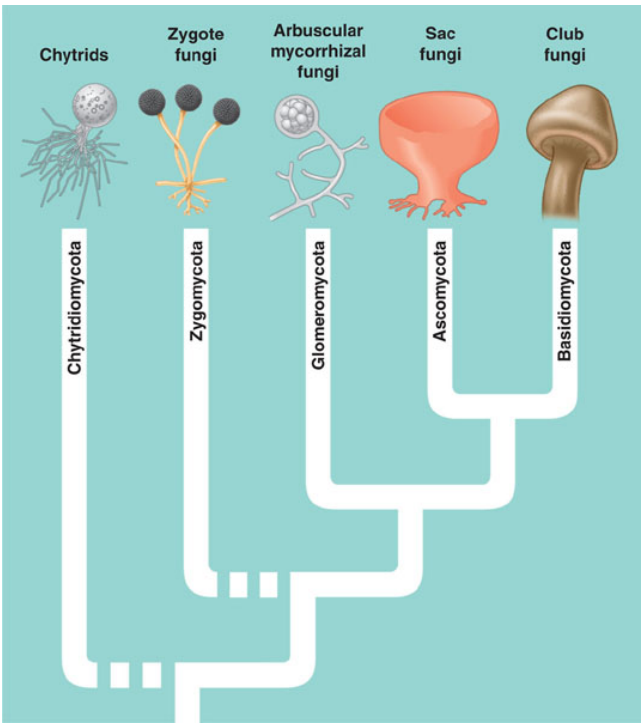
BIOL 2015 – Evolution and Diversity

Lab 6: Fungi

Introduction



The Kingdom Fungi consists of the Divisions Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. The latter three groups are primarily terrestrial. As decomposers, with rapid growth and high surface-to-volume ratio, fungi have an intimate contact with their environment that is very different from most other organisms. An extensive mycelium can bind soil particles and litter and penetrate most of the upper soil volume. Maintenance of this intimate fungal environment requires that all parts of the fungus be metabolically active. The Chytridiomycota we typically order is occasionally unavailable so we may not have the opportunity examine this group.

Fungal associations: Lichens and Mycorrhizae

All fungi are **heterotrophic** and most are saprotrophic, but the higher fungi display a diversity of other relationships. Several species of Ascomycetes and several Basidiomycetes combine with green algae or even cyanobacteria to form lichens. Lichens are extremely widespread in nature and can tolerate severe environments. They are even found close to the South Pole, one of the drier, colder sites in the world. They are also abundant in deserts and alpine areas. For example, *Cladonia rangiferina*, a light-colored fruticose lichen belonging to the Cladoniaceae family, is an extremely important food for caribou and other large grazers in the arctic. Despite being tolerant of severe environments, lichens are very sensitive to air pollution. Lichens have almost disappeared from the Los Angeles basin and there is evidence that the level of pollution has crossed the injury threshold of the native lichens in the San Bernardino Mountains and the southern aspect of the Sierra Nevada range.

Certain fungi play a crucial role in ability of higher plants to acquire mineral nutrients. These fungi form associations with plant roots called **mycorrhizae**. The fungus obtains carbohydrates from the plant while the plant benefits because the fungus essentially enlarges their root system, thereby allowing them to access to the mineral nutrients from a larger area.

Evolutionary relationships and distinguishing characters

The higher fungi appear to be related to one another. Each has cell walls made of **chitin**. The most primitive of the three divisions, Zygomycota, is **coenocytic** (multinucleate cell which can result from multiple nuclear divisions). In Ascomycota, porous septations (cell walls) occur, but the pores are large enough that nuclei can actually pass through. The septations of the basidiomycetes have pores generally too small for this to occur. All three groups have no flagellated or ciliated stages and contain no centrioles. Additionally, the mitotic spindle forms inside the nuclear membrane, which does not break down during nuclear division

Sexual Reproduction

Sexual reproduction usually involves the fusion of two distinct hyphae. In the two more advanced divisions the two nuclei do not fuse immediately; rather, a filament with more than one nucleus per cell from different individuals is formed. This is **heterokaryosis**. True diploidy occurs when the nuclei fuse. Meiosis usually occurs immediately afterwards resulting in the formation of haploid spores. The resulting haploid spores germinate to form new mycelia. Members of all three groups often produce special structures to aid spore dispersal. For example, a mushroom is an example of a "fruiting" body (composed of many heterokaryotic cells) from which spores disperse in some basidiomycetes.

Examples

Some important or familiar genera include: *Rhizopus*, the common bread mold, a Zygomycete; *Claviceps purpurea*, ergot, a plant parasite that causes severe illness or death in humans, an ascomycete; *Penicillium*, an ascomycete genus within which some fermenting species form cheeses, while others are the source of the antibiotic penicillin. Yeasts (*Saccharomyces*) are unicellular ascomycetes which ferment sugars in the production of alcoholic beers and wines, as well as bread; truffles and morels, reproductive structures of various ascomycete species, are prized as gourmet foods. *Amanita* is a familiar basidiomycete that kills amateur mushroom collectors each year after the collectors eat the fruiting body, unaware that it is highly toxic.

During this lab focus on the appearance and structure of the hyphae, as this is the basic form of the organism. Examine carefully the reproductive structures. Because fungi are usually filamentous and grow in soil, wood, and detritus materials, much of our familiarity with them is limited to their reproductive structures.

Zygomycota

Rhizopus

This fungus is distributed throughout the world. One species is used to ferment rice into sake. Another species is used to inoculate soybeans to make tempeh. The genus is usually saprophytic but frequently attacks fruits and vegetables that have been injured. It occupies non-aquatic habitats and never produces motile spores. Sexual reproduction is by **conjugation**.

(A) Examination of prepared slide

Find a prepared slide labeled "*Rhizopus* conjugation". When you observe the prepared slide, try to observe the different stages in the development of zygosporangia – especially the stage where the gametangia have formed.

(B) Culture examination

(1) Examine a culture of *Rhizopus*. Note the appearance of the colony. What color are the hyphae? What other structures can you see?

(2) Examine the culture with a dissecting microscope. Is the mycelium branched? Are cross walls present? Note the characteristic formation of rhizoids, the small root-like branches along the hyphae growing on the substrate.

(3) Focus on the dark colored structures. These are **sporangia**. The dark ones contain masses of mature **sporangiospores** (Figure 1). The whitish ones are not yet mature. The sporangial wall is very fragile and is frequently ruptured when preparing slides. The branch supporting the sporangium is called a **sporangiophore**.

Figure 1

(C) Wet Mount

Prepare a wet mount of some of the mycelium with sporangia and examine with a compound microscope. Note the coenocytic mycelium.

Locate a sporangiophore and sporangium. What color are the spores? Is the spore wall smooth or roughened?

(D) Sexual Reproduction

(1) To demonstrate sexual reproduction, 2 different strains of *Rhizopus* have been grown together. Examine a plate with a dissecting microscope. Find an early stage of conjugation in which short, lateral branches arise from separate hyphae and come into contact. The branches typically become enlarged and have a relatively dense mass of cytoplasm. They are the **progametangia**.

(2) Next find stages in which a cross wall has divided the progametangia into a terminal portion, the **gametangium** (Figure 2a, left panel, top arrow) and a supporting cell called **suspensor**.

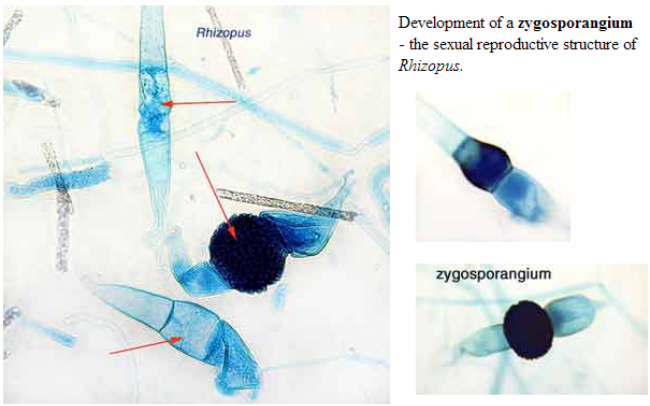


Figure 2a

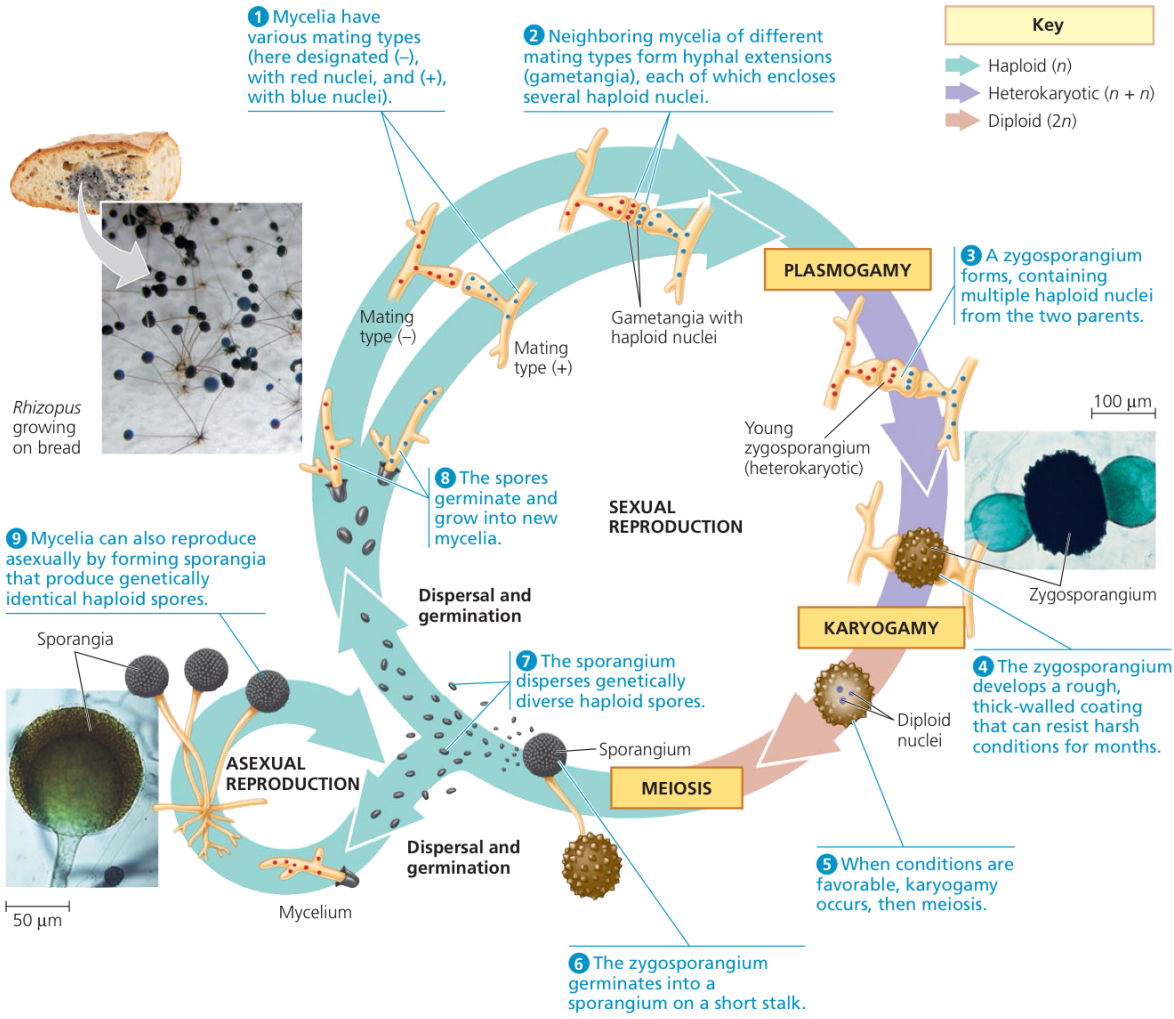
(3) The wall separating the plus (+) and minus (-) gametangia dissolves, the cytoplasm mixes, and nuclei pair. Nuclear fusion occurs and the cell formed by the former gametangia develops a thick, dark colored, rough wall. This is the **zygospore** (Figure 2a, left panel, middle arrow). It can remain dormant, but eventually meiosis occurs. A **sporangium** arises from the zygospore, after which the haploid spores disperse.

Pilobolus

This fungus is commonly found in pastures growing on dung (Figure 2b). The sporangiophores are phototrophic and can shoot the sporangia up to 2-3 meters in the direction of a bright light source.

(1) Examine the living cultures of *Pilobolus crystallinus*. What is the adaptive significance of having phototrophic sporangiophores?

Figure 2b



The life cycle of the zygomycete *Rhizopus stolonifer* (black bread mold).

Ascomycota

Saccharomyces - yeast

Yeasts consist of a single, non-motile cell. These organisms reproduce **asexually** by **budding** in which daughter cells are formed at one or both ends of the parent cell. In sexual reproduction the entire cell becomes an ascus with either four or eight ascospores. Many of the yeasts such as this one are of considerable economic importance to humans – they produce carbon dioxide to Levin bread under aerobic conditions or alcohol under anaerobic conditions.

Prepare a slide from one of the fermenting cultures and examine under high power or oil immersion.

(1) Note the shape and size of the cells. Make sure you will be able to distinguish them from other cells.

(2) Look for budding cells. The small cell continues to increase in size until it reaches the size of the parent and eventually breaks free. Before becoming free, however, it might have begun to bud; thus it isn't uncommon to see chains of 2-4 cells in various stages of budding.

Penicillium and *Aspergillus*

These two kinds of fungi are extremely common and are, in fact, laboratory "weeds". Although they are ascomycetes, only asexual stages will be examined.

Find a prepared slide labeled "Mold Types" or "Penicillium, Aspergillus, Rhizopus"

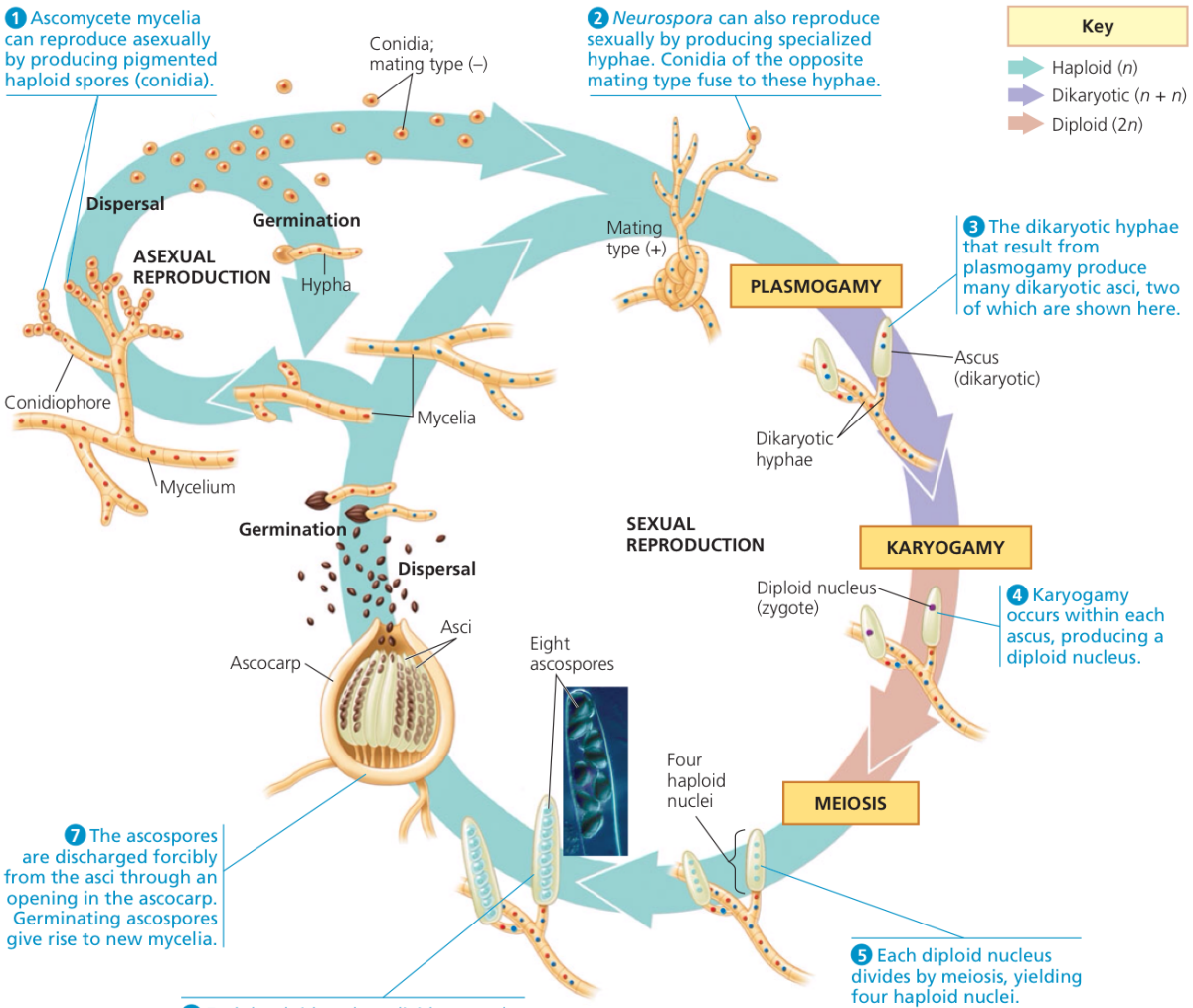
Figure 3

(1) Examine the demonstration cultures set up for you. Is the mycelium septate? Asexual reproduction occurs with the formation of **conidiospores** at the tips of an erect branch called the **conidiophore** (Figure 3). Are they the same in both genera? In some ascomycetes, the tip of the conidiophore has an enlarged cell called the **vesicle**, to which are attached short branches called **sterigmata** (Figure 3, arrow). The conidiospores can be found attached to the ends of the sterigmata. In others the sterigmata may be branched as well.

(2) What is the relationship between *Penicillium* and penicillin?

Eurotium chevalieri

Find a living culture. You will see small, round structures yellow in color under a dissecting microscope. These are the **cleistothecia** - ascocarps that completely enclose the mature asci and release the ascocpores when they rupture or degrade. You may also see small fuzzy structures (conidia and conidiophores like those in *Aspergillus*).



The life cycle of *Neurospora crassa*, an ascomycete. *Neurospora* is a bread mold and research organism that also grows in the wild on burned vegetation.

Basidiomycota

These fungi are regarded as among the more advanced basidiomycetes because of the **basidium** and the fleshy fruiting body.

(1) Find the following on a fresh mushroom: **pileus** (cap); **stipe** (stalk); **lamellae** (gills) ; **annulus** (collar); **volva** (cup). What is the origin of the annulus and volva? (answer: remnant of the **partial veil** - A sheet of tissue under a mushroom cap. It stretches from the cap margin to the stem, and protects the spore bearing tissues until the spores are mature). Be sure you can find them on all the fresh mushrooms, including *Agaricus bisporus*, common button mushroom.

Cut one of these mushrooms in half, vertically, and leave one half for someone else to use. Carefully remove one of the gills and mount on a slide in a drop of water, pressing down on the cover slip to keep it flat. Find the terminal branch-like structures (**sterigmata**), to which are attached the **basidiospores.** How many **basidiospores** are in each basidium? Note *differences in spore color* among different species.

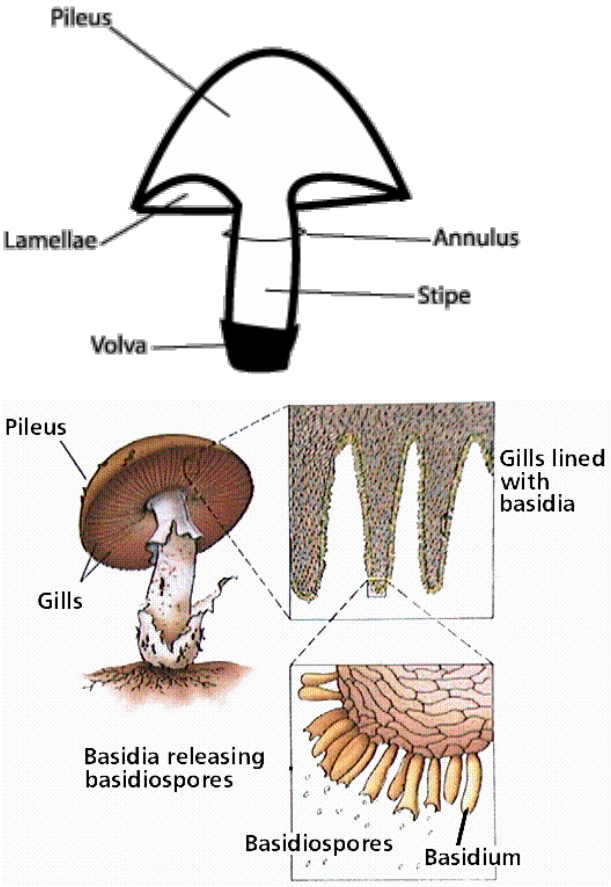
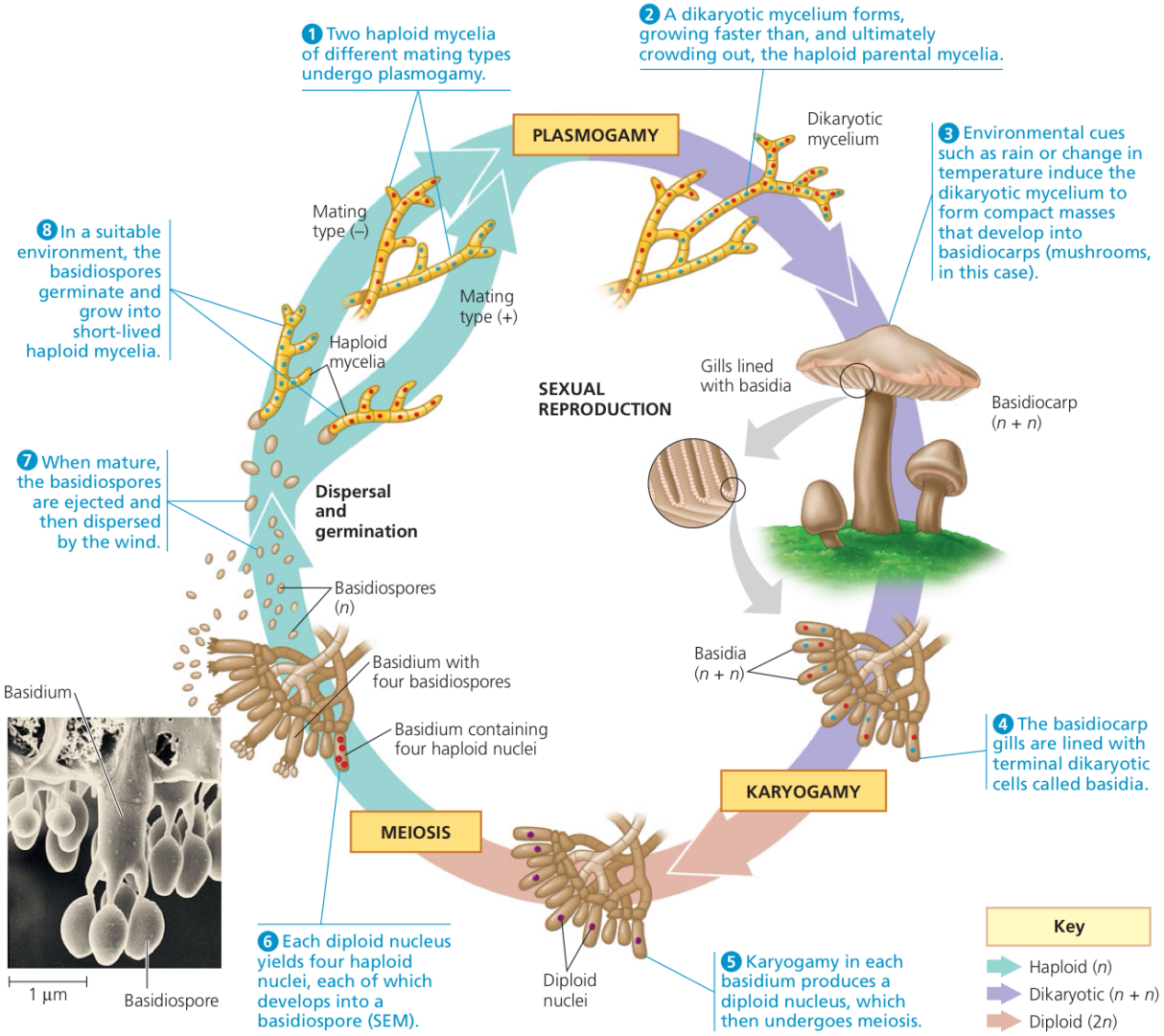


Figure 4



The life cycle of a mushroom-forming basidiomycete.

Lichens

Lichens are formed by a symbiotic association of a fungus and an alga. The fungus in most lichen species is an ascomycota, but in some lichens it is a basidiomycota. The alga is one of several distinct species of Chlorophyta or Cyanobacteria. The alga from this association can usually survive as a free-living organism, but the fungus cannot. The alga provides food for the fungus, and the fungus furnishes moisture, shelter, and minerals for the alga.

Figure 5: crustose (left), foliose (middle), and fruticose (right) morphs of lichen

Vegetative structure

Examine species of lichens that demonstrate the three general types of lichen growth forms: **crustose**, a flattened, crust-like form; **foliose**, a more or less leaf-like appearance; and **fruticose**, or a branching, shrub-like form.

Reproductive structures - Sexual reproduction of the fungal partner

On the specimens available, note whether there are any fruiting bodies associated with them. These are often brightly colored. Because most lichens are ascomycetes, these fruiting bodies are likely to be **ascocarps**, reproductive structures containing large numbers of **asci** arranged on **apothecia** (the cups). A prepared slide is available to show you a thin section of this area.

Asexual reproduction of the lichen

Carefully examine the surface of the lichens for **soredia** or **isidia**. Survey the surface of the lichen until you find an area which seems to be covered with a light dust on it and is associated with cracks or openings through the upper lichen cortex. Examine these areas carefully and you should be able to observe soredia, which are specialized fragments of the lichen containing a few algal cells and surrounded by hyphae. Other lichens display isidia, which are tiny branches on the surface of the lichen. These branches are actually immature thalli, already with an upper and lower cortex and containing algal cells. By these

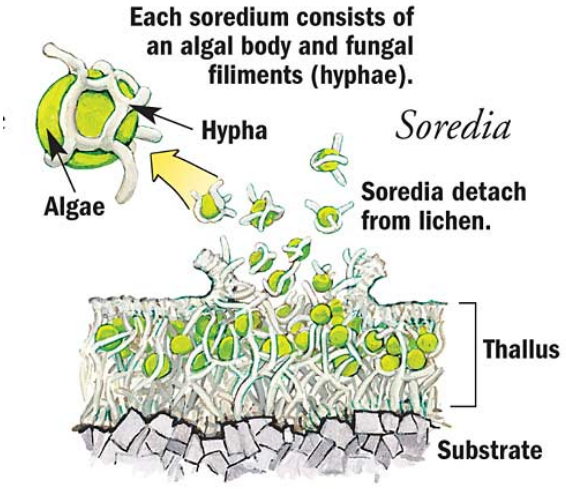


Figure 6

means, lichens are able to propagate and actually maintain both members of the symbiosis. Can you think of a way both members can sexually reproduce and disperse their progeny while continuing to maintain a symbiotic relationship?

Mycorrhizae

Certain soil fungi form associations with roots of vascular plants. The association appears to benefit both members because studies with radioactive tracers have shown that the fungus receives carbohydrates from the green plant while the roots obtain mineral nutrients that the fungus has absorbed from the soil. These mycorrhizal associations are widespread throughout the vascular plant kingdom and so far have been found in over 80% of the flowering plants examined.

(A) **Ectomycorrhizae** and **Endomycorrhizae**

The two common types of mycorrhizae are ectomycorrhizae and endomycorrhizae. Ectomycorrhizae are characteristic of certain temperate trees such as oaks, willows, and conifers. They form a sheath surrounding the root tips with hyphae penetrating between the cells of the root cortex. Root tips with ectomycorrhizae take on a characteristic appearance, shorter and stubbier than uninfected roots and often forming "Y" branches. Ectomycorrhizal fungi are usually basidiomycetes, or occasionally ascomycetes.

Find a prepared slide labeled "Ectotrophic Mycorrhiza" and note the characteristic branching pattern.

Endomycorrhizae are commonly found on herbaceous plants, many tropical and some temperate trees. The mycelium penetrates the cells of the plant root cortex in this association and forms large vesicles or branching systems within the cells. Endomycorrhizal fungi are zygomycetes. Be sure you can distinguish ecto- versus endomycorrhizae.

Find a prepared slide labeled "Endotrophic Mycorrhiza" and make sure you can distinguish the fungal hyphae among the cortical cells of the root.