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MINI-REVIEW

The phylogeny of plant and animal pathogens in the Ascomycota

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What makes a fungus pathogenic? In this review, phylogenetic inference is used to speculate on the evolution of plant and animal pathogens in the fungal Phylum Ascomycota. A phylogeny is presented using 297 18S ribosomal DNA sequences from GenBank and it is shown that most known plant pathogens are concentrated in four classes in the Ascomycota. Animal pathogens are also concentrated, but in two ascomycete classes that contain few, if any, plant pathogens. Rather than appearing as a constant character of a class, the ability to cause disease in plants and animals was gained and lost repeatedly. The genes that code for some traits involved in pathogenicity or virulence have been cloned and characterized, and so the evolutionary relationships of a few of the genes for enzymes and toxins known to play roles in diseases were explored. In general, these genes are too narrowly distributed and too recent in origin to explain the broad patterns of origin of pathogens. Co-evolution could potentially be part of an explanation for phylogenetic patterns of pathogenesis. Robust phylogenies not only of the fungi, but also of host plants and animals are becoming available, allowing for critical analysis of the nature of co-evolutionary warfare. Host animals, particularly human hosts have had little obvious effect on fungal evolution and most cases of fungal disease in humans appear to represent an evolutionary dead end for the fungus. Plants have been important in the evolution of fungi, and the rapid nature of co-evolutionary change might partially explain the lack of obvious, global characters uniting all plant pathogens.

Keywords: Ascomycota; phylogeny; ascomycete evolution; human pathogenic fungi; plant pathogenic fungi; horizontal transfer; toxin evolution; co-evolution.

INTRODUCTION

The purpose of this review is to apply phylogenetic analysis to help dissect the evolutionary origins of plant and animal pathogenicity by ascomycetous fungi. The review is divided into four sections. In the first section, the phylogenetic relationships in the Ascomycota with a tree inferred from 297 18S rRNA gene sequences from GenBank are summarized. Using the gene tree, it was explored whether gains and losses of plant and animal pathogenicity are randomly distributed through the phylogeny. In the second section, the evolutionary histories of genes that are associated with virulence in plant diseases are discussed, and the gene histories are compared with the phylogeny of the fungi. The third section considers the role of co-evolution between plants and fungi in the origins of pathogenicity. In the last section, the phylogenetic patterns evident among animal pathogens are compared with the patterns from plant pathogens.

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OVERVIEW OF THE PYHLOGENY OF THE ASCOMYCOTA WITH PARTICULAR REFERENCE TO PATHOGENS

The Ascomycota includes fungi ranging from yeasts, including baker's yeast *Saccharomyces cerevisiae* Meyen ex Hansen, to molds including Penicillium and Aspergillus. Many destructive plant pathogens, causing diseases including wheat scab and Dutch elm disease, are ascomycetes. Human pathogens including the etiological agent of thrush, the yeast *Candida albicans*, and the dermatophytes causing athlete's foot are also ascomycetes. In a larger phylogenetic perspective, the Ascomycota is one of the four phyla in the kingdom fungi. Most molecular phylogenetic analyses show the Ascomycota as a monophyletic group, descended from a common ancestral species [*6*, *38*, *46*, *47*]. The Basidiomycota is the sister group to the Ascomycota, meaning that these two largest phyla of terrestrial fungi are one another's closest relatives. Using an 18S rRNA phylogeny and assuming a molecular clock, Berbee and Taylor [*5*] estimated that the Ascomycota and Basidiomycota diverged 400 million years ago. Other analyses have

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suggested even more ancient divergence, perhaps as old as 1·2 billion years [33]. The large difference in the estimates indicates the limits to molecular clock analyses. However, both estimates are consistent with an ancient origin of the Ascomycota and Basidiomycota and suggest that plants have had to contend with fungi ever since their invasion of land perhaps 460 million years ago [28]. Land plants have probably been the main nutrient source for the Ascomycota and the Basidiomycota through much of their evolutionary history, based on the predominance of plant saprophytes, pathogens and mycorrhizal species in both phyla [6].

Phylogenetic analyses

The phylogenetic tree in Fig. 1 is the product of an alignment of 297 18S ribosomal DNA sequences from GenBank (Table 1). The figure puts the plant and animal pathogens, along with mutualistic lichenized and mycorrhizal fungi, into a possible evolutionary framework. Most of the sequences were about 1700 nucleotides in length. Sequences that represented much of the available taxonomic diversity of ascomycete 18S sequences were selected, and, when possible, species mentioned in this review were included. Partial 18S sequences were added when needed to represent important pathogens or the range of ascomycete diversity. From this alignment, PAUP 4.08 b [80] produced a majority rule consensus tree from 500 fast (without branch swapping) parsimony bootstrap replicates. The parsimony analysis clusters sequences by arranging taxa to minimize the number of sequence changes necessary to explain the observed DNA sequences of the taxa. The bootstrapping is a statistical technique used to provide some estimate of whether more data would be likely to support the same groups. In terms of interpretation, groups receiving more than 70 % bootstrap support in Fig. 1 are reasonably well-supported by the data and are usually found in other molecular phylogenetic studies using the same or different genes. On the other hand, groups with less than 50 % bootstrap support are poorly supported, and their membership changes depending on minor changes in analytical technique, or on small differences in taxon or data sampling. To provide a consistent taxonomic framework for these groups and others, the classification of Eriksson *et al*. [22] was used because it is complete and detailed and it incorporates results from molecular phylogenetics; so the classification is largely congruent with the phylogeny in Fig. 1.

Within the Ascomycota are three main subphyla, each including saprobes on plant materials as well as plant and human pathogens. The first of the three subphyla to diverge was the Taphrinomycotina (also known as Archiascomycetes) [55]. The Taphrinomycotina's genera range from obligate plant pathogens (Taphrina and Protomyces, in class Taphrinomycetes) to the animal pathogenic genus Pneumocystis and the saprobic fission yeasts in Schizosaccharomyces. The next two subphyla to split were the Saccharomycotina (also known as the Hemiascomycetes) and the Pezizomycotina (the Euascomycetes, or filamentous ascomycetes). The Saccharomycotina includes over 40 genera of ascomycetous yeasts, but only one genus pathogenic on human (Candida), and only one genus (Eremothecium) with plant pathogens.

The third group, the subphylum Pezizomycotina, with over 3000 genera, includes the majority of known ascomycete species. Not surprisingly given its large size, the Pezizomycotina includes most of the ascomycetous pathogens and mutualists. However, the color clusters in Fig. 1 show that different nutritional modes are clustered within classes. Most lichens are in the class Lecanoromycetes, although without bootstrap support. Mycorrhizal ascomycetes are concentrated in the class Pezizomycetes (operculate discomycetes). The plant pathogens are most frequent in three classes, the Sordariomycetes (= Pyrenomycetes), Dothideomycetes (= loculoascomycetes, in part), and Leotiomycetes (the inoperculate discomycetes) (Fig. 1). Obligate plant pathogens evolved three times in the subphylum Pezizomycotina [67]. All three lineages of obligate pathogens produced mildews. These mildews all form masses of hyphae, and eventually, sexual reproductive structures on the outer surfaces of green leaves. Black mildews in the genus Meliola arose in the Sordariomycetes, in a clade including Neurospora while the black mildew genus Meliolina is near Capnodium in the Dothideales. The powdery mildews in the Erysiphales are closest to the cup fungi with inoperculate asci in the Leotiomycetes [67] and to the Myxotrichaceae, a family formerly believed to be in the Eurotiomycetes that includes cellulolytic soil saprophytes and mycorrhizal fungi [79]. All the mildews obtain nutrients from living cells; but hinting at their polyphyletic origins, the structures involved in nutrient acquisition differ in details. The haustoria are produced in Meliolaceae and Erysiphales but not in Meliolina, and appressoria are two-celled in the Meliolaceae, one-celled in the Erysiphales, and instead of appressoria, *Meliolina* spp. infect using specialized hyphae (stomatopodia) that enter through the stomata [67].

Analysis of correlations between ascomycete classes and the phylogenetic distribution of plant and animal pathogens

From the phylogeny in Fig. 1, it appeared that gains and losses of animal and plant pathogenicity were concentrated in a few ascomycete classes. To evaluate whether the apparent concentration of gains and losses of plant pathogenicity could have arisen by chance, the computer program MacClade [49] was used. MacClade traces the most parsimonious evolution of characters onto a

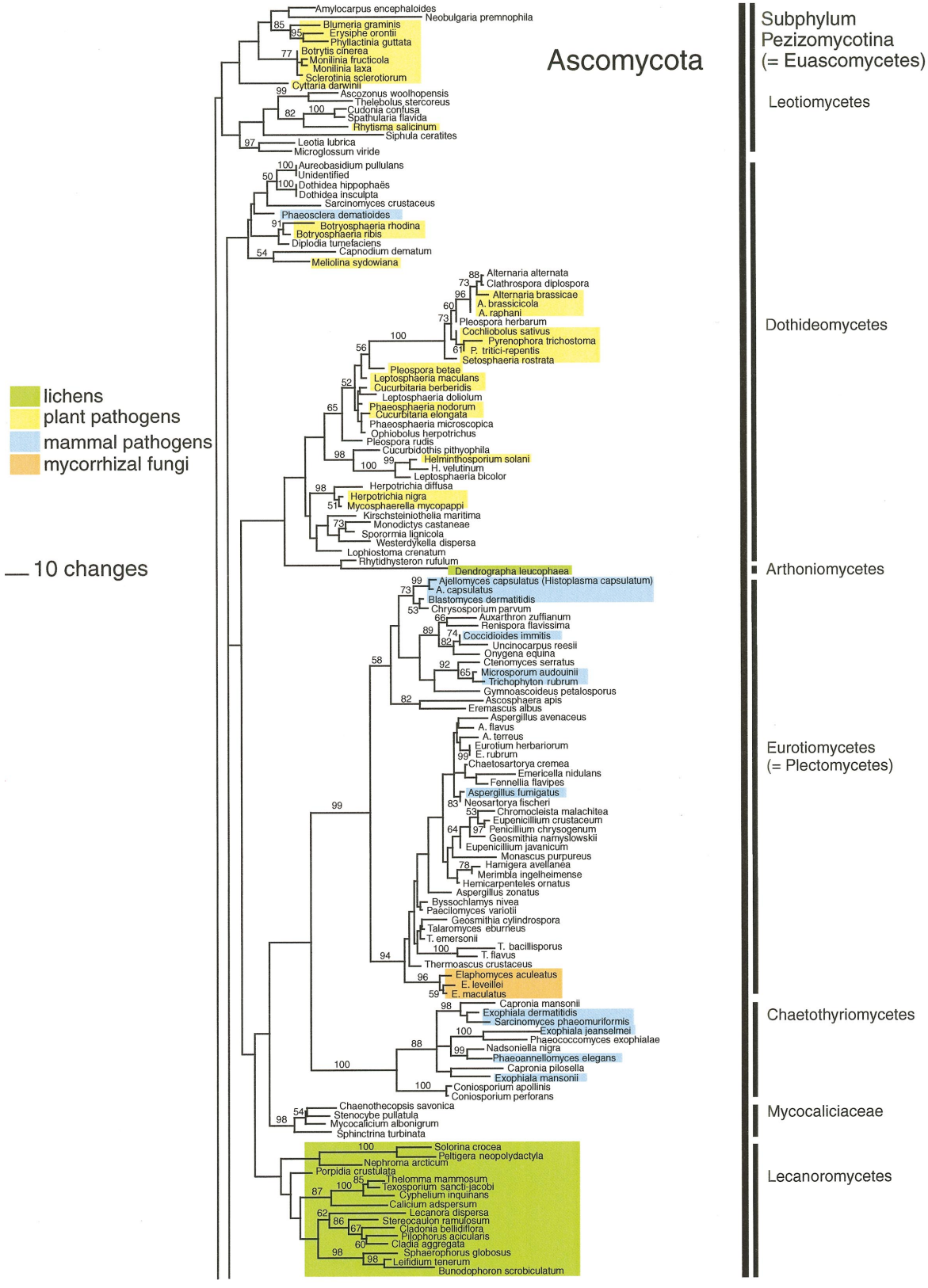
Ascomycota
Subphylum Pezizomycotina (= Euascomycetes)
Leotiomycetes
Dothideomycetes
Arthoniomycetes
Eurotiomycetes (= Plectomycetes)
Chaetothyriomycetes
Mycocaliciaceae
Lecanoromycetes
lichens
plant pathogens
mammal pathogens
mycorrhizal fungi
10 changes
Amylocarpus encephaloides
Neobulgaria premnophila
Blumeria graminis
Erysiphe orontii
Phyllactinia guttata
Botrytis cinerea
Monilinia fructicola
Monilinia laxa
Sclerotinia sclerotiorum
Cyttaria darwinii
Ascozonus woolhopensis
Thelebolus stercoreus
Cudonia confusa
Spathularia flavida
Rhytisma salicinum
Siphula ceratites
Leotia lubrica
Microglossum viride
Aureobasidium pullulans
Unidentified
Dothidea hippophaës
Dothidea insculpta
Sarcinomyces crustaceus
Phaeosclera dematioides
Botryosphaeria rhodina
Botryosphaeria ribis
Diplodia tumefaciens
Capnodium dematum
Meliolina sydowiana
Alternaria alternata
Clathrospora diplospora
Alternaria brassicae
A. brassicicola
A. raphani
Pleospora herbarum
Cochliobolus sativus
Pyrenophora trichostoma
P. tritici-repentis
Setosphaeria rostrata
Pleospora betae
Leptosphaeria maculans
Cucurbitaria berberidis
Leptosphaeria doliolum
Phaeosphaeria nodorum
Cucurbitaria elongata
Phaeosphaeria microscopica
Ophiobolus herpotrichus
Pleospora rudis
Cucurbidothis pithyophila
Helminthosporium solani
H. velutinum
Leptosphaeria bicolor
Herpotrichia diffusa
Herpotrichia nigra
Mycosphaerella mycopappi
Kirschsteiniothelia maritima
Monodictys castaneae
Sporormia lignicola
Westerdykella dispersa
Lophiostoma crenatum
Rhytidhysteron rufulum
Dendrographa leucophaea
Ajellomyces capsulatus (Histoplasma capsulatum)
A. capsulatus
Blastomyces dermatitidis
Chrysosporium parvum
Auxarthron zuffianum
Arthroderma flavissima
Coccidioides immitis
Uncinocarpus reesii
Onygena equina
Ctenomyces serratus
Microsporum audouinii
Trichophyton rubrum
Gymnoascoideus petalosporus
Ascosphaera apis
Eremascus albus
Aspergillus avenaceus
A. flavus
A. terreus
Eurotium herbariorum
E. rubrum
Chaetosartorya cremea
Emericella nidulans
Fennellia flavipes
Aspergillus fumigatus
Neosartorya fischeri
Chromocleista malachitea
Eupenicillium crustaceum
Penicillium chrysogenum
Geosmithia namyslowskii
Eupenicillium javanicum
Monascus purpureus
Hamigera avellanea
Merimbla ingelheimense
Hemicarpenteles ornatus
Aspergillus zonatus
Byssochlamys nivea
Paecilomyces variotii
Geosmithia cylindrospora
Talaromyces eburneus
T. emersonii
T. bacillisporus
T. flavus
Thermoascus crustaceus
Elaphomyces aculeatus
E. leveillei
E. maculatus
Capronia mansonii
Exophiala dermatitidis
Sarcinomyces phaeomuriformis
Exophiala jeanselmei
Phaeococcomyces exophialae
Nadsoniella nigra
Phaeoannellomyces elegans
Capronia pilosella
Exophiala mansonii
Coniosporium apollinis
Coniosporium perforans
Chaenothecopsis savonica
Stenocybe pullatula
Mycocalicium albonigrum
Sphinctrina turbinata
Solorina crocea
Peltigera neopolydactyla
Nephroma arcticum
Porpidia crustulata
Thelomma mammosum
Texosporium sancti-jacobi
Cyphelium inquinans
Calicium adspersum
Lecanora dispersa
Stereocaulon ramulosum
Cladonia bellidiflora
Pilophorus acicularis
Cladia aggregata
Sphaerophorus globosus
Leifidium tenerum
Bunodophoron scrobiculatum

FIG. 1. Phylogram showing the distribution of plant and mammal pathogens, lichens and mycorrhizal fungi in the Ascomycota. Note that the distribution varies by class. The Sordariomycetes, for example, has a high proportion of plant pathogens but no mycorrhizal representatives and few animal pathogens. The Lecanoromycetes has a high proportion of lichenized taxa, but no plant or animal pathogens are known from this group. The Pezizomycetes has a high proportion of mycorrhizal fungi, but no animal pathogens and a single known plant pathogen. Animal pathogens are concentrated in the Eurotiomycetes and Chaetothyriomycetes. This tree is from a consensus of 500 parsimony bootstrap replicates, without branch swapping. Bootstrap percentages are provided for branches receiving 50 % or more support.

phylogeny. By using MacClade, it could be inferred whether the ancestors of the classes of ascomycetes were likely to have been pathogens (Fig. 2). For tests on the distributions of pathogens in relationship to ascomycete classes, an additional four-character data set for the 297 taxa was prepared. The characters and their binary character states were as follows.

(1) Whether or not a species is known to be plant pathogenic (as in Fig. 1).
(2) Whether or not a species belonged to the apparently plant pathogen rich classes, the Leotiomycetes, Dothideomycetes (which appear divided among two clades in this analysis), Sordariomycetes, or Taphrinomycetes. These were the four Ascomycota classes each represented, in this analysis, by more than two plant pathogenic genera.
(3) Whether or not a species is a human pathogen (Fig. 1).
(4) Whether or not the species belongs to the apparently animal pathogen rich classes, the Chaetothyriomycetes and the Eurotiomycetes, each represented here by sequences from two or more human pathogenic genera.

There is ambiguity about whether or not some fungal species are pathogens. In cases of doubt, if a fungus was cited in the Biological Abstracts in the last 5 or 6 years as causing a plant disease, it was coded as a plant pathogen. Fungi that only rarely and opportunistically infect humans, for example, Fusarium species, were not coded as human pathogens. For the purpose of studying the distribution of fungi causing disease, the author did not distinguish among different kinds of pathogens or levels of virulence.

The ideal phylogenetic tree for an analysis of character correlation would be a perfect reconstruction of the evolutionary relationships among the taxa. However, because available data are insufficient to resolve all the evolutionary relationships, a tree such as the one in Fig. 1 is likely to be wrong in at least some respects. The results of an analysis of character correlation depend on the tree topology, although predictions about character correlation may be robust to minor errors in the tree. To enable further testing of predictions with alternative tree topologies, the data matrix including both the 18S gene sequences and the pathogenicity characters is in TreeBASE, under accession number S662.

Using the tree in Fig. 1 and the characters in the additional data set, MacClade applied parsimony to infer where ancestors in the Ascomycota were saprobes, or plant or animal pathogens (Fig. 2). As a statistical test of whether the gains and losses of plant and animal pathogenicity might, in spite of appearances, be randomly distributed throughout the phylogeny, the concentrated changes test in MacClade was then used. The inferred number of gains and losses of pathogenicity in the classes that had more than two pathogenic genera were compared with the expected number calculated from 1000 simulated random distributions. The random distributions were estimated from the tree in Fig. 1 and from the character data matrix using the conservative "minstate" option. If the inferred number of gains and losses occurred in fewer than 5 % of the simulations, then this indicated, with $P = 0{\cdot}95$, that the distribution was not random. When the point of origin of pathogenicity was equivocal as in Fig. 2, for example, two analyses were performed. In the first analysis, it was specified that the ancestral state for the equivocal branches was not pathogenic and the resulting number of gains and losses of pathogenicity was counted. In the second analysis, it was assumed that the equivocal ancestral states were pathogenic. In Fig. 2, MacClade applied parsimony to reconstruct the ancestor of the plant pathogenic genera Cryphonectria and Magnaporthe as having been, unequivocally, a plant pathogen. However, MacClade recorded that the common ancestor of this clade and the Ophiostoma clade could have equally well been either a pathogen or not (Fig. 2). In the first analysis, the ancestor was specified as having been a pathogen, and in the second analysis, the ancestor was specified as having been non-pathogenic.

Results from analysis of correlations between classes and pathogens

Plant pathogens. MacClade reconstructed the ancestral character state as "pathogenic" for class Taphrinomycetes, because Protomyces and Taphrina, the only two genera representing the class, are both pathogens (Fig. 1). For the Sordariomycetes, the ancestor could equally parsimoniously have been a pathogen or a non-pathogen. For the Dothideomycetes and the Leotiomycetes (Fig. 1), the ancestors were, most parsimoniously, not plant pathogens.

The concentrated changes tests indicated that the transitions to and from plant and animal pathogenicity were not randomly distributed through the phylogenetic tree of the Ascomycota. Evolutionary changes in plant pathogenicity were concentrated in the Leotiomycetes, the Dothideomycetes (which in some analyses, for example, in Fig. 1 appeared as two groups), the Sordariomycetes and the Taphrinomycetes. Assuming that "equivocal" ancestors were not plant pathogens, for the Ascomycota, 20 gains of plant pathogenicity and eight losses would most parsimoniously have been required to account for the current distribution of pathogens throughout the tree in Fig. 1. Of these, 18 gains and all the losses were reconstructed as occurring in the pathogen-rich classes (defined here as the classes that are represented in Fig. 1 by more than two plant pathogenic genera). That

TABLE 1. *18S ribosomal DNA gene sequences used in this study*

Accession No.	Species
Z75306	*Ajellomyces capsulatus* (Kwon-Chung) McGinnis & Katz ssp. duboisii
X58572	*Ajellomyces capsulatus*
U53371	*Aleuria aurantia* (Pers. : Fr.) Fuckel.
AF201453	*Aliquandostipite khaoyaiensis* Inderbitzin
U05194	*Alternaria alternata* (Fr.) Keissler
U05196	*Alternaria brassicae* (Berk.) Bolle
U05197	*Alternaria brassicicola* (Schw.) Wiltshire
U05199	*Alternaria raphani* Groves & Skolko
U45438	*Amylocarpus encephaloides* Currey
AJ001983	*Arthrobotrys conoides* Drechsl.
AJ001997	*Arthrobotrys dactyloides* Drechsl.
AJ001988	*Arthrobotrys robusta* Duddlington
L37533	*Ascobolus lineolatus* Brumm.
U53372	*Ascodesmis sphaerospora* Obrist
X69849	*Ascosphaera apis* (P. Claussen) Olive & Spiltoir
U85087	*Ascovaginospora stellipala* Fallah, Shearer & W. Chen
AF010590	*Ascozonus woolhopensis* (Renny) Boud.
AB008395	*Aspergillus avenaceus* G. Sm.
X78537	*Aspergillus flavus* Link
AB008401	*Aspergillus fumigatus* Fresen.
X78540	*Aspergillus terreus* Thom
AB008413	*Aspergillus zonatus* Kwon-Chung & Fennell
M55639	*Aureobasidium pullulans* (De Bary) Arnaud
U29395	*Auxarthron zuffianum* (Morini) G.F. Orr & Kuehn
U77179	*Balansieae* sp.
U42656	*Balsamia magnata* Harkn.
AF054905	*Balsamia vulgaris* Vittad.
U42657	*Barssia oregonensis* Gilkey
M55624	*Blastomyces dermatitidis* Gilchrist & W.R. Stokes
L26253	*Blumeria graminis* (DC.) Speer
U42476	*Botryosphaeria rhodina* (Cooke) Arx
U42477	*Botryosphaeria ribis* Grossb. & Duggar
Z73711	*Botrytis cinerea* Pers.
U53373	*Boudiera acanthospora* Dissing & T. Schumach.
U70958	*Bunodophoron scrobiculatum* (C. Bab.) Wedin
M83256	*Byssochlamys nivea* Westling
Z30241	*Byssonectria terrestris* (Alb. & Schwein.) D. Pfister
U86694	*Calicium adspersum* Pers.
U62009	*Caloscypha fulgens* (Pers.) Boud.
Z49783	*Camarops microspora* (P. Karst). Shear
X53497	*Candida albicans* (C.P. Robin) Berkhout
AF006724	*Capnodium dematum* (V.A.M. Mill. & Bonar) D.R. Reynolds
X79318	*Capronia mansonii* (Schol-Schwartz) E. Müll., Petrini, P.J. Fisher, Samuels & Rossman
U42473	*Capronia pilosella* (P. Karst.) E. Müll.
U42666	*Cazia flexiascus* Trappe
U43777	*Ceratocystis fimbriata* Ellis & Halst.
U86691	*Chaenothecopsis savonica* (Räsänen) Tibell
M83257	*Chaetomium elatum* Kunze
AB003786	*Chaetopsina fulva* Rambelli
AB008399	*Chaetosartorya cremea* Subram.
AF054904	*Choiromyces meandriformis* Vittad.
U42661	*Choiromyces venosus* (Fr.) Th. Fr.
D88323	*Chromocleista malachitea* Yaguchi & Udagawa
U29390	*Chrysosporium parvum* (Emmons & Ashburn) Carmichael
U72713	*Cladia aggregata* (Sw.) Nyl.
U60900	*Cladonia bellidiflora* (Ach.) Schaer.
U43464	*Clathrospora diplospora* (Ellis & Everh.) Wehm.
U44040	*Claviceps purpurea* (Fr.) Tul.
M55627	*Coccidioides immitis* Stiles
U42479	*Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dasturin

Table 1 continued opposite

TABLE 1. *Continued*

Accession No.	Species
Y11713	*Coniosporium apollinis* Sterflinger
Y11714	*Coniosporium perforans* Sterflinger
U62010	*Cookeina sulcipes* (Berk.) Kuntze
U44041	*Cordyceps capitata* (Holmskj.) Link
M94338	*Cronartium ribicola* J.C. Fischer
L42439	*Cryphonectria cubensis* (Bruner) Hofges
L42440	*Cryphonectria havanensis* (Bruner) M. Barr
L42441	*Cryphonectria parasitica* (Murrill) M. Barr
L42442	*Cryphonectria radicalis* (Schwein.:Fr.) M. Barr
U29391	*Ctenomyces serratus* Eidam
U42480	*Cucurbidothis pithyophila* (J.C. Schmidt & Kunze) Petr.
U42481	*Cucurbitaria berberidis* (Pers.) Gray
U42482	*Cucurbitaria elongata* (Fr.) Grev.
Z30240	*Cudonia confusa* Bres.
U86695	*Cyphelium inquinans* (Sm.) Trevis.
U53369	*Cyttaria darwinii* Berk.
AJ001993	*Dactylella oxyspora* (Sacc. & Marchal) Matsushima
AJ001992	*Dactylella rhopalota* Drechsl.
AF279381	*Dendrographa leucophaea* f. minor (Tuck.) Darb.
U53377	*Desmazierella acicola* Lib.
U32403	*Diatrype disciformis* (Hoffm.) Fr.
U42659	*Dingleya verrucosa* Trappe
AF148916, AF148917	*Diplodia tumefaciens* (Shear) Zalasky
AF038876	*Diploschistes ocellatus* (Vill.) Norman var. almeriensis Llimona
U00969	*Dipodascopsis uninucleata* (Biggs) Batra & P. Millner
U42651	*Discina macrospora* Bubák
U42643	*Disciotis venosa* (Pers.) Arnaud
U42475	*Dothidea hippophaeos hippophaës* (Pass.) Fuckel
U42474	*Dothidea insculpta* Wallr.
AJ001895	*Duddingtonia flagrans* (Duddlngton) R.C. Cooke
U45439	*Elaphomyces aculeatus* Vittad.
U45441	*Elaphomyces leveillei* Tul.
U45440	*Elaphomyces maculates* Vittad.
U77377	*Emericella nidulans* (Eidam) Vuill.
D86913	*Endomyces* sp.
L42443	*Endothia gyrosa* (Schwein.:Fr.)Fr.
U57662	*Epichloe baconii* J. F. White
M83258	*Eremascus albus* Eidam
AF113137	*Eremothecium gossypii* (Ashby & Nowell) Kurtzman
AB033483	*Erysiphe orontii* Cost. emend. Braun.
D88324	*Eupenicillium crustaceum* Ludwig
U21298	*Eupenicillium javanicum* (F.H. Beyma) Stolk & D.B. Scott
AB008402	*Eurotium herbariorum* Weber: Fr.
U00970	*Eurotium rubrum* Bremer
X79317, X79315, X79316	*Exophiala dermatitidis* (Kano) de Hoog
X80705	*Exophiala jeanselmei* (Langeron) McGinnis & Y.A Padhye
X78480	*Exophiala mansonii* (Castell.) de Hoog
AB008400	*Fennellia flavipes* B.J. Wiley & E.G. Simmons
U42646	*Fischerula subcaulis* Trappe
AF141947	*Fusarium cerealis* R. J. Cooke
AF141948	*Fusarium culmorum* (W. G. Smith) Sacc.
AF141949	*Fusarium equiseti* (Corda) Sacc.
AF141950	*Fusarium merismoides* Corda
AF141951	*Fusarium oxysporum* Schlecht.: Fr.
AF277125	*Gaeumannomyces graminis* var. *graminis* (Sacc.) Arx & D.L. Oliver
U62011	*Geopyxis carbonaria* (Alb. & Schwein.) Sacc.
D88320	*Geosmithia cylindrospora* (G. Smith) Pitt
D14405	*Geosmithia lavendula* (Raper & Fennell) Pitt
D88319	*Geosmithia namyslowskii* (Zaleski) Pitt
D88318	*Geosmithia putterillii* (Thom) Pitt

Table 1 continued over page

TABLE 1. *Continued*

Accession No.	Species
AF141946	*Gibberella avenacea* R. J. Cooke
AF081467	*Gibberella pulicaris* (Fr.) Sacc.
Z49753	*Glaziella aurantiaca* (Berk. & Curtis) Cooke
M55640	*Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk
U63138	*Glomerella glycines* F. Lehm & F.A. Wolf
AF038878	*Graphis scripta* (L.) Ach.
U43907	*Graphium tectonae* Booth
U29392	*Gymnoascoideus petalosporus* G.F. Orr, A.K. Roy & G.R. Ghosh
Z30238	*Gyromitra esculenta1* (Pers.) Fr.
U42648	*Gyromitra esculenta2* (Pers.) Fr.
U42653	*Gyromitra melaleucoides* (Seaver) Pfister
U42652	*Gyromitra montana* Harmaja
D14406	*Hamigera avellanea* (Thom & Turresson) Stolk & Samson
AF120253	*Helminthosporium solani* Durieu & Mont.
AF120254	*Helminthosporium velutinum* Link:Fr.
U42654	*Helvella lacunosa* Afz.
AB008406	*Hemicarpenteles ornatus* (Subram.) Arx
U42484	*Herpotrichia diffusa* (Schwein.) Ellis & Everh.
U42483	*Herpotrichia nigra* Hartig
U53443	*Holleya sinecauda* (Holley) Yamada
U42649	*Hydnotrya cerebriformis* Harkn.
U53379	*Hydnotrya tulasnei* (Berk. & Broome) Berk. & Broome
D14407	*Hypocrea lutea* (Tode) Petch
M89993	*Hypomyces chrysospermus* Tul.
U32410	*Hypomyces polyporinus* Peck
AF346563	*Hypoxylon nummularium* Bull.:Fr.
AB003789	*Kionochaeta spissa* P.M. Kirk & B. Sutton
AF053726	*Kirschsteiniothelia maritima* (Linder) D. Hawksw.
U42662	*Labyrinthomyces varius* (Rodway) Trappe
L37535	*Lecanora dispersa* (Pers.) Sommerf.
U70959	*Leifidium tenerum* Wedin
L37536	*Leotia lubrica* Pers.
U04202	*Leptosphaeria bicolor* D. Hawksw., W. Kaiser & Ndimande
U43457	*Leptosphaeria doliolum* (Pers.) Ces. & De Not.
U04233	*Leptosphaeria maculans* (Desm.) Ces. & De Not.
U42647	*Leucangium carthusianum* (Quél.) Paol.
U43910	*Lomentospora prolificans* Hennebert & B.G. Desai
U42485	*Lophiostoma crenatum* (Pers.) Fuckel
AB026819	*Magnaporthe grisea* (Hebert) M.E. Barr
AF021793	*Meliola juddiana* F. Stevens
AF021794	*Meliola niessliana* G. Winter
AF021795	*Meliolina sydowiana* F. Stevens
D14408	*Merimbla ingelheimense* (van Beyma) Pitt
AF280631	*Metarhizium anisopliae* (Metschn.) Sorokin
M89994	*Microascus cirrosus* Curzi
U46031	*Microglossum viride* (Pers.) Gillet
Z34924	*Microsporum audouinii* Gruby
U53395	*Microstoma protracta* (Fr.) Kanouse
AJ001995	*Monacrosporium ellipsospora* (Grove) R.C. Cooke & C.H. Dickinson
AJ001996	*Monacrosporium gephyropaga* (Drechsl.) Subram.
AJ001990	*Monacrosporium haptotylum* (Drechsl.) X.Z. Liu & K.Q. Zhang
M83260	*Monascus purpureus* Went
Y14211	*Monilinia fructicola* (Winter) Honey
Y14210	*Monilinia laxa* (Aderh. & Ruhl) Honey
Y11715	*Monodictys castaneae* (Wallr.) S. Hughes
U42641	*Morchella elata* Fr. aggr.
U42642	*Morchella esculenta* (L.) Pers.
L37538	*Mycocalicium albonigrum* (Nyl.) Tibell
U43449	*Mycosphaerella mycopappi* A. Funk & Dorworth
X80706	*Nadsoniella nigra* Issatsch

Table 1 continued opposite

TABLE 1. *Continued*

Accession No.	Species
U32412	*Nectria cinnabarina* (Tode) Fr.
AF141952	*Nectria haematococca* Berk. & Br.
U45445	*Neobulgaria premnophila* F. & H. Roll-Hansen
Z47721	*Neolecta irregularis* (Peck) Korf & J.K. Rogers
Z27393	*Neolecta vitellina* (Bres.) Korf & J.K. Rogers
U21299	*Neosartorya fischeri* (Wehmer.) Malloch & Cain
X89219	*Nephroma arcticum* (L.) Torss.
X04971	*Neurospora crassa* Shear & B.O. Dodge
Z49784	*Obolarina dryophila* (Tul. & C. Tul.) Pouzar
U45442	*Onygena equina* (Willd.) Pers.
U43453	*Ophiobolus herpotrichus* (Fr.:Fr) Sacc.
M85054	*Ophiostoma stenoceras* (Robak) Nannf.
M83261	*Ophiostoma ulmi* (Buisman) Nannf.
U72603	*Orbilia delicatula* (P. Karst.) Sacc.
U53381	*Otidea leporine* (Batch) Fuckel
AF054899	*Pachyphloeus melanoxanthus* Tul.
D85136	*Paecilomyces tenuipes 2* (Peck) Samson
Y13996	*Paecilomyces variotii* Bainier
U53382	*Paurocotylis pila* Berk.
X89218	*Peltigera neopolydactyla* (Gyeln.) Gyeln.
M55628	*Penicillium chrysogenum* Thom
U43908	*Petriella setifera* (Alf. Schmidt) Curzi
L37539	*Peziza badia* Pers.
U42665	*Peziza quelepidotia* Korf & O'Donnell
U53383	*Peziza succosa* Berk.
X80708	*Phaeoannellomyces elegans* McGinnis & Schell
X80709	*Phaeococcomyces exophialae* (de Hoog) de Hoog
Y11716	*Phaeosclera dematioides* Sigler, Tsuneda & J.W. Carmich.
U04235	*Phaeosphaeria microscopica* (P. Karst.) O.E. Erikss.
U04236	*Phaeosphaeria nodorum* (E. Müll.). Hedj.
AF021796	*Phyllactinia guttata* (Wallr.:Fr.) Lév.)
X58055	*Pichia membranaefaciens* E. C. Hansen
U70960	*Pilophorus acicularis* (Ach.) Th. Fr.
U43466	*Pleospora betae* (Berl.) Nevod.
U43458	*Pleospora herbarum* (Pers.) Rabenh.
U00975	*Pleospora rudis* Berl.
X12708	*Pneumocystis carinii* P. Delanoë & Delanoë
X54864	*Podospora anserina* (Ces.) Niessl
L37540	*Porpidia crustulata* (Ach.) Hertel & Knoph.
D11377	*Protomyces inouyei* Hennings
M89782	*Pseudallescheria boydii* (Shear) McGinnis
U43911	*Pseudallescheria ellipsoidea* (Arx & Fassatiová) McGinnis, A.A. Padhye & Ajello
U42650	*Pseudorhizina californica* (Phillips) Harmaja
U62012	*Pulvinula archeri* (Berk.) Rifai
U43459	*Pyrenophora trichostoma* (Fr.) Fuckel
U42486	*Pyrenophora tritici-repentis* (Died.) Drechsl.
U53385	*Pyronema domesticum* (Sow.) Sacc.
U42660	*Reddellomyces donkii* (Malençon) Trappe
U29393	*Renispora flavissima* Sigler *et al.*
U42664	*Rhizina undulata* Fr.
AF201452	*Rhytidhysteron rufulum* (Spreng.) Speg.
U53370	*Rhytisma salicinum* Fr.
X97778	*Saccharomyces barnettii* Vaughan-Martini
D12530	*Saitoella complicata* S. Goto, J. Sugiyama, M. Hamamoto & K. Komagata
Y11355	*Sarcinomyces crustaceus* Lindner
X80710	*Sarcinomyces phaeomuriformis* Matsumoto *et al.*
U53392	*Sarcoscypha austriaca* (Beck ex Sacc.) Boud.
U53386	*Sarcosoma globosum* (Schmidel) Rehm
Z32848	*Schizosaccharomyces japonicus* Yukawa & Maki
X69850	*Sclerotinia sclerotiorum* (Lib.) De Bary

Table 1 continued over page

TABLE 1. *Continued*

Accession No.	Species
U53387	*Scutellinia scutellata* (L.) Lamb.
U42487	*Setosphaeria rostrata* K.J. Leonard
U72712	*Siphula ceratites* (Wahlenb.) Fr.
X89220	*Solorina crocea* (L.) Ach.
X69851	*Sordaria fimicola* (Roberge) Ces. & De Not.
Z30239	*Spathularia flavida* Pers.
L37532	*Sphaerophorus globosus* (Huds.) Vain.
U86693	*Sphinctrina turbinata* (Pers.) De Not.
U69890	*Spicellum roseum* Nicot & Roquebert
U42478	*Sporormia lignicola* W. Phillips & Plowr.
M85053	*Sporothrix schenckii* Hektoen & Perkins
U86692	*Stenocybe pullatula* (Ach.) Stein
U70961	*Stereocaulon ramulosum* (Sw.) Rausch
D55719	Symbion of *Hamiltonaphis styrac*
D49656	symbiont of *Lasioderma serricor*
D14409	*Talaromyces bacillisporus* (Swift) C. R. Benjamin
D88322	*Talaromyces eburneus* Yaguchi, Someya & Udagawa
D88321	*Talaromyces emersonii* Stolk
M83262	*Talaromyces flavus* (Klöcker) Stolk & Samson
D14166	*Taphrina californica* Mix
U00971	*Taphrina deformans* (Berk.) Tul.
D12531	*Taphrina wiesneri* (Ráthay) Mix
U53389	*Tarzetta catinus* (Holmskj.) Korf & J.K. Rogers
AF054898	*Terfezia arenaria* (Moris) Trappe
AF054900	*Terfezia terfezioides* (Matt.) Trappe
U53390	*Terichophaea hybrida* (Sow.) T. Schumach.
U86696	*Texosporium sancti-jacobi* (Tuck.) Nádv.
AF010589	*Thecotheus holmskjoldii* (E.C. Hansen) Eckblad
U49936	*Thelebolus stercoreus* Tode
U86697	*Thelomma mammosum*
M83263	*Thermoascus crustaceus* (Apinis & Chesters) Stolk
AF049153	*Tolypocladium cylindrosporum* W. Gams
X58570	*Trichophyton rubrum* (Castell.) Semon
U69892	*Trichothecium roseum* (Pers.:Fr.) Link
AF054902	*Tuber borchii* Vittad.
X98089	*Tuber excavatum* Vittad.
U42663	*Tuber gibbosum* Tul.
AF054901	*Tuber magnatum* Lév.
AF054903	*Tuber panniferum* Tul. & C. Tul.
Z49755	*Tuber rapaeodorum* Tul.
L27991	*Uncinocarpus reesii* Sigler & Orr
U42658	*Underwoodia columnaris* Peck
AF031923	Unidentified
Z49754	*Urnula hiemalis* Nannf.
X62396	*Ustilago maydis* (DC) Corda
M83259	*Valsa leucostoma* (Pers.) Fr.
U42645	*Verpa bohemica* (Krombh.) Schröt.
U42644	*Verpa conica* (O. Müll.) Swartz
U33637	*Verticillium dahliae1* Kleb.
U33637	*Verticillium dahliae2* Kleb.
AF049155	*Verticillium lecanii* (Zimm.) Viégas
AF049158	*Verticillium psalliotae* Treschow
U42488	*Westerdykella dispersa* (Clum) Cejp & Milko
U62014	*Wilcoxina mikolae* (C.S. Yang & Wilcox) C.S. Yang & Korf
U42655	*Wynnella silvicolor* (Beck) Nannf.
M94340	*Xerocomus chrysenteron* (Bull.) Quél.
Z49785	*Xylaria carpophila* (Pers.) Fr.

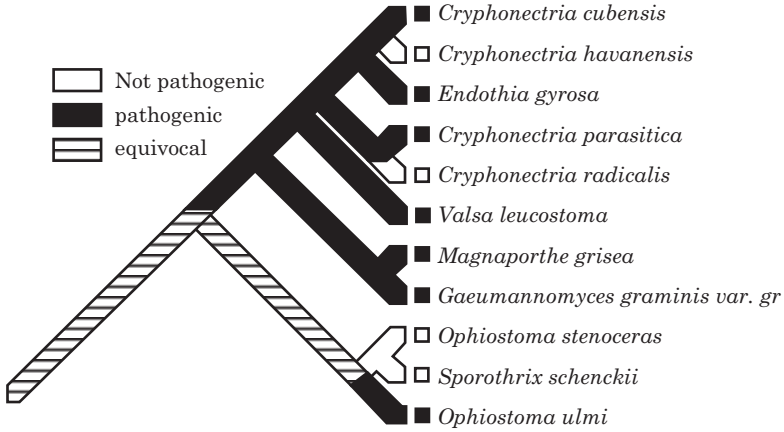

FIG. 2. Cladogram showing the evolutionary changes in pathogenicity on plants, in a small group of species, as reconstructed using parsimony analysis and the computer program MacClade. At least two and possibly three losses of plant pathogenicity would be required to explain the observed distribution of plant pathogens. This cluster of taxa includes the plant pathogens *Cryphonectria parasitica*, the cause of chestnut blight, *Magnaporthe grisea*, the cause of rice blast disease, and *Gaeumannomyces graminis*, the cause of take-all root rot of wheat and other cereals. The common ancestor to these four pathogens was, most parsimoniously, also inferred to have been a plant pathogen. Another prominent pathogen is *Ophiostoma ulmi*, one of the fungi causing Dutch elm disease. However, the Dutch elm disease fungus is also related to some non-pathogenic species so that using parsimony, it was unclear whether or not the ancestor to Ophiostoma and allies was pathogenic.

many gains in these clades were not reconstructed in any of the 1000 simulations in MacClade. In only 3 % of the simulations, 14 or more gains of pathogenicity were detected, allowing any number of losses. Making the alternative assumption, that ''equivocal'' ancestors were plant pathogens, 13 gains and 15 losses of plant pathogenicity would most parsimoniously be required to account for the current distribution of pathogens in the Ascomycota. All the losses and 11 of the gains were reconstructed as occurring in the classes with more than two plant pathogenic genera. Again, this number of gains and losses was never reconstructed in the random simulations. In only 2 % of the 1000 simulations were 10 or more gains of pathogenicity detected in the specified classes. In only 3 % of the simulations, 12 losses or more took place in these specified classes. In summary, the number of evolutionary gains and losses of plant pathogenicity, in the Leotiomycetes, the Dothideomycetes, the Sordariomycetes, and the Taphrinomycetes, was greater than expected by chance alone.

Human pathogens. In MacClade's reconstructions, pathogenicity on mammals never appeared as the ancestral character in classes of Ascomycota, implying that mammal pathogens originated recently. Animal pathogens were concentrated in the Eurotiomycetes and the Chaetothyriomycetes. These two classes often appeared as sister taxa in ribosomal DNA gene trees (Fig. 1). Evolutionary gains of human pathogenicity were more concentrated in these clades than predicted based on MacClade's simulations of random distributions. Assuming that in all equivocal cases, ancestors were not mammal pathogens, eight gains and no losses would have had to occur in the evolution of the mammal pathogens in the Eurotiomycetes and the Chaetothyriomycetes. However, six or more gains only appeared in 2 % of the 1000 random MacClade simulations. The second assumption, that in the equivocal cases, ancestors were mammal pathogens, required six gains and three losses of pathogenicity in the Eurotiomycetes and the Chaetothyriomycetes. The six gains and three losses did not appear in any of the simulations, and five or more gains were only observed in these clades in 4 % of the simulations. These results indicate that evolutionary gains of human pathogenicity were more concentrated in these clades than would be expected by chance alone.

Possible effect of more data on inferences about evolution of pathogenicity. As a qualification to this analysis, more data on the pathogenicity of fungi and a better representation of the total diversity of ascomycetes could alter the number of reconstructed gains and losses of pathogenicity. The number of transitions to and from pathogenicity would change if fungi coded as ''non-pathogens'' were instead coded as ''pathogens''. For example, in Fig. 2, one fewer loss of pathogenicity would be required if *Cryphonectria havanensis* were considered a ''pathogen'' rather than a ''non-pathogen''. Many fungal species are known only from fruiting bodies produced on dead plant material. Some of these species probably colonized living plants. If they occasionally caused disease of non-economic hosts, this might have escaped detection. The scattered nature of the pathogens in the tree (Fig. 1) could reflect the scattered opportunities to observe fungi in their parasitic phases.

Alternatively, because the alignment used in this analysis is biased in favor of plant and animal pathogens, estimates of the number of independent origins of plant and animal pathogenicity might increase with improved taxon sampling. Known animal and plant pathogens are much better represented than non-pathogens in culture collections, the genetic databases, and in Fig. 1. In June 2001, DNA sequences representing roughly 50 genera in the Dothideomycetes were available through GenBank. Almost a fourth of these genera included known plant pathogenic species. In Fig. 1, almost half the species representing the Dothideomycetes are plant pathogens. However, Eriksson *et al*. [*22*] list 727 genera of the Dothideomycetes, but few plant pathogenic genera outside of those already represented in GenBank. Similarly, a much higher proportion of saprobes than known plant pathogens are included among the 788 genera in the Sordariomycetes and 485 members of the Leotiomycetes [*22*]. From available sequences, it appears, given the high proportion of known plant pathogens in the Sordariomycetes, that the common ancestor of the class might have been plant pathogenic. However, imagine adding sequences from the 750 or so other, mostly saprobic genera of Sordariomycetes to the trees in Fig. 1 or Fig. 2. This more complete sampling would very likely surround the plant pathogens with large numbers of non-pathogenic taxa, and point to a more recent origin of plant pathogenicity, and to a higher frequency of independent origins of pathogens than can be detected at present. Future analyses, with more complete knowledge of the fungi, would contribute much to understanding the phylogenetic origins of the pathogens.

GENE EVOLUTION AND CHARACTERS OF PLANT PATHOGENICITY AND VIRULENCE

The pattern of high concentration of the pathogens in the Taphrinomycetes, Sordariomycetes, Leotiomycetes, and Dothideomycetes, coupled with the relative absence of plant pathogens from other groups including the Eurotiomycetes and the Pezizomycetes implies class-wide differences in genetic predisposition to pathogenicity. The classes are ancient groups of fungi. Are the characters involved in pathogenicity also ancient? Not necessarily. Predisposing characters need not be the same for all ascomycetes. The predisposition for some fungi to be pathogenic could result from a succession of traits coevolved in response to the evolution of plant defences. The original traits unifying the pathogens within a class in the Ascomycota may have been lost as layers of new characters, unique to genera and species, evolved in a plant/fungus arms race. Consistent with this possibility, the genes so far known to be associated with pathogenicity or virulence appear to be either too old or too young to explain the differences in pathogenicity in different classes. Ancient genes are involved in basic enzymatic processes (cellulose degradation, for example) that are probably essential for pathogenicity but may also be essential for saprobic living. Genes specifically linked to virulence or pathogenicity (toxin genes, for example) have narrow phylogenetic distributions with functions that evolved recently. The next section of this review traces the origins of some new and some old genes, associated with pathogenicity and virulence.

Enzymes of primary metabolism

Fungi pathogenic on plants must produce enzymes for degradation of plant cell walls and for the digestion of plant tissues. Based on their wide phylogenetic distribution, many of these enzymes were ancestral characteristics of ascomycete saprobes as well as pathogens. Enzymes common to the Chytridiomycota, the Ascomycota, and even bacteria degrade cellulose, hemicellulose, and glucans [*2*, *27*, *62*]. Particular properties of a set of enzymes might be important in pathogenesis. However, because genes for these enzymes are often present in multiple copies with overlapping functions, demonstrating their specific roles in pathogenesis is not simple [*32*].

Sometimes a gene for an enzyme with a general metabolic function is, in the arms race between fungi and plants, modified to take on a more readily demonstrable role in plant pathogenesis. Plants elaborate secondary metabolites toxic to the fungi. In some cases, fungi detoxify these antibiotics with an enzyme borrowed from their primary metabolism. Morrissey and Osbourn [*52*] review the mechanisms of fungal detoxification of several antibiotics produced by plants. Tomatoes, for example, produce an alpha-tomatine toxic to fungi, perhaps acting by destroying the integrity of fungal membranes. *Septoria lycopersici* Speg. detoxifies alpha-tomatine using a glycosyl hydrolase from the beta-glucosidase family of enzymes to remove one sugar residue from the toxin [*53*]. *Fusarium oxysporum* f. sp. *lycopersici* also detoxifies alpha-tomatine, but borrows a xylanase from a different family of glycosyl hydrolases to cleave four sugar residues from the toxin molecule [*65*]. *Gaeumannomyces graminis* detoxifies the defensive avenacins produced by oats, also using a beta-glucosidase to remove one sugar residue from a toxin molecule [*53*].

Also widely distributed across the Ascomycota are proteases. A trypsin-like protease from *Phaeosphaeria nodorum* [*Stagonospora nodorum* (Berk.) Castellani & EG Germano] was able to degrade wheat cell walls, possibly attacking the extensin proteins in the wall and releasing hydroxyproline [*14*], although it remains to be demonstrated that the enzyme is a virulence factor. Cytochrome P450s are another large family of enzymes with diverse functions. *Nectria heamatococca* uses pisatin demethylase, a

cytochrome P450, to detoxify a phytoalexin from peas, its host [*20*]. The use of all these enzymes in plant pathogenesis appears to be a recent innovation, based on the lack of evidence for evolutionary conservation of function.

Toxins

Toxin synthesis is important in virulence [*85*, *87*, *90*] and sometimes even in pathogenicity [*92*]. Toxin genes are also, to some extent, amenable to phylogenetic analysis. Walton and Panaccione [*87*] suggested that the evolutionary history of genes involved in plant/pathogen interactions may be analogous to the enzymes of the isoflavenoid biosynthesis pathway in plants in that the basic core of the pathway is common to all plants, but the enzymes at the endpoints of the pathways produce the diverse spectrum of flavenoids unique to different plants. Similarly, toxin production may build on a core pathway common to many organisms, with specific toxins resulting from gene duplication, gene rearrangement, and functional divergence of enzymes at the endpoints of pathways. Keller and Hohn [*39*] pointed out that homologous genes produce 1,3,8-trihydroxynaphthalene reductase, an enzyme involved in melanin production in Aspergillus, and versicolorin A reductase in the sterigmatocystin and aflatoxin pathways. These reductases are an example of genes that must have evolved through gene recycling and functional divergence.

Genes encoding fungal toxins are diverse. Some are associated with large DNA insertions absent in non-toxin producing isolates. Some are multifunctional enzymes, and many are arranged in gene clusters. High virulence in species of Fusarium, Alternaria, and Cochliobolus is clearly linked to the presence of host specific toxins [*91*]. Closely related fungi may use very different toxins. All the phytopathogenic Cochliobolus species are close relatives [*4*], but T-toxin in *Cochliobolus heterostrophus* (Drechsler) Drechsler is a polyketol; *Cochliobolus victoriae* Nelson's victorin is a chlorinated polypeptide; *Cochliobolus carbonum* Nelson's HC-toxin is a cyclic tetrapeptide, and *Bipolaris sacchari* (E. Butler) Shoem.'s HS-toxin is a sesquiterpene with galactose side units [*73*, *91*]. Given the lack of homology of these toxins, their roles in virulence must have arisen independently. Toxins unique to a species or to isolates within a species must also have arisen recently. The lack of obvious homology between toxin genes and other genes from the same species, along with their unusual size and organization, has led to the speculation that the genes arose by horizontal transfer from other organisms, such as bacteria, living in close proximity to the fungus [*85*, *90*]. Most of the known genes for toxins can, however, be traced phylogenetically to a fungal origin.

Walton [*86*] suggested that hyphal anastomosis might contribute to gene transfer between species, and that selection for transfer as a functional unit might be strong enough to maintain the clustering that is often associated with genes for secondary metabolites. However, as reviewed by Keller and Hohn [*39*], other kinds of genes involved in functions ranging from nitrogen metabolism to conidiation are also clustered, and so selection for horizontal transfer cannot be the only force driving gene clustering in fungi. In theory but not in practice, it is straightforward to distinguish between horizontal transmission of genes (that is, gene exchange between different species, outside the usual life cycle process of cell fusion and meiosis) and vertical transmission of the genes (from parent to offspring) through phylogenetic analysis (Fig. 3) [*77*]. If the phylogeny of toxin genes tracks the phylogeny of the fungi and the most closely related fungi have the most closely related toxin genes, then this is strong support for origin through the usually vertical transmission from generation to generation. On the other hand, if the toxin gene phylogenies unite unrelated fungi, this might indicate horizontal transfer. Complicating the picture, many toxin genes evolve quickly, erasing evidence of their evolutionary origin. Many are present as part of a family of duplicated genes, and if genes that evolved from different family members are inadvertently compared in a phylogeny, horizontal transfer may be inferred when it did not really occur. In the next section of this paper, a few examples of relationships among the genes that code for toxins will be briefly reviewed.

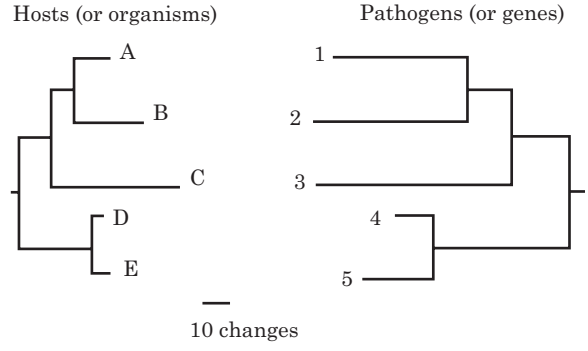

FIG. 3. Co-evolution resulting in correlated phylogenies. Similar correlated phylogenies could result from a parasite tracking its host during co-evolution, or from the congruent evolutionary patterns of a gene tree and a species tree in the absence of horizontal gene transfer. If, for example, a fungus and its host engaged in phylogenetic tracking, speciation in the host (for example, the split between "A" and "B") might result in speciation of the parasitic fungus (resulting in separation of parasite species "1" from species "2"). If this kind of tracking occurred, then, as in this diagram, host and pathogen phylogenies parallel one another. Branch lengths might also be correlated. Horizontal branch lengths are proportional to evolutionary change. The pathogens have longer branch lengths, indicating they might have had a faster rate of change than their hosts. However, the branch lengths of host and pathogen are more closely proportional than expected by chance, if host and pathogen speciation had been synchronized.

Polyketide synthases. Polyketide synthases help produce a range of biologically active molecules, including T-toxin. T-toxin, produced by race T *C. heterostrophus*, caused southern corn leaf blight to explode to epidemic status on male sterile corn in 1970 in the U.S.A. [*90*]. Other products of polyketide synthases include melanins, various toxins that are not involved in plant disease (such as the carcinogenic aflatoxins) and the cholesterol-lowering drug lovastatin [*40*]. Although structurally similar to fatty acid synthases, polyketide synthases make more diverse products because they are able to use substrates other than acetyl-CoA or malonyl-CoA and because they can suppress some or all of the regular sequence of reduction and dehydration steps that occur in fatty acid biosynthesis [*40*]. Like peptide synthetases, the last domain (closest to the carboxy terminus) in fungal polyketide synthetases is a thioesterase, which functions in chain termination and release of the peptide or polyketide [*13*]. Both sequence similarity and the arrangement of catalytic domains in polyketide synthases in fungi and fatty acid synthases in mammals point to a common evolutionary origin [*40*]. In technological applications, designer drugs are created by the use of genetic engineering to rearrange or recombine the domains in polyketide synthase genes [*59*]. In nature as well as in the laboratory, the domains within polyketide synthase genes may be reshuffled, producing novel polyketide synthases with new synthetic capabilities.

Polyketide synthase genes evolve quickly. To provide some comparisons, homologous 6-methylsalicylic acid synthases (polyketide synthases) from the closely related species *Aspergillus parasiticus* (accession No. AAC23536) and *Aspergillus terreus* (accession No. BAA20102) differed at 42 % of their amino acid positions. In contrast, these two species differed only at about 9·5 % of their nucleotide sites across their rapidly evolving, ribosomal internal transcribed spacer regions (accession No. AB008418 and AJ001333, respectively). As another example, *Penicillium griseofulvum* (accession No. P22367) and *Penicillium frei* (accession No. CAA65133) have different amino acids at 15 % of sites in the relatively highly conserved ketoacyl synthase domains in their 6-methylsalicylic acid synthases, but their internal transcribed spacer region sequences (accession No. AF033468 and PFA005479, respectively) are 98·6 % identical. Their rapid sequence evolution makes new polyketide synthase genes difficult to detect by southern hybridization [*7*] or even through PCR approaches [*63*].

Melanins might serve as markers for the phylogenetic distribution of polyketide synthases. This is because, where characterized in the Ascomycota, polyketide synthases have been among the genes involved in melanin production. Melanins are widely distributed through the fungi, including the Ascomycota, which could indicate that almost all ascomycetes have at least one polyketide synthase gene. Melanins can be essential for fungal cell wall strength, for protection from UV radiation, and for pathogenicity to plants and animals {reviewed by Butler *et al*. [*12*]}. It is speculated that these genes involved in pigment production may also serve as the precursors for toxin gene evolution. Evidence that fungi can ''borrow'' genes from melanin production, and through gene duplication and divergence create toxins is presented by the homology and 30–40 % amino acid sequence identity among polyketide synthases involved in melanin production in the Sordariomycetes fungus *Colletotrichum lagenarium* (Pass.) Ellis et Halsted (accession No. S60224, teleomorph *Glomerella*?), in production of green pigment in conidia of the Eurotiomycetes species *Emericella* (*Aspergillus*) *nidulans* (accession No. Q03149), and in production of the toxin sterigmatocystin in *E. nidulans* (accession No. Q12397). Although without strong bootstrap support, the polyketide synthases associated with T-toxin in *C. heterostrophus* clustered phylogenetically in a larger group including both pigment-producing polyketide synthases and aflatoxin and sterigmatocystin genes from Aspergillus [*7*]. If toxin evolved in *C. heterostrophus* by gene duplication and divergence, then homologues to the toxin genes should be found in the ancestral, non-toxigenic race O isolates [*89*, *90*]. So far, a homologue in race O remains undetected [*89*, *90*]. A critical analysis of the evolution of the T-toxin genes and other members of families of polyketide synthase genes will become possible when more complete genome sequences are available for the Ascomycota and a complete picture emerges of the range of genes from one species.

Peptide synthetases. Peptide synthetases, like polyketide synthases, help produce a diverse assortment of molecules that range from toxins (HC-toxin in *C. carbonum*, victorin in *C. victoriae*) to important drugs (penicillin and cyclosporin A) [*13*]. The production of the cyclic tetrapeptide HC-toxin in *C. carbonum* leads to a highly virulent leaf spot disease in susceptible maize cultivars. The HC-toxin synthetase is a typical peptide synthetase in that it is a large (in this case, 5217 amino acid) multidomain protein, consisting of an assembly line of individual units that add a succession of amino acids to form a peptide chain, without the need for a ribosome or transfer RNAs [*87*].

Peptide synthetases are known from both bacteria and fungi. Their multidomain structure suggested possible origin in fungi by horizontal transfer from bacteria [*43*]. However, transfer from bacteria would have had to have been either ancient or repeated many times to explain their broad distribution among the fungi. They occur in diverse ascomycetes and basidiomycetes and may have been an ancestral feature in both. The evolutionary building blocks of peptide synthetases include fatty acid synthases [*13*], which are present in both animals and fungi,

and which were also therefore present in the first fungi. In BLAST searches, the peptidyl carrier protein domains, which tether the growing peptide chain during its synthesis, show homology to acyl carrier proteins from fatty acid biosynthesis in animals and to acyl carrier proteins in polyketide biosynthesis in fungi. Unlike the polyketide synthases where the melanin biosynthesis pathway is one plausible source of raw material for duplication and functional divergence to produce toxin genes, a plausible source for peptide synthetases, other than distantly related fatty acid synthases, has yet to be identified.

As with polyketide synthases, the extreme divergence of known peptide synthetases, their large size, their unknown range of function, and the limited knowledge of possible paralogous, duplicate genes within a genome makes sorting out their phylogeny in more than general terms exceedingly difficult [*66*]. Amino acid sequences from the HC-toxin synthetase were only about 40 % similar to the inferred amino acid sequences of the peptide synthetases from other fungi that could be detected in PCR based searches. The closest matches to the amino acid sequence of the HC-toxins are from AM-toxin from an Alternaria species, and a peptide synthetase from *Metarhizium anisopliae*. Based on a BLAST search, the amino acid sequences of all three toxin synthetases were only about 30 % identical over about 4/5 of the gene. Alternaria and *C. carbonum* are close relatives in the same family while *M. anisopliae* is distantly related in a different class (Fig. 1), but these differing degrees of relationship are not reflected in differing percent similarities of the toxin genes. An explanation for the failure of percent similarity to track phylogeny would lead to improved understanding of gene evolution. Rosewich and Kistler [*66*] pointed out that there are often several equally plausible explanations for the presence of an unusual gene in a fungus. The genes in these three species may have evolved quickly, with loss of phylogenetic signal. They may be part of families of genes, and the large genetic distance between the Alternata and *C. carbonum* genes may indicate that different gene family members (or in other words, genes that are not orthologues) are being compared. The peptide synthetases among different filamentous ascomycetes appear to be homologues with a common evolutionary origin, based on their sequence similarity across the subphylum. However, exchange of toxin genes among ascomycetes by horizontal transfer cannot be ruled out.

AK-toxins. AK-toxins are virulence factors for Alternaria species causing Alternaria black spot of pear and of strawberry, as well as brown spot of tangerines. Each of the four genes that have been linked to toxin production is a member of a cluster of homologues [*81*]. Tanaka and Tsuge [*81*] could detect one of the toxin genes, *AKTR-2*, only in Alternaria strains causing black spot of pear. Suggesting that this gene evolved rapidly with functional divergence following gene duplication, *AKTR-2* differed from multiple copies of its homologues at 26 % of its amino acid positions [*81*]. The homologues were more widely distributed than *AKTR-2* and were also detected in isolates of Alternaria causing disease in strawberry and tangerines. Although the function of *AKTR-2* is unknown, the presence of a leucine zipper in the gene suggests that it is a transcriptional regulator. *AKTR-2* may be another example of a gene with a function in ordinary growth and development that was ''borrowed'' for a new role in toxin production and pathogenesis.

Trichothecenes. Trichothecene production appears to be an idiosyncratic ability to produce toxic sesquiterpenoids that evolved within the Hypocreales. Desjardins *et al*. [*21*] demonstrated that trichothecenes are virulence factors in *Gibberella zeae* (Schwien.) Petch. Trapp *et al*. [*83*] compared three genes needed for trichothecene production and found high levels of similarity in the genes from closely related genera, specifically, from *Fusarium sporotrichioides* Sherbakoff and *Myrothecium roridum* Tode : Fr. Although the genes in both species were clearly homologues, the distance between genes and direction of transcription were different, indicating that genetic rearrangement had occurred within the cluster. The gene encoding trichodiene synthase, the first enzyme in the synthesis pathway, has as of this writing, no obvious homologue to reveal its early evolutionary origins. The trichothecene 3-*O*-acetyltransferase gene product protects the fungi from their own toxin. The trichothecene 3-*O*-acetyltransferase gene has been proposed as a candidate for origin by horizontal transfer in the trichothecene B producing *Fusarium graminearum* because it is not part of the co-regulated trichothecene gene cluster and because its activity could not be detected in the trichothecene A producer, *F. sporotrichioides* [*41*]. O'Donnell *et al*. [*58*] used the trichothecene 3-*O*-acetyltransferase gene as a phylogenetic marker and concluded that the gene tracked phylogeny, for different species that do produce B trichothecenes, ruling out the occurrence of horizontal transfer after the radiation of these species. Future studies of whether or not the gene tracks the species phylogeny outside of the B trichothecene-producing Fusarium species will resolve whether horizontal gene transfer could have occurred earlier.

RARITY OF PLANT PATHOGENS IN THE SACHAROMYCOTINA, THE PEZIZOMYCETES, THE LECANOROMYCETES AND THE EUROTIOMYCETES

The paucity of plant pathogens in some clades poses a puzzle. Understanding why these clades do not produce pathogens might lead to an understanding of the

metabolic or structural features that enable other fungi to be pathogens. However, the non-pathogenic clades differ from each other and from the pathogens in many features. Just as there is no single feature that makes a fungus a pathogen, there is no obvious suite of features absent from all the non-pathogens.

In the Ascomycota, as in the Basidiomycota, groups with a high proportion of mutualistic symbionts do not usually give rise to plant pathogens. Within the filamentous ascomycetes, cup fungi in the class Pezizomycetes were perhaps the first to diverge and the group includes about 1000 known species [31]. Some of these fungi are ectomycorrhizal on woody plants in the Pinaceae and in the angiosperms. Having had a great deal of time to evolve and living in close proximity to plant roots, the Pezizomycetes might have been expected to spawn some aggressive plant pathogens. However, the only fungus in the class that can act as a plant pathogen is *Rhizina undulata*, causing root rots in the Pinaceae. Similarly, Hibbett *et al*. [34] concluded that in the Basidiomycota, ectomycorrhizal associations arose and were lost multiple times, usually with a transition to or from saprobism.

Production of large numbers of dispersed conidia that can stick to plants and initiate infection seems to be a widely distributed feature of plant pathogens. Lichens and the cup fungi in the Pezizomycetes rarely produce conidia, and this might contribute to the lack of pathogens in these groups. On the other hand, Eurotiomycetes including Penicillium and Aspergillus produce enormous numbers of conidia but are rarely associated with diseases of living plants. Losses of conidiation may be related to the lack of pathogens in some groups, but cannot be a global explanation for the class-specific low numbers of plant pathogens.

The ability to grow within plant material may be one of the several features necessary for the evolution of plant pathogenicity. Indicating that at least parts of their mycelia are closely associated with plants, sexual fruiting bodies from most fungi in the pathogen-rich classes are formed on or in woody or herbaceous plant material. Most fungi in the non-pathogenic clades, on the other hand, are found fruiting on other substrates. Morels and other fruiting bodies of the largely non-pathogenic Pezizomycetes are usually collected from the soil. Lichen fruiting bodies are (obviously) seated on the lichen thallus, and the fruiting bodies of the Eurotiomycetes are often collected on keratinous substrates or bits of substrate high in starches. The Chaetothyriomycetes seems to be an exception. The class includes no known plant pathogens, but specimens are usually found fruiting on rotten wood or on leaf surfaces [79]. In some but not all non-plant pathogenic fungal lineages, an inability to produce mycelia within plant material may have reduced the probability of evolution to plant pathogenicity.

Having lost enzymes for plant cell-wall breakdown could explain the rarity of plant pathogens in some groups of fungi. Baker's yeast, *S. cerevisiae*, lacks key enzymes for degrading plant cell walls. In lichens, fungi obtain nutrients from algae rather than vascular plants. If algal association and pathogenesis on vascular plants selected for different degrading enzymes, then lichenized fungi may have evolved away from the ability to cause plant disease. In a phylogenetic study emphasizing the distribution of lichenized species, Lutzoni *et al*. [48] suggested that the Eurotiomycetes are derived from lichens. The Eurotiomycetes must still have some of the enzymes needed for attack of plants, based on the ability of some species to decay stored fruits and seeds. However, perhaps their lichenized ancestors lost or modified some of the enzymes necessary for plant pathogenicity.

CO-EVOLUTION OF FUNGI AND PLANTS

Fungi and plants have a long history of opportunities for co-evolution. Most species in the Ascomycota, as well as most of the Basidiomycota, live off plants as saprobes, parasites, or symbionts. Together with fossil evidence of ancient plant/fungal association, the broad phylogenetic distribution of association with plants suggests that the Ascomycota depended on plants, dead or alive, for nutrients since its earliest evolution. Plants and fungi exert reciprocal evolutionary effects on one another. Fungal traits involved in pathogenesis have variance and respond to selection by plants, as is regularly demonstrated when pathogens overcome the disease resistance bred into crop plants. Plant populations vary in their resistance to fungi, as shown recently in the geographical variation in resistance by beans to *Colletotrichum lindemuthianum* (Saccardo & Magnus) Briosi & Cavara [26]. Plant chitinase genes, as well as other genes involved in resistance to fungi, evolved under positive selection, probably in adaptation to defence against fungi [8]. As another example of evolution of plant defences to fungal attack, Laugé *et al*. [44] showed that some tomato cultivars initiate resistance to the pathogen *Cladosporium fulvum* Cooke by detecting a virulence gene produced by the fungus. If plants and fungi have had reciprocal selective effects on one another over time, evidence of co-evolution in the phylogenies of fungi and their plant hosts is to be expected.

The association of an individual ascomycete and its host is often intimate, with the fungus growing exclusively in the plant. Thompson [82] argued that long-term, intimate association of a parasite with a single host favors specialization to a single host species. However, other outcomes are not only possible, but also likely, especially over long periods of time, such as the 400 or so million years the Ascomycota have had to evolve. Multiple

parasites may attack the same host, so that host evolution involves trade-offs rather than optimization of resistance to a single parasite [64]. Specialist parasites may evolve into generalists, jump hosts, or go extinct, and such events can erase the phylogenetic evidence of episodes of host tracking. Thompson [82] reviews many examples of specialist parasites that do not track host phylogenies but few of those that do. This potential for variation in evolutionary outcome is consistent with the varying patterns of host–pathogen phylogeny for the fungi in general and Ascomycota in particular.

Phylogenetic tracking of fungi and their plant hosts: do plant pathogens evolve early in the evolutionary history of their hosts?

If host/parasite tracking occurred, host and parasite phylogenies should show evidence of correlation (Fig. 3). Pairwise co-evolutionary relationships, where speciation in fungus and host are linked, have been proposed, particularly for the rust fungi and the smuts in the Basidiomycota, and for Taphrina and members of the Clavicipitaceae in the Ascomycota [16, 70, 71]. These fungal candidates for phylogenetic tracking all depend on living plants to complete their life cycles. Some candidates have not held up well to molecular phylogenetic scrutiny. Others should be re-examined with future critical molecular systematic studies. Savile [69] and others have speculated that the ferns (which he considered ancient plants) hosted the most ancient rusts while angiosperms hosted the most derived rusts. Unfortunately, this interpretation has not received support from molecular phylogenetics and the fern rusts turn out to be recent in origin and closely related to angiosperm rusts [74]. The smuts, or Ustilaginomycetes, include a high proportion of obligate plant parasites and hypotheses about co-evolution should be re-examined in the light of improved systematics. Smut fungi have few morphological taxonomic characters and unrelated smut fungi had been classified together, obscuring relationships between smut and host phylogenies. Taxonomic revision based on DNA sequences and more critical evaluation of morphological characters reveal that phylogenetically related smuts are more consistently associated with phylogenetically related hosts. The Ustilaginales, for example, parasitize grasses, while the Exobasidiales and Entylomales infect dicots. In the Ustilaginomycetes, each of the 10 orders proposed by Bauer *et al*. [3] has a clear preference for either monocot or dicot hosts.

As in the Basidiomycota, the Ascomycota primitive hosts do not usually have primitive parasites. The class Taphrinomycetes is made up of obligate biotrophic parasites that diverged hundreds of millions of years ago from other groups of filamentous ascomycetes with a high proportion of plant pathogens (Fig. 1) [6]. Taphrina was proposed as a candidate for an ancient lineage that coevolved with the first vascular plants [70]. Consistent with ancient co-evolution with vascular plants, ''Taphrina'' species have been described from highly divergent hosts, from both ferns and angiosperms [70]. However, molecular phylogenetic analysis casts doubt on the ascomycetous affinity of the ''Taphrina'' species on ferns. Several ''Taphrina'' species are reported from ferns, but DNA sequence analysis of the 18S genes of one such species, ''*T*''. *californicum*, revealed that the fungus is a basidiomycete and not a Taphrina species at all [75]. Very possibly, the other ''Taphrina'' species from ferns will also prove to be basidiomycetes, leaving only parasites of dicots in a carefully defined genus Taphrina. Based on 18S sequences, 11 species of Taphrina from dicots were correctly classified to genus, but, suggesting relatively recent origin, their sequences were too similar for resolution of their phylogenetic branching order [75]. Species of Protomyces, the sister group to Taphrina, are most often on herbaceous dicots, on Apiaceae or Asteraceae. The lack of molecular diversity and the dicot host preferences of confirmed Taphrina and Protomyces species point to a radiation long after the origin of angiosperms rather than phylogenetic tracking during angiosperm origins.

Fungi in the Clavicipitaceae, in genera including Claviceps, Balansia, and Epichloë are biotrophic on grasses and carry out most of their life cycles on their hosts. Most species in these genera are specialized to one or a few host species. *Balansia* spp. do not exhibit obvious phylogenetic tracking of their host grasses [42]. However, the beta-tubulin intron phylogeny of Epichloë species is congruent with a phylogeny from chloroplast restriction fragment length polymorphisms of temperate, cool-season host grasses [72]. The evolution of *Claviceps* spp. may have tracked the evolution of the tropical grasses that are frequently their hosts [61]. Further testing of phylogenetic tracking for these species would be welcome, with evaluation of whether parallels in host and fungus branching order are greater than expected by chance, and whether branch lengths in host and pathogen phylogenies from homologous genes are more closely proportional than expected by chance alone.

Beyond host tracking: host use and co-evolution in the Ascomycota

Although the Ascomycota probably evolved in association with plants, most of its evolution did not track plant evolution through continuing strong, pairwise associations. However, fungal species and host species are not randomly distributed either, and related fungi frequently infect related hosts. Geographical overlap between fungus and host is important in determining the host range. In Thompson's words [82], the Ascomycota appear to be very good ''ecological opportunists'' operating under ''phylogenetic constraints'' and geographical limitations.

Monilinia (Sclerotiniaceae, Leotiomycetes) provides a good example of a genus in which host jumping occurred occasionally, but related species usually attack related hosts [36]. Many of the Monilinia species infect fruits of plants in the Rosaceae or Ericaceae. Some host jumps may have been related to host fruit type and biochemistry. The Monilinia species that infect cherries and other fleshy fruits in the Rosaceae are more closely related to the Monilinia species infecting fleshy fruits like blueberries in the Ericaceae than they are to the species infecting members of the Ericaceae with dry, capsular fruits [36].

The Pleosporaceae offer another example of a common pattern among the Ascomycota in which the related species generally infect geographically or phylogenetically related hosts. However, a few species break the host preference rules. Alcorn [1] found that the Pleosporaceae genus Pyrenophora and its Drechslera anamorphs are predominantly associated with temperate grasses in the Pooideae (Avena, Bromus, Triticum etc.) [88]. Of 37 species of Drechslera/Pyrenophora, 24 are recorded from grasses in the Pooideae, while only seven are recorded from hosts in the Chloridoideae or Panicoideae. The statistics are reversed with Cochliobolus species with Bipolaris anamorphs (also in the Pleosporaceae) that are predominantly associated with tropical grasses in the Panicoideae (Zea, etc.) or Chloridoideae (Cynodon, Chloris, etc.) Of 89 species in Cochliobolus/Bipolaris, 66 are recorded from Panicoid or Chloridoid hosts, and only nine are on hosts in the Pooideae. Although species in the genus Cochliobolus/Bipolaris are usually associated with herbaceous monocots, *Bipolaris heveae*, one very close relative of the rice pathogen, *Cochliobolus miyabeanus*, jumped hosts dramatically and causes disease on rubber trees, which are woody dicots [93].

Improved taxonomic understanding helps to reveal the geographical distribution and host use patterns in the large and complex genus Fusarium and its teleomorphs in genera including Gibberella. For these fungi, O'Donnell and collegues have been using multiple gene genealogies to delimit potentially recombining phylogenetic species. Closely related Fusarium species often originate from the same geographical region. Species in the *Gibberella fujikuroi* complex are distributed into three monophyletic groups, one containing isolates from the Americas, one with African isolates, and one with the Asian isolates [56]. In a study of *F. graminearum*, isolates were clustered into seven monophyletic clades so that all isolates within a clade were from the same continent [58]. Although geography seems to be a good predictor of relationship, shared host specificity surprisingly is not. *Fusarium oxysporum* f. sp. *cubense* is defined by its ability to cause Panama disease of banana. However, this ability arose independently, multiple times among isolates of the *F. oxysporum* complex [57].

As obligate plant pathogens, powdery mildews might be expected to show higher levels of host specificity than the facultative pathogens in the Pleosporaceae or Fusarium, but, as Savile [71] pointed out, the powdery mildews can have surprisingly broad host ranges. Isolates of *Podosphaera fusca* infecting various hosts (from the Cucurbitaceae, Malvaceae, Fabaceae, Gesneriaceae, Verbenaceae and Asteraceae) had identical sequences in the highly variable ribosomal internal transcribed spacer regions [35]. Closely related powdery mildews must have jumped hosts recently to explain that, in ''Erysiphe Clade 3'' of Saenz and Taylor [68], three closely related fungal species (*Erysiphe cichoracearum*, *Erysiphe sordida*, and *Erysiphe orotii*) attack hosts in distantly related dicot families (respectively, Asteraceae, Plantaginaceae, and Brassicaceae). The mildews seem to be highly capable ecological opportunists.

One constraint on host switching in the powdery mildews may involve a requirement for the right kind of fungal fruiting body appendages to facilitate dispersal between hosts. Many powdery mildews surround their cleistothecia (closed, spherical fruiting bodies enclosing the sexual asci and ascospores) with dichotomously branched or hooked appendages. These branches or hooks may help the cleistothecium to adhere to twigs or the bark of woody hosts, after dispersal from the leaf surfaces on which they are produced. Mori *et al.* [51] concluded that powdery mildews on woody hosts were basal members of their phylogenetic clades and that occasional convergent switching from complex to simple hyphal appendages paralleled jumps from woody deciduous to herbaceous hosts. The appendages, they argued, are important in dispersal of the entire cleistothecium from the leaf of a woody host to its twigs, before the cleistothecia on the leaves would fall to the ground. If this view is correct, a mildew with simple appendages on an herbaceous host would need to evolve complex appendages before it could efficiently parasitize a deciduous, woody host.

Among the fungi in the Clavicipitaceae, Clay [16] proposed the evolution of Epichloë species and their asexual relatives as an example of a transition from sexuality and parasitism towards asexuality and mutualism. Selection related to mutualism may have favored asexual transmission of co-adapted sets of genes in the fungus. From recent phylogenetic studies, it is not completely clear that a progression from parasite to mutualist took place [42]. Whatever their origin, Epichloë and allied species of fungi do act as mutualistic symbionts, producing alkaloids that offer their host grasses protection against herbivores at the cost of reduced seed survival [16]. The origin of some endophytes appears to be through the hybridization of parents in different haploid, sexual species of Epichloë. Instead of a single copy of beta-tubulin genes, the endophytes have two to three copies, suggesting that they are diploid, triploid, or perhaps aneuploid. Supporting hybrid origin of the endophytes,

the beta-tubulin gene copies from a single individual endophyte clustered with different sexual species [*50*, *84*]. Possibly, asexuality in these fungi is the result of irregular chromosomal complements.

PHYLOGENY OF ANIMAL PATHOGENS

Animal pathogens are concentrated in the Eurotiomycetes and the Chaetothyriomycetes. Together, these two classes form a monophyletic group. They include common molds like Penicillium and Aspergillus, as well as a high proportion of fungi that are preferentially isolated from nature from hair, skin, feathers, nails, or other animal products high in keratin (Fig. 1), [*15*, *17*]. The animal pathogens are phylogenetically intermingled with soil-dwelling saprophytes, suggesting multiple, convergent origins of the ability to cause disease in animals [*11*, *45*, *60*, *79*]. The concentration of animal pathogens in two classes suggests that these fungi share characteristics that facilitated the repeated origin of pathogenicity on mammals. As with plant pathogens, it is not clear exactly which characteristics predispose towards the evolution of pathogens.

Plant pathogens and their host plants influence one another's reproductive success and many plant pathogens can invade healthy, living tissue and reproduce successfully on plant materials. In contrast, most fungi lack a means of transmission from one mammal to another, and most have difficulty overcoming barriers to infection including high body temperature, intact skin, and the immune system. When fungi do breach skin or mucous membranes to cause internal disease, it usually appears to be a chance infection with a negligible effect on reproductive success of either the animal or the fungal populations. In contrast to most plant pathogens, where the life cycle is usually understood, little is known about the biology of the many human pathogenic fungi outside the human body. For example, little is known about the ecology of human pathogenic species of the Chaetothyriomycetes [*30*]. *Pneumocystis carinii* has been a major killer of AIDS patients, but again, little is known about its life history. The source of *P. carinii* infections remains unclear and infections of humans may originate from a free-living form of the fungus that has yet to be discovered [*18*].

Coccidioides immitis is unusual among fungi in that it can cause fatal lung infections (Valley Fever) in otherwise healthy individuals [*24*]. Until recently, its life history was as opaque as that of *P. carinii*, but molecular population genetic studies are beginning to provide more definitive information about its natural history and evolution. *C. immitis* did not evolve with humans; high genetic diversity in *C. immitis* sister species points to an evolutionary history that predates humans. Until recently, *C. immitis*, like *P. carinii*, was known only from animal hosts. However, as expected of a saprobe, *C. immitis* has been isolated from soils [*29*]. If humans were important to *C. immitis* as a source of nutrients, then outbreaks of human disease would likely be associated with single clones of unusually virulent pathogens. Instead, Fisher *et al*. [*24*] determined, using a combination of single nucleotide polymorphisms and multi-allelic, short tandem repeats, that the *C. immitis* isolates involved in epidemic disease levels in California from 1991–1994 were not clonal. The outbreak may have been linked to environmental conditions including alternating drought and high rainfall that increased the saprobic fungal population. Earlier outbreaks of Valley Fever have been linked to earthquakes and wind storms that increased human exposure to dust carrying the fungus. The opportunistic nature of infections suggests that colonizing humans may be accidental, contributing little to the genetics and evolution of the fungal species.

Humans may also influence fungal evolution by carrying pathogens from one place to another. The athlete's foot fungus *Trichophyton rubrum* only occurred frequently in Europe after the World War II, and humans may have brought the disease from either Asia or Africa [*19*]. *Histoplasma capsulatum* var. *farciminosum* causes lesions in horses in Europe, Africa and Asia, but is phylogenetically nested among *Histoplasma capsulatum* var. *capsulatum* isolates from South America [*37*]. Kasuga *et al*. [*37*] speculated that horses were infected with the disease in South America, and then returned to the Old World. Fisher *et al*. [*23*] argued that limited genetic diversity of strains of *C. immitis* in South America could best be explained by a population bottleneck in the fungus, resulting from its long-distance dispersal by a mammalian host. Possible hosts during dispersal, they argued, were the first humans migrating south. The timing of the dispersal inferred from estimates of evolutionary rates would be consistent with dispersal by Amerindians, but the evidence here is equivocal. If humans were important in the early dispersal of *C. immitis*, it is not clear why human movement has not homogenized the distribution of *C. immitis* in the U.S.A. Two sibling species of *C. immitis* show strong geographical structuring, one mainly in California, and one primarily in Texas [*23*]. If humans were particularly important to genetic structuring of populations, both species of *C. immitis* would be widely distributed wherever the humans travel and the climate favors the fungus.

Just as the ability to degrade plant cell-wall materials is insufficient to explain the restricted phylogenetic distribution of plant pathogens, the ability to degrade keratin for carbon nutrients may be too widespread among the fungi to explain the concentration of animal pathogens in the Eurotiomycetes and the Chaetothyriomycetes. Beyond these two classes, keratin-utilizing fungi are scattered among the Ascomycota and the Zygomycota [*25*], and

keratinous bait substances are commonly used to attract and isolate chytridiomycetes [78]. Based on this broad phylogenetic distribution, the ability to degrade keratin may well have been ancestral in the fungi. Possibly, one link between reproduction on hair or feathers and pathogenicity has to do with bringing high densities of fungal spores into contact with high mammal populations, leading to opportunistic infections. High spore loads near dense mammal populations might also be related to the origin of pathogenicity in fungi that are associated with dung or faeces, such as *Ajellomyces capsulatus* (*Histoplasma capsulatum* Darling).

Proximity between fungus and host may also have been important in the evolution of fungal pathogens of insects. Like the fungi that attack mammals, the fungi attacking insects are phylogenetically concentrated in a few Ascomycota groups. A concentration of insect parasites, as well as biotrophs of other fungi and of living plants, is found among the Hypocreales [76], in genera including Cordyceps, Tolypocladium and Metarhizium (Fig. 1). The close phylogenetic relationships of insect parasites including 14 species of Cordyceps and four related anamorphs suggest that these fungi evolved and radiated on their insect hosts [54]. The specialization on insects did not preclude further dramatic host jumping, and one species of Cordyceps parasitic on fungi (specifically, on truffles in the genus Elaphomyces) is nested among species of Cordyceps parasitic on insects [54]. Nikoh and Fukatsu [54] speculated that the common subterranean habit of hosts allowed a Cordyceps species on cicada larvae to evolve to parasitism of the cleistothecia of Elaphomyces.

The Laboulbeniales may be another example of a group of fungi where proximity between insect and fungus led to parasitism [9, 10]. In this case, an ancestral fungal species similar to Pyxidiophora, a primitive genus in the Laboulbeniales, may have used arthropods for dispersal from one patch of nutrients to another. The extant Pyxidiophora species probably parasitize other fungi as their primary nutrient source, but they are dispersed by mites which in turn are carried by beetles or flies. Possibly, the other members of the Laboulbeniales that are obligate parasites of arthropods originated from ancestors like Pyxidiophora that had switched from using arthropods primarily for transportation to using arthropods as a primary nutrient source [9].

CONCLUSIONS

Since their origin hundreds of millions of years ago, the Ascomycota have been associated with plants. Plant pathogens in the Ascomycota are concentrated in four classes in the filamentous ascomycetes. The ancestral species within these groups may themselves have been plant pathogens or they may have been saprobes decaying plant material, pre-adapted for pathogenicity. The extant, highly virulent pathogens originated recently and independently. Traits associated with virulence, including toxin production, have in some cases arisen through duplication of genes with routine cellular functions, followed by gene divergence. Human pathogens are concentrated in two monophyletic clades, the class Eurotiomycetes and class Chaetothyriomycetes, that include the athlete's foot fungus, Penicillium, and black yeasts, but essentially no plant pathogens. Unlike the plant pathogens that depend on their hosts for survival, most animal pathogens probably exist primarily as saprobes and their level of reproductive success on animals may be negligible. Animals and fungi may not have had major reciprocal evolutionary effects.

On the other hand, fungi and plants do exert mutual evolutionary selection on genetically based, variable traits, resulting in at least periods of co-evolution. Related fungi often concentrate on phylogenetically related hosts. Opportunistic host jumping has also been frequent, however. As a result, comparison of host and fungus phylogenies shows minimal evidence of long-term phylogenetic tracking and primitive fungi are not noticeably concentrated on primitive hosts.

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