

REVIEW

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Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequences

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Abstract Powdery mildew fungi (Erysiphales, Ascomycota) are obligate biotrophs that infect a wide range of angiosperms. Phylogenetic analyses based on the nucleotide sequences of the nuclear ribosomal DNA revealed that the powdery mildew fungi are divided into five major lineages. The respective lineage was well defined by the morphology of conidial stage, but not of ascomata. In this fungal group, tree-parasitic taxa are generally ancestral, and multiple events of host expansion from trees to herbs have occurred within the respective lineage. Accompanying the host expansion to herbs, simplification of appendage morphology has occurred multiple times. The simple, mycelioid appendages are thus a result of convergence. The host expansion route was investigated in detail in the tribe Cystothecae. Two sections of the genus *Sphaerotheca* were derived from the genus *Podosphaera* separately. The section *Magnicellulatae* was derived from a *Podosphaera* species parasitic to *Prunus* (Rosaceae), acquired parasitism to the Scrophulariaceae, and then expanded host range into the Asteraceae. After genetic radiation on the Asteraceae, they further expanded their host ranges into other plant families. According to the molecular clock (1.26%/100 million years, myr) reported by Berbee and Taylor, splitting of the Erysiphales and the Myxotrichaceae and first divergence within the Erysiphales was calculated to have occurred 100 and 76 myr ago, respectively.

Key words Convergence · Erysiphaceae · Host range · Molecular clock · rDNA

Introduction

Powdery mildew is the general name of the fungi belonging to the Erysiphales, Ascomycota. All the powdery mildew

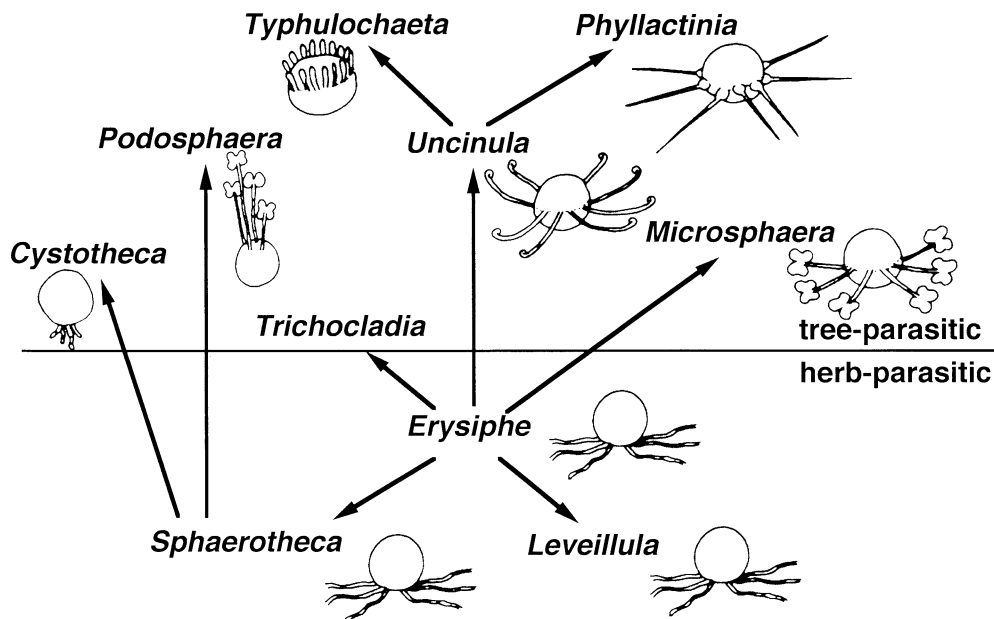
fungi are obligate biotrophs that infect a wide range of angiosperm plants and appear as white, powdery material on the leaves, stems, or fruits. Braun (1987) described 18 genera and 435 species of the powdery mildews in his monograph. Up to 9838 species in 1617 genera, 169 families, and 44 orders of angiosperms have been recorded as the host plants of powdery mildews (Amano 1986), in which a number of economically important plants are involved. Thus, the powdery mildews are one of the most important plant pathogens. The host range of the fungi is strictly restricted to angiosperms, and they never infect ferns and gymnosperms. Of the 9838 host plants, 9176 host species belong to the dicots, and only the remaining 662 hosts are monocots, of which 634 (96%) belong to the Apaceae. The powdery mildews can be summarized as the fungi mainly parasitic to dicots of the angiosperms and specifically to the Apaceae in monocots.

This fungal group has the following unique characteristics as plant pathogenic fungi.

1. Obligate biotrophy: Biotope of the fungi is strictly limited to living plants and lacks saprophytic life stages. Several plant pathogenic fungi such as rusts and downy mildews are also known as obligate biotrophs. Although the obligate biotrophic nature is not unique to the powdery mildews, it is an important characteristic in considering the evolutionary history of this fungal group.
2. Ectotrophy: All but a few genera of the powdery mildews are ectotrophic. Their mycelia usually distribute on the host surface and put only haustorium, a nutrient-absorbing organ, into the epidermal cells of the infected plants. Ectotrophy is unique to the species of the Erysiphales and Meliolales. Only three genera, *Leveillula*, *Phyllactinia*, and *Pleochaeta*, are endotrophic, in which they penetrate into host tissues through stomata and put the haustorium in parenchyma cells. *Phyllactinia* and *Pleochaeta* are partly endotrophic, in which most of the mycelia are exposed on the surface of plants. *Leveillula* is the only genus that has a true endotrophic nature among the powdery mildews. There are contradictory hypotheses as to which of the ectotrophic

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Fig. 1. Hypothetic evolution scheme from herb-parasitic genera to tree-parasitic genera proposed by Amano (1986)



and endotrophic natures are ancestral in this group of fungi.

3. Xerophytism: Almost all plant pathogenic fungi require free water for their spore germination and infection. The powdery mildews can germinate vigorously and infect host plants under low atmospheric humidity (Yarwood 1957). The question of how these fungi obtained this xerophytic nature is an interesting subject for considering evolution of the powdery mildews.

Traditional evolutionary speculations

There have been a few arguments on evolution of the powdery mildew fungi based on morphological characteristics and host plants. Most of these arguments could be simply put as to the points of which characteristics are primitive or derived. Some definite points are as follows: (1) number of asci in an ascoma, single or plural; (2) number of ascospores in an ascus, eight or less than eight; (3) morphology of appendage; (4) conidiogenesis, produce conidia in chains (*Euoidium* type) or solitary (*Pseudoidium* type); and (5) parasitism, ectotrophic or endotrophic. Of these, most researchers are consistent in regarding eight-spored asci and mycelioid appendages as primitive characteristics.

Neger (1901) regarded single-ascial ascomata as a primitive character, placing the genus *Sphaerotheca* at the base of his system and deriving *Erysiphe* and *Podosphaera*. In contrast, most other researchers considered the polyascial form as primitive.

Arnaud (1921) and Katumoto (1973) regarded the endotrophic genus *Leveillula* as primitive and the ectotrophic genera as derivatives from *Leveillula*. On the other hand, Raymond (1927), Blumer (1933), and Braun (1987) considered the endotrophic habit of *Leveillula* as xerophytic adaptation and not as a primitive feature.

Powdery mildew fungi can be divided into two conidiogenesis types, *Pseudoidium* type and *Euoidium* type. There is disagreement on the evolutionary polarization of these two conidiogenesis types. Blumer (1933) and Heluta (1981) treated the *Euoidium* type as primitive and the *Pseudoidium* type as derived. In contrast, Braun (1981, 1987) regarded the *Pseudoidium* type as primitive and the *Euoidium* type as derived.

Braun (1987) postulated that fungi with mycelioid appendages, polyascial ascoma, eight-spored ascus, and a simple structure of the ascoma wall represent the most primitive extant type of powdery mildews. Based on this hypothesis, he mentioned a few species of the genus *Erysiphe* as the most primitive in the powdery mildews.

Hirata (Amano) discussed evolution of the powdery mildew fungi with the relationships of their host plants (Amano 1986, 1992; Hirata 1968, 1969, 1971a,b, 1972, 1975, 1976, 1980). He pointed out that the powdery mildews are divided into two distinct groups of genera based on their host ranges, i.e., tree-parasitic genera and herb-parasitic genera (Hirata 1976). All the herb-parasitic genera have mycelioid appendages, whereas tree-parasitic genera represent various types of appendages such as dichotomously branched, uncinately to circinate, clavate, or bristle like. Because mycelioid appendages have long been regarded as the most primitive type, herb-parasitic genera were placed at the primitive and tree-parasitic genera at the derived position in his evolutionary hypothesis. From the results, Hirata (1976) postulated that the powdery mildews were originally herb parasitic and laterally expanded their host ranges into trees (Fig. 1). Moreover, he proposed that there are two major evolutionary routes from herb-parasitic to tree-parasitic forms (Hirata 1971a,b): one is the route from *Erysiphe* to *Microsphaera*, and the other from *Sphaerotheca* to *Podosphaera*. He postulated that a particular plant family served as a key for the respective evolutionary route, because the powdery mildews depend on living host plants

for their whole life cycle. He surveyed plant families that include both trees and herbs and have hosts of both fungal genera in a single family. As a result, he found the Fabaceae in the evolutionary route from *Erysiphe* to *Microsphaera* and the Rosaceae in the route from *Sphaerotheca* to *Podosphaera* as postulated stages of evolution (Hirata 1971a,b). For instance, the Fabaceae includes many tree and herbaceous species in the family and is commonly infected by both *Erysiphe* and *Microsphaera*. Moreover, there are many species having intermediate morphological characteristics between *Erysiphe* and *Microsphaera* in the taxa parasitic to the Fabaceae.

Amano (1986) carried out a comprehensive survey of the literature on occurrence of powdery mildews from all over the world and listed the host plants with their geographical distributions in his memorial work "Host range and geographical distribution of the powdery mildew fungi." He proposed many hypotheses on evolution of the powdery mildews with relation to host plants and geographical distribution, and also raised many questions. A serious problem in his contributions may be that his discussion was constructed on the basis of the traditional evolutionary hypotheses. Because the traditional hypotheses do not always represent precise phylogeny of the powdery mildews as described in the following sections, some of his hypotheses will be rejected. His comprehensive list will exhibit its real value when true phylogenetic relationships of the fungi are revealed.

Phylogeny and evolution based on molecular data

Phylogenetic placement of the Erysiphales

Erysiphales have been placed in Plectomycetes based on their cleistothecial ascomata without ostiole (Ainsworth et al. 1971; Webster 1980). On the other hand, the production of asci from a basal hymenium and the forcible discharge of ascospores are used to classify Erysiphales with Pyrenomycetes (Alexopoulos and Mims 1979; Yarwood 1973, 1978). To resolve the ambiguities of the taxonomic placement of the Erysiphales, Saenz et al. (1994) determined the nucleotide sequences of the 18S rDNA of *Blumeria graminis* (DC.) Speer and reported that the fungus was neither Pyrenomycetes nor Plectomycetes. Instead, they reported a close relationship between *B. graminis* and *Sclerotinia sclerotiorum* (Lib.) de Bary (Leotiales). Sugiyama et al. (1999) determined the 18S rDNA sequences for 19 taxa of the onygenalean fungi including the Myxotrichaceae, and reported that the Myxotrichaceae has its own lineage distantly related to the other onygenalean fungi and closely related to the Leotiales and Erysiphales. Both reports used only one nucleotide sequence of *B. graminis* as representative Erysiphales for their analyses. *Blumeria graminis* is regarded as an isolated fungus among the Erysiphales, because of its unique morphological characteristics and also its unique parasitism to monocots, especially cereal plants, in contrast to the dicot parasitism of

most other Erysiphales. The nucleotide sequence data from other species of the Erysiphales were required to clarify the phylogenetic relationship of the Erysiphales among Ascomycota more precisely. Mori et al. (2000b) determined the nucleotide sequences of the 18S rDNA for ten taxa of the powdery mildews representing all major genera of the Erysiphales to reconstruct the phylogenetic placement of the fungi. They confirmed that the Erysiphales is the sister of the Myxotrichaceae of the Onygenales and is closely related to the Leotiales, Cyttariales, and Pezizales (Fig. 2). Further, it was reported that some taxa of the Eurotiales are closely related to the Erysiphales (Suh and Blackwell 1999). These results suggest the possible existence of a novel evolutionary pathway from cleistothecial discomycetous fungi to Erysiphales and Myxotrichaceae.

Based on these results, the Erysiphales has been regarded as an isolated, poorly resolved group among filamentous ascomycetes in *Introductory Mycology* (Alexopoulos et al. 1996), and was placed into the monotypic subclass Erysiphomycetidae of the class Ascomycetes in Ainsworth and Bisby's *Dictionary of Fungi*, 9th edition (Kirk et al. 2001). Pfister and Kimbrough (2001) described the Erysiphales in the assemblage of the Discomycetes.

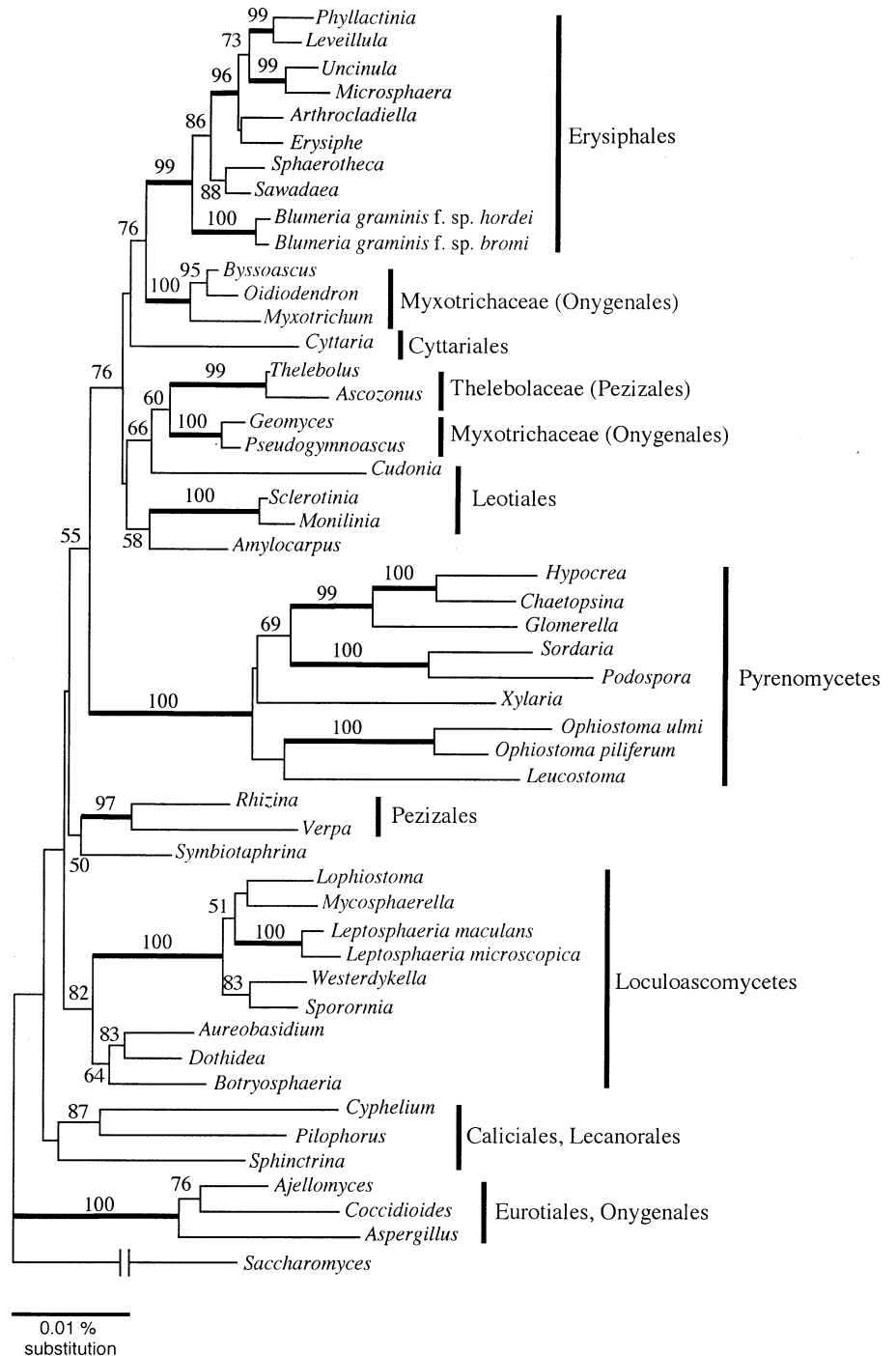
Phylogenetic relationships within the Erysiphales

Zeller (1995) and Zeller and Levy (1995) tried to apply cladistic analysis to phylogenetic study of the Erysiphales based on the morphological characteristics and polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) of rDNA internal transcribed spacer (ITS) regions. Takamatsu et al. (1998) and Saenz and Taylor (1999) determined the nucleotide sequences of the rDNA ITS region, and reported that the Erysiphales is divided into five and four phylogenetic groups, respectively. Takamatsu et al. (1998) pointed out that the rDNA ITS region is too variable to infer the phylogenetic relationships throughout the Erysiphales, and that analyses of more conserved DNA regions are required. Mori et al. (2000a) carried out phylogenetic analysis of the Erysiphales using the sequence data of the 18S, 28S, and 5.8S rDNA. They reported that the Erysiphales is divided into five distinct lineages, and the respective lineage is well defined by the morphology of the conidial stage (Boesewinkel 1980; Cook et al. 1997), but not of the ascomata (Fig. 3). The respective lineage was recognized as a taxonomic unit, a tribe, by Braun and Takamatsu (2000). *Uncinula septata*, a parasite of the Fagaceae, was placed in the primitive base to the large clade composed of all other powdery mildew taxa.

Evolution of appendages and host plants

Detailed examination within the respective lineage revealed that fungi with uncinulate-circinate appendages are placed at the base of the lineage in three of the five lineages, i.e., *Uncinula* in the tribe Erysipheae, *Pleochaeta* in the tribe Phyllactineae, and *Sawadaea* in the tribe Cystothecaceae. This result, as well as the basal placement of *U. septata* in the

Fig. 2. Neighbor-joining analysis of the 18S ribosomal RNA gene for 50 ascomycetes. The neighbor-joining tree was found using Kimura's two-parameter model for nucleotide substitutions to correct for multiple hits. Branch lengths correspond to pairwise distances between taxa. *Saccharomyces cerevisiae* was used to root the tree. Percentages of neighbor-joining analysis of 1000 bootstrapped data sets supporting specific branches are indicated at the respective nodes. **Bold lines** indicate the nodes supported with more than 95% bootstrap values. Bootstrap values below 50% are not shown. (From Mori et al. 2000b, with permission)



Erysiphales, suggests that the uncinately-circinate appendages are the most primitive morphology of appendage in the Erysiphales. The genera *Uncinula*, *Pleochaeta*, and *Sawadaea* might have an old origin, and other taxa included in the respective lineage might have diverged from those genera.

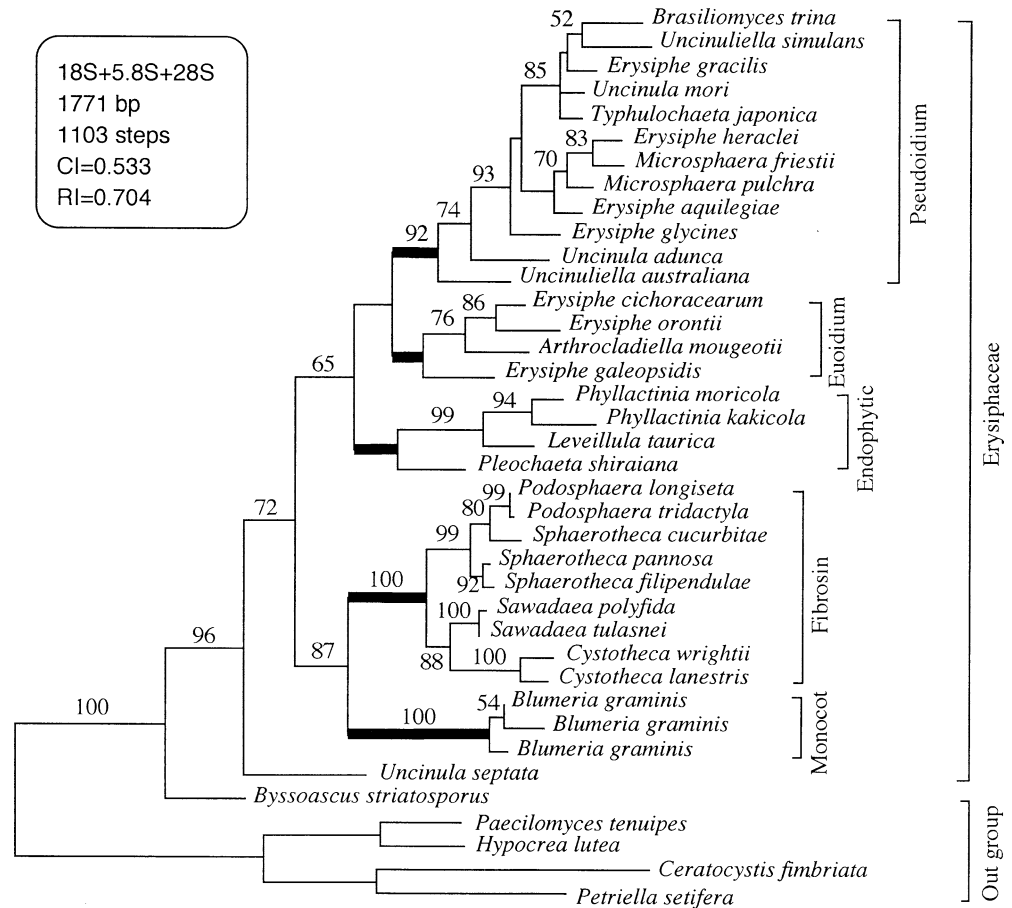
Although the fungal taxa with mycelioid appendages, which have long been regarded as a primitive character, are distributed in all the five lineages, they are placed at the derived positions at least in these three tribes. The mycelioid appendage may be a derived character having recent origin, but not a primitive character. More detailed discus-

sion on evolution of the appendage within the respective lineages follows.

Tribe Erysipheae

In the tribe Erysipheae, the genus *Uncinula*, having uncinately-circinate appendages, is placed at the base of the lineage, and the genus *Microsphaera* with dichotomously branched appendages is diverged from *Uncinula*. This result indicates that the uncinately-circinate appendage is primitive in the lineage and that the dichotomously

Fig. 3. One of the 30 most-parsimonious trees inferred from the combined 18S, 5.8S, and 28S rDNA datasets. The tree topology is nearly identical to the neighbor-joining tree, except for the placement of *Pleochaeta shiraiana*. The numbers above the branches represent the proportion (percent) of 1000 bootstrap replications in which the groups to the right were placed together. Bold lines indicate the nodes supported with more than 95% bootstrap values. (From Mori et al. 2000a, with permission)



branched appendage is derived from the uncinete-circinate appendage.

It is interesting that the genus *Erysiphe*, having mycelioid appendages, does not group into a clade. Instead, they form several small groups together with the taxa of *Microsphaera*, and some taxa of *Erysiphe* are placed in the *Uncinula* group. This result indicates that the genus *Erysiphe* is a polyphyletic fungal group that diverged multiple times independently.

As stated earlier, genera of the powdery mildews are divided into two large groups, i.e., tree-parasitic genera and herb-parasitic genera. *Uncinula* is a typical tree-parasitic genus and *Microsphaera* is also tree- or shrub parasitic. *Erysiphe* is mainly a herb-parasitic genus. In the tribe Erysipheae, powdery mildews were originally tree parasitic, then expanded their hosts to shrubs, and finally to herbs. Polyphyly of the genus *Erysiphe* suggests that expansion of the host range to herbs occurred multiple times independently in this tribe. Moreover, simplification of appendages may have occurred accompanying host expansion to herbs. Therefore, the mycelioid appendage is a derived character that occurred as a result of convergence.

Tribe Cystothecaceae

The tribe Cystothecaceae consists of four genera: *Cystotheca*, *Podosphaera*, *Sawadaea*, and *Sphaerotheca*. *Sawadaea* has

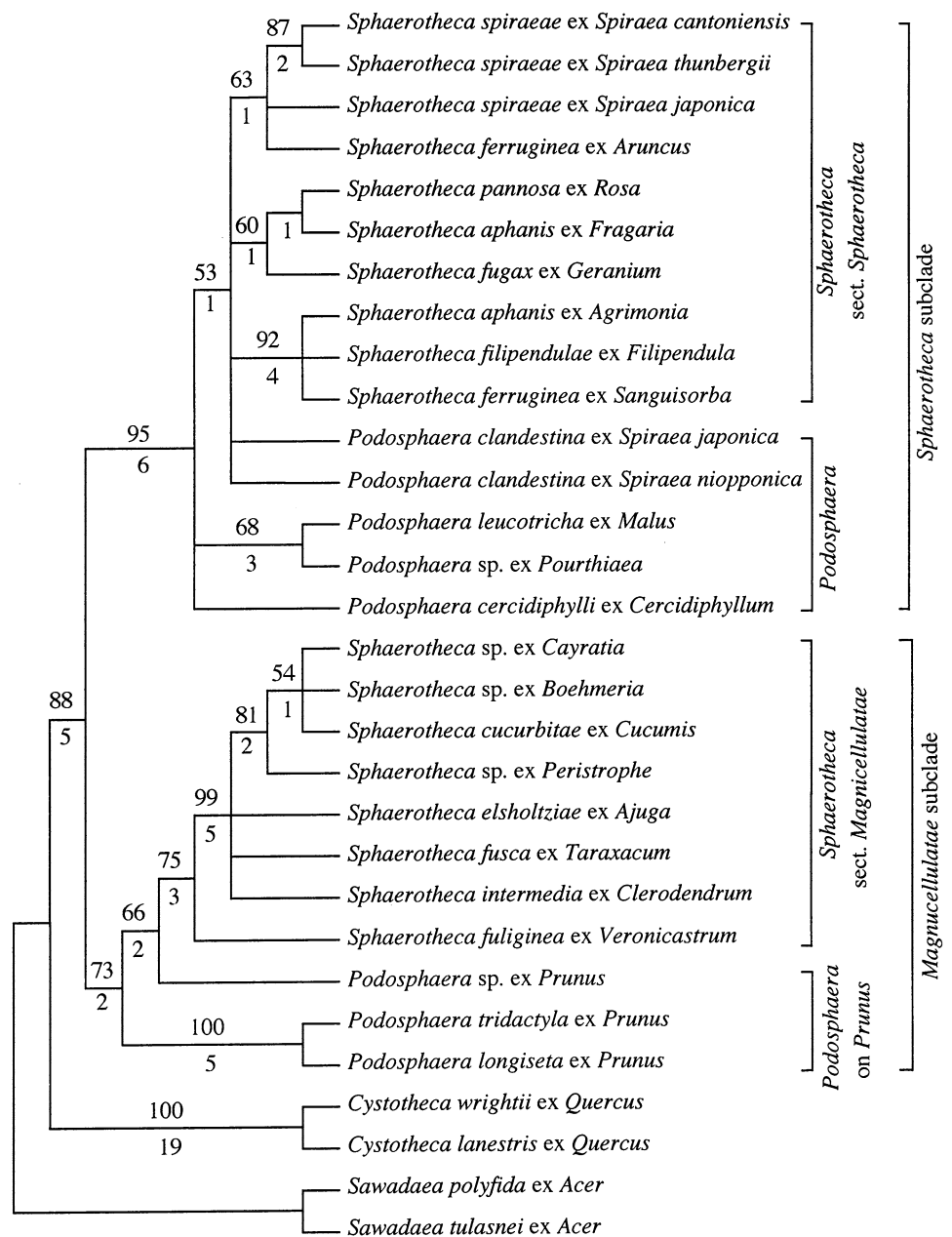
uncinate-circinate appendages, *Podosphaera* has dichotomously branched appendages, and *Cystotheca* and *Sphaerotheca* have mycelioid appendages. In the context of host plants, *Sawadaea* and *Cystotheca* are tree parasitic, i.e., Aceraceae for *Sawadaea* and Fagaceae for *Cystotheca*. *Podosphaera* parasitizes trees or shrubs of Rosaceae, and *Sphaerotheca* is parasitic mainly to herbs, rarely to shrubs. It is obvious also in this tribe that host expansion has occurred from trees to shrubs and herbs, and that morphological convergence (simplification) has occurred accompanying host expansion to herbs. The difference for the case of the tribe Erysipheae is that the host expansion to herbs occurred only twice in this tribe. That is, two sections of *Sphaerotheca* form each separate monophyletic clade to be derived from *Podosphaera* independently (Fig. 4).

Cystotheca has mycelioid appendages although the genus parasitizes the trees of the Fagaceae. This reason is discussed later.

Other tribes

The tribe Phyllactineae consists of three genera: *Leveillula*, *Phyllactinia*, and *Pleochaeta*. *Pleochaeta* has uncinete-circinate appendages and parasitizes the trees of the Ulmaceae and Fabaceae. *Phyllactinia* has unique bristle-like appendages and parasitizes the trees of a wide range of plant families. *Leveillula* has mycelioid appendages and

Fig. 4. Strict consensus of 17 equally parsimonious trees inferred from sequences of ITS1, ITS2, and the 5.8S rRNA gene from 28 tribe Cystothecae DNAs plus two outgroup taxa, using “gaps = newstate” option of PAUP version 3.1.1. The bootstrap values of 1000 replications are shown on the respective branch. Decay indices are shown *below* the branches. The consistency index (CI) is 0.716; the retention index (RI) is 0.850; and the rescaled consistency index (RC) is 0.609. (From Takamatsu et al. 2000, with permission)



mainly parasitizes herbs. Because *Pleochaeta* is placed in the base of this tribe, the host expansion route from trees to herbs is obvious in this tribe. Because *Leveillula* forms a distinct monophyletic clade, host expansion to herbs may have occurred only once in this tribe.

As discussed earlier, the host expansion from trees to shrubs and herbs may have commonly occurred in the respective lineages. On the other hand, the tribes Golovinomycetaceae and Blumerieae have only herbs as their hosts, excepting the genus *Arthrocladiella*. If this kind of host expansion had occurred in all the five tribes, how could we explain the case of the tribes Golovinomycetaceae and Blumerieae? One of the possible explanations may be that there are tree-parasitic taxa that became extinct or have not yet been used for phylogenetic analysis in these

tribes. The tribe Blumerieae is a unique group in the Erysiphales that has only the Apaceae of monocots as their hosts. If the original hosts of the Erysiphales could be assumed to be dicots, there should be a dicot species that mediated host switching to monocots. What was the dicot species? What were the ancestral taxa of the tribe Golovinomycetaceae?

Why did the convergence occur?

As already described, the mycelioid appendage is not a primitive character. It is a derived character that occurred as a result of convergence accompanying host expansion to herbaceous plants. Why did the convergence occur?

A key to address the question is in the difference in overwintering behavior of the powdery mildews on trees and herbs. The ascoma is considered as an organ for enduring the winter season for the powdery mildews. Overwintering behavior by ascomata is well known in the genus *Phyllactinia* (Blumer 1933; Cullum and Webster 1977; Itoi et al. 1962; Neger 1901; Yarwood 1957). *Phyllactinia* has two types of appendages, bristle like and penicillate (penicillate cell). The bristle-like appendages bend downward and lift the ascomata off the leaf surface. These ascomata are easily dislodged and blown off the leaf surface by wind or rain, whereupon they adhere to the bark of twigs by the sticky penicillate cells, and function as primary infection sources for the next year (Fig. 5). Similarly, mature ascomata of the grape powdery mildew *Uncinula necator* readily disperse in rain from infected tissues, adhere to the bark of the vine by their appendages, and overwinter there (Cortesi et al. 1995; Gadoury and Pearson 1988). For powdery mildews of deciduous trees, overwintering on the bark of twigs may be far more advantageous than falling to the ground as primary inoculum for the next year (Itoi et al. 1962; Pearson and Gadoury 1987). By contrast, the ascomata of the herb- and evergreen tree-parasitic genera remain on the leaf surface even after maturing. Thus, overwintering behavior of the ascomata is quite different between herb- and evergreen tree-parasitic genera and deciduous tree-parasitic genera. Appendages have an important role for ascomatal behavior, which suggests the possibility that the morphology of the appendages is subjected to a selection pressure depending on the type of host. However, there are several examples where mildews have mycelioid appendages and infect deciduous hosts, i.e., *Cystotheca lanestris* on *Quercus* spp., *E. weigelae* Z.X. Chen & Luo on *Weigela* spp., *E. aggregata* (Peck) Farl. and *E. vernalis* Karst. on *Alnus* spp., *Sphaerotheca spiraeae* Sawada on *Spiraea* spp., *S. filipendulae* on *Filipendula* spp., and *S. japonica* (Salm.) Homma on *Stephanandra incisa* (Thunb.) Zabel. These fungi are known to form dense mycelial mats on the young twigs or female catkins of their host plants and produce a large number of ascomata on the mycelia (Braun 1987; Gardner et al. 1972; observations by the author). These fungi may be able to pass the winter as ascomata on the young twigs without dispersal and readhering of mature ascomata. These findings further support the idea that the morphology of appendages is a result of adaptation for overwintering behavior of ascomata. Polyphyly of the powdery mildew taxa having mycelioid appendages may indicate that the mycelioid appendages are derived characters as a result of the simplification of appendages that occurred multiple times independently.

Host expansion of the powdery mildews

The powdery mildews are obligate parasitic fungi of plants, and all stages of their life cycles depend on living host plants. This requirement means that living host plants are just like the earth for powdery mildews, and their evolution must have occurred on living hosts. Specialization of host

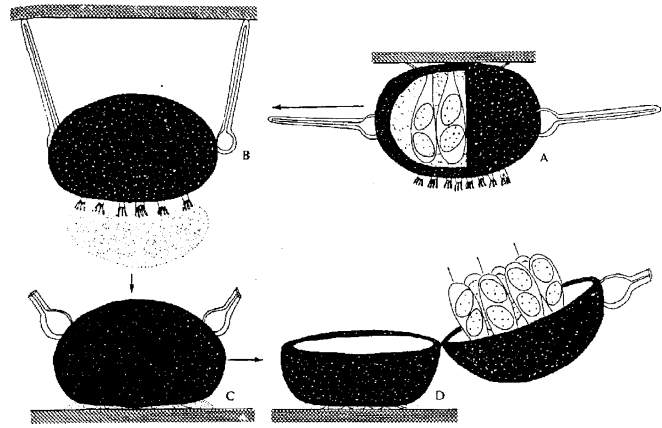


Fig. 5. Diagrammatic sequence of events during ascoma development and dehiscence in *Phyllactinia guttata*. *Phyllactinia* has two types of appendages, bristle-like and penicillate (A). The bristle-like appendages bend downward and lift the ascoma off the leaf surface (B). This ascoma is dislodged and blown off the leaf surface by wind or rain, whereupon it adheres to the bark of twigs by the sticky penicillate cells (C), and functions as a primary infection source for the next year (D). (From Cullum and Webster 1977, with permission)

range is niche separation for powdery mildews. Therefore, evolution of powdery mildews must have occurred closely related to the evolution of their hosts. In this section, I discuss evolution of the powdery mildews with special reference to host relationship.

In the previous section, I mentioned that the two sections of the *Sphaerotheca*, sections *Sphaerotheca* and *Magnicellulatae*, were derived from the genus *Podosphaera* separately (Takamatsu et al. 2000). The genus *Podosphaera* is a fungal group parasitic to the Rosaceae in which 86% of hosts of the genus belong to the Rosaceae. The ITS sequence analysis revealed that the section *Magnicellulatae* of the genus *Sphaerotheca* was derived from a *Podosphaera* species parasitic to the genus *Prunus* (Fig. 4; Takamatsu et al. 2000). Although *Prunus* consists of tree species, the *Magnicellulatae* is herb parasitic. The *Magnicellulatae* is known to occur on herbaceous plants of more than 1000 plant species ranging among 40 families, such as Asteraceae, Scrophulariaceae, Cucurbitaceae, and Fabaceae, in the world. Interestingly, a rosaceous plant is not included in the hosts of the *Magnicellulatae*, although *Magnicellulatae* was derived from Rosaceae-parasitic species. How was the *Magnicellulatae* evolved from Rosaceae-parasitic species? To address the question, we determined the nucleotide sequences of the rDNA ITS region for 79 powdery mildew isolates of the *Magnicellulatae* that occurred on 60 plant species and performed phylogenetic analysis based on the sequences (Fig. 6; Hirata et al. 2000).

The *Magnicellulatae* formed a distinct monophyletic group in the phylogenetic tree, which indicates that the evolution of the *Magnicellulatae* from Rosaceae-parasitic species has occurred only once. With the exception of two species occurring on the Scrophulariaceae, all isolates formed a large, well-defined clade. Genetic diversity among species on the Scrophulariaceae was much larger than the diversity among all other taxa, and the first split of the

Haplotype

- 9:** *Matricaria*, *Taraxacum* (Asteraceae)
15: *Calendula*, *Euryops*, *Farfugium*, *Syneilesis* (Asteraceae)
Impatiens (Balsaminaceae)
16: *Coreopsis*, *Cosmos* (Asteraceae)
18: *Bidens*, *Carthamus*, *Helianthus* (Asteraceae)
22: *Arctium*, *Lactuca*, *Tussilago*, *Youngia* (Asteraceae)
Verbena (Verbenaceae), *Physalis* (Solanaceae)
25: *Petasites*, *Rudbeckia* (Asteraceae)
26: *Vernonia* (Asteraceae), *Solanum* (Solanaceae),
Acalypha (Euphorbiaceae), *Dunbaria*, *Vigna*
(Fabaceae), *Hibiscus* (Malvaceae)
27: *Helianthus*, *Zinnia* (Asteraceae), *Saintpaulia* (Gesneriaceae)
Lycopus (Lamiaceae), *Verbena* (Verbenaceae), *Crotalaria*,
Glycine, *Vigna* (Fabaceae), *Cucumis*, *Cucurbita*,
Gynostemma, *Melothria*, *Trichosanthes* (Cucurbitaceae),
Abelmoschus (Malvaceae)

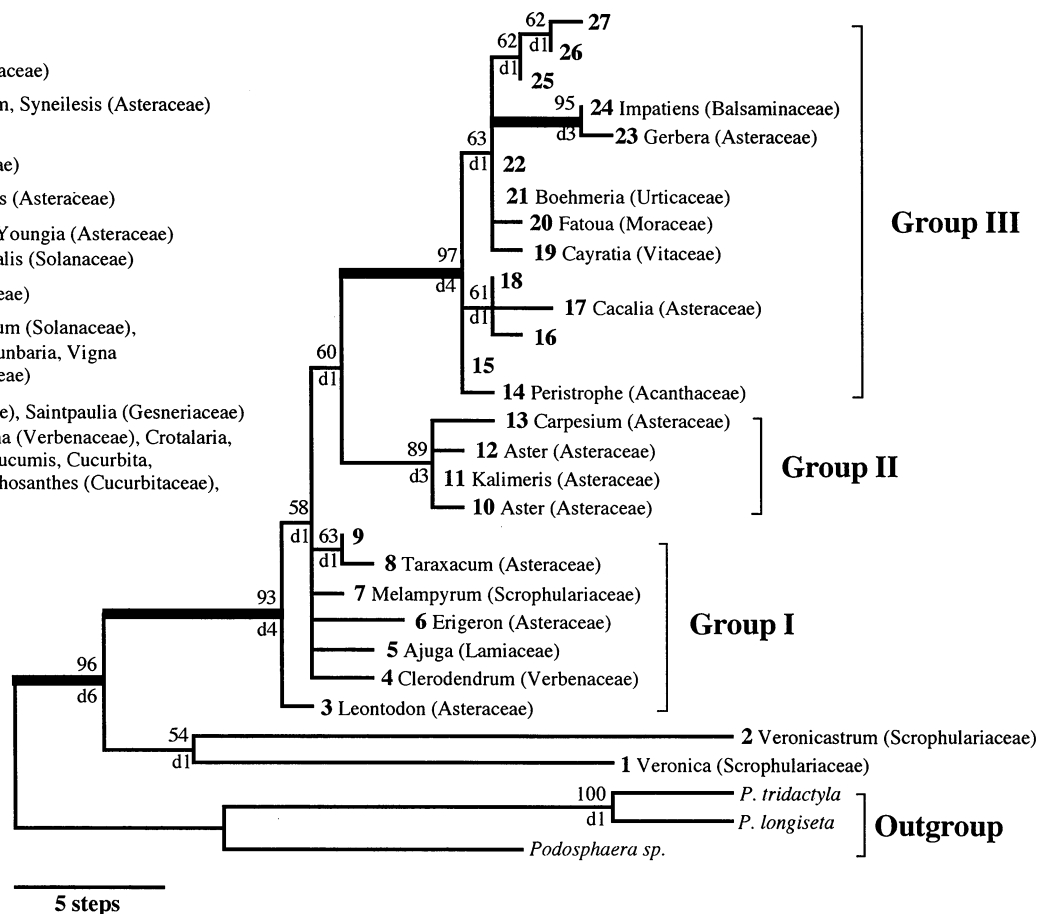


Fig. 6. A single most parsimonious tree reconstructed by unweighted analysis of ITS sequence data from 27 haplotypes of *Sphaerotheca fuliginea* s. lat. plus three outgroup taxa with gaps treated as missing. Numerals beside the respective terminal branch shows haplotype number. Host genera and families included in the respective haplotype are shown at right side of the haplotype number. For the haplotypes that include more than one host genus, the host genera and families are

shown at the left of the phylogenetic tree with haplotype number. The bootstrap values of 1000 replications are shown on the respective branch. Bold lines indicate the nodes supported with more than 95% bootstrap values. Decay indices are shown below the branches. The consistency index (CI) is 0.868; the retention index (RI) is 0.898; and the rescaled consistency index (RC) is 0.779. (From Hirata et al. 2000, with permission)

Magnicellulatae clade was shared by these species. This result suggests that the Scrophulariaceae is the earliest host of the section *Magnicellulatae*. Isolates from the Asteraceae shared the largest sequence diversity in the large clade and were represented in all major groups. Most members of the basal groups consisted of isolates from the Asteraceae, which suggests that an early radiation in the large clade occurred on the Asteraceae. Based on the small sequence diversity and placement at the terminal end of the phylogenetic tree of isolates from the Cucurbitaceae and the Fabaceae, it is suggested that the ability of section *Magnicellulatae* to infect these families was acquired more recently.

Summarizing these results, it is suggested that a *Prunus*-parasitic species shifted its host range to the Scrophulariaceae and then expanded its host range into the Asteraceae. After occurrence of genetic radiation on the Asteraceae and expansion of geographical distributions, some Asteraceae-parasitic taxa further expanded their host ranges into other plant families. They may have ac-

quired parasitism to the Cucurbitaceae and the Fabaceae recently.

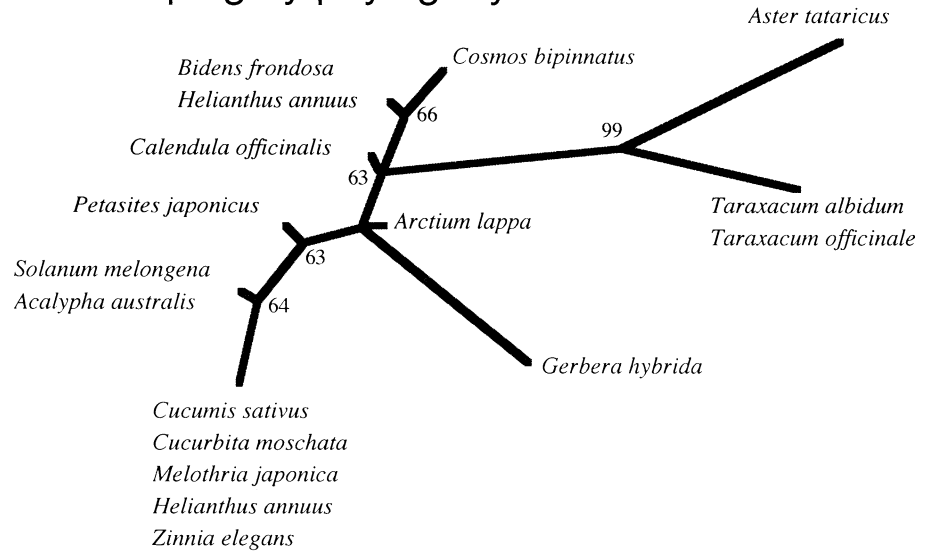
The *Magnicellulatae* is a powdery mildew group exceptionally well characterized in their host ranges. We compared the groupings based on molecular phylogeny with grouping by their infectivity. As a result, the groupings of fungal taxa by molecular phylogeny showed general agreement with groupings by infectivity (Fig. 7). The powdery mildews are obligate parasitic fungi of plants, and living host plants are the only niche for them. Host specialization is a kind of niche separation for powdery mildews and may trigger genetic divergence in these fungi.

Divergence time of the powdery mildews

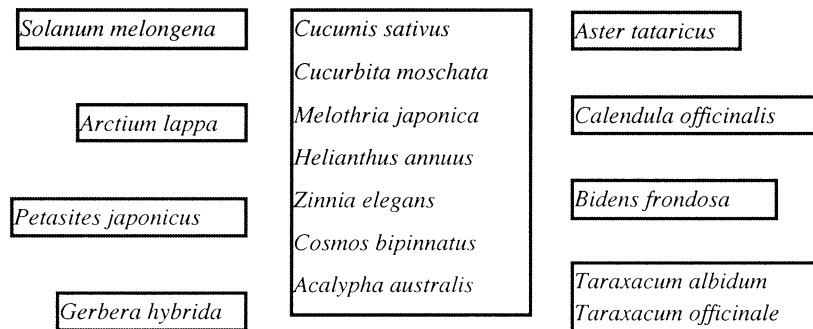
Evolutionary timings have been largely based on fossil records. We have many fossil records in the animals, fewer in the plants, and very few in the fungi. Especially, we have no reliable fossil record for powdery mildews. Estimation of evolutionary timing based on the fossil record is thus almost

Fig. 7. Grouping of *Sphaerotheca fuliginea* s. lat. based on molecular phylogeny and infectivity. The fungal strains are shown as scientific names of host plants. **A** The most parsimonious tree based on the reduced data set of rDNA ITS sequences. The bootstrap values of 1000 replications are shown beside the respective branch. A completely identical tree topology was yielded by the neighbor-joining method. **B** Grouping based on the cross-inoculation test of Abiko (1978, 1982a,b). Fungal strains on the hosts within a box are coinfectious to each other but to those in other boxes. Note that the groupings based on two quite different methods show similar results with only a few exceptions. (From Hirata et al. 2000, with permission)

A. Grouping by phylogeny



B. Grouping by infectivity

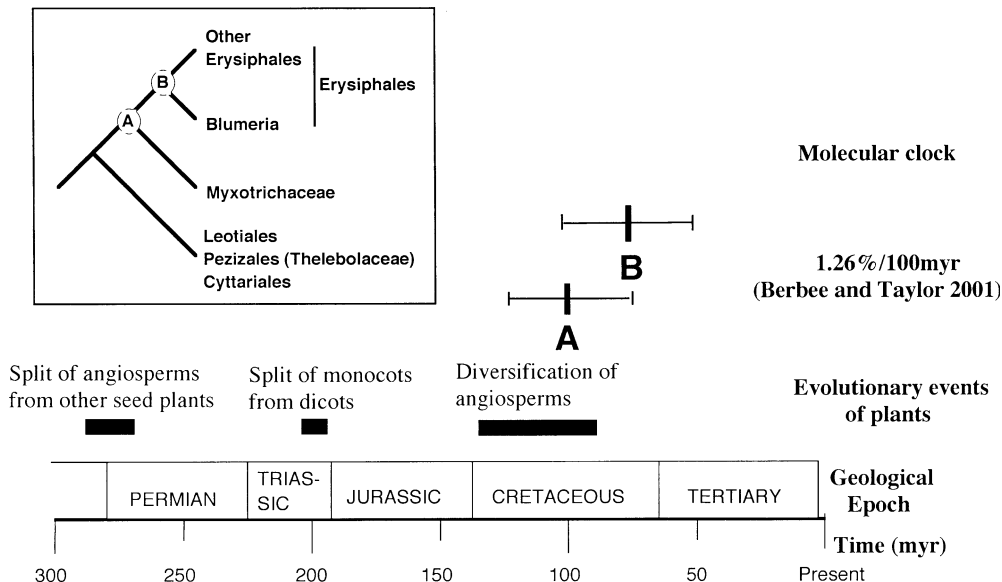


impossible in powdery mildews. Recent development of molecular phylogenetic studies made it possible to estimate evolutionary timing using a molecular clock. Mori et al. (2000b) tried to estimate divergence time of the powdery mildews based on the molecular clock. Calibration of the molecular clock has been done in 18S rDNA sequences by Simon et al. (1993) and Berbee and Taylor (1993). Mori et al. (2000b) determined the nucleotide sequences of 18S rDNA for ten powdery mildew taxa. The phylogenetic tree constructed by the sequence data of the powdery mildews and other ascomycetous fungi revealed that the Myxotrichaceae of Onygenales is the sister group of the Erysiphales. The genetic distances were 2.53% between the Erysiphales and the Myxotrichaceae, and 1.85% within the Erysiphales. Based on the molecular clock (0.67%/100myr) of Simon et al. (1993), the split of the Erysiphales and the Myxotrichaceae occurred 190 million years (myr) ago, and the first split within the Erysiphales occurred 140myr ago. Similarly, based on the molecular clock (1%/100myr) of Berbee and Taylor (1993), the split of the

Erysiphales and the Myxotrichaceae occurred 130myr ago, and the first split within the Erysiphales occurred 90myr ago. Simon et al. (1993) calculated the molecular clock based on the split time between dicots and monocots. Because there is no evidence to consider that the evolution rate of fungi is same with that of plants in 18S rDNA, their calibration could be unreliable. Therefore, the molecular clock of Berbee and Taylor (1993) may be more reliable than that of Simon et al. (1993).

Berbee and Taylor (2001) corrected their molecular clock to 1.26%/100myr based on new fossil records. According to this new molecular clock, the splitting time of the Erysiphales and the Myxotrichaceae could be corrected as 100myr ago and the first split within the Erysiphales as 76myr ago (Fig. 8). Therefore, the powdery mildews may have originated between mid- and late Cretaceous. Interestingly, this period is consistent with the timing of the first large-scale radiation of the angiosperms. The powdery mildews may have been born with the beginning of the flourishing of the angiosperms, and have been

Fig. 8. Estimated dates of origin and divergence of the Erysiphales based on the nucleotide sequences of the 18S rDNA. *A* is the splitting of the Erysiphales and Myxotrichaceae, and *B* is the first radiation within the Erysiphales. *myr*, million years. (From Mori et al. 2000b, with permission)



evolving for about 100myr along with the evolution of angiosperms.

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References

- Abiko K (1978) Studies on the specialization of parasitism of *Sphaerotheca fuliginea* (Schlecht.) Pollacci I. Powdery mildew fungi parasitic on cucurbits, eggplant, edible burdock and Japanese butterbur. *Ann Phytopathol Soc Jpn* 44:612–618
- Abiko K (1982a) Studies on the specialization of parasitism of *Sphaerotheca fuliginea* (Schlecht.) Pollacci II. Powdery mildew fungi on flowering plants. *Bull Veg Ornamental Crops Res Stn Ser A* 10: 57–62
- Abiko K (1982b) Studies on the specialization of parasitism of *Sphaerotheca fuliginea* (Schlecht.) Pollacci II. Powdery mildew fungi parasitic on weeds. *Bull Veg Ornamental Crops Res Stn Ser A* 10: 63–67
- Ainsworth GC, James PW, Hawksworth DL (1971) Ainsworth and Bisby's dictionary of the fungi including the lichens, 6th edn. CAB International, Kew
- Alexopoulos CJ, Mims CW (1979) *Introductory mycology*, 3rd edn. Wiley, New York
- Alexopoulos CJ, Mims CW, Blackwell M (1996) *Introductory mycology*, 4th edn. Wiley, New York
- Amano K (1986) Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo
- Amano K (1992) Notes on the host range and geographical distribution of *Podosphaera*. *Trans Mycol Soc Jpn* 33:139–148
- Arnaud G (1921) Etude sur les champignons parasites. *Ann Epiphyt (Paris)* 7:1–116
- Berbee ML, Taylor JW (1993) Dating the evolutionary radiation of the true fungi. *Can J Bot* 71:1114–1127
- Berbee ML, Taylor JW (2001) Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) *The Mycota VII. Systematics and evolution, part B*. Springer-Verlag, Berlin, pp 229–245
- Blumer S (1933) Die Erysiphaceen Mitteleuropas unterbesonderer Berücksichtigung der Schweiz. *Beitr Kryptogamenflora Schweiz* 7(1):1–483
- Boesewinkel HJ (1980) The morphology of the imperfect states of powdery mildews (Erysiphales). *Bot Rev* 46:167–224
- Braun U (1981) Taxonomic studies in the genus *Erysiphe* I. Generic delimitation and position in the system of the Erysiphaceae. *Nova Hedwigia* 34:679–719
- Braun U (1987) A monograph of the Erysiphales (powdery mildews). *Beih Nova Hedwigia* 89:1–700
- Braun U, Takamatsu S (2000) Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysiphaceae) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (Cystothecaceae) inferred from rDNA ITS sequences: some taxonomic consequences. *Schlechtendalia* 4:1–33
- Cook RTA, Inman AJ, Billings C (1997) Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range. *Mycol Res* 101:975–1002
- Cortesi P, Gadoury DM, Seem RC, Pearson RC (1995) Distribution and retention of cleistothecia of *Uncinula necator* on the bark of grapevines. *Plant Dis* 79:15–19
- Cullum FJ, Webster J (1977) Cleistocarp dehiscence in *Phyllactinia*. *Trans Br Mycol Soc* 68:316–320
- Gadoury DM, Pearson RC (1988) Initiation, development, dispersal, and survival of cleistothecia of *Uncinula necator* in New York vineyards. *Phytopathology* 78:1413–1421
- Gardner MW, Yarwood CE, Duafala T (1972) Oak mildews. *Plant Dis Rep* 56:313–317
- Heluta VP (1981) Mesto roda *Podosphaera* Kunze v sheme vozmozhnyh filogeneticeskikh vzaimosvjazej rodov mucnistoro-sjanyh gribov. *Nov Sist Nizshikh Rast* 1979:200–209
- Hirata K (1968) Notes on host range and geographic distribution of the powdery mildew fungi. *Trans Mycol Soc Jpn* 9:73–88
- Hirata K (1969) Notes on host range and geographic distribution of the powdery mildew fungi II. *Trans Mycol Soc Jpn* 10:47–72
- Hirata K (1971a) Host range and geographic distribution of Erysiphaceae as viewed from the families of angiosperms, and comparison with Meliolineae. *Sydowia* 25:100–118
- Hirata K (1971b) Notes on host range and geographic distribution of the powdery mildew fungi III. *Trans Mycol Soc Jpn* 12:1–13
- Hirata K (1972) Notes on host range and geographic distribution of the powdery mildew fungi IV. *Trans Mycol Soc Jpn* 13:1–21
- Hirata K (1975) Notes on host range and geographic distribution of the powdery mildew fungi V. Uneven distribution of the powdery mildew fungi in the temperate zone of the northern hemisphere, especially of Eurasia. *Trans Mycol Soc Jpn* 16:113–127

- Hirata K (1976) Notes on host range and geographic distribution of the powdery mildew fungi VI. Distribution of the hosts of powdery mildew fungi in the families of angiosperms. *Trans Mycol Soc Jpn* 17:35–62
- Hirata K (1980) Host plants of powdery mildew fungi collected at the Royal Botanic Gardens, Kew, England, during August and September in 1978. *Trans Mycol Soc Jpn* 21:245–258
- Hirata T, Cunningham JH, Paksiri U, Limkaisang S, Shishkoff N, Grigaliunaite B, Sato Y, Takamatsu S (2000) Evolutionary analysis of subsection Magnicellulatae of *Podosphaera* section *Sphaerotheca* (Erysiphales) based on the rDNA ITS sequences with special reference to host plants. *Can J Bot* 78:1521–1530
- Itoi S, Nakayama N, Kubomura K (1962) Studies on the powdery mildew disease of mulberry tree caused by *Phyllactinia moricola* (P. Henn.) Homma. *Bull Sericult Exp Stn* 17:321–445
- Katamoto K (1973) Notes on the genera *Lanomyces* Gäum. and *Cystotheca* Berk. et Curt. *Rep Tottori Mycol Inst* 10:437–446
- Kirk PM, Cannon PF, David JC, Stalpers JA (2001) *Ainsworth and Bisby's dictionary of the fungi*, 9th edn. CAB International, Kew
- Mori Y, Sato Y, Takamatsu S (2000a) Evolutionary analysis of the powdery mildew fungi (Erysiphales) using nucleotide sequences of the nuclear ribosomal DNA. *Mycologia* 92:74–93
- Mori Y, Sato Y, Takamatsu S (2000b) Molecular phylogeny and radiation time of Erysiphales inferred from the nuclear ribosomal DNA sequences. *Mycoscience* 41:437–447
- Neger FW (1901) Beiträge zur Biologie der Erysipheen. *Flora* 88:333–370
- Pearson RC, Gadoury DM (1987) Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. *Phytopathology* 77:1509–1514
- Pfister DH, Kimbrough JW (2001) Discomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) *The Mycota VII. Systematics and evolution*, part A. Springer, Berlin, pp 257–281
- Raymond J (1927) Le blanc du chêne. *Ann Epiphyt (Paris)* 13:94–129
- Saenz GS, Taylor JW (1999) Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer (ITS) ribosomal DNA sequences. *Can J Bot* 77:150–169
- Saenz GS, Taylor JW, Gargas A (1994) 18S rRNA gene sequences and supraordinal classification of the Erysiphales. *Mycologia* 86:212–216
- Simon L, Bousquet J, Lévesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature (Lond)* 363:67–69
- Sugiyama M, Ohara A, Mikawa T (1999) Molecular phylogeny of onygenalean fungi based on small subunit ribosomal DNA (SSU rDNA) sequences. *Mycoscience* 40:251–258
- Suh SO, Blackwell M (1999) Molecular phylogeny of the cleistothecial fungi placed in Cephalothecaceae and Pseudeurotiaceae. *Mycologia* 91:836–848
- Takamatsu S, Hirata T, Sato Y (1998) Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae). *Mycoscience* 39:441–453
- Takamatsu S, Hirata T, Sato Y (2000) A parasitic transition from trees to herbs occurred at least twice in tribe Cystothecae (Erysiphaceae): evidence from nuclear ribosomal DNA. *Mycol Res* 104:1304–1311
- Webster J (1980) *Introduction of fungi*, 2nd edn. Cambridge University Press, Cambridge
- Yarwood CE (1957) Powdery mildews. *Bot Rev* 13:235–301
- Yarwood CE (1973) Pyrenomycetes: Erysiphales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) *The fungi: an advanced treatise*, vol IV A. Academic Press, New York, pp 71–86
- Yarwood CE (1978) History and taxonomy of the powdery mildews. In: Spencer DM (ed) *The powdery mildews*. Academic Press, New York, pp 1–37
- Zeller KA (1995) Phylogenetic relatedness within the genus *Erysiphe* estimated with morphological characteristics. *Mycologia* 87:525–531
- Zeller KA, Levy M (1995) Intraspecific differentiation in the powdery mildew *Erysiphe cichoracearum* determined with rDNA RFLPs. *Mol Ecol* 4:277–283