

Topic 19. Fungi

Historically fungi were considered to be plants. Molecular evidence, however, indicates that they are actually more closely allied with the animals. Fungi are all **heterotrophic**, and live either as **saprophytes**, **parasites**, or in **mutualistic partnerships**. Fungi have **cell walls made of chitin** and, with the exception of some unicellular species, have bodies composed of filaments called **hyphae**. A mass of hyphae is called a **mycelium**. Until recently, the taxonomy of the fungi has largely been based on their sexual, spore bearing structures. If the organism formed **zygosporangia**, then it was a **zygomycete** in the phylum, **Zygomycota**; if **asci** were found, then the organism was an **ascomycete**, in the phylum **Ascomycota**; if it bore **basidia** then it was a **basidiomycete**, phylum **Basidiomycota**. If the organism was asexual, it was placed in an explicitly artificial group, the **imperfect fungi**.

Recent molecular research has enriched our understanding of fungal evolution, but has complicated the taxonomy of the fungi. The **chytrids** and **microsporidians** are now included in the kingdom, and these do not conform to the rules for grouping outlined above. Further, we now know that the zygomycetes are polyphyletic. The basidiomycetes and ascomycetes, however, are monophyletic, and together constitute a larger clade, the **Dikarya**. In today's lab, we will study some common examples of terrestrial fungi. Two of these are zygomycetes in the sense that they form zygosporangia. These do not have a persistent dikaryotic stage and are **coenocytic**. We will also study examples of the clade Dikarya including examples of ascomycetes and of basidiomycetes.

Domain: Eukarya - Organisms with nucleated cells

Kingdom: Fungi

Zygomycetes

Genus *Rhizopus*

Ascomycycetes

Genus *Sordaria*

Yeasts

Genus *Penicillium*

Powdery Mildews

Genus *Morchella*

Basidiomycotes

Common woodland fungi

Rusts and Smuts

I. Zygomycetes: Terrestrial Fungi Without a Persistent Dikaryotic State.

These have bodies made up of **coenocytic** hyphae and produce **zygosporangia** during sexual reproduction. Plasmogamy (the union of the cytoplasm) is followed by karyogamy (the union of the nuclei) without any intervening mitotic divisions.

Ia. Sexual Structures Leading up to the Formation of Zygosporangia.

Take a sexual culture of *Phycomyces* to your seat. Note the two points of inoculation on either side of the plate. *Phycomyces* is heterothallic - that is it has genetically determined mating types which must out-cross with strains of a different type. In this case there are two strains, “+” and “-”. Sexuality is manifested by a line running midway between where each strain was inoculated where the two strains met. Remove the cover and observe this region of sexuality more closely using an old Olympus compound microscope. In the zone of sexuality, certain hyphae from opposite strains grow together and touch. These hyphae are called **progametangia**.

Draw a pair of progametangia.

Eventually a septation will form behind where each of the progametangia touch forming two gametangia.

Draw a pair of gametangia.

During the next stage the wall between the two gametangia breaks down and the cytoplasm of the two gametangia unite in a process called **plasmogamy**. Eventually this results in the formation of a zygosporangium still attached, on either side, to each of the original mating filaments.

Draw any of the later stages after plasmogamy visible.

Ib. Genus *Rhizopus*.

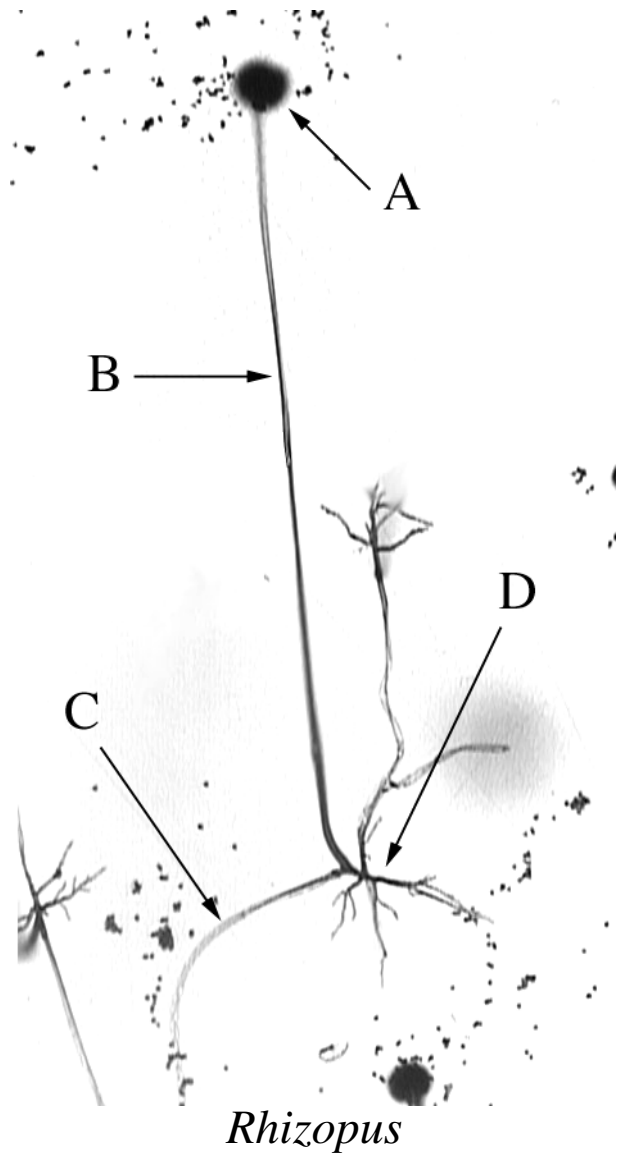
Asexual cultures: Take a culture of *Rhizopus* growing in a mini petri dish located on the side bench to your seat. Observe using the Olympus microscope with the 4x objective or with the Meiji dissecting microscope. Identify the various types of hyphae illustrated to the right. Note that the organism is coenocytic except where the spores are formed in the sporangium.

A = _____

B = _____

C = _____

D = _____



Prepared slide of *Rhizopus*: View this slide with your microscope. Note the different asexual hyphae, but look for zygosporangia and gametangia.

Draw a zygosporangium and gametangia of *Rhizopus*, and gametangia .

II. Dikarya.

In the dikarya, plasmogamy is followed by mitotic divisions and a unique type of cytokinesis before karyogamy resulting in a persistent dikaryotic stage, the **dikaryon**. Each cell of a dikaryon has two, genetically different, haploid nuclei. At some stage of the life cycle, some or all of these cells will undergo karyogamy to form a diploid nucleus. This often occurs in fleshy structures which we will simply call **fruiting bodies**. These diploid nuclei then undergo meiosis. With the basidiomycetes, these haploid cells form **basidiospores** borne external to the mother cell wall. With the ascomycetes, these haploid cells typically undergo one mitotic division, resulting in **eight ascospores** which are borne inside the mother cell wall.

IIa. Ascomycetes.

Hyphae of the ascomycetes are cellular but have incomplete septa. Members typically form **asci**, which are often associated with fleshy fruiting bodies. Each fruiting body generally results from one plasmogamy event. This explains why, in the exercise on tetrad analysis, each fruiting body of *Sordaria* contained asci all of which were interstrain, or, all of which were intrastrain.

Many members reproduce asexually by **conidia** borne on **conidiophores**. The group is important as decomposers, parasites, and symbionts. They are the primary group of fungi that form **lichens** and are important in **mycorrhizal** associations.

IIa1. Genus *Sordaria*. Cultures on the side bench. *Sordaria* is homothallic. Any genetic type can mate with itself as we observed in the tetrad analysis activity.

Procedure. Crush a fruiting body as outlined in the meiosis lab and observe the asci.

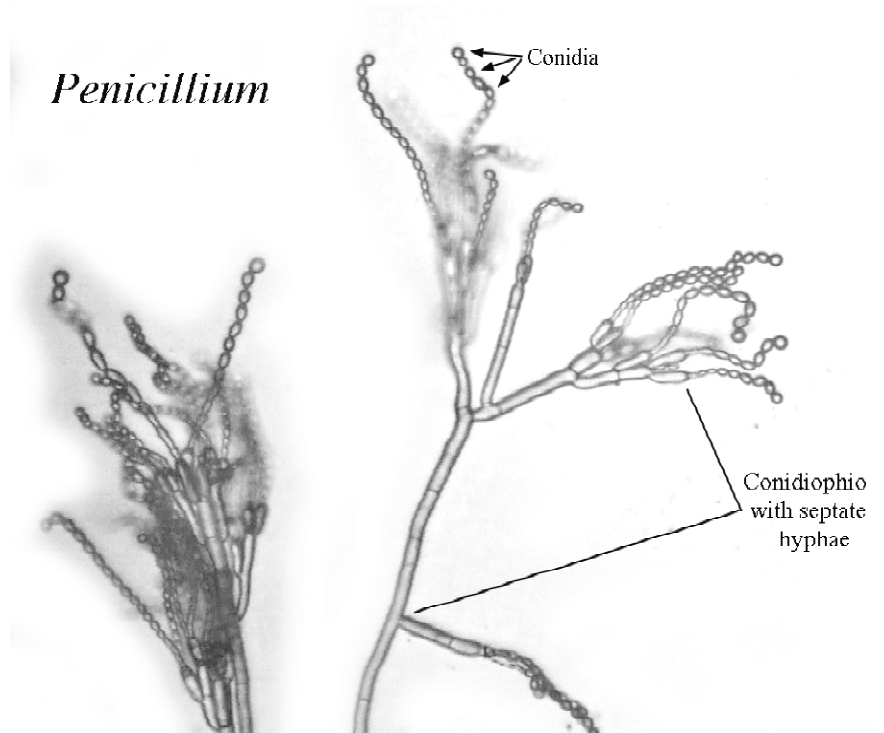
Draw an ascus

IIa2. Genus *Penicillium*.

Members of the genus *Penicillium*, have all lost the ability to reproduce sexually. Molecular evidence, though, clearly shows that the genus is grouped in the ascomycetes. Members of the genus are the source of penicillin, the first antibiotic to be identified and used in medicine. Other members are commercially important in their role in food spoilage, and also for their role in the aging of blue cheeses such as Roquefort and Stilton.

Cultures are on the side bench. The blue coloration is due to a type of asexual spore called **conidia**.

Procedure. Observe the conidia on intact conidiophores in the cultures through the microscope. We also have prep slides for viewing conidia.



IIa3. Yeast. budding culture at the front.

The type of yeast used in baking and brewing is an ascomycete. Yeasts are unusual in that they are unicellular. Yeast cells divide unequally and this pattern of division is termed budding.

Procedure. Prepare a wet mount using a drop of the culture.

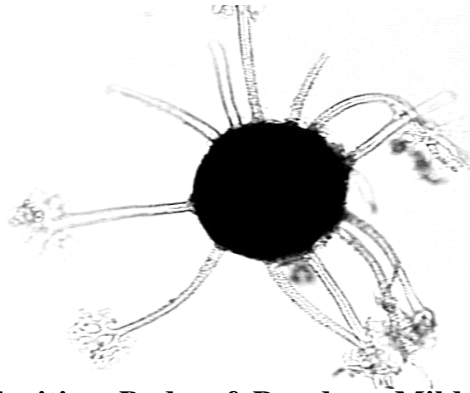
Draw budding yeast cells.

IIa4. Powdery mildew - a parasitic ascomycete.

Procedure. Observe the infected leaves of nannyberry available at the front of each student bench. Note the white areas on the leaf that give the parasite its common name. These are areas where hyphae have broken through the leaf and bear conidia.

See the demonstration microscope of conidia of a powdery mildew.

Using a dissecting microscope, identify the black dots found within the white areas on an infected leaf. These are fruiting bodies that totally enclose the asci. Typically these remain intact through the winter.



Fruiting Body of Powdery Mildew

Flood an area containing these fruiting bodies with 70% alcohol and scrape a number of fruiting bodies off onto a microscope slide and into a drop of water. Observe at 40x. See the illustration above.

While observing through your 4x objective (your shortest lens), crush the fruiting body using the tip of a teasing needle by applying pressure directly over it. Apply only enough pressure to crack the outer surface! The asci inside will imbibe water and swell and emerge from the ascoma. If you experience difficulties ask your TA for help.

Draw a fruiting body with emerging asci.

Draw an ascus of powdery mildew with ascospores.

IIa5. Genus *Morchella*. - Preserved fruiting bodies on each bench and a prepared slide.

Morels have large eatable fruiting bodies. These consist of three types of hyphae, two are from the different mating strains, the third is the product of plasmogamy between these strains and is dikaryotic. While observing a preserved fruiting body (morel), take a prepared slide of the fruiting body and determine where on the ascoma the section was cut. Observe the prepared slide with your microscope and find the red stained ascospores residing in asci.



Draw an ascus with ascospores.

Label where the asci are borne on the fruiting body pictured above.

IIb. Basidiomycetes.

Like the ascomycetes, basidiomycetes have **cellular hyphae** with incomplete septa, and, like the ascomycetes, plasmogamy is separated in time from karyogamy resulting in a persistent dikaryotic stage. Unlike the ascomycetes, members produce **basidiospores** borne externally on a **basidium**. In the basidiomycetes, the dikaryon is not usually associated with the generation of a single fruiting body. The vegetative hyphae for most of the mushrooms, puffballs and other common basidiomycetes, are dikaryotic and persist from one season to the next.

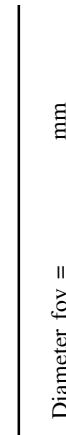
Objectives. Recognize basidia and basidiospores, and the **cap, stipe and gills** of a mushroom. Recognize all the examples of fruiting bodies seen in lab as coming from basidiomycetes. **Know where basidiospores are borne on a mushroom.**

Ib1. Diversity of the fleshy basidiomycota. With a few notable exceptions, the fleshy fungi we typically observe outside are basidiomycetes. Mushrooms, jelly fungi, tooth fungi, shelf fungi, stinkhorns and puffballs are all basidiomycetes (the structures we observe are all fruiting bodies). See the display of various examples in your lab room.

Ib2. Observations of a Mushroom. Fresh mushrooms at the front and prepared slide (section through a cap of *Coprinus*).

Take a mushroom and observe its gross morphology. Identify the stalk (stipe), and cap with the gills on the underside. Take the prepared slide of the section through the cap of *Coprinus* and, while using your mushroom as a reference, determine where the section was made. Now observe your prepared slide with your microscope and locate where the basidia are borne. Indicate on the illustration to the right where they are located.

Draw a fruiting body with basidiospores. Label **basidium**, **basidiospores** and **sterigma**.



Ib3. Parasitic Basidiomycetes.

Rusts and smuts are parasitic basidiomycetes. Observe the examples of wheat rust and corn smut in your lab room.

III. Lichens - a dual organism. Lichens consist of a fungus (almost always an ascomycete) associated with either a cyanobacterium or a green alga (a eukaryote with chloroplasts).

Activity. Observe the lichens attached to the pieces of bark on the side bench. Break off a small piece using your forceps, place the material in a drop of water on a microscope slide and tease the material apart using your needles. Add a cover slip and observe with your microscope. Look closely at the smaller fragments to view the phycobiont associated with the fungus.

Is this organism a green alga (eukaryotic) or a cyanobacterium (prokaryote)?

Draw algal cells viewed in the macerated lichen. Label any fungal hyphae still attached.

Diameter fov = _____ mm

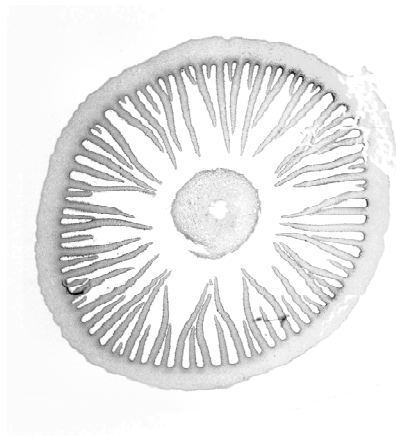
Draw the intact lichen growing on bark.



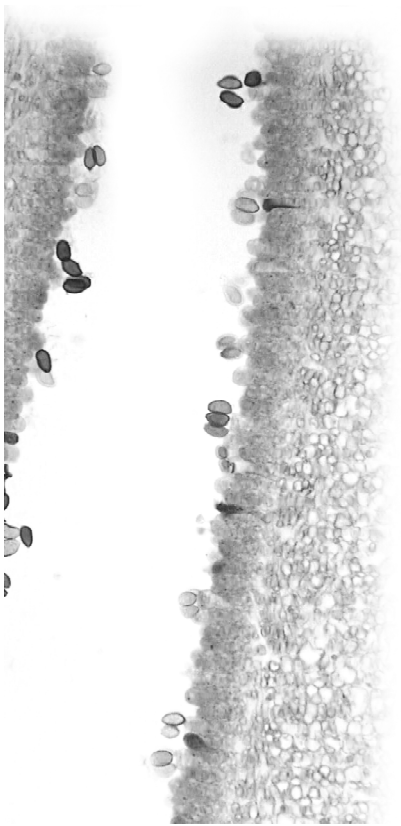
View of Commercial Mushroom *Coprinus*



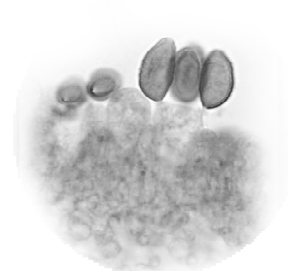
View of gills of a wild mushroom.



Cross Section Of the Cap of a Mushroom



Magnified view of a mushroom gill showing basidia.



Basidium with Basidiospores.