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Review

Origin and evolution of the powdery mildews (Ascomycota, Erysiphales)

*Susumu Takamatsu**

Department of Bioresources, Graduate School, Mie University, 1577 Kurima-Machiya, Tsu 514-8507, Japan

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ABSTRACT

Molecular phylogeny suggests a close relationship of Asteraceae to the early evolution of *Golovinomyces*. The family Asteraceae, with a geographic origin in South America, expanded into the Northern Hemisphere, where it may have been infected by an ancestor of *Golovinomyces*, thus starting a close host–parasite relationship. Using this event as a calibration point, we designed molecular clocks for powdery mildews using the 28S rDNA D1/D2 and internal transcribed spacer (ITS) regions. According to these clocks, the powdery mildews originated in the Late Cretaceous and the first radiation of the major lineages occurred at the Cretaceous/Paleogene boundary. Ancestral powdery mildews may have first radiated on broad-leaved deciduous trees in the high latitudes of the Northern Hemisphere, and continued further speciation whilst migrating to southward during the world cooling in the Paleogene and Neogene periods. The cradle of four herb infecting genera, viz. *Blumeria*, *Golovinomyces*, *Leveillula*, and *Neoërysiphe* may be within the area extending from Central/West Asia to the Mediterranean.

1. Introduction

Powdery mildews are an important group of plant pathogenic fungi forming a white, powdery film on leaves, stems, flowers, and fruits of Angiosperms. Sixteen genera and ca 900 species are presently known worldwide (Braun and Cook 2012). All of the species are exclusively obligate biotrophs of plants. Molecular phylogenetic analyses so far reported indicate that the powdery mildews form a distinct monophyletic group (Mori et al., 2000a,b; Wang et al., 2006a,b). They may be a fungal group that acquired an obligate biotrophic nature only once in their ancestry and have retained it ever since. Up to 9838 plant species have been recorded as hosts of powdery mildews, all of which are Angiosperms but not Gymnosperms or Ferns (Amano 1986). Of the total host species, 9176 are dicotyledons, the remaining 662 being monocotyledons. Of the monocotyledons, 634 species belong to the subfamily Pooideae of Poaceae (the grass family).

Several scientific speculations on phylogenetic relationships and evolution of powdery mildews based on morphology and host relationships have been published (Neger 1901; Arnaud 1921; Raymond 1927; Blumer 1933; Katumoto 1973; Braun 1987, 1995). Most of these arguments simply devolved around characteristics thought to be primitive or derived. Some critical characteristics are as follows: (1) number of asci in an ascoma, e.g. one or several; (2) number of ascospores in an ascus, e.g. eight or fewer; (3) morphology of appendages; (4) conidiogenesis, conidia maturing in chains,

* Tel.: +81 59 231 9497; fax: +81 59 231 9540.
E-mail address: Takamatu@bio.mie-u.ac.jp.

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catenescent (*Euoidium*-type) or maturing one at a time (*Pseudoidium*-type); (5) mycelium, ectotrophic or endotrophic. Of these, most scientists consistently regarded eight-spored asci and mycelioid appendages as primitive characters. These arguments were reviewed in Braun (1987, 1995) and Braun and Cook (2012).

Hirata (1966) and Amano (1986) constructed a comprehensive, worldwide list of powdery mildews and their host plants based on nearly 4000 references. It consists of 14,647 powdery mildew/host combinations, and the countries where they were recorded (Amano 1986). This list has been utilized by many scientists as a comprehensive database of powdery mildews and their hosts. The biogeography, phylogeny and evolution of the powdery mildews were discussed with reference to the data (Hirata 1968, 1969, 1971a,b, 1972, 1975, 1976, 1980; Amano 1986, 1992, 2002). These papers provide a unique insight into these subjects because other scientists have mostly based such arguments solely on morphology. Since previous studies on molecular phylogeny of the powdery mildews have already been reviewed in Braun et al. (2002), Takamatsu (2004), Glawe (2008) and Braun and Cook (2012), this review is focused on two other aspects, viz. evolutionary dating and geographic origin of the powdery mildews.

2. Evolutionary dating of powdery mildews

The evolution of an organism is mostly driven by the Earth's environmental changes and the concurrent evolution of other organisms. Host plants are an absolute prerequisite for the survival of an obligate biotroph like a powdery mildew. So we cannot discuss the evolution of powdery mildews without relating them to the evolution of their hosts. Thus Earth's environmental changes influence the evolution of powdery mildews either directly or indirectly via their effects on host plants. Therefore, an estimation of the date of evolutionary events is essential to discuss the evolution of powdery mildews in relation to their hosts and environmental factors.

2.1. *Fossil record of powdery mildews*

Whilst fossil records are commonly used as direct evidence of evolution, they are relatively rare in fungi compared with those of animals and plants and there is no reliable fossil record, especially for powdery mildews (Braun and Cook 2012). According to a summary of fungal fossils by Tiffney and Barghoorn (1974), so far four records have been listed, of which the most reliable, named *Protoascon missouriensis* was discovered in the mid Pennsylvanian Epoch of the Permian Period in strata laid down ca 300 million years ago (Ma) (Batra et al. 1964). However, subsequent studies re-interpreted this record as a kind of zygomycete fungus (Pirozynski 1976; Taylor et al. 2005). Stubblefield and Taylor (1983) re-investigated the fossil *Traquairia* found in coal balls of the same epoch, and reported that it is most similar to the Eurotiales or Erysiphales. However, as far as I can see from the photo, there is no strong evidence to consider it a member of the Erysiphales. A comprehensive recent overview on fossil fungi was published by Taylor and Krings (2005).

2.2. *Speculation on evolutionary dating in the absence of a molecular clock*

Since all members of powdery mildews are obligate biotrophs of plants and their hosts are restricted to Angiosperms, the appearance of a typical powdery mildew cannot pre-date the Angiosperms. Also, since the monocotyledonous hosts are mostly restricted to the subfamily Pooideae of Poaceae, it is likely that a dicot infecting powdery mildew spread to an ancestor of Pooideae after the monocot/dicot split. From these assumptions it may be possible to speculate the earliest origin of powdery mildews.

The origin of Angiosperms based on the molecular clock is not congruent with the estimates of fossil ages. Calibration by molecular clock suggests Angiosperms first appeared in the Early-Middle Jurassic Period [179–158 Ma] (Wikström et al. 2001). On the other hand, fossil records indicate that Angiosperms first appeared in northern Gondwana during the Early Cretaceous Period, approximately 135 Ma (Barrett and Willis 2001). The oldest fossil of a monocot was found in the United States from Late Cretaceous sediments [90 Ma] (Gandolfo et al. 1998). The molecular clock suggests that monocots have originated in Late Jurassic (Wikström et al. 2001). Based on these reports, it is unlikely that powdery mildews originated earlier than the Jurassic Period.

2.3. *Design of molecular clocks for powdery mildews*

Mori et al. (2000b) determined the nucleotide sequences of the 18S and 28S rDNA for ten powdery mildew species and used them to determine the phylogenetic placement of powdery mildews in the Ascomycota. They used the molecular clocks of 18S rDNA reported by Simon et al. (1993) (0.667% per lineage per 100 Ma) and Berbee and Taylor (1993) (1.0% per lineage per 100 Ma). Simon et al. (1993) used the monocot/dicot split [200 Ma] as a calibration point. However, subsequent studies estimate the split to have occurred later than 200 Ma (Gandolfo et al. 1998; Wikström et al. 2001). Thus, the molecular clock of Simon et al. (1993) may be too slow. It is noteworthy that Berbee and Taylor used several fossil records as calibration points for their molecular clock that was later changed to 1.26% per 100 Ma based on a re-assessment of fossil records (Berbee and Taylor's 2001), and so their molecular clock may be more reliable than that of Simon et al's. 1993 clock. Berbee and Taylor's 1993 molecular clock and 18S rDNA sequences estimated the split of powdery mildews from Myxotrichaceae (putative sister group of powdery mildews) as 127 ± 24 Ma, and the first divergence within the powdery mildews as 92 ± 24 Ma, both of which suggest powdery mildews originated in the Cretaceous Period. Berbee and Taylor's 2001 clock (1.26% per 100 Ma) resulted in the split and divergence dates of 101 and 73 Ma, respectively, still suggesting a cretaceous origin of powdery mildews (Fig. 1).

The maximum genetic divergence of the 18S rDNA within the powdery mildews was only 1.85% and the maximum substitutions were only 37 bases. This rate of change of 18S rDNA may be too slow to calibrate evolutionary dating within powdery mildews. Furthermore, Berbee and Taylor's 2001 clock did not include fossil records of powdery mildews, and there is no evidence that their 18S rDNA evolves at the same

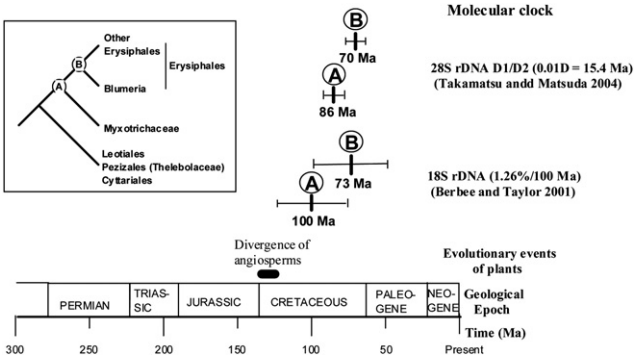

Fig. 1 – Estimated dates of origin and divergence of the powdery mildews based on the nucleotide sequences of the 28S rDNA D1/D2 region and 18S rDNA. A is the splitting of the Erysiphales and Myxotrichaceae, and B is the first radiation within the Erysiphales. Ma: million years ago.

rate as other fungal groups. Therefore, it was necessary to design a molecular clock of DNA regions that evolve faster than 18S rDNA, i.e. the variable region of 28S rDNA or internal transcribed spacer (ITS) region, using a revised calibration point for powdery mildews.

A molecular clock needs at least one calibration point and there are three options for obtaining it using: 1) fossil records, 2) geological events like the breakup of a continent, orogeny, the appearance of a big river, etc., 3) co-speciation of hosts and parasites. The first option may be impossible at present because there is no reliable fossil record of powdery mildews as mentioned above. Whilst the second option may be effective for organisms lacking long distance mobility like *Damaster* ground beetles (Su et al. 1998), powdery mildews may travel longer distances by means of air-borne spores. Although it is not clear exactly how efficient long distance dispersal is with powdery mildews, there is a report that the cereal powdery mildew, *Blumeria graminis*, has flown about 650 km from England to Denmark (Hermansen et al. 1978). In the United States, conidia of *Golovinomyces cichoracearum* on lettuce flew 190 km northward from Salinas Valley, California (Schnathorst 1959). Therefore, there is some doubt about the validity of using the second option due to powdery mildews being able to surmount certain physical barriers. The third option as used for the relationships with long-term co-speciation such as insects and their endosymbiotic bacteria (Moran et al. 1993) may, however, be applicable. In powdery mildews the first candidate for studies on co-speciation was *B. graminis* and its grass hosts (subfamily Pooideae). As stated above, the present form of *B. graminis* probably originated after the first appearance of the grasses, i.e. due to infection of an ancestor of the subfamily. If co-speciation between *B. graminis* and its hosts started after this infection, it may be possible to estimate calibration points based on this relationship. However, this approach proved difficult as Japan has only a few grass hosts. Meanwhile, phylogenetic analyses of *Golovinomyces* revealed that all clades situated at the base of the tree were occupied by isolates from asteraceous hosts, and isolates from single tribes of Asteraceae occupied several basal clades (Matsuda and Takamatsu 2003). This result suggests a close affinity of asteraceous hosts with *Golovinomyces* in early evolution. The branching order of the *Golovinomyces* tree was similar, although not identical, to the branching order of the tribes of the Asteraceae, suggesting co-speciation between *Golovinomyces* and Asteraceae. The geographic origin of the Asteraceae was in South America from whence they expanded into the Northern Hemisphere. All the asteraceous hosts used in Matsuda and Takamatsu (2003) belong to tribes that diverged in the Northern Hemisphere. It is possible that the ancestor of *Golovinomyces* first infected asteraceous hosts in South America making this region the origin of the genus. There is a report that *Oidium mutisiae*, being a *Golovinomyces*-like anamorph, occurs on two *Mutisia* species of the tribe Mutisieae of the Asteraceae in Argentina (Havrylenko 1993). Mutisieae is a tribe mainly distributed in South America, but is sister to a group of tribes that radiated in the Northern Hemisphere (Bremer 1994). If an ancestor of *Golovinomyces* infected an asteraceous host before the Asteraceae expanded into the Northern Hemisphere, the *Oidium* on *Mutisia* should occupy the very base of the *Golovinomyces* tree. However, the phylogenetic analyses gave us an unexpected result (Takamatsu et al. 2006). The powdery mildew isolates on *Mutisia* were not placed at the base of *Golovinomyces*. Instead, they nested in clades consisting of isolates from the Northern Hemisphere and furthermore, they separated into two different clades, indicating that the isolates from each *Mutisia* species were derived from different ancestors. This result suggests that an ancestor of *Golovinomyces* occurring in the Northern Hemisphere first infected Asteraceae after the family had migrated to the Northern Hemisphere and before it split off the Cardueae, the first tribe radiated in the Northern Hemisphere (Takamatsu et al. 2006). Then *Golovinomyces* may have migrated from the Northern into the Southern Hemisphere and infected *Mutisia* on two separate occasions. If the timing of the divergence of the Cardueae was known, it would provide a calibration point for the design of a molecular clock for powdery mildews.

Unfortunately, there was no report of the timing of this divergence. Therefore, we used the molecular clock of the *rbcL* gene (Bremer and Gustafsson 1997), and this suggested that the Cardueae split from other asteraceous tribes 25.2 Ma (Takamatsu and Matsuda 2004). This result was congruent with the report of Kim et al. (1998) that the Asteraceae underwent an explosive radiation and dispersal during or near the transition between the Oligocene and Miocene Epochs [ca 23 Ma]. We used this date in the *Golovinomyces* trees constructed by sequences from the ITS region including the 5.8S rDNA, and the 5′ end of the 28S rDNA including the D1 and D2 regions (Takamatsu and Matsuda 2004). When the node of the first split was dated 25.2 Ma, the nucleotide substitution rates were found to be $2.52 \pm 0.11 \times 10^{-9}$ per site per year [0.01D = 3.97 Ma; D = genetic distance calculated by the Kimura's two-parameter model (Kimura 1980)] in the ITS region and $6.5 \pm 0.4 \times 10^{-10}$ per site per year (0.01D = 15.4 Ma) in the D1 and D2 regions of the 28S rDNA (Takamatsu and Matsuda 2004). Using this clock, date of the first radiation of the major tribe was estimated to be ca 70 Ma (Fig. 1). Based on complete sequences of the 18S rDNA and the molecular clock (1.26% per 100 Ma) reported by Berbee and Taylor (2001), the first radiation of the major tribe was dated ca 73 Ma, which agrees well with the date calculated by our molecular clock. Oberhaensli et al. (2011) calculated the

emergence of *B. graminis* on wheat and barley using a molecular clock of a transposable element. The result was in good agreement with our calculation.

2.4. *Evolutionary dating using molecular clocks of powdery mildews*

Calculations of evolutionary dating using the molecular clocks of 28S rDNA (Takamatsu and Matsuda 2004) and 18S rDNA (Berbee and Taylor 2001) suggested that the split of powdery mildews and Myxotrichaceae occurred in the Middle Cretaceous Period [ca 100 Ma] and first split within powdery mildews occurred in the Late Cretaceous [90–80 Ma] (Takamatsu 2004; Takamatsu et al. 2005). This dating is clearly later than the oldest angiosperm fossils [ca 135 Ma], supporting the speculation that powdery mildews infected Angiosperms after they split from the Gymnosperms. Radiation of the five major lineages of the Erysiphaceae occurred in the Cretaceous/Paleogene boundary [70–58 Ma] (Takamatsu and Matsuda 2004). Radiation of herb-parasitic powdery mildews began in the Miocene Epoch of the Neogene Period with the first radiation within *Golovinomyces* and *Neoërysiphe* [25–10 Ma] (Takamatsu et al. 2008a). This was followed by the formation of the subsection *Magnicellulatae* (formerly *Sphaerotheca fuliginea*) of *Podosphaera* section *Sphaerotheca* in the Middle Miocene [15–10 Ma] (Takamatsu et al. 2010), and by the first split within *Leveillula* in the Pliocene [≤5 Ma] (Takamatsu et al. 2008b).

The global cooling of the Earth and an increase in the arid area followed Alpine and Himalayan orogenies caused an increasing area of steps containing grasses and asteraceous herbs (Tiffney 1985a,b; Tiffney and Manchester 2001; Takahashi 2006). This may have triggered the transfer of powdery mildews to herbaceous plants and the divergence of these herb-parasitic species. Thus, the estimation of evolutionary dating using the molecular clock suggests that the evolution of powdery mildews is closely related to the changes in the Earth's environment and its flora.

3. Origin of powdery mildews

3.1. *Closest relatives of powdery mildews*

A survey of closest relatives is important for speculation on the origin of powdery mildews. Powdery mildews have previous often been placed in Plectomycetes based on their cleistothecial ascomata, i.e. closed fruiting bodies 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 have been used to classify powdery mildews as Pyrenomycetes (Yarwood 1973, 1978; Alexopoulos and Mims 1979). Saenz et al. (1994) pointed out that powdery mildews belong to neither Pyrenomycetes nor Plectomycetes based on phylogenetic analysis of 18S rDNA sequences. Instead, they reported a close relationship between *B. graminis* and *Sclerotinia sclerotiorum* (Leotiales). Based on closed, non-ostiolate ascomata as in cleistothecia, but with asci arranged in hymenial arrangement as in perithecia as well as the phylogenetic position distant of true Plectomycetes and Pyrenomycetes, the new term chasmothecium was introduced for ascomata of powdery mildews (Braun et al. 2002; Braun and Cook 2012). Sugiyama et al. (1999) reported that the Myxotrichaceae has its own lineage distantly related to the other onygenalean fungi and closely related to the Leotiales and Erysiphales. Mori et al. (2000b) confirmed the report of Sugiyama et al. (1999) by using newly determined 18S and 28S rDNA sequences from 10 powdery mildew species. Wang et al. (2006a,b) reported that *Chlorociboria* (Helotiaceae) and *Cyttaria* (Cyttariaceae) are the closest relatives of powdery mildews based on combined data of 18S, 28S and 5.8S rDNA. Peterson and Pfister (2010) conducted a phylogenetic analysis of the Leotiomycetes including powdery mildews using combined data of five DNA regions including *EF1*-alpha gene. In this analysis, *Cyttaria* and *Chlorociboria* were near to the powdery mildews but not the closest relatives. Instead, Myxotrichaceae and *Pleuroascus* (Pseudeurotiaceae) proved to be the closest relatives.

Cyttaria, an obligate pathogen of plants, has *Nothofagus*, a Gondwanan plant, as host and is distributed only in the Southern Hemisphere. The species of *Cyttaria* have been in the focus of evolutionary biologists since Charles Darwin in terms of co-evolution with *Nothofagus* and their biogeography (Peterson et al. 2010). Although it is interesting that powdery mildews are closely related to *Cyttaria*, it may be difficult to find direct morphological affinities between *Cyttaria* and powdery mildews. Since *Chlorociboria* is a fungal group to produce typical apothecia, it is also difficult to find common morphological characteristics with powdery mildews. On the other hand, both Myxotricaceae and *Pleuroascus* produce cleistothecia (ascomata without ostiole) similar to the chasmothecia of powdery mildews. In addition, some members of *Myxotrichum* have appendages with circinate tips (Alexopoulos et al. 1996), which are similar to the uncinuloid appendages of some powdery mildews. Members of the Myxotricaceae have cellulolytic characters and colonize plant debris (Sugiyama et al. 1999; Tsuneda and Currah 2004). It is known that powdery mildews also encode cellulase genes (Pryce-Jones et al. 1999). Thus, it might be possible that an ancestral fungus colonizing on plant debris has obtained biotrophic ability, leading to obligate parasitism. Tsuneda and Currah (2004) reported that ascomata of *Myxotrichum arcticum* bear a striking resemblance to apothecia in morphogenesis, supporting the derivation of the Myxotrichaceae from a helotialean ancestor. Detailed morphogenetic observations of powdery mildews might support the results of molecular phylogenetic analyses.

3.2. *Geographic origin of powdery mildews*

Publications discussing the geographic origin of powdery mildews are very rare. Heluta (1992) speculated southern China as the geographic origin. Unfortunately, I do not have sufficient data to evaluate his speculation. Thus, it is at present only possible to speculate on geographic origin of powdery mildews below.

3.2.1. *Physical geography, climate and flora in Tertiary (Paleogene and Neogene periods)*

Physical geography, climate and flora in the Tertiary Period (65.5–2.6 Ma, now split into Paleogene and Neogene periods of

the Cenozoic Era), are described below using the following publications: Tiffney (1985a,b; 2000); Wen (1999); Manchester (1999); Tiffney and Manchester (2001); Takahashi (2005, 2006). These references can be consulted to obtain more detailed information.

The Mesozoic/Cenozoic boundary [65.5 Ma] is well known as the point of extinction of the dinosaurs. However, many other organisms also became extinct at this time. The Cenozoic Era is the period of mammals and Angiosperms. It begins with the Paleogene Period containing three successive epochs, Paleocene, Eocene and Oligocene. The Paleocene Epoch started with a warm climate, which continued to the Early Eocene Epoch [ca 50 Ma]. Tropical and subtropical flora extended to lat. 65°N. Broadleaf deciduous trees like the genera *Alnus, Betula, Quercus, Juglans, Populus*, and *Acer* were distributed in the Arctic area at latitude above 70°N (Takahashi 2006).

A climate deterioration involving a decrease in world temperature and an increase in seasonality occurred during the Late Eocene [37.2–33.9 Ma] or at the Eocene–Oligocene boundary [33.9 Ma] (Tiffney 1985b). Thermophilic and evergreen taxa became less common and restricted to favourable sites in much of Europe and North America. Temperate forests with evergreen and deciduous trees were distributed in Eurasia, North America and North Africa in the Oligocene [33.9–23.0 Ma] (Takahashi 2005). The genera *Carya, Liquidambar, Cercidiphyllum, Alnus, Corylus, Carpinus, Nyssa, Quercus*, and *Ulmus* were the major components of the temperate forests.

Towards the boundary with the Neogene Period which contains the Miocene and then the Pliocene Epochs, a gradual warming that extended into the Early Miocene, led to the expansion of several evergreen and thermophilic lineages in Europe and coastal margins of North America. Starting in the middle Miocene [ca 15 Ma], cooling returned and progressed stepwise into the Quaternary Period containing the Pleistocene Epoch and the ice ages (Tiffney and Manchester 2001). This led to sequential modernization of the flora in Europe and North America, involving the spread of deciduous trees and herbs and the loss of thermophilic, evergreen elements (Tiffney and Manchester 2001). Back in the Miocene Epoch of the Neogene Period in Western and Central Asia, a temperate flora evolved at the southern end of the Turgai Depression near the Paratethys, while to the east in Kazakhstan, a series of great lakes developed, surrounded by mesic (moderately moist) vegetation of *Pterocarya, Betula, Alnus, Salix, Nyssa*, and so forth. Adjacent Alpine and Himalayan orogenies shut off the southern source of moisture, allowing these areas to become arid, further isolating forest communities and contributing to the change in climate and vegetation in Central Asia and to the north (Tiffney and Manchester 2001). In the central and northern Turgai Depression, the diversity of Angiosperms decreased during the Miocene Epoch. Open vegetation began to appear in the area by the Early Miocene [23–16 Ma], and by the Early Pliocene Epoch [5.3–3.6 Ma], the area was a mosaic of deciduous forests (*Tilia, Quercus, Carpinus, Cotylus*) and open steppe dominated by grasses, Asteraceae, and Chenopodiaceae (Tiffney and Manchester 2001).

The Bering land bridge (BLB) has connected north-eastern Asia and north-western North America several times since the Mesozoic Period (Wen 1999; Tiffney 2000; Tiffney and Manchester 2001). The latitude of the BLB in the Paleogene Period [65.5–23 Ma] was approximately 75°N, then moving to its present latitude (ca 65°N) as the Paleogene progressed and North America rotated counterclockwise. During the Miocene Epoch [23–5.3 Ma] of the Neogene Period, the BLB was suitable for the exchange of temperate deciduous plants, such as *Castanea, Juglans, Magnolia*, and *Quercus*, and remained available for floristic exchanges until about 3.5 Ma (Wen 1999; Tiffney 2000; Tiffney and Manchester 2001).

The North Atlantic land bridge (NALB) was connected to Europe in the Paleogene Period via Greenland, with a northern connection in the high Arctic region and a southern connection through southern Greenland. The latitude of the southern route was approximately 60°N, thus further south than the BLB. Floristic migration via the NALB was possible during the Paleocene and Eocene Epochs [65.5–34 Ma], the next epoch in the Paleogene Period (Wen 1999; Tiffney 2000; Tiffney and Manchester 2001).

The Turgai Straits separated Europe from Western Siberia from the mid-Mesozoic Era to the end of the Eocene Epoch [ca 34 Ma] in the Paleogene Period and are generally presumed to have formed a biogeographic barrier to animals (Tiffney 1985b; Tiffney and Manchester 2001). It clearly separated European and Asian Faunas until its demise in the Oligocene [34–23 Ma].

3.2.2. *Northern Hemisphere origin or Southern Hemisphere origin?*

When discussing the origin of powdery mildews, it is important to consider the geographic origin of Angiosperms due to the close host/parasite relationships. Although there are several different hypotheses as to the origin of Angiosperms, North Gondwana in the Early Cretaceous Period (ca 135 Ma) may be the most likely (Barrett and Willis 2001; Takahashi 2006).

The genus *Uncinula* (now *Erysiphe* sect. *Uncinula*) has a relatively old origin among powdery mildew genera (Mori et al. 2000a). In the monograph of Braun (1987), 86 *Uncinula* species are listed. Most of them (54 species) are distributed in East Asia and only 10 in South America, of which 8 are endemic to South America. Four species of *Erysiphe* sect. *Uncinula* have been recorded on *Nothofagus* (Braun 1987; Havrylenko and Takamatsu 2003; Meeboon and Takamatsu 2012), which is a typical Gondwanan genus, having a relatively old origin within broad-leaved deciduous trees. Three of the *Erysiphe* sect. *Uncinula* species parasitic to *Nothofagus*, *E. nothofagi*, *E. patagoniaca* (Havrylenko and Takamatsu 2003), and *E. havrylenkoana* (Meeboon and Takamatsu 2012) have unique appendages spiral coiling like a spring, which are unknown in powdery mildews of the Northern Hemisphere. Assuming genuine co-speciation between Angiosperms and powdery mildews, these three species are candidates for an old origin. To address this possibility, Niinomi et al. (2008) determined rDNA sequences for the *Nothofagus* powdery mildews. The sequences were compared in a phylogenetic analysis with those from Northern Hemisphere species of *Erysiphe* sect. *Uncinula*. Surprisingly, the sequences from the powdery mildews on *Nothofagus* were not placed at the base of the tree, instead they were closely related to those of the Northern Hemisphere (Niinomi et al. 2008), indicating that they are not ancestral in the Erysiphales. An ancestor of the

species may have infected *Nothofagus* after migrating into South America from the Northern Hemisphere after the breakup of the Gondwana continent. This possibility is supported by the fact that *Uncinula*-like *Erysiphe* species on *Nothofagus* are reported only from South America, and not from Australia, New Zealand, or New Guinea. The co-evolution of *Golovinomyces* and Asteraceae described above also does not support a Southern Hemisphere origin of *Golovinomyces* as well (Takamatsu et al. 2006).

Hirata (1972) surveyed host species of powdery mildews and their distribution for 167 angiosperm families excluding small families composed of less than 30 species and the families which have special nutrient requirements, and/or an ecological habit making them parasitic, insectivorous or aquatic. It was shown that all 13 families distributed only in the Northern Hemisphere were host to a powdery mildew. Moreover, the most popular host families, the Salicaceae, Betulaceae, Brassicaceae, Geraniaceae, and Aceraceae, belonged to this distribution type. Of the 52 families endemic to tropical areas, only 23 (44%), were hosts and of the seven families endemic to the Southern Hemisphere, only two (29%) were hosts. When a similar investigation was done with species of plants belonging to families confined to the Northern Hemisphere, 11.8% were hosts. By contrast, only 0.4% and 0.1% of the species confined respectively to tropical areas and the Southern Hemisphere were hosts. Therefore, powdery mildews frequently infect plants found in the Northern Hemisphere, but are less frequent on tropical plants and those in the Southern Hemisphere. Taking into consideration these uneven distributions of powdery mildews and the papers cited above, a Southern Hemisphere or tropical origin of powdery mildews seems unlikely.

3.2.3. *Cradle of powdery mildews*

Because powdery mildews are obligate biotrophs of plants, their niche is strictly confined to the surface of living plants. Geographic distributions of the early hosts of powdery mildews in the Paleogene Period when their first radiation occurred may provide a base to speculate on the location of the cradle of these pathogens. Powdery mildews are divided into five large lineages (tribes) and two basal genera, *Parauncinula* and *Caespitotheca* (Mori et al. 2000a). Of the five tribes, three, viz. Erysipheae, Phyllactinieae, and Cystotheceae, include both tree-parasitic and herb-parasitic taxa, whereas the remaining two tribes, viz. Blumerieae and Golovinomyceteae, include only herb-parasitic taxa with only a few exceptions. In the first-mentioned three tribes, tree-parasitic taxa usually occupy basal positions in the lineages and herb-parasitic species a higher (derived) position. In addition, both the two totally basal genera are tree-parasitic. This evidence suggests that powdery mildews were originally tree-parasitic and subsequently expanded their host ranges to herbaceous plants (Takamatsu et al. 2000; Mori et al. 2000a; Takamatsu 2004). This conforms well to the evolutionary history of Angiosperms (Tiffney and Manchester 2001; Takahashi 2006). Among tree-parasitic taxa, those with teleomorphs (chasmothecia) having uncinate-circinate appendage tips (uncinuloid type) usually occupy the very base of the respective lineages, and the two basal genera, *Parauncinula* and *Caespitotheca*, also have uncinuloid appendages. Thus, among the tree-parasitic taxa, those having uncinuloid appendages may be the most ancestral.

In the tribe Cystotheceae, *Sawadaea*, which also has uncinuloid appendages, occupies the basal position. So, Aceraceae, a main host family of *Sawadaea*, may be one of the early host families of powdery mildews. The base of the tribe Phyllactinieae is occupied by *Pleochaeta* and *Queirozia*, both with uncinuloid appendages. Therefore, Ulmaceae, a main host family of *Pleochaeta*, may be an additional early host family. Although the host family of *Queirozia* is Fabaceae, it is not likely that this represents an early host family of powdery mildews. Aceraceae and Ulmaceae are also the main host families of *Erysiphe* sect. *Uncinula* (previously the genus *Uncinula*). This section comprises hosts belonging to 39 host plant families (Amano 2002), most of them are members of the families Salicaceae, Betulaceae, Fagaceae, Moraceae, Aceraceae, Vitaceae, and Anacardiaceae. In addition, taxa occurring on *Liquidambar* (Hamamelidaceae) and *Lagerstroemia* (Lythraceae) occupy the very base of *Erysiphe* sect. *Uncinula*. It is noteworthy that all involved plant families and/or genera encompass broad-leaved deciduous trees.

Calibration by molecular clock suggested that powdery mildews originated in the Late Cretaceous Period and the major lineages diverged at the Cretaceous/Paleogene Period boundary [70–57 Ma] (Takamatsu and Matsuda 2004). The temperature of the Earth was warmer than present time from this boundary to Early Eocene [ca 50 Ma], and broad-leaved deciduous trees, the early hosts of powdery mildews, were distributed in the Arctic area at a latitude higher than 70°N (Tiffney 1985b; Manchester 1999; Tiffney and Manchester 2001; Takahashi 2005, 2006). Therefore, it is likely that the early divergence of powdery mildews occurred in this region. A climatic deterioration involving a decrease in world temperature and an increase in seasonality started to occur from the Middle Eocene [ca 50 Ma] and continued until the Glacial Ages in the Pleistocene Epoch of the Quaternary Period [ca 2.6 Ma]. This may have resulted in a southward migration of deciduous trees together with their associated powdery mildews. These climatic as well as geological changes in the Neogene Period may have triggered the further divergence of powdery mildews.

3.2.4. *The basal genera* Parauncinula *and* Caespitotheca

Parauncinula is a small genus consisting of only two species, *P. septata* and *P. curvispora*. Both species infect Fagaceae and their distributions are restricted to East Asia (Japan and China). The monotypic genus *Caespitotheca* is only known in tropical Argentina on *Schinopsis* (Anacardiaceae) (Braun and Cook 2012). One possible scenario to explain why these two basic genera are isolated in narrow areas of two different continents may be as follows. Ancestors of these genera originated in high latitudes of the Northern Hemisphere. They then accompanied the southward migrations of their hosts and having survived many extinction events, *Parauncinula* and *Caespitotheca* now remain as relict distribution in East Asia and Argentina, respectively.

3.2.5. *Comparisons of powdery mildew distributions among Europe, North America, Central & West Asia, and East Asia*

Hirata (1971b) divided the Northern Hemisphere into four regions, viz. Europe, North America, East Asia, and Central &

West Asia, and counted the number of powdery mildews which occur in two of the regions but are not found in the other two regions. He found that the distribution in Europe was closely related to that in North America and Central & West Asia, but not to that in East Asia. However, the distribution in North America was somewhat related to East Asia (Fig. 2). From these results, he concluded that the distribution in East Asia is unique in the Northern Hemisphere and that of North America is intermediate between Europe and East Asia. This uneven distribution of powdery mildews in the Northern Hemisphere may be partly explained by the formation of Turgai Strait, the NALB and BLB. Until end of the Paleogene Period, Europe and East Asia were separated by the Turgai Strait which may have barred the exchange of powdery mildews and their host plants. On the other hand, the NALB and BLB may have allowed the exchange of powdery mildews between Europe and North America, and between North America and East Asia, respectively.

Heluta et al. (2010) conducted molecular phylogenetic analyses of *Neoërysiphe*. Firstly, a *N. galeopsidis* clade split off from a clade composed of other *Neoërysiphe* species. The latter clade then split into a clade of *Neoërysiphe* spp. confined to Europe and a clade of those distributed in North & South America (Fig. 3). Then, the latter clade split off *N. hiratae* now distributed in East Asia. The following scenario may be postulated. *Neoërysiphe* spp. except for *N. galeopsidis* split across the NALB into a European and a North American group. Then, a part of the North American group migrated into East Asia via the BLB. However, an estimation of dating by molecular clock suggests that these events occurred after the disappearance of the Turgai Strait (Takamatsu et al. 2008a). Therefore, there might have been additional barriers for plant exchange like large arid areas between Europe and East Asia after the disappearance of the Turgai Strait.

According to Hirata (1969, 1971b) and Amano (1986), the ratio of tree-parasitic to herb-parasitic taxa is high in East Asia

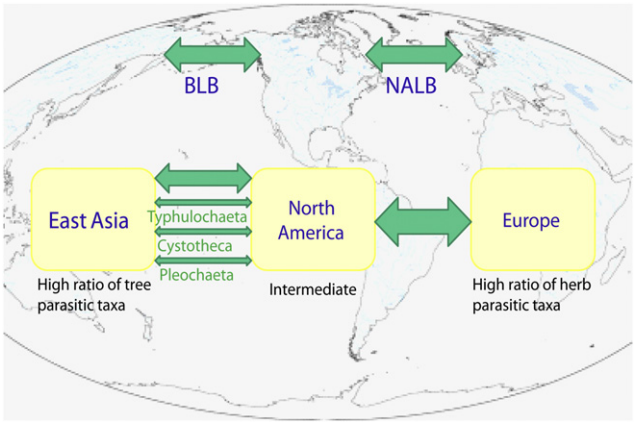

Fig. 2 – Schematic diagram showing comparisons of powdery mildew flora among East Asia, North America, and Europe. East Asia was isolated from Europe by the Turgai Strait during the Paleogene Period. Europe and East Asia were connected to North America by the North Atlantic Land Bridge (NALB) and the Bering Land Bridge (BLB), respectively, in the Paleogene Period.

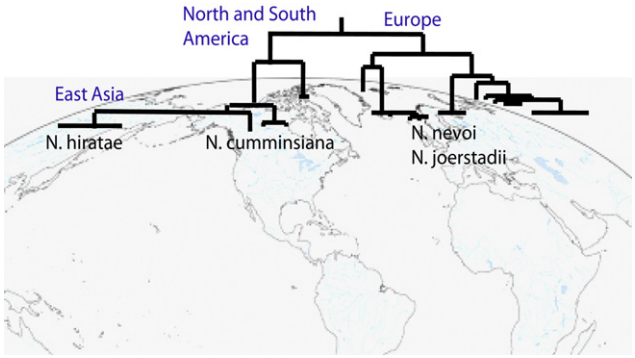

Fig. 3 – Phylogenetic tree of *Neoërysiphe* except for *N. galeopsidis*. European group split from the North & South American group. Then, East Asian group split from a part of the North & South American group.

(Fig. 2). On the other hand, herb-parasitic taxa are more common in Europe and Central & West Asia than in East Asia. These two main groups based on host ranges, viz. the tree-parasitic and herb-parasitic groups of genera, were recognized in the taxonomic system of Braun (1987). For instance, *Cystotheca, Podosphaera, Microsphaera, Uncinula, Typhulochaeta, Pleochaeta*, and *Phyllactinia* are tree-parasitic, whilst *Blumeria, Erysiphe s. l.* (containing *Golovinomyces* and *Neoërysiphe*), *Leveillula*, and *Sphaerotheca* are herb-parasitic. The number of host species was calculated using Table 4 of Amano (1986) based on the assumption that all hosts of tree-parasitic genera and herb-parasitic genera were actually trees and herbs, respectively. The ratio of tree hosts was 34.0%, 31.0%, and 9.8% in East Asia, North America, and Europe, respectively. When the ratio of tree hosts was calculated for each powdery mildew genus, the ratio of hosts of *Uncinula* was about two times higher in East Asia than North America. On the other hand, the ratio of hosts of *Microsphaera* was higher in North America than in East Asia.

In East Asia, 51 *Uncinula* species infect 28 host genera containing 171 species. In North America, on the other hand, only eight *Uncinula* species infect seven host genera containing 90 species. Likewise in Europe, only six *Uncinula* species infect six genera containing 88 species. Therefore, both number of *Uncinula* species and number of hosts are much more abundant in East Asia than in either North America or Europe. A similar but less conspicuous tendency is also found in *Phyllactinia*. Since tree-parasitic genera generally have an origin older than that of herb-parasitic genera, the above results suggest that powdery mildews of older origin have flourished in East Asia whilst those of more recent origin have flourished in Europe. In this respect, North America is intermediate between the two regions.

Powdery mildew genera can also be divided into two other groups, viz. genera with many species, many hosts and wide distributions, and genera with small numbers of species and hosts, and narrow distributions. The former group consists of *Podosphaera, Microsphaera, Uncinula, Phyllactinia, Erysiphe s. l.* (containing *Golovinomyces* and *Neoërysiphe*), *Leveillula*, and *Sphaerotheca*, and the latter includes *Cystotheca, Pleochaeta, Typhulochaeta, Brasiliomyces, Parauncinula*, and *Caespitotheca*.

Because *Brasiliomyces* is a polyphyletic genus it is excluded from the subsequent consideration. It is noteworthy that all genera belonging to the latter group are tree-parasitic. *Cystotheca*, *Pleochaeta*, and *Typhulochaeta* are distributed in Asia and North & South America, but not in Europe (Hirata 1975) (Fig. 2). *Parauncinula* and *Caespitotheca* are distributed in East Asia and South America, respectively, but not in Europe. In summary, tree-parasitic genera with a small number of species and hosts, and probably of old origin, are distributed in Asia and North & South America, but not in Europe.

As described above, the major radiation of powdery mildews occurred in the Paleogene and Neogene periods, a period of climate deterioration and large scale orogeny which formed the Alps, Himalayas, and Rockies. As the earth cooled the broad-leaved deciduous trees growing in high latitudes in the Northern Hemisphere moved to middle latitudes and survived in several refugia where speciation occurred. The Western part of North America and Europe also may have had a rich Paleogene flora until the beginning of the Neogene Period when large scale extinction occurred due to global cooling and large scale desertification. Finally, most Neogene flora became extinct in Europe during the Ice Ages of the Quaternary Period. On the other hand, East Asia became the largest refugium of this flora in the world due to its warm and humid climate (Tiffney 1985a,b; Manchester 1999; Wen 1999; Tiffney and Manchester 2001). The Eastern part of North America also functioned as a refugium. Thus both East Asia and North America became refugia for ancestral powdery mildews and would have avoided a possible mass extinction of powdery mildews in Europe during the Neogene and Quaternary periods. The richer flora of ancestral powdery mildews in East Asia compared with those in North America reflects well the difference in the sizes of their refugia.

3.2.6. *Unexpected phylogeny and distributions of endophytic genera*

The genera *Phyllactinia, Pleochaeta, Queirozia*, and *Leveillula*, which present a monophyletic group within the Erysiphales, are characterized by the formation of endophytic mycelium. The most ancestral genera of this group are *Pleochaeta* and the closely allied *Queirozia* which form a grade (not clade) at the base of this lineage. *Phyllactinia* diverged from a part of the *Pleochaeta* grade, and then *Leveillula* diverged from *Phyllactinia*.

Hirata (1968), Amano (1986), and Palti (1988) mentioned the uneven distribution of *Leveillula* in the world. *Leveillula* has been recorded on many host species in Central & West Asia and the Mediterranean region, but much fewer hosts are recorded in East Asia, South Asia, North & South America, Oceania, and Northern Europe. Hirata (1968) and Amano (1986) speculated that the geographic origin of *Leveillula* is Central & East Asia or the Mediterranean region, from where *Leveillula* is in the process of expanding its distribution in the world. Our molecular phylogenetic analysis indicated that *Leveillula* has the most recent origin in Erysiphaceae, thus supporting this speculation (Takamatsu et al. 2008b).

The closest relative of *Leveillula* is *Phyllactinia adesmiae* (Khodaparast et al. 2012) recorded only in Argentina (South America). The next closest relative is *Ovulariopsis obclavata* (anamorph of *Phyllactinia*) also recorded only in South America. Furthermore, all *Phyllactinia* species that are sister to the *Leveillula* clade are distributed in North & South America, and none of them are distributed in Central & West Asia or the Mediterranean region (unpublished data). Several explanations would be possible to interpret this strange relationship between *Leveillula* and its sister group. One explanation could be as follows: *Leveillula* originated in North or South America, and later it migrated to Central & West Asia or the Mediterranean region where it radiated, whilst the ancestral *Leveillula* became extinct in South and North America. Another possible explanation could be: The closest relatives of *Leveillula* in *Phyllactinia* are distributed in Central & West Asia or the Mediterranean region. However, we accidentally did not use these species in the current analysis. However, there is a third possible explanation that I prefer, viz. an ancestral *Phyllactinia* species originating in the higher latitudes of the Northern Hemisphere migrated southward to North America and Europe during the global cooling of the world. A part of the North American population migrated further to East Asia via the BLB. *Leveillula* diverged from an ancestral species of *Phyllactinia* that migrated to Central & West Asia or the Mediterranean region due to an adaptation to arid environments. The ancestral *Phyllactinia* species became extinct with its host due to further climate and environmental changes in this area. Further investigations are required to evaluate this hypothesis.

3.2.7. *Host shift from trees to herbs*

A host shift from trees to herbs may have occurred many times during the evolutionary history of powdery mildews. For instance, it occurred at least twice in the tribe Cystotheceae (Takamatsu et al. 2000, 2010). One of these may have been a shift of a *Podosphaera* sp. from the tribe Amygdaloideae (Rosaceae) to herbs of Scrophulariaceae, leading to the creation of sect. *Sphaerotheca* subsect. *Magnicellulatae*. Another one was a shift from rosaceous trees (tribe Maloideae of Rosaceae) to rosaceous herbs, where the pathogen formed the subsect. *Sphaerotheca* of *Podosphaera* sect. *Sphaerotheca*. In the tribe Phyllactinieae, evolution from *Phyllactinia* (tree parasite) to *Leveillula* (herb parasite) may have occurred only once. It is noteworthy that *Leveillula* species parasitic to Asteraceae usually occupy the basal part of the *Leveillula* tree (Khodaparast et al. 2001, 2012; Voytyuk et al. 2009). In the tribe Erysipheae, herb-parasitic taxa form many small groups scattered mainly towards the upper half of the Erysipheae tree (Takamatsu et al. 1999), suggesting that host shift from trees to herbs occurred many times independently in this tribe. A comprehensive phylogenetic tree of this lineage is urgently required to discuss evolution of this group.

Among the three genera in the tribe Golovinomyceteae, *Golovinomyces* and *Neoërysiphe* mainly infect herbs and only *Arthrocladiella* infects shrubs. Because *Arthrocladiella* does not occupy the basal part of this lineage, an ancestral tree-parasitic taxon of the Golovinomyceteae is not known yet. As mentioned above, Asteraceae may be closely related to the early evolution of *Golovinomyces* (Matsuda and Takamatsu 2003). *Neoërysiphe* is a relatively small genus infecting 336 host species in eight families (Amano 1986). Of these host species, 267 (79.5%) belong to the Lamiaceae, suggesting *Neoërysiphe* has a close relationship with this family. In its

phylogenetic tree, a group of isolates from Lamiaceae (*N. galeopsidis* group) split firstly from a group consisting of isolates from other plant families (Takamatsu et al. 2008a; Heluta et al. 2010). However, genetic diversity of the *N. galeopsidis* group is quite low, that is, most isolates have an identical ITS sequence and maximum substitutions within the group are only 4 bases. This low genetic divergence of *N. galeopsidis* suggests that infection of *Neoërysiphe* to Lamiaceae was a relatively recent event despite *Neoërysiphe* having such a high proportion of Lamiaceae hosts. Furthermore, many Asteraceous hosts are included in the primary group which forms several distinct clades with a high genetic diversity depending on the regions where they were collected. From these results, Asteraceae seems to be closely related to the early evolution of *Neoërysiphe* as well as to the early evolution of *Leveillula* and *Golovinomyces*. Voytyuk et al. (2004, 2006) and Heluta et al. (2010) pointed out that many *Neoërysiphe* specimens on Asteraceae may have been erroneously deposited in European herbaria as *Golovinomyces* specimens. Therefore, the number of Asteraceous hosts of *Neoërysiphe* should increase following re-assessments of herbarium specimens. It is noteworthy that such erroneous identification occurred mainly in the Mediterranean region.

Blumeria is the only genus in the Erysiphaceae that infects monocotyledons. Host range of this genus is restricted to the subfamily Pooideae of Poaceae. Apart from *Leveillula allii*, all other powdery mildew genera infect dicotyledons. So, it is likely that the ancestor of *Blumeria* underwent a host shift from dicotyledons to Pooideae. The *Blumeria* lineage consists only of a single species *B. graminis* and is only distantly related to all other powdery mildew lineages. Thus, the ancestral taxa leading to *Blumeria* are presently unknown.

3.2.8. *Geographic origin of herb-parasitic powdery mildews*

As discussed above, the family Asteraceae may be closely related to the early evolution of at least three herb-parasitic genera, viz. *Golovinomyces, Leveillula*, and *Neoërysiphe*. These were unexpected results because the Asteraceae is of relatively recent origin among Angiosperms (Bremer 1994). South America is considered to be the geographic origin of the Asteraceae (Bremer 1994). Then, this family expanded into the Northern Hemisphere and underwent explosive radiation and dispersal during or near the Oligocene and Miocene of the Paleogene and Neogene periods (Kim et al. 1998). The tribe Cardueae was the first major group of the Asteraceae to disperse throughout the Northern Hemisphere (Bremer 1994; Kim and Jansen 1995), where it differentiated into genera with hundreds of species in the Mediterranean region and Central & West Asia (Bremer 1994).

Hirata (1968) noted that *Podosphaera xanthii* (currently in subsect. *Magnicellulatae* of *Podosphaera* sect. *Sphaerotheca*) [= *S. fuliginea* in Hirata (1968)] is commonly reported as a causal agent of powdery mildew of cucumber and cucurbit in Japan, whereas *Golovinomyces orontii* [= *Erysiphe cichoracearum* in Hirata (1968)] is recorded as the main cause of powdery mildew of Cucurbitaceae in many other countries. The latter was first found in Japan in 2002, and within several years expanded to cover all parts of the country (Hoshi et al. 2009; Uchida et al. 2009). Hirata (1955, 1968) compared records of powdery mildews and their hosts between Japan and USA. He was aware that *G. orontii* was recorded on many host species in the USA whilst *Po. xanthii* was recorded in Japan but not in the USA. In order to determine whether there was a similar tendency between East Asia and Europe, we compared the number of hosts of *G. orontii* and *Po. xanthii* among East Asia, North America, and Europe based on Amano's book (1986). About four times more host species of *Golovinomyces* are recorded in Europe than in Asia either in total or as hosts of Asteraceae (Table 1). Thus *Golovinomyces* seems to be more closely associated with Europe than with East Asia. On the other hand, the difference is not so large in the number of host species of *P.* sect. *Sphaerotheca* subsect. *Magnicellulatae* (mainly *Po. xanthii*) between these two regions, although Europe still has the most species.

Since *Blumeria* may have originated due to a host shift from a dicotyledon to an ancestor of Pooideae, the geographic origin of the latter might be relevant to the geographic origin of *Blumeria*. Several reports discussing the origin of Pooideae mostly agree that the subfamily originated in the region from the Mediterranean to Southwest Asia (Blattner 2006; Inda et al. 2008) and so these regions could be the geographic origin of *Blumeria*.

As mentioned above, the early evolution of the three herb-parasitic genera, *Leveillula, Golovinomyces*, and *Neoërysiphe* seems, like *Blumeria*, to be closely associated with the region from the Mediterranean to Central & West Asia. One notable fact is that none of these share an ancestor with tree-parasitic taxa. In the tribes Erysipheae, Cystotheceae and Phyllactinieae, both tree-parasitic taxa and herb-parasitic taxa are represented in their respective lineages, although tree-parasitic taxa usually occupy the basal parts. However, with the exception of *Arthrocladiella* in the tribe Golovinomyceteae there is no other tree-parasitic taxon in this tribe or in Blumerieae. In the case of *Leveillula*, it is unclear whether it shares a direct ancestor with its sister, *Ph. adesmiae*. Extinction of woody plants in this region could be a possible explanation for the lack of tree hosts in these lineages. Central & West Asia was a region surrounded by the Turgai Strait and Tethys Sea in the Paleogene Period and vegetated by *Pterocarya, Betula, Alnus,*

Table 1 – Number of host species of the genus *Golovinomyces* and of *Podosphaera* sect. *Sphaerotheca* subsect. *Magnicellulatae* in East Asia, North America, and Europe.

	Number of host species[a]			
	World	East Asia	North America	Europe
Golovinomyces				
Total host species	2283	287 (12.6%)	685 (30.0%)	1108 (48.5%)
Asteraceae host species	1242	167 (13.4%)	401 (32.3%)	642 (51.7%)
P. sect. *Sph.* subsect *Magnicellulatae*				
Total host species	1110	291 (26.2%)	228 (20.5%)	426 (38.4%)
Asteraceae host species	499	152 (30.5%)	110 (22.0%)	191 (38.3%)

a Numbers based on the list of Amano (1986).

Salix, *Nyssa*, and so forth (Tiffney and Manchester 2001). The subsequent Alpine and Himalayan orogenies shut off the southern source of moisture, allowing this area to become arid during the Miocene of the Neogene Period when the diversity of Angiosperms decreased. However, information on the changes in Central & West Asian flora is not adequate to discuss this subject in detail. Further investigations are required.

With regard to *P.* sect. *Sph.* subsect. *Magnicellulatae*, Hirata (1969) found a relatively high proportion of this subsect. as well as *B. graminis* amongst powdery mildews in latitudes above 60°N. Junell (1967) also reported frequent distribution of this subsect. in the northern part of Sweden. Worldwide it infects 206 species of Scrophulariaceae, of which 113 (54.9%) were recorded in Europe. On the other hand, only 27 (13.1%) host species were recorded in East Asia and only 41 (19.9%) in North America. Therefore, the area from Europe to Central Asia might be closely associated with the early evolution of subsect. *Magnicellulatae*.

Other herb-parasitic powdery mildews belonging to *Erysiphe* sect. *Erysiphe* in the tribe Erysipheae show a complicated polyphyly, which suggests that host shifts to herbaceous plants have occurred many times at different places in this lineage. There are not enough phylogenetic analyses of the tribe Erysipheae to discuss the origin of herb-parasitic taxa in this group.

4. Prospects

Discussions in this review are focused on two significant subjects: 1) estimation of evolutionary dating using molecular clock newly designed for powdery mildews, and 2) geographic origin of powdery mildews. Calibration of evolutionary dating is essential to discuss evolution of organisms like powdery mildews whose life cycle is completely dependent on other organisms. Fortunately, we were able to find a calibration point for a molecular clock based on host–parasite relationships, which we used in a newly designed molecular clock employing ITS and 28S rDNA D1/D2 regions. This clock enabled us to discuss various aspects of the evolution of powdery mildews. It is necessary to re-evaluate these aspects from a different viewpoint or using a new molecular clock constructed with a different base.

The discussion on the geographic origin of powdery mildews was challenging due to the scarcity of usable evidence. Thus, the present discussion has to be mainly based on a lot of speculation, the evaluation of which requires further molecular data. The following approaches are necessary in future to improve the situation:

1) Comprehensive molecular phylogenetic analyses of more (maybe all) powdery mildews. Because the nucleotide sequence data so far determined are only from a part of the powdery mildew genome, many more sequence data are required to infer comprehensive phylogenetic relationships of this fungal group on a worldwide basis.
2) Multigene phylogenetic analyses. Although ribosomal DNA regions like ITS or 28S rDNA D1/D2 regions have been extremely useful for the broad scale elucidation of phylogenetic relationships of powdery mildews, multigene analyses including protein-coding regions are necessary to evaluate the phylogeny of powdery mildews more accurately. Multigene analyses of powdery mildews have currently been initiated by several research groups. I hope that the discussion conducted in this review will be a trigger for further discussion on the origin and evolution of the powdery mildews.

Disclosure

All the experiments undertaken in this study comply with the current laws of Japan.

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