Exercise 8
INTRODUCTION TO FUNGI (MYCOLOGY)
and Fungus-like Protista

Introduction

The science or study of fungi is called **mycology**, and often deals with organisms having similar characteristics and sharing a way of life rather than evolutionary origin. Fungi (singular fungus) are **eucaryotic** organisms, so are larger than bacteria and structurally more complex. They are nutritionally categorized as **chemoheterotrophs** (no fungi are photoauto-trophs), and secrete digestive enzymes that degrade a wide variety of materials. Because they absorb rather that ingest food, they are sometimes said to be **osmotrophic**. Ecologically fungi are usually considered decomposers or **saprotrophs**, but some are **parasites** and some are **pathogens**. Many fungi have a strictly **respiratory** type of metabolism, but some are **fermentative**. As a group they are wide spread in the environment and can tolerate wide ranges in osmotic pressure and pH. Morphologically fungi can be divided into three groups; those with filamentous bodies such as mildews, rusts and smuts are called **molds**, those with filamentous bodies producing large, fleshy, reproductive structures are called **fleshy fungi**, and those occurring as single cells are called **yeasts** (however there is much overlap, and many forms do not fit consistently into one or the other of these categories).

The **thallus** (body) of a mold-type or a fleshy fungus is made up of many individual thread-like structures called **hyphae** (singular **hypha**). These may be **septate** (with cross walls) or **aseptate** (without cross walls). When growing conditions are favorable, multiple hyphae will twine together to form a mat called a **mycelium** that is visible to the naked eye. The portion of a mycelium that extends into the medium (secretes enzymes and absorbs nutrients) is called the **vegetative mycelium**, while the portion that extends into the air and produces spores (or other reproductive structures) is called the **aerial** or **reproductive mycelium**. The hyphae of fleshy fungi are often not recognizable as individual strands whereas those of molds remain separate, are easily observed (microscopically), and are often given specific names depending upon their function. Horizontal hyphae that grow along the surface of the medium and allow molds to spread rapidly are called **stolons**. Those appearing as short root-like branches that attach the fungus to the medium or to surfaces (such as glass) are called **rhizoids**. Rhizoids secrete enzymes and absorb nutrients, so are vegetative in function. Some parasitic fungi form hyphae called **haustoria** that penetrate host cells and absorb nutrients. Fungi that form symbiotic relationships with plant roots form **mycorrhizae**, specialized hyphae that help the plant obtain minerals and water from soil. Hyphae that extend away from the culture medium (typically upward) and support spores are called **sporangiophores** if the spores are contained within a sac-like **sporangium**, and **conidiophores** if the spores are free (like beads on a string). The asexual spores (reproductive structures) may be **sporangiospores** (produced and contained within a sac), **conidiospores** (free bead-like spores in strings), **arthrospores** (fragments of hyphae), **chlamydospores** (thick-walled spores within hyphae) or **blastospores** (formed as buds along or at the ends of hyphae). **Note** - Arthrospores, chlamydospores and blastospores are sometimes referred to as **arthroconidia**, **chlamydoconidia** and **blastoconidia**, respectively. Fungi within the dermatophyte group also produce reproductive structures known as **micro** and/or **macroconidia**)

The classification of fungi was initially based on method of reproduction (types of spores produced and manner of sporulation); however, other features were also utilized. This system left much to be desired, as many forms remained poorly or essentially unclassified. A major obstacle to students studying fungi classification is the inconsistency in terminology used. Although some current schemas for the classification of fungi place all terrestrial organisms within the kingdom **Fungi** (***Myceteae***), some divide fungi into two kingdoms, the ***Eumycota*** and ***Chromista***. The names used for individual taxa within the

kingdom(s) vary considerably. For example, fungi producing sexual spores contained in a "sac" may be placed within the phylum ***Ascomycotina*** or ***Ascomycota*** or within the class ***Ascomycetes***, depending upon the source. In addition to this, many newer texts will include some organisms previously classified as fungi within the kingdom ***Protista*** along with the algae and protozoa. This classification change is due to new information relating to genetic composition and metabolism.

In addition to this, fungi known to cause mycoses were often referred to by different names depending upon their mode of reproduction. Fungi that reproduce only by asexual means are said to be in their **anamorph** state, while those that reproduce sexually are in their **teleomorph** state (a fungus showing both is called a **holomorph**). A single fungus might therefore be given two different names and be placed into more than one taxon. For example, *Trichophyton mentagrophytes,* a dermatophyte recognized as a common cause of Athletes foot, can be placed within the phylum (division) *Deuteromycota* (*Deuteromycotina*) because it reproduces by asexual means when acting as an infective agent. This same fungus can be placed into the genus *Arthroderma,* phylum *Ascomycota* (*Ascomycotina*), when it reproduces sexually. During July of 2011, the International Botanical Congress, meeting in Melbourne, Australia, changed the International Code of Nomenclature for fungi that adopted the principle "one fungus, one name".[After January, 1, 2013, each different type of fungus can have only one correct technical name.

Microorganisms usually considered as fungi may also be divided into three general categories on the basis of their physiological and ecological characteristics. These three categories are: 1) The slime molds (fungi with complex life cycles that sometimes resemble protozoa), 2) the flagellated lower fungi (most of which live in water and have walls of cellulose), and 3) the terrestrial fungi (common mushrooms, puff-balls, molds and yeasts). Fungi for which we often have examples in this laboratory include:

Slime molds: (Sometimes classified within the kingdom *Protista*, phylum *Amoebazoa*)

Phylum ***Acrasiomycota*** (Class ***Acrasiomycetes***) **-** cellular slime molds

Phylum ***Myxomycota*** (Class ***Myxomycetes***) **-** acellular slime molds

Flagellated lower fungi: (Sometimes classified within the kingdom Protista)

Phylum ***Oomycota*** (Class ***Oomycetes***) **-** Fungi with sexual spores called **oospores**.

Terrestrial fungi:

Phylum ***Zygomycota*** (Class ***Zygomycetes***) **-** Fungi with sexual spores called **zygospores**.

Phylum ***Ascomycota*** (Class ***Ascomycetes***) **-** A large and important group of Fungi that produce sexual spores called **ascospores** within a sac-like structure called an **ascus**.

Phylum ***Basidiomycota*** (Class ***Basidiomycetes***) **-** Fungi that produce sexual spores called **basidiospores** upon a structure called a **basidium**. The *Basidiomycota* are probably the best known of the fungi since their fruiting bodies form the typically macroscopic mushrooms, puffballs, and bracket fungi observed throughout the natural environment.

A forth phylum of fungi known as the ***Deuteromycota*** (Class ***Deuteromycetes*** or Fungi imperfecti) is sometimes included in the terrestrial group. This phylum is made up of fungi of various types for which sexual reproductive structures are seldom or never observed. Most of the fungi within this group have

been reclassified as their sexual cycles have been discovered, however maintain their asexual (anamorph) identity because they are frequently referred to by such names in medical literature. Recent research has revealed that most members of the phylum *Deuteromycota* actually belong to the phyla *Ascomycota* or *Basidiomycota*.

Note - The phylum names for fungi are sometimes spelled with a cota ending, and sometimes with a cotina ending. You may see both versions in various textbooks.

Procedure:

1. Working as a small group of two or three students, **aseptically** prepare one fungus (mold) slide culture chamber as explained below:

   a. Obtain a pre-sterilized slide culture chamber and a slant culture of the fungus specified. (Several groups will also share a single tube of potato dextrose or sabouraud's dextrose agar.)

   b. Heat the tube of agar in a boiling water bath until it is thoroughly melted. Then using a sterile pipette, transfer one or two drops of molten agar onto the surface of the glass slide.

   c. Allow the agar a few minutes to solidify and then inoculate the top surface of the agar with the desired fungus culture. **Note** - It is not critical that the agar remain in one piece during inoculation since the fungi will grow throughout the medium.

   d. Carefully place the sterile coverslip onto the inoculated surface of the agar drop and press it **gently** against the slide to reduce the distance between the glass surfaces.

   e. Using the tube of sterile distilled water provided, thoroughly wet the filter paper covering the bottom of the chamber. Do not pour water on the slide preparation.

   f. Incubate the culture chamber (right side up) at room temperature until the next laboratory period, or until instructed to make observations.

   Note - In order to observe spore production the fungi must be allowed to grow for more than 48 hours.

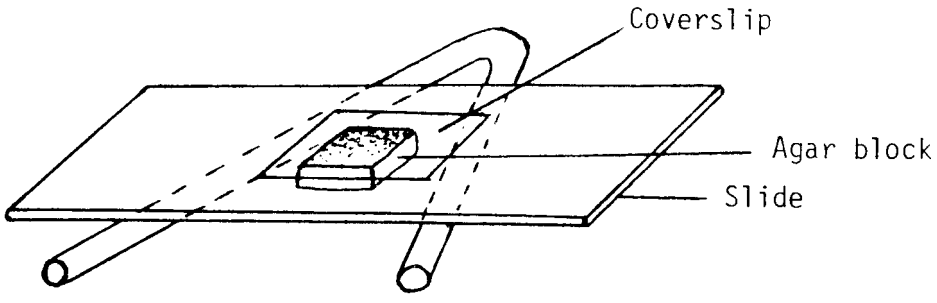


Fig. 8.1 - Slide Culture Setup

2. After 3 or 4 days of incubation, prepare a stained slide of the fungi cultured as follows or observe the culture unstained.

    a. Remove the slide preparation from the fungus culture chamber and wipe away any moisture on the lower surface.

    b. Place one or two drops of methylene blue at the edge of the coverslip and tip the slide so that the stain runs under the coverslip. If the culture is young and you would like it to remain viable, do not apply stain. Observe the slide only briefly before returning it to the culture chamber.

    c. Observe the slide preparation with the low power and high dry objectives of your microscope. Do not try to use the oil immersion lens as it will probably contact the coverslip and might break it.

3. Make a sketch of the fungus growing on your slide. Label the various structures present as accurately as possible. Mold type fungi will have most or all of the structures listed in the introduction, while yeast-type fungi will have few.

4. Set up a stereomicroscope and arrange the slant culture of the fungus used for the chamber preparation so that its cultural characteristics (size, shape, color, etc.) can be readily observed.

5. Observe the slides and plate cultures of **all** the fungi grown in culture chambers. Compare the general morphology and detailed structures of these fungi.

6. If time permits, follow the procedure outlined below to make a scotch-tape mount of the fungi indicated.

    a. Obtain a strip of clear cellophane tape about 5 cm long and hold the two ends together, **sticky-side-out**. If the fungus culture being sampled is in a tube, it will be necessary to use a pair of forceps.

    b. Bring the sticky surface of the tape into contact with the fungus culture such that some of the hyphae and spores adhere to the tape. Light contact is sufficient.

    c. Place a drop of methylene blue on a clean dry glass slide. Bring the tape surface containing the fungus sample into contact with the drop, and then lay the tape out flat so that the sticky ends attach to the glass.

    d. Place the slide, tape-side-up, on your microscope and view the fungus preparation.

7. Examine the prepared slides of the various fungi listed below. Be able to recognize and identify different genera as well as sexual and asexual reproductive structures.

    a. Phylum Oomycota (Oomycotina):

    Albugo bliti (white rust)

    Saprolegnia (water mold)

b. Phylum Zygomycota (Zygomycotina):

Rhizopus stolonifer (bread mold)

c. Phylum Ascomycota (Ascomycotina):

Saccharomyces cerevisiae (brewers yeast)

Morchella esculenta

Penicillium notatum and ***chrysogenum***

Aspergillus niger, flavus and ***glaucus***

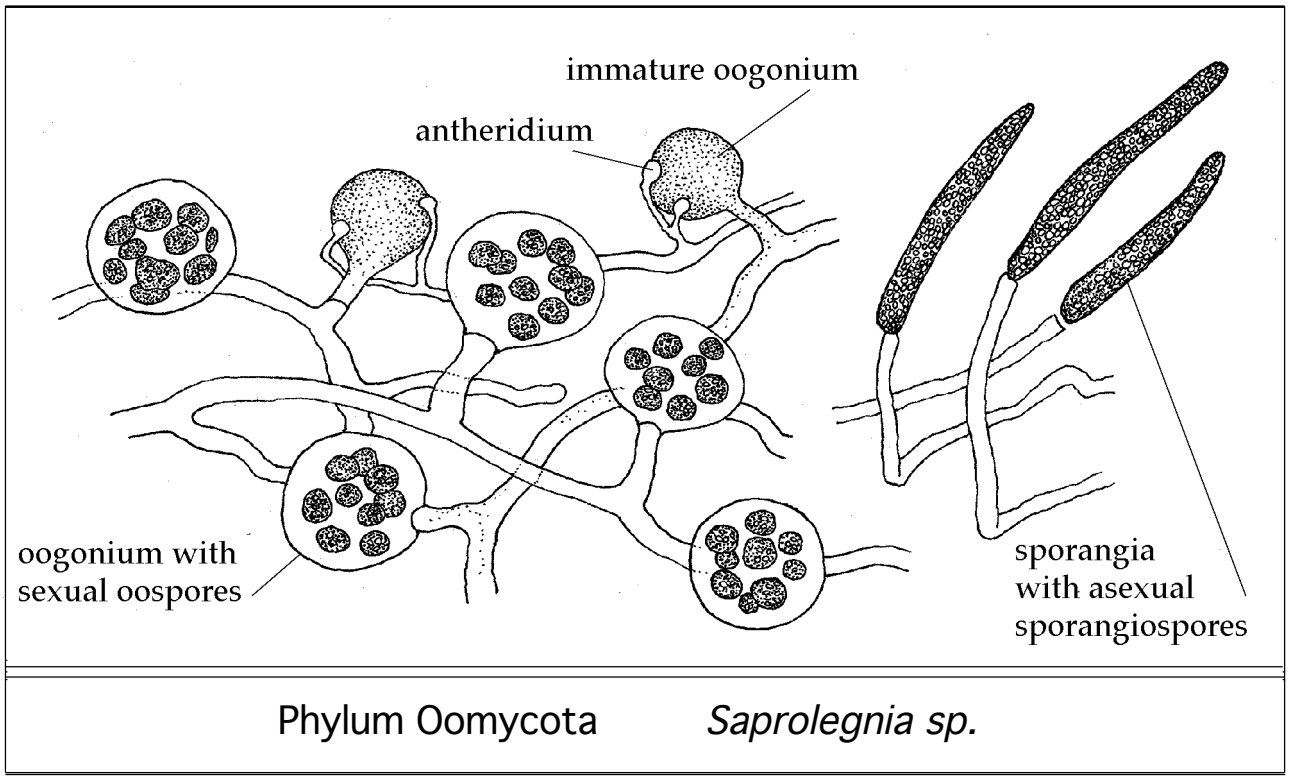
Claviceps purpurea

d. Phylum Basidiomycota (Basidiomycotina):

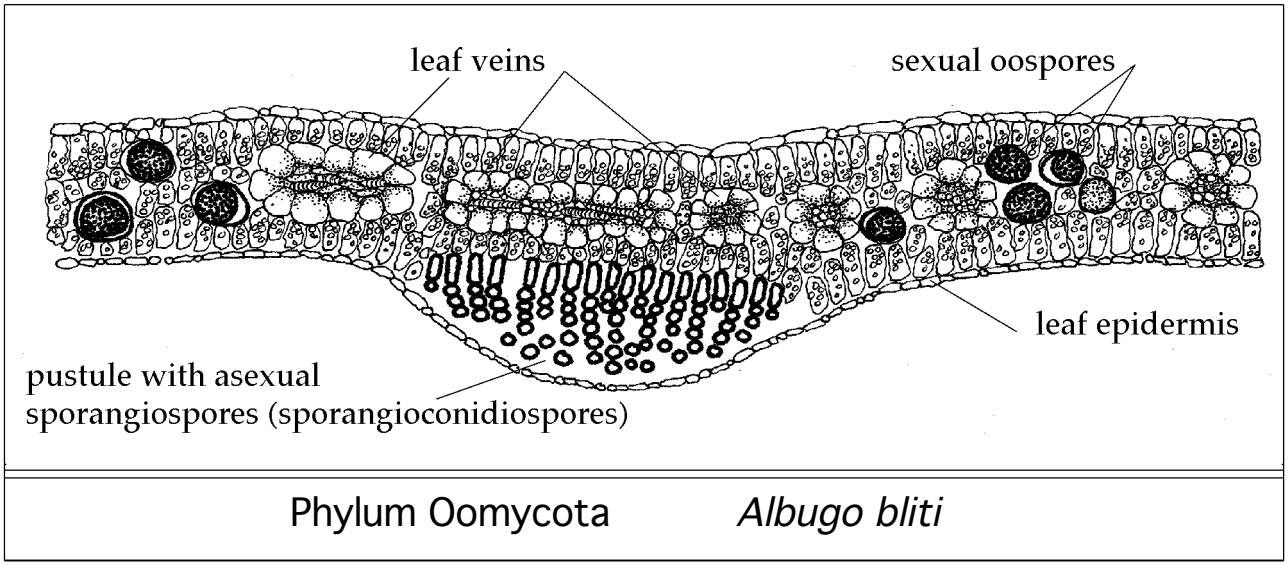
Coprinus comatus

Note – The names *Penicillium* and *Aspergillus* are anamorphic names, but both of these genera have been reclassified within the phylum Ascomycota. These fungi reproduce sexually only under special circumstances, so their more commonly recognized anamorphs names are now official.

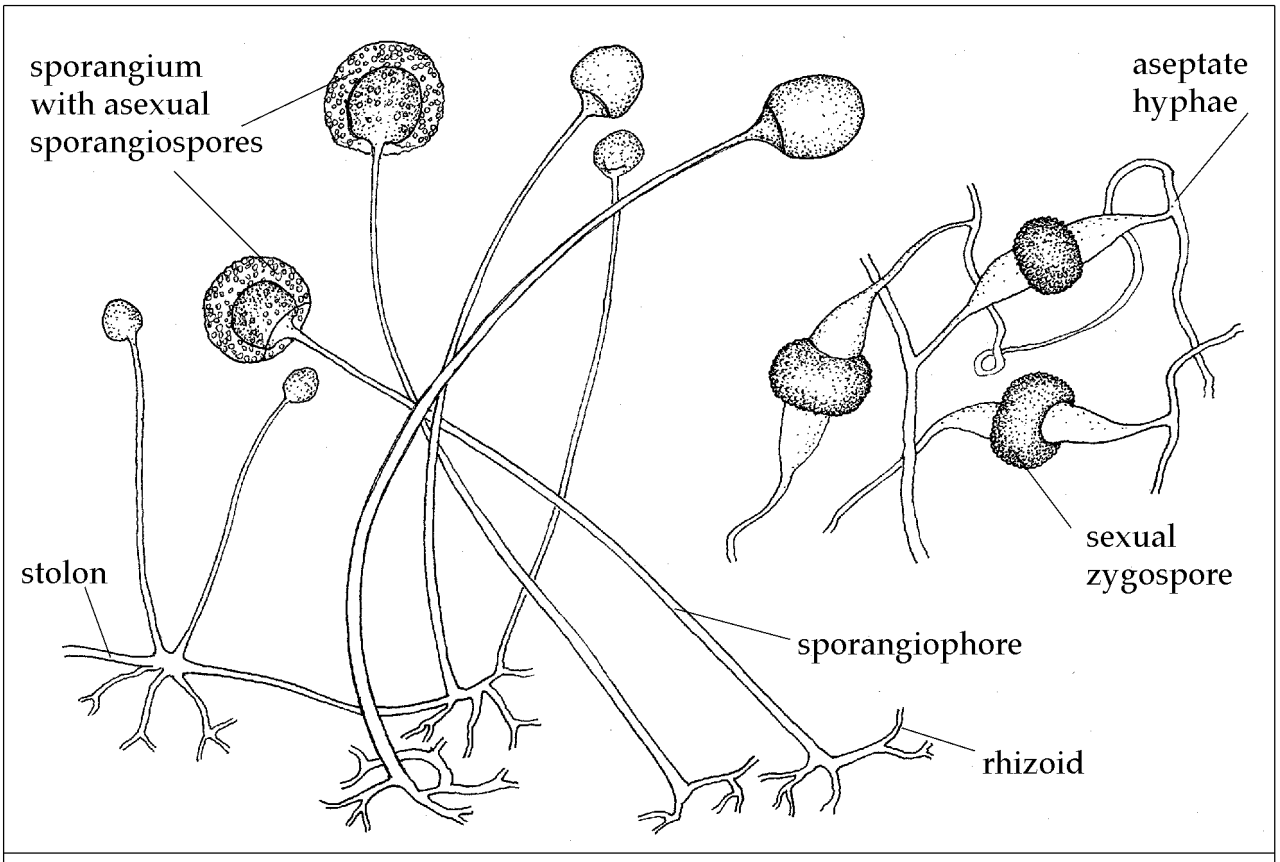
SOME REPRESENTATIVE FUNGI



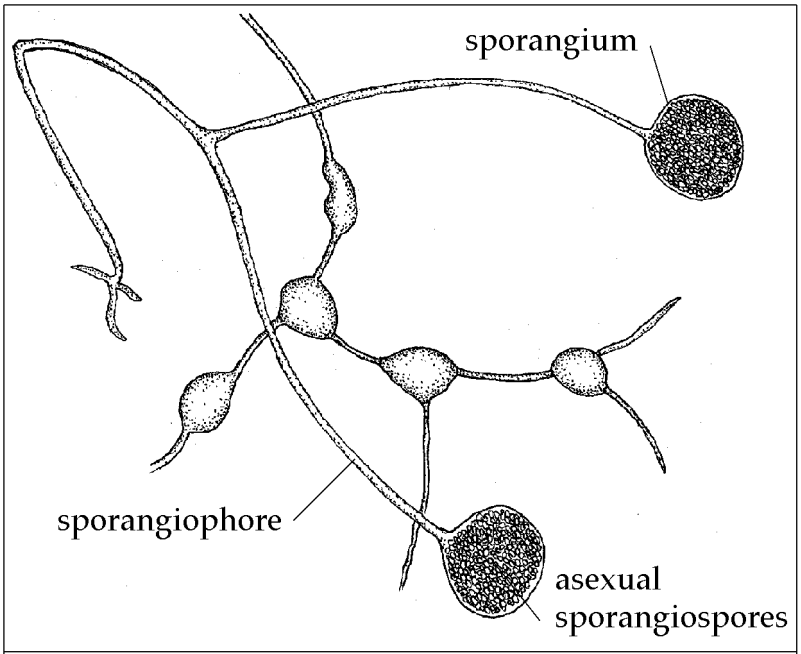
Phylum Oomycota *Saprolegnia sp.*



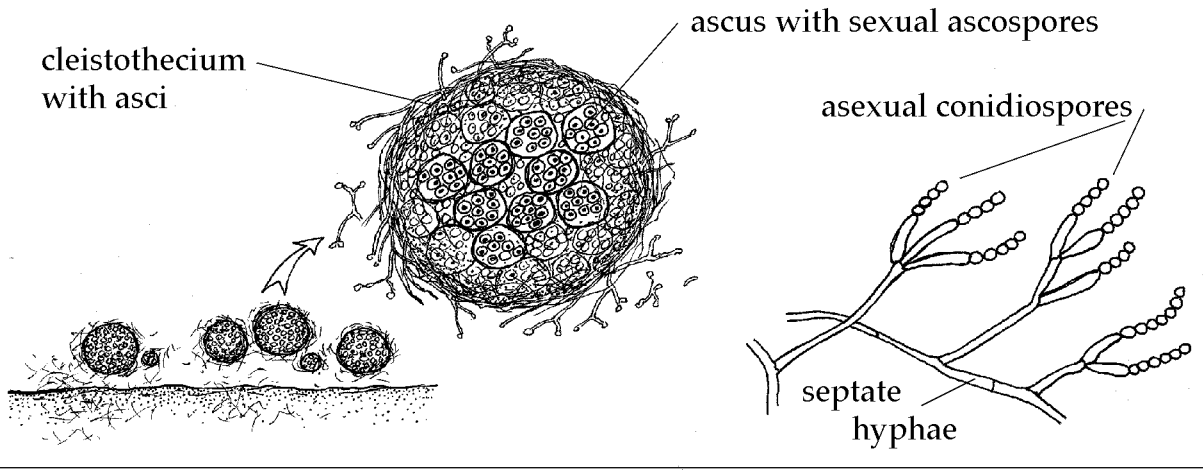
Phylum Oomycota *Albugo bliti*



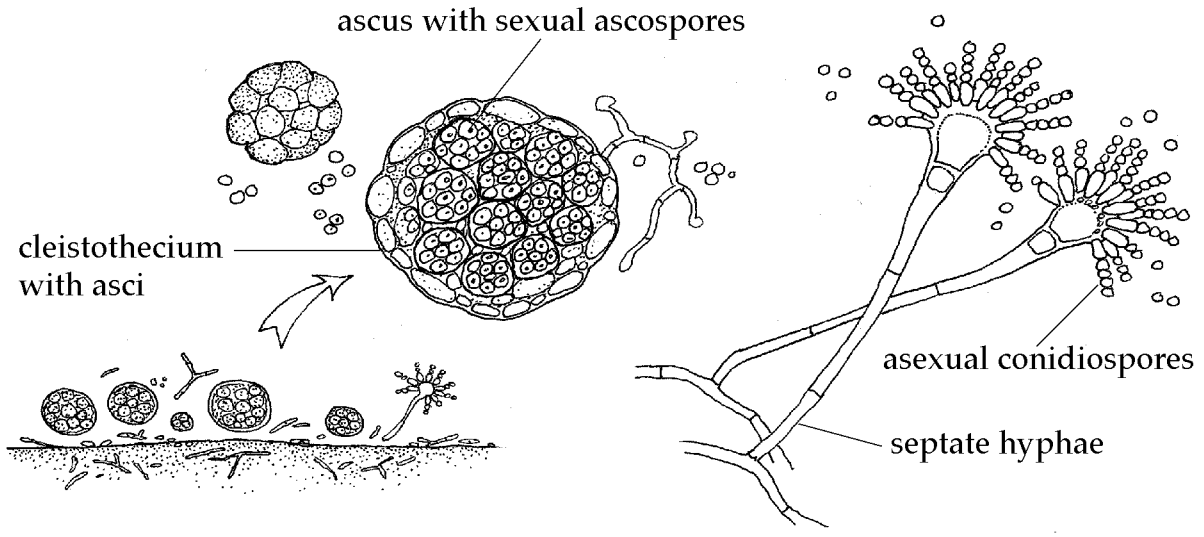
Phylum Zygomycota *Rhizopus stolonifer*



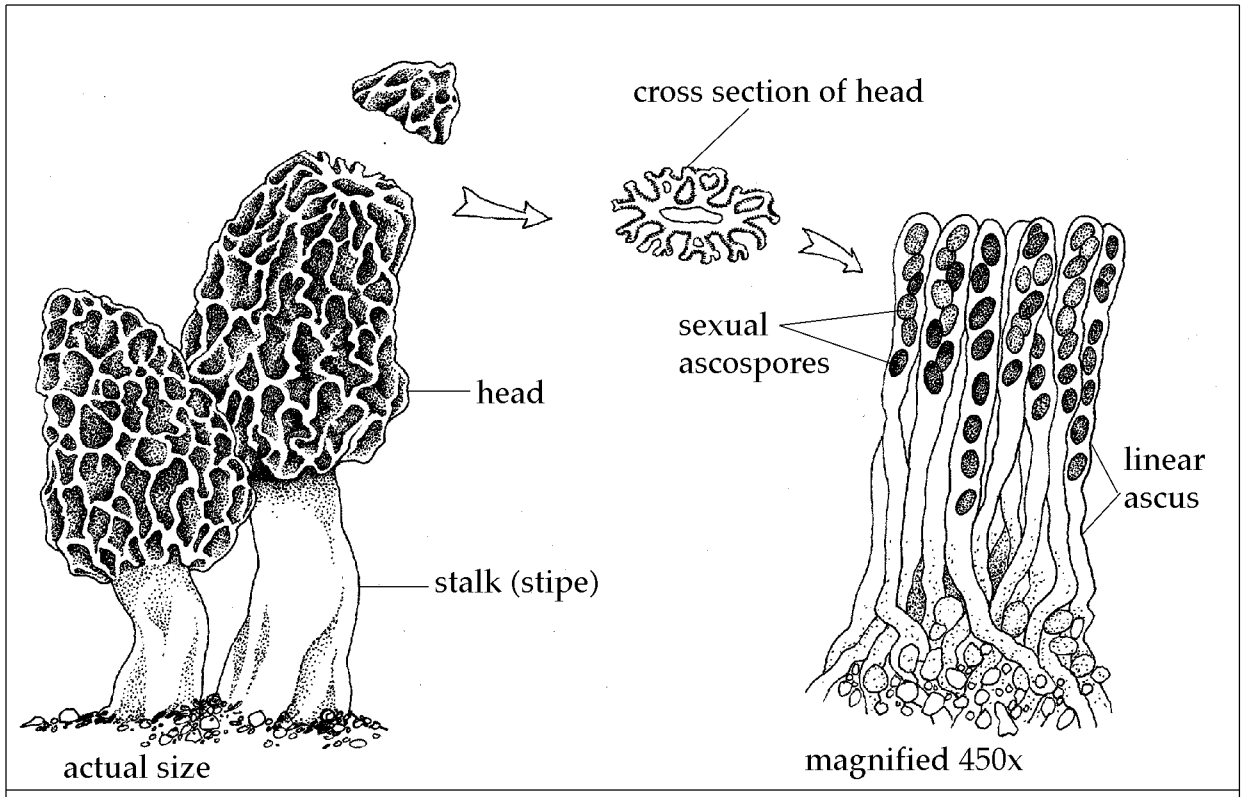
Phylum Zygomycota *Rhizomucor sp.*



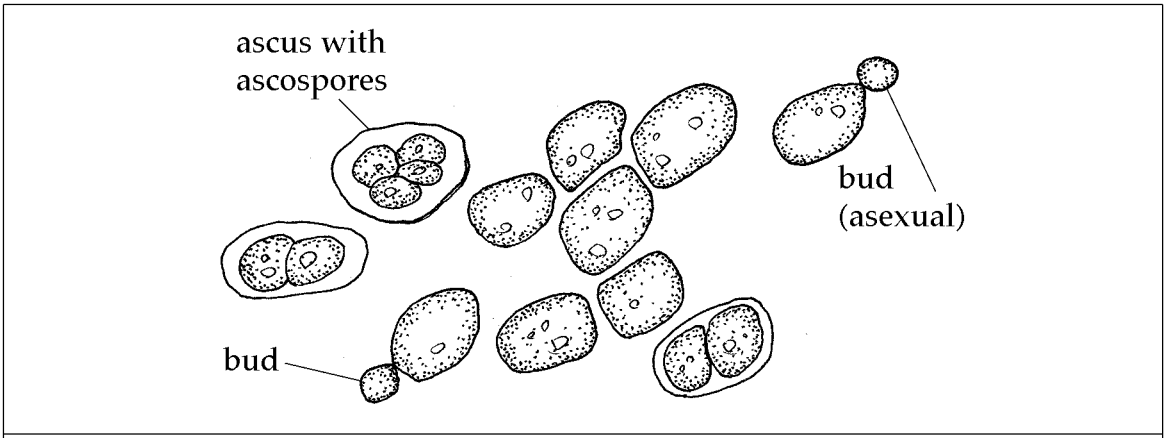
Phylum Ascomycota *Penicillium sp.*



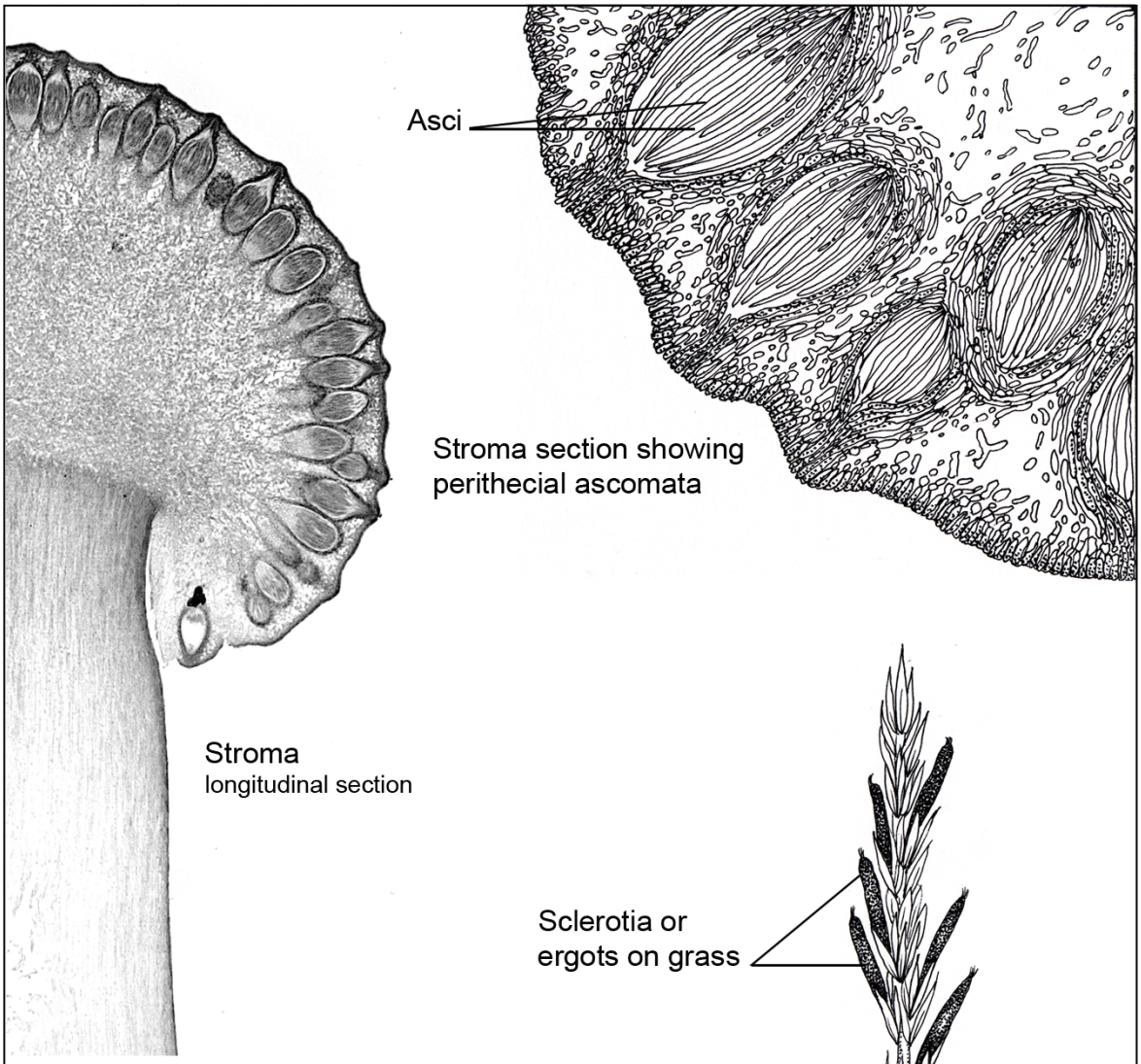
Phylum Ascomycota *Aspergillus sp.*



Phylum Ascomycota *Morchella esculenta*



Phylum Ascomycota *Saccharomyces cerevisiae*



Phylum Ascomycota *Claviceps purpurea*

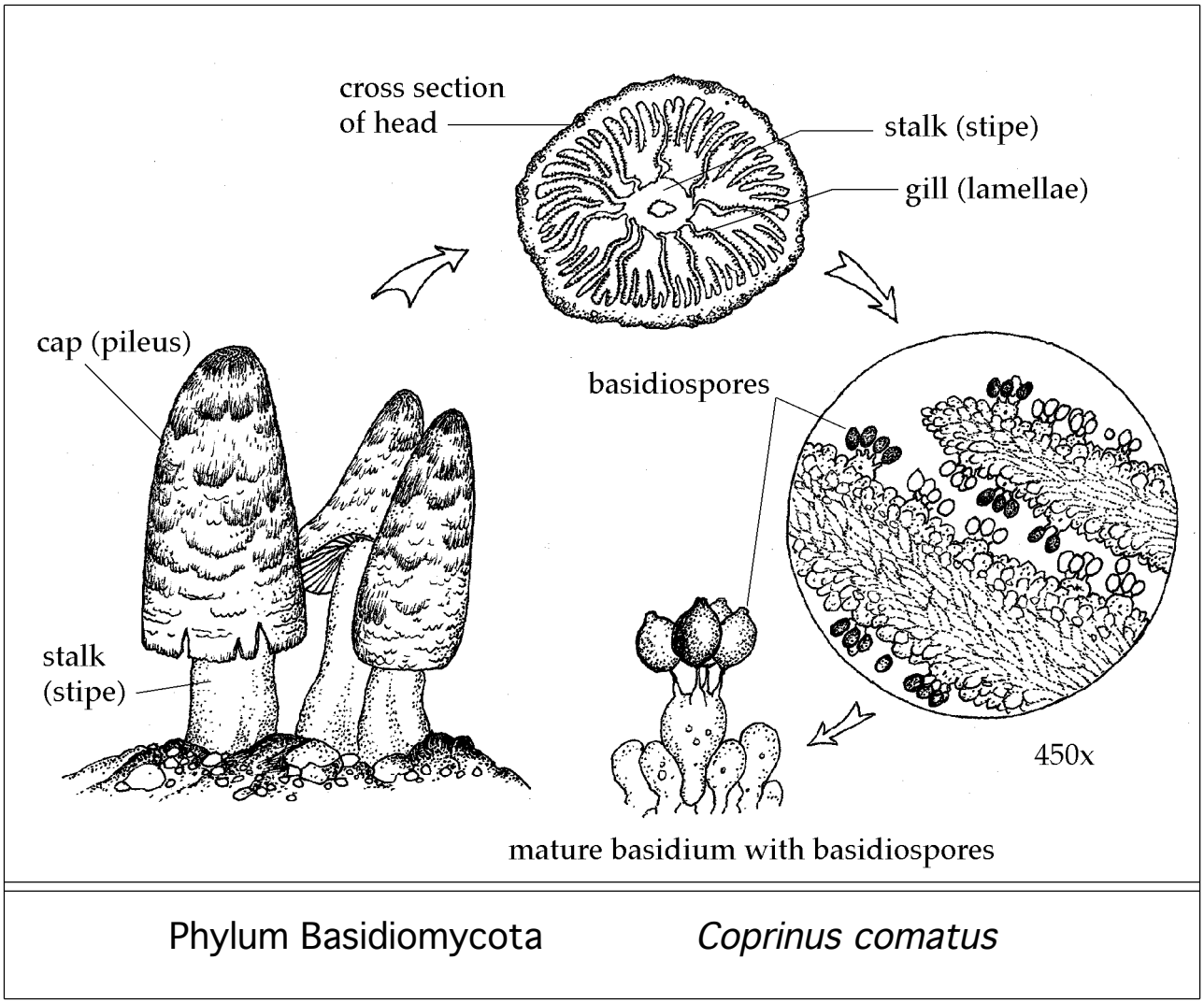


Fig. 8.2 - Illustrations of Reproductive and Vegetative Structures

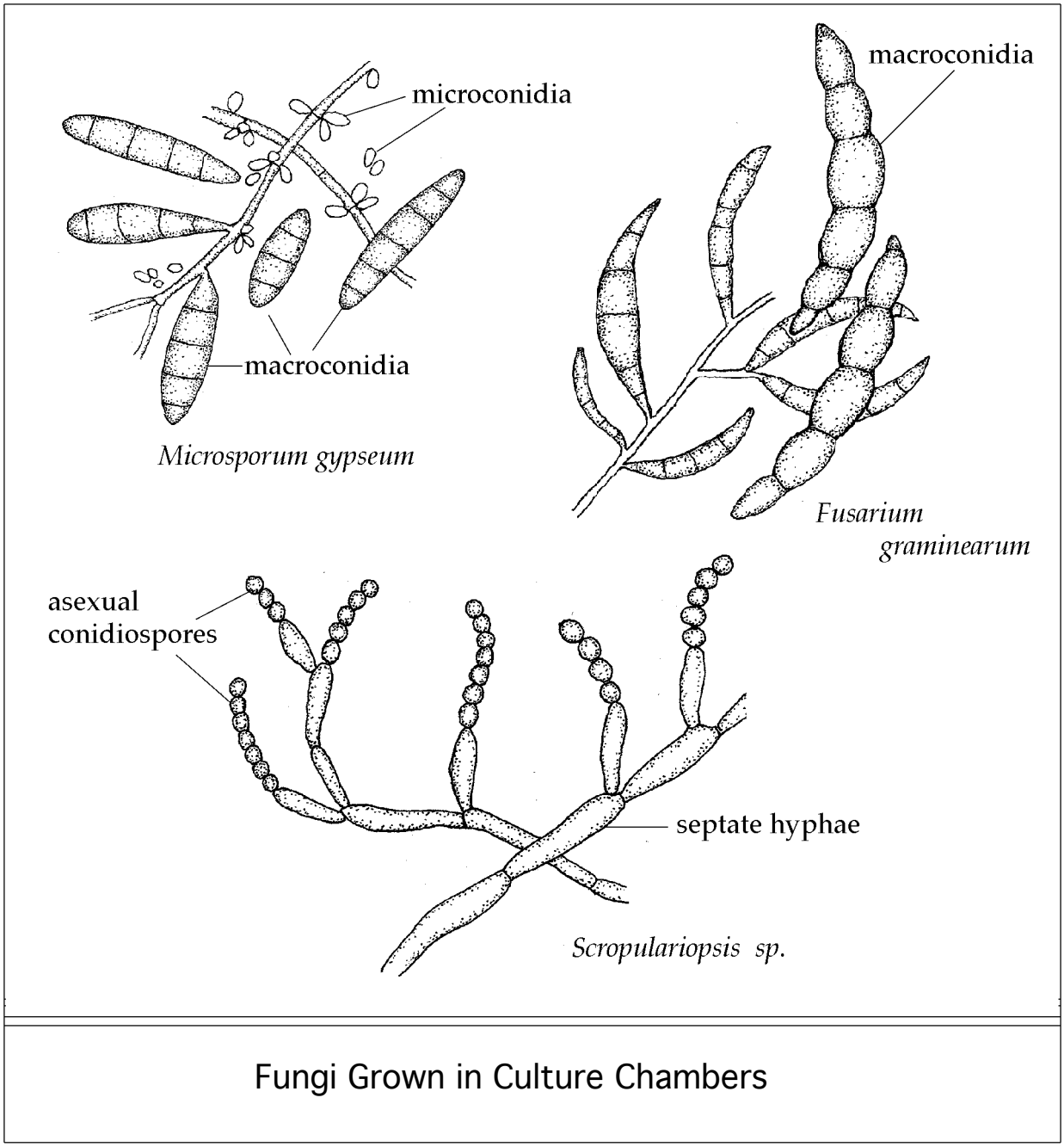


Fig. 8.3 - Additional Illustrations of Fungi Structures

7. Make wet mounts of *Saccharomyces* cultures grown on glucose-acetate slants and stain with malachite green stain for at least 5 minutes. *Saccharomyces* grown on glucose-acetate media will usually produce ascospores. Examine the slides made and look for ascospores.

8. Observe the demonstration materials provided.

a. Plates that have been inoculated with (+) and (-) strains of *Rhizopus*. **Note** that there is a region down the center of the plate where the thallus appears darkened. Observe this region using a dissecting microscope and try to observe the union of hyphae and minute zygospores.

b. Evidence of dermatophyte infection including fluorescence under Wood's lamp and hair perforation.

c. Unusual examples of fungal growth.

9. Read the material provided about cellular slime molds (*Dictyostelium*) and true slime molds (*Physarum*). Observe the samples provided. **Note** – The true slime molds and cellular slime molds are categorized as protozoa in some references. The phylum names used here apply only if these groups are classified as fungi.

Cellular Slime Molds (Phylum - *Acrasiomycota*)

The cellular slime molds are not true slime molds but belong to the same general group. Though considered here within the Kingdom fungi, slime molds are unique and more closely related to protozoa. During their vegetative phase, cellular slime molds closely resemble protozoa in terms of their wall-less anatomy, their motility, and their ingestion of particulate nutrients. It is only during the phase of their life cycle when they form sporangia bearing walled spores, that they resemble fungi.

Life Cycle

The life cycle of *D. discoideum* starts with the dispersal of spores from a sporangium. Upon the absorption of water, the wall of each spore cracks open and a single amoeba-like cell, a **myxamoeba**, emerges. These cells live independently, and as they move about feeding on bacteria, grow and reproduce by fission.

When available nutrients are depleted, the free-living feeding stage ends, and numerous myxamoeba stream to an aggregation center where they aggregate into a sausage-shaped slug, or **Pseudoplasmodium**. For a period of time, this multicellular slug may migrate as a unit, leaving behind a trail of slime. When the migration ceases, the cells of the slug undergo complex differentiation to form a stalk-like structure. The cells from the rear, or posterior, region eventually move up the stalk as a ball of cells and become the tip of the stalk. These cells then transform into spores, each capable of initiating another slime mold life cycle.

True Slime Molds (Phylum – *Myxomycota*, or Phylum *Myxogastria*)

The *Myxomycota* (also classified as phylum *Myxogastria*), are true slime molds. All of the true slime molds have a naked acellular assimilative body called a **plasmodium**, and all form fungus-like fruiting bodies. Within the fruiting body, numerous spores form and are eventually released. Under suitable conditions, these spores germinate to produce amoeboid or flagellated cells.

Life Cycle

Physarum is a true slime mold that grows in areas having cool temperatures, high humidity, and dead organic matter. Its life cycle includes an alternation of **haploid** and **diploid** generations or phases. The diploid (2n) reproductive phase is plant-like while the haploid (n) phase is animal-like.

The yellow-colored active phase of the diploid plasmodium is like a giant amoeba of naked protoplasm surrounded by a thin plasma membrane. It moves across the agar surface in a fan-like manner, ingesting its food by secreting enzymes into the food vacuoles it has created. Within the fanning area, the mass of vein-like strands carries fluid protoplasm (sol) in various directions (moving in one direction for a minute or so, and then reversing). This protoplasmic streaming is probably associated with metabolism more than with movement of the organism from place to place. As the plasmodium travels, it leaves behind a slime track that is the remains of its outer sheath.

After a period of time, the plasmodium may produce a number of stalked fruiting structures (**sporocarps or sporangia**) containing masses of spores. The fragile sporangial walls containing the spores are called **peridia** (singular **peridium**). In nature the spores are generally dispersed by air. If they land in areas with proper environmental conditions, they germinate. During germination, the spore walls crack open and usually one flagellated swarm cell or one myxamoeba emerges from each spore. Each of these cells then grows and multiplies. The emerging myxamoebae or swarm cells are haploid since **meiosis** (reduction division) took place within the very young spores. Within 24 hours, pairs of these haploid cells fuse to form **zygotes** and grow into plasmodia.

Should the environmental conditions begin to dry out while the organism is in its plasmodial stage prior to fruiting, the plasmodium will form multi-nucleated hardened masses of tissue. These dry resting stages, called **sclerotia** (singular **sclerotium**), can yield new plasmodia under certain conditions.

Questions:

1. What are hyphae, rhizoids, haustoria, mycorrhizae and conidiophores?

2. What are the distinctive features of the various phyla of fungi observed?

3. From your observations, would you conclude that the formation of spores occurs more frequently among the molds or the yeasts?

4. The yeasts used in baking (*Saccharomyces*) belong to which phylum of fungi?

5. If you had a mixed culture of fungi and bacteria, what selective plating procedure could you use to isolate each in a pure culture?

6. Which do you think you would be more likely to observe in the natural environment, cellular or true slime molds?