2006 Summer Workshop in Fungal Biology for High School Teachers
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Introduction to Fungal Biology—Morphology, Phylogeny, and Ecology

General features of Fungi

Fungi are very diverse. It is hard to define what a fungus is using only morphological criteria.

Features shared by all fungi:

- Eukaryotic cell structure (but some have highly reduced mitochondria)
- Heterotrophic nutritional mode—meaning that they must ingest organic compounds for their carbon nutrition (but some live in close symbioses with photosynthetic algae—these are lichens)
- Absorptive nutrition—meaning that they digest organic compounds with enzymes that are secreted extracellularly, and take up relatively simple, small molecules (e.g., sugars).
- Cell walls composed of chitin—a polymer of nitrogen-containing sugars that is also found in the exoskeletons of arthropods.
- Typically reproduce and disperse via spores

Variable features of fungi:

- Unicellular or multicellular—unicellular forms are called yeasts, multicellular forms are composed of filaments called hyphae.
- With or without complex, multicellular fruiting bodies (reproductive structures)
- Sexual or asexual reproduction
- With or without flagella—if they have flagella, then these are the same as all other eukaryotic flagellae (i.e., with the "9+2" arrangement of microtubules, ensheathed by the plasma membrane)
- Occur on land (including deserts) or in aquatic habitats (including deep-sea thermal vent communities)
- Function as decomposers of dead organic matter or as symbionts of other living organisms—the latter include mutualists, pathogens, parasites, and commensals (examples to be given later)

Familiar examples of fungi include mushrooms, molds, yeasts, lichens, puffballs, bracket fungi, and others.

There are about 70,000 described species of fungi. The actual number of species is a matter of conjecture. One popular estimate is that there are 1.5 million species of fungi.

Fungal Phylogeny

A phylogenetic definition of fungi:

Fungi form a monophyletic group (a single branch of the tree of life, including an ancestor and all of its descendants—also called a clade).

The closest relatives of the fungi include animals and slime molds (Mycetozoa). Fungi have traditionally been studied by "botanists" but they are not closely related to plants.

Some organisms that have traditionally been considered fungi are now known not to be in the fungus clade, including slime molds and Oomycetes. The latter includes the organism that caused the Irish potato famine, *Phytophthora infestans*. *Phytophthora* is a member of the Stramenopiles, a group that also includes kelps (including the largest seaweeds), diatoms (unicellular phytoplankton with glass cell walls) and others.

Some organisms that have not traditionally been considered fungi are now known to be Fungi, including *Pneumocystis* (an intracellular parasite—infects lungs of mammals—a serious pathogen of AIDS patients), and microsporidia (another obligate intracellular parasite, with highly reduced mitochondria).

The major groups of fungi:

- Chytridiomycota*—flagellate, mostly aquatic, unicellular or filamentous. ca 1000 described spp.
- Zygomycota*—non-flagellate, mostly terrestrial, filamentous, no multicellular fruiting body, zygospores or asexual, aseptate hyphae. ca 1000 described spp.
- Ascomycota—non-flagellate, mostly terrestrial, filamentous or unicellular (yeasts), ascospores or asexual spores, septate hyphae, many with multicellular fruiting bodies. ca 45,000 described spp.
- Basidiomycota—non-flagellate, mostly terrestrial, filamentous or unicellular, basidiospores or asexual spores, septate hyphae—often with "clamp connections", multicellular fruiting bodies. ca 22,000 described spp.
  *non-monophyletic groups

Ecological Roles of Fungi

All fungi are heterotrophs, which means that they must use organic compounds as sources of carbon nutrition. Fungi have evolved diverse strategies for obtaining carbon, however. Broadly, fungi are either saprotrophs (saprobes), which decay dead organic matter, or symbionts, which obtain carbon from living organisms. A few fungi attack small living organisms so aggressively that they have been called "carnivorous". Many fungi defy easy categorization, acting as saprotrophs, symbionts, or carnivores at various stages of their life cycles, or in interactions with different organisms. Necrotrophic fungi act both as saprotrophs and symbionts—such forms infect a living host, which they kill, then continue to feed off of the dead tissues of the host.

Saprotrophs.

Saprotrophic fungi decompose diverse carbon sources. They have negative economic impacts when they attack substances that are produced and used by humans, such as fabric, leather goods, petroleum products, and especially food and wood products.

Fungi play a pivotal role in the decomposition of plant matter, where most of the carbon in terrestrial ecosystems is sequestered. Aerobic respiration by fungi returns much of this carbon to the atmosphere as CO_2.

One ecological group of fungi that decays plant tissues is the litter decomposers, which decay leaves and other relatively ephemeral substrates. Leaf litter is patchy, ephemeral, and heterogeneous. Litter decomposers employ different strategies to gain access to their substrates. Some reproduce quickly, producing many spores that can colonize news substrates, while others employ rhizomorphs, which allow them to explore the forest floor. Many mushrooms that grow on the forest floor are litter decayers. **Examples**: *Gymnopus dryophilus, Marasmius sp*. (basidiomycetes).

Another major ecological assemblage is the wood decay fungi. Wood is composed mostly of cellulose, which is a polymer of sugars, and lignin, which is a complex, heterogeneous polymer that is made up of several phenol-containing compounds. Lignin is extremely resistant to decay. Fungi, mostly basidiomycetes, are among the few organisms that are capable of degrading lignin. Species that decay massive woody substrates produce the largest fruiting bodies in the fungi. **Example**: *Inonotus dryadeus* (basidiomycete).

There are two major categories of wood decay fungi:

1. White rot fungi are capable of degrading both lignin and cellulose. They leave the wood bleached and with a stringy consistency. White rot fungi can degrade diverse organic compounds besides lignin. They are therefore of potential use in bioremediation (reduction of environmental pollutants using living organisms) and biopulping (treatment of wood fibers in paper production). **Example**: *Trametes versicolor* ("turkey tails", basidiomycete)
2. Brown rot fungi degrade cellulose but leave the lignin behind. Brown-rotted wood has a crumbly consistency, breaking up into cubical fragments, and has a red-brown color. Brown rot residues are highly enriched in lignin and are very resistant to further decay—they make up a major component of humic soils in some forest types. **Examples**: *Piptporus betulinus, Fomitopsis pinicola* (basidiomycetes). *Serpula lacrimans*, mentioned above, is also a brown-rot fungus.

In addition to plant tissues, saprotrophic fungi degrade chitin (including arthropod exoskeletons and remains of other fungi), dung, and other naturally occurring substrates.

Carnivorous fungi.

About 150 species of fungi attack, kill, and digest small organisms including bacteria and nematodes. **Examples**:

1. *Pleurotus ostreatus* (oyster mushroom, basidiomycete). This is primarily a wood decay fungus (white rot), but it traps nematodes using droplets of toxin that paralyze the animal. The nematodes may provide a nitrogen supplement for the fungus—wood is a nitrogen-poor environment. *Pleurotus* and many other wood-decay fungi will also attack and digest colonies of bacteria in agar cultures.
2. *Arthrobotrys* and *Dactyllella* spp. (ascomycetes). These fungi trap nematodes by diverse mechanisms, including constricting and non-constricting rings of hyphae that snare nematodes. Some of these have been shown to be anamorphs of the sexual ascomycete *Orbilia*, which grows on wood.
3. *Catenaria* (chytridiomycete) infects nematodes by means of swimming spores.

Symbiotic fungi.

Symbioses are intimate associations involving two or more species. Fungi have evolved numerous symbioses involving diverse eukaryotes and prokaryotes. Traditionally, symbioses are categorized according to the relative "benefit" or "harm" that the partners experience (properly conceived in terms of fitness—reproductive success) as a consequence of the interactions. In parasitism one partner benefits from the association, but the other partner is harmed. In mutualisms both symbionts benefit from the interaction. In commensalism, one partner benefits, but there is no (perceived) effect on the other partner.

The categories given above are useful for conceptualizing the diversity of symbioses, but they oversimplify the nature of the interactions, especially "mutualisms". It is now appreciated that even in the most benign associations there is a basic conflict of interest among the partners, both of which are trying to maximize their reproductive output at the expense of the other partner. Thus, many ecologists and evolutionary biologists now regard mutualisms and other symbioses as "reciprocal parasitism". That being said, the following discussion provides examples of selected fungal symbioses, divided into the traditional (if flawed) categories of parasitism, mutualism, and commensalism.

Commensalism

These are symbioses in which there is no obvious effect on the host. Some biologists reject the concept of commensalism, insisting that every interaction must have an effect on fitness, positive or negative, no matter how slight.

1. Laboulbeniales (ascomycetes) are symbionts that occur on the exoskeleton of insects. 1869 species are known, despite the fact that they have been studied by only a handful of mycologists.
2. Trichomycetes (zygomycetes) live in the hindgut of aquatic arthropods. They are most commonly known from the stream-dwelling larvae of insects such as stoneflies, blackflies, and mayflies, but one has been discovered in the gut of a galatheid squat lobster in a deep-sea hydrothermal vent.
3.

Parasites and Pathogens

Parasitism. Selected examples, of many possible:

1. Plant pathogens, e.g. *Cryphonectria parasitica*, the chestnut blight fungus
2. Animal pathogens: *Batrachochytrium dendrobatidis* (chytridiomycete) causes amphibian chytridiomycosis—a recently discovered disease that is implicated in the worldwide decline of amphibians. *Cordyceps militaris* (ascomycete) is an insect pathogen.
3. Fungal parasites: *Asterophora parasitica* (basidiomycete) is a mushroom that grows on mushrooms. *Hypomyces lactifluorum* (ascomycete) is a parasite of fleshy mushrooms (basidiomycetes). The parasitized mushrooms are edible and are sold as "lobster mushrooms".

Mutualism

Many fungal mutualisms are driven by the ability of the fungus to decompose organic substrates that are inaccessible to its host. Again, it is often not clear to what extent the two partners benefit (experience enhanced fitness), which calls into question the classification of these interactions as "mutualisms".

1. Attine ants and cultivated fungi. The attine "leaf-cutter" ants are major herbivores of the neotropics. These insects cut pieces of plant tissue which they carry to underground nests and feed to cultivated basidiomycetes. The ants then feed on the fungi. When winged female ants disperse to found new nests they carry the fungal inoculum with them.
2. Termites and *Termitomyces* (basidiomycete). In Africa, termites in the subfamily Macrotermitinae cultivate basidiomycetes, much like attine ants. The termites consume plant material and deposit it as fecal material in the nest. Fungi in the next digest the plant tissue and the termites consume the fungi. Fungal cellulases remain active in the termite's gut. As in attine ants, females that disperse to found new colonies bring fungal inoculum with them.
3. Bark beetles and woodwasps and their cultivated fungi. These distantly related insects share the habit of colonizing dead trees. In both cases, the fungi inoculate the chambers where the eggs are laid with wood-decaying fungi, including ascomycetes and basidiomycetes. Larvae feed on fungal hyphae.

4. Gut symbionts (*Neocallimastix* spp.; chytridiomycetes) are found in the stomachs of cows and other ruminant herbivores. Along with bacteria and other microorganisms, these anaerobic fungi help cows digest plant matter.
5. Lichens are symbioses involving fungi and unicellular algae. The fungi are mostly ascomycetes, but there are also a few “basidiolichens”. The algae are mostly eukaryotic green algae, but there are also some cyanobacterial symbionts. The fungi obtain carbohydrates from the algae, which are photosynthetic and contribute the green color to the lichen thallus. Many lichens are sensitive to air pollution and are indicators of air quality.
6. Mycorrhizae are symbioses involving fungi and the roots of plants. In these associations, the fungi derive photosynthetic sugars from the plants, and they assist the plant by facilitating the uptake of mineral nutrients and water. Approximately 70-80% of all plants have mycorrhizae. Mycorrhizae have evolved repeatedly in different groups of fungi. There are two major forms of mycorrhizae:
    - Arbuscular mycorrhizae are formed by zygomycetes called Glomales (150 species). Although there are relatively few known species of Glomales, these symbioses are extremely widespread, involving roughly 70% of plants, including many herbaceous plants. Here the fungal hyphae penetrate into the cells of the root cortex, where they produce characteristic branched structures called arbuscules. Reproduction is by asexual spores that are produced underground—there are no aboveground structures or multicellular fruiting bodies, which makes Glomales very difficult to study. May have been important in colonization of land by plants.
    - Ectomycorrhizae are formed primarily by basidiomycetes (about 5000 species), and also a few ascomycetes. A sheath of hyphae called a mantle envelops the plant root and hyphae penetrate into the cortex. These symbioses involve mostly forest trees, including oaks, birches, willows, pines, dipterocarps, and eucalypts. Many choice edible fungi are ectomycorrhizal.

The monotrope symbiosis

- *Monotropa uniflora* is a non-photosynthetic plant—it does not make sugars via photosynthesis
- Yet it is mycorrhizal—in this case the plant gets the sugars from the fungus, which gets sugars from a neighboring green plant
- Monotropes are “cheaters”—their association with fungi is a <u>parasitism</u>

It is difficult to identify mycorrhizal partners of plants, because the connection between the fungus and the plant is underground, and the identity of the fungus is only apparent when it produces a mushroom.

In our project, we will attempt to identify the fungal symbionts of indian pipes plants. To do so, we will obtain sequences of ribosomal genes from fungi and monotropes and we will compare them to each other, as well as to sequences of fungi from the GenBank database.

References/resources:

Books:

Campbell, N. A., and J. B. Reece. 2002. *Biology, Sixth Edition*. Benjamin Cummings. This text has the most up-to-date discussion of fungal phylogeny that I have seen in a general biology text. Most of the material on fungi is in Chapter 31 (Fungi), but there is also information about mycorrhizae in Chapter 36 (Transport in Plants), and decomposers in Chapter 54 (Ecosystems). The introduction to eukaryote phylogeny, including fungi, is also very good (Chapter 28).

Alexopoulos, A., C. W. Mims, and M. Blackwell. 1996. *Introductory Mycology, Fourth Edition*. Wiley. This is an encyclopedic treatment of fungal diversity. It is loaded with discussions of the ecological roles and practical significance of fungi, with copious life cycles, illustrations, and primary source references. The classification is a bit out of date, but the descriptions of individual groups are unmatched. An essential reference for anyone who gives more than a few lectures on fungi in a year.

Kendrick, B. 1992. *The Fifth Kingdom, Second Edition*. Mycologue Publications. This paperback provides very readable discussions of many aspects of the diversity, ecology, and practical significance of fungi. Primary sources are referenced. The taxonomy is out of date, but this is still a useful text. Out of print?

Benjamin, D. R. 1995. *Mushrooms: Poisons and Panaceas*. Freeman. Good overview of fungal toxins and the groups of fungi that produce them. Includes discussion of the physiology and chemistry of mushroom poisoning. The author is a physician.

Hudler, G. W. 1998. *Magical Mushrooms, Mischievous Molds*. Princeton University Press. Very entertaining popular text treating miscellaneous topics on plant/animal pathogens, edible and hallucinogenic mushrooms and other subjects. Should be accessible for high school students. Includes references to other “mainstream” publications relating to fungal topics.

Wasson, R. G. 1968. *Soma: The divine mushroom of immortality*. Harcourt Brace Janovich. The author advances his theory that the soma described in the Rig Vedas was actually *Amanita muscaria*. A classic text in ethnomycology.

Cracraft, J., and M. J. Donoghue. 2004. Assembling the Tree of Life. Oxford University Press. This edited volume summarizes current knowledge about the phylogeny of all of life. The Fungi chapter provides an excellent overview of the current picture of fungal diviersity. This is a very useful general reference on biodiversity.

Web sites:

Mycological Society of America. The main professional mycological organization in the United States. The links pages include many professional and amateur organizations, laboratories, etc. http://MSAfungi.org/.

Tom Volk’s Fungi. Tom is a professor at the University of Wisconsin, La Crosse. His web site has an incredible diversity of photos, essays, and links. http://botit.botany.wisc.edu/toms_fungi/.

Boston Mycological Club. Local group of serious amateurs—and the oldest mushroom club in the country. Holds regular forays open to the public during the fruiting season. This club provides great opportunities to learn about mushroom identification. http://www.bostonmycologicalclub.org/.

Berkshire Mycological Society. Another local mushroom group. They also hold regular forays. http://www.bms.iwarp.com/.

Friends of the Farlow. This is a group of amateur and professional cryptogamic botanists, who support the Farlow Herbarium and Reference Library of Cryptogamic Botany at Harvard University. The FoF includes members who are interested in fungi, including lichens, bryophytes (mosses and liverworts), and algae. They hold an annual meeting with a lecture on cryptogamic botany, and host the annual Clara Cummings Walk, usually in the springtime. Both are open to the public and provide informal opportunities to learn about cryptogams. http://www.huh.harvard.edu/collections/fof/fof.html.

APSnet article on *Stachybotrys* mycotoxins and sick building syndrome: http://www.apsnet.org/online/feature/stachybotrys/

Tree of Life Web Project. The ToL is an ambitious project to provide an overview of the diversity of all of life in a phylogenetic framework. Each page on the ToL is for a particular node (clade) in the tree. The ToL is intended to be accessible to students and other non-specialists. There are several pages on fungi, including Ascomycota and Basidiomycota, and more should appear over the next few years. Begin at the root node for fungi and surf upwards: http://tolweb.org/tree?group=Fungi&contgroup=Eukaryotes