tabulation, underscoring the prevalence of host conservatism. The distributions of host family shift frequencies, strongly right-skewed, have medians of 0.08 (maximum frequency) and 0.03 (minimum frequency). Statistical tests of the hypothesis of non-random phylogenetic conservatism in host genus or family use have now become routine within studies of the kind tabulated here. These most often use the so-called PTP test (Permutation Tail Probability; Faith and Cranston 1991), in which the null distribution is generated by random re-distribution of the observed host family associations across the insect phylogeny. Significant “phylogenetic signal” has been detected in nearly every instance (see e.g., Table 2.2). In addition, several authors have used randomization tests on frequencies of shift among different host families or groups thereof to show that these preferentially involve related high-rank host taxa (Janz and Nylin 1998, Ronquist and Liljeblad 2001); conservatism at the level of major angiosperm clades (APGII 2003) is probably common as well.

Fig. 2.1. Frequency of host shifts per speciation event for 93 phytophagous insect phylogenies, calculated by dividing number of host family shifts observed on phylogeny by number of included ingroup species (max. host shift freq., solid bars), and by total number of described species in the ingroup clade (min. host shift freq., hatched bars). For references and taxa included, see Supplementary Table S2.



It is widely accepted that conserved host taxon associations primarily reflect conserved recognition of and other adaptations to plant secondary chemistry, but this assumption has been difficult to test because of the generally close correlation of chemistry with plant taxonomy. Several cases of mismatch between host chemical and taxonomic similarity have now been examined phylogenetically, and shown closer correspondence of insect phylogeny to chemistry than plant relatedness (Becerra 1997, Wahlberg 2001, Kergoat et al. 2005). Recent studies include re-examination of classic examples (Dethier 1941, Feeny 1991) of repeated shifts by lepidopterans between unrelated host families bearing similar secondary compounds (e.g., Lauraceae, Rutaceae, and/or Apiaceae; Berenbaum and Passoa 1999, Zakharov et al. 2004, Berenbaum and

Feeny 2008). This subject is by no means exhausted, as many more such syndromes surely await documentation. It should be noted, however, that herbivore groups feeding on plants without distinctive chemical defenses or on undefended plant parts can also show similarly specialized, conserved host associations (e.g., leafhoppers; Nickel 2003).

Variation in Rates of Major Host Shift

Although conservatism is pervasive, phylogenetic studies continue to document great variation among phytophage lineages in the frequency of “major” host shifts (e.g., to different plant families). Establishing patterns to this variation will be a key step toward understanding the constraints on diet evolution. Many predictors for differential host shift rates have been advanced (review in Mitter and Farrell 1991), some invoking properties of plant taxa and/or communities, others invoking traits of the phytophages. Attempts to test these, however, remain few, and the subject seems ripe for further synthesis. In one of the few explicit analyses, Janz and Nylin (1998) present evidence that among butterflies, shifts among major angiosperm clades are less frequent in herb feeders than tree feeders. Nyman et al. (2006) found that internally-feeding nematine sawfly clades have colonized significantly fewer plant families than their externally-feeding sister groups. Radiations on oceanic islands have been suggested to undergo exaggerated divergence in niches, including host plant use, compared to continental relatives (e.g., Schluter 1988). In the only test for phytophages, the eight genera of delphacid planthoppers endemic to various Pacific islands were found to have a significantly higher mean rate of host family shift (2X higher), and frequency of polyphagy, than the 52 continental genera (Wilson et al. 1994); systematic work in progress will permit re-

analysis with better control for phylogeny. Possible explanations for elevated host shift rates on islands include limited availability of preferred hosts of colonizers, reduced chemical distinctiveness among host species due to relaxed natural enemy pressure, and absence of continental competitors and/or insect natural enemies (review in Wilson et al. 1994). Further comparisons to insular radiations may help to identify causes of the prevailing host specificity and conservatism of mainland phytophages.

Compilations of host shift rates as in Supplementary Table S2 should permit further tests of hypotheses about differential host conservatism. Following Fagan et al. (2002), we used phylogenies from the literature to concatenate all the groups in the table into a single meta-phylogeny (presented in Supplementary Figure S3). One can then map onto the phylogeny the inferred host shift frequencies plus the distribution of traits postulated to affect them, e.g. internal versus external feeding. The meta-tree can then be divided into a maximal number of independent regions (contrasts), each consisting of a set of contiguous branches and containing an inferred evolutionary change in the putative predictor trait. For each contrast, a single response measure is calculated, e.g. the difference in mean host shift frequency between groups having the opposing states of the predictor variable. Paired comparisons are then used to test for a consistent effect of the predictor variable on host shift frequency. In a first analysis, strong support was found for elevated mean frequency of host family shifts inferred from just the oligophagous species (i.e., polyphages not scored) in lineages which include one or more polyphagous species, as opposed to lineages lacking polyphages (12/12 contrasts differing in the same direction, $P < 0.0001$, sign test). This finding supports the conjecture (e.g. Janz and Nylin

2008) that rapid shift among host taxa and polyphagy of individual species are related phenomena.

It has often been suggested (e.g., Farrell and Mitter 1990) that dependence on host-derived toxins for larval and/or adult phytophage defense should reduce the likelihood of major host shifts. This postulate has had no formal comparative test. However, recent phylogenetic evidence suggests that use of such defenses itself is in general not so conservative, or so intimately tied to larval diet, as might be supposed (Dobler et al. 1996, Dobler 2001), probably because herbivores often have multiple defenses. Thus, in the chrysomelid beetle subtribe Chrysomelina (Termonia et al. 2001, Kuhn et al. 2004) the ancestral larval defense is entirely autogenous, but there have been two independent origins, within Salicaceae-feeding lineages, of dependence on host-derived salicin. Within one of these groups there has been subsequent addition of a second type of defense, based on a combination of autogenous and host derived pathways, followed by multiple host shifts to another family (Betulaceae) from which salicin is not available. Availability of more than one defense-metabolism pathway may likewise have facilitated repeated host family shifts in other groups, such as the tropical chrysomeline genus *Platyphora* (Termonia et al. 2002). Moths of the typically aposematic family Arctiidae are one of several groups which have converged on defensive use of plant-derived pyrrolizidine alkaloids (PAs), while producing endogenous other toxins as well. A recent phylogeny for arctiids implies a single origin of larval feeding on PA-containing plants and sequestration of PAs that are retained into the adult stage (Weller et al. 1999). In a species-rich subclade of the ancestrally PA-plant-

feeding lineage, there have been repeated shifts to non-PA larval hosts, implying lack of constraint by chemical dependence. Adult defense, however, shows strong apparent phylogenetic inertia, as adults in this subclade have evolved to actively collect and use PAs. A similar “constraint” explanation was proposed for the propensity of adults in one African and one New World galerucine chrysomelid subtribe to feed on, and use in courtship and defense, toxic cucurbitacins from Cucurbitaceae, which are at present fed on by larvae in just a single genus in each subtribe. Recent phylogenetic evidence (Gillespie et al. 2003, 2004), however, strongly supports independent New World and Old World origins for both larval and adult use of cucurbits, and points, albeit less strongly, to adult use arising first.

Other Conserved Aspects of Host Use

Most discussion of the impact of host plant use on insect diversification has focused on host taxon differences, but other conserved dimensions of the feeding niche have also been recognized (e.g., Powell 1980, Powell et al. 1998), including host growth form and habitat, plant part exploited, mode of insect feeding, and phenology of oviposition and feeding. Most herbivorous insects are specialized to particular host tissues, such as leaves, flowers, fruits, seeds, stems, or roots, in addition to particular host taxa. On any one plant part, moreover, insects are typically specialized for one of a great variety of feeding modes. For example, a partial list of feeding behaviors exhibited by insects that eat leaves includes galling, mining, leaf rolling or tying, and external folivory. The relative rates of evolution of the various niche dimensions are fundamental to assessing their roles in phytophage diversification.

Several authors have begun to quantify these rates and their variation. Cook et al. (2002) used a maximum likelihood approach to show that a genus of cynipid gall wasps shifts among host plant organs more often than among sections of their host genus, oaks. Farrell and Sequeira (2004) used similar methods in demonstrating, conversely, that in chrysomeloid beetles, shift among major host clades outpaces shift among host tissues. Other reports reinforce this latter trend at the host species level (Condon and Steck 1997, Favret and Voegtlin 2004). However, studies of galls are mostly consistent in finding rapid shift among host tissues (e.g., Yang and Mitter 1994, Plantard et al. 1997, Nyman et al. 2000, Dorchin et al. 2004, Joy and Crespi 2007); shifts in gall location, shape and timing, often on the same host species, may be important facilitators of galler speciation. Host growth form (i.e., trees vs. herbs) often shows very strong phylogenetic conservatism relative to host clade (Ronquist and Liljeblad 2001, Bucheli et al. 2002, Lopez-Vaamonde et al. 2003), but not always (Janz and Nylin 1998, Schick et al. 2003). Timing of oviposition or development with respect to host phenology is another dimension of host use which may frequently contribute to speciation, either on the same host or on a novel host (e.g., Wood 1993, Pratt 1994, Whitcomb et al. 1994, Harry et al. 1998, Filchak et al. 2000, Weiblen and Bush 2002, Sachet et al. 2006).

A special form of conserved host use, occurring in some groups of aphids and gall wasps (Cynipidae), is obligate alternation between different host taxa in successive generations. Host alternation may have originated multiple times in aphids (Moran 1988, Moran 1992, von Dohlen and Moran 2000, von Dohlen et al. 2006), though this inference rests mostly on differences in the mode of host alternation and other life history features,

as the phylogenetic evidence cannot adequately distinguish between gains and losses of host alternation per se. Regardless, this kind of complex host association has clearly evolved only a few times, while the loss of one or the other host has occurred repeatedly within ancestrally host-alternating lineages (Moran 1992; see also Cook et al. 2002). The degree to which host alternation (as opposed to simply shifting to a different host) reflects constraint versus adaptation has been debated (Moran 1988, Mackenzie and Dixon 1990, Moran 1990, Moran 1992).

Parallelism, Reversal, and Genetic Constraints on Host Shift

Although conservatism of host use traits can suggest the influence of phylogenetic "constraint" or "inertia" (Blomberg and Garland 2002), this interpretation is not automatic, as stabilizing selection is a plausible alternative (Hansen and Orzack 2005). The "constraint" interpretation would receive powerful support if one could demonstrate limitations on within-population genetic variation, for traits determining host use, that corresponded to the actual history of shifts undergone by the larger clade to which the test populations belonged. In a series of studies deserving wide emulation, Futuyma and colleagues (review in Futuyma et al. 1995; see also Gassman et al. 2006) reconstructed the history of host use in oligophagous *Ophraella* leaf beetles, then screened four species for genetic variation in larval and adult ability to feed and survive on the hosts (various genera of Asteraceae, in several tribes) fed on by their congeners. In only 23 of 55 tests (species x host) was there any detectable genetic variation for ability to use the alternative host. Such variation as did appear was mainly for use of hosts of closely-related beetle species; these plants were themselves closely related to the normal host. Thus, lack of

available variation for use of alternative hosts is probably much of the explanation for the conserved association of this genus with Asteraceae. Other lines of evidence, less direct, point to an analogous conclusion for other clades and traits. Many authors have noted (e.g., Janz and Nylin 1998, Hsiao and Windsor 1999, Janz et al. 2001, Swigoňová and Kjer 2004, Zakharov et al. 2004) that host family use is often highly homoplasious (i.e., showing multiple independent origins of the same habit), sometimes with repeated colonizations of a single plant family inferred to be an ancestral host. Janz et al. (2001) tested the long-standing hypothesis that such a propensity reflects retained ability to use former hosts, finding that Nymphalini butterfly larvae of most species were willing to feed on the ancestral host (*Urtica*), regardless of what host they normally fed on. Some specific kinds of phylogenetic pattern also strongly suggest genetic constraint. Thus, in several unrelated groups of galling insects, it has been found that features such as gall structure or gall position on the plant follow an ordered multi-step progression on the phylogeny, for example from simple to successively more complex (Nyman et al. 2000, Ronquist and Liljeblad 2001). If the evolution of such traits were not limited by genetic variation, it is hard to see why it should nonetheless follow the presumptive path of “genetic least resistance” (Schluter 2000). The nature and extent of genetic constraints, critical to a full understanding of host use evolution, is an under-explored subject on which modern genetic/genomic approaches hold promise for rapid progress (e.g. Berenbaum and Feeny 2008).

Conservatism, Host Shifts, and Speciation

Given the pervasive conservatism of higher-host-taxon use, one might wonder whether diet conservatism on a finer scale has been underestimated, and shifts to different host species consequently assigned too large a role in phytophagous insect speciation. One requisite for answering this question is a broad estimate of the proportion of speciation events which are accompanied by a change in host species. To our knowledge, no such survey has been published. We provide an estimate based on 145 presumptive sister species pairs found within 45 phylogenies of phytophagous insect genera or species groups in our data base for which information about hosts and geographic distribution was available. Taxa other than confirmed species (e.g., host races or unconfirmed sibling species) were excluded. Each species pair was scored as sharing a host plant species or not; pairs were also scored as having hosts from the same genus, family, or higher angiosperm clade (defined in APGII 2003). To contrast the frequency of host differences to that of differences in distribution, each sister pair was also scored as having distributions overlapping by 10% or more (subjectively estimated) versus <10%. No characterization of the accuracy of these phylogenies was attempted. A possible source of bias is that island radiations, which show a somewhat greater frequency of allopatry between sister species than continental forms and (surprisingly) a somewhat lower mean proportion of host differences, comprise over 25% of our data set. Therefore, we also present results with and without island lineages. Our tabulation and its sources are given in Supplementary Table S4 (online), and the results are summarized in Table 2.1.

Overall, about 48% of the divergence events we tabulated are associated with an apparent change in host species. This is our best estimate of the fraction of speciation events which could have been driven by host shifts (though of course we have no way of knowing whether the host differences actually accompanied speciation, rather than arising after speciation). Our results are consistent with a major role for host shifts in phytophage speciation, but not a ubiquitous one; we estimate that about half of all speciation events are unaccompanied by a host shift. Of course, many of the latter could have involved change in tissue fed upon or other aspects of host use.

Greater circumspection is required in interpreting our compilation of differences in distribution, which potentially bear on the controversial question of sympatric speciation (Lynch 1989). The utility of phylogenetic evidence on this issue has been doubted, even dismissed, because species' distributions can shift rapidly (Barraclough and Vogler 2000, Losos and Glor 2003, Fitzpatrick and Turelli 2006). Thus, the proportion of sister species which are sympatric might reflect dispersal ability rather than frequency of sympatric speciation (Chesser and Zink 1994, Losos and Glor 2003). Indeed, allopatric speciation has recently been suggested to play a prominent role even in the *Rhagoletis pomonella* group, the poster child for sympatric speciation (Barraclough and Vogler 2000). Nonetheless, we follow Berlocher (1998) in holding the comparative approach worthy of further exploration. Berlocher suggested that there should be a higher frequency of sympatry between sister species in host-shifting than in non-host shifting taxa, if host differences are commonly important in allowing species to originate, or at least remain distinct, in sympatry. In our compilation, however, extant

Table 2.1. Summary of host and distribution overlap versus non-overlap for 145 sister species pairs from 45 phytophagous insect phylogenies.

	Host species:	overlap	overlap	disjunct	disjunct	Total, hosts disjunct	Total, distr. disjunct
	Distributions:	overlap	disjunct	overlap	disjunct		
All pairs	145	27	48	26	44	48%	63%
Continental pairs only	101	22	27	22	30	52%	56%
Island pairs only	44	5	21	4	14	41%	80%

Host species overlap = members of pair sharing at least one host species; Host species disjunct = sharing no host species; Distributions overlap = with >10% area overlap in geographic distribution; Distributions disjunct = with <10% overlap in geographic distribution. Details, including sources, are in Supplementary Table S4.

sister species using different host species were sympatric only slightly (and not significantly) more often than those not differing in host, 37% (n =70) vs. 36% (n=75). This result seems to cast doubt on the ubiquity of divergence by sympatric host shift, but that interpretation may be too conservative. For example, among-group variation in dispersal ability, which we did not correct for, might obscure the “signal” for host-associated sympatric divergence in our tabulation. Moreover, the probability of sympatric divergence may depend strongly on how different the hosts are. Thus, sister species which differ in host genus used show a markedly higher frequency of sympatry (50%) than pairs whose hosts are congeneric if they differ at all (33%), though this difference was not statistically significant ($P=0.189$, χ^2 test). This observation is at least consistent both with a role for “major” host differences in promoting sympatric divergence, and with the postulate that shifts to distantly related hosts are more likely in sympatry, which allows for prolonged prior adaptation (Percy 2003). We should note, finally, that the study of phytophagous insect speciation and host shift mechanisms is being revolutionized by, among other advances, the advent of fine-scale, intra-specific

molecular phylogenetics including phylogeography sensu Avise (2000), which is not treated here.

Phylogenesis of Host Range

Special attention has focused on the evolution of diet breadth, i.e. the diversity of host plants fed on by a single herbivore species. Restriction to a small subset of the available plants is a dominant feature of phytophagous insect ecology. In addition to demanding an explanation in its own right (Bernays and Chapman 1994), it has made herbivorous insects a leading exemplar for investigating the ecological and evolutionary consequences of specialization (Schluter 2000, Funk et al. 2002). Phylogenies can potentially serve three roles in the study of host range. First, they delimit independent contrasts for identifying traits or circumstances whose occurrence is correlated with evolutionary changes in host range, facilitating both comparative and experimental studies of the adaptive significance and consequences of those changes. Second, the rate and direction of changes in host range inferred on a phylogeny can point to genetic/phylogenetic constraints or lack thereof on host range evolution. Third, phylogenies can in principle detect differential effects of broad versus narrow host range on diversification rates. Analyses of the second and third kinds could potentially support non-adaptive, macroevolutionary explanations for the predominance of host specificity, such as more frequent speciation in specialists than in generalists, in contrast to hypotheses invoking a prevailing individual advantage (Futuyma and Moreno 1988).

The study of host range evolution is still something of a conceptual and methodological tangle. A fundamental question is how to define host range. Although broad, somewhat arbitrary categories of relative specialization may often suffice to reveal evolutionary patterns (e.g., Janz et al. 2001), objective, quantitative measures may yield greater statistical power and allow more meaningful comparisons across studies (Symons and Beccaloni 1999). However it is defined, host range is surely a composite feature likely to reflect different combinations of (typically unknown) adult and immature traits in different groups. It is probably subject to a heterogeneous mix of influences that vary in relative strength with the scale of comparison. Small-scale changes in host range might reflect behavioral plasticity or local adaptation in response to differences in host abundance or quality, or host-associated assemblages of competitors, predators or parasitoids (e.g., Singer et al. 2004, Bernays and Singer 2005). Such changes could also represent short-lived intermediate steps in the evolution of new specialist species (e.g., Hsiao and Pasteels 1999, Janz et al. 2001, Janz et al. 2006). In contrast, changes evident mainly on longer time scales, and spanning a greater range of diet breadths, could reflect less frequent but more pervasive evolutionary shifts involving multiple component adaptations. At any scale of examination, broader host range could result from different causes in different lineages.

Given the heterogeneity of potential causes, evolutionary patterns of host range are likely to differ widely among groups. Phylogenetic evidence has begun to accumulate, but we are far from having an adequate characterization of that variation, let alone an explanation. The most useful studies will be those in which (a) unambiguous

distinctions are evident in host range, reflecting intrinsic differences among species (not the collective range of hosts used by higher taxa as in Berenbaum and Passoa [1999], contra Nosil [2002] and Nosil and Mooers [2005]), and (b) taxon sampling is dense enough to permit detection of evolutionary trends if these exist. Only a handful of the studies in our data base appear to meet these criteria. We summarize the nine which we judged to come closest in Table 2.2. No criticism is implied of any work not included in this somewhat subjective selection, particularly since the tracing of diet breadth has only rarely been an explicit goal.

The strongest generalization evident so far is that host range is quite evolutionarily labile, much more so than use of particular host taxa. As a gauge of that lability, we tabulated the results of PTP tests (Faith and Cranston 1991) on degree of host specificity treated as a binary character with changes in the two directions equally weighted (one versus more than one host family, or other criteria specified by the authors or otherwise appropriate to the study group; about half these analyses were performed by the authors). In seven of nine cases, this test cannot reject a random distribution of host range on the phylogeny, whereas in each case but one, use of individual host taxa is significantly conserved. As several authors have noted, host range is clearly not subject to strong forms of phylogenetic constraint or “inertia” (Blomberg and Garland 2002) such as absolute irreversibility (Nosil and Mooers 2005, Yotoko et al. 2005). In fact, the paucity of obvious phylogenetic signal may complicate further characterization of host range evolution, by limiting the utility of some standard strategies of phylogenetic character analysis. Thus, when a two-state likelihood model is applied to estimate the relative rates

Table 2.2. Synopsis of nine recent studies bearing on phylogenetic patterns of host range.

INSECT TAXON	Taxon sample	#specialists vs. #generalists	Criterion for "specialist" vs. "generalist"	forms of directionality reported	Significant host taxon conservation?	significant host range conservation?	Source
Coleoptera: Chrysomelidae: <i>Oreina</i>	12/24 spp., spanning all ecological variation in genus	6 vs. 6	1 vs. > 1 host tribe	none	host family & tribe conserved, P=0.01 PTP	no: P=0.47, PTP	1
Coleoptera: Curculionidae: Scolytinae: <i>Dendroctonus</i>	18/19 spp. (position of remaining species taken from literature)	6 vs. 13	using < 1/2 vs. > 1/2 of available host species	specialists limited to tips of phylogeny	host genus conserved, P=0.01, PTP (authors)	no: P=1.00, PTP (authors)	2
Coleoptera: Bruchidae: <i>Stator</i>	21/22 spp. with known hosts	4 vs. 16	1 vs. >1 host tribe	generalists derived	use of 1 of 4 host genera conserved, P=0.03, PTP (authors)	no: P=1.00, multiple tests (authors)	3
Lepidoptera: Nymphalidae: Melitaeini	10/10 gen., 65/250 spp.; sparse sampling in one large Neotropical clade	51 vs. 14	1 vs. >1 host family	host family gains > losses	host family use conserved, P=0.003 (author)	no for gen. vs. spec. (P = 0.34 PTP; yes (P=0.02) for # host families (1-6)	4
Lepidoptera: Nymphalidae: Nymphalini	27/70 spp.; most taxa in some Nearctic lineages	17 vs. 10	1 vs. >1 host order	specialist ancestor; host gains > losses	host family use conserved, P=0.01, PTP (authors)	marginal: P=0.06, PTP	5
Lepidoptera: Nymphalidae: <i>Polygonia</i>	14/16 spp.	7 vs. 5	1 vs. >1 host order	specialist ancestor; host gains > losses	host order use conserved, P=0.001, PTP	no for gen. vs. spec. and # host orders (1-5; P>0.30, PTP)	6
Lepidoptera: Saturniidae: <i>Hemileuca</i>	22 populations in 17 spp./ 28 spp. total; excluded taxa may be synonyms	15 vs. 7	using primarily 1 vs. >1 host family	none	host family use conserved, P=0.02, PTP	no: P=1.00, PTP (authors)	7

INSECT TAXON	Taxon sample	#specialists vs. #generalists	Criterion for "specialist" vs. "generalist"	forms of directionality reported	Significant host taxon conservation?	significant host range conservation?	Source
Diptera: Tephritidae: <i>Tomoplagia</i>	19/59 spp.; sampling limited to part of Brazil	11 vs. 8, or 14 vs. 5, or 15 vs. 4	1 vs. >1 host genus, or subtribe, or tribe	depends on criterion	no: P > 0.18 (PTP) for host subtribe use	no: P>0.50, PTP, all criteria	8
Phasmida: Timematidae: <i>Timema</i>	14 spp. (17 taxa)/21 spp. (remainder described subsequently)	11 vs. 6	1 (95% of records) vs. >1 host genus	generalist ancestor	host genus conserved, P < 0.04 (authors)	no: P=0.59, PTP	9

Abbreviations: gen. (generalist); PTP (Permutation Tail Probability test); spec. (specialist); spp. (species); vs. (versus).

Sources: 1. Dobler et al. (1996), 2. Kelly and Farrell (1998), 3. Morse and Farrell (2005), 4. Wahlberg (2001), 5. Janz et al. (2001), 6. Weingartner et al. (2006), 7. Rubinoff and Sperling (2002), 8. Yotoko et al. (2005), 9. Crespi and Sandoval (2000).

of transition to and from specialization, the rates can most often be closely predicted from just the proportions of specialists and generalists among the terminal taxa (Nosil 2002, Nosil and Mooers 2005). This outcome, intuitively expected if the states are distributed randomly on the tree, might be taken to suggest that phylogenies have little to contribute to the understanding of host range evolution. And indeed, it is possible that much of the variation in host range analyzed so far is in fact phylogenetically “random” in the sense of reflecting idiosyncratic local fluctuation, for example in the availability of, and/or selective advantage of using, particular hosts. This may be especially true when all the species within the study group are specialists in the broad sense of feeding on plants in, for instance, the same family.

As several authors have noted, however, it is plausible that larger-scale phylogenetic regularities remain to be discovered, through the elaboration of more detailed, process-oriented models of host range evolution (Stireman 2005). Multiple approaches can be distinguished. Thus, host range might be thought of as a trait phylogenetically ephemeral in itself, but with probabilities of change predictable from the states of other, more conserved features, inviting use of the “comparative method.” For example, distribution of the use of two versus more than two tribes of legumes appears by itself to be random on a phylogeny of the seed beetle genus *Stator*. Closer inspection, however, shows that independent origins of broader host range are significantly concentrated in lineages which oviposit on pre-dispersal seeds, rather than on intact seed pods or dispersed seeds (Morse and Farrell 2005).

An alternative approach focuses on the genetic and ecological mechanisms by which host range changes. Thus, Crespi and Sandoval (2000; see also Nosil et al. 2003) conclude that host specialization in *Timema* walking sticks comes about when host-associated color polymorphism in polyphagous ancestors is converted into species differences under disruptive selection by predators. Phylogenetic evidence by itself is consistent with but does not strongly establish ancestral polyphagy. However, that interpretation is supported by abundant experimental and other evidence. Similar logic is reflected in the elaboration of a novel hypothesis about butterfly host range (e.g., Janz et al. 2001, Weingartner et al. 2006, Janz and Nylin 2008). A phylogeny for the nymphalid tribe Nymphalini suggests ancestral restriction to Urticales followed by repeated host range expansions as well as contractions, with multiple ostensibly independent colonizations of a set of disparate plant families. Complementary experiments show that larvae of many species are able to feed on hosts not presently used by that species, but characteristic of their inferred ancestors and/or extant relatives. Retained latent feeding abilities may help to explain rapid expansions (and hence observed lability) of host range. Polyphagy may also facilitate radical host shifts (and/or further broadening of host range), given that less specialized species seem to generally make more oviposition mistakes (Janz et al. 2001), and has been suggested to thereby promote diversification (Weingartner et al. 2006, Janz et al. 2006, Janz and Nylin 2008). This postulate stands in direct contrast to the prediction that specialization promotes faster speciation, for which evidence is currently lacking (see below).

Several of the foregoing hypotheses may apply to a broad phylogenetic pattern of host range in the noctuid moth subfamily Heliothinae (Mitter et al. 1993, Fang et al. 1997, Cho 1997, S. Cho, A. Mitchell, C. Mitter, J. Regier, M. Matthews, submitted). A paraphyletic basal assemblage, species rich and almost entirely oligophagous or monophagous (80% on Asteraceae), contrasts sharply with an advanced “*Heliothis* clade” containing a much higher proportion of polyphages. Host range is correlated with phylogeny, albeit weakly, but the most dramatic difference is in its much higher rate of change in the *Heliothis* clade. That lineage appears to have a set of conserved life history features (higher fecundity, body size and other traits) which are relatively permissive of changes in host range, while the low fecundity, small size, low vagility and other traits of the more basal species may strongly disfavor host range expansion. Phylogenetically controlled analyses of the life history correlates of diet breadth are still too few, but the number is growing (e.g., Beccaloni and Symons 2000) and further synthesis seems imminent (Jervis et al. 2005).

With so many promising recent leads at hand, we can look forward to rapid progress in understanding of the phylogenetic patterns of host range evolution.

Signatures of Long-Term History in Extant Insect-Plant Interactions

Strong conservatism of host taxon or other aspects of host plant use raises the possibility that the current distribution of insects across plant species reflects some form of long-term synchrony in the diversification of those associates. One extreme form of synchronous evolution would be strict parallel phylogenesis or cospeciation, in which

descendant lineages of the insect ancestor maintain continuous and exclusive association with the descendants of the ancestral plant species; the expected signature is a characteristic form of correspondence between the phylogenetic relationships, and the absolute ages, of the extant associates (ref?). Extensive methodological and empirical work on this general issue over the past 15 years, in many groups of organisms, has established that strict or nearly-strict parallel phylogenesis is almost entirely limited to parasites and other symbionts which are directly transmitted between host parent and offspring individuals (e.g., Page 2003). However, variants of this scenario more likely for free-living phytophages have also been envisioned, involving intermittent and/or less specific association of insect species with particular host plant taxa, and producing corresponding forms of incomplete phylogeny matching. Under escape and radiation coevolution, for example, the closest match is expected not between phylogenies per se, but between phylogenetic sequences of escalating plant defenses and insect counter-adaptations (Mitter and Brooks 1983). The marks of other forms of shared evolutionary history might lie primarily elsewhere. For example, it has been proposed that differences in the predominant host associations of major phytophagous insect clades reflect differences in which plant groups dominated the global flora in the different eras in which those phytophages arose (Zwölfer 1978). The critical evidence on such postulates will often be absolute datings. For the full range of questions considered in this section, a combined approach from phylogenetics and paleontology is proving especially powerful (review in Labandeira 2002a; see also Grimaldi and Engel 2005). There is currently a surge of interest in molecular dating studies, driven in part by the increasing sophistication of methods for combining evidence from fossils and molecular divergence

(reviews in Magallón 2004, Welch and Bromham 2005), though the reliability of such datings is still poorly understood.

In this section we attempt to sketch out and evaluate the evidence for several forms of historical imprint on insect/plant associations. Such inquiry matters for two reasons. First, traces of shared long-term history imply that there has been at least the opportunity for prolonged reciprocal evolutionary influence – coevolution in a broad sense – and may even provide evidence on the nature and extent of that coevolution. Second, from the ecological point of view, unique marks of history imply that the assembly of extant insect/plant communities cannot be fully explained by just the current properties of local or regional species pools or even the evolutionary propensities of these; one may need also to invoke the contingent historical sequence in which particular insect and plant lineages appeared on earth (Farrell and Mitter 1993).

Early in the current era of phylogenetic studies, there was much interest in the possibility of parallel phylogenesis between insect and host plant clades. There is now enough evidence to state with confidence that correspondence of phytophagous insect and host phylogeny is rare on the taxonomic scale at which it has most often been examined, namely within and among related insect genera. Even groups involved in obligate pollination mutualisms show much less correspondence with host phylogeny than previously assumed (Pellmyr 2003, Kawakita et al. 2004, Machado et al. 2005, Kawakita and Kato 2006). An early compilation (Mitter and Farrell 1991) examined 14 studies, in only one of which was there unambiguous support for parallel phylogenesis.

Here we tabulate a subset of 18 of the many relevant studies appearing since then, limited to papers in which the authors themselves drew conclusions about parallel cladogenesis (Table 2.3). In the great majority of these, there is little evidence, from either cladogram concordance or datings, for parallel diversification. Our sample undoubtedly underestimates the true prevalence of such negative evidence, as we did not include the many papers in which parallel cladogenesis is implicitly ruled out at the start. One exception to the rule is particularly instructive: a group of psyllids showed significant phylogeny concordance with its legume hosts, but molecular clock and fossil datings indicate that host diversification was likely complete before the group was colonized by these phytophages (Percy et al. 2004). Presumably, host shifts in these herbivores have been governed by plant traits correlated with plant phylogeny; it is less clear why colonization should start at the base of the host phylogeny. In light of this finding, it seems especially important that newly discovered instances of possible cladogram match, e.g. as reported for a group of gracillariid moths which obligately pollinate their hosts (Kawakita et al. 2004), be investigated for equivalence of ages.

The few plausible cases for both cladogram match and equivalence of ages include two genera of herb-feeding beetles (leaf beetles on skullcap mints, Farrell and Mitter 1990; longhorn beetles on milkweeds, Farrell and Mitter 1998, Farrell 2001). The vast assemblage of figs and their mutualist wasp pollinators, the subject of many recent phylogenetic studies (Silvius et al. 2008), shows clear elements of parallel diversification, although it now appears that host specificity and parallel speciation are much less strict than was formerly thought (Machado et al. 2005).

Table 2.3. Synopsis of 18 recent studies testing for parallel insect/plant phylogenesis at lower taxonomic levels.

Insect Order and Family	Insect Clade	Host Clade(s)	Overall Phylogeny Correspondence Plausible?	Equivalent Ages Plausible?	Sources
Coleoptera: Cerambycidae	<i>Tetraopes</i>	<i>Asclepias</i> (Apocynaceae)	yes - significant cladogram similarity	yes	1,2
Coleoptera: Chrysomelidae	<i>Ophraella</i>	Asteraceae	no - cladograms do not match	no - beetles younger than hosts	3
Coleoptera: Chrysomelidae	<i>Blepharida</i>	Burseraceae	maybe - depends on analysis	yes	4,5
Coleoptera: Curculionidae	<i>Anthonomus grandis grp.</i>	<i>Hempia</i> (Malvaceae)	no - cladograms do not match	not tested	6
Hymenoptera: Tenthredinidae	Euurina (Nematinae)	<i>Salix</i> (Salicaceae)	no - cladograms do not match	not tested	7,8
Hymenoptera: Cynipidae	major lineages of cynipids	Asteraceae, Lamiaceae, Fagaceae, Rosaceae, Papaveraceae	no - cladograms not significantly similar	maybe (based on fossils, biogeography)	9
Hymenoptera: Agaonidae	Agaoninae	<i>Ficus</i> (Moraceae)	yes, but correspondence not universal	yes	10,11
Hymenoptera: Agaonidae	<i>Apocryptophagus</i> (non-pollinators)	<i>Ficus</i> (Moraceae)	no - cladograms not significantly similar	not tested	12
Diptera: Tephritidae	<i>Urophora</i>	Cardueae (Asteraceae)	no - cladograms not significantly similar	no - flies younger than hosts	13
Lepidoptera: Gracillariidae	<i>Epicephala</i>	<i>Glochidion</i> (Phyllanthaceae)	maybe - depends on type of analysis	not tested	14
Lepidoptera: Gracillariidae	<i>Phyllonorycter</i>	>30 families of angiosperms	no - cladograms not significantly similar	no (individual moth/host radiations tested)	15,16
Lepidoptera: Geometridae	Lithinini	ferns, multiple families	no - multiple shifts to distantly related hosts	no - moths younger than hosts	17
Hemiptera: Aphididae	<i>Uroleucon</i>	Asteraceae	no - multiple shifts to distantly related hosts	no - aphids much younger than hosts	18
Hemiptera: Psyllidae	Arytaininae	Fabaceae; Genisteae of Macaronesia	yes - significant cladogram similarity	no - psyllids much younger than hosts	19

Insect Order and Family	Insect Clade	Host Clade(s)	Overall Phylogeny Correspondence Plausible?	Equivalent Ages Plausible?	Sources
Hemiptera: Psyllidae	<i>Calophya</i> , <i>Tainarys</i>	<i>Schinus</i> (Anacardiaceae)	maybe - depends on group and analysis	not tested	20
Hemiptera: Delphacidae	Tribes of delphacids	Various monocots	Little evidence for cladogram match	maybe (ages uncertain)	21
Hemiptera: Delphacidae	<i>Nesosydne</i>	Hawaiian silverswords (Asteraceae: Heliantheae, 3 genera)	maybe - depends on analysis (sampling incomplete)	yes	22
Acari: Eriophyiidae	<i>Cecidophyopsis</i>	<i>Ribes</i> (Grossulariaceae)	no - cladograms do not match	no - mites younger than hosts	23

† Machado et al. (2005) found fig and pollinator wasp phylogeny congruence to be nonsignificant, and point out the paucity of evidence for cladogram matching of figs and their pollinating wasps at lower levels, as well. However, it is evident that substantial overall codivergence has occurred (Rønsted et al. 2005), and widespread (but not universal) congruence at lower levels still seems plausible (see also Weiblen and Bush 2002, Silvieus et al. 2008, and references therein).

Sources: 1. Farrell (2001), 2. Farrell and Mitter (1998), 3. Funk et al. (1995), 4. Becerra (1997), 5. Becerra (2003), 6. Jones (2001), 7. Nyman et al. (2000), 8. Roininen et al. (2005), 9. Ronquist and Liljeblad (2001), 10. Machado et al. (2005), 11. Ronsted et al. (2005), 12. Weiblen and Bush (2002), 13. Brändle et al. (2005), 14. Kawakita et al. (2004), 15. Lopez-Vaamonde et al. (2003), 16. Lopez-Vaamonde et al. (2006), 17. Weintraub et al. (1995), 18. Moran et al. (1999), 19. Percy et al. (2004), 20. Burekhardt and Basset (2000), 21. Wilson et al. (1994), 22. Roderick (1997), 23. Fenton et al. (2000).

Datings based on fossils, molecular clocks and biogeography also continue to identify other patterns suggesting long-continued, not necessarily coevolutionary interactions (e.g., von Dohlen et al. 2002). One of the most elaborate apparent historical interaction signatures involves *Blepharida* alticine leaf beetles and related genera. Beetle phylogeny shows only tenuous concordance with that of the chief hosts, *Bursera* and relatives (Burseraceae/Anacardiaceae), but much stronger match to a phenogram of leaf extract gas chromatography profiles (compounds not specified; Becerra 1997). Shared geographic disjunction between the New World and African tropics implies comparable overall ages (112 MY; but see Davis et al. 2002) for the interacting clades, and molecular clocks point to similar, younger ages for two associated beetle and plant subsets marked by corresponding innovations in resin canal defense and counter-defense (Becerra 2003). This case, an exemplar of the broad syndrome of parallel origins of resin/latex canal defenses and counter-adaptations thereto (Farrell et al. 1991), is perhaps the most detailed to date for long-term insect/plant “arms race” sequences as envisioned by Ehrlich and Raven (1964; but see Berenbaum 2001), though evidence for the accelerated diversification expected with each innovation is lacking.

We digress here to note that such putative escalations of plant defense are under-investigated and possibly rare. Aside from resin/latex canals, the two most strongly stated hypotheses involve evolutionary trends toward chemical complexity in coumarins and other secondary compounds in Apiaceae (review in Berenbaum 2001) and in cardenolides of milkweeds (*Asclepias*; review in Farrell and Mitter 1998). Although the modern revolution in plant phylogeny has underscored the conservatism of some major

secondary chemistry types (e.g., Rodman et al. 1998), phylogenetic studies directed explicitly at the evolution of plant defense are still few (but see, e.g., Armbruster 1997, Wink 2003, Rudgers et al. 2004). Agrawal and Fishbein (2006) mapped an array of putative defense traits that included total cardenolides (though not the hypothesized ‘arms race’ aspects thereof) onto a molecular phylogeny for 24 *Asclepias* species. Rather than reflecting plant phylogeny, these traits appear to define three distinct, convergently evolved defense syndromes, each possibly optimal in the right circumstances. This implicit optimality/equilibrium view of plant defense is very different from the historically contingent view inherent in the “arms race” hypothesis. Under the latter, we expect some lineages to have acquired novel defenses that confer, at least temporarily, a ubiquitous fitness advantage over relatives lacking those innovations. The relative applicability of these two views of defense evolution across the diversity of plants and their defensive traits has yet to be determined.

Reinforcing the view that ancient host associations may have left widespread, if not numerically dominant traces on contemporary assemblages is the increasing evidence for broad-scale correspondence between the ages of currently-associated insect and plant groups, over time spans encompassing major evolutionary changes in the global flora. The case for this long-standing postulate (see e.g., Zwölfer 1978) is best developed for the beetle clade Phytophaga (Chrysomeloidea + Curculionoidea, ~ 135,000 species), whose hosts span the chief lineages of seed plants (Farrell 1998, Marvaldi et al. 2002, Farrell and Sequeira 2004). Recent phylogeny estimates show most of the basal phytophagous lineages in both superfamilies to feed exclusively on conifers or cycads,

the most basal seed plants. The five gymnosperm-associated clades, totaling about 220 species, have apparently Gondwanan-relict distributions, and several are known as Jurassic fossils from the same deposits as are members of their present-day host groups. Within both superfamilies, moreover, there are early splits between monocot and (eu)dicot feeders, possibly established during the early divergence between these two main lineages of angiosperms (Farrell 1998). A similar pattern is evident, in abbreviated form, in the Lepidoptera, first known from the early Jurassic (Grimaldi and Engel 2005). Larvae of the most basal lineage (Micropterigidae) inhabit riparian moss and liverwort beds, apparently feeding on these and/or other plant materials. Their habits match those of the inferred common ancestor of Lepidoptera and their sister group Trichoptera (Kristensen 1997). Recent morphological and molecular phylogenies (Kristensen 1984; Wiegmann et al. 2000, 2002) firmly establish that the most basal lineage of the remaining Lepidoptera, which are otherwise mostly restricted to advanced angiosperms, consists of two Australasian species that feed inside cones of the conifer *Araucaria*. This association, which parallels basal gymnosperm feeding (specifically within reproductive structures) in Phytophaga (Farrell 1998), is quite plausibly viewed as pre-dating the availability (or at least the dominance) of angiosperm hosts. It is however the only obvious such relictual habit in Lepidoptera. While other primitive lineages also have apparent Gondwanan-relict distributions, suggesting mid-Mesozoic ages, they feed on advanced (mainly eurosid) dicots, and their phylogenetic relationships correspond not at all to those of their chief host plant taxa (Powell et al. 1998). Host use appears to have evolved considerably faster in Lepidoptera than in Phytophaga, thus traces of earlier feeding habits are probably more quickly obliterated.

Ancient host associations in other phytophagous lineages that date to the early Mesozoic and before, less well characterized, await clarification by modern studies. Recent progress on phylogeny of sawflies (basal hymenopterans; e.g., Schulmeister 2003), modern families of which date to the early Jurassic or even Triassic, should permit elucidation of the degree to which the multiple conifer (& fern) feeding lineages, totaling several hundred species, represent ancestral habits. We can hope for similar enlightenment about the Aphidomorpha (aphids and relatives), probably Triassic in age, in which the phylogenetic positions of the few extant gymnosperm-associated lineages are still obscure (Heie 1996, Normark 2000, von Dohlen and Moran 2000, Ortiz-Rivas et al. 2004). Moreover, documentation of such deep-level relictual host associations may prompt re-examination of some younger groups for which synchronous diversification with hosts seems at first glance implausible. Thus, analysis of the 1000+ species of cynipid gall wasps detected no significant overall phylogeny match with their host plant families, mostly woody rosids and herbaceous asterids (Ronquist and Liljeblad 2001). However, recently-discovered taxa have raised the possibility that the ancestral gall wasps, like one basal extant lineage, fed on Papaveraceae, a member of the most basal eudicot lineage, Ranunculales (but see Nylander 2004, Nylander et al. 2004). Fossils date the gall wasps to at least the late Cretaceous, thus it is possible that this habit has been retained since before the rise to prominence of the host groups commonly used today (Ronquist and Liljeblad 2001). A similar history is possible for some genera of leafmining agromyzid flies (Spencer 1990).

Aphids, agromyzids and other groups may participate in another broad historical pattern that is receiving increased attention. Insect groups whose chief diversity is associated with modern (especially poaceous or euasterid) herbaceous plants in temperate regions might well have diversified in parallel with the great Tertiary expansion of open habitats and herbaceous vegetation, driven by global cooling, drying and latitudinal climate stratification trends (Behrensmeyer et al. 1992, Graham 1999). This postulate, in need of rigorous test, shares some elements with escape-and-radiation coevolution, including the ascription of diversification to ecological opportunity, and the distribution of insect lineages across plants to long-term historical trends. The hypothesis predicts that phylogenies of these herbivores should exhibit trends toward use of successively younger host groups (and/or perhaps from trees to herbs), and subclade ages should roughly match those of their hosts and/or biomes (von Dohlen and Moran 2000, von Dohlen et al. 2006, Dietrich 1999). One among many candidate lineages is the so-called trifine Noctuidae (Noctuidae sensu stricto; 11,000+ species). Trifines have a markedly higher ratio of temperate to tropical species than any other large family of Macrolepidoptera, and unlike those families, are mostly herb feeders instead of tree feeders. Recent phylogenies confirm that the trifine groups most closely adapted to open, boreal habitats, which are often ground dwelling “cutworms” as larvae, are among the most derived (Holloway and Nielsen 1998, Mitchell et al. 2006).

Diversification of Phytophagous Insects

The extraordinary species richness of plant-feeding insects is a salient feature of terrestrial biodiversity (Strong et al. 1984). It is therefore not surprising that insect-plant

interactions have been a prominent model in the modern revival of interest in diversification (Wood 1993, Schluter 2000, Coyne and Orr 2004). Full understanding of the diversification of phytophagous insects will require both detailed analysis of speciation (and extinction) mechanisms, and comparative study of broad diversification patterns. These enterprises are of course intertwined, and phylogeny is relevant to both. Our review, however, will focus mainly on the comparative aspect.

A fundamental question to be asked is whether the apparent exceptional diversity of phytophagous insects is actually the result of consistent clade selection (Williams 1992), rather than a coincidental impression created by a few groups whose hyperdiversity could reflect some other cause. Sister group comparisons between independently originating phytophagous insect clades and their non-phytophagous sister groups, which control for clade age and other traits possibly influencing diversification rate, show that phytophages have consistently elevated diversities (Mitter et al. 1988). This conclusion is at least consistent with the results of an analysis screening for significant variation in diversification rate across the insect orders (Mayhew 2002). It should be noted that the finding rests at present on only a small fraction of the potential evidence, as the phylogenetic positions of most originations of insect phytophagy are only now beginning to be resolved. Thus, further test of this hypothesis is desirable.

Why should phytophagous insects have elevated diversification rates? Several broad hypotheses have been advanced. One possibility is adaptive radiation (Simpson 1953), re-defined loosely by Schluter (2000) as “evolution of ecological diversity in a

rapidly multiplying lineage” (pg. 1). Vascular plants might constitute an “adaptive zone” providing an extraordinary diversity of underutilized, distinct resources on which insect specialization is possible. A contributing factor might be that more niches supporting a sustainable population size are available at the primary consumer level than to higher levels or to decomposers, no matter how those niches are filled. Diversification could be accelerated still further if plant diversity continually increases due to coevolution sensu Ehrlich and Raven (1964). In a contrasting though complementary hypothesis (Price 1980), phytophage diversity reflects instead a broad propensity of the “parasitic lifestyle” for rapid diversification, due in part to the ease with which populations of small, specialized consumers can be fragmented by the patchy distribution of hosts.

Some progress has been made toward sorting out these alternatives. The finding that insect groups parasitic on animals are, if anything, less diverse than their non-parasitic sister groups (Wiegmann et al. 1993) casts strong doubt on the primacy of the “parasitic lifestyle” hypothesis. The leading hypothesis, adaptive radiation, makes two chief predictions. One of these, the subject of a vigorous area of research (Via 2001, Berlocher and Feder 2002, Rundle and Nosil 2005), is that shifts to new plant resources should be a major contributor to the origin of new species. Earlier, we estimated that about 50% of speciation events in phytophagous insects involve shifts to a different host plant species. This is an underestimate of the importance of plant resource diversity to speciation, because niche shifts within the same host plant species (e.g., to different host organs or tissues) and changes in host range (with retention of at least one previous host) are not included. Comparative data, then, are at least consistent with a major role for

host-related divergence in phytophage diversification. It should be noted that ecological differences between sister species can arise by multiple mechanisms before, during, or after speciation (Futuyma 1989, Schluter 2000). Even if host-related differences were incidental to speciation, however, a broad form of the adaptive zone or radiation hypothesis could be said to hold, if those differences produced higher net diversification rate by forestalling extinction due to competition for resources or enemy-free space. As the foregoing suggests, hypotheses attributing diversification to ecological differentiation have rarely been explicit about which of the many possible mechanisms are involved (review in Allmon 1992). Ongoing ecological study of the importance of competition and natural enemies to phytophage fitness and host use (e.g., Denno et al. 1995, Murphy 2004) should help to distinguish among plausible candidate mechanisms.

A second prediction of the adaptive radiation hypothesis is that the diversification rate of a phytophagous lineage should be correlated with the number of plant resource niches available to it. The strongest evidence on this question so far comes from studies of the beetle clade Phytophaga. In each of ten contrasts identified so far (Farrell 1998, Farrell et al. 2001), beetle groups feeding on conifers or other gymnosperms were less diverse than their angiosperm-feeding sister groups. To these can be added the contrast in Lepidoptera between the basal conifer-feeding lineage Agathiphagidae (two species) and its almost entirely angiosperm-feeding sister group Heterobathmiidae + Glossata (~160,000 spp.; Wiegmann et al. 2000). Although exceptions will undoubtedly be found (e.g., probably lachnine aphids, Normark 2000; xyelid sawflies, Blank 2002), elevated diversity of angiosperm feeders seems likely to remain one of the strongest

diversification effects known (Coyne and Orr 2004) as the numerous additional contrasts are examined. Ascription of this trend to the much greater taxonomic and chemical diversity of flowering plants, rather than some unique historical circumstance or the global biomass difference between angiosperms and gymnosperms, gains credibility from the great variation in ages and geographic distributions among the contrasted lineage pairs, and the fact that some represent secondary return to gymnosperms (Farrell et al. 2001). It will now be of great interest to determine whether association of enhanced insect diversification with more diverse host groups holds on smaller plant-taxonomic scales as well.

Ehrlich and Raven (1964) speculated that diversification of the angiosperms was promoted by their novel and diverse secondary chemistry, which improved protection from herbivores. Correspondingly greater diversity in angiosperm-feeding insects than in related relict gymnosperm feeders is at least consistent with their hypothesis. Broad-scale escape and radiation coevolution is also lent credence by recent evidence that adaptations to and interaction with insects (and other organisms) have marked influence on plant diversification rates. Plant clades bearing latex or resin canals, one of the most elaborate plant defense syndromes known, were shown to be consistently more diverse than sister groups lacking such canals (Farrell et al. 1991). More recently, several types of innovations in reproductive structures, affecting pollinator fidelity or fruit dispersal, have also been shown to be associated with more rapid plant diversification (Sargent 2004, Bolmgren and Eriksson 2005; review in Coyne and Orr 2004). Thus, mounting evidence supports a central tenet of the New Synthesis, implicit in escape and radiation

coevolution, namely that adaptations to biotic interactions have major influence on diversification.

While substantial progress has been made in establishing phytophage diversification patterns at the broadest scale, countless questions remain, particularly at shorter evolutionary timescales. There is almost no unambiguous evidence on whether repeated counter-adaptations to plant defenses have accelerated insect diversification, as predicted under escape-and-radiation coevolution (but see Farrell et al. 2001 regarding mutualism with ambrosia fungi in bark beetles; parallel examples of fungal mutualism in cecidomyiid gall midges discussed by Bisset and Borkent [1988] and Gagné [1989] await further phylogenetic study). Numerous other causes have been postulated for differential diversification of phytophages, including, among others: species richness, secondary chemical diversity, growth form and geographic distribution of the host group (e.g., Price 1980, Strong et al. 1984, Lewinsohn et al. 2005); mode of feeding, including plant tissue attacked, internal versus external feeding, and gallmaking (and advanced forms thereof; Ronquist and Liljeblad 2001); trenching and other forms of herbivore “offense” (Karban and Agrawal 2002); degree of food plant specialization; host shift frequency; and various traits (often host-use-related) rendering phytophages less susceptible to natural enemies (Singer and Stireman 2005). Indeed, just about any trait that might be conserved on phylogenies becomes a plausible candidate. Ideally, one would like to determine the relative importance of and interactions among these factors, and compare them to other types of influence on diversification. In the Lepidoptera, for example, the most pervasive differential influence on diversification may prove to be the repeated evolution of ultra-

sound detectors allowing adults to avoid bat predation (e.g., Yack and Fullard 2000), rather than any “bottom-up” factor having to do with host plants.

Progress on testing such hypotheses has been quite limited so far, probably for several reasons. First, although phylogenies are accumulating rapidly, the detailed phylogenetic resolution needed to detect correlates of diversification rates is still lacking within most families of phytophagous insects; in some cases, even species diversities are not yet well characterized. Second, we are only beginning to understand the phylogenetic distributions of most candidate traits. Many of these appear to be much more evolutionarily labile than the relatively conserved features reviewed earlier. Rapid trait evolution can frustrate estimation of ancestral states, particularly when life history information is incomplete, making reliable sister group comparisons hard to identify. For example, our scan of published studies uncovered essentially no unambiguous contrasts between lineages with broader versus narrower species host ranges, though sister clades often differed in average host range (cite Janz paper?). Moreover, the groups characterized by labile traits, when identifiable, will often be so recent that dissecting deterministic from stochastic influences on diversification would require a large number of comparisons. Sister-group comparisons remain the most robust and straightforward method for detecting traits correlated with diversification rate (Vamosi and Vamosi 2005). But, unless traits that vary mostly at lower taxonomic levels are to be dismissed as unlikely to influence diversification rates, additional approaches will be needed (Ree 2005).

Fortunately, there is now a diverse, rapidly growing literature on diversification rate analysis, a full survey of which is beyond the scope of this paper. Any of several approaches might prove useful for testing the association of relatively labile traits with diversification rates, depending on the nature of the data. If the chief difficulty is that inferred trait origins do not clearly define sister group comparisons, one might identify comparisons a priori, then score sister groups simultaneously for diversity and some appropriate measure of frequency of the predictor trait. To select potentially informative comparisons, one might employ one of the various model-based methods proposed for identifying significant shifts in rates of diversification (Sanderson and Donoghue 1994, Magallón and Sanderson 2001, Moore et al. 2004); possible drawbacks include the need for well resolved phylogenies and high variance of trait frequency estimates in extremely asymmetrical comparisons. For quantitative predictor variables (e.g., average host range) a variant of the independent contrasts method is available (Isaac et al. 2003). When lack of deeper-level phylogeny resolution limits identification of sister groups, one might make independent comparisons among groups of different ages, using estimates of absolute or relative diversification rates (Purvis 1996, Bokma 2003; application in Nyman et al. 2006). For relatively recent radiations, average time between speciation events may be a more sensitive estimator of diversification rate than species numbers per se (Ree 2005). Clock-based temporal analyses of diversification can in principle also detect changes in diversification rate over time (e.g., Nee et al. 1992, Nee et al. 1996, Paradis 1997), allowing test of such refinements of the adaptive zone hypothesis as the postulated slowing of diversification as niches are filled (Simpson 1953, Schluter 2000). Recently, this and other approaches have been used to identify periods of accelerated

insect diversification and correlate these with potential causes such as radiation of particular plant clades, or particular biogeographic events (e.g., McKenna and Farrell 2006, Moreau et al. 2006, but see Brady et al. 2006). Caution must be exercised in interpreting such correlations, however; single examples do not constitute strong evidence of causation.

While phytophage diversification rate variation at lower levels is a daunting problem, even the analysis of relatively conserved traits remains under-developed. To underscore this point, we end with a summary of progress on one much-discussed issue that bears on the puzzle of phytophagous insect diversity, namely, the macroevolutionary consequences of internal versus external feeding. Both habits are widespread, although their frequencies differ markedly across insect phylogeny. Most hemi-metabolous insect herbivores, in orders such as Orthoptera, Phasmida, Hemiptera and Thysanoptera, are free-living external feeders, though some (e.g., thrips) may hide in flowers or other plant structures; the chief exceptions are gall formers, which have evolved repeatedly in the piercing/sucking lineages. In contrast, larvae of a large fraction of phytophagous Holometabola, including the basal members of nearly all the major lineages, actively bore or mine inside living plants. External phytophagy has arisen infrequently in most holometabolous orders, or not at all (e.g., higher Diptera), while return to endophagy has occurred somewhat more often. Overall, the opposing traits seem sufficiently conserved, yet also sufficiently labile, to permit replicated sister group comparisons.

Opposing predictions have been made about diversification under these contrasting feeding modes, drawing on broader theories about ecological specialization (reviews in Wiegmann et al. 1993, Yang and Mitter 1994). Although analyses controlled for phylogeny are needed (Nyman et al. 2006), internal feeders appear to be more host specific than external feeders (e.g., Gaston et al. 1992). Greater specialization, as argued earlier, could promote speciation by increasing the strength of population subdivision and diversifying selection (e.g., Miller and Crespi 2003). Internal feeding could also be viewed as an adaptive zone providing escape from pathogens and some parasites, and desiccation or other physical stresses (Connor and Taverner 1997). Conversely (Powell et al. 1998, Nyman et al. 2006), one could predict that external feeding, by providing release from constraints on body size, voltinism and leaf excision, might typically increase individual and (thereby) clade fitness. Moreover, by lowering the barriers to colonization of alternative hosts and habitats, exophagy might open more opportunities for speciation.

Sister-group contrasts between internal and external feeders are potentially numerous. For example, there is strong evidence for several to many independent transitions between internal and external larval feeding within Lepidoptera (Powell et al. 1998), Coleoptera-Phytophaga (Marvaldi et al. 2002, Farrell and Sequiera 2004), and basal Hymenoptera (sawflies), and between galling and free-living habits within Aphidoidea (von Dohlen and Moran 2000), Coccoidea (Cook and Gullan 2004), Psylloidea (Burckhardt 2005) and Thysanoptera (Morris et al. 1999). Surprisingly, however, from our literature survey we are able to extract at most eight unambiguous

comparisons (Table 2.4). The only phylogenetic study directed specifically at this question is that of Nyman et al. (2006); others are clearly needed.

Disregarding the one tie, five of the seven sister group comparisons we identified show the external feeding lineage to be more diverse than its internal feeding closest relatives. Nyman et al. (2006), in a non-overlapping set of comparisons within the sawfly subfamily Nematinae (Tenthredinidae), found external feeders to be more diverse in 10 of 13 sister group contrasts. Taken together, these compilations yield a result just significant by a two-tailed sign test (external feeders more diverse in 15 of 20 pairs, $P = 0.042$), corroborating the trend in an earlier, more limited compilation by Connor and Taverner (1997).

Although progress is evident, continued study of this question is desirable. The statistical significance of the observed trend is still marginal; several of the comparisons in Table 2.4 are based on provisional phylogenies, and in several the diversity differences are small; it will also be of much interest to separately test the effects of different categories of internal feeding (e.g., gallers vs. miners), and of gains versus losses of external feeding. At the least, however, the current evidence appears to firmly reject the hypothesis of consistently faster diversification by internal feeders. The result parallels previous rejection of the hypothesis of higher diversification in animal-parasitic than free-living insects due to their exceptionally specialized lifestyles (Wiegmann et al. 1993). Together, these observations suggest that, even if phytophages are more ecologically specialized in some sense than other insects, specialization per se is an

Table 2.4. Sister group diversity comparisons between endo- and exophytophage lineages

Higher Taxon	Internally-feeding clade	Diversity	Externally-feeding clade	Diversity	Sources
Coleoptera: Chrysomelidae	Bruchinae + Sagriinae	3,300	Chrysomelinae + Criocerinae + others, minus 2° internal feeders	10,000	1
Hymenoptera	Cephidae + Siricidae + Anaxyelidae + Xiphydriidae, with parasitic subclade Vespina excluded	280	Pamphilidae + Megalodontesidae	350	2,3,4
Hymenoptera	Blasticotomidae	9	Remaining Tenthredinoidea	7,000+	4
Hymenoptera: Xyelidae	Xyelinae	71	Macroxyelinae	11	4,5
Lepidoptera	Cossoidea	1,873	Zygaenoidea	2,115	6
Lepidoptera	Obtectomera minus Macrolepidoptera (part or all)	<22,0000	Macrolepidoptera	87,000	6
Lepidoptera: Heliodinidae	<i>Lamprolophus</i> + 9 genera	56	<i>Epicroesa</i> + <i>Philocoristis</i>	6	7
Thysanoptera: Phlaeothripidae	<i>Kladothrips</i>	22	<i>Rhopalothripoides</i> (+ 5 possibly related genera)	22	8,9

Compilation excludes nematine tenthredinid sawflies, studied by Nyman et al. (2006).

Sources: 1. Farrell and Sequeira (2004), 2. Brown (1989), 3. Heitland (2002), 4. Schulmeister (2003), 5. Blank (2002), 6. Powell et al. (1998), 7. Hsu and Powell (2004), 8. Crespi et al. (2004), 9. Morris et al. (2002).

unlikely explanation for their exceptional diversity. Rather, the evidence increasingly points to the importance of the sheer diversity of niches available to insects feeding on plants, particularly flowering plants.

Synopsis and Conclusions

In this essay we have attempted to compile and synthesize the recent literature (mainly since 1993) treating aspects of the phylogenesis of associated insects and plants.

We have focused on phylogenies at the among-species level and higher, mostly for insects, and on their bearing on three general questions posed implicitly by Ehrlich and Raven's hypothesis of coevolution. These are: (1) the degree to which the various traits governing use of host plants are conserved during phylogenesis; (2) the degree to which contemporary associations show evidence, from phylogenies and other sources, of long-continued interactions between particular insect and plant lineages; and (3), the degree to which evolution in traits affecting their interactions affects the diversification rates of interacting insect and plant lineages.

Our main conclusions are follows:

1. Ubiquitous conservation of plant higher taxon use during insect phylogenesis is confirmed and quantified in a compilation of 93 phylogenies of mostly oligophagous insect groups. The median frequency of shift to a different plant family is estimated to be about 0.03 - 0.08 per speciation event. Important initial insights have been gained on the reasons for this conservatism.

2. There are many hypotheses to explain among-clade variation in the frequency of among-plant-family shift, but few quantitative tests. The strongest evidence to date is for more frequent host shifting in tree feeders than in herb feeders among butterflies, and among oligophages within lineages that contain one or more polyphagous species than in lineages which do not (across 95 insect phylogenies). Recent case studies suggest that

reliance on plant-derived compounds for insect defense poses less of a barrier to larval host shift than was formerly thought.

3. In contrast to the prevailing broad-scale host conservatism, shifts to a different host species have accompanied about 50% of 145 phytophage speciation events tabulated, consistent with a substantial but not universal role for host shifts in phytophage speciation. There is a suggestive but not statistically significant tendency for greater host differentiation between sympatric than allopatric species pairs.

4. The as-yet limited evidence on phylogenetic patterns of host plant range provides no support for directionality or other strong constraints, but suggests an important distinction between ephemeral, phylogenetically random fluctuation, and larger-scale trends interpretable using experimental approaches combined with phylogenetic “comparative methods.”

5. It is now clear that with very few exceptions, the host use variation within and among phytophagous insect genera, in contrast to that in some vertically-transmitted parasites and symbionts, reflects colonization of already diversified hosts rather than any form of strict parallel phylogenesis. At the same time, however, evidence is increasing that associations established in the distant past, especially the Mesozoic, have left widespread if not numerically dominant marks on contemporary insect/plant assemblages; the full range of such historical “signatures” is only beginning to be explored.

6. Because phylogenetic studies directed specifically at plant defense evolution are still few, we do not yet know whether that evolution is characterized more by sequential coevolutionary “escalations,” or by stably co-existing syndromes reflecting optimal adaptations for differing environments.

7. Replicated sister group comparisons have established elevated diversification rates for phytophagous over non-phytophagous insects, and for angiosperm over non-angiosperm feeders among phytophages, both at least consistent with diffuse insect-plant coevolution sensu Ehrlich and Raven. Recent studies on plant diversification rates demonstrate a role for interaction with insects and other animals, likewise consonant with that theory, though most examples do not involve defense. Evidence on most phytophage diversification hypotheses (including “offense” innovations), however, has been slow to accumulate, and diversification studies at finer taxonomic scales, mostly lacking, may face methodological obstacles. A progress report on sister group comparisons of internal versus external feeders effectively negates the hypothesis of faster radiation by endophages, thought to be more specialized, and strongly suggests the opposite trend.

Given the range of questions mapped out, the tools available, and the cornucopia of phylogenetic studies now ongoing in nearly all major herbivorous insect groups and their host plants, we can look forward to spectacular near-future advances in understanding of the evolution of insect/plant interactions, with increasing integration between phylogenetic and other perspectives.

Note: Online supplementary tables and figures are available at

www.chemlife.umd.edu/entm/mitterlab. These include the following:

S1: Database of insect/plant phylogeny studies (Access, FileMaker formats).

S2: Compilation of host shift frequencies on phylogenies (Excel format).

S3: Meta-phylogeny of taxa included in table S2 for comparative analysis of host shift frequency vs. host range (PDF format).

S4: Compilation of host and distribution differences for speciation events (Excel format).

Chapter 3: Phylogeny of the Leaf-mining Fly Genus *Phytomyza* Fallén s.lat. (Diptera: Agromyzidae), with Comments on Life History Traits and on the Status of *Chromatomyia* Hardy

Introduction

Leaf-mining flies (Diptera: Agromyzidae) comprise a species-rich family of internally feeding phytophagous insects, with over 2,800 described species feeding on plants in over 140 families (Spencer 1990, Benavent-Corai et al. 2005, Scheffer et al. 2007). Most species are highly host specific and mine leaves of herbaceous angiosperms, but host use in agromyzids is remarkably varied, with some species feeding in stems, seeds, and roots, and even in twig galls and cambium of young trees. A few are widely polyphagous crop pests (Spencer 1973). *Phytomyza* Fallén is the largest agromyzid genus, including over 530 described species. Host use in *Phytomyza* plus the closely related and possibly synonymous *Chromatomyia* Hardy (>110 spp.) spans much of the variation observed for the family (Spencer 1990). Although most *Phytomyza/Chromatomyia* species are not economically important, a few have been recorded as occasionally serious pests in Europe, e.g., *P. gymnostoma* Loew on leek, *P. rufipes* Meigen on *Brassica* spp., and *C. fuscula* (Zetterstedt) on cereals (Spencer 1973, Dempewolf 2004). *Chromatomyia syngenesiae* Hardy is a Holarctic species that can be a major pest on flowers, including in greenhouses (Spencer 1973). Its highly polyphagous close relative *C. horticola* (Goureau) is a major pest of peas and other agricultural crops and ornamentals across much of the Old World (Griffiths 1967, Spencer 1973). Other

species are common pests of ornamental plants, including hollies (*Ilex*) (Kulp 1968) and columbines (*Aquilegia*) (Spencer 1973, Braman et al. 2005).

Herbivorous insects provide some of the most spectacular cases of adaptive radiation, and collectively comprise over one quarter of macroscopic biodiversity (Strong et al. 1984, Mitter et al. 1988). However, our understanding of the link between host use evolution and patterns of speciation in phytophagous insects is far from complete, despite several decades of concerted work (Berlocher and Feder 2005, Winkler and Mitter 2008; see Chapter 2). Because *Phytomyza* is a species-rich group with diverse and relatively well-known host associations, it is a good candidate clade in which to study host-plant-associated patterns of diversification. As for many phytophagous insects, diversification of host use is suspected as a major factor explaining the inordinate species richness of *Phytomyza* (Scheffer and Wiegmann 2000). Understanding evolutionary diversification, however, necessitates first a knowledge of phylogenetic relationships, which is largely lacking for *Phytomyza*. Although a number of distinct species groups have been identified in *Phytomyza* (Spencer 1990), the classification is still incomplete, and a number of outstanding questions about relationships exist, including the status of *Chromatomyia*. These questions must be resolved, and a clearer understanding of *Phytomyza* phylogeny developed, before any detailed evolutionary hypotheses can be tested.

This study aims to identify and test the monophyly of host-associated species groups in *Phytomyza* and the closely related genus *Chromatomyia* and to investigate

phylogenetic relationships between these groups as a prelude to a more thorough analysis of diversification and host use evolution in this group (Chapter 4). To this end, we present a phylogenetic analysis of one mitochondrial and two nuclear gene regions (CO-I, CAD, PGD) totaling 3,076 b.p., sequenced in 113 species, including nearly all previously-recognized species groups of *Phytomyza* and *Chromatomyia* plus related, outgroup genera. Based on these results, we test the monophyly of both *Phytomyza* and *Chromatomyia* and revise the species group classification of *Phytomyza* insofar as the data permit.

The genus *Phytomyza sensu lato* has long been recognized as morphologically distinct (Fallén 1810), and can be distinguished from most other agromyzids by a combination of the following characters: fronto-orbital setae proclinate, costa extending only to vein R4+5, and crossvein dm-cu usually absent (Spencer and Steyskal 1986, Spencer 1987). Some of these characters, however, are shared by species now placed in the genera *Aulagromyza* Enderlein (= *Paraphytomyza* Enderlein of earlier authors (Tchirnhaus 1991)) and *Napomyza* Westwood, as well as the small genera *Ptochomyza* Hering and *Gymnophytomyza* Hendel. These genera have been confirmed as the closest relatives of *Phytomyza* by both morphological (Dempewolf 2001) and molecular (Scheffer et al. 2007) phylogenetic studies.

The status of *Chromatomyia*, in contrast, has long been uncertain. *Chromatomyia* was originally erected for species of *Phytomyza* with characteristic slipper-shaped pupae that remain in the leaf mine (Hardy 1849); most agromyzids instead leave the mine to

pupate in the soil. It was treated as a subgenus of *Phytomyza* by Brashnikov (1897), but in a somewhat different sense. It was later recognized, however (e.g. Griffiths 1974a), that species pupating in this manner belong to several possibly unrelated groups of *Phytomyza*. The name *Chromatomyia* was widely overlooked or rejected (e.g. Collin 1911, Hendel 1931-36, Frick 1952) until revived by Griffiths (1974a), who further characterized the genus as possessing apomorphic male genitalia, with the distiphallus reduced and lying below a dorsal lobe or sclerite. This definition excluded one species (*P. ilicis* Curtis) originally placed in *Chromatomyia* that is not closely related to the others. Subsequently, Spencer expanded the limits of *Chromatomyia* to include species that do not correspond completely with other *Chromatomyia* either in mode of pupation or in structure of the genitalia (Spencer 1981, 1990, Spencer and Steyskal 1986; see also Godfray 1985), suggesting that the generic limits require further clarification. Spencer (1990: 406) also questioned the validity of *Chromatomyia* on nomenclatural grounds, noting that application of the same name to a genus of Tephritidae had priority. Because adults of *Chromatomyia* are externally indistinguishable from *Phytomyza* (Spencer and Steyskal 1986), some recent faunal lists (e.g. Papp 1984) have also not recognized the genus. Even Griffiths (1974a) suggested that *Chromatomyia* could optionally be considered a subgenus of *Phytomyza* to avoid breaking up the latter. Dempewolf (2001), in a morphological analysis of Agromyzidae focusing on larval characters, did recover a monophyletic *Chromatomyia*, supported by one larval and one pupal character. However, his data did not support the monophyly of *Phytomyza*, or resolve the relationships among *Phytomyza*, *Chromatomyia*, *Napomyza*, and *Ptochomyza*. Recent molecular evidence (Scheffer et al. 2007) suggests that neither *Phytomyza* nor

Chromatomyia alone is monophyletic, but that these together form a monophyletic unit (excluding *C. scolopendri* (Goureu)), which can be considered *Phytomyza* in the broad sense (*s. lat.*). Both studies, however, included a very limited sample of species of *Phytomyza* and *Chromatomyia*; the present work seeks to provide a more robust test of monophyly for both genera.

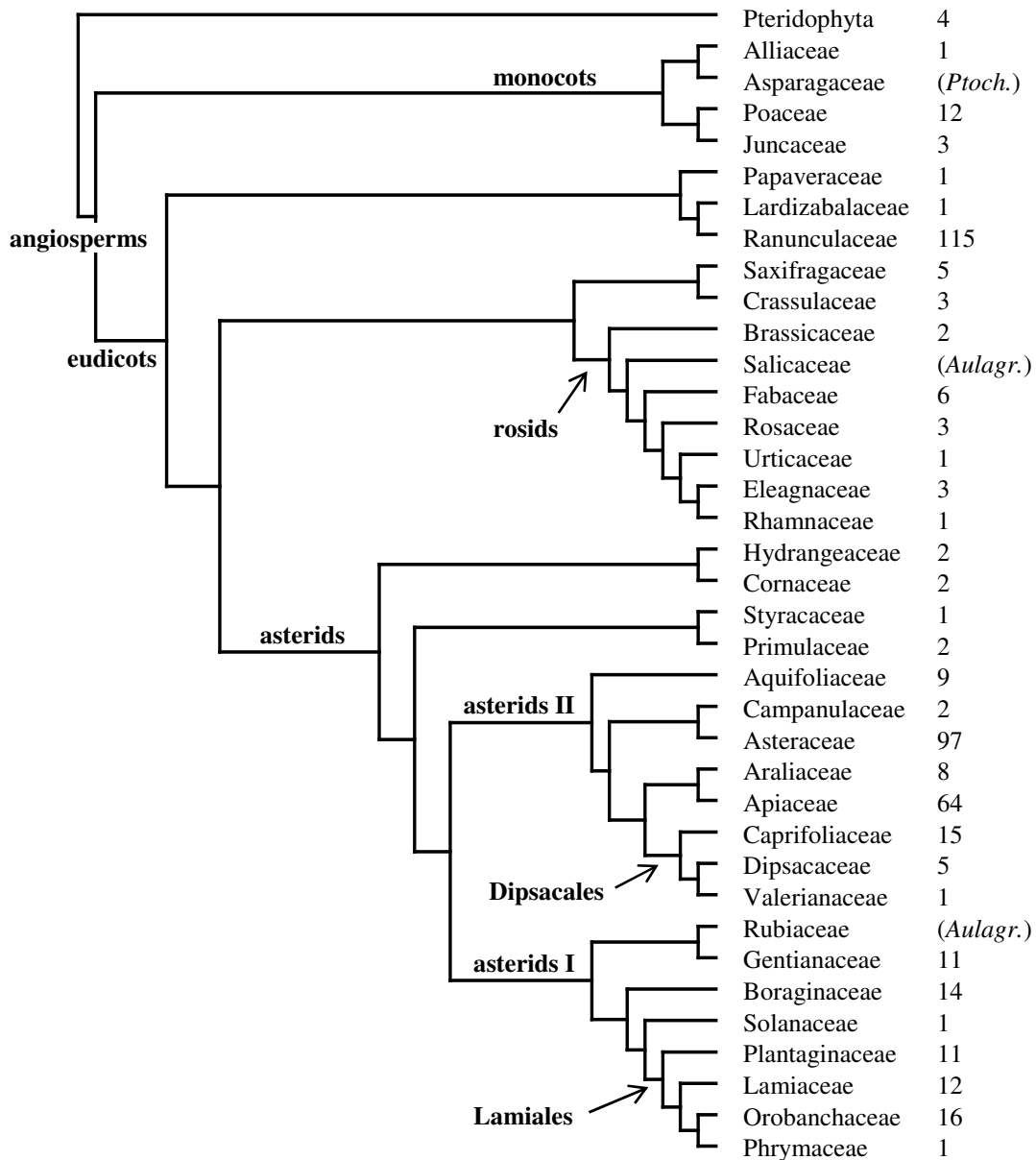
More than a dozen species groups have been recognized in *Phytomyza* (see Table 3.1), each consisting of species with similar male genital morphology and feeding on related host plants (usually in the same family; Spencer 1990). We have cataloged and attempted to test the monophyly of as many such proposals as possible. References to some species groups can be found scattered in earlier taxonomic literature (e.g. Hendel 1927), based on host plant data and external morphology or coloration. However, some of these group names were never formalized, and their circumscription has been somewhat fluid. Some early groupings have since been corroborated, but unrelated species were also sometimes grouped (or even considered as conspecific!) until genital morphology was widely examined and natural groupings further explored (e.g. Nowakowski 1959, 1962, Griffiths 1964, 1972b, 1973, Spencer 1976a, Zlobin 1994, 1997). Species groups of *Phytomyza* were individually discussed by Spencer (1990) in the context of host plant association; this is the main source used to identify presumptive species groups for this study. A few additional groups of species, not explicitly recognized by Spencer, can also be identified by perusal of illustrations of male genitalia and host plant data from some of Spencer's comprehensive works (esp. Spencer and Steyskal 1986, Spencer 1990). A number of species groups ("superspecies") have been

proposed in *Chromatomyia* as well (summarized in Griffiths 1980), each composed of closely related species feeding on plants in a single family (e.g. Griffiths 1967, 1972a, 1974a, 1976a, 1976b, 1980; Spencer, 1990).

While the aforementioned groups account for a large majority of the species, a substantial number of *Phytomyza* species (at least 100) for which male genitalia have been examined do not appear fit into any of the named species groups. Illustrations of the male genitalia are unavailable for approximately another 50, precluding any assignment to a species group.

About half of the species groups and the majority of species of *Phytomyza*, as well as many groups of *Chromatomyia*, feed on plants included in the group “asterids” (sensu Angiosperm Phylogeny Group 2003; hereafter APGII), including Orobanchaceae and Plantaginaceae (sensu Olmstead et al. 2001), Lamiaceae, Boraginaceae, Apiaceae, Aquifoliaceae, and especially Asteraceae (Spencer 1990; see also Figs. 3.1, 3.3, Appendix A). Most of the remaining species and species groups feed on hosts in the family Ranunculaceae, which belongs to the oldest lineage of the “eudicot” clade of angiosperms (APGII 2003). The Ranunculaceae feeders appear to be more morphologically heterogeneous than asterid feeders, though some distinct groups are recognized. It is tempting to suppose, as did Spencer (1990), that this pattern reflects an ancestral host association with the Ranunculaceae, followed by later radiations on more recently evolved asterid plants.

Fig. 3.1. Phylogeny of host plant families of *Phytomyza* and related genera, showing major clades referred to in text. The number of *Phytomyza* species feeding on each host family are listed at right (including *Chromatomyia*, except the polyphagous *C. horticola*). Species numbers are listed in Scheffer et al. (2007), and were compiled from Spencer (1990) and Benavent-Corai et al (2005). The plant phylogeny is taken largely from Stevens (2007), with tentative relationships in the “asterid I” clade reflecting those found by Bremer et al. (2002)



Methods

Taxon sampling

Through our own collecting and contributions from colleagues, we were able to obtain material for sequencing from 102 species of *Phytomyza* and *Chromatomyia*, mostly from North America and Europe, representing nearly all putative species groups and host plant family associations (Table 3.1). Specimens were mainly obtained as adults by sweep netting, but a substantial number were collected as larvae or pupae from host plants and reared. A few immature specimens were sequenced when rearing was unsuccessful or impractical. All species groups of *Phytomyza* discussed in Spencer (1990) and other recent literature were represented except for the *buhriana* group, comprising three species in the Palearctic and Oriental Regions (Zlobin 2002). The latter group feeds on Ranunculaceae and is thought to be related to the holarctic *hendeli* group. Species not belonging to named species groups, possibly representing distinct lineages, were included whenever possible, but material was unavailable for many of these. Of our ingroup species sample, 33 species are unplaced to species group, representing about a third of these unaffiliated species. For *Chromatomyia*, all of Griffiths' (1980) "superspecies" were represented with the exception of the *erigontophaga* and *opacella* superspecies (the latter closely related to the *milii* superspecies). Several species added subsequently to *Chromatomyia* were also represented. Thirteen species of *Napomyza*, *Aulagromyza*, *Gymnophytomyza*, and *Ptochomyza* were included as outgroups. Twenty-six of the species, including all outgroup taxa (except *Ptochomyza*), were included in the family-level analysis of Scheffer *et al.* (2007), while the remaining specimens were

Table 3.1. Species sampled for this study, arranged by species group. Species group names and delimitation largely follow Spencer (1990), with some additional groups from more recent literature (Zlobin, 1994, 1997), and minimum species group diversities estimated approximately from the taxonomic literature. Unless indicated, hosts are taken from Spencer (1990) and Benavent-Corai et al. (2005). Abbreviations used are as follows: **Author- R.-D.:** Robineau-Desvoidy; **Locality-** (besides commonly accepted US state abbreviations)- UK: United Kingdom, Swi.: Switzerland, Fra.: France, Jap.: Japan, Lith.: Lithuania, Nor.: Norway, N.Z.: New Zealand, Mon.: Mongolia; **Collector-** ISW: Isaac Winkler, SJS: Sonja Scheffer, BMW: Brian Wiegmann, SP: Saulius Pakalniškis, US: Urs Schaffner, HH: H. Heinz, PI: Povilas Iviniskis, AA: Arild Andersen, CRN: Riley Nelson, GZ: Greg Zolnerowich et al., HCJG: Charles Godfray, WNM: Wayne Mathis, NAM: Nick Martin, WEC: William Chaney; **Stage - M:** adult male, F: adult female, P: pupa, L: larva, * indicates dry pinned material; **Hosts -** underlined hosts represent material reared for this study, * indicates new host records; GenBank Accession: NS, no sequence obtained.

Species	Author	Locality	Collector	Stage	hosts	GenBank Accession #			
						COI	CADI	CADIV	PGD
OUTGROUPS (<i>Phytomyza</i> group of genera)									
<i>Aulagromyza</i> [50 spp: Rubiaceae: 8 spp., Caprifoliaceae: 10 spp., Salix: 5 spp., other hosts: 10 (generic placement of some uncertain)]									
<i>A. discrepans</i>	(van der Wulp)	UK	ISW	M	stems of <i>Galium</i> (Rubiaceae)	EF104672	EF104757	EU367802	EU367913
<i>A. luteoscutellata</i>	(de Meijere)	NY	SJS	M	<i>Lonicera</i> , <i>Symphoricarpos</i> (Caprifoliaceae)	EF104673	EF104758	EU367795	EU367905
<i>A. nitida</i>	(Malloch)	MD	SJS	M	unknown	EF104674	EF104759	NS	EU367904
<i>A. orbitalis</i>	(Malloch)	NC	BMW	M	<i>Lonicera</i> (Caprifoliaceae)	EF104675	EF104760	EU367797	EU367907
<i>A. tridentata</i>	(Loew)	CO	ISW	M	<i>Salix</i> (Salicaceae)	EF104676	EF104761	EU367803	EU367914
<i>Gymnophytomyza</i> [2 spp. on Rubiaceae (seed feeders)]									
<i>G. heteroneura</i>	(Hendel)	UK	ISW	M	seeds of <i>Galium</i> (Rubiaceae)	EF104698	EF104783	EU367805	EU367916
<i>Napomyza</i> [55 spp.; 1 of 3 groups represented; most known hosts Asteraceae (stem & flower head feeders)]									
<i>N. lateralis</i>	(Fallén)	Swi.	US	F	seeds of many Asteraceae (including <i>Tripleurospermum</i>)	EF104710	EF104796	EU367799	EU367909
<i>N. montanoides</i>	Spencer	CO	SJS	M	unknown	EF104711	EF104797	EU367800	EU367911
<i>N. plumea</i>	Spencer	CO	ISW	M	stems of <i>Achillea</i> (Asteraceae)	EF104712	EF104798	EU367798	EU367908
<i>N. cichorii</i>	Spencer	Fra.	HH	M	stems of <i>Cichorium</i> , <i>Lactuca</i> * (Asteraceae)	EF104708	EF104794	NS	EU367910
<i>Ptochomyza</i> [4 spp; Asparagaceae: 2 spp., Apiaceae: 1 sp., Ranunculaceae: 1 sp.]									
<i>Pto. asparagi</i>	Hering	Lith.	SP	M*	<i>Asparagus</i> (Asparagaceae)	EU367605	EU367695	EU367783	EU367892

Species	Author	Locality	Collector	Stage	hosts	GenBank Accession #			
						COI	CADI	CADIV	PGD
INGROUPS									
<i>Chromatomyia</i> [112 spp.]									
<i>Chromatomyia</i> s.s. [after Griffiths (1974a)]									
<i>C. aprilina</i>	(Goureau)	UK	ISW	M	<i>Lonicera</i> (Caprifoliaceae)	EF104691	EF104776	EU367804	EU367915
<i>C. fricki</i>	Griffiths	UT	CRN	M	<i>Symphoricarpos</i> (Caprifoliaceae)	EU367528	EU367617	NS	EU367813
<i>C. fuscula</i>	Zetterstedt	Nor.	AA	M	many Poaceae	EF104692	EF104777	EU367791	EU367900
<i>C. gentianae</i>	(Hendel)	Lith.	PI	M*	<i>Gentiana</i> (Gentianaceae)	EU367530	EU367619	EU367708	EU367815
<i>C. lactuca</i>	(Frost)	NC	SJS	M	<i>Lactuca</i> (Asteraceae)	EF104693	EF104778	EU367793	EU367902
<i>C. nr. luzulae</i>		UT	CRN	M	probably Juncaceae	EU367525	EU367614	EU367704	EU367810
<i>C. milii</i>	(Kaltenbach)	UK	ISW	M	many Poaceae	EU367524	EU367613	EU367703	EU367809
<i>C. nigra</i>	(Meigen)	UK	ISW	M	many Poaceae	EU367607	EU367697	EU367792	EU367901
<i>C. primulae</i>	(R.-D.)	UK	ISW	P	<i>Primula</i> (Primulaceae)	EU367533	EU367622	EU367711	EU367818
<i>C. shepherdiana</i>	Griffiths	WA	ISW	M	<i>Shepherdia</i> (Eleagnaceae)	EU367531	EU367620	EU367709	EU367816
<i>C. syngenesiae</i>	Hardy	CA	WEC	M	<i>Sonchus</i> , many other Asteraceae	EF104694	EF104779	EU367714	EU367821
<i>C. tiarella</i>	Griffiths	WA	ISW	F	<i>Tiarella</i> , <i>Tolmiea</i> , <i>Heuchera</i> , <i>Tellima</i> (Saxifragaceae)	EU367527	EU367616	EU367706	EU367812
additional species [described or transferred later]									
<i>C. nr. castillejae</i>		CO	ISW	M	unknown	EU367600	EU367690	EU367778	EU367887
<i>C. clematoides</i>	(Spencer)	CO	ISW	M	<i>Clematis</i> (Ranunculaceae)	EU367596	EU367686	EU367774	EU367884
<i>C. mimuli</i>	Spencer	CO	ISW	M	<i>Mimulus</i> (Phrymaceae), <i>Hydrophyllum</i> (Boraginaceae), several <i>Lamiaceae</i> *	EU367599	EU367689	EU367777	EU367886
<i>C. paraciliata</i>	Godfray	UK	HJG	F*	<i>Leucanthemum</i> (Asteraceae)	EU367532	EU367621	EU367710	EU367817
<i>C. ramosa</i>	(Hendel)	Lith.	SP	P	<i>Dipsacus</i> , <i>Knautia</i> , <i>Succisa</i> (Dipsacaceae)	EU367540	EU367629	EU367718	EU367826
<i>C. scolopendri</i>	(Goureau)	UK	ISW	F	<i>Asplenium</i> (Aspleniaceae), <i>Polypodium</i> (Polypodiaceae)	EF104695	EF104780	EU367801	EU367912
Phytomyza [532 spp.]									
<i>albipennis</i> grp. s.l. [10 spp.; Ranunculaceae (stemminers); including <i>nigritula</i> grp. of Zlobin (1994)]									
<i>P. evanescens</i>	Hendel	NC	SJS	M	stems of <i>Ranunculus</i> (Ranunculaceae)	EF104730	EF104816	EU367786	EU367895
<i>P. marginalis</i>	Frost	NY	SJS	M	stems of <i>Ranunculus</i> (Ranunculaceae)	EU367551	EU367640	EU367729	EU367837
<i>anemones</i> grp. [11 spp.; Ranunculaceae]									

Species	Author	Loc-ality	Coll-ector	Stage	hosts	GenBank Accession #			
						COI	CADI	CADIV	PGD
<i>P. fallaciosa</i>	Brischke	UK	ISW	M	<i>Ranunculus</i> (Ranunculaceae)	EU367595	EU367685	EU367773	EU367883
<i>aquilegiae</i> grp. s.s. [9 spp.; Ranunculaceae]									
<i>P. aquilegiana</i>	Frost	MD	SJS	M	<i>Aquilegia</i> (Ranunculaceae)	EF104724	EF104810	EU367784	EU367893
<i>P. aquilegioides</i>	Sehgal	CA	ISW	M	<i>Aquilegia, Thalictrum</i> (Ranunculaceae)	EU367563	EU367652	EU367741	EU367849
<i>P. columbinae</i>	Sehgal	WY	ISW	M	<i>Aquilegia, Thalictrum</i> (Ranunculaceae)	EU367562	EU367651	EU367740	EU367848
<i>P. plumiseta</i>	Frost	NY	SJS	M	<i>Thalictrum</i> (Ranunculaceae)	EU367564	EU367653	EU367742	EU367850
<i>hendeli</i> grp. [10 spp.; mostly Ranunculaceae]									
<i>P. ranunculivora</i>	Hering	UK	ISW	M	<i>Ranunculus</i> (Ranunculaceae)	EU367538	EU367627	EU367717	EU367824
<i>notata</i> grp. [16 spp.; Ranunculaceae]									
<i>P. 'Clematis'</i>		Jap.	ISW	L	<i>Clematis</i> (Ranunculaceae)	EU367594	EU367684	NS	EU367882
<i>P. notata</i>	Meigen	UK	ISW	M	<i>Ranunculus</i> (Ranunculaceae)	EU367592	EU367682	EU367771	EU367880
<i>P. ranunculi</i>	Schrank	UK	ISW	M	<i>Ranunculus</i> (Ranunculaceae)	EU367591	EU367681	EU367770	EU367879
<i>P. vitalbae</i>	Kaltenbach	Swi.	US	F	<i>Clematis</i> (Ranunculaceae)	EU367593	EU367683	EU367772	EU367881
<i>opaca</i> grp. [6 spp.; Ranunculaceae]									
<i>P. nr. calthivora</i>		CO	ISW	M	probably <i>Caltha</i> (Ranunculaceae)	EU367573	EU367662	EU367750	EU367859
<i>ranunculella</i> grp. [16 spp; Ranunculaceae (mostly stemminers, some leafminers); mostly south temperate]									
<i>P. costata</i>	Harrison	N.Z.	WNM	M	<i>Ranunculus</i> (Ranunculaceae)	EF104709	EF104795	EU367788	EU367897
<i>P. lyalli</i>	Spencer	N.Z.	NAM	M	stems of <i>Ranunculus</i> (Ranunculaceae)	EU367606	EU367696	EU367789	EU367898
<i>albiceps</i> grp. [59 spp.; Asteraceae]									
<i>P. nr. arnicae</i>		MT	ISW	M	<i>Arnica</i> (Asteraceae)	EU367583	EU367673	EU367762	EU367871
<i>P. nr. artemisiae</i>		Jap.	ISW	M	probably <i>Artemisia</i> (Asteraceae)	EU367601	EU367691	EU367779	EU367888
<i>P. cirsii</i>	Hendel	UK	ISW	P	<i>Carduus, Cirsium</i> (Asteraceae)	EU367587	EU367677	EU367766	EU367875
<i>P. erigerophila</i>	Hering	NC	SJS	M	<i>Erigeron</i> (Asteraceae)	EF104726	EF104812	EU367760	EU367869
<i>P. lappae</i>	Goureau	UK	ISW	P	<i>Arctium</i> (Asteraceae)	EU367602	EU367692	EU367780	EU367889
<i>P. ovimontis</i>	Griffiths	CA	ISW	M	<i>Erigeron</i> (Asteraceae)	EU367585	EU367675	EU367764	EU367873
<i>P. saxatilis</i>	Griffiths	CO	ISW	M	<i>Artemisia</i> (Asteraceae)	EU367586	EU367676	EU367765	EU367874
<i>P. nr. saximontana</i>		CA	ISW	M	probably Asteraceae	EU367582	EU367672	EU367761	EU367870
<i>P. solidaginophaga</i>	Sehgal	CO	ISW	M	<i>Solidago</i> (Asteraceae)	EU367584	EU367674	EU367763	EU367872
<i>angelicae</i> grp. [30 spp.; Apiaceae, Araliaceae]									
<i>P. angelicae</i>	Kaltenbach	UK	ISW	L	<i>Angelica</i> (Apiaceae)	EU367589	EU367679	EU367768	EU367877

Species	Author	Loc-ality	Coll-ector	Stage	hosts	GenBank Accession #			
						COI	CADI	CADIV	PGD
<i>P. nr. cicutella</i>		CA	ISW	M	probably Apiaceae	EU367588	EU367678	EU367767	EU367876
<i>P. ukogi</i>	Iwasaki	Jap.	ISW	M	<i>Acanthopanax</i> (Araliaceae)	EU367590	EU367680	EU367769	EU367878
atomaria grp. [44 spp.; Orobanchaceae, Plantaginaceae (stemminers, seed-feeders, and leafminers)]									
<i>P. crassiseta</i>	Zetterstedt	UK	ISW	M	<i>Veronica</i> (Plantaginaceae)	EU367549	EU367638	EU367727	EU367835
<i>P. lupini</i>	Sehgal	CO	SJS	M	stems and flower heads of <i>Lupinus</i> (Fabaceae)	EU367554	EU367643	EU367732	EU367840
<i>P. subtenella</i>	Frost	CO	ISW	M	seeds of <i>Castilleja, Pedicularis*</i> (Orobanchaceae)	EU367556	EU367645	EU367734	EU367842
<i>P. nr. superba</i>		WY	ISW	M	unknown	EU367553	EU367642	EU367731	EU367839
<i>P. trivittata</i>	Frost	CA	ISW	M	seeds of <i>Cordylanthus</i> (Orobanchaceae)	EU367557	EU367646	EU367735	EU367843
ilicis grp. [10 spp.; Aquifoliaceae]									
<i>P. dimani</i>	Kulp	DC	SJS	M	<i>Ilex decidua</i> (Aquifoliaceae)	EU367543	EU367632	EU367721	EU367829
<i>P. glabricola</i>	Kulp	FL	SJS	M	<i>Ilex glabra, I. coriacea</i> (Aquifoliaceae)	EU367544	EU367633	EU367722	EU367830
<i>P. ilicicola</i>	Loew	MD	SJS	M	<i>Ilex opaca</i> (Aquifoliaceae)	EU367542	EU367631	EU367720	EU367828
<i>P. ilicis</i>	Curtis	WA	SJS	M	<i>Ilex aquifolium</i> (Aquifoliaceae)	EU367541	EU367630	EU367719	EU367827
obscura grp. s.l. [18 spp.; Boraginaceae, Lamiaceae; including <i>symplyti</i> and <i>nepetae</i> grps.]									
<i>P. nepetae</i>	Hendel	MN	SJS	M	<i>Nepeta</i> , possibly other <u>Lamiaceae</u>	EU367608	EU367698	EU367796	EU367906
<i>P. ovals</i>	Griffiths	CO	ISW	M	<i>Mertensia</i> , other Boraginaceae	EU367568	EU367657	NS	EU367854
<i>P. tetrasticha</i>	Hendel	UK	ISW	M	<i>Mentha</i> (Lamiaceae)	EU367569	EU367658	EU367746	EU367855
petoei grp. [6 spp.; Lamiaceae]									
<i>P. glechomae</i>	Kaltenbach	UK	ISW	M	<i>Glechoma</i> (Lamiaceae)	EU367546	EU367635	EU367724	EU367832
plantaginis grp. [3 spp.; Plantaginaceae]									
<i>P. penstemonis</i>	Spencer	MD	ISW	M	<i>Penstemon</i> (Plantaginaceae)	EU367547	EU367636	EU367725	EU367833
<i>P. plantaginis</i>	R.-D.	NC	SJS	M	<i>Plantago</i> (Plantainaceae)	BF104729	BF104815	EU367790	EU367899
robustella grp. [29 spp.; Asteraceae]									
<i>P. campestris</i>	Griffiths	WA	ISW	M	<i>Arnica</i> (Asteraceae)	EU367534	EU367623	EU367712	EU367819
<i>P. continua</i>	Hendel	UK	ISW	M	<i>Cirsium</i> (Asteraceae)	EU367537	EU367626	EU367716	EU367823
<i>P. nr. major</i>		WA	ISW	M	probably Asteraceae	EU367536	EU367625	EU367715	EU367822
<i>P. 'Petasites'</i>		WA	ISW	M	<i>Petasites</i> (Asteraceae)	EU367535	EU367624	EU367713	EU367820
spondylii grp. [36 spp.; Apiaceae]									

Species	Author	Loc-ality	Coll-ector	Stage	hosts	GenBank Accession #			
						COI	CADI	CADIV	PGD
<i>P. angelicastroi</i>	Hering	UK	ISW	M	<i>Angelica</i> (Apiaceae)	EU367578	EU367668	EU367756	EU367865
<i>P. archangelicae</i>	Hering	MT	ISW	M	<i>Angelica</i> (Apiaceae)	EU367577	EU367667	EU367755	EU367864
<i>P. chaerophylli</i>	Kaltenbach	UK	ISW	M	several Apiaceae	EU367581	EU367671	EU367759	EU367868
<i>P. osmorhizae</i>	Spencer	MD	SJS	M	<i>Osmorhiza</i> (Apiaceae)	EU367579	EU367669	EU367757	EU367866
<i>P. spondylii</i>	R.-D.	UK	ISW	P	<i>Heracleum, Pastinaca</i> (Apiaceae)	EU367580	EU367670	EU367758	EU367867
unplaced species									
<i>P. aconiti</i>	Hendel	NH	SJS	M	<i>Aconitum, Delphinium</i> (Ranunculaceae)	EU367597	EU367687	EU367775	EU367885
<i>P. nr. acteae</i>		CO	ISW	M	<i>Actea</i> (Ranunculaceae)	EU367567	EU367656	EU367745	EU367853
<i>P. agromyzina</i>	Meigen	WA	ISW	L	<i>Cornus</i> (Cornaceae)	EU367526	EU367615	EU367705	EU367811
<i>P. anemonanthaeae</i>	Spencer	Lith.	SP	P	stems of <i>Anemone</i> (Ranunculaceae)	EU367570	EU367659	EU367747	EU367856
<i>P. aquilegivora</i>	Spencer	NC	BMW	M	<i>Aquilegia</i> (Ranunculaceae)	EF104725	EF104811	EU367794	EU367903
<i>P. nr. bicolor</i>		NC	BMW	M	unknown	EU367550	EU367639	EU367728	EU367836
<i>P. ceanothi</i>	Spencer	KS	GZ	M	<i>Ceanothus</i> (Rhamnaceae)	EU367529	EU367618	EU367707	EU367814
<i>P. 'Cimicifuga'</i>		Jap.	ISW	P	<i>Cimicifuga</i> (Ranunculaceae)	EU367572	EU367661	EU367749	EU367858
<i>P. davisii</i>	Walton	MD	ISW	M	<i>Clematis, Ranunculus</i> (Ranunculaceae)	EU367571	EU367660	EU367748	EU367857
<i>P. 'Escalante'</i>		UT	CRN	M	unknown	EU367552	EU367641	EU367730	EU367838
<i>P. flavicornis</i>	Fallén	MN	SJS	M	stems of <i>Urtica</i> (Urticaceae)	EU367576	EU367665	EU367753	EU367862
<i>P. glabra</i>	Hendel	Lith.	SP	P	stems of <i>Anthriscus, Angelica</i> (Apiaceae)	EU367610	EU367700	NS	EU367918
<i>P. 'Guanella'</i>		CO	ISW	M	unknown	EU367548	EU367637	EU367726	EU367834
<i>P. gymnostoma</i>	Loew	Lith.	SP	F*	<i>Allium</i> (Alliaceae)	EU367598	EU367688	EU367776	NS
<i>P. jonaitisi</i>	Pakalniškis	Lith.	SP	F*	leaf stalks of <i>Thalictrum</i> (Ranunculaceae)	EU367560	EU367649	EU367738	EU367846
<i>P. kasi</i>	Henshaw	CO	ISW	M	unknown	EU367561	EU367650	EU367739	EU367847
<i>P. loewii</i>	Hendel	MD	ISW	M	<i>Clematis</i> (Ranunculaceae)	EU367603	EU367693	EU367781	EU367890
<i>P. nr. manni</i>		UT	CRN	M	unknown	EU367555	EU367644	EU367733	EU367841
<i>P. minuscula</i>	Goureau	CO	ISW	M	<i>Aquilegia, Thalictrum</i> (Ranunculaceae)	EU367522	EU367611	EU367701	EU367807
<i>P. 'Mongolia'</i>		Mon.	CRN	M	<i>Aquilegia, Thalictrum</i> (Ranunculaceae)	EU367523	EU367612	EU367702	EU367808
<i>P. nr. nigriervis</i>		WA	ISW	M	unknown	EU367545	EU367634	EU367723	EU367831
<i>P. 'North Carolina'</i>		NC	BMW	M	unknown	EF104713	EF104799	EU367785	EU367894
<i>P. nr. oxytropidis</i>		CO	SJS	M	probably Fabaceae	EU367574	EU367663	EU367751	EU367860
<i>P. ranunculoides</i>	Spencer	NC	SJS	M	<i>Ranunculus</i> (Ranunculaceae)	EU367604	EU367694	EU367782	EU367891

Species	Author	Loc-ality	Coll-ector	Stage	hosts	GenBank Accession #			
						COI	CADI	CADIV	PGD
<i>P. 'Roosevelt'</i>		CO	SJS	M	unknown	EU367559	EU367648	EU367737	EU367845
<i>P. rufipes</i>	Meigen	CA	SJS	M	<i>Brassica</i> (Brassicaceae)	EF494670	EU367666	EU367754	EU367863
<i>P. 'Spanish Fork'</i>		UT	CRN	M	unknown	EU367558	EU367647	EU367736	EU367844
<i>P. spinaciae</i>	Hendel	UK	ISW	M	<i>Carduus, Cnicus, Serratula</i> (Asteraceae)	EF104696	EF104781	EU367787	EU367896
<i>P. subaquelegiana</i>	Zlobin	CO	SJS	M	<i>Lupinus*</i> (Fabaceae)	EU367566	EU367655	EU367744	EU367852
<i>P. subtilis</i>	Spencer	CA	ISW	M	<i>Lathyrus, Vicia</i> (Fabaceae)	EU367565	EU367654	EU367743	EU367851
<i>P. thalictrella</i>	Spencer	UT	CRN	M	<i>Thalictrum</i> (Ranunculaceae)	EU367539	EU367628	NS	EU367825
<i>P. trollii</i>	Hering	WY	ISW	M	<i>Trollius</i> (Ranunculaceae)	EU367575	EU367664	EU367752	EU367861
<i>P. urbana</i>	Spencer	AK	SJS	M	<i>Lupinus*</i> (Fabaceae)	EU367609	EU367699	EU367806	EU367917

newly obtained for this study, except for the COI sequence of *Phytomyza rufipes*, which was reported by Scheffer and Winkler (in press).

Extraction and sequencing

Procedures for DNA extraction, amplification, and sequencing largely follow Scheffer et al. (2007), and are summarized below. Adult male specimens were used for extraction in most cases, with the dissected genitalia retained as vouchers after removal of the abdomen and maceration in KOH solution. Preliminary identification was performed following keys by Griffiths (1980), Spencer (1969, 1972, 1976a, 1976b), and Spencer and Steyskal (1986). Cleared genitalia were then used to provide a final identification. Some specimens appear to represent undescribed species. Unless these could be closely associated with a described species they were given designations corresponding to hosts or localities. In cases of extraction from immature or female specimens, species identity was clear from external characters, by features of the larval mine, or the identity of the host plant (except two possibly new Japanese species). Total nucleic acids were extracted from single dissected specimens by grinding the specimen in PBS solution and following the insect protocol B of the DNeasy DNA extraction kit (Qiagen Inc., Valencia, CA). For some specimens, extracted most recently, detached abdomens were subjected to the extraction procedure without grinding, allowing the intact head and thorax, as well as the cleared genitalia, to be used as vouchers. This did not appear to affect subsequent amplification of nuclear or mitochondrial genes. This procedure was also used for a few dry, pin-mounted specimens, with times for incubation in the proteinase solution extended to 1-2 days, with mixed results. However, both

nuclear and mitochondrial genes were successfully amplified for five taxa up to fifteen years old. Vouchers will be deposited in the National Museum of Natural History in Washington, D.C.

Fragments from the mitochondrial cytochrome oxidase (COI) gene and from the nuclear genes CAD (*rudimentary*) and phosphogluconate dehydrogenase (PGD) were amplified and sequenced using primers listed in Table 3.2. For COI, a fragment representing nearly the entire coding region was amplified in one piece. Two noncontiguous fragments of CAD were amplified using primers listed in Moulton and Wiegmann (2004). For the second fragment (fragment 4 of Moulton and Wiegmann), and for a few taxa for the first fragment, nested re-amplification using internal primers was necessary. The first fragment included a small intron; an internal sequencing primer was used for most taxa that excluded this intron from the final data set. PGD was recently developed for phylogenetic use by J. Regier and C. Cunningham (Regier 2006), and amplifies relatively easily across the Diptera (J.-W. Kim, pers. comm.). Primers listed by Regier (2006) were used, along with additional primers developed for this study (Table 3.2). A small intron found in this gene was also excluded from the data set by use of internal sequencing primers. For all genes, additional, taxon-specific primers were developed to sequence problematic taxa; sequences for these primers may be obtained from the authors upon request.

A touchdown amplification protocol was used to amplify each gene, with initial denaturation at 92°C for 2 min, followed by 2 touchdown cycles from 58 to 46°C

Table 3.2. Primers sequences used for this study. Primers marked by an asterisk were used for initial amplification (some for sequencing also). Sequences of additional, taxon-specific primers can be obtained from the authors.

Gene	Primer	Sequence	Reference
COI	TY-J-1461*	TTT ACA RTT TAC CGC CTA TTR TCA GCC A	modified from Sperling and Hickey (1994)
	C1-N-2191	CCC GGT AAA ATT AAA ATA TAA ACT TC	Simon et al. (1994); Shao et al. (2001)
	C1-J-2183	CAA CAT TTA TTT TGA TTT TTT GG	Sperling and Hickey (1994)
	C1-N-2413	TCA RCT RAA AAT TTT AAT TCC TGT	this study (modified from C1-J-2441)
	C1-J-2441	CCT ACA GGA ATT AAA ATT TTT AGT TGA TTA GC	Simon et al. (1994)
	TL2-N-3014*	TCC ATT GCA CTA ATC TGC CAT ATT A	slightly modified from Simon et al. (1994)
CAD	CAD 54F*	GTN GTN TTY CAR ACN GGN ATG GT	Moulton and Wiegmann (2004)
	AG-360AR	CCA TGA TTY TGT GAR GYC AT	Scheffer et al. (2007)
	CAD 405R*	GCN GTR TGY TCN GGR TGR AAY TG	Moulton and Wiegmann (2004)
	CAD 787F	GGD GTN ACN ACN GCN TGY TTY GAR CC	Moulton and Wiegmann (2004)
	CAD 850F	RAA YAT HGG HAG TTC BAT GA	this study
	CAD 970R	TRT CRT ART CNG TGG AHA CRG TYT CNG G	this study
	CAD 1098R*	TTN GGN AGY TGN CCN CCC AT	Moulton and Wiegmann (2004)
	PGD	PGD 2F*	ATH GAR TAY GGN GAY ATG CA
PGD 2.5AF		ATGAARACCCTYGGCATGTC	this study
PGD 2.5R		ATRCAACCNCCRCGCCACAT	this study
PGD 3R*		GTR TGT GCN CCR AAR TAR TC	Regier (2006)

(10 s at 92°C, 10 s at 58–46°C, 2 min at 72°C), 29 cycles of 10 s at 92°C, 10 s at 45°C, 2 min at 72°C, and a final extension step for 10 min at 72°C. Amplification products were purified using the Qiaquick PCR Purification kit (Qiagen Inc., Valencia, CA), after which sequencing reactions were carried out using BigDye Sequencing kits (Applied Biosystems, Foster City, CA), and the products fractionated using an ABI-3130 Automated Sequencer (Applied Biosystems). Sequences were assembled using Sequencher software (GeneCodes, Ann Arbor, MI), and aligned using ClustalX (Thompson et al. 1994). Alignment of all genes was trivial except for a small (~30bp) unalignable region in CAD, which was excluded from the analysis. Alignments for individual genes were concatenated into a single sequence alignment using Winclada (Nixon). Sequences have been deposited in the GenBank database (accession numbers listed in Table 3.1).

Phylogenetic analysis

Three approaches were used for phylogenetic inference from the concatenated data set. Parsimony (MP) analysis was conducted in PAUP version 4.0b10 (Swofford 2001), using a heuristic search with 100 random addition sequences and TBR branch swapping. Branch support was estimated with 500 bootstrap replicates (20 random addition sequences each). Second, maximum likelihood (ML) analysis was carried out using GARLI version 0.951 (Zwickl 2006) under the default settings, except that *genthreshfortopoterm* was increased to 20,000. The default settings include specification of a general time reversible model with a gamma rate distribution and invariant sites (GTR+I+G). Eight separate GARLI runs were performed, yielding slightly differing

results. The tree of highest likelihood from these eight was then used as a starting tree for TBR branch swapping in PAUP, in a further search for an optimal tree. Bootstrap values were calculated in a separate GARLI run with 500 replicates, with *genthreshfortopoterm* set at the default value of 10,000. Identical analyses (including bootstrap analyses) were also performed for each gene partition separately, to gauge the level of support provided by each. Third, an analysis using Bayesian inference (BI) was performed using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003), with the data partitioned by gene and modeled separately for each partition. The GTR+I+G model was used for each gene partition, following results obtained from the program MrModelTest 2.2 (Nylander 2004), a modified version of Modeltest 3.6 (Posada and Crandall 1998). Two concurrent runs with four chains each were continued for ten million generations and sampled every 100 generations, with the first 25% discarded as burn-in. All trees were rooted with *Aulagromyza* (excepting *A. tridentata*) and *Gymnophytomyza*, following the results of Dempewolf (2001) and Scheffer et al. (2007).

As *Chromatomyia* proved not to be monophyletic on any resulting tree, we applied the definitions of Farris (1974) to determine the form of non-monophyly of this genus (poly- versus paraphyly) as circumscribed by Griffiths (1974a) and by later authors. Farris invokes a two-state pseudo-character denoting membership versus non-membership in the group of interest, scored for each species on the tree. Under Farris's definition, the group is polyphyletic if and only if, under parsimony optimization of this "membership" variable, multiple origins of membership from non-membership must be

postulated on the tree. If membership is inferred to arise only once, but to be lost in one or more lineages, the group is deemed paraphyletic.

Results

The final alignment consisted of 3,076 characters (COI: 1446 b.p.; CAD: 1211 b.p.; PGD: 418 b.p.), of which nearly half were parsimony informative (see Table 3.3). Approximately two-thirds of the informative characters were at third codon positions. Overall pairwise distances (p) ranged from 1.9% to 21%. All three genes were similarly variable at third positions, with COI slightly less variable, but the nuclear genes were noticeably more variable at first and second positions than COI (Table 3.3).

Parsimony analysis yielded three most parsimonious trees (length 16853) resulting in a nearly completely resolved strict consensus tree (not shown). Bootstrap values were generally high for nodes defining species groups, and moderate to strong support was also found for some deeper nodes, but parsimony support values were

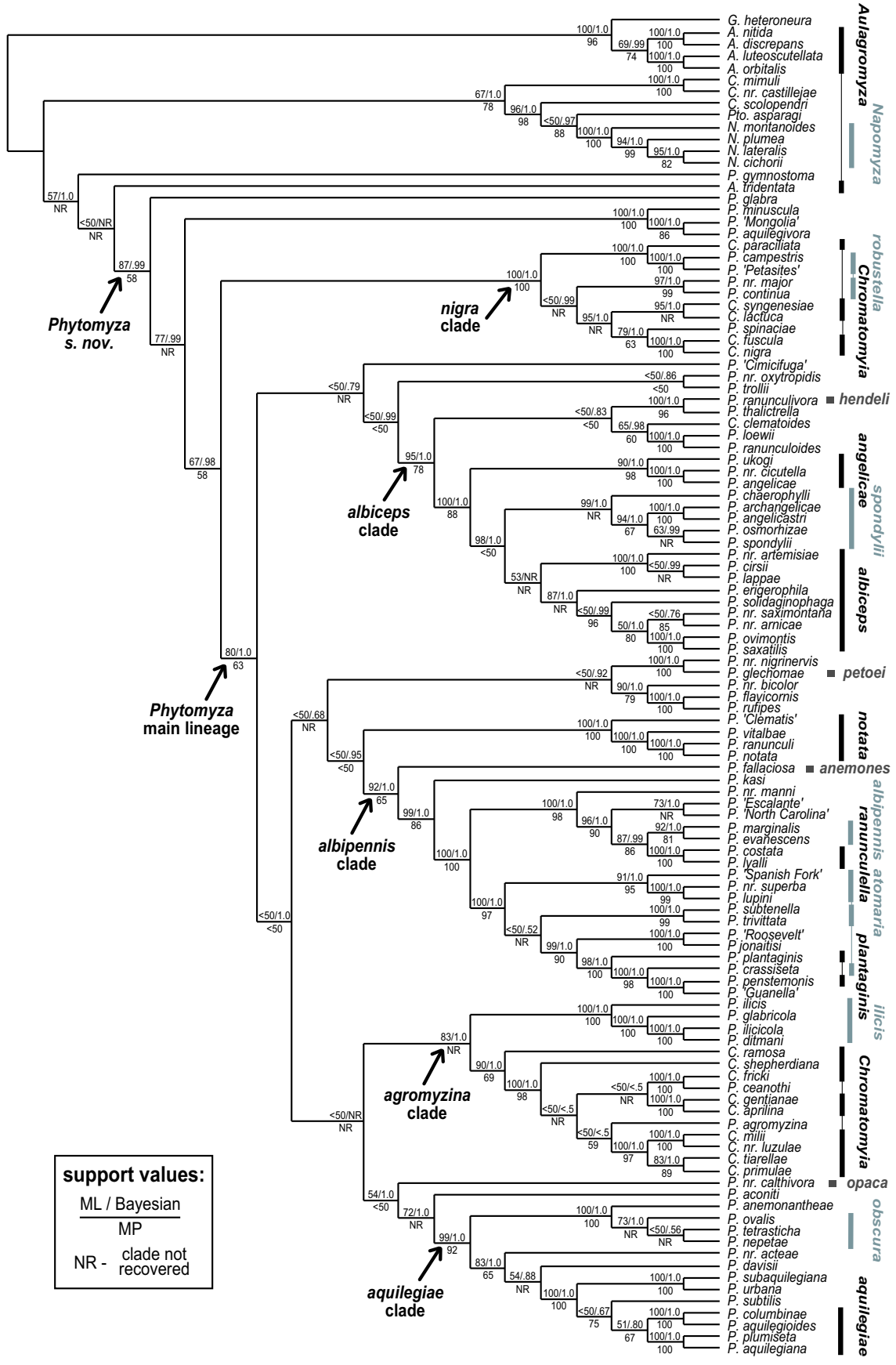
Table 3.3. Number of parsimony informative sites and average pairwise distances for the three gene partitions used in this study.

	COI	CAD	PGD
total base pairs	1446	1212	418
parsimony informative sites (pos. 3 only)	574 (434)	637 (387)	204 (131)
ave. pairwise dist. (p) – pos. 1+2	0.035	0.067	0.050
ave. pairwise dist. (p) – pos. 3	0.278	0.340	0.315

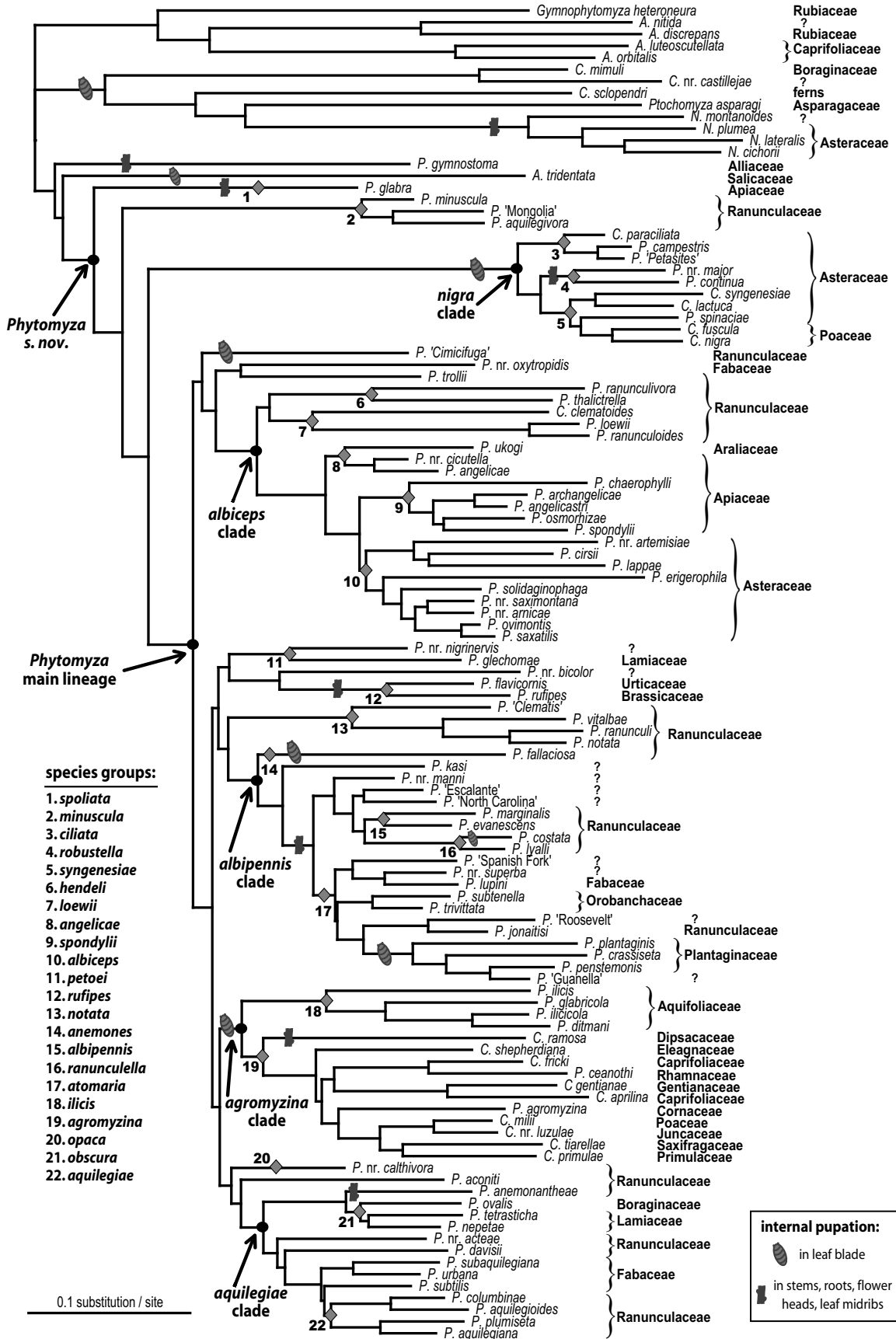
below 50% for many deeper nodes of the *Phytomyza/Chromatomyia* radiation. Further branch swapping on the GARLI tree in PAUP failed to produce a higher likelihood topology. Topologies resulting from the maximum likelihood (Figs. 3.2, 3.3) and Bayesian analyses (not shown) were very similar to each other, differing in only a few poorly supported nodes. These were also similar to the parsimony results, although the arrangement of several important branches differed. Both the Bayesian majority-rule consensus and MP strict consensus trees showed lack of resolution in only one clade – the *agromyzina* group (Fig. 3.2). Bootstrap support values for species groups in the ML analysis were comparable to those under parsimony, while some deeper nodes had markedly higher under ML, and were in no case strongly contradicted by parsimony, suggesting that likelihood does a somewhat better job overall at extracting phylogenetic signal from these data. Bayesian posterior probabilities were generally much higher than both likelihood and parsimony bootstrap percentages. This difference could reflect in part the effect of modeling genes separately, but many recent studies suggest that the posterior probabilities produced by current Bayesian phylogenetic methods generally

Fig. 3.2. (following page) Maximum likelihood phylogeny of *Phytomyza* and *Chromatomyia* species and outgroups from three genes, using GARLI v.0.951 (Zwickl 2006). ML bootstrap values and Bayesian posterior probabilities are listed above branches, with MP bootstrap values below nodes. “NR” indicates that a given clade was not recovered in the MP strict consensus or Bayesian majority rule consensus. Major clades (see text) are labeled within the tree, and generic and species group names, as used in the taxonomic literature (Spencer 1990, and other references in text), are listed at right.

Fig. 3.3. (p. 77) Phylogram of ML phylogeny from Fig. 3.2. Host family for each species is listed at right, where known. Major clades (see text) are labeled, and species groups, as recognized in this study, are labeled 1-22. Lineages which pupate internally in the host plant, either in leaf tissue or in other tissues, are labeled as shown in the key at lower right. Remaining taxa (where known) are all leafminers which leave the mine to pupate in the soil.



support values:
 ML / Bayesian
 MP
 NR - clade not recovered



overestimate branch support (e.g. Suzuki et al. 2002). Thus, we consider ML bootstrap values to provide the best estimates of clade reliability in this study. In the narrative below we distinguish among levels of support using the following somewhat arbitrary conventions: Bootstrap percentage (BP) of 70-79% = moderate support; 80-89% = moderately strong support; BP of 90-100% = strong support.

On no tree was the putative ingroup, *Phytomyza* plus *Chromatomyia*, entirely monophyletic. However, there was moderately strong support (87% ML BP; Fig. 3.2) for a clade consisting of all except a few aberrant *Phytomyza* and *Chromatomyia* species. We term this clade *Phytomyza sensu novo*. The four species that consistently fell outside *Phytomyza sensu novo* were *P. gymnostoma*, *C. scolopendri*, *C. mimuli*, and *C. nr. castillejae*. The latter three species were consistently recovered as part of a clade with *Napomyza* and *Ptochomyza*. *Phytomyza gymnostoma* was recovered either as sister group to *Phytomyza sensu novo* plus *A. tridentata* (ML), or comprising with *A. tridentata* the sister group to *Phytomyza s. nov.* (MP, Bayesian). Relationships among the outgroups were identical to those found by Scheffer et al. (2007), except for the position of *Gymnophytomyza*. This similarity is unsurprising, since all outgroups in this study were included by Scheffer *et al.* except *Ptochomyza asparagi*. This species was found to be sister to *Napomyza* in this analysis, a result consistent with Dempewolf (2001), who placed it in an unresolved clade also including *Phytomyza*, *Chromatomyia*, and *Napomyza*.

Within *Phytomyza sensu novo*, the two earliest-branching lineages, whose sequence of origin is only weakly to moderately supported, are (a) *Phytomyza glabra* and (b) a strongly supported trio of species related to *P. minuscula*. Most of the remaining species and species groups fall within one of five major, well-supported clades of *Phytomyza* and *Chromatomyia* (BP ML = 83-100). These clades, marked on Figs. 3.2 and 3.3 using the names we propose for them, are as follows: 1) The *nigra* clade, consisting of members of the *P. robustella* group and Asteraceae-feeding *Chromatomyia*, together with two Poaceae-feeders and *P. spinaciae*; 2) The *albiceps* clade, including the *angelicae*, *albiceps*, and *spondylii* groups, as well as the *hendeli* group and another small group including *P. loewii*; 3) The *albipennis* clade, consisting of the *anemones*, *plantaginis*, *atomaria*, *ranunculella*, and *albipennis* groups, plus *P. jonaitisi* and several undescribed North American species; 4) The *agromyzina* clade, containing the *ilicis* group and *Chromatomyia* species feeding on non-asteraceous host plants, as well as *P. agromyzina* and *P. ceanothi*; 5) The *aquilegiae* clade, containing the *obscura* and *aquilegiae* species groups, as well as *P. anemonantheae* and a cluster of previously-unplaced species allied to the *aquilegiae* group. Clades 2-5, along with the *notata* and *petoei* groups and the remaining unplaced species of *Phytomyza*, form a monophyletic group with moderately strong support (BP=80, ML) that we term the *Phytomyza* main lineage (Fig. 3.2). The unaffiliated species were scattered throughout this lineage, with mostly low nodal support (see Fig. 3.2). Relationships within the five major clades, reflecting divergences within and among species groups (see below) were well resolved, with 51 of the 69 total contained nodes (74%) having greater than 80% ML bootstrap support.

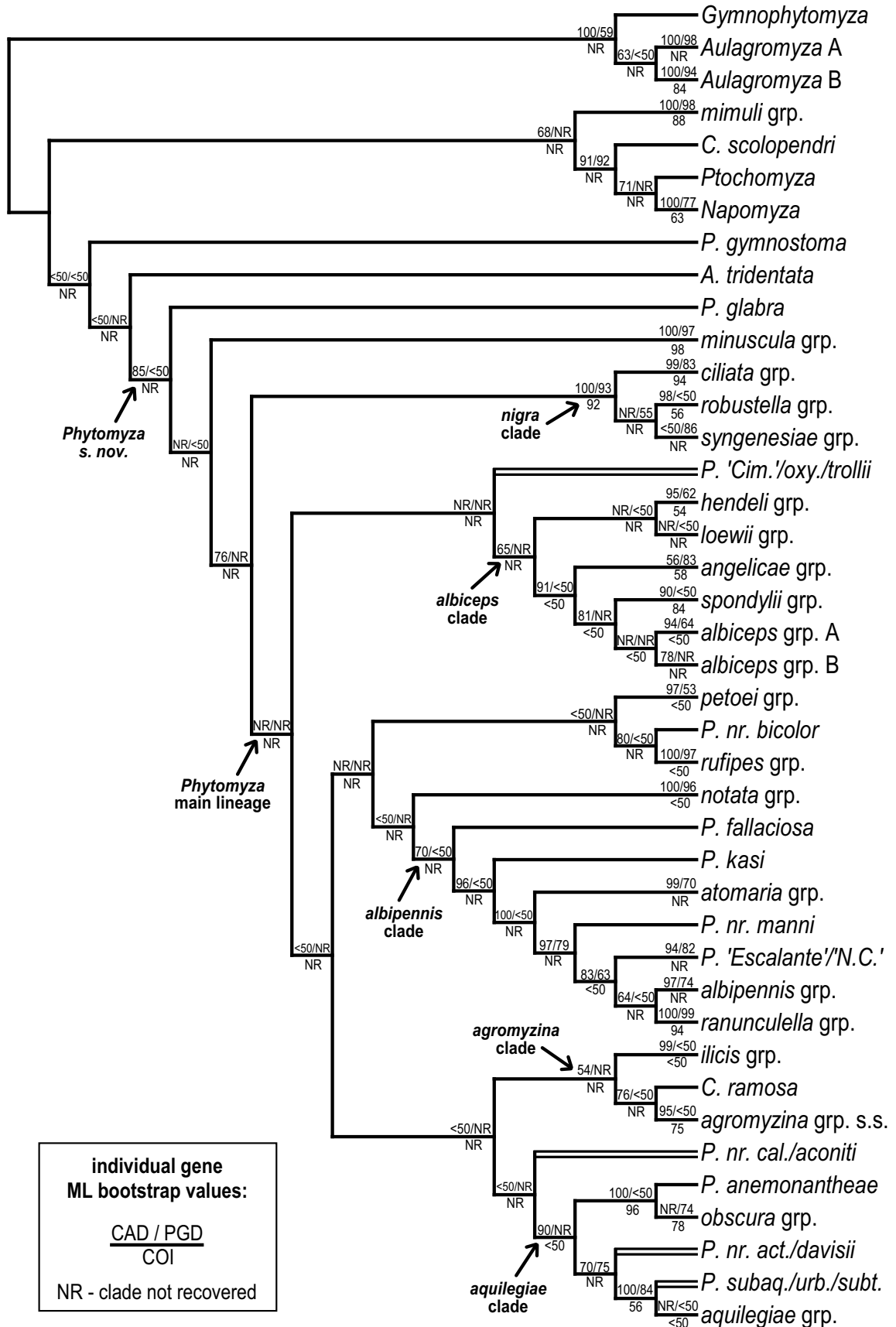
Species belonging to *Chromatomyia* as defined by Griffiths (1974a), here termed *Chromatomyia sensu stricto* (Table 3.1), always fell into two distantly-related lineages, the *syngenesiae* and *agromyzina* groups. *Chromatomyia s.s.* is inferred to be polyphyletic in the definition of Farris (1974) on the tree of Fig. 3.2, even if that tree is trimmed to remove weakly-supported groupings. This reflects the fact that at least two intervening nodes (subtending species assigned to *Phytomyza*) between the subsets of *Chromatomyia s.s.* have moderately strong support. Each subset considered by itself also fails the test of monophyly, because in each case, at least one species of *Chromatomyia* is more closely related to a *Phytomyza* species than it is to other *Chromatomyia*.

Nearly all previously recognized species groups of *Phytomyza* were recovered as monophyletic in one or more analyses, under the original definition or with slight emendation (Fig. 3.2), often with strong support. The *ilicis*, *angelicae*, *notata*, *aquilegiae*, and *ranunculella* groups were strongly corroborated by all analyses. The *spondylii* and *obscura* groups were also monophyletic in the Bayesian and ML analyses, though not under parsimony. Likewise, the large *albiceps* group was monophyletic under ML (albeit with weak support), but paraphyletic to the very similar *spondylii* group under MP and BI. An unusually long branch leading to *P. erigerophila* seemed to contribute to inconsistency in this region of the tree, as this taxon moved significantly in exploratory analyses with fewer data. *Phytomyza evanescens* and *P. marginalis*, representing Zlobin's (1994) *albipennis* and *nigritula* groups, respectively, strongly clustered together, forming what we term the *albipennis* group *s.l.* The *plantaginis* group, defined by Zlobin (1997) to consist of *P. penstemonis*, *P. plantaginis* and one species not sampled here,

was monophyletic with strong support if re-defined to include *P. crassiseta* and an undescribed species from Colorado. If *P. crassiseta* is excluded, Zlobin's *atomaria* group was monophyletic under parsimony but paraphyletic under ML and BI, in each case with weak support. Similarly, the *robustella* group is monophyletic under parsimony if *C. paraciliata* Godfray is included, but paraphyletic under ML and BI, with weak support in each case.

ML Bootstrap analysis with single gene partitions showed that support for most species group and higher relationships comes largely from CAD. These support values are shown in Fig. 3.4 on a simplified phylogeny; values corresponding to relationships within species groups are not shown. Many nodes were poorly supported or not recovered with PGD and/or COI, in some cases even when strongly supported by CAD. Reduced performance of PGD compared to CAD is expected, since this gene partition includes only about one third as many base positions as the CAD partition. However, COI, with slightly more data than sequenced for CAD, performed even more poorly than PGD. Of 28 nodes in Fig. 3.4 for which PGD showed >50% bootstrap support, PGD showed higher support than COI for 23 nodes. Interestingly, CAD showed anomalously low support for three nodes that were more strongly supported by PGD (and also in one case by COI), including the *syngenesiae*, *angelicae*, and *obscura* groups. In the first

Fig. 3.4. (following page) Simplified phylogeny of *Phytomyza* and outgroups, based on the ML phylogeny (Figs. 3.2 and 3.3), showing ML support values (obtained in GARLI v.0.951; Zwickl 2006) for each of the three individual gene partitions (above branches: CAD / PGD; below branches: COI). Branch support values are generally in the order CAD > PGD > COI. Species group names match those listed in Fig. 3.3 and Table 3.4. "NR" indicates that a clade was not recovered in the ML topology.



case, the *syngenesiae* group was not even recovered as monophyletic with CAD, despite moderately strong support (86%) with PGD. Low support of the *obscura* group by CAD may be related to our inability to obtain sequence for one of the two regions of CAD for *P. ovalis*. However, this cannot wholly explain the discrepancy, as the sequence available for this species from CAD was still nearly 300 bases greater than that of PGD. Preliminary data exploration suggests that this inconsistency is likely due to base compositional bias. Third positions (but not first and second) of all partitions were found to be significantly biased when tested in PAUP* (Swofford 2001), but this bias was most pronounced in CAD. In particular, members of the *nigra* clade were found to exhibit a much higher G+C content at third positions than other species: 57.7% in CAD, compared to the mean of 32.5%. This trend was seen to a lesser extent in PGD (51.3% vs. 36.6%), but hardly at all in COI (11.5% vs. 10.2%), probably due to the extreme A+T bias found in all insect mitochondrial genomes. Interestingly, for CAD this bias in the *nigra* clade is least pronounced in the *syngenesiae* group, and especially in *C. syngenesiae*, which is recovered as sister to remaining members of the *nigra* clade in the CAD only ML analysis (not shown).

Discussion

Delimitation of Phytomyza

We propose that the definition of *Phytomyza* be amended to include all species presently placed in *Phytomyza* and *Chromatomyia*, with the exception of *P. gymnostoma* and two small groups of species related to *C. mimuli* and *C. scolopendri* (see Table 3.4, Appendix A). Our justification is as follows. Our trees provide strong evidence against

monophyly of the entities *Phytomyza*, *Chromatomyia* and *Phytomyza* + *Chromatomyia* as currently defined. Second, the branch subtending our proposed *Phytomyza* sensu novo (Fig. 3.2, *Phytomyza* s. nov.) is the only well-defended node (BP=87%, ML) in our analysis that comes close to including all species currently in *Phytomyza* and *Chromatomyia*. While our proposal would require change and temporary instability for the names of a few excluded species, it would provide a firm phylogenetic foundation for the definition of this largest genus of Agromyzidae. The implied synonymization of *Chromatomyia* with *Phytomyza* is treated in a later section (see also Appendix A).

Of the groups excluded from *Phytomyza sensu novo*, *P. gymnostoma* differs from other *Phytomyza* in some characters (mainly of the male postabdomen) which led Spencer (1976a) to remove it to *Napomyza*. In contrast, while acknowledging these plesiomorphic characters, Zlobin (1994) returned *P. gymnostoma* to *Phytomyza* because it lacks synapomorphies of his more precisely defined *Napomyza*. This species may deserve separate generic status once its phylogenetic position is more securely established. At the moment, however, we decline to assign a new generic name, partly to avoid confusion in the literature treating the recently expanding range and pest status of *P. gymnostoma* (Zlobin 1994, Kahrer 1999, Dempewolf 2004, Collins and Lole 2005).

The *mimuli* and *scolopendri* groups of *Chromatomyia* should probably also be given generic status when a more complete morphological diagnosis is possible. Although the habitus of *C. scolopendri* is unusual for the *Phytomyza* group, more like a

typical *Liriomyza*, *C. mimuli* and relatives appear externally in all respects to be typical *Phytomyza* species.

Perhaps the strongest evidence for the monophyly of our newly delimited *Phytomyza* is a six base pair insertion in a variable region of fragment I of CAD (beginning at position 710 of the *Drosophila melanogaster* reference sequence, Genbank #NM078653). This variable region was not included in the phylogenetic analyses, but is shown for selected taxa in Fig. 3.5, which may be consulted for the following discussion. Later insertions or deletions in this variable region in some *Phytomyza* species may complicate detection of this insertion, as may the fact that sequences for this variable region cannot be confidently aligned between *Phytomyza* and outgroup taxa. However, this insertion is accompanied by a consistent change in the amino acid sequence: the first bases in the variable region for outgroup taxa, as well as *C. mimuli* and *C. scolopendri*, (CCT, CCA, or CCC) code for proline (P), while the inserted bases which initiate the variable region in *Phytomyza* (mostly GAA or GAT, with substitutions or deletions in some taxa) mostly code for glutamic acid (E) or aspartic acid (D), but never for phenylalanine. Inspection of other agromyzid CAD sequences (not shown) used in the analysis of Scheffer et al. (2007) show these changes to be unique for *Phytomyza* within the Agromyzidae; of 71 non-*Phytomyza* agromyzids included in this study, only two (*Cerodontha capitata* and *Aulagromyza tridentata*) did not exhibit a proline at this position, and a proline is also present here in the *Drosophila* sequence. *A. tridentata* deserves some note as it represents a possible sister group of *Phytomyza sensu novo*. Instead of an insertion, *A. tridentata* and *P. gymnostoma* (also excluded from *Phytomyza*

Fig. 3.5. Nucleotide sequence of variable portion of CAD gene (marked by brackets) and flanking regions for selected taxa, showing amino acid translation below. *Phytomyza s. nov.* (below dotted line) exhibits a six-base insertion relative to outgroup taxa which is a putative synapomorphy of this newly defined clade.

<i>G. heteroneura</i>	ATT GTT TAT GAA ATT [--- --- CCT ACA AAT TTG AAA AAC TTA CAA] TTT AAT GAT I V Y E I - - P T N L K N L Q F N D
<i>A. discrepans</i>	ATT GTS TAT GAG GAT [--- --- CCA CTG GGT TCA CTA GAT CTG AAA] TTT AAT GAT I V Y E D - - P L G S L D L K F N D
<i>A. luteoscutellata</i>	ATT GTT TAT GAA AAA [--- --- CCA CTG ACA TTG CAA GGG CAG CAA] TTT CAA GAT I V Y E K - - P L T L Q G Q Q F Q D
<i>C. mimuli</i>	ATT GTT TAT GAG AAA [--- --- CCT GCA AAT TTG AAT AAT TTA AAA] TTC ATT GAT I V Y E K - - P A N L N N L K F I D
<i>C. scolopendri</i>	ATA ACT TAT GAA AAG [--- --- CCC ACA AAT ATA CAA AAT TTG CAA] ATT AAT GAT I T Y E K - - P T N I Q N L Q I N D
<i>Pt. asparagi</i>	ATT ATT TAT GAG TTA [--- --- CCA ACC AAT ATG AAA GTT ATG AAA] TTT ACT GAT I I Y E L - - P T N M K V M K F T D
<i>N. plumea</i>	ATC ACT TAT GTG GAG [--- --- CCA CTT ACT --- AAG ATA TTA AAA] TTC AAT GAT I T Y V E - - P L T - K I L K F N D
<i>P. gymnostoma</i>	ATT GTT TAT GAA AAT [--- --- --- AGC TCT GTG CAG GAA ATC CAA] TTC AAT GAT I V Y E N - - - S S V Q E I Q F N D
<i>A. tridentata</i>	ATT GTT TAT GAA AAA [--- --- --- GCT TCT GTT AAG GGA CTG AAA] TTT AAT GAT I V Y E K - - - A S V K G L K F N D
<hr/>	
<i>P. glabra</i>	ATA ATA TAC AAT AAA [GAA GAA GCC AAT GTC AAG AAG GGT ATG CAA] TTT AAT GAT I I Y N K E E A N V K K G M Q F N D
<i>P. minuscula</i>	ATT ATT TAT AAG GTG [GAA GAT GCC GCC ATT CAG AAT AAC ATG CGA] TTT GTT GAT I I Y K V E D A A I Q N N M R F V D
<i>P. continua</i>	GTG ATT TAT GAG AAG [GAA AAT --- --- ATT --- AAG ACC CTG AAG] TTC ATC GAT V I Y E K E N - - I - K T L K F I D
<i>P. ranunculivora</i>	ATT ATT TAT GAT AAG [AAT TTA AAT CCC AAC ATT AAA TCA ATG CAA] TTC AAT GAT I I Y D K N L N P N I K S M Q F N D
<i>P. angelicae</i>	ATT ATT TAT GAG AAT [GAT AAT AAT GTT AAC AAA AAA TCC ACG CAA] TTC AAT GAT I I Y E N D N N V N K K S T Q F N D
<i>P. angelicastris</i>	ATT ATT TAT GAG AAT [--- --- AAT GCT ATA AAA AAA ACC ATG CAA] TTT AAT GAT I I Y E N - - N A I K K T M Q F N D
<i>P. solidaginophaga</i>	ATT ATT TAT GAG AAT [GAT GAT AAT GCT ATA AAA AAA TCC ATG CAA] TTC AAT GAT I I Y E N D D N A I K K S M Q F N D
<i>P. ranunculi</i>	ATT ATT TAT GAA AAG [GAT AAT AAT GCT ACC AAA --- --- --- CAA] TTT AAT GAT I I Y E K D N N A T K - - - Q F N D
<i>P. fallaciosa</i>	ATT ATT TAT AAT AAG [AAT AAT GAT GAT GTC AAG CAA TTA ATT AAA] TTC AAT GAT I I Y N K N N D D V K Q L I K F N D
<i>P. penstemonis</i>	ATT ATT TAT GAA AAG [GAA AAT ATT GAT ACA AAA GAA ATT ATG AAA] TTC AAT GAT I I Y E K E N I D T K E I M K F N D
<i>P. glabricola</i>	ATT ATT TAT GAT AAT [GAT AGT AAT GCC ATT AAG AAA GCA ATG ACA] TTT AAT GAT I I Y D N D S N A I K K A M T F N D
<i>C. ramosa</i>	ATA ATA TAT GAT AAT [--- ATA AAT GAT ATT GAG AAA TTA ATG CAA] TTC AAT GAT I I Y D N - I N D I E K L M Q F N D
<i>C. milii</i>	ATT ATT TAT GAA AAT [CAA AAT AAT GCT ATT CAT GAA TCC TTG CAA] TTC AAA GAT I I Y E N Q N N A I H E S L Q F K D
<i>P. ovalis</i>	ATT GTT TAT GAA AAG [GAA AAT AAT GCT ATT AAT AAC TCC ATG CAA] TTC AAT GAT I V Y E K E N N A I N N S M Q F N D

here) show a three base deletion in the variable region, and have an amino acid sequence different from both other outgroup taxa and typical *Phytomyza*.

Although there is solid molecular evidence for a monophyletic *Phytomyza sensu novo*, at the moment we can provide no morphological definition for this clade that excludes the *Chromatomyia mimuli* group and *P. gymnostoma*, as well as the remaining *Phytomyza* group genera. The previous diagnosis of *Phytomyza/Chromatomyia* involved a combination of three adult characters (Spencer and Steyskal 1986): 1) costa ending at vein R4+5, 2) orbital setulae proclinate, and 3) crossvein dm-cu usually absent. However, each of these character states occurs in other *Phytomyza* group genera, and in other agromyzids as well. Thus, an shortened costa is found in all genera of the *Phytomyza* group plus some other agromyzids; proclinate orbital setulae are found also in *Ptochomyza* and *Napomyza*, as well as some species of the distantly related *Phytoliriomyza*; and the dm-cu crossvein is also absent in *Ptochomyza*, *Gymnophytomyza*, some *Aulagromyza*, and a few other genera.

Even combinations of these three characters are insufficient to consistently distinguish *Napomyza* and *Ptochomyza* from *Phytomyza*. As mentioned below, several species and species groups have been recently transferred between *Napomyza* and *Phytomyza*. It has also long been recognized that additional characters (i.e. genitalia) are required to separate *Napomyza* from a few otherwise typical species of *Phytomyza* that possess a dm-cu crossvein, such as *P. davisii*, *P. aprilina*, and *P. glechomae* (Nowakowski 1962). A new genus (*Indonapomyza* Singh and Ipe) was even erected to

accommodate the incongruous character combinations of one of these species (Singh and Ipe 1971), though the genus was not subsequently recognized (Sasakawa 1977). The tiny *Ptochomyza* has traditionally been separated from *Phytomyza* by the loss of one notopleural seta, but this character actually applies to *P. asparagi* only, and is variable even within this species (Süss 2002, Dempewolf 2004). Furthermore, the Californian *Phytomyza minutissima* Spencer also lacks one notopleural bristle (Spencer 1981). Spencer (1990: 403), in transferring the Ranunculaceae-feeding *Ptochomyza mayeri* (Spencer) to this genus, implied that larval and genitalic characters may more adequately delimit *Ptochomyza*.

In short, precise diagnosis of *Phytomyza* on external adult characters is already impractical. Larval characters have also been shown by Dempewolf (2001) to provide little resolution among genera of the *Phytomyza* group. It may be that genitalic characters will be found to adequately delimit *Phytomyza*. Spencer (1976a) justified transfer of some *Phytomyza* species to *Napomyza* on the basis of certain characters of the male postabdomen, but the position of some of these taxa (*P. glabra*, *albipennis* and *ranuculella* groups) firmly in *Phytomyza* in our analysis suggests that these genitalic characters were not interpreted correctly. Our results instead corroborate Zlobin (1994), who showed that most of these characters were plesiomorphic for the Agromyzidae and thus not indicative of generic relationships. In any case, additional morphological study will be necessary for a complete generic revision of the *Phytomyza* group, but given the current state of knowledge, lack of morphological diagnosability does not seem a strong argument against our generic delimitation of *Phytomyza*.

Species groups, major clades and infra-generic classification of Phytomyza

Our results show that previous concepts of species groups, based on characters such as male genitalia and host plant use, generally correspond at least approximately to clades supported by molecular evidence, corroborating their utility in summarizing the variation in this very large genus. To maximize that utility we have attempted a preliminary “revision” of species group classification in *Phytomyza*, cataloging the species group concepts known to us and, where our evidence permits, critiquing and modifying their definitions to increase their correspondence to phylogeny. The revised classification, summarized in Table 3.4 (see also Fig. 3.3), includes a number of groups noted by Spencer (1990) or other authors but first explicitly named by us. It also includes several new groups first suggested by strong support from our molecular results; with one exception, we declined to recognize new groupings of species unless they were supported by at least 80% BP.

Potential users of this classification will need to keep in mind its provisional nature and incompleteness. The evidence on monophyly and composition of most groups still rests partly or entirely on morphology. For example, the *opaca*, *anemones*, and *spoliata* groups were represented by only one specimen each in our sample, and the *knowtoniae* and *buhriana* groups not at all. We reiterate, moreover, that over 100 species belong to no obvious species group based on morphology, and over 50 more have yet to be examined for the traits defining those groups.

Table 3.4. Revised species groups of *Phytomyza*. Minimum diversities of described species were estimated from the taxonomic literature; a complete listing of species placed in each is in Appendix A. Unless listed, the primary reference for all groups is Spencer (1990). Some groups newly named here were identified, but not named by Spencer (1990) or other authors. These groups are not comprehensive, as at least 170 described species are unplaced by this classification. Abbreviations: l.m., leaf-miner; s.m., stem-miner; s.f., seed-feeder.

group	min. diversity	biology	notes	references
<i>spoliata</i>	3	l.m. of Asteraceae, s.m. of Apiaceae	<i>grp. nov.</i>	Zlobin (1994)
<i>minuscula</i>	3	l.m. of Ranunculaceae	<i>grp. nov.</i>	Spencer (1969)
<i>ciliata</i>	11	l.m. of Asteraceae; internal pupation	<i>grp. nov.</i> ; included by Griffiths in <i>robustella</i> grp.; 3 spp. formerly in <i>Chromatomyia</i>	Griffiths (1972b, 1974b)
<i>robustella</i>	19	most feed in leaf midribs of Asteraceae, often causing gall-like swellings		Griffiths (1964)
<i>sygenesiae</i>	33	l.m. of Asteraceae, Poaceae; internal pupation	formerly in <i>Chromatomyia</i> ; grp. circumscription wider than Griffiths	Griffiths (1967, 1974c, 1980)
<i>hendeli</i>	10	l.m. (mostly of Ranunculaceae)	added <i>P.</i> <i>thalictrella</i>	Nowakowski (1962)
<i>loewii</i>	6	l.m. of Ranunculaceae	<i>grp. nov.</i> ; 3 spp. formerly in <i>Chromatomyia</i>	
<i>angelicae</i>	30	l.m. of Apiaceae		Griffiths (1973)
<i>spondylii</i>	36	l.m. of Apiaceae	optionally included in <i>albiceps</i> grp.; includes <i>obscurella</i> subgroup	
<i>albiceps</i>	59	l.m. of Asteraceae	Spencer's narrow circumscription retained	
<i>petoei</i>	6	l.m. of Lamiaceae		

group	min. diversity	biology	notes	references
<i>rufipes</i>	5	s.m. (known hosts: Urticaceae, Brassicaceae)	composition uncertain	
<i>notata</i>	15	l.m. of Ranunculaceae		
<i>anemones</i>	11	l.m. of Ranunculaceae; some internal pupation		
<i>albipennis</i>	10	s.m. of Ranunculaceae	<i>nigritula</i> grp. of Zlobin optionally included	Zlobin (1994)
<i>ranunculella</i>	16	s.m. (some l.m.) of Ranunculaceae	mostly S. temperate; inclusion of Chilean species uncertain	Zlobin (1994)
<i>atomaria</i>	52	s.m., s.f., l.m. (Orobanchaceae, Plantaginaceae, Ranunculaceae)	includes <i>plantaginis</i> grp. of Zlobin + Ranunculaceae-feeding cluster	Zlobin (1997)
<i>ilicis</i>	10	l.m. of Aquifoliaceae; internal pupation		Kulp (1968), Scheffer & Wiegmann (2000)
<i>agromyzina</i>	71	l.m. of many herbs and shrubs; internal pupation	<i>grp. nov.</i> , most formerly in <i>Chromatomyia</i>	Griffiths (1972a, 1974a, 1976a, 1980)
<i>opaca</i>	6	l.m. of Ranunculaceae		Süss (1989)
<i>obscura</i>	18	l.m. of Boraginaceae, Lamiaceae	includes <i>obscura</i> , <i>nepetae</i> , <i>symphyti</i> subgroups	Nowakowski (1959)
<i>aquilegiae</i>	9	l.m. of Ranunculaceae		
<i>buhriana</i>	3	l.m. of Ranunculaceae		Zlobin (2002)
<i>knowltoniae</i>	4	l.m. of Ranunculaceae	<i>grp. nov.</i> ; South African	

excluded from <i>Phytomyza</i>				
group	min. diversity	biology	notes	references
<i>mimuli</i>	5	l.m. of several families of Lamiales	<i>grp. nov.</i>	
<i>scolopendri</i>	4	l.m. of several ferns	<i>grp. nov.</i>	
<i>gymnostoma</i>	1	l.m. of Alliaceae	no known relatives; placed in <i>Napomyza</i> by Spencer (1976a)	Zlobin (1994)

Despite these shortcomings, nearly 75% of the species of *Phytomyza sensu novo* can now be placed in a named species group (see appendix A). To further increase the utility of infra-generic classification in *Phytomyza*, we have added the more inclusive informal category of “major clades.” Our goals in delimiting the five such clades we name were to erect groups which (a) were moderately to strongly-supported (BP at least 80%), (b) were non-overlapping, and (c) collectively encompassed as many species as possible, without creating “empty” concepts encompassing a single species or species group. Of the 33 previously “unaffiliated” species included in the present study, 17 are now placed in species groups, and a further 10 are placed at least to major clade, while 6 remain unaffiliated. In the remainder of this section we present an annotated review of our classification/phylogeny of *Phytomyza sensu novo*, following approximately the order (top to bottom) in which the taxa occur on the trees in Figs. 3.2 and 3.3.

Our results do answer the question of which lineages branch first within *Phytomyza sensu novo*. *Phytomyza glabra* was placed in our ML analysis as sister to remaining *Phytomyza* species. This species possesses several aedeagal characters that set it apart from most *Phytomyza*, and was thus placed by Spencer (1976a) in *Napomyza*, though later returned to *Phytomyza* by Zlobin (1994). Zlobin (1994) also noted that this species is quite similar to *P. bupleuri* Hering and *P. spoliata* Strobl; we term this small group the *spoliata* group. *Phytomyza minuscula* and two related species were found to branch next from remaining *Phytomyza*. Spencer (1969, 1990) noted the relatedness of these species, plus *P. thalictrivora* Spencer, which we propose to name as the *minuscula* group. Apart from noting their distinctiveness, Spencer (1990) did not note any

particularly primitive characteristics of *P. minuscula* or related species. However, Sasakawa (1961) noted two characters of his “*P. minuscula*” (= our *P.* ‘Mongolia’) that he considered plesiomorphic with respect to other *Phytomyza*: a “cruciate” female ninth tergite (i.e. with a medial transverse unsclerotized area; see Sasakawa 1961, fig. 133g), and an elongate “processus longus” (hyandrial lobe) in the male. It is not known if these characters also apply to the *spoliata* group or to taxa excluded here from *Phytomyza*. The placement of these two groups as sister to remaining *Phytomyza* received only moderate support, and could possibly change with further data.

The *nigra* clade is one of the most strongly supported groups in our analysis, and appeared with high support in preliminary analyses even with single genes (Fig. 3.4). Nearly all members of this clade feed on Asteraceae (except for several grass feeders) and pupate internally in the mine. This clade includes one group of species placed in *Chromatomyia* by Griffiths (1974a), all belonging to a lineage we call the *syngenesiae* group (more widely circumscribed than the *syngenesiae* group of Griffiths (1967)). Characters of this group are those defining *Chromatomyia* and are discussed further in the next section (see below). Possible inclusion of *P. spinaciae* in this group was predicted by Spencer (1990), though its genitalia do not exactly match Griffith’s concept of *Chromatomyia*, and it was previously thought to be related to members of the *ciliata* group (Godfray 1985). Our definition of the *robustella* group departs somewhat from previous authors. *Phytomyza campestris* and *P.* sp. ‘*Petasites*’ represent a cluster of leafmining species that, though added by Griffiths (1972b, 1974b) to the *robustella* group, are instead grouped strongly by our data with *Chromatomyia paraciliata*; we term

this cluster the *ciliata* group. The *robustella* group in its original sense (Griffiths 1964) is here represented by *P. continua*, and consists of large, *Napomyza*-like species that mine leaf midribs of asteraceous plants, usually forming gall-like swellings (Spencer 1990, Dempewolf 2005). Our analyses strongly ally *Phytomyza* nr. *major* and, by implication, the very similar Palearctic *P. rufescens* von Roser, with *P. continua*. We therefore include these in the *robustella* group, with which they agree in life history (known from *P. rufescens* only, which Spencer (1990) also listed in the *robustella* group) and genital morphology despite their very different adult external appearance. Relationships between the three groups of the *nigra* clade are not well resolved, possibly due to base compositional bias in this clade (see above).

In the large *albiceps* clade nearly all of the 140+ described species feed on Apiaceae or Asteraceae. However, the inclusion of the *hendeli* and *loewii* groups at the base of this clade suggests that there was an early shift from Ranunculaceae. Groupings in the *albiceps* clade have a complex history. Parts of the clade were recognized quite early based on host use and external morphology. For example, Hendel (1927) included species from the *angelicae*, *albiceps*, and *spondylii* groups as defined here, as well as *P. aconiti*, in his key to the “*albiceps* group”, though he excluded species of the *obscurella* subgroup (not sampled here) which, unlike others now placed in the *spondylii* group, have a dark frons. Nowakowski (1962) largely followed Hendel in defining his “*albiceps* complex”, but divided it into Apiaceae- and Asteraceae-feeding groups, and the former further into four subgroups, including separate subgroups for species now placed in the *spondylii* and *angelicae* groups). Griffiths (1972b) instead defined the *albiceps* group as

including species feeding on either Asteraceae or Apiaceae (including the *obscurella* subgroup) plus having an apomorphic form of the male genitalia, with rows of spines usually present on the basiphallar membrane. The Apiaceae-feeding species which do not have this genitalic form Griffiths (1973) placed in a separate *angelicae* group. Spencer (1990) preferred to split Griffiths' *albiceps* group into an Asteraceae-feeding *albiceps* group and an Apiaceae-feeding *spondylii* group. Spencer gave no justification beyond host affiliation, but his division, tentatively followed here, is supported by our data except that the trio of species centered on *P. cirsii* is only weakly joined to the rest of our *albiceps* group.

Our data strongly place Spencer's *albiceps* and *spondylii* groups as sister taxa, with the *angelicae* group as sister to these. This result is consistent with the observation that the genitalia of Griffiths' *angelicae* group appear relatively plesiomorphic, resembling those of several of the Ranunculaceae-feeding taxa (such as *P. aconiti*; Griffiths 1973). Despite the inclusion of one of a subgroup of Araliaceae-feeding species (Iwasaki 1996, 1997), genitalia of the species of the *angelicae* group analyzed here are quite homogeneous. Other species (e.g. *P. pimpinellae* Hendel, *P. chaerophylliana* Hering) placed in the *angelicae* group by Spencer (1990) are more derived, and were excluded by Nowakowski (1962) from of his *angelicae* subgroup; these should be included in future studies before the limits of the *angelicae* group are considered certain.

Inclusion of the *hendeli* and *loewii* groups in the *albiceps* clade was unexpected, and corroborating morphological characters have yet to be demonstrated. Candidate

characters include shortening of the upper orbital bristle (found in at least some members of all groups in this clade) and dorsal deflection of the distal tubules of the aedeagus (not found in *angelicae* group). Placement of the Nearctic *P. thalictrella* in the *hendeli* group, strongly supported by our data, is concordant with aedeagal morphology, and was anticipated by Spencer (1981). In addition to the weakly differentiated Ranunculaceae-feeding species, which form the core of the *hendeli* group (= “*rectae* group” of Nowakowski (1962)), morphological evidence allows assignment to the *hendeli* group with varying degrees of confidence of several species not analyzed here feeding on other hosts (Spencer 1990). These species include *P. brischkei* Hendel (Fabaceae), *P. sedicola* Hering (Crassulaceae), *P. rubicola* Sasakawa (Rosaceae; see Sasakawa and Matsumura 1998), and possibly *P. lappivora* Hendel (Asteraceae).

Exclusion of *P. nr. oxytropidis* from the *albiceps* clade by our data is somewhat enigmatic, as this species shares derived genitalic features with many *albiceps/spondylii* group members, including a strongly reduced distiphallus and the presence of spines on the basiphallus. The hosts of *P. oxytropidis* Sehgal, the related *P. lupinivora* Sehgal (both not included in this study), and most likely of the very similar species (*P. nr. oxytropidis*) which we did include, are in Fabaceae (Spencer 1969, Sehgal 1971). The position of this latter species removed from the *albiceps/spondylii* groups probably indicates an early, rather than recent host shift to the Fabaceae.

In the strongly supported *albipennis* clade, most species apart from the *anemones* group share a strongly projecting frons, an unpaired distiphallus (probably due to

reduction of the paired tubules of the distiphallus), and the habit of feeding in stems or seed heads. All three characters are also found in *Napomyza* species, leading Spencer (1976a, 1990) to move species in the *albipennis* and *ranunculella* groups to that genus, though these were transferred back to *Phytomyza* by Zlobin (1994). However, these traits are not constant even within the *albipennis* clade. For example, the *plantaginis* group, plus some members of the *ranunculella* group, have reverted to leafmining, while the paired distal tubules of the aedeagus are reduced, but not absent in most of the *ranunculella* group.

The *atomaria* group as characterized by Zlobin (1997) is not monophyletic, as at least *P. crassiseta* is placed strongly within the *plantaginis* group in which Zlobin (1997) only included *P. plantaginis*, the closely related *P. griffithsi* Spencer (not sampled here), and *P. penstemonis*. Each of these species (including *P. crassiseta*) are leafminers on Plantaginaceae sensu Albach et al. (2005; includes genera formerly in Scrophulariaceae) and differ in male genitalia from typical members of the “*atomaria* group”, which are seed- and stem-feeders mostly on Orobanchaceae (also including genera formerly in Scrophulariaceae; Olmstead et al. 2001). Based on this result, it seems likely that most of the other leafmining species that Zlobin placed in the *atomaria* group also belong with the *plantaginis* group, including, among taxa not analyzed here, *P. digitalis* Hering, *P. veronicicola* Hering, *P. globulariae* Hendel, and *P. atomaria* Zetterstedt itself, the last of which was reported by Zlobin (1997) from *Veronica*. Even excluding *P. crassiseta*, Zlobin’s “*atomaria*” group was still not recovered as monophyletic (except in the MP analysis), but consisted of two separate lineages. It should be noted that although species

here included in one of the two *atomaria* group lineages (*P. lupini* + two undescribed species) are not known to feed on Orobanchaceae, it is probable based on genitalic resemblance that some species placed by Zlobin in the *atomaria* group and recorded as feeding on hosts in the Orobanchaceae also belong in this lineage. Because Zlobin's (1997) *atomaria* group is not monophyletic, we propose to enlarge the concept of the group to include the *plantaginis* group. This necessitates also the inclusion of *P. jonaitisi* and probably also a number of other related species feeding in the stems, leaf stalks, or seed pods of Ranunculaceae (Spencer 1990, Pakalniškis 1998, 2003). Thus, we also tentatively place the unsampled species *P. krygeri* Hering, *P. thalictri* Escher-Kündig, *P. aquilegiophaga* Spencer, *P. murina* Hendel, and *P. clematadi* Watt in the *atomaria* group. It may be preferable later to split this wide concept of the *atomaria* group, as some natural groups are evident even within the Orobanchaceae feeders (Gaimari et al. 2004) but this will require additional species sampling and morphological study.

The inclusion of *P. fallaciosa* (*anemones* group) at the base of the *albipennis* clade was surprising, as no such relationship had been previously proposed. No obvious morphological characters unite *P. fallaciosa* with the remainder of this clade. However, *P. kasi* (= *P. latifrons* Spencer; see Henshaw and Howse 1989), which branches off second in this clade, may be a morphological intermediate. *Phytomyza kasi* has distinctly sclerotized, paired tubules of the distiphallus (see fig. 1191 of Spencer and Steyskal 1986), as in *P. fallaciosa* and most other *Phytomyza*, but is externally similar to many other species of the *albipennis* clade.

For reasons including the need to clarify species group limits, the *albipennis* clade would obviously benefit from further descriptive and life history study, especially in North America, as several species in the current study are undescribed and/or appear to represent distinctive lineages with uncharacterized host associations. The stem-mining habits of this clade make collection and rearing more difficult, impeding the accumulation of taxonomic and life history data. *Phytomyza* species from temperate Chile and Argentina (Spencer 1982), unavailable for this study, may be allied to the *ranunculella* group. It is probable that still other Ranunculaceae-feeding species not included in our sample belong to the *albipennis* clade. For example, several species known to feed on stems and seed heads of *Anemone* (e.g. *P. nigricoxa* Hendel, *P. soenderupiella* Spencer, *P. anemonivora* Spencer) may be included here. The biogeography of the *albipennis* clade, which includes many boreal/alpine species as well as some south temperate elements, is also worthy of further study.

Allied to the *albipennis* clade in our analyses, though with weak support, is the *notata* group. Monophyly for this group, which feeds on several genera of Ranunculaceae, is supported by the highly apomorphic male aedeagus: the distiphallus in some species is extremely elongate and coiled. The *notata* group is also marked by an unusually wide geographic distribution; it includes species in Australia, New Guinea and Indonesia, Africa, and the Canary Islands, in addition to common Palearctic and Nearctic species (Spencer 1990).

Also placed near the *albipennis* clade, with even less support, is a loosely-associated pair of strongly-supported sister groups, one containing *P. glechomae* (*petoei* group) and *P. nr. nigrinervis*, and the other containing *P. flavicornis*, *P. rufipes*, and *P. nr. bicolor*. Members of the *petoei* group are markedly similar to certain other *Phytomyza* species, notably the *opaca* group, while *P. flavicornis* and *P. rufipes* were considered by Spencer (1990) to be isolated, possibly primitive species. This designation reflects their distinctive morphology, but also their relatively large size and their habit of mining the stems (*P. flavicornis*) or leaf midribs (*P. rufipes*) of rosid hosts, both traits which Spencer considered to be primitive in the Agromyzidae. These two species (*rufipes* group) may be related to several similar Nearctic species with unknown biology (see Scheffer and Winkler, in press), and possibly also to *P. alyssi* Nowakowski (Nowakowski 1975) and *P. aulagromyzina* Pakalniškis (Pakalniškis 1994). Because of morphological similarity, we tentatively place *P. nr. nigrinervis* in the *petoei* group, and predict that its hosts may also be in the family Lamiaceae. However, as there are no obvious similarities between *P. nr. bicolor* and the related *rufipes* groups, we decline to place *P. nr. bicolor* in a species group.

The monophyly of the *agromyzina* clade (BP =83%, ML), consisting of the *ilicis* group (holly leafminers) plus the *agromyzina* group, is corroborated by similarities in external though not internal morphology. Species in these groups were placed in the same morphogroup in keys by Sasakawa (1961) and Spencer (1972), on the basis of characters including dark coloration of the head. These groups further are nearly unique within *Phytomyza* in feeding on woody plants; the hosts of nearly all other *Phytomyza*

are herbaceous. The inclusion of *P. agromyzina* with typical *Chromatomyia* species, while not expected, is concordant with morphological characters, but the inclusion of *P. ceanothi* is more surprising. Several unsampled Japanese species representing further unique host associations with woody plants also probably belong to this clade. For example, membership in the *agromyzina* group is also apparent from the genitalic form of *C. actinidiae* Sasakawa (feeding on Actinidiaceae; Sasakawa and Matsumura 1998), and Sasakawa (1956) also predicted a close relationship between *P. hydrangeae* Sasakawa (host in Hydrangeaceae) and members of the *agromyzina* group. Even within the relatively homogeneous holly leafminer (*ilicis*) group, some frequency of host shifts to unrelated woody plant families is suggested by the discovery that species feeding on Illiciaceae, Gelsemiaceae, and Styracaceae also belong to this group (Sasakawa 1961, Scheffer and Wiegmann 2000, Sasakawa 1993).

The probable independent acquisition of Caprifoliaceae-mining in two *Chromatomyia* species included here in the *agromyzina* group was anticipated by Griffiths (1974a, 1980), who placed *C. aprilina* outside of the *periclymeni* superspecies which includes most Caprifoliaceae-mining congeners. Griffiths (1980) also anticipated the separation of *C. milii* (*agromyzina* group) from other grass-feeding species (*C. nigra* and *C. fuscula*; *syngenesiae* group), implying independent colonizations of Poaceae. Griffiths' (1980) hypotheses regarding the nearest relatives to the Poaceae-feeding groups (*luzulae* superspecies and Saxifragaceae-feeders to the *milii+opacella* superspecies, *syngenesiae* superspecies to the *fuscula* superspecies and *C. nigra*) closely match our results.

Monophyly of the the *aquilegiae* clade, finally, is at least consistent with genitalic similarity, in that the aedeagus typically has paired, elongate distal tubules and a bulb-shaped mesophallus with well-developed lateral sclerites (“paramesophalli”), though this form is modified in a few taxa. A roughly similar form is found in the *opaca* group (e.g. *P. nr. calthivora*; see Süss 1989) and in *P. aconiti*, both clustered with the *aquilegiae* clade. However, weak support precludes confident assignment of these latter species. Inclusion in the *aquilegiae* clade is even more unclear for, other, non-sequenced Ranunculaceae feeders of a similar genitalic type, because this general genitalic form, possibly plesiomorphic, is also found in other groups found here to be only distantly related (e.g. the *petoei*, *anemones*, *ilicis* and *angelicae* groups).

Delimitation of and full resolution of relationships within the *aquilegiae* clade will thus require increased gene and taxon sampling, though two previously-recognized lineages are supported by our data. *P. nepetae*, *P. ovalis*, and *P. tetrasticha* represent the small *nepetae*, *symphyti*, and *obscura* groups, respectively, which were united by Nowakowski (1959) in the *obscura* group *sensu lato*. These groups feed only on Lamiaceae and Boraginaceae, both belonging to the “Euasterids I” clade (APGII 2003). The *aquilegiae* group, as delimited by Spencer (1990), includes at least eight species, all feeding on the closely related genera *Aquilegia* and *Thalictrum* (Ranunculaceae). Though monophyletic, this group received only weak support in our analyses, and the cluster of species affiliated with the *aquilegiae* group deserves closer study to clarify species groups and host-use evolution. The finding that *P. subaquilegiana* and *P. urbana* feed on legumes (*Lupinus*; S.J. Scheffer, unpubl. data), like the similar *P. subtilis*

(Spencer 1969), is surprising, but placement of these species within an otherwise mainly Ranunculaceae-feeding lineage is concordant with morphology. The remaining species allied with the *aquilegiae*-group lineage, *P. nr. acteae* and *P. davisii*, are typical of a set of at least nine species which feed on several genera of Ranunculaceae other than *Aquilegia* or *Thalictrum*, and share a unique aedeagal form in which the distiphallus exhibits long, tortuous tubules.

The isolated position of some species feeding on Ranunculaceae in our results suggests that these probably represent lineages distinct from other Ranunculaceae-feeding species groups. The genitalia of some of these species show marked resemblance, however, to those of species groups feeding on other plant families. For example, the *petoei* group on Lamiaceae (*P. glechomae*) and the *opaca* group on Ranunculaceae have strikingly similar genitalia, prompting Spencer (1990) to suggest a common origin. *P. aconiti* was likewise suggested by Griffiths (1973) to belong to the *angelicae* group on Apiaceae, while the unique genitalia of *P. trollii* most closely resemble those of some leafminers of the Asteraceae-feeding *ciliata* group (e.g. *P. crepidis* Spencer). Finally, the genitalia of the *ilicis* group (holly leafminers) are much like those of some of the of the *aquilegiae* clade of Ranunculaceae feeders. That none of these suspected relationships were recovered in our trees is thus somewhat surprising, for which one possible explanation is that the similarities represent shared plesiomorphy. This postulate is consistent with Spencer's (1990) hypothesis of an early radiation on Ranunculaceae, though neither phylogenetic error nor morphological convergence can be ruled out at present. Further sampling of Ranunculaceae-feeding taxa, especially in the

Palearctic, is desirable, as it seems evident that some distinct lineages were not sampled in this study.

The status of Chromatomyia

In light of our results, it is evident that *Chromatomyia* Hardy as defined by Griffiths (1974a) is (a) nested within the main lineage of *Phytomyza*, (b) paraphyletic with respect to some *Phytomyza* species, and (c) polyphyletic. Thus, there seems little point to maintaining any version of this genus name or concept. We consider *Chromatomyia* sensu Griffiths (1974a) to be a synonym of *Phytomyza*. All species transferred to or described in *Chromatomyia* (e.g. Griffiths 1974a, Spencer and Martinez 1987, Spencer and Steyskal 1986, Spencer 1990), and placed phylogenetically within *Phytomyza sensu novo* as defined here, should be moved to *Phytomyza* (see Appendix A). Species described or placed in *Chromatomyia* by other authors, and falling phylogenetically outside *Phytomyza sensu novo*, will need new generic assignments, as noted earlier.

The polyphyly of *Chromatomyia* is somewhat unexpected, and suggests remarkable convergences in life history and/or morphology, particularly among species of the *syngenesiae* and *agromyzina* groups formerly placed in *Chromatomyia*. Specifically, these species were grouped together based upon a) a derived form of the aedeagus (male intromittent organ) with the sclerites of the distiphallus completely reduced and a presumably newly derived set of dorsal “supporting sclerites” present, and

b) a slipper-shaped, usually lightly sclerotized puparium which remains in the leaf mine with spiracles protruding (Griffiths 1974a; discussed in following section).

Griffiths (1972a, 1974a; following Tchirnhaus 1969) considered the dorsally projecting sclerites in *Chromatomyia* to represent newly derived (and synapomorphic) “supporting sclerites”, and this character arguably represents the strongest evidence for monophyly of *Chromatomyia*. However, our results suggest the possibility that these supporting sclerites may not have evolved de novo, but are independently derived from the distiphallus (and/or mesophallus) as originally suggested by Griffiths (1967) and Steyskal (1969). The clearest evidence for this is in the close relationships between the *syngenesiae* group and the *robustella* and *ciliata* groups. Members of these latter two groups have a bifid distiphallus which is dorsally oriented, and sometimes partially reduced (e.g. *P. wahlgreni* Rydén; figs. 1011, 1012 of Spencer 1990). The supporting sclerites in the *agromyzina* group may also be derived from the distiphallus, as suggested by the position of *C. ramosa* as the sister to the remaining species of this group. This and other Dipsacaceae-feeding species have aedeagal structures which show less reduction than other members of the *agromyzina* group. Significantly, two of these species (*C. scabiosarum* (de Meijere) and *C. succisae* (Hering), not included here) appear to have a dorsally positioned, bilobed (though partially reduced) distiphallus, complete with associated sclerites (“paramesophalli”; see Spencer 1990, figs. 919, 920). If the dorsal sclerites are, in fact, derived from the distiphallus, the ejaculatory duct must have become independently disassociated with the sclerites of the distiphallus in these groups.

However, the dorsal sclerites in at least some species (in addition to the Dipsacaceae-

feeders) are still associated basally with the ejaculatory duct (see e.g. *C. erigontophaga* Griffiths (figs. 31-33, Griffiths 1976b), *C. periclymeni* (fig 14, Griffiths 1974a); the former species was singled out by Griffiths as possibly important in interpreting the aedeagal structure of *Chromatomyia*).

Admittedly, some problems remain with our interpretation of the dorsal sclerites. For example, the derivation of the distal sclerotization of the ejaculatory duct in some members of the *syngenesiae* group remains uncertain. Tchirnhaus (1969) called this structure the distiphallus, but Griffiths (1967, 1972a) instead surmised that it represents modification of the mesophallus, or a secondary sclerotization. In addition, the position of the little-studied *P. ceanothi* nested within the *agromyzina* group suggests a possibly different intermediate aedeagal form: the distal tubules of the phallus in this species are visible and posterodorsally directed, though weakly sclerotised and indistinct, and the “dorsal sclerites” are lacking (see fig. 565 in Spencer and Steyskal 1986). The marked reduction of the aedeagus in both the *syngenesiae* and *agromyzina* groups makes interpretation of remaining aedeagal sclerites difficult, and more detailed work must be done to determine if there is corresponding morphological evidence for the polyphyly of *Chromatomyia*.

It is possible that independent reduction of the male distiphallus in the *syngenesiae* and *agromyzina* groups may reflect parallel shifts in life history or mating system.

Griffiths (1967) points out that reduction of the aedeagus in *Chromatomyia* is accompanied in some groups by a reduction in the size of the male sperm pump and

apodeme, and possibly also by a reduction in the female spermathecal size (recorded by Sasakawa (1961) for “*P. atricornis* Meigen” and *C. nigra*).

Our results show that taxa added to *Chromatomyia* by subsequent authors are not closely related to either of the two major groups of species included therein by Griffiths (1974a), with some even falling outside *Phytomyza s. nov.* For instance, *C. paraciliata* and the closely related (and unsampled) *C. ciliata* (Hendel) belong to the *nigra* clade, but not to the *syngesesiae* group therein which contains other former *Chromatomyia*. These two species, as well as others which cluster here in the *ciliata* group, are *Chromatomyia*-like in pupating internally after mining leaves of Asteraceae, and were placed by Godfray (1985) and Spencer (1990) in *Chromatomyia* despite sharing distinct paired distiphallar tubules with the *Phytomyza robustella* group, the other member of our *nigra* clade. Spencer’s decision in this case reflects his opinion (1990: 405) that mode of pupation should be more strongly considered in delimiting *Chromatomyia*, following Hardy’s (1849) original concept. Paradoxically, however, Spencer and Steyskal (1986) and Spencer (1990) placed *C. clemativora* (Coquillett) and the related *C. clematoides* in *Chromatomyia* despite the fact that neither pupates internally, because they show a reduction of the aedeagus analogous to that in some other *Chromatomyia* species. The placement of *C. clematoides* found here is instead consistent with its genitalic similarities to the *Phytomyza loewii* group, which had been previously noted by Spencer and Steyskal (1986). Lastly, two small species groups placed in *Chromatomyia* by Spencer were found here to be more closely related to *Napomyza* and *Ptochomyza* than to *Phytomyza sensu novo* and included former *Chromatomyia*. Of these, the *C. scolopendri* group,

comprising four palearctic species feeding on ferns, shares only internal pupation with other *Chromatomyia* (Spencer 1990). *C. mimuli* and relatives share in addition a reduction of the male genitalia, though not of the same form as more typical *Chromatomyia*.

Evolution of life history and host use

As the above discussion suggests, the slipper-shaped puparium of species formerly placed in *Chromatomyia*, which is formed in the leaf mine with spiracles projecting out of the leaf epidermis, must also represent parallelism if the molecular phylogeny is correct. As noted by Griffiths (1972a, 1974a) leaf-mining species of *Phytomyza* with internally-formed puparia, often quite similar to those of “*Chromatomyia*”, are found in several additional species groups (see Fig. 3.3), including the *atomaria*, *anemones*, *ciliata*, and *ilicis* groups. Of these, internal pupation in the *ciliata* and *ilicis* groups would appear from the phylogeny to share a common origin with the *syngenesiae* and *agromyzina* groups, respectively, of former *Chromatomyia*. In fact, we estimate that this mode of pupation must have evolved at least eight times in the *Phytomyza* group (six in *Phytomyza s. nov.*; see Fig. 3.3), although this has not been followed by significant proliferation of species except in the *nigra* and *agromyzina* clades. In addition to those groups mentioned above, *Chromatomyia*-type pupation is also found in the unidentified *P.* ‘*Cimicifuga*’, collected as larvae and pupae in *Actaea* (= *Cimicifuga*; Ranunculaceae) in Japan (ISW), and possibly associated with *P. tamui* Sasakawa on *Coptis* (also Ranunculaceae), which also pupates internally (Sasakawa 1957). Two unsampled Ranunculaceae-feeders (*P. rydeni* Hering and *P. ranunculicola*

Hering), both possibly associated with the *aquilegiae* clade, have similar pupation (Pakalniškis 2003). Also, some members of the *Aulagromyza populicola* group (leafminers on Salicaceae) pupate internally, including the species (*A. tridentata*) included here; we found this to be the sister group to *Phytomyza s. nov.* Finally, *Ptochomyza* species which feed in the finely divided leaves of *Asparagus* (Asparagaceae) pupate internally (Spencer 1990). Facultative internal pupation is also present in the *petoei* group and a few other species (Spencer 1976a), but pupation in these species is qualitatively different; an exit slit is first cut, as in most agromyzids, and spiracles do not protrude from the leaf epidermis. In contrast, external pupation has evolved from internal pupation very few times in leaf-mining lineages; in *Phytomyza* the only known examples are *C. alpigenae* (Groschke) and *C. chamaemetabola* Griffiths (Griffiths 1974a), both in the *periclymeni* superspecies of the *agromyzina* group, and possibly *P. hydrangeae* Sasakawa (Sasakawa 1956), whose relationships have not been confirmed. The apparent parallel evolution of *Chromatomyia*-type pupation raises the question of why and how internal pupation has repeatedly evolved in *Phytomyza* and related taxa. More specifically, is there some adaptive advantage to internal pupation that is driving this transition, and are there any additional life-history factors that are connected to internal pupation?

The advantages of pupation in the leaf mine are unclear, but it is possible that this could give some additional protection from natural enemies (Connor and Taverner 1997). It is not known if pupation in the leaf actually facilitates avoidance of predators, pupal parasitoids, or pathogenic fungi which attack soil-pupating species. On the other hand,

pupation in a conspicuous leaf mine could heighten susceptibility to some predators and parasitoids (e.g. Owen 1975). However, most known agromyzid parasitoids, including those which specialize on internally-pupating *Phytomyza* species (e.g. Griffiths 1966), attack the larval stages, which are presumably equally vulnerable regardless of pupation site. Abiotic factors may also be important. An interesting parallel is found in asphondyliine gall midges, which have apparently evolved internal pupation several times from an externally pupating condition (Möhn 1961). Although galls (and stem mines) may provide much more substantial protection from natural enemies than leaf mines, Möhn points out that this adaptation also allowed the Asphondyliini and other internally pupating genera to flourish in climates where soil conditions are not favorable for pupation, such as in arid areas, or areas prone to seasonal flooding. A similar scenario is possible for members of the *nigra* clade and *agromyzina* group, which are especially diverse in boreal and alpine regions (Griffiths 1972-1980), including many habitats with especially dry or saturated soils. Habitat may have also been important in the evolution of internal pupation in the genus *Cerodontha*, species of which mine grasses, sedges, or rushes, and are often abundant in dry grasslands, as well as marshy areas. In contrast, many other agromyzids feeding on semiaquatic plants (including some *Phytomyza* species) have developed characteristically elongate spiracles which help the pupa cling to the host plant (Nowakowski 1962).

Regardless of any possible adaptive advantage of pupation inside a leaf mine, it may be that internal pupation is precipitated largely by other life-history traits. For example, most agromyzid species feeding in stems, flower heads, and leaf midribs also

pupate internally in the host plant. This assertion is difficult to quantify because the relative difficulty in locating and rearing these species has resulted in a paucity of life-history data compared to leaf-mining taxa. However, it appears to be true for most such members of the *Phytomyza* group, except for some Orobanchaceae-feeding members of the *atomaria* group (e.g. *P. affinis* Fallén and sometimes *P. subtenella*; Spencer 1976a; Gaimari et al. 2004), and also for stem-mining species of *Aulagromyza* and the seed-feeding *Gymnophytomyza*, both of which feed on *Gallium* (Rubiaceae) and pupate externally (see Spencer 1976a; Zlobin 1999). Spencer (1990: 29,41) suggested that a progression from stem feeding to leaf-mining with a retention of internal pupation had occurred in the *ranunculella* group; this group mostly consists mostly of stem-miners, but at least two non-sister species (*P. clematidicolla* Spencer in Australia and *P. costata* in New Zealand) are obligate leaf-miners, and *P. lyalli* also occasionally moves into the leaf blade to feed. Despite our limited sampling of the *ranunculella* group, our phylogeny strongly supports this hypothesis, in that the *ranunculella* group is nested within the *albipennis* clade, which largely consists of stem- and flower head- feeding species. A similar scenario must also account for internal pupation in the leaf-mining species of the *atomaria* group (including the *plantaginis* group of Zlobin (1997)), also nested in this clade. In most other cases, species with *Chromatomyia*-type pupation in the leaf blade are also phylogenetically proximate to species with atypical feeding habits (i.e. feeding in tissues other than leaf parenchyma), though a clear progression is not evident. For example, the *anemones* group (internally-pupating leaf-miners) is also associated with the *albipennis* clade. Likewise, the internally pupating *mimuli* and *scolopendri* groups and *Ptochomyza* are allied with the stem- and seed-feeding genus *Napomyza*, though in this

case, parsimony predicts the opposite transition, from leaf-mining to stem- and seed-feeding (Fig. 3.3). Finally, we found *A. tridentata* to branch between *P. gymnostoma* and *P. glabra* at the base of *Phytomyza*. The former species is a leafminer in onion (*Alliaceae*), but mines downward and pupates in or near the root; *P. glabra* is a stem-miner, but the related *P. bupleuri* and *P. spoliata* are typical, externally-pupating leafminers (Spencer 1990).

What of the *nigra* and *agromyzina* clades, for which internal pupation is ancestral, but do not have any stem- or seed-mining members or close relatives (except for *P. hasegawai* Sasakawa, of the *robustella* group; Sasakawa 1981)? In the *nigra* clade, feeding habits of the *robustella* group are in some ways analogous to stem-mining; as mentioned above, typical members of this group mine leaf midribs, often causing gall-like swellings. Our ML phylogeny (Fig. 3.3) predicts a transition from feeding in typical leaf-mines to feeding in leaf midribs in this clade, but relationships between the three species groups of the *nigra* clade are poorly supported (Fig. 3.2), and position of the *robustella* group as sister to the other two may be more concordant with morphological characters. Similarly, examination of habits of *C. ramosa* and other *Dipsacaceae*-miners may again provide insight into evolution of the *agromyzina* clade. Both *C. ramosa* and *C. scabiosarum* (which Spencer does not believe to be closely related) mine in the leaf midrib, with offshoots into the leaf blade (Spencer 1990). However, like the *robustella* group, pupation occurs in the midrib (Spencer 1976a). Mines of the other *Dipsacaceae*-feeding species are more typical of the *agromyzina* group.

The phylogenetic position of *P. gymnostoma* and *P. glabra* at the base of *Phytomyza* suggests the possibility that stem-mining and/or other atypical feeding behaviors may be ancestral for *Phytomyza*. Spencer (1990) considered large size and stem-mining habits to be ancestral for *Phytomyza* and for other agromyzids, and believed that progression from stem- to leaf-mining was a general trend for the family. Accordingly, Spencer suggests that species such as *P. rufipes*, *P. gymnostoma*, or members of the *robustella* group represent “primitive” *Phytomyza* species. These species are also generally similar in habitus to *Napomyza*, which Spencer (1990: 392) thought to be the most primitive among the “*Napomyza* group” of genera (roughly corresponding with Dempewolf’s (2001) *Phytomyza* group, except Spencer excluded *Aulagromyza* and *Gymnophytomyza*, and included *Pseudonapomyza*). However, Dempewolf (2001, 2005) and Scheffer et al. (2007) showed that leaf-mining is probably the ancestral habit for agromyzids in general. Our results cannot resolve this question for *Phytomyza*, but the *albipennis* clade is nested within a predominately leaf-mining lineage, so stem- and seed-feeding in at least this clade is probably secondarily derived. It now seems unlikely that the large size of some species in the *atomaria*, *robustella*, and *rufipes* group species, as well as *Napomyza* and *P. gymnostoma*, reflects a shared plesiomorphic trait; this may instead result from relaxation of size constraints imposed on other species by existence within the narrow leaf plane.

As noted for other phytophagous insects (Winkler and Mitter 2008; see Chapter 2), shifts between host families in *Phytomyza* have been generally more frequent than shifts in other life history traits. Nevertheless, such shifts are relatively rare in some

lineages; for example, some Ranunculaceae-feeding groups may have retained this habit for many millions of years, and the large *albiceps* group (at least 60 species) has repeatedly colonized hosts only within the Asteraceae (except for two species on the closely related Campanulaceae) (Spencer 1990). In contrast, the *agromyzina* group exhibits a uniquely accelerated apparent rate of host family shifts, including to host families not colonized by any other *Phytomyza* species, and in some cases not used by any other agromyzids. Its collective host list includes at least ten angiosperm plant families, spanning the rosid, asterid, saxifragalean, and commelinid clades (APGII 2003), as well as both woody and herbaceous hosts. This result may corroborate a long-standing general hypothesis that colonization of novel hosts is more likely for insects which feed on woody plants (Feeny 1975; see also Winkler and Mitter 2008, Chapter 2). Also notable is the exceptional case of *C. horticola* (*syngenesiae* group), which has been recorded on hosts in over 35 different plant families (Griffiths 1967, Spencer 1973, Spencer 1990, Benavent-Corai et al. 2005). Scheffer et al. (2007) suggested that the incidence of polyphagy in *Liriomyza* may be related to frequent host shifts to unrelated plant families by related specialist species. However, the broad polyphagy of *C. horticola* evidently reflects a different evolutionary phenomenon, as the *nigra* clade to which it belongs shows only two shifts to plant families other than Asteraceae (Poaceae and Valerianaceae; Griffiths 1974c, 1980) during the evolution of over 60 species. Instead, the precursor of extreme host range expansion in *C. horticola* is likely indicated by the biology of the related *C. syngenesiae*, which is broadly oligophagous within Asteraceae, and is known to rarely feed on plants in other families (Griffiths 1967, Spencer 1990, Benavent-Corai et al. 2005).

Although a detailed investigation of host relationships and their evolution is beyond the scope of this chapter, the present results generally support the scenario elaborated by Spencer (1990) of many separate lineages feeding on Ranunculaceae, with a few, larger radiations onto asterid hosts. However, it is not clear if Ranunculaceae is in fact the ancestral host of *Phytomyza*, as predicted by Spencer (1990), because asterid- and Ranunculaceae- feeding lineages are both dispersed throughout the tree (Fig. 3.3). In addition, the larger clade to which *Phytomyza* belongs is very unlikely to have Ranunculaceae as an ancestral host, given the predominant modern association of *Aulagromyza* and *Napomyza* with the asterid families Rubiaceae, Caprifoliaceae, and Asteraceae (Spencer 1990). At least some asterid-feeding lineages are probably derived from Ranunculaceae-feeding ancestors, including the those in the *albiceps*, *albipennis*, and *aquilegiae* clades. These shifts to asterid plant families seem to have been very important in spurring species diversification, by opening new “adaptive zones” for colonization. This is evidenced by the overall pattern seen of distinctive species groups representing shifts to novel hosts, followed by varying degrees of morphological differentiation and speciation. These themes will be explored in more detail in Chapter 4. We also anticipate that more detailed sampling of individual clades and species groups will lead to more pointed insights into the link between host use and speciation in *Phytomyza* flies.

Chapter 4: The History of a Temperate Adaptive Radiation: Diversification and Host Use Evolution in *Phytomyza* Leaf-mining Flies (Diptera: Agromyzidae)

Introduction

Phylogenetic patterns in insect/plant evolution

Over the past several decades, the widespread use of phylogeny has revolutionized study of the evolution of insect/plant and other trophic interactions (reviewed in Chapter 2; see also Mitter and Brooks 1983, Page 2003, Winkler and Mitter 2008). Phylogenies can offer two principle lines of evidence on such questions. First, they allow reconstruction of the temporal sequence of associations between interacting species and of the origin of traits affecting these interactions. Combined with evidence from fossils and biogeography, they also can permit estimation of the absolute times of such events. Secondly, phylogenies, with or without calibration by absolute dates, can be used to estimate absolute or relative rates of diversification. The histories established by such analyses then can be used to test hypotheses about the evolution and evolutionary consequences of interactions.

A variety of ideas have been advanced to indicate how insect/plant interactions might evolve over the long time scales, and how the effects of those processes might be manifest in the reconstructed histories of present day interactions (see Labandeira 2002a). For example, if an insect species retained strict fidelity to a particular host plant species over evolutionary time, the associated lineages might undergo cladogenesis in concert, e.g. through simultaneous geographic isolation, resulting ultimately in matching

phylogenies between the plant species and their associated herbivore species (Mitter and Brooks 1983). This is frequently referred to as the cospeciation model. Alternatively, if a pair of insect and plant lineages has evolved under the “escape and radiation” model (Thompson 1988) initially envisioned by Ehrlich and Raven (1964), one expects not a detailed phylogeny match, but rather parallel phylogenetic sequences of plant defense traits and of corresponding insect counter-adaptations, with each step accompanied by accelerated diversification. Recent studies confirm that insect host use is strongly conserved in a broad sense; about 80% of the time, for example, sister species of phytophages feed on the same plant family (Winkler and Mitter 2008; see Chapter 2). However, patterns of the kind sketched above, suggesting closely parallel diversification between particular insect and plant species or groups thereof, have proven to be rare. Most differences in host use between related insect species instead result from colonization of plant species which had diverged long before.

There is some evidence, on the other hand, that on a very broad scale, the current distribution of insects over plant clades does partly reflect a long-term history of interaction. For example, differences in host-use trends among major phytophagous insect clades have been argued to reflect retention of associations with the plant groups which were dominant during the different eras in which those clades arose (Zwölfer 1978). An example is the beetle clade Phytophaga (Curculionoidea + Chrysomeloidea), in which several ancient, species-poor lineages feeding on conifers or cycads, the apparent ancestral hosts, were found to be sister groups to large, diverse angiosperm-feeding lineages (Farrell 1998). Within these beetle lineages, both phylogenetic

sequences of host associations and differential rates of diversification parallel the origin and rise to dominance of the highly diverse plant clade Angiospermae.

Earth history, niche conservatism, and “biome tracking”

In addition to biotic interactions, changes in the environment may be an important factor promoting both extinction and speciation. This dichotomy between biotic vs. environmental causation of evolution was emphasized by Vrba (1985), who suggested that although biotic factors (e.g. resource use; see Vrba 1987) may ultimately determine the degree of diversification, extinction and speciation events may be concentrated during periods of environmental change (i.e. tectonic events or climate change). With the advent of new molecular phylogenetic methods for estimating divergence dates, these questions have been receiving increased attention from evolutionary biologists (Megens et al. 2004, Becerra 2005, Bell and Donoghue 2005, McKenna and Farrell 2006, Yamamoto and Soto 2007).

There has been particular focus of late on the Cenozoic history of the northern hemisphere, which includes dramatic episodes of both warming and cooling, and a strong overall trend toward cooling, drying, seasonality and latitudinal climate stratification. In particular, two rapid, global cooling events, in the early Oligocene (33 Ma) and the mid-Miocene (~13 Ma), have been hypothesized to have played a large role in the evolution of modern temperate biomes (Wolfe 1978, 1994b, Zachos et al. 2001). Largely during the course of these two events, plant communities in middle latitudes of the northern hemisphere underwent a net shift from warm-adapted toward cool-adapted modes, with

pronounced expansion of open habitats and herbaceous vegetation including grasses and composites (Retallack 2001). During the last 33 million years, many plant lineages adapted to the new conditions (especially herbaceous groups) have undergone dramatic diversification (Tiffney 1981, Niklas et al. 1985). Similarly, many temperate insect groups specializing on herbaceous plants also experienced major diversification (e.g. Heie 1996, Dietrich 1999, Mitchell et al. 2006). Because tropical/subtropical conditions had prevailed at middle and high latitudes since the mid Cretaceous, a majority of lineages colonizing cool temperate biomes during the Cenozoic may have had tropical ancestors (Farrell et al. 1992, Latham and Ricklefs 1993, Wiens and Donoghue 2004), requiring them to develop new adaptations to both biotic and abiotic aspects of the new environment.

The process of colonizing and evolving in newly-available environments by plants and their insect herbivores might be predicted to exhibit several distinctive features, under a scenario that I term “biome tracking.” Specifically, I hypothesize that the dominant plant and phytophagous insect clades in the open habitats characteristic of the north temperate zone will show: (a) significant overlap in the timing of their diversification with each other and with the expansion of those habitats; (b) character changes coinciding with or following colonization of these habitats, which confer improved adaptation to those habitats; and (c) increased diversification rates associated with those new adaptations. The “biome tracking” hypothesis bears several parallels with “escape and radiation” coevolution, for example in ascribing differential diversities of insect and plant lineages to key adaptations, while differing in assigning ultimate

causation to abiotic change. This hypothesis does not exclude the possibility of traditional pairwise or diffuse coevolution with specific plant lineages, but asserts that these interactions are part of the larger process of adaptation to newly available biomes. However, predictions may be made about the sequence of association with broad categories (e.g. growth form, habitat type) of plants. For example, an original association with broadleaved evergreen trees might be followed by associations with deciduous trees, and then herbs, as these forms in turn became dominant in central North America.

Study group: leaf-mining flies

In this chapter I explore the utility of the “biome tracking” hypothesis for explaining diversity, distribution and host associations for a large temperate clade of leaf mining flies in the family Agromyzidae (Diptera). The Agromyzidae consist of over 2,800 species in approximately 29 genera (Scheffer et al. 2007). Larvae feed internally in tissues of many different (mostly herbaceous) plant families, usually in the leaves, but also in stems, seeds, and even (in the case of *Phytobia*) trunk cambium of trees (Spencer 1990). Although much undescribed diversity exists in both tropical and temperate regions, worldwide collection and description of agromyzids by the late Kenneth Spencer and others in the last 50 years has confirmed that agromyzids in general, and especially leaf-mining taxa, are more diverse and abundant in north temperate regions than in tropical, subtropical, or south temperate regions (e.g. Spencer 1969, 1977). This trend is most marked in (and largely driven by) the *Phytomyza* group of genera (Dempewolf 2001), consisting of *Phytomyza sensu lato* (including *Chromatomyia*; see Chapter 3), *Napomyza*, *Aulagromyza*, and the small genera *Ptochomyza* and *Gymnophytomyza*. Out

of about 750 described species in these genera (640 of which are in *Phytomyza*), less than 75 (10%) are found outside of the Nearctic and Palearctic regions, and the majority of these occur in south temperate areas or at high altitudes, or are recent introductions from north temperate regions. Sufficient taxonomic and faunal data now exists, especially from the neotropics, to confirm that *Phytomyza* and related genera are extremely depauperate in tropical and subtropical areas (Sasakawa 1977, Cogan 1980, Spencer 1989, Martinez and Etienne 2002; see Table 4.1); the agromyzid fauna in these regions instead is dominated by *Melanagromyza* and (in the neotropics) *Liriomyza* and *Calycomyza*. In contrast, *Phytomyza* is the most diverse genus of agromyzids at high latitudes and altitudes in the northern hemisphere. For instance, 141 *Phytomyza* species are recorded from Scandinavia (Spencer 1976a; including Finland and Denmark), with at least 37 species north of the Arctic Circle. This diversity gradient is apparent across North America as well, though individual species distributions are not yet well known (Spencer 1969, Spencer and Stegmaier 1973, Spencer and Steyskal 1986; Table 4.1).

Hosts of *Phytomyza* species are predominantly herbaceous plants in families that are diverse and abundant in temperate regions, especially the families Asteraceae, Ranunculaceae, and Apiaceae, but also Lamiaceae, Boraginaceae, Orobanchaceae, Plantaginaceae, Caprifoliaceae, Gentianaceae, Saxifragaceae, Poaceae, and others (see Fig. 3.3, Appendix A). A few species feed on trees or shrubs, but these are mostly concentrated in a single species group (see Chapter 3). Spencer (1990) emphasized the strong association of *Phytomyza* species with the primitive angiosperm family Ranunculaceae, which hosts a heterogeneous assemblage of species in many groups,

Table 4.1. Number of *Phytomyza* species (including *Chromatomyia*) reported in various regional catalogues, surveys, or revisions. Because some areas are better-studied than others, the number of total agromyzid species reported from each of these areas and the percentage of these represented by *Phytomyza* are shown for comparison. *Phytomyza* species are nearly absent from tropical regions, are sparsely represented in south temperate regions, diverse in north temperate regions, and disproportionately represented relative to other agromyzid genera at high latitudes.

Region	# total described agromyzid spp.	# <i>Phytomyza</i> spp. (% <i>Phytomyza</i>)	Source
Australia & New Zealand	185	13 (7%)	Spencer (1976b, 1977)
India	130	11 (8%) ¹	Singh and Ipe (1973)
Africa (except S. Africa)	181	11 (6%)	Cogan (1980)
South Africa	116	12 (10%)	Cogan (1980)
Chile, Argentina	146	11 (8%)	Martinez and Etienne (2002)
Colombia, Venezuela, Ecuador, Peru, Brazil	205	6 (3%)	Martinez and Etienne (2002)
Antilles (Caribbean)	111	1 (1%)	Martinez and Etienne (2002)
Florida	86	7 (8%)	Spencer and Stegmaier (1973)
California	252	58 (23%)	Spencer (1981)
Alberta	170	82 (35%)	Sehgal (1971)
Canary Islands	68	18 (26%)	Martinez (2004)
Italy	203	74 (36%)	Süss (1995)
France	359	132 (37%)	Martinez (2004)
Hungary	209	53 (25%)	Martinez (2004)
Switzerland	232	82 (35%)	Martinez (1998), Černý (2005)
Britain and Ireland	368	133 (36%)	Chandler (1998)
Lithuania	377	127 (34%)	Pakalniškis et al. (2000)

¹ not including 17 unidentified species listed from larval records only. Indian *Phytomyza* species are primarily in the Himalayan region, and show Palearctic affinity.

Region	# total described agromyzid spp.	# <i>Phytomyza</i> spp. (% <i>Phytomyza</i>)	Source
Germany	552	213 (39%)	Tchirnhaus (1999)
Scandinavia (including Finland, Denmark)	385	141 (37%)	Spencer (1976a)
Iceland, Greenland, Faroes	26	18 (69%)	Griffiths (1964, 1966, 1968)

including nearly all south temperate *Phytomyza* species. Hosts of most other *Phytomyza* species are members of the large “asterid” clade of angiosperms (sensu Angiosperm Phylogeny Group 2003; hereafter APGII; see also Fig. 3.1); however, these species mostly fit into a few large, morphologically homogeneous species groups. This led Spencer (1990) to postulate that an association with Ranunculaceae may have been primary for the genus, possibly before other host families originated or rose to ecological prominence.

Scheffer et al. (2007) recently investigated the phylogeny of the Agromyzidae using sequence data from 86 species, including all major genera. Their results, along with some fossil data, allows estimation of minimum ages of agromyzid genera and other clades. We focus here on the *Phytomyza* group of genera defined by Dempewolf (2001), and found by him to be monophyletic based on morphological characters. This group was also supported by the molecular analysis of Scheffer et al. (2007). Relationships within the *Phytomyza* group were presented in some detail in Chapter 3, based on sequence data from three genes and over 100 ingroup species, and provide a framework with which to study the timing and pattern of evolution in this clade. This study mostly corroborated previous hypotheses (summarized in Spencer et al. 1990) that much of the diversity of *Phytomyza* can be partitioned into monophyletic species groups, each with a distinctive genitalic morphology and mostly restricted to feeding on a single plant family (see Table 3.4, Fig. 3.3). These species groups were found to belong mostly to five major clades, with some additional lineages present. Relationships between these major clades were not well resolved, making detailed reconstruction of shifts between host families

difficult. Despite this, and although the large size of the genus *Phytomyza* permitted only a small percentage of species (<15%) to be sampled for this study, we can make use of the species group classification developed in Chapter 3 to characterize the diversity of several major lineages of *Phytomyza* in order to test hypotheses about diversification in this group.

Fossil history

Given the strong association of species of the *Phytomyza* group with northern temperate habitats and flora, and the relatively short time period in which these habitats and hosts have been widespread, it could be expected that this group is of relatively recent Cenozoic origin. Schizophoran (“higher”) flies, the diverse clade of approximately 85 families to which agromyzids and other “acalyptrate” flies belong, have long been considered a primarily Cenozoic radiation (Rohdendorf 1974, Wiegmann et al. 2003, Grimaldi and Engel 2005), with major diversification occurring even as late as the Miocene (Wilson 1978, Blagoderov et al. 2002). Only two Cretaceous fossils have been authoritatively assigned to this clade (Grimaldi and Engel 2005): a putative calyptrate puparium from Canada (McAlpine 1970), and a Cretaceous amber specimen tentatively identified as the acalyptrate family Milichiidae (Grimaldi et al. 1989). However, the first fossil can confidently be assigned only to the more inclusive Cyclorrhapha (Grimaldi and Engel 2005). No confirmed reports of acalyptrate fly fossils from the Paleocene exist; for many families (>20/70), the earliest fossil records are from the mid Eocene (44.4 Ma) Baltic amber (Hennig 1965, Evenhuis 1994). The fossil record of the Agromyzidae itself is sparse (Evenhuis 1994), with many fossils doubtfully assigned (Spencer and Martinez

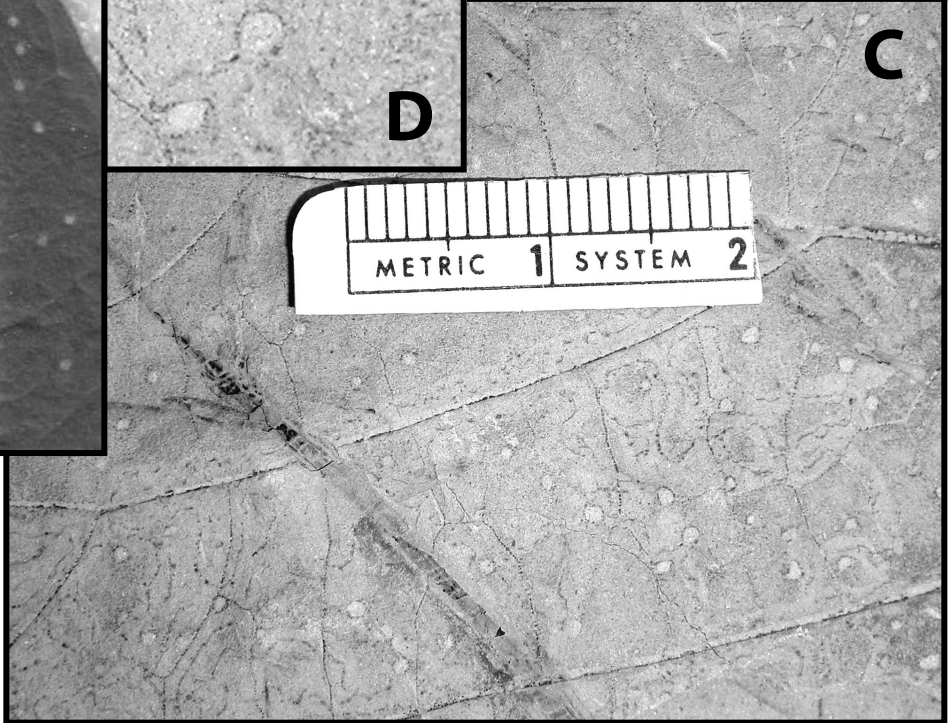
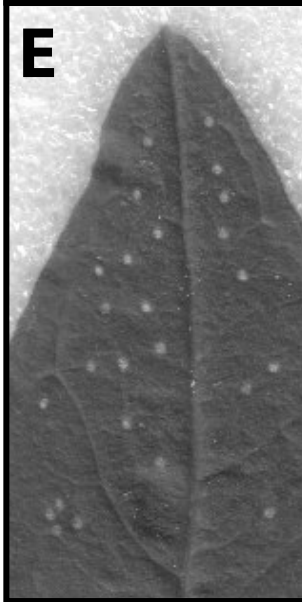
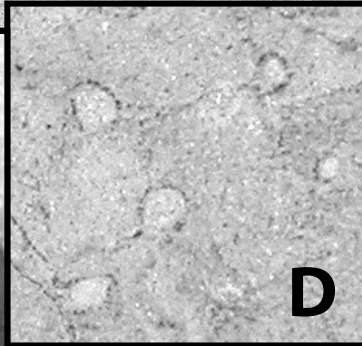
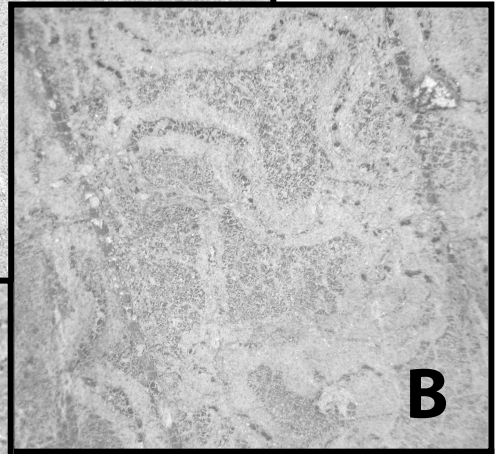
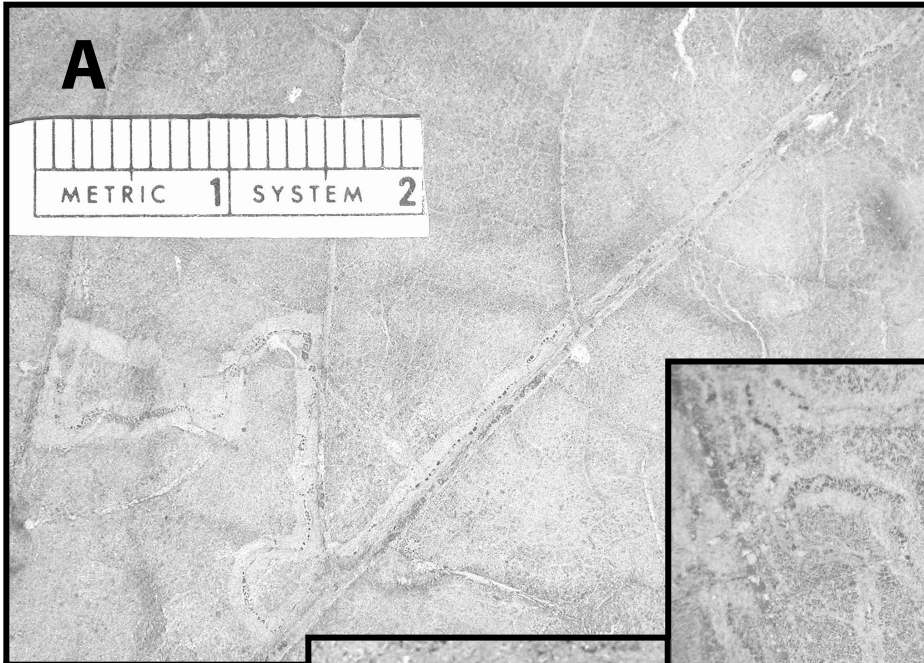
1987). Of seven fossils putatively assigned to the *Phytomyza* group (Evenhuis 1994), one is a compression fossil not reliably identifiable as an agromyzid, two are leaf mine traces for which placement is also uncertain (Spencer and Martinez 1987), and insufficient information is available for two additional fossils. Two Pliocene leaf mines on hosts of modern *Phytomyza* species likely represent *Phytomyza*, but these are too recent to be informative of earlier divergence times.

However, both trace fossils and body fossils exist for other agromyzids which are probably reliably assigned and which can be used to investigate the history of the family. In particular, three fossils were judged most relevant to early agromyzid evolution. These fossils were: 1) early Paleocene leaf mines on *Platanus* (64.4 Ma; Wilf et al. 2006), 2) *Palaeophytobia prunorum*, a fossil boring trace in wood from Yellowstone, Wyoming at the early/middle Eocene boundary (>48 Ma; Süss and Müller-Stoll 1980, Smedes and Prostka 1972), identical to traces made by modern flies of the cambium-mining agromyzid genus *Phytobia*, and 3) “*Agromyza*” *praecursor*, a body fossil from Florissant, Colorado, with the expanded basal flagellomere of the antenna characteristic of species now placed in *Cerodontha* subgenus *Dizygomyza* (~34 Ma; Melander 1949, Meyer 2003).

Taxonomic assignment of leaf mines and other trace fossils can be problematic, since insects from several insect orders and many families produce leaf mines, and insect-host plant associations may not be stable through time (Grimaldi 1999, Labandeira 2002b, Zherikhin 2002). For example, *Foliofossor cranei* was described from Paleocene

leaf mines in *Platanus* (Crane and Jarzembowski 1980, Jarzembowski 1989), which is not a host of modern agromyzids, but no justification was given for its assignment in Agromyzidae except for a resemblance to mines of modern *Phytomyza* in unrelated plants. Kozlov (1988) considered these to be made instead by a nepticulid moth. The fossil leaf mines reported by Wilf et al. (2006), also on *Platanus*, and used as the major calibration point of this study, appears to be more reliably assigned to the Agromyzidae (see Fig. 4.1). Apart from a general appearance as a typical agromyzid mine, the authors note its distinctive, fluidized frass trail, a feature not often found in lepidopterous mines (Hering 1951). Further examination of voucher specimens (Mexican Hat vouchers #501-504) at the USNM supported the authors' assignment of these fossils to Agromyzidae. Two of these specimens are illustrated in Fig. 4.1. Two additional lines of evidence suggest that these traces were made by a dipteran (as opposed to a lepidopteran) leaf miner. First, lepidopteran miners almost always form linear mines, often following secondary veins, or sometimes rounded blotch mines. Mine shapes for agromyzid flies are variable, though usually species-specific in form (Hering 1951). In addition to the above two mine types, some agromyzids form "linear-blotch" mines, in which a serpentine mine curves back upon itself in an irregular fashion, forming a blotch-like

Fig. 4.1. (following page) A-D: Early Paleocene (64.4 Ma) leaf mines assigned to Agromyzidae on *Platanus raynoldsi* from the Mexican Hat locality, Powder River Basin, Montana (Wilf et al. 2006), and used as the major calibration point for this study. Mines with otherwise identical characteristics were found both as elongate and linear (A), or winding and appressed (B,C). Small (0.3-1 mm) holes in the leaf (C,D) putatively represent feeding punctures formed by the female ovipositor; such damage is caused by many extant agromyzid species (E, punctures of *Amauromyza flavifrons* on *Saponaria officinalis* from Lakewood, Colorado), and is diagnostic of agromyzid feeding. A-B: voucher specimen MH#510; C-D: voucher specimen MH#514.



pattern. Most of the Mexican Hat specimens show this latter pattern (e.g. Fig 4.1 C), though the mine in one specimen is clearly linear (Fig. 4.1 A) in later stages. The most distinctive feature of the mines, however, and that which confirms causation by agromyzid flies, is a series of small (0.5-1 mm) puncture marks in the leaf (Fig. 4.1 C, D). Dark reaction tissue surrounding these punctures indicates that the leaf was alive when these holes were formed. Very similar marks are often caused by adult female agromyzid flies when preparing to oviposit in a host plant (e.g. Fig. 4.1 E). The female drills a hole in the leaf tissue with her ovipositor, then turns about and tastes the extruding liquid. This behavior is thought to provide nourishment for the female, but may also help to distinguish preferred host plants from non-hosts. Although these marks are small and often inconspicuous, if they are formed when the leaf is still expanding they will widen into more conspicuous holes as seen in Fig. 4.1.

The trace fossils assigned to *Palaeophytobia* were considered by Spencer (1990) to be reliably assigned, based on extensive comparison by the authors to feeding traces of *Phytobia* traces in recent wood (Süss 1980, Süss and Müller-Stoll 1980). The only other insect group that is known to form similar traces (“pith flecks”) is the little-known moth family Opostegidae (Davis 1989). The single study directly comparing opostegid to *Phytobia* traces (Kumada 1984) found that *Phytobia* mines do have distinctive features, including an elongate shape when viewed in cross-section, and mines of later instars can be easily distinguished from opostegid mines in the same host. The description of *Palaeophytobia prunorum* (Süss and Müller-Stoll 1980) notes this tangential elongation, strongly suggesting that this fossil in fact represents a typical *Phytobia* trace.

Study aims

The current study aims to use fossil-calibrated molecular divergence time estimation to fit the phylogenies of the Agromyzidae and of *Phytomyza* into a temporal framework in order to investigate the timing of shifts in diversification rate and other evolutionary events. Specifically, the following hypotheses will be addressed: (1) Ranunculaceae was the ancestral host of *Phytomyza*; (2) colonization of and diversification onto herbaceous asterid families occurred as these plant families diversified in the northern hemisphere; (3) shifts to asterid herbs resulted in higher rates of diversification attributable to an increase in available host species; (4) the *Phytomyza* group of genera originated in the north temperate zone soon after cool temperate biomes began to expand during the Eocene; and (5) global cooling events in the Oligocene and Miocene were associated with major events in the evolution of of *Phytomyza* and related temperate genera, such as a) origin of major clades, b) increases in diversification rate, or c) shifts in habitat preferences or other ecological characteristics. These hypotheses reflect the expectation that both trophic associations and external climatic influences will leave phylogenetic signatures in the sequence of observed host and biome associations and in rates of diversification.

Methods

Data sets and divergence time estimation

Because our divergence time calibrations were based on agromyzid fossils from lineages outside our focal clade (the *Phytomyza* group), and sequence data for all gene

partitions was not available from other agromyzid taxa, we used separate analyses of two molecular data sets, overlapping partly in gene and taxon sampling. One of these broadly sampled lineages across Agromyzidae, while the other is restricted to *Phytomyza* and close relatives. The across-Agromyzidae data set is an augmented version of that presented by Scheffer et al. (2007), which totaled 2,965 base pairs from the mitochondrial COI gene, the nuclear ribosomal gene 28S, and the nuclear protein-coding gene CAD, sequenced in 86 exemplars representing nearly all genera in the family. To these data we added COI and CAD sequences for an additional 13 species of *Phytomyza* from the analysis presented in Chapter 3, plus 28S data from these same species (Genbank accession numbers EU367919-EU367931), newly generated following the methods of Scheffer et al. (2007). The augmented data set was reanalyzed with maximum likelihood using the program GARLI v.0.951 (Zwickl 2006), with default parameters. Monophyly for *Phytomyza s.l.*, not initially recovered, was enforced for subsequent analyses, as this grouping was strongly established by the extensive sampling of Chapter 3, and supported by the results of Scheffer et al. (2007). Eight separate GARLI runs were performed, from which the tree of highest likelihood was selected for dating analysis. A bootstrap analysis (500 replicates) was then performed in GARLI to gauge support for monophyly of the *Phytomyza* group of genera.

Divergence time estimation was first performed on the family level tree (with non-agromyzid outgroups pruned), using three different methods: non-parametric rate smoothing (nprs; Sanderson 1997) and penalized likelihood (pl; Sanderson 2002) implemented in the program r8s v.1.71 (Sanderson 2006), and Bayesian MCMC analysis

using BEAST v.1.4.5 (Drummond and Rambaut 2006). Analyses in r8s used the logarithmic penalty, as suggested by Smith et al. (2006). Identification of the optimal smoothing parameter (s) for penalized likelihood by cross validation analysis was not straightforward, as calculations failed for some values of s . However, as the remaining calculations implied an optimal value near 10^3 , suggested in the r8s manual as an upper bound for s in usual cases, the smoothing parameter was set to 1,000. Because larger values of s also result in stronger differentiation between divergence times estimated by the nprs and pl method, this choice also served to delimit an interval of plausible date estimates for this class of methods (parametric and semiparametric rate smoothing). The BEAST analysis was performed using the same model as in GARLI (GTR+I+G), with a Yule prior on speciation rates, implementing the uncorrelated lognormal relaxed clock (Drummond et al. 2006) and using the nprs tree as a starting topology. The three gene partitions were modeled separately. The weight of several parameter operators were increased from default values to increase mixing of the Yule prior and the frequency of topology changes (swap operator on branch rate categories and wide exchange and Wilson-Balding operators to 100; uniform operator on internal node heights and narrow exchange operator to 50). In order to reach stationarity, it was also necessary to constrain the following groups as monophyletic: Agromyzinae, Phytomyzinae, and (*Phytobia* + *Amauromyza* + *Phytoliriomyza robiniae* + *Phytomyza* group). The final analysis was run for 100 million generations, sampling the chain every 1,000 generations. In addition to providing confidence intervals for divergence time estimates by integration over many possible topologies and other parameters, the BEAST analysis was useful in providing

independent estimates not based on the assumption of autocorrelation of evolutionary rates between adjacent branches, as is assumed in the nprs and pl methods.

For all three methods, divergence times were calibrated using the three relevant fossils mentioned above. Although these fossils necessarily represent only minimum ages for clades, which may be adjusted as fossil sampling becomes more complete, calibration of molecular phylogenies requires some kind of maximum age constraint, as well. Accordingly, for the nprs and pl analyses, the root of the tree was fixed at 64.4 Ma, the node subtending *Cerodontha (Dizygomyza) fasciata* was constrained at a minimum age of 34 Ma, and the node connecting *Phytobia* with *Phytomyza* and related genera was constrained at a minimum age of 48 Ma. In addition, the penalized likelihood analysis was repeated with each of these fossil constraints singly. To facilitate comparison of estimates between analyses, the root height was tightly constrained in the BEAST analysis by placing a strong prior on root height (mean = 64.4 Ma and standard deviation = 0.5 My). The remaining two calibration points were incorporated using uniform priors with minimum ages as in the r8s analysis. In addition to nodes used for calibration, divergence times were estimated for the *Phytomyza* group of genera, for *Phytomyza* itself (excluding *C. scolopendri*), and for the “main lineage” of *Phytomyza* (excluding the *nigra* clade and smaller, more basal lineages). In order to obtain meaningful estimates, these nodes were also constrained as monophyletic.

Analyses using the nprs and pl methods were next performed on the *Phytomyza* ML phylogeny from Chapter 3. Cross-validation analyses again failed, so the smoothing

parameter was again set to 1,000. In these analyses, the age of the common ancestor of *Phytomyza* (excluding *C. mimuli*, *C. nr. castillejae*, *C. scolopendri*, and *P. gymnostoma*) was set at 32.8 Ma and the root of the *Phytomyza* group at 44.0 Ma, the age of the corresponding nodes in the pl analysis of the family-level phylogeny. Preliminary analyses were also attempted using a combined data set with both the family-level and *Phytomyza* data. These two data sets have 38 overlapping taxa, and non-overlapping taxa share approximately 2,000 base pairs of data out of 3,700 total. However, these combined analyses yielded anomalous results, with estimates from BEAST much older and some estimates from r8s significantly younger than with the reduced agromyzid data set. Although the nature of these discrepancies was not explored, they suggest that both methods are sensitive to either taxon sampling density or missing data, or both, and that future studies should explore this possibility (see also Linder et al. 2005).

Ancestral host reconstruction

In order to test the hypothesis that Ranunculaceae were the ancestral hosts of *Phytomyza*, a simplified phylogeny of *Phytomyza* was prepared by pruning taxa with unknown hosts from the ML phylogeny presented in Chapter 3. Host use (see Table 3.1) was coded for each species according to high-level plant clades, delimited by APGII (2003) as follows: 0 – ranunculid, 1 – asterid, 2 – rosid, 3 – monocot, 4 – Saxifragales, 5 – ferns. Ancestral states were then reconstructed using a single rate maximum likelihood model (Schluter et al. 1997) in Mesquite v. 1.06 (Maddison and Maddison 2005). In this method, probabilities of character state changes are modeled stochastically on the phylogeny with a rate parameter that is optimized by maximum likelihood

estimation, integrating over the likelihoods of all possible character state combinations at internal nodes. The relative likelihoods of each state at any given node are then estimated as the proportional contribution of these to the overall likelihood, given the optimal value of the rate parameter. Ancestral states were also reconstructed with a similar model using Bayesian estimation (Huelsenbeck and Bollback 2001) in the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), by adding a single character representing host use to the DNA sequence data matrix as a separate morphological partition. Bayesian methods are able to account for uncertainty in phylogeny, as well as in other relevant parameters. However, because MrBayes requires constraining nodes of interest one at a time and repeating the analysis for each node of interest, ancestral states were only inferred for two nodes – the root of the genus *Phytomyza*, and a large clade representing the “main lineage” of *Phytomyza* (Fig. 4.3). Both Bayesian analyses were run for 8 million generations and sampled every 100 generations, with the first 2 million discarded as burnin.

Diversification rate analysis

Although techniques exist to study diversification rate variation which do not require any information on absolute dates (Mitter et al. 1988, Moore et al. 2004, Vamosi and Vamosi 2005), these methods are limited because they do not make use of all available information (Purvis 1996). Incorporating information about relative divergence times from molecular phylogenies can increase the precision and power of tests of diversification rate variation (e.g. Nee et al. 1992, Purvis 1996, Ree 2005). Furthermore, calculation of absolute, rather than relative, diversification rates using fossil calibrations

allows for greater flexibility in hypothesis testing when taxon sampling or phylogeny resolution is incomplete (Magallón and Sanderson 2001, Bokma 2003). Perhaps most importantly, knowledge of the timing of major bursts of diversification can lead to very different interpretations of evolutionary events and interactions than would otherwise be assumed (e.g. Schneider et al. 2004).

We considered a number of different methods for estimating rates of diversification and testing for differences in these rates between Ranunculaceae- and asterid- feeding clades. There were several inherent limitations in our study design which limited choice of methods: (a) many major clades can be characterized (i.e. delimited and approximate diversity specified) but are themselves sparsely sampled; (b) some lineages on the phylogeny cannot be adequately characterized, and an unknown number of lineages were not included; and (c) relationships between some lineages are strongly supported, but a significant lack of resolution is seen in parts of the phylogeny. These difficulties are likely to exist in many phylogenetic studies of large adaptive radiations, where taxon and character sampling are often limiting; however, it is precisely such large, rapid radiations which present the most interesting questions regarding patterns of diversification. Many current methods for testing diversification rate variation assume complete taxon sampling, a condition not met in this study, though this assumption can be relaxed to some degree (Paradis 1998, Pybus and Harvey 2000). The lack of resolution at some levels in the *Phytomyza* phylogeny, and incomplete characterization of lineages represent a further difficulty for other methods (e.g. Paradis 2003). The method of Magallón and Sanderson (2001) was finally judged to be the best suited for our data,

with supplementary results obtained using other methods. In their method, a series of well-supported, non-overlapping clades (plant families in their case) are chosen from a phylogeny, and information about clade diversities and times of origin are used to calculate absolute rates of diversification (speciation minus extinction) based on a simple birth-death model. These individual clade diversification rates can then be compared to an overall rate to identify clades with significantly higher or lower rates of diversification. Although Magallón and Sanderson did not do so, these clade rates can also be compared between two or more categories of clades, as was done by Bokma (2003), who developed a roughly similar approach.

In order to test the hypothesis that clades shifting to asterid hosts have diversified faster than those on Ranunculaceae, ten clades of *Phytomyza* were identified from the results of Chapter 3 which are relatively well-characterized, and have hosts mostly in the Ranunculaceae (n=5) or various asterid families (n=5). These clades are identified in Fig. 4.3, and are listed also in Table 4.3. Minimum diversity of each of these clades was characterized by summing the numbers of described species of each component species group. These group diversities were estimated directly from the taxonomic literature, including host plant and genitalic morphology data from Spencer (1990) and other sources (see references cited in Chapter 3). Of the included clades, only the *agromyzina* group (Fig. 4.3, A4) could not be easily characterized as having hosts of nearly all species in a single plant clade. In this case, hosts of about half the species are asterids, and this is inferred by ML to be the ancestral host; no species in this clade have hosts in the Ranunculaceae. Likewise, the delimitation of individual clades was relatively

nonproblematic, given the taxon sampling and additional taxonomic data; this is largely because species groups are each characterized by a unique form of the male genitalia, drawings of which are available for most described taxa. The major exception is a group of mainly Ranunculaceae-feeding taxa (Fig. 4.3, A5) centered on the *aquilegiae* group (Spencer 1990), for which inclusion of a number of unsampled taxa is uncertain. In this case, a wide circumscription was adopted in order to make subsequent tests of diversification rate differences more conservative.

Two additional considerations should be addressed in regards to the validity of this test. First, are the host plant categories (Ranunculaceae and asterids) comparable, biologically relevant entities, or arbitrary taxonomic categories? Although these two plant groups are very different in taxonomic rank and diversity, there are at least two reasons to consider this a biologically meaningful comparison. First, the distribution of *Phytomyza* species across plant lineages is essentially bimodal, with the majority feeding on one of these two host groups and very few on phylogenetically intermediate plant taxa. Secondly, because of the relative lack of association with other plant groups, the comparison may be viewed as essentially between two sister clades of plant hosts (Fig. 3.1): Ranunculales (including Ranunculaceae), and remaining eudicots (including asterids). An additional consideration is whether the ten clades chosen represent independent evolutionary events or are phylogenetically correlated – i.e. whether it is possible that a single increase in diversification rate led to multiple species rich clades in nested lineages. Results of the phylogenetic analysis (Figs. 3.3, 4.3) strongly suggest that shifts to asterid hosts occurred independently, and thus any overall increase in

diversification rate in these clades also occurred independently. Specifically, three of the five asterid-feeding clades have strongly-supported sister group relationships with Ranunculaceae-feeders (in the *albiceps*, *albipennis*, and *aquilegiae* clades), and the remaining two asterid-feeding clades are widely separated on the phylogeny, with at least one intervening node having moderate support.

To test for significant diversification rate variation, a maximum likelihood estimate of overall diversification (speciation minus extinction) rate was calculated for the genus *Phytomyza* using the method of Magallón and Sanderson (2001) in the geiger package (Harmon et al. 2007), using the values of $n=630$, $t=32.8$ My, and $\epsilon=0$ (no extinction). Diversification rates were then estimated separately for individual clades of *Phytomyza*, using crown group ages from the dated phylogeny. Use of crown group ages implies that taxa spanning the basal nodes of each clade were sampled; we believe our sampling to be a reasonable approximation to this assumption, especially for the asterid-feeding groups. To determine if the diversification rate in individual lineages has been significantly greater than for *Phytomyza* as a whole, probabilities of observing clade sizes as great or greater than observed, given individual times of origin and the overall diversification rate, were calculated (again in geiger, with the crown.p function). Note that this test is somewhat conservative given the possibility that some unplaced species may fall inside recognized clades, and were included in the calculation of the overall rate. Following Magallón and Sanderson (2001), we repeated these estimates under the assumption of high extinction rates ($\epsilon=0.9$). Finally, to more directly test the hypothesis of greater rates of diversification for asterid feeders than for ranunculid feeders, expected

clade sizes were calculated, again given individual times of origin and the overall estimated diversification rate. A Mann-Whitney rank test was performed on the ratio of observed to expected clade sizes for asterid- versus ranunculid-feeding clades.

The Slowinski-Guyer (SG) statistic was also calculated for basal nodes of the *Phytomyza* phylogeny to determine along which nodes significant shifts in diversification rate have occurred. This statistic represents the probability that the difference in diversity of two sister clades is greater than that expected by chance according to a simple Yule model, and is calculated as $2\ell / (\ell+r-1)$ where ℓ and r are the diversities of the smaller and larger sister clades, respectively (Slowinski and Guyer 1989). Because comparisons nearer the root are influenced by significant comparisons at nested nodes (Sanderson and Donoghue 1994), the least inclusive node with a significant value was considered to represent an actual shift in diversification rates. SG probabilities were not calculated for remaining nodes because of uncertainty due to taxon sampling within the main radiation of *Phytomyza*. Next, a list of branching times was generated using the ape package (Paradis et al. 2004) and graphed in Microsoft Excel to generate a lineage through time (LTT) plot. When the y axis (number of lineages or species) is graphed on a logarithmic scale, the slope of the plot is equal to the speciation rate, assuming extinction is negligible.

Finally, the hypothesis of an increase in diversification rates following the Oligocene cooling event was tested via the method of Paradis (1997) using a truncated phylogeny of the *Phytomyza* group extending from its origin (~44 Ma) to the global

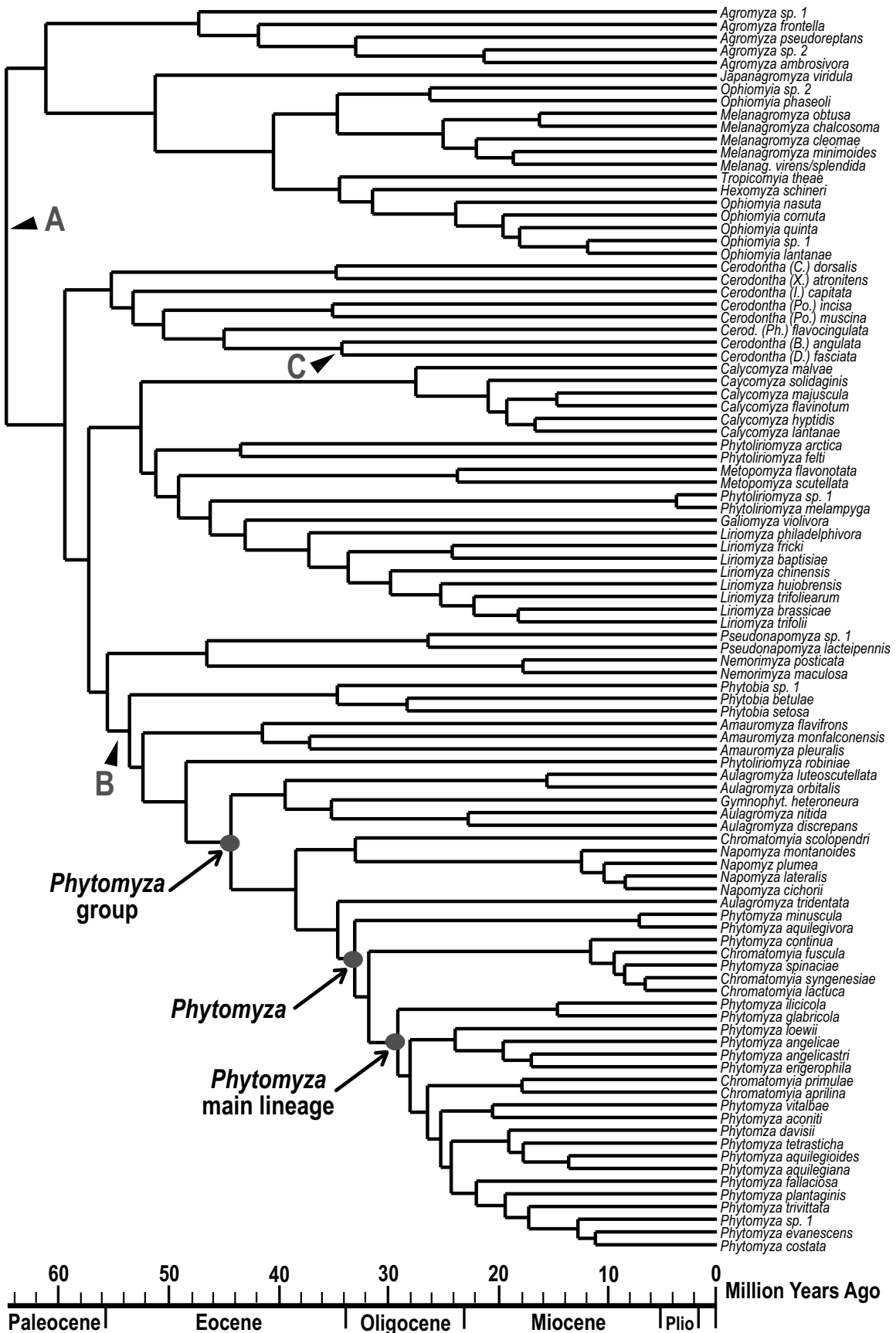
warming event of approximately 24 Ma. This method, adapted from survival analysis in ecology, uses the timing of branching events to obtain a maximum likelihood estimate of diversification rate (δ). This estimate is simply the number of observed splitting events divided by the sum of all splitting times. Two more complex models, where δ changes over time, were also derived by Paradis. We compared his model C, where the diversification rate changes abruptly at a specified time, to a model assuming a constant diversification rate. Likelihoods for both models were calculated using formulae in Paradis (1997), with divergence times generated in ape as for the LTT plot, but adjusted for an endpoint of 24 Ma. These two models were then compared by a likelihood ratio test (LRT).

Results

Divergence time estimates

ML analysis of the expanded agromyzid data set resulted in a topology (Fig. 4.2) nearly identical to the Bayesian results of Scheffer et al. (2007), except for relationships within *Phytomyza*, which more nearly matched the more densely sampled phylogeny presented in Chapter 3. The *Phytomyza* group of genera was recovered with moderately

Fig. 4.2. (following page) Time-calibrated phylogeny of the Agromyzidae, generated by penalized likelihood rate smoothing of ML phylogeny obtained using r8s v. 1.71 (Sanderson 2006) and GARLI v. 0.951 (Zwickl 2006). Sequence data is largely from Scheffer et al. (2007), with some additional taxa added, and includes data from the COI, CAD, and 28S genes. Fossil calibrations are lettered as followed: A) early Paleocene leaf mine on *Platanus* (Wilf et al. 2006), B) *Palaeophytobia prunorum* (Süss and Müller-Stoll 1980), and C) “*Agromyza*” *praecursor* (Melander 1949). The latter two were applied as minimum age constraints only.



strong (83%) support in the bootstrap analysis, and *Phytomyza* (+ *Chromatomyia*) + *Napomyza* was monophyletic with 90% bootstrap support. However, relationships between the *nigra* clade, remaining *Phytomyza*, *Napomyza*, and *C. scolopendri* were not resolved in the bootstrap tree. Divergence time estimates from the nprs, pl, and BEAST analyses were all very similar, and are listed in Table 4.2. Taking the pl results as representative estimates, the time of origin of *Phytomyza* was inferred at 32.8 Ma, and of the *Phytomyza* group of genera at 44.0 Ma. These estimates were found to be most influenced by the root calibration point; calibration with the *Dizygomyza* fossil only resulted in much older estimates, and calibration with the *Palaeophytobia* fossil only resulted in slightly younger estimates (Table 4.2). 95% confidence intervals in BEAST for these two estimates spanned approximately eight million years (*Phytomyza*) and ten million years (*Phytomyza* group), respectively. Examination of BEAST log files using TRACER v. 1.4 (Rambaut and Drummond 2007) showed that the posterior reached approximate stationarity after approximately 40 million generations; data previous to this point were discarded as burnin. Significant fluctuations in the prior probability even after this point resulted in a low effective sample size (ESS) for both the prior and posterior. This may be due to a lag in adjustment of the yule prior parameters after major topology proposals are accepted (A. Drummond, pers. comment), though this problem was mostly alleviated by fixing three basal divergences as noted above. However, all other parameters (including *Phytomyza* group divergence times) had high ESS values (>400), appeared to be approximately normally distributed around a stationary mean, and were not correlated with fluctuations in the prior. The exceptional parameters that were not normally distributed were divergence times for the two nodes with minimum age

Table 4.2. Divergence times of *Phytomyza* and other clades estimated by non-parametric rate smoothing and penalized likelihood (using r8s v.1.71; Sanderson 2006), and Bayesian MCMC analysis (in BEAST v.1.4.5; Drummond and Rambaut 2006). The rightmost three columns represent penalized likelihood results using single calibration points only. Times all represent millions of years before present; dates in parentheses represent nodes fixed for a given analysis.

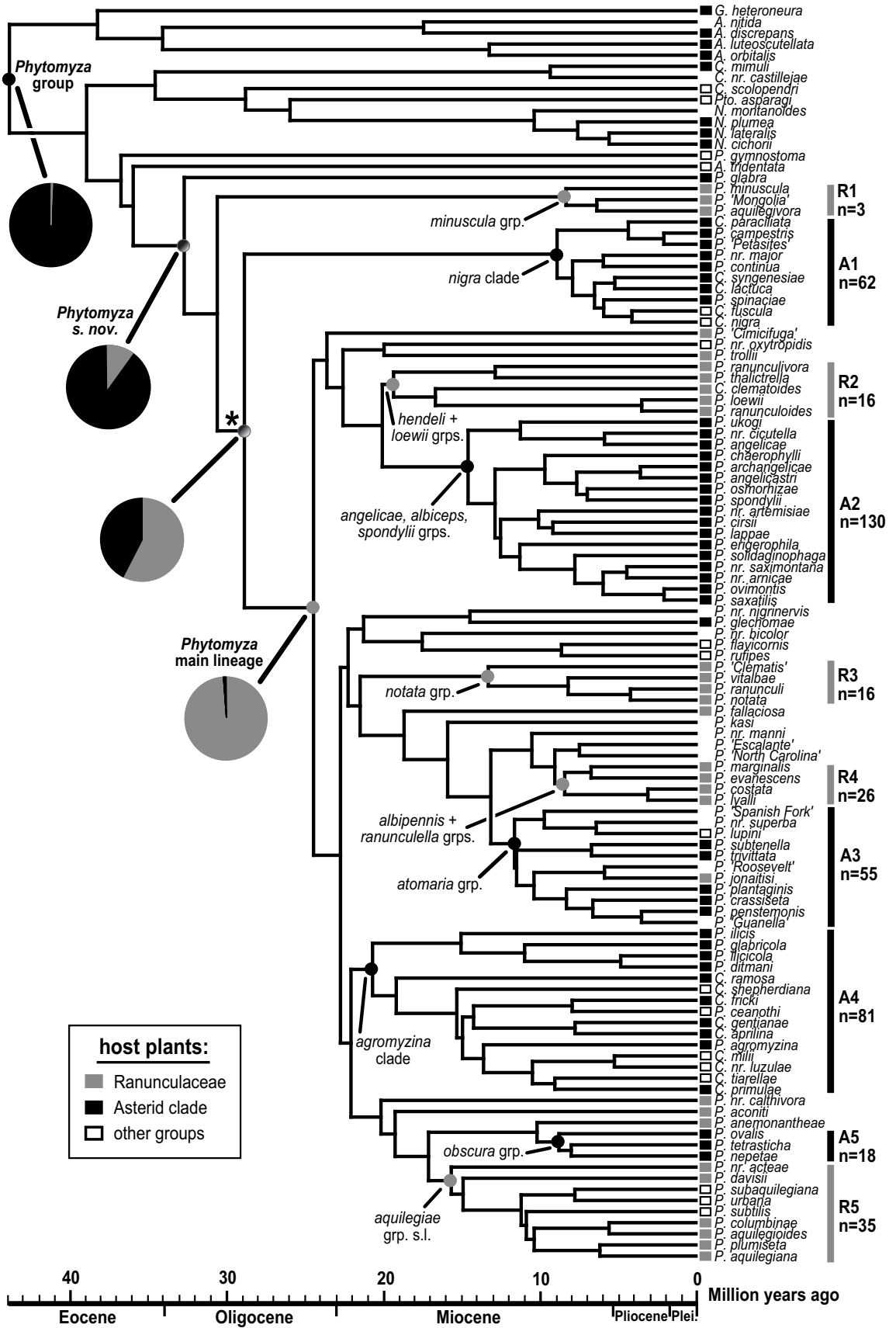
clade	nprs	pl	BEAST (95% interval)	root only	<i>Dizygomyza</i> only	<i>Palaeophytobia</i> only
Agromyzidae	(64.4)	(64.4)	64.5 (63.5- 65.5)	(64.4)	100.7	59.9
<i>Dizygomyza</i> stem	34.0	34.0	35.2 (34.0- 37.6)	21.7	(34.0)	20.2
<i>Phytobia</i> stem	54.2	53.2	53.6 (49.2- 57.8)	51.6	80.8	(48.0)
<i>Phytomyza</i> group	44.2	44.0	42.7 (37.9- 47.2)	42.7	66.8	39.7
<i>Phytomyza</i>	32.5	32.8	32.1 (28.3- 35.8)	31.9	49.8	29.6

constraints, and this was expected given the nature of the constraint. Our divergence date estimates thus appear to be unaffected by nonstationarity in the prior. Divergence times for the selected clades of *Phytomyza* estimated by penalized likelihood are found in Table 4.3 (see also Fig. 4.3), and are mostly between eight and nineteen million years ago, suggesting a Miocene origin for most species groups and major clades of *Phytomyza*.

Table 4.3. Selected clades of *Phytomyza*, with minimum ages estimated by penalized likelihood, minimum diversities estimated from the taxonomic literature, and the probability of obtaining a clade of equal or greater size given the crown group age and assuming the same diversification rate as inferred for *Phytomyza* as a whole. Clade probabilities were calculated using the geiger package (Harmon et al. 2007), under assumptions of both zero extinction and high extinction rates.

clade	included groups	crown group age (pl)	min. diversity	prob. of clade size ($\epsilon=0$)	prob. of clade size ($\epsilon=0.9$)
R1	<i>minuscula</i> grp.	8.33	3	0.946	0.907
R2	<i>hendeli</i> , <i>loewii</i> grps.	19.38	16	0.912	0.872
R3	<i>notata</i> grp.	13.32	16	0.567	0.724
R4	<i>albipennis</i> , <i>ranunculella</i> grps.	8.41	26	0.013*	0.300
R5	<i>aquilegiae</i> grp. s.l.	15.68	37	0.321	0.578
A1	<i>nigra</i> clade	8.9	62	<0.001*	0.067
A2	<i>angelicae</i> , <i>albiceps</i> , <i>spondylii</i> grps.	14.61	130	<0.001*	0.100
A3	<i>atomaria</i> grp.	11.64	55	0.005*	0.221
A4	<i>agromyzina</i> clade	20.72	81	0.372	0.545
A5	<i>obscura</i> grp.	8.78	18	0.093	0.465

Fig. 4.3. (Following page) Time calibrated phylogeny of the *Phytomyza* group of genera, focusing on *Phytomyza*, generated from sequence data from the COI, CAD, and PGD genes (see Chapter 3). The phylogeny was ultrametricized using penalized likelihood (pl) in r8s 1.71 (Sanderson 2006) with age constraints for the *Phytomyza* group and *Phytomyza sensu novo* taken from the family-wide pl analysis (Table 4.2). Host clades of each species (where known) are listed at right, as follows: grey – Ranunculaceae, black – Asterid families, open box – other families. The relative likelihoods (based on a single rate ML model in Mesquite; Maddison and Maddison 2005) of Ranunculaceae-feeding vs. asterid-feeding for four basal nodes is indicated in the pie graphs at left. The site of an inferred diversification rate shift, according to the Slowinski-Guyer statistic, is marked by an asterisk. Ten major clades selected for the diversification rate analysis are also labelled in the text, and at right as R1-R5 and A1-A5, with the minimum number of described species estimated from the taxonomic literature (see Chapter 3, Appendix A). Generic, clade, and group names follow those in Chapter 3.



Ancestral host reconstruction

Ancestral state estimation using maximum likelihood returned a high relative likelihood (89%) corresponding to an asterid host at the root of *Phytomyza* (Fig. 4.3). This likelihood was even higher (98%) at the node connecting *Aulagromyza* to *Phytomyza*, representing the common ancestor of the *Phytomyza* group of genera. In contrast, for a large clade of *Phytomyza* representing the main lineage (excluding the *nigra* clade and more basal branches), Ranunculaceae was inferred as the ancestral host with a very high relative likelihood (99%). Bayesian ancestral state estimation, which accounts for phylogenetic and other sources of error, gave similar results, but with less certainty; the ancestor of *Phytomyza* was inferred to have fed on an asterid host with a 80% posterior probability and that of its major radiation on Ranunculaceae with an 81% posterior probability.

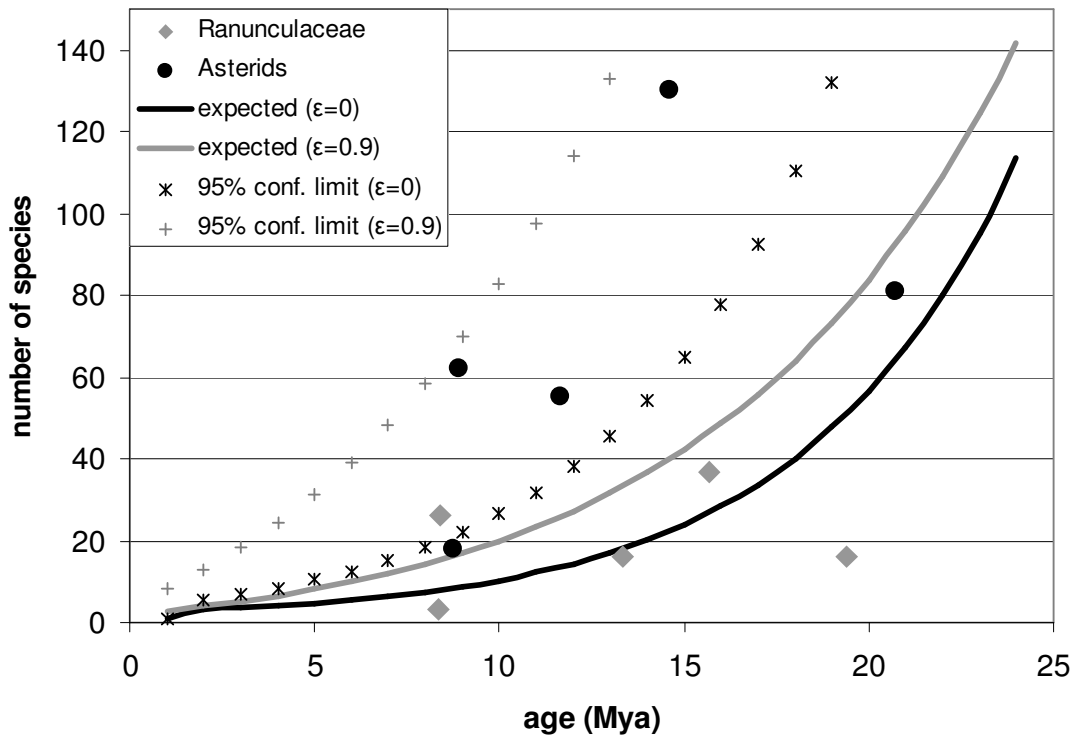
Diversification rate analysis

Estimates of minimum clade diversities from the ten focal clades are listed in Table 4.3. In total, these ten clades include over 440 of the 640 described species of *Phytomyza*, with the positions of an additional 50 species approximately known from available data, and of the remaining 150 species unknown (and thus possibly belonging to one of the identified clades). Although this degree of uncertainty in assigning species diversity is undesirable, and is compounded by a presumably significant number of undescribed species, it is difficult to avoid in a group as large, complex, and understudied as *Phytomyza*. We believe that these remaining species are probably dispersed

throughout the phylogeny, that our figures realistically represent relative clade diversities, and that it is unlikely that excluding them will substantially bias our results.

The overall rate of diversification (speciation minus extinction) for *Phytomyza* was estimated to be $r=0.175$ / m.yr. under no extinction and $r=0.125$ / m.yr. under high extinction rates. Individual asterid-feeding clades mostly had higher rates of diversification than these overall rates, reflecting higher than expected clade sizes (see Table 4.3; Fig. 4.4), while ranunculid-feeding groups mostly had lower rates. Three of these asterid-feeding clades were shown to be significantly more diverse than expected at a 0.05 significance level under the assumption of no extinction, and one ranunculid-feeding clade was also significantly diverse. However, under the assumption of high extinction rates, none of these clades were found to be significantly more diverse than expected, due mostly to a larger variance when extinction is considered. Paradoxically, expected clade sizes are larger under the assumption of high extinction rates; this is because a higher speciation rate is necessary to account for observed present diversity, and surviving clades will thus be on average larger, if a number of unobserved clades have gone extinct (Magallón and Sanderson 2001; see also Nee et al. 1994). The Mann-Whitney test showed the trend of higher than expected diversity for asterid feeders to be significant with a one-tailed test ($p = 0.025$). Results of this rank test were not affected by differing assumptions about extinction rates. Four adjacent basal nodes showed a significant imbalance in diversity by the Slowinski-Guyer statistic, beginning with the node where *P. gymnostoma* branches, and ending with the node where *P. minuscula* and relatives diverged from remaining *Phytomyza*. This final comparison was then judged to

Fig. 4.4. Clade size vs. clade age for ten selected clades of *Phytomyza* feeding primarily on Ranunculaceae (diamonds) or asterids (circles). Expected diversity vs. age, assuming the same overall diversification rate inferred for *Phytomyza* as a whole, is shown for the case of no extinction ($\epsilon=0$; dark line) and high extinction rates ($\epsilon=0.9$; grey line), with 95% confidence limits (one-tailed) are also indicated for each case. The expected clade sizes are larger under the assumption of high extinction rates because a higher speciation rate is necessary to account for observed present diversity, and surviving clades will thus be on average larger, if a number of unobserved clades have gone extinct. See Table 4.3 for estimates of clade ages and diversities.

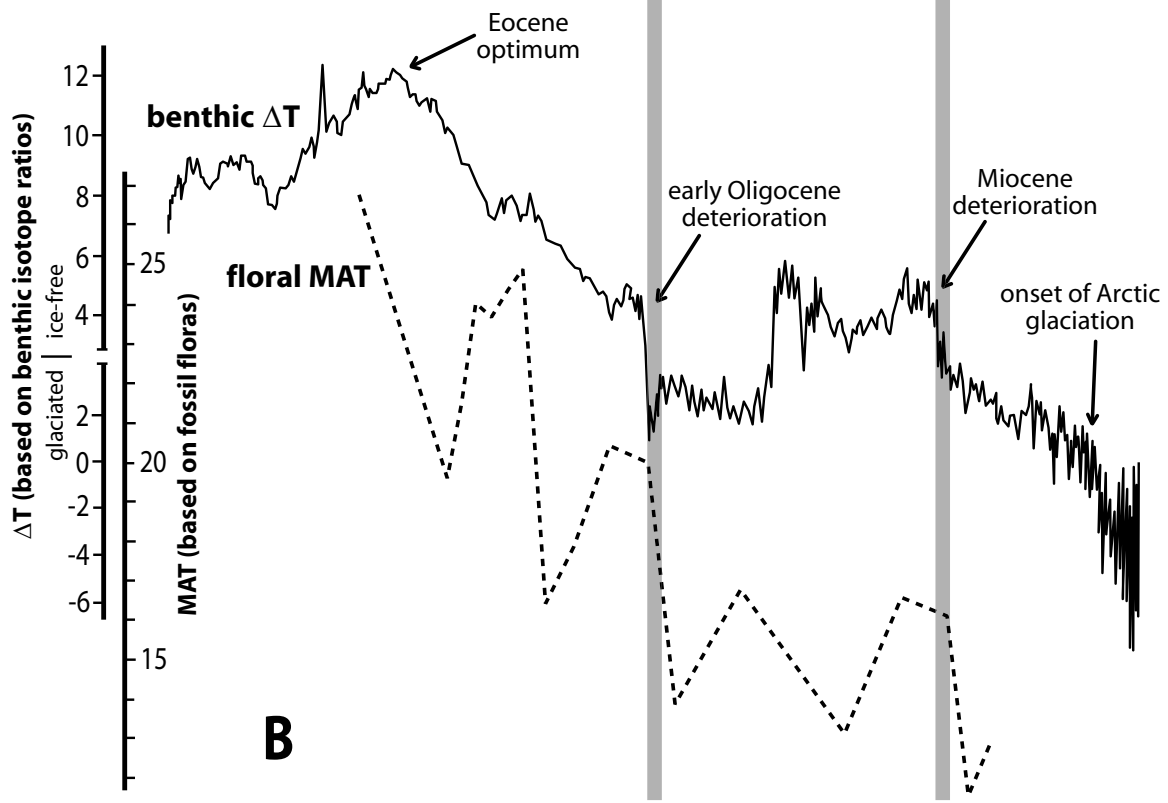
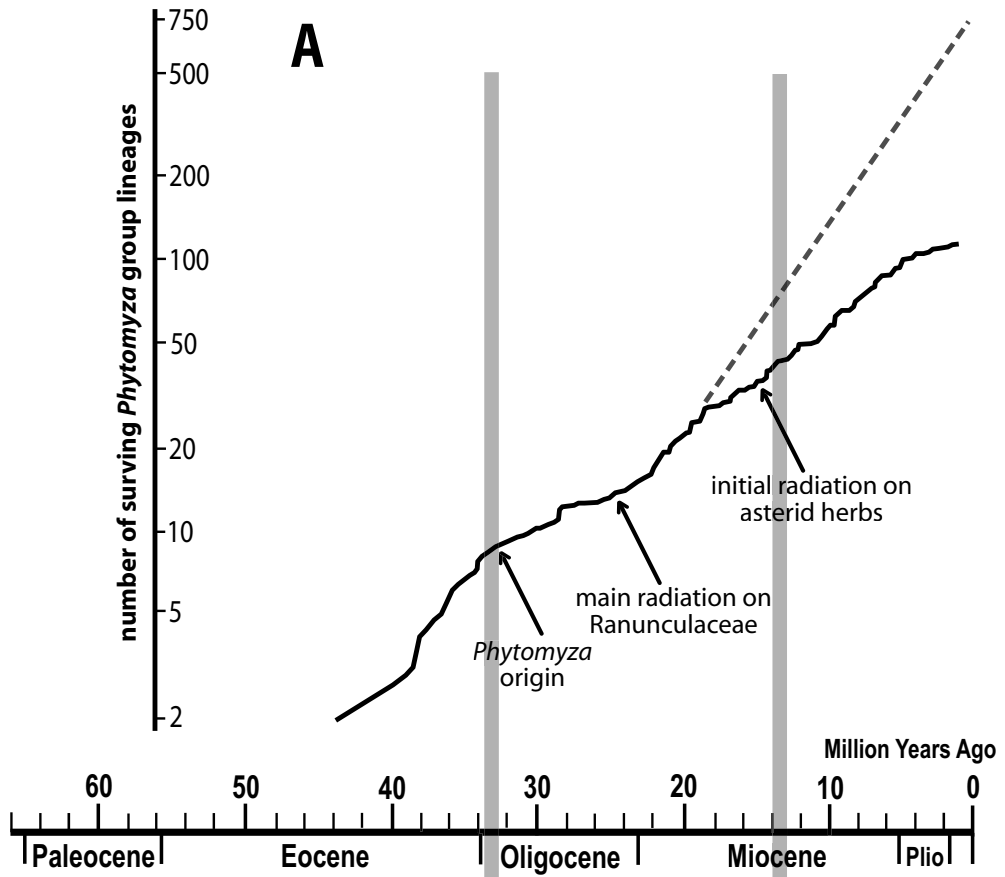


indicate an actual diversification rate shift along the nested branch ($p = 0.01$; location of shift labeled with an asterisk in Fig. 4.3).

The LTT plot showed relatively constant rates of diversification after initial divergences (Fig. 4.5). However, the curve appears to flatten somewhat around the initial divergences within *Phytomyza* (35-25 Ma), and diversification appears to accelerate slightly at the divergence of lineages within the main radiation of *Phytomyza* (22 Ma).

The use of a LTT plot is admittedly problematic for such a sparsely sampled clade (less than 1/6 of extant species sampled). However, it is evident that the great majority of unsampled species belong to the main lineage of *Phytomyza* (see Chapter 3, Appendix A), and most of these (probably at least 4/5) belong to species groups represented in our species sample. As nearly all of these species groups are inferred to have originated between 8 and 15 Ma, the actual accumulation of lineages was probably similar to that inferred by the LTT plot up to at least 20 Ma, but after this was probably much more rapid (see Fig. 4.5, dashed line). The likelihood ratio test comparing a model where speciation rate changed at the Oligocene cooling event versus a constant rate model strongly favored the former ($p < 0.001$). However, as inspection of the LTT plot showed, the diversification rate was higher prior to the event ($\delta = 0.22$ / m.yr.) than after ($\delta = 0.07$ / m.yr.).

Fig. 4.5. (following page) **A.** Logarithmic lineage through time (LTT) plot of *Phytomyza* group species generated using the APE package (Paradis et al. 2004), based on 113 species (of ~750 total) and the chronogram in Fig. 4.3. The dashed portion of the LTT plot represents a possible trajectory of the curve if all *Phytomyza* species were included, assuming most unsampled species fit into the major recognized species groups. **B.** Paleoclimatic curves derived from benthic foraminifera isotope data (thin solid line, Zachos et al. 2001, modified from www.globalwarmingart.com) and physiognomic analysis of North American floras (dashed line, Wolfe 1994b). The early Oligocene and mid-Miocene climate deteriorations (grey bars) represent significant drops in mean global temperatures concurrent with the onset of major Antarctic glaciations, but were not as dramatic as appears in the graph because calibration of temperature curves from the isotopic ratio curve differs between glaciated and non-glaciated conditions (see scale bars at left).



Discussion

Fossil calibrations and age of the Agromyzidae

The age of origin here suggested for the Agromyzidae and its component clades is earlier than expected by some (e.g. Rohdendorf 1974), given its characterization as a “young” family with highly derived phytophagous habits, and is also significant in its implications for broader acalyprate origin. The leaf mine fossil used as our major calibration point may represent the earliest known evidence for an acalyprate fly in the fossil record, apart from one Cretaceous amber specimen (Grimaldi et al. 1989).

Although the probable existence of acalyprates in the late Cretaceous can be inferred, it has been generally assumed, based on the fossil record (or lack thereof), that many acalyprate families diversified during the Eocene or later. It is hoped that further study of potential agromyzid fossils will confirm our results, but for the present it is suggested that these fossils provide convincing evidence for the presence of agromyzid flies in the early Paleocene. What this means for the age of origin of the broader schizophoran clade will depend on the phylogenetic position of agromyzids within this diverse group, a difficult question that cannot be confidently answered with current morphological data (McAlpine 1989), but will hopefully be soon addressed with molecular approaches.

Npr analyses using single calibration points (see Table 4.2) support the inference of an early Paleocene origin of agromyzids, and largely corroborate divergence times inferred for *Phytomyza* and the *Phytomyza* group. These differed only slightly when only the root calibration point was used, and were only inferred as 2-3 million years later with the *Phytobia* calibration. However, this difference is well within Bayesian confidence

intervals (Table 4.2), especially given the uncertain phylogenetic position of *Phytobia* (which was not allowed to vary in the BEAST analysis). Furthermore, the *Phytobia* calibration was applied conservatively by assigning it to the common ancestor of *Phytobia* and the *Phytomyza* group. Given its typical *Phytobia*-like biology and the occurrence of modern *Phytobia* on the same host genus (*Prunus*; Spencer 1990), it is likely that this fossil in fact belongs to the crown group radiation of *Phytobia*. The much older ages inferred when the *Cerodontha* (*Dizygomyza*) fossil was used alone as a calibration point suggests that this fossil may not have been a good choice for calibration. There are several possible reasons for this. First, using single, recent divergences to date deep nodes is known to be problematic (e.g. Near et al. 2005, Hug and Roger 2007), and this may have been exacerbated in this case by the substantial rate variation apparent in the genus *Cerodontha* (see fig. 4 of Scheffer et al. 2007), which is greater than for other agromyzid genera. Alternatively, the phylogenetic position of this fossil may have been mistakenly assigned by us. Although the distinctive trait (enlarged basal flagellomere of the antenna) which led Melander to postulate a relationship with *Cerodontha* (as “*Agromyza*”) *luctuosa* is generally diagnostic of the subgenus *Dizygomyza*, there is a chance that it may have been independently derived in older lineages, as it has been recently in a few species (e.g. *Phytomyza lactuca*, *Liriomyza commelinae*; see Spencer and Steyskal 1986). Melander’s (1949) description and illustration are not sufficiently detailed to confirm the placement of this species with other characters. Regardless, since inclusion of this last calibration with the other two did not change divergence time estimates substantially, our conclusions are not affected by this discrepancy. A somewhat more relevant discrepancy (4-5 My) occurs in the age of the

main *Phytomyza* lineage estimated from the family-level versus genus-level data set. This is evidently an artifact of taxon sampling, but it is unclear which result should be expected to be more accurate.

Ancestral host of Phytomyza and early adaptive radiation

Our results corroborate Spencer's (1990) hypothesis of an early shift to Ranunculaceae. However, this host was probably not ancestral for the genus, but derived secondarily from association with more "advanced" asterid plants. The node representing the main lineage of *Phytomyza* (Figs. 3.2, 3.3) probably represents a shift to ranunculaceous plants, followed by adaptive radiation on this family, unless the shift occurred earlier, at the node subtending the *minuscula* group. Patterns of association between *Phytomyza* lineages and Ranunculaceae hosts are also consistent with an early adaptive radiation, in that major lineages of Ranunculaceae-feeders usually show predominant associations with specific clades of Ranunculaceae (see Hoot 1995 and Jensen et al. 1995 for phylogeny and tribal classification, respectively). Implicit in this argument is the premise (plausible but largely untested) that larger shifts (i.e. between more distantly related taxa) are more likely early in an adaptive radiation (Schluter 2000). For example, several radiations are restricted to the chemically distinctive Isopyreae (*Aquilegia/Thalictrum*; see Jensen 1995), including the *minuscula* group, *aquilegiae* group (s.s.), and one subclade of the *atomaria* group. Lineages feeding on the taxonomically isolated genera *Actaea* (Cimicifugeae), *Delphinium/Aconitum* (Delphinieae), *Caltha* (Caltheae), and *Trollius* (Adonideae) are often themselves isolated (i.e. at the base of the *albiceps* and *aquilegiae* clades). Several lineages (e.g.

albipennis/ranunculella grps., *notata* grp., *loewii* grp.) are concentrated on the abundant and widespread genera *Ranunculus* (Ranunculaceae) and *Clematis* (Anemoneae), which are in the same clade of Ranunculaceae; there seem to have been more host shifts between these two genera. The *Phytomyza* fauna of *Anemone* (closely related to *Clematis*) is somewhat more distinctive, though with a few evident connections. It will be desirable to further explore these patterns with increased sampling of Ranunculaceae-feeding lineages of *Phytomyza* (see Chapter 3).

Similar to the probable early shift from asterid to ranunculaceous plants in the *Phytomyza* group, a shift from feeding on “advanced” (angiospermous) plants to feeding on more primitive plants is also undoubtedly the case for members of the *C. scolopendri* group, which feed on ferns, as well as for “primitive” species of *Liriomyza* and *Phytoliriomyza* feeding on ferns, horsetails, or liverworts (Spencer 1990). An analogous case was noted by Sequeira et al. (2000) and Sequeira and Farrell (2001), who noted that bark beetles feeding on the ancient conifer genus *Auracaria*, although quite old, were probably derived secondarily in primarily angiosperm-feeding lineages.

Insect/host plant evolution: delayed colonization and co-diversification

We next address one of our central questions: how the timing of evolution in the *Phytomyza* group corresponds to that of its plant hosts. Ranunculaceae, which belongs to an early-branching lineage of eudicot angiosperms (APGII 2003; see Fig. 3.1), was present long before *Phytomyza*, and fossils assigned to *Thalictrum* (a common host of several modern *Phytomyza*) are known from the early Cretaceous (Friis et al. 1994). As

noted by Spencer (1990) for agromyzids, and by many others for phytophagous insects generally (Percy et al. 2004, Lopez-Vaamonde et al. 2006, Winkler and Mitter 2008; see Chapter 2), individual lineages of *Phytomyza* seem to have also originated significantly after the origin of the host families they are associated with. For example, fossil fruits similar to *Ilex* (Aquifoliaceae) are present in Cretaceous strata in Europe (Collinson et al. 1993), and the genus was present in North America at least by the Paleocene (Graham 1999). *Phytomyza* colonized *Ilex* relatively early in its evolution, but this was not earlier than the earliest Miocene (20 Ma). Fossils indicate the presence of Caprifoliaceae, including *Lonicera*, in North America and Europe from at least the middle Eocene onwards (Collinson et al. 1993, Graham 1999), but the family may have been present much earlier than this in the Cretaceous (Bell and Donoghue 2005). This family may have been colonized by *Aulagromyza* as early as the late Eocene, but the later shift to this family in the *agromyzina* clade (sister group of the holly leafminers) was also delayed until at least the early Miocene.

Most relevant to the current discussion is the age of the species-rich families of herbaceous asterids which are hosts of a majority of *Phytomyza* species. However, the fossil record of herbaceous angiosperms is notoriously incomplete, due to a lower frequency of fossilization, and molecular dating of asterid lineages infers divergence times far older than the fossil record for many clades (Bremer et al. 2004, Wikström et al. 2001). For example, fossils of the diverse asterid order Lamiales are not known from prior to the Eocene (Magallón et al. 1999), but the history of the order probably extends well into the Cretaceous (Bremer et al. 2004). The origin of the mainly herbaceous

clades of Lamiales (e.g. Orobanchaceae, Plantaginaceae, Lamiaceae) used by *Phytomyza* is somewhat obscure; at least Plantaginaceae and Lamiaceae probably originated during the latest Cretaceous or early Paleocene (Bremer et al. 2004), but were not colonized by *Phytomyza* until the most recent nine million years (*plantaginis* and *obscura* groups). The colonization of Lamiales by the *C. mimuli* group may have occurred much earlier, but certainly was no earlier than the mid-Eocene. Similarly, Apiaceae was probably colonized by the ancestor of the *angelicae* group 12-15 Ma (middle Miocene). Macrofossils of Apiaceae are not known from earlier than the Miocene, though lower Eocene pollen records are present (Collinson et al. 1993). However, the closely related family Araliaceae (host of *P. ukogi* and a few other members of the *angelicae* group) is known from the Cretaceous, and Apiaceae may predate the Cenozoic as well (Bremer et al. 2004).

The evolution of the large family Asteraceae is of special interest, both because of its dominant position in the modern temperate flora and because it is the host of many *Phytomyza* species. Until recently, the fossil record of this family was thought to be restricted to the late Oligocene onwards (Raven and Axelrod 1974, Collinson et al. 1993). Re-examination of pollen records (Graham 1996) presents the possibility that some middle Eocene pollen remains from South America represent the first record of this family; one possible late Eocene record from North America exists also. However, dating and/or identity of these Eocene records are all unconfirmed. Molecular data (Bremer et al. 2004) also suggests an Eocene origin for the family, but Kim et al. (2005) suggest that the origin of major lineages which include most north temperate

representatives did not occur until the Oligocene. Most of these lineages probably originated in warmer regions (Funk et al. 2005) and the timing of their spread to north temperate regions is unknown. Regardless, asteraceous plants were present in the north temperate region at least by the late Oligocene (23 Ma; Graham 1996), and were probably diverse before they were colonized by the ancestor of the *albiceps* group at 13 Ma. The timing of colonization of the Asteraceae by the *nigra* group cannot be precisely estimated, but could have been soon after their spread to the temperate region or much later.

Although we have established that most host plant families were probably present long before they were colonized by *Phytomyza* leaf-miners, it is possible that a period of substantial concurrent diversification could have occurred, as predicted by both the coevolutionary and biome tracking hypotheses. This possibility is especially appealing given the long-standing notion that herbaceous plant taxa may themselves have especially high rates of diversification (Niklas et al. 1985, Eriksson and Bremer 1992, Dodd et al. 1999), and that much of this diversification may have occurred during the latter half of the Miocene. This applies especially to the “advanced” asterid groups, but even the ancient family Ranunculaceae probably underwent significant diversification concurrent with cooling climates in the Neogene (Ziman and Keener 1989). From the chronogram in Fig. 4.3, at least four independent shifts to asterids can be inferred within the main lineage of *Phytomyza*. For the *agromyzina* clade, it seems likely that the ancestral host was a woody asterid plant, and this shift occurred early in the Miocene. However, for the

remaining three clades (A2, A3, A5), the shift to asterid herbs occurred in the mid-Miocene (15 Ma) or later.

Comparison of diversification rates between asterid- and Ranunculaceae-feeding clades strongly suggests that these shifts to asterid plants led to an increase in diversification rates. If speciation in insect herbivores is driven largely by shifts between related host plants, as is probably true for *Phytomyza* (Scheffer and Wiegmann 2000), one could predict that insect lineages feeding on more diverse host clades would usually generate higher diversity. To our knowledge, this intuitive hypothesis has been explicitly tested only at the broadest scale for phytophagous insects, between angiosperm vs. gymnosperm-feeding clades of the beetle clade Phytophaga (Farrell 1998). Further tests of this kind for other phytophagous insects are desirable at finer taxonomic scales to see if this reflects a general pattern. It may be, for example, that enhanced diversification is more likely on plant clades that are especially abundant or otherwise ecologically apparent (e.g. cynipid gall wasps on oaks; Ronquist and Liljeblad 2001), or those that are chemically diverse.

Evolutionary context: history of north temperate climate and flora

Assuming that the time scale for agromyzid evolution is approximately correct, how does the evolution of *Phytomyza* correlate with Cenozoic climatic and floristic history? In order to address this question, it is desirable to first summarize what is known about the climatic and floristic history of the north temperate zone. Several excellent

reviews of this topic are available (Wolfe 1978, 1985, Matthews 1979, Potts and Behrensmeyer 1992, Graham 1999, Willis and McElwain 2002)

As mentioned in the introduction, the evolution of the modern temperate flora was strongly affected by two dramatic global cooling events, both concurrent with the onset of major glaciations in Antarctica, and documented by ocean isotopic records, as well as by analysis of fossil floras (Wolfe 1978, 1992, 1994b, Prothero 1994, Graham 1999, Zachos et al. 2001). During much of the Eocene, “boreotropical” forests consisting largely of broadleaved evergreen trees covered most of North America, probably reaching the Arctic circle in coastal Alaska (Wolfe 1977, 1985). Although the climatic deterioration was possibly not as abrupt or dramatic elsewhere (Collinson 1992, Wing 1998), Wolfe (1992, 1994b) estimates that during the early Oligocene event (33 Ma), mean annual temperatures plunged 6-8° C in the Pacific Northwest (U.S.) in less than half a million years, mostly due to colder winter temperatures (greater seasonality). This climatic deterioration was associated with “catastrophic” extinctions of warm temperate/subtropical trees at high latitudes (Wolfe 1992), as well as turnover in vertebrate faunas in North America and especially Europe (Janis 1993, Prothero 1994). Following the cooling event, cool-adapted deciduous forests dominated much of North America throughout the Oligocene. The Oligocene/Miocene transition was followed by a gradual global warming and a re-expansion of warm-adapted floras, peaking at the middle Miocene (approx. 15 Ma; Wolfe 1978, 1985, 1994b, Zachos et al. 2001). Temperatures then dropped significantly in the second half of the Miocene to approximately modern levels (Fig. 4.5; Zachos et al. 2001). This second major cooling

event may have also occurred very rapidly, at 13-14 Ma (Wolfe 1994b). Just as significant for biotic evolution were regional trends towards reduced precipitation, which resulted in the spread of habitats dominated by grasses and other low-biomass vegetation (Wolfe 1985, Potts and Behrensmeyer 1992, Graham 1999, Jacobs et al. 1999). Herbs of any kind are not abundant in North American fossil assemblages before the Miocene event (Graham 1999; except for the high arctic Banks Island flora). This applies especially to the Asteraceae; Graham (1996) notes that before the Miocene asteraceous pollen is very rare, and it is not until the middle Miocene (14 Ma) that the Asteraceae become diverse and abundant in fossil assemblages. Thus, early Oligocene climatic deterioration resulted in widespread climatic conditions favorable to *Phytomyza*, whereas the Miocene climatic events resulted in an increase in the ecological abundance and diversity of herbaceous plants acceptable as *Phytomyza* hosts.

It has been generally assumed that the tropics have been the major centers of diversification for many kinds of animals and plants (Darlington 1959, Eskov 2002, Wiens and Donoghue 2004, Hawkins et al. 2006, Jablonski et al. 2006), with later adaptation of selected groups independently to temperate climates. Indeed, many temperate clades of angiosperms are clearly evolved from tropical ancestors (Latham and Ricklefs 1993, Judd et al. 1994). However, some groups may have adapted to cool climates fairly early in the Cenozoic, or even during the Mesozoic. This latter premise was the basis for the once influential concept of the “Arcto-Tertiary Geoflora” (Chaney 1947; see reviews in Wolfe 1977, Graham 1999, Wen 1999). This theory held that plant taxa characteristic of Oligocene and later broadleaf deciduous forests across much the

northern hemisphere (many of which persist in eastern North America and eastern Asia today) were derived from Paleocene or even Cretaceous deciduous polar forests which spread southwards gradually as global climate cooled in the Cenozoic and replaced the warm-adapted flora. The Geoflora theory has been roundly criticized first because it was partly based on incomplete or inaccurate paleontological data (Wolfe 1977). Instead, the polar forests of the Paleocene and early Eocene had a much different composition than later “arcto-tertiary” floras, at times nearly tropical, and taxa comprising the “arcto-tertiary flora” do not have a single history, but adapted to temperate climates at different times and different places in geological history (Wolfe 1977, Tiffney 1985, Manchester 1999, Donoghue and Smith 2004). In particular, the Rocky Mountains were also an important center of origin for many plant groups which were widespread later in the Cenozoic (Wing 1987, Wolfe 1987). Other plant genera characteristic of disjunct temperate forests today probably existed as (or evolved from) elements in the warm “boreotropical” forests of the Eocene (Wolfe 1975, 1977).

However, Wolfe (1994a) also notes that some predictions of the Arcto-tertiary concept have been borne out by modern data, in that many typical “arcto-tertiary” elements (including *Metasequoia*, *Platanus*, *Alnus*, *Betula*, *Carya*, *Castanea*, *Fagus*, *Quercus*, *Ulmus*, *Prunus*, and *Acer*) do exist in some middle Eocene inland polar floras (notably the Rex Creek flora, 45 Ma), and these may have been spread southward to become dominant temperate elements later in the Cenozoic. However, assemblages of a similar character probably existed at high altitudes but lower latitudes in western North America somewhat earlier than this (Wolfe 1987, Wing 1987). This and other

paleontological data, as well as some molecular systematic studies, demonstrate that some essentially temperate clades were probably associated with microthermal (cool temperate) climates before such climates became widespread, including *Acer* (Wolfe and Tanai 1987), *Cornus* (Xiang et al. 2005), and the order Dipsacales (Bell and Donoghue 2005). Many of these cool-adapted taxa date from prior to the Paleocene/Eocene global warming, and even from before the Cenozoic. Some temperate herbaceous lineages (in addition to the Ranunculaceae) may also have a history extending to the Eocene or earlier. However, because the fossil record for most herbaceous plant groups is incomplete, estimates of divergence times based on molecular phylogenies will be critical in documenting this antiquity. For example, a species-rich temperate clade of legumes (IRLC clade) originated in the Eocene, about 39 Ma (Lavin et al. 2005), although the origin of the most diverse herbaceous genera was not until the Miocene (<15 Ma). More robust estimates for the ages of other temperate herb radiations are also anticipated, and will help form a clearer picture of the evolution of northern hemisphere biomes.

Origin of the Phytomyza group and timing of Cenozoic temperate radiation

We inferred the origin of the primarily temperate *Phytomyza* to be during the middle Eocene, when the climate of the Northern Hemisphere was much warmer than today and cool temperate biomes were much more limited in distribution. As noted by Bell and Donoghue (2005) for the older, also largely temperate plant order Dipsacales, this implies that either (1) the ancestor of the *Phytomyza* group existed in cool regions, but had a limited diversity and distribution until cool temperate biomes expanded in the Oligocene, or (2) the ancestor of the *Phytomyza* group lived in warmer regions than most

present species, but failed to generate many surviving lineages until colonization of the expanding temperate zone. The presence of microthermal plant taxa in the middle Eocene by 45 Ma suggests that early representatives of the *Phytomyza* group could have been associated with cool temperate habits at this time. If so, it is likely that they had a limited distribution at high latitudes or altitudes. This presumed long association of the *Phytomyza* group with cool temperate climates begs the question: what factors have constrained colonization of and diversification in warmer climates? Besides possible physiological constraints, the distribution of preferred hosts also is probably important. Many of the host plant families fed upon by *Phytomyza* species are largely limited to temperate and/or montane regions; this is especially true of the Ranunculaceae (Tamura 1966, Ziman and Keener 1989), which probably was the ancestral host to the main lineage of *Phytomyza* (see above).

Alternatively, the *Phytomyza* group ancestor may have inhabited warmer habitats than most modern descendants. *Phytomyza* and related genera do include a few species found in subtropical or tropical regions. Most of these seem to be nested within groups with predominantly temperate distribution and thus more recent colonists of warm climates; this is true, for example, of several members of the *syngenesiae*, *notata*, *loewii*, *atomaria*, and *ranunculella* groups. However, the more southerly distributions of some species in the heterogeneous grade related to *Napomyza* may be relevant. This grade includes the *mimuli* and *scolopendri* groups and *Ptochomyza*, the distribution of which extends southwards into warm Mediterranean climates, and beyond into the tropics in the case of the *mimuli* group and *Ptochomyza*. These three lineages are among only a few in

the *Phytomyza* group which are not widespread across the Northern Hemisphere; the *mimuli* group is mostly distributed in the New World (with one possible Japanese representative; Sasakawa 1993), while *Ptochomyza* and the *scolopendri* group are entirely Old World in distribution. One possible interpretation of this observation is that thermal preferences of these groups were not compatible with the climate of the Bering land bridge, which was probably cool temperate in character through most of the post-Eocene Cenozoic (Wolfe 1977, 1985). This could indicate a preference for warmer climates among early members of the *Phytomyza* group. Given the above listed exceptions, what is most remarkable about the *Phytomyza* group in warm climates is that there appears to have been little or no speciation of these lineages even when species are present. This is true even for the early lineages which could have had ample time to diversify into tropical regions.

Finally, a third alternative is that the temperate-tropical dichotomy evident in modern distributions is not applicable to Eocene environments, which were markedly less seasonal than today, and exhibited a significantly weaker latitudinal temperature gradient compared to the present (Wolfe 1978, Greenwood and Wing 1995). For example, freezing winter temperatures are unlikely to have existed in a more equable Eocene climate. In this case especially, understanding the evolution of latitudinal distribution in *Phytomyza* may depend on determining specifically (a) which adaptations allow tolerance of cold temperatures and when these evolved, and (b) what ecological or other factors currently limit distributions. As an example of the first kind of evidence for a plant group, Karlson et al. (2004) showed that extreme cold tolerance in *Cornus* dogwoods was

related to clade-specific evolution of certain proteins. In general, it is not known what limits the distribution of individual agromyzid species. In one of the few empirical studies of this question for agromyzids, Klok et al. (2003) found that the distribution of *Phytomyza ilicis* was probably not limited by temperature at either extreme, but rather by the distribution of its host in the north, and possibly by the abundance of a specific parasitoid wasp in the south. Sasakawa (1953) tested the range of temperature tolerance for six co-occurring Japanese agromyzid species, and found that minimum, maximum, and optimal temperatures for these species differed. The three *Phytomyza* species tested tolerated a larger range of temperatures than did three members of the more tropically distributed subfamily Agromyzinae, suggesting that some variation may be due to clade-specific adaptive effects.

The early colonization of and high diversity in the temperate zone observed for the *Phytomyza* group of genera may be relevant to the evolution of latitudinal diversity gradients in general. It is a well-noted fact that most organisms are more diverse in tropical regions (Hillebrand 2004). Differences in speciation or extinction rates have often been postulated to explain this trend (Mittelbach et al. 2007, Weir and Schluter 2007). Wiens and Donoghue (2004; see also Farrell et al. 1992, Latham and Ricklefs 1993, Wiens and Graham 2005) instead ascribe this trend to the more recent appearance of modern temperate climates and habitats, and to a tendency for species to retain ancestral ecological characteristics (niche conservatism). Wiens and Donoghue dub their model the “tropical conservatism model”, and note that this model can explain diversity gradients without postulating differences in either speciation or extinction rates because

tropical lineages have simply had more time to generate diversity. Wiens and Donoghue (2004) also point out that their model can also account for exceptional groups with greater diversity at temperate latitudes, if these groups originated in cool climates. According to their paradigm, such groups should simply be younger on average (and thus less diverse) than groups originating in tropical latitudes.

However, a number of species rich herbivorous insect groups, including aphids (Dixon 1987), sawflies (Kouki et al. 1994), deltocephaline leafhoppers (Dietrich 1999), and trifine noctuid moths (Mitchell et al. 2006), exhibit a “reverse latitudinal gradient” with more species found in the temperate zone than in the tropics. History (i.e. place of origin) is probably an important factor in the distributions of these groups, as evidenced, for example, in the observations that some groups (e.g. aphids) are overwhelmingly distributed in the northern hemisphere and are largely absent from climatically similar south temperate regions (Heie 1994; but see von Dohlen and Teulon 2003). The *Phytomyza* group represents one further example of such a diverse north temperate clade, and many more examples probably remain obscure in the literature. Contrary to the expectations of the tropical conservatism model, many temperate genera and higher groups are not recently derived from tropical groups, and instead seem to have retained a preference for cool climates from the early Cenozoic or before (Brundin 1966, Crowson 1980, Downes and Kavanaugh 1988, Holloway and Nielsen 1998, Sanmartín et al. 2001). Like these other insect groups, diversification in the *Phytomyza* group has not followed the pattern generally expected from the tropical conservatism model. That is, its origin was at a time when the preferred habitat was not widely distributed, and diversification in

this group does not appear to be constrained by time since colonization of the temperate region. These groups represent remarkable examples of “niche conservatism” (Wiens and Graham 2005), in that they have mostly failed to diversify in tropical areas over long spans of geologic time and through dramatic changes in global climate.

Biome tracking in Phytomyza?

Finally, we ask whether major events in the evolution of *Phytomyza* and related genera are correlated with the Oligocene and Miocene global climatic deteriorations. The origin of the genus *Phytomyza* (as newly defined by us; see Chapter 3) is inferred to have occurred very close to the early Oligocene cooling event (Figs. 3.3, 3.5). The SG shift statistics imply that a major increase in diversification rate also occurred not long after this, at about 30 Ma (Fig. 4.3, marked by an asterisk) on the branch leading to the *nigra* clade and the main radiation of *Phytomyza*. However, the significance of this correlation is unclear, and an increase in diversification rate did not occur at this time. Inspection of the chronogram (Fig. 4.3) and LTT plot (Fig. 4.5) shows that few surviving lineages arose in the Oligocene, and comparison of diversification models indicates instead a significant reduction in the rate of diversification at this time. An increase in diversification rates may have occurred much later, with the divergence of major clades within the main lineage of *Phytomyza* (Figs. 4.3, 4.5). Estimates for the origin of this lineage differed between our two analyses: 28.9 Ma with the family-level data set and 24.5 Ma with the genus-level data set. The LTT plot (based on the second of these analyses) indicates a possible increase in diversification rates shortly following this (~22 Ma), and roughly corresponding to a warming period. Our taxon sampling does not

allow us to reliably estimate the shape of the LTT curve past this point, but diversification rates must have been generally high (Fig. 4.5, dashed line).

Our initial expectation, that diversification rates of *Phytomyza* would increase with the advent of global cooling and expansion of temperate forests, is thus negated by the evidence. What would cause diversification rates to fall at a time when suitable habitats have become widespread? Extinction is one possibility; an analogous case may be the extinction of many microthermal tree taxa during the Oligocene event (Wolfe 1992). In that case, it appears that many plant species were not able to track suitable climate zones during the cooling period, and Wolfe (1992) notes that diversity of cold-tolerant floras did not recover until the end of the Oligocene. Conversely, the possibility of an increase in diversification during the early Miocene, concurrent with overall global warming is difficult to explain with the biome tracking model. However, rapid diversification during warm periods has been noted for at least one other temperate animal group, plethodontid salamanders (Vieites et al. 2007), and such a phenomenon was predicted by Vrba (1985). Vrba's prediction was based on the assumption that greater climatic variability at high latitudes will lead to increased rates of speciation, as long as the extremes are mild enough to avoid extinction episodes.

There is also no clear evidence for a shift in habitat or climate preference during either of the major cooling events. Lineages across the *Phytomyza* group seem to have mostly retained a preference for cool temperate regions, herbaceous plant hosts, and mesic habitats since their origin in the Eocene. For some temperate herbivore groups

(e.g. Dietrich 1999, Mitchell et al. 2006) which are abundant in open, semi-arid grasslands, major diversification is thought to be associated with regional drying and the spread of grasslands in the mid- to late Miocene. However, *Phytomyza* species are not diverse in grasslands or more arid regions; in general, grass-feeding species in other agromyzid genera dominate in grasslands, while the few *Phytomyza* species feeding on grasses are often abundant in the forest understory (Winkler, pers. obs.). Given the preference of most *Phytomyza* species for mesic habitats, including riparian areas, moist meadows or forests, and montane regions, it is not clear how this climatic trend influenced the diversification of *Phytomyza*. As the diversity of available host plants did increase with the mid- to late Miocene climatic changes, these environmental changes may have had a largely indirect effect on the evolution of *Phytomyza*.

Conclusion

Most of the historical signatures hypothesized initially to occur in the *Phytomyza* phylogeny were not found, or were found to be more complex than initially thought. The strong association noted by Spencer (1990) with ranunculaceous plants does probably reflect an early shift to this plant family, but is not ancestral to *Phytomyza* and is predated by an association with more derived asterid plants. In most cases, secondary shifts to herbaceous asterid families occurred long after these families appeared in the north temperate region. However, a period of rapid co-diversification may have occurred for both leaf-miners and their hosts during the mid- to late Miocene. This inference is suggested by the increase in diversification rates which we document associated with asterid-feeding groups, which originated around this time. The correlation between

widespread climate change and *Phytomyza* evolution is likewise not straightforward. The temperate *Phytomyza* group of genera probably originated not long after the Eocene thermal maximum, and much before the Oligocene global cooling and expansion of temperate deciduous forests. Although the origin of *Phytomyza* itself probably closely corresponds to the early Oligocene climatic deterioration, this resulted in a decrease in the rate of diversification for *Phytomyza* during this period, despite an expansion of suitable habitats. The origin of some species groups roughly corresponds to the mid-Miocene cooling event, and this event may have precipitated radiation onto asterid herb families. However, a possible increase in overall diversification rates in *Phytomyza* cannot be confirmed at this time.

Our results provide stronger evidence for diversification driven by biotic interactions, rather than by environmental changes. However, biotic evolution cannot be really understood without considering the environmental context. Thus, we envision a complex connection between host plant evolution, climate change, and herbivore diversification. This complexity partly reflects the idiosyncratic nature of adaptation and adaptive radiation, which is often characterized by unpredictable lags between the origin of a lineage or availability of a resource or habitat, and the onset of rapid diversification (Donoghue 2005, Labandeira 2006). The role of extinction is another unknown in the equation, and we purposely referred to diversification rates generally in the foregoing report, instead of separating the components of speciation and extinction. One striking result of this study is that members of the *Phytomyza* group appear to have retained a strong association with cool temperate environments in the northern hemisphere through

over 40 million years of evolution, a period spanning dramatic shifts in climate. This kind of evolutionary stasis has been called “phylogenetic niche conservatism” (Wiens and Graham 2005), and is probably an important influence on the evolution of species and their distributions (DiMichele et al. 2004, Wiens and Donoghue 2004).

Diversification (i.e. patterns of speciation) in phytophagous insects has previously been mostly studied in small groups of closely related species, where species and even populations can be thoroughly sampled, and strong inferences about speciation made. On the other end of the evolutionary scale, broad trends in diversification have been documented for some large insect clades with host differences at the largest scale (e.g. angiosperm vs. gymnosperm hosts; Farrell 1998). However, very few studies explicitly consider diversification at an intermediate evolutionary scale in sizeable adaptive radiations, where significant ecological variation may have occurred, but lineages of somewhat homogeneous host use can still be characterized and sampled (but see McKenna and Farrell 2006, Nyman et al. 2006). Significant challenges may accompany study of such groups, including insufficient taxonomic or ecological data, and difficulty obtaining adequate taxon sampling or phylogenetic resolution. Despite these challenges, it is hoped that this study demonstrates both the utility and feasibility of studying diversification in large adaptive radiations of herbivorous insects.

As noted by Donoghue and Moore (2003), divergence time estimation is central to understanding the context of evolution and patterns of diversification. Ross (1953) characterized the current state of knowledge of the origins of the North American insect

fauna as “a host of intriguing questions,” but little data. Progress since that time has been dramatic, with an explosion of phylogenetic data for many groups, and some valuable synthesis of biogeographic patterns (Sanmartín et al. 2001). Knowledge of climatic and floristic evolution during the Cenozoic has also blossomed in the last fifty years. However, many of these “intriguing questions” remain unanswered, and not until a substantial body of reliably dated phytophagous insect and plant phylogenies are available will a complete and coherent picture of temperate plant-insect evolution emerge.

Appendix A

Species of *Phytomyza* listed by species group. Species originally described in *Chromatomyia* are transferred here to *Phytomyza*, and species described in *Phytomyza* but subsequently considered as *Chromatomyia* are also noted (*Chrom.*). The species group classification here represents a partial revision of previously recognized groups (Spencer 1990) based on available literature and species obtained for this study; it is not meant to be a comprehensive list. Therefore, many species listed as “unplaced” may belong in listed groups or in additional, unlisted groups, and some species placed in groups may only tentatively belong to these. In addition, three new names in the agromyzina group are here proposed for secondary homonyms created by the synonymy of *Chromatomyia*. Geographic regions from which each species is known are listed in brackets, and host plant family (where known) is listed following this in parentheses. Additional notes on classification, including subgroup or possible clade assignments are also listed (n.m. = male genitalia not described; ? = assignment tentative or suspect).

Phytomyza Fallén

Chromatomyia Hardy, **syn. nov.**

1. *spoliata* group

- Phytomyza bupleuri* Hering, 1963 [PA] (Apiaceae)
- Phytomyza glabra* Hendel, 1935 [PA] (Apiaceae)
- Phytomyza spoliata* Strobl, 1906 [PA] (Asteraceae)

2. *minuscula* group

- Phytomyza aquilegiovora* Spencer, 1969 [NE] (Ranunculaceae)
- Phytomyza minuscula* Goureau, 1851 [NE,PA] (Ranunculaceae)
- Phytomyza thalictrivora* Spencer, 1969 [NE] (Ranunculaceae)

3. *ciliata* group

- Phytomyza arnicivora* Sehgal, 1971 [NE] (Asteraceae)
- Phytomyza aurata* Griffiths, 1974 [NE] (Asteraceae)
- Phytomyza campestris* Griffiths, 1974 [NE] (Asteraceae)
- Phytomyza ciliata* Hendel, 1935 (*Chrom.*) [PA] (Asteraceae)
- Phytomyza crepidis* Spencer, 1981 [NE] (Asteraceae)
- Phytomyza farfarae* Hendel, 1935 [PA] (Asteraceae)
- Phytomyza hyperborea* Griffiths, 1972 [NE] (Asteraceae)
- Phytomyza hypophylla* Griffiths, 1972 [NE] (Asteraceae)
- Phytomyza integerrimi* Griffiths, 1974 [NE] (Asteraceae)
- Phytomyza lugentis* Griffiths, 1972 [NE] (Asteraceae)
- Phytomyza montereyensis* Spencer, 1981 [NE]
- Phytomyza orbitella* (Spencer, 1981), **comb. nov.** [NE]
- Phytomyza oreas* Griffiths, 1974 [NE] (Asteraceae)
- Phytomyza paraciliata* (Godfray, 1985), **comb. nov.** [PA] (Asteraceae)

4. *robustella* group

- Phytomyza achilleaececis* Süss, 1984 [PA] (Asteraceae; ? n.m.)
- Phytomyza affinalis* Frost, 1924 [NE] (?)
- Phytomyza araciocecis* Hering, 1958 [PA] (Asteraceae)
- Phytomyza buhriella* Spencer, 1969 [PA] (Asteraceae)

Phytomyza cecidonomia Hering, 1937 [PA] (Asteraceae)
Phytomyza cinerea Hendel, 1920 [PA] (Asteraceae; ?)
Phytomyza continua Hendel, 1920 [PA] (Asteraceae,
Phytomyza ferina Spencer, 1971 [PA]
Phytomyza flavens Spencer, 1986 [NE] (?)
Phytomyza flaviventris Zetterstedt, 1848 [PA] (? n.m.)
Phytomyza hasegawai Sasakawa, 1981 [PA] (Asteraceae)
Phytomyza hedingi Rydén, 1953 [PA]
Phytomyza major Malloch, 1913 [NE]
Phytomyza penicilla Hendel, 1935 [PA] (Asteraceae)
Phytomyza picridocesis Hering, 1957 [PA] (Asteraceae)
Phytomyza rhabdophora Griffiths, 1964 [PA]
Phytomyza robustella Hendel, 1936 [PA] (Asteraceae)
Phytomyza rufescens von Roser, 1840 [PA] (Asteraceae)
Phytomyza wahlgreni Rydén, 1944 [NE,PA] (Asteraceae)

5. *syngenesiae* group

Phytomyza alopecuri (Griffiths, 1980), **comb. nov.** [NE] (Poaceae; *fuscula* supersp.)
Phytomyza anonera Seguy, 1951 (*Chrom.*) [AF] (Asteraceae)
Phytomyza aragonensis Griffiths, 1967 (*Chrom.*) [PA] (Asteraceae; *syngenesiae* supersp.)
Phytomyza asteris Hendel, 1934 (*Chrom.*) [PA] (Asteraceae; *syngenesiae* supersp.)
Phytomyza autumnalis Griffiths, 1959 [PA] (Asteraceae; nr. *spinaciae*)
Phytomyza elgonensis (Spencer, 1985), **comb. nov.** [AF]
Phytomyza erigerontophaga Spencer, 1969 (*Chrom.*) [NE] (Asteraceae; *erigontophaga* supersp.)
Phytomyza farfarella Hendel, 1935 (*Chrom.*) [PA] (Asteraceae; *syngenesiae* supersp.)
Phytomyza fuscula Zetterstedt, 1838 (*Chrom.*) [NE,PA] (Poaceae; *fuscula* supersp.)
Phytomyza griffithsiana (Beiger, 1977), **comb. nov.** [PA] (Asteraceae; nr. *lactuca*)
Phytomyza hebronensis Spencer, 1969 [NE] (nr. *spinaciae*)
Phytomyza hirsuta Spencer, 1976 (*Chrom.*) [PA]
Phytomyza horticola Goureau, 1851 (*Chrom.*) [PA] (polyphagous; *syngenesiae* supersp.)
Phytomyza ixeridopsis (Griffiths, 1977), **comb. nov.** [NE] (Asteraceae; *syngenesiae* supersp.)
Phytomyza kluanensis (Griffiths, 1974), **comb. nov.** [NE] (Valerianaceae; *syngenesiae* supersp.)
Phytomyza lactuca Frost, 1924 (*Chrom.*) [NE] (Asteraceae)
Phytomyza lindbergi Spencer, 1957 (*Chrom.*) [PA] (*syngenesiae* supersp.)
Phytomyza montella (Spencer, 1986), **comb. nov.** [NE]
Phytomyza nigra Meigen, 1830 (*Chrom.*) [NE,PA] (Poaceae; *nigra* supersp.)
Phytomyza nigrissima (Spencer, 1985), **comb. nov.** [AF] (*nigra* supersp.?)
Phytomyza notopleuralis Spencer, 1969 [NE] (nr. *spinaciae*)
Phytomyza poae (Griffiths, 1980), **comb. nov.** [NE] (Poaceae; *fuscula* supersp.)
Phytomyza puccinelliae Spencer, 1969 (*Chrom.*) [NE] (Poaceae; *fuscula* supersp.)
Phytomyza senecionella Sehgal, 1971 (*Chrom.*) [NE] (Asteraceae; *syngenesiae* supersp.?)
Phytomyza seneciophila (Spencer, 1985), **comb. nov.** [AF] (Asteraceae)
Phytomyza seneciovora Spencer, 1959 (*Chrom.*) [AF] (Asteraceae)
Phytomyza spinaciae Hendel, 1928 [PA] (Asteraceae)
Phytomyza subnigra (Spencer, 1985), **comb. nov.** [AF] (*nigra* supersp.)
Phytomyza syngenesiae (Hardy, 1849), **comb. nov.** [NE,PA] (mostly Asteraceae; *syngenesiae* supersp.)
Phytomyza thermanum (Griffiths, 1976), **comb. nov.** [NE] (Asteraceae; *erigontophaga* supersp.)

6. *hendeli* group

Phytomyza albimargo Hering, 1925 [PA] (Ranunculaceae; ?)
Phytomyza brischkei Hendel, 1922 [PA] (Fabaceae; ?)
Phytomyza hendeli Hering, 1923 [PA] (Ranunculaceae)
Phytomyza linguae Lundquist, 1947 [PA]
Phytomyza multifidae Sehgal, 1971 [NE] (Ranunculaceae)
Phytomyza pulsatillae Hering, 1924 [PA] (Ranunculaceae)
Phytomyza ranunculivora Hering, 1932 [PA] (Ranunculaceae)
Phytomyza rectae Hendel, 1924 [PA] (Ranunculaceae)

Phytomyza rubicola Sasakawa , 1998 [PA] (Rubiaceae)
Phytomyza sedicola Hering, 1924 [PA] (Crassulaceae)
Phytomyza thalictrella Spencer, 1981 [NE] (Ranunculaceae)

7. loewii group

Phytomyza clemativora Coquillett, 1910 (*Chrom.*) [NE] (Ranunculaceae)
Phytomyza clematoides Spencer, 1986 (*Chrom.*) [NE] (Ranunculaceae)
Phytomyza compta Spencer, 1986, **comb. nov.** [NE] (?)
Phytomyza fulgens Hendel, 1920 [PA] (Ranunculaceae)
Phytomyza loewii Hendel, 1923 [NE,NT] (Ranunculaceae)
Phytomyza ranunculoides Spencer, 1986 [NE] (Ranunculaceae)

8. angelicae group

Phytomyza acanthopanicis Sasakawa, 1961 [PA] (Araliaceae)
Phytomyza aegopodii Hendel, 1923 [PA] (Apiaceae)
Phytomyza angelicae Kaltenbach, 1872 [NE,PA] (Apiaceae)
Phytomyza angelicivora Hering, 1924 [PA] (Apiaceae)
Phytomyza araliae Sasakawa, 1955 [PA] (Araliaceae)
Phytomyza aralivora Spencer, 1969 [NE] (Araliaceae)
Phytomyza athamantae Hering, 1943 [PA] (Apiaceae)
Phytomyza bifida Sasakawa, 1961 [PA] (Araliaceae)
Phytomyza chaerophylliana Hering, 1931 [PA] (Apiaceae; ?)
Phytomyza cicutella Spencer, 1981 [NE] (Apiaceae)
Phytomyza cicutivora Hering, 1931 [PA] (Apiaceae)
Phytomyza conjuncta Iwasaki, 1996 [PA] (Araliaceae)
Phytomyza dioni Boucher & Wheeler, 2001 [NE]
Phytomyza elsae Hendel, 1927 [PA] (Apiaceae)
Phytomyza facialis Kaltenbach, 1872 [PA] (Apiaceae)
Phytomyza heracleana Hering, 1937 [PA] (Apiaceae)
Phytomyza kalopanacis Iwasaki, 1997 [PA] (Araliaceae)
Phytomyza kibunensis Sasakawa, 1953 [PA] (Apiaceae)
Phytomyza latifolii Groschke, 1957 [PA] (Apiaceae)
Phytomyza libanotidis Hering, 1928 [PA] (Apiaceae)
Phytomyza mylini Hering, 1954 [PA] (Apiaceae)
Phytomyza pauliloewii Hendel, 1920 [PA] (Apiaceae)
Phytomyza peucedani Rydén , [PA] (Apiaceae; ?)
Phytomyza pimpinellae Hendel, 1924 [PA] (Apiaceae)
Phytomyza riparia Sehgal, 1971 [NE] (?)
Phytomyza selini Hering, 1922 [PA] (Apiaceae)
Phytomyza silai Hering, 1935 [PA] (Apiaceae)
Phytomyza suwai Iwasaki, 1996 [PA] (Araliaceae)
Phytomyza thysselinivora Hering, 1924 [PA] (Apiaceae; ?)
Phytomyza ukogi Iwasaki, 1996 [PA] (Araliaceae)
Phytomyza zarzyckii Nowakowski, 1975 [PA] (Apiaceae)

9. spondylii group

Phytomyza abiskensis Spencer, 1976 [PA]
Phytomyza adjuncta Hering, 1928 [PA] (Apiaceae, *obscorella* subgrp.)
Phytomyza angelicastris Hering, 1932 [PA] (Apiaceae)
Phytomyza archangelicae Hering, 1937 [NE,PA] (Apiaceae)
Phytomyza arnaudi Sasakawa, 1955 [PA] (Apiaceae)
Phytomyza astantiae Hendel, 1924 [PA] (Apiaceae)
Phytomyza aurei Hering, 1931 [PA] (Apiaceae)
Phytomyza biseta Hering, 1954 [PA] (Apiaceae)
Phytomyza brevituba Sasakawa , 1998 [PA] (Apiaceae)
Phytomyza brunnipes Brischke, 1881 [PA] (Apiaceae)
Phytomyza chaerophylli Kaltenbach, 1856 [PA] (Apiaceae)
Phytomyza cicutae Hendel, 1922 [PA] (Apiaceae)
Phytomyza cnidii Griffiths, 1973 [NE] (Apiaceae, *obscorella* subgrp.)

Phytomyza conii Hering, 1931 [PA] (Apiaceae)
Phytomyza coniopais Hering, 1931 [PA] (Apiaceae)
Phytomyza conioselini Griffiths, 1973 [NE] (Apiaceae, *obscurella* subgrp.)
Phytomyza ferulae Hering, 1927 [PA] (Apiaceae)
Phytomyza ferulivora Griffiths, 1956 [PA] (Apiaceae)
Phytomyza lanati Spencer, 1966 [NE] (Apiaceae)
Phytomyza melana Hendel, 1920 [PA] (Apiaceae, *obscurella* subgrp.)
Phytomyza mutellinae Beiger, 1961 [PA] (Apiaceae)
Phytomyza obscurella Fallén, 1823 [PA] (Apiaceae, *obscurella* subgrp.)
Phytomyza oenanthes Sasakawa, 1955 [PA] (Apiaceae)
Phytomyza oenanthoides Spencer, 1981 [NE] (Apiaceae)
Phytomyza osmorhizae Spencer, 1969 [NE] (Apiaceae, *obscurella* subgrp.)
Phytomyza pastinacae Hendel, 1923 [NE,PA] (Apiaceae)
Phytomyza podagrariae Hering, 1954 [PA] (Apiaceae, *obscurella* subgrp.)
Phytomyza polycladae Sasakawa, 1955 [PA] (Apiaceae, *obscurella* subgrp.)
Phytomyza saniculae Spencer, 1981 [NE] (Apiaceae)
Phytomyza sii Hering, 1930 [PA] (Apiaceae)
Phytomyza sitchensis Griffiths, 1973 [NE,PA] (Apiaceae, *obscurella* subgrp.)
Phytomyza sphondyliivora Spencer, 1957 [PA] (Apiaceae)
Phytomyza spondylii Robineau-Desvoidy, 1851 [NE,PA] (Apiaceae)
Phytomyza thysselini Hendel, 1923 [PA] (Apiaceae, *obscurella* subgrp.)
Phytomyza tlingitica Griffiths, 1973 [NE] (Apiaceae)
Phytomyza umanomitsubae Sasakawa, 1993 [PA] (Apiaceae)
Phytomyza vilnensis Pakalniškis, 1998a [PA] (Apiaceae)

10. *albiceps* group

Phytomyza achilleae Hering, 1932 [PA] (Asteraceae)
Phytomyza adenostylis Hering, 1926 [PA] (Asteraceae)
Phytomyza alaskana Griffiths, 1974 [NE] (Asteraceae)
Phytomyza albiceps Meigen, 1830 [NE,PA] (Asteraceae)
Phytomyza alpina Groschke, 1957 [NE,PA] (Asteraceae)
Phytomyza anserimontis Griffiths, 1976 [NE] (Asteraceae)
Phytomyza aposeridis Groschke, 1957 [PA] (Asteraceae)
Phytomyza arnicae Hering, 1925 [NE,PA] (Asteraceae)
Phytomyza arnicicola Lundquist, 1949 [NE,PA] (Asteraceae)
Phytomyza aronici Nowakowski, 1962 [PA] (Asteraceae)
Phytomyza artemisivora Spencer, 1971 [PA] (Asteraceae)
Phytomyza asterophaga Spencer, 1969 [NE] (Asteraceae)
Phytomyza astotinensis Griffiths, 1976 [NE] (Asteraceae)
Phytomyza bellidina Hendel, 1934 [PA] (Asteraceae)
Phytomyza bipunctata Loew, 1858 [PA] (Asteraceae)
Phytomyza burchardi Hering, 1927 [PA] (Asteraceae)
Phytomyza californica Griffiths, 1974 [NE] (Asteraceae)
Phytomyza campanulae Hendel, 1920 [PA], Campanulaceae)
Phytomyza carpasicola Sasakawa, 1955 [PA] (Asteraceae; ?)
Phytomyza ciliolati Spencer, 1969 [NE] (Asteraceae)
Phytomyza cirsii Hendel, 1923 [PA] (Asteraceae)
Phytomyza cirsiphaga Hendel, 1935 [PA] (Asteraceae)
Phytomyza columbiana Griffiths, 1977 [NE] (Asteraceae)
Phytomyza conyzae Hendel, 1920 [PA] (Asteraceae)
Phytomyza corvimontana Hering, 1930 [PA] (Asteraceae)
Phytomyza demissa Spencer, 1969 [NE] (Asteraceae)
Phytomyza despinosa Griffiths, 1976 [NE] (Asteraceae)
Phytomyza doronici Hendel, 1923 [PA] (Asteraceae)
Phytomyza erigerophila Hering, 1927 [NE,PA] (Asteraceae)
Phytomyza eupatorii Hendel, 1927 [PA] (Asteraceae)
Phytomyza helianthi Sasakawa, 1955 [PA] (Asteraceae)

Phytomyza hiemalis Griffiths, 1974 [PA] (Asteraceae)
Phytomyza homogyneae Hendel, 1927 [PA] (Asteraceae)
Phytomyza hoppi Hering, 1925 [PA] (Asteraceae)
Phytomyza japonica Sasakawa, 1953 [PA] (Asteraceae)
Phytomyza kyffhusana Hering, 1928 [PA] (Asteraceae)
Phytomyza lappae Goureau, 1851 [PA] (Asteraceae)
Phytomyza leucanthemi Hering, 1935 [PA] (Asteraceae)
Phytomyza marginella Fallén, 1823 [PA] (Asteraceae)
Phytomyza monori Groschke, 1957 [PA] (Asteraceae)
Phytomyza montana Groschke, 1957 [PA] (Asteraceae)
Phytomyza ovimontis Griffiths, 1976 [NE] (Asteraceae)
Phytomyza peregrini Griffiths, 1976 [NE] (Asteraceae)
Phytomyza phalangites Griffiths, 1976 [NE] (Asteraceae)
Phytomyza pieninica Nowakowski, 1963 [PA] (Asteraceae)
Phytomyza ptarmicae Hering, 1937 [PA] (Asteraceae)
Phytomyza pullula Zetterstedt, 1848 [NE,PA] (Asteraceae)
Phytomyza rapunculi Hendel, 1927 [PA] (Campanulaceae)
Phytomyza saxatilis Griffiths, 1974 [NE] (Asteraceae)
Phytomyza saximontana Griffiths, 1974 [NE] (Asteraceae)
Phytomyza scopulina Griffiths, 1976 [NE] (Asteraceae)
Phytomyza senecionis Kaltenbach, 1869 [PA] (Asteraceae)
Phytomyza solidaginis Hendel, 1920 [PA] (Asteraceae)
Phytomyza solidaginivora Spencer, 1969 [NE] (Asteraceae)
Phytomyza solidaginophaga Sehgal, 1971 [NE] (Asteraceae)
Phytomyza tanacetii Hendel, 1923 [PA] (Asteraceae)
Phytomyza tottoriensis Kuroda, 1960 [PA] (Asteraceae)
Phytomyza tundrensis Spencer, 1969 [NE] (Asteraceae)
Phytomyza tussilaginis Hendel, 1925 [NE,PA] (Asteraceae)
Phytomyza virgaureae Hering, 1926 [PA] (Asteraceae)

11. *petoei* group

Phytomyza glechomae Kaltenbach, 1862 [PA] (Lamiaceae)
Phytomyza nigrinervis Frost, 1924 [NE]
Phytomyza petoei Hering, 1924 [PA] (Lamiaceae)
Phytomyza salviae (Hering, 1924) [PA] (Lamiaceae)
Phytomyza scotina Hendel, 1920 [PA] (Lamiaceae)
Phytomyza thymi Hering, 1928 [PA] (Lamiaceae)

12. *rufipes* group

Phytomyza alyssi Nowakowski, 1975 [PA] (Brassicaceae; ?)
Phytomyza aulagromyzina Pakalniškis, 1994 [PA] (?)
Phytomyza coquilletti Spencer, 1986 [NE] (?)
Phytomyza flavicornis Fallén, 1823 [NE,PA] (Urticaceae)
Phytomyza genalis Melander, 1913 [NE] (?)
Phytomyza ruficeps Zlobin, 1997 [NE]
Phytomyza rufipes Meigen, 1830 [PA] (Brassicaceae)

13. *notata* group

Phytomyza anthoceridis Spencer, 1977 [AU], Solanaceae
Phytomyza aquilonia Frey, 1946 [NE,PA] (Ranunculaceae)
Phytomyza callianthemi Hering, 1944 [PA] (Ranunculaceae)
Phytomyza caulinaris Hering, 1949 [PA] (Ranunculaceae)
Phytomyza cortusifolii Spencer, 1965 [PA] (Ranunculaceae)
Phytomyza dalmatiensis (Spencer, 1961) [PA] (Ranunculaceae)
Phytomyza humilis Spencer, 1969 [NE] (Ranunculaceae)
Phytomyza infelix Spencer, 1969 [NE]
Phytomyza modocensis Spencer, 1981 [NE]
Phytomyza multifidi Spencer, 1985 [AF] (Ranunculaceae)
Phytomyza notata Meigen, 1830 [PA] (Ranunculaceae)

Phytomyza orientalis Spencer, 1962 [AU] (Ranunculaceae)
Phytomyza ranunculi (Schrank, 1803) [NE,PA] (Ranunculaceae)
Phytomyza stolonigena Hering, 1949 [PA] (Ranunculaceae)
Phytomyza varii Spencer, 1964 [AF] (Ranunculaceae)
Phytomyza vitalbae Kaltenbach, 1872 [PA], [AU] (Ranunculaceae)

14. *anemones* group

Phytomyza aldrichi Spencer, 1986 [NE] (?)
Phytomyza anemones Hering, 1925 [PA] (Ranunculaceae)
Phytomyza buhri Hering, 1930 [PA] (Ranunculaceae)
Phytomyza clematisella Spencer, 1986 [NE] (Ranunculaceae; ?)
Phytomyza fallaciosa Brischke, 1881 [PA] (Ranunculaceae)
Phytomyza flavofemoralis Sasakawa, 1955 [PA] (Ranunculaceae; ?)
Phytomyza hellebori Kaltenbach, 1872 [PA] (Ranunculaceae)
Phytomyza ignota Pakalniškis, 1994 [PA] (Ranunculaceae)
Phytomyza kaltenbachii Hendel, 1922 [PA] (Ranunculaceae)
Phytomyza palionisi Pakalniškis, 1998 [PA] (Ranunculaceae)
Phytomyza paniculatae Sasakawa, 1953 [PA] (Ranunculaceae; ?)
Phytomyza philactaeae Hering, 1932 [PA]

15. *albipennis* group

Phytomyza albipennis Fallén, 1823 [PA] (Ranunculaceae)
Phytomyza aristata Hendel, 1934 [PA] (*nigritula* subgrp.)
Phytomyza blairmorensis Sehgal, 1971 [NE] (?)
Phytomyza cineracea Hendel, 1920 [PA]
Phytomyza enigmatica Zlobin, 1994 [PA]
Phytomyza enigmoides Hering, 1937 [PA] (Ranunculaceae)
Phytomyza evanescens Hendel, 1920 [NE,PA] (Ranunculaceae)
Phytomyza marginalis Frost, 1927 [NE] (Ranunculaceae, *nigritula* subgrp.)
Phytomyza nigritula Zetterstedt, 1838 [PA] (Ranunculaceae, *nigritula* subgrp.)
Phytomyza zinovjevi Zlobin, 1994 [PA]

16. *ranunculella* group

Phytomyza cameronensis Spencer, 1982 [NT] (?)
Phytomyza clematidicolla Spencer, 1963 [AU] (Ranunculaceae)
Phytomyza clematidis Kaltenbach, 1859 [PA] (Ranunculaceae)
Phytomyza costata Harrison, 1959 [AU] (Ranunculaceae)
Phytomyza drakensbergensis (Spencer, 1963) [AF]
Phytomyza enigma Malloch, 1934 [NT] (?)
Phytomyza eximia Spencer, 1964 [AF]
Phytomyza improvisa Spencer, 1976 [AU] (Ranunculaceae)
Phytomyza lyallii Spencer, 1976 [AU] (Ranunculaceae)
Phytomyza meridialis Spencer, 1982 [NT] (?)
Phytomyza munroi Spencer, 1960 [AF]
Phytomyza placita Spencer, 1977 [AU]
Phytomyza pulchella Spencer, 1977 [AU]
Phytomyza ranunculella Spencer, 1974 [PA] (Ranunculaceae)
Phytomyza ranunculicaulis Spencer, 1977 [AU] (Ranunculaceae)
Phytomyza renovata Spencer, 1960 [AF] (Ranunculaceae)
Phytomyza strana Spencer, 1960 [AF]
Phytomyza subeximia Spencer, 1985 [AF] (Ranunculaceae)

17. *atomaria* group

Phytomyza affinis Fallén, 1823 [NE,PA] (Orobanchaceae)
Phytomyza aquilegiophaga Spencer, 1969 [NE] (Ranunculaceae)
Phytomyza atomaria Zetterstedt, 1848 [PA]
Phytomyza banffensis Spencer, 1969 [NE]
Phytomyza brevifacies Hendel, 1934 [PA] (Plantaginaceae)
Phytomyza bulbiseta Zlobin, 1997 [NE]
Phytomyza carbonensis Spencer, 1981 [NE]

Phytomyza chelonei Spencer, 1969 [NE] (Plantaginaceae)
Phytomyza clematadi Watt, 1923 [AU] (Ranunculaceae; ?)
Phytomyza crassiseta Zetterstedt, 1860 [NE,PA] (Plantaginaceae)
Phytomyza dasyops Hendel, 1920 [PA]
Phytomyza digitalis Hering, 1925 [PA] (Plantaginaceae)
Phytomyza diversicornis Hendel, 1927 [PA] (Orobanchaceae)
Phytomyza dreisbachi Steyskal, 1972 [NE] (?)
Phytomyza eumorpha Frey, 1946 [PA]
Phytomyza euphrasiae Kaltenbach, 1860 [PA] (?)
Phytomyza flavofemorata Strobl, 1893 [PA] (Orobanchaceae)
Phytomyza franzi Hering, 1944 [PA] (?)
Phytomyza gelida Spencer, 1969 [NE]
Phytomyza globulariae Hendel, 1935 [PA] (Plantaginaceae)
Phytomyza griffithsi Spencer, 1963 [PA] (Plantaginaceae)
Phytomyza hirta Rydén, 1957 [PA]
Phytomyza isais Hering, 1937 [PA] (Orobanchaceae)
Phytomyza jasperensis Sehgal, 1971 [NE]
Phytomyza jonaitisi Pakalniškis, 1996 [PA] (Ranunculaceae)
Phytomyza krygeri Hering, 1949 [PA] (Ranunculaceae)
Phytomyza kurilensis Iwasaki, 2000 [PA]
Phytomyza lupini Sehgal, 1968 [NE] (Fabaceae)
Phytomyza majalis Zlobin, 1994 [PA]
Phytomyza melampyri Hering, 1934 [PA] (Orobanchaceae)
Phytomyza misella Spencer, 1969 [NE]
Phytomyza nigella Zlobin, 1997 [NE]
Phytomyza nigrifemur Hering, 1934 [PA]
Phytomyza nigroorbitalis Rydén, 1956 [PA] (Orobanchaceae)
Phytomyza oblita Spencer, 1969 [NE]
Phytomyza orindensis Spencer, 1981 [NE]
Phytomyza orlandensis Spencer, 1973 [NE]
Phytomyza orobanchia Kaltenbach, 1864 [PA] (Orobanchaceae)
Phytomyza pedicularicaulis Spencer, 1969 [NE] (Orobanchaceae)
Phytomyza pedicularidis Spencer, 1969 [NE] (Orobanchaceae)
Phytomyza pedicularifolii Hering, 1960 [PA] (Orobanchaceae)
Phytomyza penstemonella Spencer, 1981 [NE] (Plantaginaceae; ? n.m.)
Phytomyza penstemonis Spencer, 1969 [NE] (Plantaginaceae)
Phytomyza plantaginis Robineau-Desvoidy, 1851 [PA,NE,AU] (Plantaginaceae)
Phytomyza ringdahli Rydén, 1937 [PA]
Phytomyza rostrata Hering, 1934 [PA] (Orobanchaceae)
Phytomyza schlicki Spencer, 1976 [PA]
Phytomyza subalpina Sehgal, 1971 [NE] (?)
Phytomyza subtenella Frost, 1924 [NE] (Orobanchaceae)
Phytomyza superba Spencer, 1969 [NE]
Phytomyza tenella Meigen, 1830 [NE,PA] (Orobanchaceae)
Phytomyza tenuis Spencer, 1969 [NE]
Phytomyza thalictri Escher-Kündig, 1912 [PA] (Ranunculaceae)
Phytomyza trivittata Frost, 1924 [NE] (Orobanchaceae)
Phytomyza varipes Macquart, 1835 [NE,PA] (Orobanchaceae)
Phytomyza veronicicola Hering, 1925 [PA] (Plantaginaceae)

18. *ilicis* group

Phytomyza ditmani Kulp, 1968 [NE] (Aquifoliaceae)
Phytomyza glabricola Kulp, 1968 [NE] (Aquifoliaceae)
Phytomyza ilicicola Loew, 1872 [NE] (Aquifoliaceae)
Phytomyza ilicis Curtis, 1846 [NE,PA] (Aquifoliaceae)
Phytomyza jucunda Frost & Sasakawa, 1954 [PA] (Aquifoliaceae)
Phytomyza kisakai Sasakawa, 1954 [PA] (Styracaceae)

Phytomyza nemopanthe Griffiths & Piercey-Normore, 1995 [NE] (Aquifoliaceae)

Phytomyza opacae Kulp, 1968 [NE] (Aquifoliaceae)

Phytomyza verticillatae Kulp, 1968 [NE] (Aquifoliaceae)

Phytomyza vomitoriae Kulp, 1968 [NE] (Aquifoliaceae)

19. *agromyzina* group

Phytomyza abeliae Sasakawa, 1961 [PA] (Caprifoliaceae)

Phytomyza actinidiae (Sasakawa, 1998), **comb. nov.** [PA] (Actinidiaceae)

Phytomyza agromyzina Meigen, 1830 [NE,PA] (Cornaceae)

Phytomyza aizoon Hering, 1932 (*Chrom.*) [PA] (Saxifragaceae)

Phytomyza alpigenae Groschke, 1957 (*Chrom.*) [PA] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza aprilina Goureau, 1851 (*Chrom.*) [PA] (Caprifoliaceae)

Phytomyza arctagrostidis (Griffiths, 1980), **comb. nov.** [NE] (Poaceae; *milii* supersp.)

Phytomyza beigeriae (Griffiths, 1980), **comb. nov.** [PA] (Juncaceae; *luzulae* supersp.)

Phytomyza blackstoniae (Spencer, 1990), **comb. nov.** [PA] (Gentianaceae)

Phytomyza californiensis Winkler, 2008, **nom. nov.** [NE] (new name for *C. montana* Spencer, 1981)

Phytomyza caprifoliae Spencer, 1969 (*Chrom.*) [NE] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza ceanothi Spencer, 1986 [NE] (Rhamnaceae)

Phytomyza centaurii (Spencer, 1990), **comb. nov.** [PA] (Gentianaceae)

Phytomyza chamaemetabola (Griffiths, 1974), **comb. nov.** [NE] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza cinnae (Griffiths, 1980), **comb. nov.** [NE] (Poaceae; *milii* supersp.)

Phytomyza crawfurdiae Sasakawa, 1954 (*Chrom.*) [PA] (Gentianaceae)

Phytomyza cygnicollina (Griffiths, 1980), **comb. nov.** [NE] (Juncaceae; *luzulae* supersp.)

Phytomyza deirdreae Griffiths, 1972 (*Chrom.*) [NE,PA] (Saxifragaceae)

Phytomyza doolittlei (Spencer, 1986), **comb. nov.** [NE]

Phytomyza flavida (Spencer, 1986), **comb. nov.** [NE]

Phytomyza fricki (Griffiths, 1974), **comb. nov.** [NE] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza furcata (Griffiths, 1980), **comb. nov.** [PA] (*milii* supersp.)

Phytomyza gentianae Hendel, 1920 (*Chrom.*) [PA] (Gentianaceae)

Phytomyza gentianella Hendel, 1932 (*Chrom.*) [PA] (Gentianaceae)

Phytomyza gentii Hendel, 1920 (*Chrom.*) [PA] (Gentianaceae)

Phytomyza glacialis Griffiths, 1964 (*Chrom.*) [PA] (*opacella* supersp.)

Phytomyza gregaria Frick, 1954 (*Chrom.*) [NE] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza griffithsella Winkler, 2008, **nom. nov.**, [NE] (new name for *C. griffithsi* Spencer, 1986)

Phytomyza hoppiella (Spencer, 1990), **comb. nov.** [PA] (Gentianaceae)

Phytomyza involucratae Spencer, 1969 (*Chrom.*) [NE] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza isicae Hering, 1962 (*Chrom.*) [PA] (*milii* supersp.)

Phytomyza leptargyreae (Griffiths, 1976), **comb. nov.** [NE] (Elaeagnaceae; *merula* supersp.)

Phytomyza linnaeae (Griffiths, 1974), **comb. nov.** [NE] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza loniceriae Robineau-Desvoidy, 1851 (*Chrom.*) [NE,PA] (Caprifoliaceae)

Phytomyza luzulae Hering, 1924 (*Chrom.*) [PA] (Juncaceae; *luzulae* supersp.)

Phytomyza luzulivora (Spencer, 1986), **comb. nov.** [NE] (*luzulae* supersp.)

Phytomyza merula Spencer, 1969 (*Chrom.*) [NE] (Elaeagnaceae; *merula* supersp.)

Phytomyza milii Kaltenbach, 1864 (*Chrom.*) [PA] (Poaceae; *milii* supersp.)

Phytomyza mitchelli (Spencer, 1986), **comb. nov.** [NE] (*luzulae* supersp.)

Phytomyza mitellae Griffiths, 1972 (*Chrom.*) [NE] (Saxifragaceae)

Phytomyza nervi Groschke, 1957 (*Chrom.*) [PA] (Caprifoliaceae)

Phytomyza nigrilineata (Griffiths, 1974), **comb. nov.** [NE] (Caprifoliaceae; *periclymeni* supersp.)

Phytomyza norwegica Rydén, 1957 (*Chrom.*) [NE,PA] (Poaceae; *milii* supersp.)

Phytomyza opacella Hendel, 1935 (*Chrom.*) [NE,PA] (Poaceae; *opacella* supersp.)

Phytomyza periclymeni de Meijere, 1924 (*Chrom.*) [PA] (Caprifoliaceae; *periclymeni* grp.)

Phytomyza primulae Robineau-Desvoidy, 1851 (*Chrom.*) [PA] (Primulaceae)

Phytomyza pseudogentii Beiger, 1972 (*Chrom.*) [PA] (Gentianaceae)

Phytomyza pseudomilii (Griffiths, 1980), **comb. nov.** [NE,PA] (Poaceae; *milii* supersp.)

Phytomyza qinghaiensis (Gu, 1991), **comb. nov.** [OR]

Phytomyza ramosa Hendel, 1923 (*Chrom.*) [PA] (Dipsacaceae)

Phytomyza regalensis Steyskal, 1972 (*Chrom.*) [NE] (*periclymeni* supersp.)

Phytomyza rhaetica (Griffiths, 1980), **comb. nov.** [PA] (*luzulae* supersp.)
Phytomyza saxifragae Hering, 1924 (*Chrom.*) [PA] (Saxifragaceae)
Phytomyza scabiosae Hendel, 1935 (*Chrom.*) [PA] (Dipsacaceae)
Phytomyza scabiosarum de Meijere, 1934 (*Chrom.*) [PA] (Dipsacaceae)
Phytomyza scabiosella (Beiger, 2001), **comb. nov.** [PA] (Dipsacaceae)
Phytomyza shepherdiana (Griffiths, 1976), **comb. nov.** [NE] (Elaeagnaceae; *merula* supersp.)
Phytomyza skuratowiczi Beiger, 1972 (*Chrom.*) [PA] (Gentianaceae)
Phytomyza soldanellae Starý, 1950 (*Chrom.*) [PA] (Primulaceae)
Phytomyza spenceriana (Griffiths, 1980), **comb. nov.** [PA] (*luzulae* supersp.)
Phytomyza styriaca (Griffiths, 1980), **comb. nov.** [PA] (*milii* supersp.)
Phytomyza succisae Hering, 1922 (*Chrom.*) [PA] (Dipsacaceae)
Phytomyza suikazurae (Sasakawa, 1993), **comb. nov.** [PA] (Caprifoliaceae)
Phytomyza swertiae Hering, 1937 (*Chrom.*) [PA] (Gentianaceae)
Phytomyza symphoricarpi (Griffiths, 1974), **comb. nov.** [NE] (Caprifoliaceae; *periclymeni* supersp.)
Phytomyza tiarellae Griffiths, 1972 (*Chrom.*) [NE] (Saxifragaceae)
Phytomyza torrentium (Griffiths, 1980), **comb. nov.** [NE] (Poaceae; *milii* supersp.)
Phytomyza tschirnhausi (Griffiths, 1980), **comb. nov.** [PA] (*luzulae* supersp.)
Phytomyza vernalis Groschke, 1957 (*Chrom.*) [PA] (Gentianaceae)
Phytomyza vockerothi Winkler, 2008, **nom. nov.**, [NE] (new name for *C. nigrella* Spencer, 1986)

20. *opaca* group

Phytomyza calthivora Hendel, 1934 [PA] (Ranunculaceae)
Phytomyza calthophila Hering, 1931 [PA] (Ranunculaceae)
Phytomyza nigripennis Fallén, 1823 [NE,PA] (Ranunculaceae; ?)
Phytomyza opaca Hendel, 1920 [PA]
Phytomyza pummankiensis Spencer, 1976 [PA]
Phytomyza soenderupi Hering, 1941 [PA] (Ranunculaceae)
Phytomyza subrostrata Frey, 1946 [PA] (Ranunculaceae)?
Phytomyza trolliicaulis Süss, 1989 [PA] (Ranunculaceae)

21. *obscura* group

Phytomyza beringiana Griffiths, 1975 [NE] (Boraginaceae; *symphyti* subgrp.)
Phytomyza kugleri Spencer, 1974 [PA] (?)
Phytomyza lithospermi Nowakowski, 1959 [PA] (Boraginaceae; *symphyti* subgrp.)
Phytomyza lycopi Nowakowski, 1959 [PA] (Lamiaceae; *nepetae* subgrp.)
Phytomyza malaca Spencer, 1981 [NE] (?)
Phytomyza mertensiae Sehgal, 1971 [NE] (Boraginaceae; *symphyti* subgrp.)
Phytomyza myosotica Nowakowski, 1959 [PA] (Boraginaceae; *symphyti* subgrp.)
Phytomyza nepetae Hendel, 1922 [NE,PA] (Lamiaceae; *nepetae* subgrp.)
Phytomyza nowakowskiana Beiger, 1975 [PA] (Boraginaceae; *symphyti* subgrp.)
Phytomyza obscura Hendel, 1920 [PA] (Lamiaceae; *obscura* subgrp.)
Phytomyza origani Hering, 1931 [PA] (Lamiaceae; *obscura* subgrp.)
Phytomyza ovalis Griffiths, 1975 [NE] (Boraginaceae; *symphyti* subgrp.)
Phytomyza petiolaris Griffiths, 1975 [NE] (Boraginaceae; *symphyti* subgrp.)
Phytomyza phaceliae Spencer, 1981 [NE] (Boraginaceae; *symphyti* subgrp.)
Phytomyza pulmonariae Nowakowski, 1959 [PA] (Boraginaceae; *symphyti* subgrp.)
Phytomyza rhodopaea Beiger, 1979 [PA] (Boraginaceae; *symphyti* subgrp.)
Phytomyza symphyti Hendel, 1935 [PA] (Boraginaceae; *symphyti* subgrp.)
Phytomyza tetrasticha Hendel, 1927 [PA] (Lamiaceae; *obscura* subgrp.)

22. *aquilegiae* group

Phytomyza aquilegiae Hardy, 1849 [PA] (Ranunculaceae)
Phytomyza aquilegiana Frost, 1930 [NE] (Ranunculaceae)
Phytomyza aquilegioides Sehgal, 1971 [NE] (Ranunculaceae)
Phytomyza camuna Süss & Moreschi, 2005 [PA] (Ranunculaceae)
Phytomyza columbinae Sehgal, 1971 [NE] (Ranunculaceae)
Phytomyza platonoffi Spencer, 1976 [PA]
Phytomyza plumiseta Frost, 1924 [NE] (Ranunculaceae)
Phytomyza sonorensis Spencer, 1981 [NE] (Ranunculaceae)

Phytomyza thalictricola Hendel, 1925 [PA] (Ranunculaceae)

23. buhriana group

Phytomyza buhriana Hering, 1949 [PA] (Ranunculaceae)

Phytomyza malaisei Zlobin, 2002 [OR]

Phytomyza yasumatsui (Sasakawa, 1955) [PA] (Ranunculaceae)

24. knowltoniae group

Phytomyza clematissi Spencer, 1964 [AF] (Ranunculaceae)

Phytomyza knowltoniae Hering, 1957 [AF] (Ranunculaceae)

Phytomyza natalensis Spencer, 1964 [AF] (Ranunculaceae)

Phytomyza philoclematidis Hering, 1957 [AF] (Ranunculaceae; ?)

Phytomyza ranunculina Spencer, 1963 [AF] (Ranunculaceae)

Phytomyza vitalbella Hering, 1957 [AF] (Ranunculaceae; ?)

unplaced species in *Phytomyza*

Phytomyza abdita Hering, 1927 [PA] (Lamiaceae; isolated)

Phytomyza abdominalis Zetterstedt, 1848 [PA] (Ranunculaceae; *aquilegiae* clade)

Phytomyza aconitella Hendel, 1934 [PA] (Ranunculaceae; possibly *aquilegiae* clade)

Phytomyza aconitii Hendel, 1920 [NE,PA] (Ranunculaceae; possibly *aquilegiae* clade)

Phytomyza aconitophila Hendel, 1927 [PA] (Ranunculaceae; *aquilegiae* clade)

Phytomyza actaeae Hendel, 1922 [PA] (Ranunculaceae; *aquilegiae* clade)

Phytomyza africana Spencer, 1959 [AF] (n.m.)

Phytomyza akebiae (Sasakawa, 1954) [PA] (Lardizabalaceae; n.m., possibly *agromyzina* grp.)

Phytomyza alamedensis Spencer, 1981 [NE] (?)

Phytomyza albifrons Groschke, 1957 [PA] (Ranunculaceae; possibly *aquilegiae* clade)

Phytomyza alpestris Hendel, 1920 [PA]

Phytomyza alpigenae Hendel, 1925 [PA]

Phytomyza alysicarpi Singh & Ipe, 1968 (*Chrom.*) [OR] (*syngenesiae* or *agromyzina* grp.)

Phytomyza anderi (Rydén, 1952) [PA] (*aquilegiae* clade)

Phytomyza anemonantheae Spencer, 1969 [PA] (Ranunculaceae; *aquilegiae* clade)

Phytomyza anemonivora Spencer, 1969 [NE] (Ranunculaceae; *albipennis* clade?)

Phytomyza antennata Spencer, 1960 [PA]

Phytomyza aphyllae Beiger, 1964 [PA] (Plantaginaceae; isolated)

Phytomyza atripalpis Aldrich, 1929 [NE] (Ranunculaceae; n.m.)

Phytomyza auricornis Frost, 1927 [NE] (*aquilegiae* clade)

Phytomyza bicolor Coquillett, 1902 [NE] (nr. *rufipes* grp.)

Phytomyza boulderella Spencer, 1986 [NE] (?)

Phytomyza brevicornis Hendel, 1934 [PA]

Phytomyza burmensis Zlobin, 2002 [OR] (n.m.)

Phytomyza caffra Macquart, 1846 [AF]

Phytomyza calthae Hering, 1924 [PA] (Ranunculaceae; possibly *aquilegiae* clade)

Phytomyza canadensis Spencer, 1969 [NE] (n.m.)

Phytomyza catalaunica Spencer, 1960 [PA]

Phytomyza ceylonensis Spencer, 1975? [OR] (n.m.)

Phytomyza chrysocera Hendel, 1935 [PA]

Phytomyza cirrhosae Spencer, 1969 [PA] (Ranunculaceae; nr. *hendeli* grp.?)

Phytomyza clematidella Spencer, 1959 [AF] (Ranunculaceae; n.m.)

Phytomyza clematidicaulis Hering, 1958 [PA] (Ranunculaceae; possibly *aquilegiae* clade)

Phytomyza clematidophoeta Spencer, 1969 [NE] (Ranunculaceae; *aquilegiae* clade)

Phytomyza clematiphaga Spencer, 1969 [NE] (Ranunculaceae; possibly *aquilegiae* clade)

Phytomyza clematisana Spencer, 1981 [NE] (Ranunculaceae; possibly *aquilegiae* clade)

Phytomyza coloradella Spencer, 1986 [NE] (?)

Phytomyza conglomerata Boucher & Wheeler, 2001 [NE] (?)

Phytomyza cornuta Hendel, 1935 [PA]

Phytomyza cytisi Brischke, 1881 [PA] (Fabaceae; isolated)

Phytomyza davisii (Walton, 1912) [NE] (Ranunculaceae; *aquilegiae* clade)

Phytomyza deflecta Hendel, 1920 [PA]

Phytomyza delphinivora Spencer, 1969 [NE] (Ranunculaceae; isolated)
Phytomyza deutziae Sasakawa, 1957 [PA] (Hydrangeaceae; n.m., *agromyzina* grp.?)
Phytomyza disjuncta Sasakawa, 1961 [PA]
Phytomyza disjunctivena Gu, 1991 [OR] (*albiceps/spondylii* grps.)
Phytomyza dryas Hering, 1937 [PA]
Phytomyza duplex Spencer, 1986 [NE] (*aquilegiae* clade)
Phytomyza edmontonensis Sehgal, 1971 [NE] (?)
Phytomyza epistomella Hendel, 1935 [PA]
Phytomyza esakii Sasakawa, 1955 [PA] (Ranunculaceae, possibly *aquilegiae* clade)
Phytomyza evansi Spencer, 1986 [NE] (*albiceps/spondylii* grps.)
Phytomyza exilis Hering, 1937 [PA]
Phytomyza felix Spencer, 1981 [NE] (isolated)
Phytomyza fennoscandiae Spencer, 1976 [PA] (nr. *murina*; *atomaria* grp.?)
Phytomyza ferruginea Hendel, 1935 [PA]
Phytomyza fimbriata Sasakawa, 1955 [PA] (Balsaminaceae; n.m., *Phytoliriomyza*?)
Phytomyza flaviantennalis Spencer, 1981 [NE] (?)
Phytomyza flavifacies Hendel, 1935 [PA]
Phytomyza flavinervis Frost, 1924 [NE] (?)
Phytomyza flexuosa Spencer, 1986 [NE] (*aquilegiae* clade)
Phytomyza formosae Spencer, 1966 [OR]
Phytomyza gilva Spencer, 1971 [PA] (?)
Phytomyza grisescens Hendel, 1920 [PA] (isolated)
Phytomyza hedickei Hering, 1927 [PA]
Phytomyza heringiana Hendel, 1922 [PA] (Rosaceae; *agromyzina* grp.?)
Phytomyza heterophyllii Bland, 1997 [PA] (Asteraceae; isolated)
Phytomyza himachali Singh & Garg, 1970 [OR]
Phytomyza hyaloposthia Sasakawa, 1986 [PA]
Phytomyza hydrangeae Sasakawa, 1956 [PA] (Hydrangeaceae; *agromyzina* grp.?)
Phytomyza jugalis Hendel, 1935 [PA]
Phytomyza kamtschatkensis Hendel, 1935 [PA]
Phytomyza kareliensis Spencer, 1976 [PA] (?)
Phytomyza kasi Henshaw, 1989 [NE] (*albipennis* clade)
Phytomyza klondikensis Boucher & Wheeler, 2001 [NE] (*aquilegiae* clade)
Phytomyza kumaonensis Singh & Ipe, 1968 [OR] (Ranunculaceae)
Phytomyza lappivora Hendel, 1927 [PA] (Asteraceae; nr. *hendeli* grp.?)
Phytomyza latifrons Hendel, 1935 [PA]
Phytomyza ligusticifoliae Spencer, 1981 [NE] (Ranunculaceae; n.m.)
Phytomyza lupinivora Sehgal, 1968 [NE] (Fabaceae; n.m.)
Phytomyza lusatica Hering, 1955 [PA]
Phytomyza manni Spencer, 1986 [NE] (*albipennis* clade)
Phytomyza masoni Spencer, 1986 [NE] (*aquilegiae* clade)
Phytomyza melanella Frost, 1924 [NE] (*albiceps/spondylii* grps.)
Phytomyza melanogaster Thomson, 1869 [NT] (?)
Phytomyza melanosoma Hendel, 1920 [PA]
Phytomyza meridionalis Spencer, 1972 [PA]
Phytomyza minutissima Spencer, 1981 [NE] (isolated)
Phytomyza miranda Spencer, 1969 [NE] (isolated)
Phytomyza modica Spencer, 1969 [NE] (*aquilegiae* clade)
Phytomyza murina Hendel, 1935 [PA] (Ranunculaceae; *atomaria* grp.?)
Phytomyza nagvakensis Spencer, 1969 [NE] (*aquilegiae* clade)
Phytomyza narcissiflorae Hering, 1928 [PA] (Ranunculaceae; n.m.)
Phytomyza nepalensis Spencer, 1965 [OR]
Phytomyza nervosa Loew, 1869 [NE] (?)
Phytomyza nigrella Hendel, 1935 [PA]
Phytomyza nigricoxa Hendel, 1935 [PA] (Ranunculaceae; *albipennis* clade?)
Phytomyza nigrita Spencer, 1960 [PA]

Phytomyza nigrifolia Zetterstedt, 1848 [PA] (?)
Phytomyza nigrociliata Sasakawa, 1961 [PA,OR]
Phytomyza nigroclypea Hendel, 1935 [PA]
Phytomyza nilgiriensis Ipe, 1971 [OR] (Asteraceae)
Phytomyza nishijimai Sasakawa, 1955 [PA] (Cornaceae; possibly *aquilegiae* clade)
Phytomyza novitzkyi Hering, 1958 [PA] (Ranunculaceae; *aquilegiae* clade)
Phytomyza obscurata Hendel, 1920 [PA]
Phytomyza obscuriceps Hendel, 1935 (*Chrom.*) [PA] (*syngenesiae* or *agromyzina* grp.)
Phytomyza ochracea Hendel, 1920 (*Chrom.*) [PA] (*syngenesiae* or *agromyzina* grp.)
Phytomyza oenanthica Hering, 1949 [PA] (Apiaceae; n.m.)
Phytomyza oreophila (Franz, 1947) [PA]
Phytomyza oxytropidis Sehgal, 1971 [NE] (Fabaceae)
Phytomyza pallipes Spencer, 1969 [NE] (n.m.)
Phytomyza palpata (Hendel, 1920) [PA]
Phytomyza pampeana Blanchard, 1954 [NT] (Ranunculaceae; isolated)
Phytomyza parvicella (Coquillett, 1902) [NE,PA] (Papaveraceae; isolated)
Phytomyza perangusta Sasakawa, 1972 (*Chrom.*) [OR] (*syngenesiae* or *agromyzina* grp.)
Phytomyza permutata Hering, 1962 [PA]
Phytomyza persicae Frick, 1954 [NE] (Rosaceae; *agromyzina* grp.?)
Phytomyza phellandrii Hering, 1957 [PA] (Apiaceae; n.m.)
Phytomyza phillyreae Hering, 1930 [PA] (Oleaceae; *Aulagromyza*?)
Phytomyza pilescens Singh & Ipe, 1973 [OR]
Phytomyza platystoma (Hendel, 1920) [PA]
Phytomyza polysticha Hendel, 1935 [PA] (n.m.)
Phytomyza poppii Rydén, 1951 [PA] (n.m.)
Phytomyza prava Spencer, 1969 [NE] (*aquilegiae* clade)
Phytomyza pubicornis Hendel, 1920 [PA] (Apiaceae; isolated)
Phytomyza pulchelloides Henshaw, 1989 [NE] (possibly *aquilegiae* clade)
Phytomyza pulchra Hendel, 1920 [PA]
Phytomyza pulsatillicola Hering, 1962 [PA] (Ranunculaceae; *aquilegiae* clade)
Phytomyza pusilla (Forster, 1891) [PA]
Phytomyza quadriseta Sasakawa, 1972 [OR] (*albiceps/spondylii* grps.)
Phytomyza queribunda Spencer, 1969 [NE] (*aquilegiae* clade)
Phytomyza ranunculicola Hering, 1949 [PA] (Ranunculaceae; *aquilegiae* clade)
Phytomyza ranunculiphila Zlobin, 1993 [PA] (*aquilegiae* clade)
Phytomyza rhodiolae Griffiths, 1976 [NE,PA] (Crassulaceae; possibly *aquilegiae* clade)
Phytomyza rydeni Hering, 1934 [PA] (Ranunculaceae; *aquilegiae* clade)
Phytomyza rydeniella Spencer, 1976 [PA] (n.m.)
Phytomyza saskatoonensis Spencer, 1969 [NE] (*aquilegiae* clade)
Phytomyza scaligerae Hering, 1967 [PA]
Phytomyza schuetzei Hering, 1955 [PA]
Phytomyza sedi Kaltenbach, 1869 [PA] (Crassulaceae; possibly *aquilegiae* clade)
Phytomyza sehgalii Spencer, 1969 [NE] (*spondylii* grp.?)
Phytomyza seseleos Hering, 1957 [PA] (Apiaceae; n.m.)
Phytomyza sibirica Hendel, 1935 [PA]
Phytomyza smyrnii Spencer, 1954 [PA] (Apiaceae; n.m.)
Phytomyza socia Brischke, 1881 [PA] (Ranunculaceae; *aquilegiae* clade)
Phytomyza soenderupiella Spencer, 1976 [PA] (Ranunculaceae; *albipennis* clade?)
Phytomyza sorosi Zlobin, 1994 [PA]
Phytomyza splendida Spencer, 1981 [NE] (?)
Phytomyza subaquilegiana Zlobin, 1997 [NE] (Fabaceae; *aquilegiae* clade)
Phytomyza subtilis Spencer, 1969 [NE] (Fabaceae; *aquilegiae* clade)
Phytomyza takasagoensis Sasakawa, 1972 [OR]
Phytomyza takhiniensis Boucher & Wheeler, 2001 [NE] (*aquilegiae* clade)
Phytomyza tamui Sasakawa, 1957 [PA] (Ranunculaceae; possibly *aquilegiae* clade)
Phytomyza timida Spencer, 1969 [NE] (*aquilegiae* clade)

Phytomyza tomentella Sasakawa, 1972 [OR]
Phytomyza trichopsis Hendel, 1935 [PA]
Phytomyza trollii Hering, 1930 [PA] (Ranunculaceae; isolated)
Phytomyza trolliophila Hering, 1949 [PA] (Ranunculaceae; possibly *aquilegiae* clade)
Phytomyza trolliovara Hering, 1935 [PA] (Ranunculaceae; *aquilegiae* clade?)
Phytomyza tropica Spencer, 1961 [OR]
Phytomyza tucumana Blanchard, 1954 [NT]
Phytomyza uncinata Sasakawa, 1986 [PA]
Phytomyza urbana Spencer, 1969 [NE] (Fabaceae; *aquilegiae* clade)
Phytomyza valida Sasakawa, 1972 [OR]
Phytomyza venerabilis Spencer, 1977 [AU]
Phytomyza veratri Hering, 1941 [PA]
Phytomyza virosae Pakalniskis, 2000 [PA] (Apiaceae)
Phytomyza williamsoni Blanchard, 1938 [NT] (Ranunculaceae; isolated)
Phytomyza xiphochaeta Hendel, 1935 [PA]
Phytomyza xiphochaetoides Zlobin, 1999 [PA]

excluded from *Phytomyza*

Phytomyza castillejae Spencer, 1973 (*Chrom.*) [NE,NT] (Orobanchaceae; *mimuli* grp.)
Chromatomyia eriodictyi Spencer, 1981 [NE] (Boraginaceae; *mimuli* grp.)
Chromatomyia mimuli Spencer, 1981 [NE] (Boraginaceae, Phrymaceae, Lamiaceae; *mimuli* grp.)
Chromatomyia omphalivora Sasakawa, 1993 [PA] (Boraginaceae; *mimuli* grp.?)
Phytomyza platensis Brèthes, 1923 (*Chrom.*) [NT] (Lamiaceae; *mimuli* grp.)
Phytomyza cheilanthus Garg, 1971 (*Chrom.*) [OR] (Adiantaceae; *scolopendri* grp.)
Phytomyza dorsata Hendel, 1920 (*Chrom.*) [PA] (Aspleniaceae; *scolopendri* grp.)
Phytomyza dryoptericola Sasakawa, 1961 (*Chrom.*) [PA] (Dryopteridaceae, Aspleniaceae; *scolopendri* grp.)
Phytomyza scolopendri Goureau, 1851 (*Chrom.*) [PA] (Polypodiaceae, Aspleniaceae; *scolopendri* grp.)
Phytomyza gymnostoma Loew, 1858 [PA] (Alliaceae)

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