

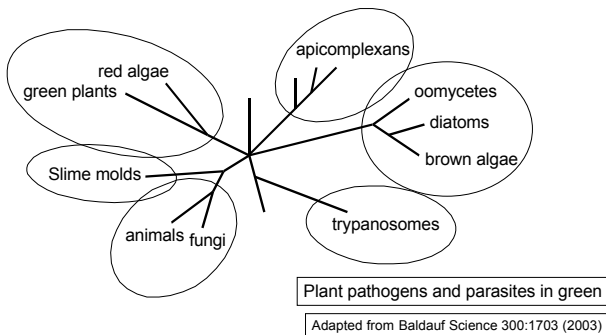
Eukaryotic plant pathogens

A diversity of eukaryotes (including plants) evolved the ability to infect and parasitize plants with varying degrees of specificity and severity.

Major phylogenetic groups of eukaryotic plant pathogens

- **Fungi:** (ascomycetes and basidiomycetes- *Fusarium*, powdery mildews, rusts etc...)
- **Stramenopiles (heterokonts):** Oomycetes (*Phytophthora*, *Pythium*, downy mildews etc...)
- **Metazoa (animals):** Nematodes
- **Green plants:** Parasitic plants (witchweed (*Striga*), mistletoe etc...)

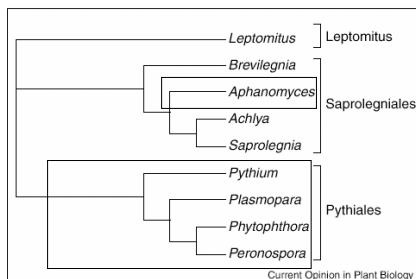
The ability to infect plants evolved multiple times in the eukaryotes



Major phylogenetic groups of eukaryotic plant pathogens

- The modern view of eukaryote evolution suggests several (8) major distinct lineages (Kingdoms?)
- Fungi are more closely related to animals than to plants
- Two distinct lineages of eukaryotic microbial pathogens of plants: Fungi and Oomycetes
- Plant pathogenesis evolved repeatedly and independently in eukaryotes: A diversity of pathogenic strategies!

Plant pathogenesis evolved at least twice in the oomycetes



Fungal infections

Pre-penetration events - may include:

- A. Chemotaxis
- B. Zoospore encystment
- C. Spore adhesion
- D. Spore germination
- E. Germ tube orientation and appressoria formation

Penetration of fungal pathogens

1. Active penetration can involve

- * Appressoria (some form, some don't)
- * Infection pegs (sharp or blunt)
- * Enzymatic or physical penetration events

2. Penetration through openings

- * most common is through stomata (*Cladosporium fulvum*)
- * can involve chemical or physical signals

Penetration of fungal pathogens

3. Penetration through wounds

large group of fungi that can only penetrate through wounds (*Fusarium*, *Sclerotinia*, *Ceratocystis*, and facultative pathogens)

Colonization and host cell "penetration"

Some fungal biotrophs penetrate into individual cells

Others (most biotrophs) grow between cells and later penetrate cells

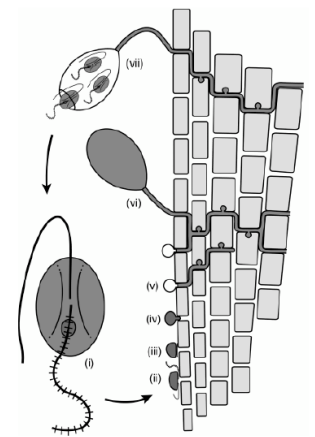
Some directly penetrate cells with their hyphae and form no specialized feeding structures

Others (rusts, mildews, smuts, some *Phytophthora*, *Pernospora*, etc) form specialized feeding structures called "haustoria"

Infection cycle of a soilborne *Phytophthora* in the root of susceptible plant

Example: *Phytophthora sojae* on soybean

From: A. Hardham Australasian Plant Pathology 30:9, 2001



Infection cycle of a soilborne *Phytophthora* in the root of susceptible plant A. Hardham Aus Plant Path 30:9, 2001

Fig. 1. Schematic diagram showing the infection cycle for a soilborne *Phytophthora* species establishing disease in a root of a susceptible plant. Motile zoospores (i) are chemotactically attracted to the root surface where they settle and encyst with their ventral surface facing the root (ii). Adhesive material is secreted during the first few minutes of encystment (iii). The cyst germinates (iv) and the germ tube penetrates the epidermis intercellularly (v) or intracellularly (not shown). As colonisation proceeds, haustoria may be formed in cortical cells (v). Within 2–3 days, multinucleate sporangia may develop on the root surface (vi) and cleave to form uninucleate zoospores that are released through an apical pore in the sporangium (vii). The zoospore in (i) and the rest of the diagram are not at the same scale. Zoospores and cysts are approximately 7–10 μm in diameter; sporangia are approximately 50 μm in diameter.

Bacterial vs. Fungal Plant Pathogens

- Fungi produce various types of spores to survive overwinter and disperse
- Infections frequently start from spores that germinate on plant surfaces
- Fungi use a number of degradative enzymes, such as cutinase, to make openings in plant cell walls
- Specialized penetration structures: appressoria and haustoria allow fungi to directly penetrate the host cytoplasm to gain nutrients
- Production of toxins is a common virulence mechanism

Induced Resistance Mechanisms

Hypersensitive Response

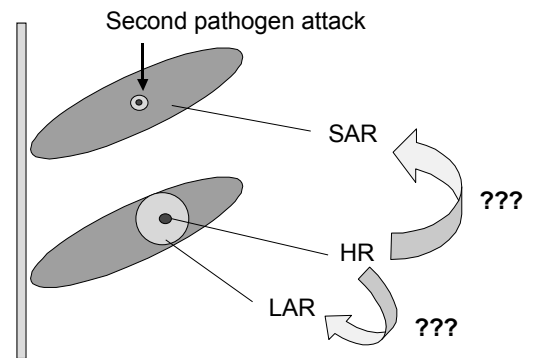
Local Induced Resistance

Local Acquired Resistance, LAR
Proximal Cell Resistance

Systemic Induced Resistance

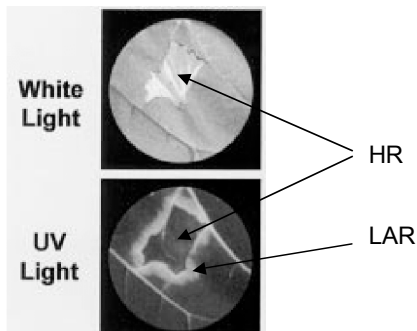
Systemic Acquired Resistance (SAR)
Induced Systemic Resistance (ISR)

Induced Resistance Mechanisms



Hypersensitive Response and LAR

Costet et al MPMI 12:655 (1999)



The hypersensitive response (HR)

A form of localized programmed cell death (PCD) which occurs in cells immediately adjacent to the invading organism - somewhat analogous to other PCD events in animals and plants

Thought to be a self-sacrifice reaction to contain the pathogen - it is complemented by a rapidly formed matrix of local oxidative, antibiotic, protein and barrier type responses

Also may generate secondary signals for other resistance responses (Proximal, Distal, LAR, SAR)

Local Acquired Resistance (LAR) and Proximal Cell Responses

Occur in healthy cells immediately adjacent to the HR

Critically important in preventing spread of the pathogen from the HR lesion

Some aspects are quite common to many plants, others more dependent on species

Relatively common elements:

Cell wall strengthening (e.g., lignin deposition)
Cell wall appositions (e.g., callose deposition)

Systemic acquired resistance (SAR)

First described in tobacco after infection of resistant plants with tobacco mosaic virus

Involves salicylic acid (SA) as a signal component, but this is not thought by most to be the systemic inducing compound

Resistance is due to accumulation of many of the PR proteins as well as possible potentiation of LAR responses

Induced Systemic Resistance (ISR)

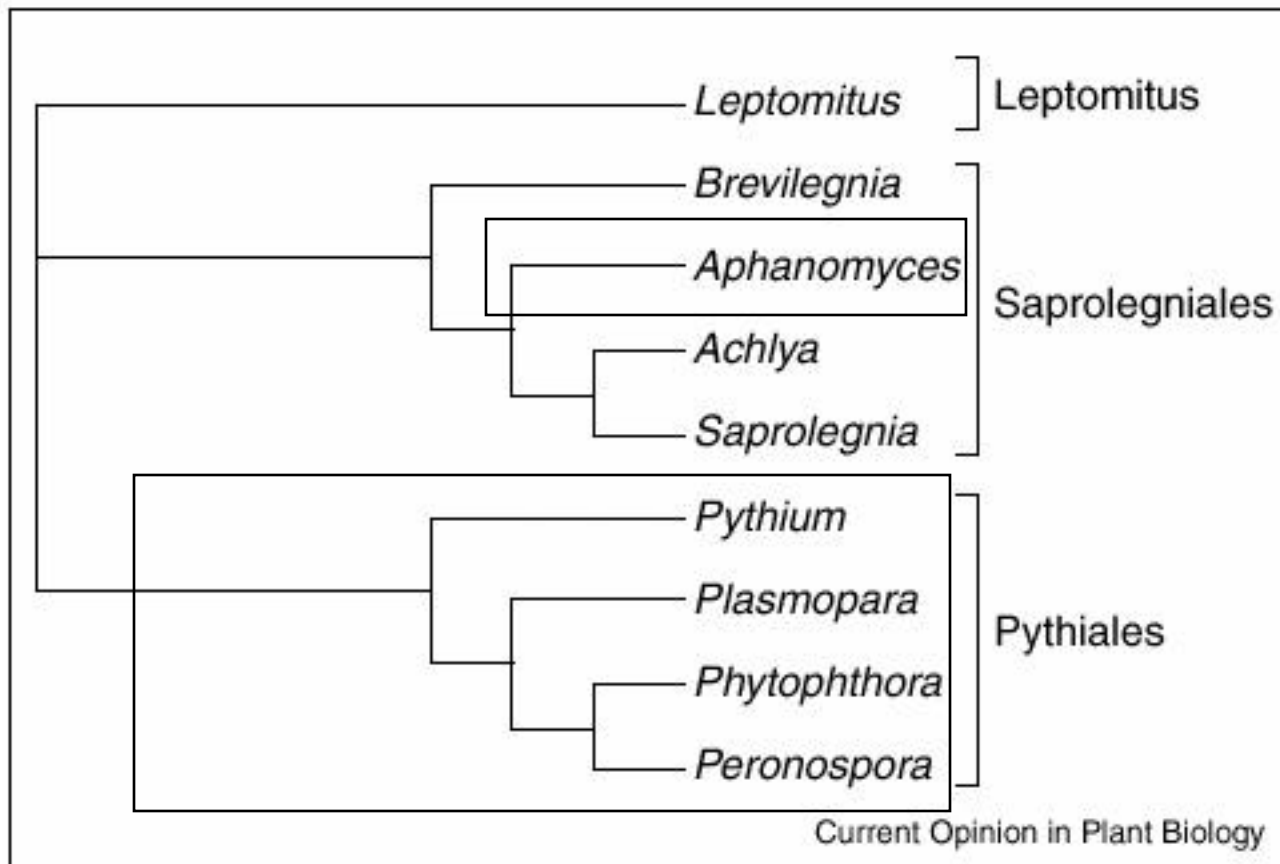
Induced in plants by rhizobacteria (biological control agents)

Induction seems to involve lipopolysaccharides, chelating agents, etc?

Is not mediated by SA, but involves ethylene and jasmonic acid as signals

Involves the accumulation of PR proteins?

Plant pathogenesis evolved at least twice in the oomycetes



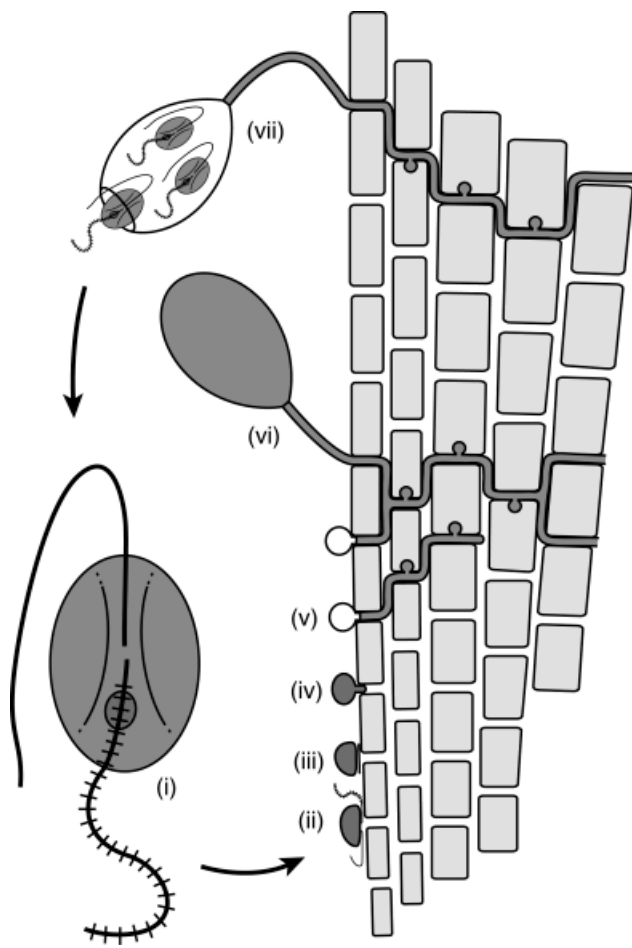


Fig. 1. Schematic diagram showing the infection cycle for a soilborne *Phytophthora* species establishing disease in a root of a susceptible plant. Motile zoospores (i) are chemotactically attracted to the root surface where they settle and encyst with their ventral surface facing the root (ii). Adhesive material is secreted during the first few minutes of encystment (iii). The cyst germinates (iv) and the germ tube penetrates the epidermis intercellularly (v) or intracellularly (not shown). As colonisation proceeds, haustoria may be formed in cortical cells (v). Within 2–3 days, multinucleate sporangia may develop on the root surface (vi) and cleave to form uninucleate zoospores that are released through an apical pore in the sporangium (vii). The zoospore in (i) and the rest of the diagram are not at the same scale. Zoospores and cysts are approximately 7–10 μm in diameter; sporangia are approximately 50 μm in diameter.

that are effective against *Phytophthora* species (Erwin and Ribeiro 1996) but the development of resistance to them can be quite rapid (see Davidse 1986). Resistant plant germplasm for some crop species subject to *Phytophthora* diseases is sometimes available, but may be limited and is subject to loss of effectiveness as the pathogen evolves methods of overcoming resistance. Management protocols must be based on a sound understanding of disease epidemiology and etiology. If we can identify the key features of *Phytophthora* pathogenicity at a cell and molecular level, it may then be possible to improve our

capabilities in each of the four above-mentioned areas of integrated pest management. This short review article aims to summarise our knowledge of the cell biology underlying the infection of plants by *Phytophthora* species. Much of the information also applies to *Pythium* species.

The Phytophthora infection cycle

The infection process is a cycle. The pathogen makes contact with the plant and becomes firmly attached to the plant surface. It then penetrates the host surface and colonises the plant, in the process acquiring the nutrients it needs for growth and sporulation. Spores are produced and released, and the cycle begins again. Fig. 1 shows a schematic representation of the infection cycle for a soilborne *Phytophthora* species and cell biological aspects of each of the main stages are discussed in more detail in the following five sections.

Initial contact with a potential host plant

For the majority of *Phytophthora* species, initial contact with a potential host plant is made by motile, biflagellate zoospores (Duniway 1983) (Fig. 2). Zoospores are wall-less cells whose outer surface is the plasma membrane. Lacking a cell wall, zoospores are unable to build up turgor pressure as part of their osmoregulation and instead, like many other protists, contain a contractile, water expulsion vacuole which takes up water from the cytoplasm and cyclically pumps it out of the cell. The water expulsion vacuole consists of a reticulum of tubular membranes, the spongiome, surrounding a central bladder (Patterson 1980; Cho and Fuller 1989). Although the molecular basis of contractile vacuole function is, as yet, not fully understood in any organism, it is believed that the spongiome accumulates osmolytes that draw in water from the cytoplasm down its chemo-osmotic gradient. In protists such as *Dictyostelium* and *Paramecium*, this process is thought to be powered by proton-pumping vacuolar ATPases (Fok *et al.* 1995; Temesvari *et al.* 1996) and in recent studies, similar ATPases have also been shown to be concentrated on the spongiome membranes of *P. nicotianae* zoospores (Mitchell and Hardham 1999). Treatment of *P. nicotianae* zoospores with 2 mM potassium nitrate, a known inhibitor of vacuolar H^+ -ATPases, slows the pulsing of the water expulsion vacuole cycle to nearly half the rate in untreated cells and leads to premature encystment (Mitchell and Hardham 1999).

Most fungal spores are blown passively in the wind but *Phytophthora* zoospores swim actively in water and, although they are less than 10 μm in diameter, can travel 25–35 mm in waterlogged soils (Duniway 1976). The anterior flagellum projects in front of the cell and pulls the cell forward (Carlile 1983). The posterior flagellum acts like a rudder to turn the zoospore as it swims. The anterior flagellum propagates sinusoidal waves from base to tip and would thus be expected to propel the zoospore backwards. It

is either Archaea (nuclear housekeeping-gene trees) or Bacteria (mitochondrial gene trees). The antiquity of these relationships makes them almost by definition the mother of all long branches. Thus, although most rooted molecular trees place the amito-excavates nearest the root [e.g., (2, 3, 30, 31); but see (24)], these taxa, also largely obligate parasites or symbionts, also tend to have very long branches. This makes their deep position look suspiciously like a long-branch attraction to the long branches of the distant outgroup (6).

An alternative approach to quantitatively calculating trees is to use macromolecular characters such as gene fusions, genomic rearrangements, or large insertions and deletions in conservative genes. These can be powerful phylogenetic markers because they are rare, complex, and largely irreversible and therefore unlikely to arise independently and be shared by unrelated taxa. A particularly dramatic example is the fusion of dihydrofolate reductase and thymidylate

synthase (11), recently identified in nearly all eukaryotes except opisthokonts (animals, fungi, and their allies). Because gene fusions are rare, particularly among eukaryotes that lack operons, these genes probably fused only once, meaning that most eukaryotes share a unique common ancestor exclusive of opisthokonts.

Thus, these data place the root of the eukaryote tree between opisthokonts and nearly all the other major eukaryote taxa. Essentially, it turns the tree on its head, rooting it within the former "crown radiation." This is a radical reinterpretation and would mean that opisthokonts branched off very early from the main line of eukaryote descent. The LCA of all extant eukaryotes would then have been a far more complex organism than previously envisioned, and any similarities between, e.g., animals and plants would simply be universal eukaryote traits. It also suggests that opisthokonts may be older than previously thought, consistent with the diver-

sity of single-celled protists now thought to be closely allied to animals and/or fungi (32).

There are many caveats. While compelling, this gene fusion is still only a single character and unsupported by any robust molecular trees, most of which still place the root close to or within amito-excavates. It is particularly disconcerting that these genes are missing altogether from amoebozoans and amito-excavates, which occupy pivotal positions in the two competing scenarios (Fig. 1). The antiquity of this event (1 to 2 billion years) allows alternative explanations such as reversal of the gene fission in an opisthokont ancestor (33) or replacement of the fused genes by lateral gene transfer from bacteria (34), where these genes are adjacent in an operon.

Future Prospects

The discovery, much less the characterization, of ultrasmall eukaryotes is barely in its infancy. Few habitats have been reported on, and so

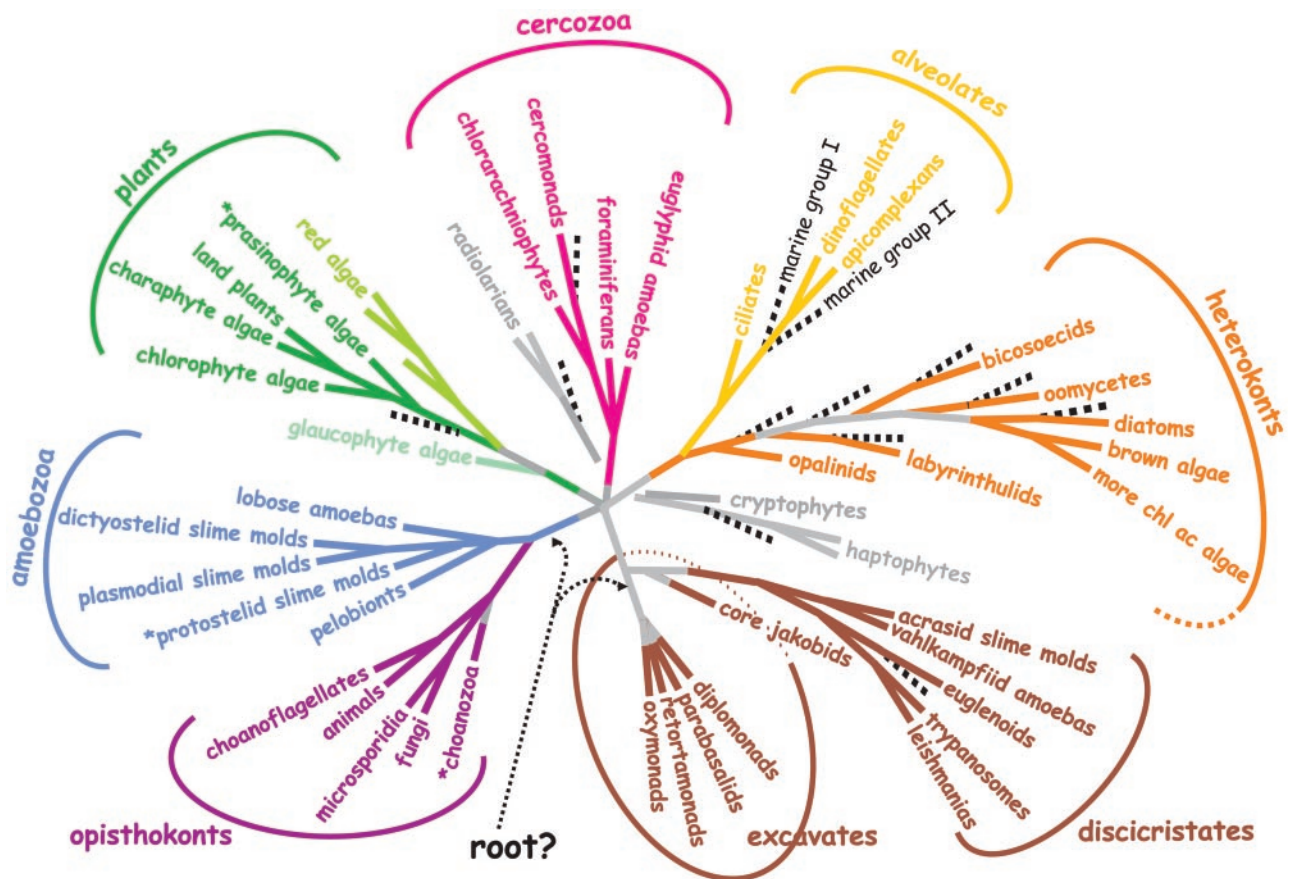


Fig. 1. A consensus phylogeny of eukaryotes. The vast majority of characterized eukaryotes, with the notable exception of major subgroups of amoebae, can now be assigned to one of eight major groups. Opisthokonts (basal flagellum) have a single basal flagellum on reproductive cells and flat mitochondrial cristae (most eukaryotes have tubular ones). Eukaryotic photosynthesis originated in Plants; theirs are the only plastids with just two outer membranes. Heterokonts (different flagellae) have a unique flagellum decorated with hollow tripartite hairs (stramenopiles) and, usually, a second plain one. Cercozoans are amoebae with filose pseudopodia, often living within tests (hard outer shells), some very elaborate (foraminiferans). Amoe-

bozoa are mostly naked amoebae (lacking tests), often with lobose pseudopodia for at least part of their life cycle. Alveolates have systems of cortical alveoli directly beneath their plasma membranes. Discicristates have discoid mitochondrial cristae and, in some cases, a deep (excavated) ventral feeding groove. Amitochondrial excavates lack substantial molecular phylogenetic support, but most have an excavated ventral feeding groove, and all lack mitochondria. The tree shown is based on a consensus of molecular (1-4) and ultrastructural (16, 17) data and includes a rough indication of new ciPCR "taxa" (broken black lines) (7-11). An asterisk preceding the taxon name indicates probable paraphyletic group.