EXERCISE 1
The Protists

In this exercise you will investigate the diversity of organisms traditionally considered protists. To organize your study, protists can be divided into three categories (not taxonomic groups) according to their mode of nutrition. The *protozoa* are **heterotrophic** protists that ingest their food by **phagocytosis** (the uptake of large particles or whole organisms by the pinching inward of the plasma membrane). Some protozoa, euglenoids for example, are **mixotrophic**, capable of photosynthesis and ingestion. The *algae* include all photosynthetic (**autotrophic**) unicellular organisms and their multicellular relatives. Traditionally, dinoflagellates have been considered algae, but recent evidence suggests that they are closely related to the protozoa, specifically ciliates. The green algae are included in the protists, although, as you will see, evidence indicates that land plants originated from ancestral green algae, and some classifications place green algae (or at least those known as charophytes) in the kingdom Plantae. The third category includes the *funguslike slime molds* that are heterotrophic, obtaining their nutrition by absorbing nutrients from decomposing organic material. Some scientists propose placing the slime molds in a separate kingdom, Mycetozoa, a name that reflects their close relationship with fungi (*myco*) and animals (*zoa*).

These three general categories—protozoa, algae, and slime molds—provide a framework for the study of the diverse organisms referred to as protists. For each organism that you investigate, you will find a brief statement about protistan classification that incorporates molecular and cytological evidence. (For further discussion of protistan classification, see Campbell and Reece, 2002.)

Lab Study A. Heterotrophic Protists—Protozoa

Materials

compound microscope
slides and coverslips
prepared slides of foraminiferans
prepared slides of radiolarians skeletons (demonstration only)
prepared slides of *Trypanosoma levisi*
cultures of living *Paramecium caudata*
Protoslo® or other quieting agent
solution of yeast stained with Congo red
cultures of *Paramecium caudata* that have been fed yeast stained with Congo red (optional)
dropper bottle of 1% acetic acid
transfer pipettes
freshwater and marine plankton tows

Introduction

Traditionally, single-celled heterotrophic protists are called *protozoa*. There are three categories of protozoa based on their mode of locomotion. In one group, organisms move and feed using cellular extensions called **pseudopodia.** Included in this group are amoebas, foraminiferans, and actinopods. Other protozoa move using **flagella,** motile structures supported by microtubules. The third means of locomotion in protozoa is by **cilia,** short cellular extensions supported by microtubules.

Protozoa That Move Using Pseudopodia

Although some taxonomists group all protists that move using pseudopodia into one phylum, others divide the group into several phyla. Given the present uncertainty of the protistan classification, we have selected three protozoans with pseudopodia that were traditionally in different phyla.

Rhizopods (Amoebas)

The rhizopod *Amoeba proteus* is a protozoan species of organisms that move using **pseudopodia**. Rhizopod is derived from *rhizo* or root and *pod* or foot; thus, rootlike foot. In this group, organisms have no fixed body shape, and they are naked; that is, they do not have a shell. Different species may be found in a variety of habitats, including freshwater and marine habitats. Recall that pseudopodia are cellular extensions. As the pseudopod extends, endoplasm flows into the extension. By extending several pseudopods in sequence and flowing into first one and then the next, the amoeba proceeds along in an irregular, slow fashion. Pseudopods are also used to capture and ingest food. When a suitable food particle such as a bacterium, another protist, or a piece of detritus (fragmented remains of dead organisms) contacts an amoeba, a pseudopod will flow completely around the particle and take it into the cell by phagocytosis.

Foraminiferans (Forams)

Foraminiferans, commonly called **forams**, are another example of organisms that move and feed using pseudopodia. Forams are marine planktonic (freely floating) or benthic (bottom-dwelling) organisms that secrete a shell-like *test* (a hard outer covering) made up of chambers. In many species, the test consists of chambers secreted in a spiral pattern, and the organism resembles a microscopic snail. Although most forams are microscopic, some species, called *living sands*, may grow to the size of several centimeters, an astounding size for a single-celled protist. Pseudopodia extend through special pores in the calcium carbonate test. The test can persist after the organism dies, becoming part of marine sand. Remains of tests can form vast limestone deposits.

Procedure

1. Obtain a prepared slide of representative forams (Figure 1a).
2. Observe the organisms first on the lowest power of the compound microscope and then on intermediate and high powers.
3. Note the arrangement and attempt to count the number of chambers in the test. In most species, the number of chambers indicates the relative ages of the organisms, with older organisms having more chambers. Which are more abundant on your slide, older or younger organisms?

   Chambers can be arranged in a single row, in multiple rows, or wound into a spiral. Protozoologists determine the foram species based on the appearance of the test. Are different species present?

Results

Sketch several different forams in the margin of your lab manual. Note differences in the organisms on your slide and those depicted in Figure 1a.

3. Compare the appearance and rate of locomotion in amoeboid, flagellated, and ciliated organisms observed in this exercise.

4. Describe mechanisms for defense in the organisms studied.

5. Give examples of modifications in cell shape or distribution of cilia or flagella allowing the organism to adapt to its environment.

6. Complete Table 1, summarizing characteristics of organisms in the heterotrophic protistan phyla.

Table 1
Characteristics of Heterotrophic Protists—Protozoa

Group	Nutritional Mode	Means of Locomotion	Protective Adaptations	Habitat or Lifestyle	Other Unusual Characteristics
Rhizopods					
Foraminiferans					
Actinopods					
Flagellates					
Ciliates					

Lab Study B. Autotrophic Protists—Algae

Materials

compound microscope
slides and coverslips
living cultures or prepared slides of dinoflagellates
living cultures of diatoms
prepared slides of diatomaceous earth, demonstration only
marine plankton samples
transfer pipettes for all cultures
demonstration materials of brown algae and red algae

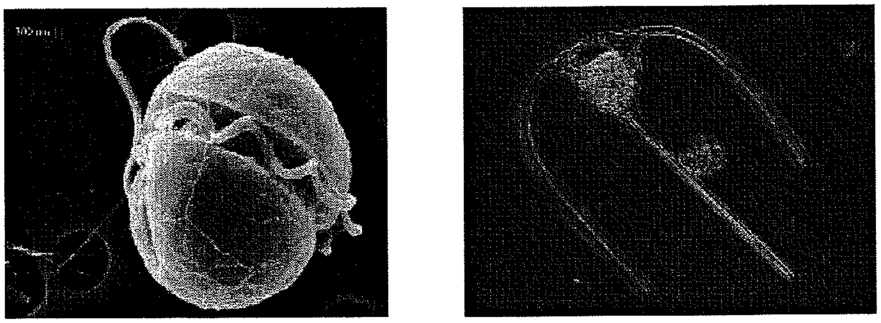
Introduction

In this lab study you will investigate representatives of four phyla of algae. Body form, flagella, and photosynthetic pigments can distinguish the algae. In **unicellular** organisms, the body is only one cell, and daughter cells separate from each other after division. In **filamentous** organisms, cell division takes place in the same plane, and the daughter cells remain attached, resulting in a long line of cells—a filament. Whereas **aggregates** are random, temporary clusters of cells, **simple** and **complex colonies** are predictable organizations of cells either without physiological connections (a simple colony) or with them (a complex colony). In aggregates, simple colonies, and complex colonies, cell divisions take place in many planes. The complex bodies of **multicellular** algae are differentiated into specialized structures for photosynthesis, flotation, and anchorage.

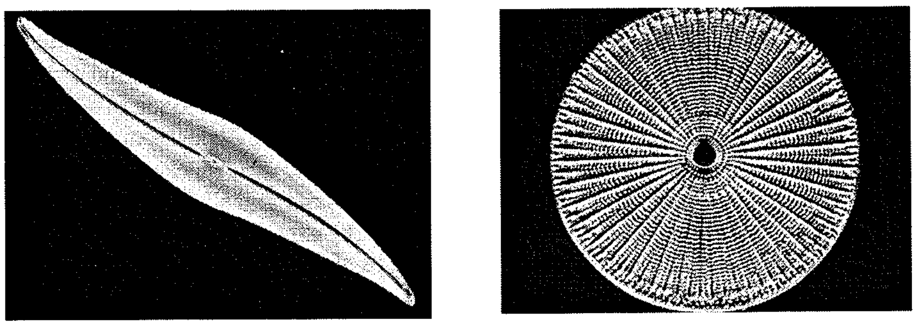
The phyla of algae are characterized by differences in cellular structure and pigmentation. The exclusively microscopic algae demonstrate differences in cell walls (diatoms) and flagella (dinoflagellates). All the algae have chlorophyll *a* as their primary photosynthetic pigment, but brown and red algae have additional accessory pigments. Green algae, like land plants, have chlorophyll *b* as an accessory pigment. Accessory pigments increase the spectrum of light available for photosynthesis. This is particularly important for aquatic organisms because many wavelengths of light, including those absorbed by chlorophyll *a*, are absorbed as they pass through ocean waters. Finally, the red algae lack flagella at any stage in their life cycle. In this lab study, you will examine some of the most common and ecologically important protistan algae, learning the characteristics, ecological roles, and economic importance of each.

Dinoflagellates (Dinoflagellata)

Swirl your hand through tropical ocean waters at night, and you may notice a burst of tiny lights. Visit a warm, stagnant inlet and you might notice that the water appears reddish and dead fish are floating on the surface. Both of these phenomena may be due to activities of dinoflagellates, single-celled organisms that are generally photosynthetic. Some are able to bioluminesce, or produce light. They sometimes can *bloom* (reproduce very rapidly) and cause the water to appear red from pigments in their bodies. If the organisms in this "red tide" are a species of dinoflagellate that releases toxins, fish and other marine animals can be poisoned. Red tides in the Chesapeake Bay are thought to be caused by *Pfiesteria,* a dinoflagellate that produces deadly toxins resulting in invertebrate and fish kills, and that also may be implicated in human illness and death. Dinoflagellates have a cellulose cell wall that is often in the form of an armor of numerous plates with two perpendicular



a. Dinoflagellates



b. Diatoms

Figure 3.
Autotrophic protists (algae). (a) Dinoflagellates have a cellulose cell wall in the form of plates with two grooves that house flagella. (b) Diatoms have a cell wall made of silica consisting of two valves. Species can be pennate forms or centric forms.

grooves, each containing a flagellum. These organisms play an important role in **primary productivity** in oceans, photosynthesis that ultimately provides food for all marine organisms.

Dinoflagellates have traditionally been considered algae, but as previously mentioned, they are now thought to share a common ancestor with ciliates, as evidenced by the presence of subsurface cavities called alveoli. In the future, dinoflagellates and ciliates may be placed in a single group, the Alveolata.

Procedure

1. Obtain a prepared slide or make a wet mount of dinoflagellates (Figure 3a).
2. Focus the slide on low power and attempt to locate the cells. You may have to switch to intermediate power to see them.
3. Switch to high power.
4. Identify the perpendicular **grooves** and the **cellulose plates** making up the cell wall. Are the plates in your species elongated into spines? **Flagella** may be visible in living specimens.

Results

1. Draw several examples of cell shapes in the margin of your lab manual. Note differences between the species on your slide and those in Figure 3a.

2. Summarize distinguishing characteristics of dinoflagellates.

3. Describe the ecological role and economic importance of these organisms.

Diatoms (Bacillariophyta)

Diatoms also play an important role in primary productivity in oceans. In fact, they are the most important photosynthesizers in cold marine waters. They can be unicellular, or they can aggregate into chains or starlike groups. Protoplasts of these organisms are enclosed by a cell wall made of silica that persists after the death of the cell. These cell wall deposits are mined as **diatomaceous earth** and have numerous economic uses, for example, in swimming pool filters and as an abrasive in toothpaste and silver polish. Perhaps the greatest value of diatoms, however, is the excess carbohydrate and oxygen they produce that can be utilized by other organisms. Ecologists are concerned about the effects of acid rain and changing climatic conditions on populations of diatoms and their rate of primary productivity.

Diatom cells are either elongated, boat-shaped, bilaterally symmetrical **pennate** forms or radially symmetrical **centric** forms. The cell wall consists of two valves, one fitting inside the other, in the manner of the lid and bottom of a petri dish. As scientists continue to utilize new information in classifying the protists, the diatoms, brown algae (in the next section), and golden algae (not included in this lab topic) are placed in a single group, **Stramenopila,** based on unique flagella structure usually observed in sex cells.

Procedure

1. Prepare a wet mount of diatoms (Figure 3b) from marine plankton samples or other living cultures.
2. Observe the organisms on low, intermediate, and high powers.
3. Describe the form of the diatoms in your sample. Are they centric, pennate, or both?

4. If you are studying living cells, you may be able to detect locomotion. The method of movement is uncertain, but it is thought that contractile fibers just inside the cell membrane produce waves of motion on the cytoplasmic surface that extends through a groove in the cell wall. What is the body form of motile diatoms?

5. Observe a single centric form on high power and note the intricate geometric pattern of the cell wall. Can you detect the two valves?
6. Look for chloroplasts in living forms.

7. Observe diatomaceous earth on demonstration and identify pennate and centric forms.

Results

1. Sketch several different shapes of diatoms in the margin of your lab manual.
2. Summarize distinguishing characteristics of diatoms.

3. Describe the ecological role and economic importance of these organisms.

Discussion

1. Compare dinoflagellates and diatoms. What important ecological role is shared by these two groups?

2. What is one characteristic that you could observe under the microscope to distinguish diatoms and dinoflagellates?

Brown Algae (Phaeophyta)

Some of the largest algae, the **kelps**, are brown algae. The Sargasso Sea is named after the large, free-floating brown algae *Sargassum*. These algae appear brown because of the presence of the brown pigment **fucoxanthin** in addition to chlorophyll *a*. Brown algae are perhaps best known for their commercial value. Have you ever wondered why commercial ice cream is smoother in texture than homemade ice cream? Extracts of **algin,** a polysaccharide in the cell wall of some brown algae, are used commercially as thickening or emulsifying agents in paint, toothpaste, ice cream, pudding, and in many other commercial food products. *Laminaria,* known as *kombu* in Japan, is added to soups, used to brew a beverage, and covered with icing as a dessert. As previously noted, the brown algae and diatoms may share a common ancestor and therefore be placed together in the Stramenopila.

Procedure

Observe the examples of brown algae that are on demonstration.

Results

In Table 2 on the next page, list the names and distinguishing characteristics of each brown algal species on demonstration. Compare the examples with those illustrated in Figure 4.

Table 2
Representative Brown Algae

Name	Body Form (single-celled, filamentous, colonial, leaflike; broad or linear blades)	Characteristics (pigments, reproductive structures, structures for attachment and flotation)

Red Algae (Rhodophyta)

The simplest red algae are single-celled, but most species have a macroscopic, multicellular body form. The red algae, unlike all the other algae, do not have flagella at any stage in their life cycle. Some scientists suggest that the red algae represent a monophyletic (having a single origin) group and should be placed in their own kingdom. Red algae contain chlorophyll *a* and the accessory pigments **phycocyanin** and **phycoerythrin** that often mask the chlorophyll, making the algae appear red. These pigments absorb

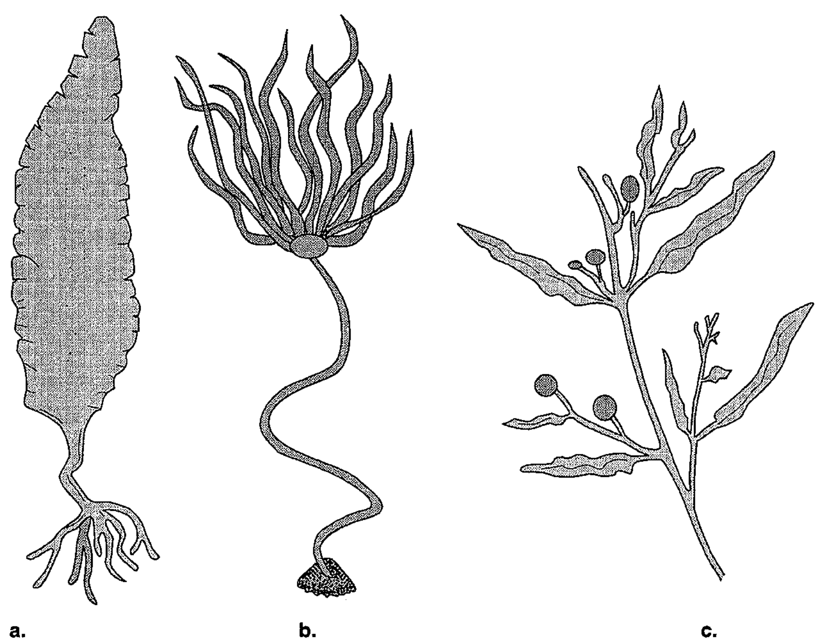


Figure 4.
Examples of multicellular brown algae (phylum Phaeophyta). The body of a brown alga consists of a broad **blade**, a stemlike **stipe**, and a **holdfast** for attachment. These body parts are found in the kelps (a) *Laminaria* and (b) *Nereocystis*. Rounded air bladders for flotation are seen in (c) *Sargassum* and other species of brown algae.

green and blue wavelengths of light that penetrate deep into ocean waters. Many red algae also appear green or black or even blue, depending on the depth at which they are growing. Because of this, color is not always a good characteristic to use when determining the classification of algae. **Agar** is a polysaccharide extracted from the cell wall of red algae. Another extract of red algae cell walls, **carrageenan,** is used to give the texture of thickness and richness to foods such as dairy drinks and soups. In Asia and elsewhere, the red algae *Porphyra* (known as *nori*) are used as seaweed wrappers for sushi. The cultivation and production of *Porphyra* constitute a billion-dollar industry.

Procedure

Observe the examples of red algae that are on demonstration.

Results

In Table 3, list the names and characteristics of the red algae on demonstration. Compare the demonstration examples with those illustrated in Figure 5.

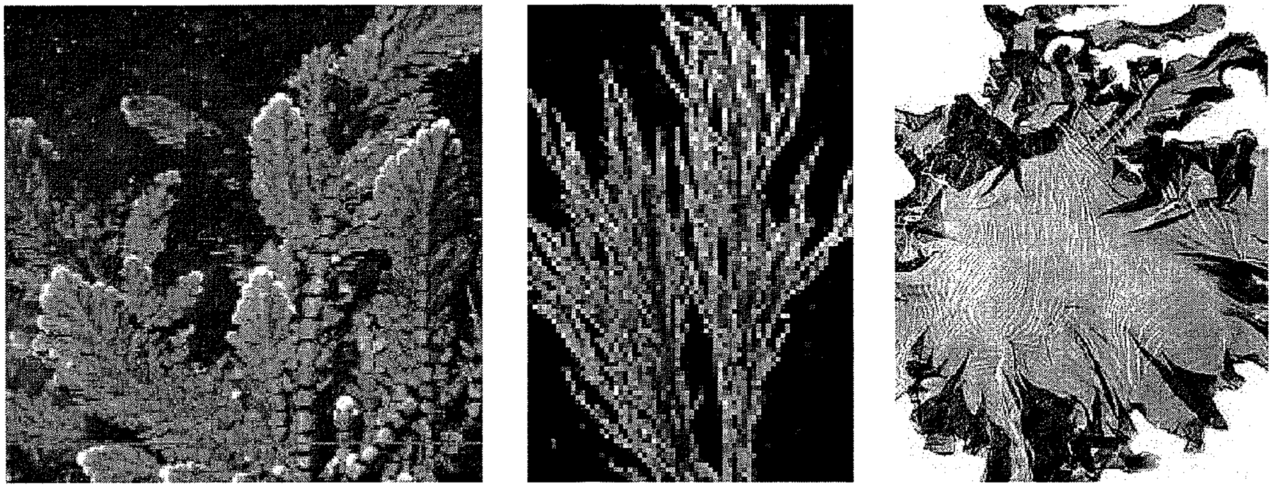
Table 3
Representative Red Algae

Name	Body Form (single-celled, filamentous, colonial, leaflike)	Characteristics (reproductive structures, structures for attachment or flotation, pigments)

Discussion

1. What important ecological role is shared by the macroscopic algae (green, red, and brown)?

2. Based on your observations in the laboratory, what two characteristics might you use to distinguish brown and red algae?



a. b. c.

Figure 5.
Examples of multicellular red algae (phylum Rhodophyta). (a) Some red algae have deposits of carbonates of calcium and magnesium in their cell walls and are important components of coral reefs. (b) Most red algae have delicate, finely dissected blades. (c) *Porphyra* (or *nori*) is used to make sushi.

Lab Study C. The Green Algae (Chlorophyta)—The Protist-Plant Connection

Materials

cultures or prepared slides of *Spirogyra* sp.
preserved *Ulva lactuca*
preserved *Chara* sp.

Introduction

The green algae include unicellular motile and nonmotile, colonial, filamentous, and multicellular species that inhabit primarily freshwater environments. Because green algae share many characteristics with land plants, including storage of starch and the presence of chlorophylls *a* and *b*, photosynthetic pathways, and organic compounds called flavonoids, most botanists support the hypothesis that plants evolved from green algae. Results of recent work in sequencing ribosomal and transfer RNA genes confirm the close relationship between green algae and land plants, and have led some scientists to propose that green algae, or at least those known as charophytes, be included in the Plant kingdom. In this exercise you will view several body forms of green algae on demonstration: single-celled, filamentous, colonial, and multicellular. Finally, you will observe the multicellular, branched green algae *Chara* (the stonewort), believed to be most similar to the green algae that gave rise to land plants over 460 million years ago.

If you completed the Microscopes and Cells lab, you may remember observing aggregates of single-celled algae, *Protococcus,* and the colonial green algae *Volvox.* In this lab study you will observe the filamentous alga *Spirogyra* sp. and the multicellular algae *Ulva* sp. and *Chara* sp.

Procedure

1. Using your compound microscope, observe living materials or prepared slides of the filamentous alga *Spirogyra* sp. (Figure 6a). This organism is common in small, freshwater ponds. The most obvious structure in the cells of the filament is a long chloroplast. Can you determine how the alga got its name? Describe the appearance of the chloroplast.

   Can you see a nucleus in each cell of the filament?
2. Observe the preserved specimen of *Ulva* sp., commonly called sea lettuce. This multicellular alga is commonly found on rocks or docks in marine and brackish water.
   a. Describe the body form of Ulva.

   b. Are structures present that would serve to attach *Ulva* to its substrate (dock or rock)? If so, describe them.

   c. Compare your specimen of *Ulva* with that shown in Figure 6b.
3. Examine the preserved specimen of the multicellular green alga *Chara.* This alga grows in muddy or sandy bottoms of clear lakes or ponds. Its body form is so complex that it is often mistaken for a plant, but careful study of its structure and reproduction confirms its classification as a green alga.

   Note the cylindrical branches attached to nodes. Compare your specimen to Figure 6c. Sketch the appearance of your specimen in the margin of your lab manual.

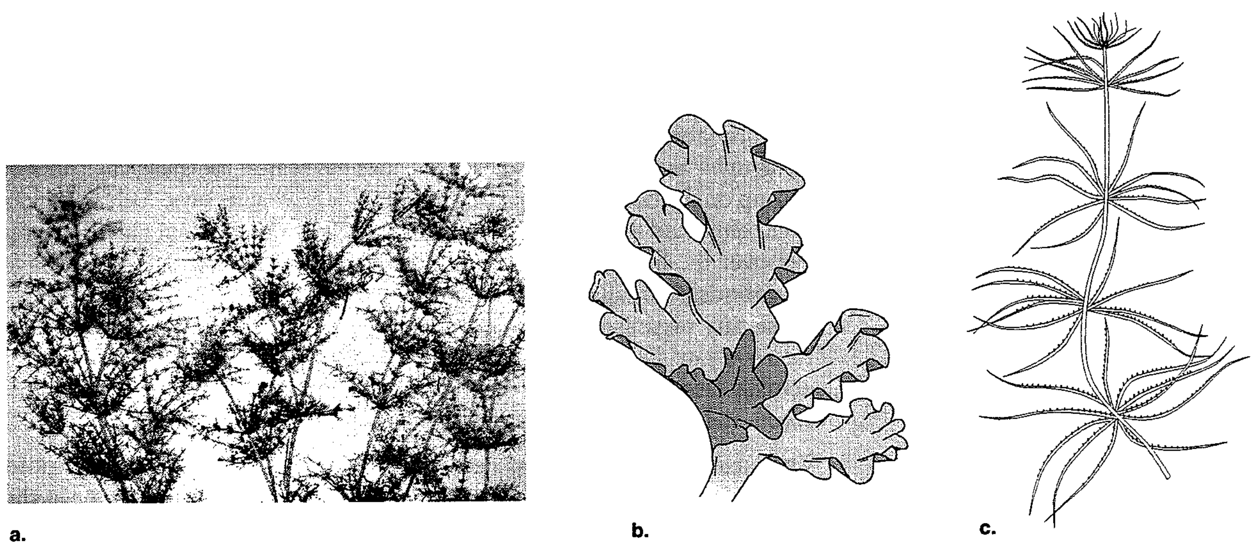


Figure 6.
Examples of multicellular green algae (phylum Chlorophyta). (a) A filamentous green alga, *Spirogyra.* (b) Some green algae are multicellular as in *Ulva,* sea lettuce. (c) A multicellular, branched green alga, *Chara.*

Results

In Table 4, list the names and characteristics of each green algal species studied. Compare these examples with those illustrated in Figure 6.

Table 4
Representative Green Algae

Name	Body Form (single-celled, filamentous, colonial, leaflike)	Characteristics (pigments, specialized structures, flagella, structures for attachment)
Spirogyra		
Ulva		
Chara		

Lab Study D. Funguslike Protists—Slime Molds (Mycetozoa)

Materials

stereoscopic microscopes
Physarum growing on agar plates

Introduction

The organisms you will investigate in this lab study have been called plants, fungi, animals, fungus animals, protozoa, Protoctista, Protista, Mycetozoa, and probably many more names. William Crowder, in a classic *National Geographic* article (April 1926), describes his search for these creatures in a swamp on the north shore of Long Island. This is what he says: "Behold! Seldom ever before had such a gorgeous sight startled my unexpectant gaze. Spreading out over the bark [of a dead tree] was a rich red coverlet . . . consisting of thousands of small, closely crowded, funguslike growths. . . . A colony of these tiny organisms extended in an irregular patch . . . covering an area nearly a yard in length and slightly less in breadth. . . . Each unit, although actually less than a quarter of an inch in height, resembled . . . a small mushroom, though more marvelous than any I have ever seen."

The creatures Crowder was describing are commonly called **slime molds.** Many place them in the kingdom Fungi, but this classification causes difficulties because, whereas slime molds are phagocytic like protozoa, fungi are never phagocytic but obtain their nutrition by absorption. Characteristics other than feeding mode, including cellular ultrastructure, cell wall chemistry, and molecular studies, indicate that slime molds fit better with amoeboid protists than with fungi. However, the general consensus among taxonomists is that slime molds represent a separate kingdom, the **Mycetozoa** or "fungus-animals."

There are two types of slime molds, plasmodial slime molds and cellular slime molds. In this lab study, you will observe the plasmodial slime mold *Physarum*. The vegetative stage is called a **plasmodium,** and it consists of a multinucleate mass of protoplasm totally devoid of cell walls. This mass feeds on bacteria as it creeps along the surface of moist logs or dead leaves. When conditions are right, it is converted into one or more reproductive structures, called **fruiting bodies,** that produce spores. You may choose to investigate slime molds further in Exercise 3.

Figure 7.
Slime mold. Slime molds are protists that share some characteristics with both protozoa and fungi. The vegetative stage of a plasmodial slime mold includes an amoeboid phase consisting of a multinucleate mass known as a plasmodium.

Procedure

1. Obtain a petri dish containing *Physarum* and return to your lab bench to study the organism. Keep the dish closed.
2. With the aid of your stereoscopic microscope, examine the plasmodium (Figure 7). Describe characteristics such as color, size, and shape. Look for a system of branching veins. Do you see any movement? Speculate about the source of the movement. Is the movement unidirectional or bidirectional—that is, flows first in one direction and then in the other? Your instructor may have placed oat flakes or another food source on the agar. How does the appearance of the plasmodium change as it contacts a food source?
3. Examine the entire culture for evidence of forming or mature fruiting bodies. Are the fruiting bodies stalked or are they sessile, that is, without a stalk? If a stalk is present, describe it.

Results

Sketch the plasmodium and fruiting bodies in the margin of your lab manual. Label structures where appropriate.

Discussion

Slime molds were once placed in the kingdom Fungi. What characteristics suggest that these organisms are protistan?

EXERCISE 2

The Kingdom Fungi

Introduction

The kingdom Fungi includes a diverse group of organisms that play important economic and ecological roles. These organisms are unicellular (yeasts) or multicellular, heterotrophic organisms that obtain their nutrients by absorption, digesting their food outside their bodies and absorbing the digestion products into their cells. They often have complex life cycles with alternating sexual and asexual (vegetative) reproduction. They may produce spores either asexually by mitosis or sexually by meiosis.

Fungi are beneficial to humans in many ways. We have long used fungi to make wine and bake leavened bread. Yeast, a single-celled fungus, is used in the production of wine, beer, and bread; and other fungi are used to produce other foods. *Penicillium* is a fungus that is used to produce antibiotics. In ecosystems, fungi share with bacteria the essential role of decomposition, returning to the ecosystem the matter trapped in dead organisms.

Although many fungi are beneficial, others play destructive roles in nature. Some species parasitize animals and plants. Athlete's foot and ringworm are diseases commonly known to humans, and potato late blight and wheat rust are common plant diseases caused by fungi. The ergot fungus that parasitizes rye causes convulsive ergotism in humans who eat bread made with infested grains. The bizarre behavior of young women who were later convicted of witchcraft in Salem Village, Massachusetts, in 1692 has been attributed to convulsive ergotism. Fungi are also a source of food in many cultures, with truffles being the most expensive. Truffles are dark, edible subterranean fungi that sell for $200 per pound, with an annual harvest of 30 tons. Truffles cannot be grown in a lab or greenhouse, and are located by specially trained truffle-sniffing pigs or dogs.

In this exercise, you will learn about the structure of typical fungi and the characteristics of four important phyla of fungi: Zygomycota, Ascomycota, Basidiomycota, and Deuteromycota. You will see examples of lichens that are associations between fungi and algae. As you observe these examples, consider interesting questions that might be asked about fungi diversity or ecology. You can choose one of these questions to design a simple experiment in Exercise 3.

Lab Study A. Zygote Fungi—Zygomycota

Materials

compound microscope
stereoscopic microscope
cultures of *Rhizopus stolonifer* with sporangia
cultures of *Pilobolus crystallinus* on demonstration
forceps, ethyl alcohol, alcohol lamp
slides and coverslips
dropper bottles of water

Introduction

One common organism in the phylum Zygomycota is probably growing in your refrigerator right now. The common bread mold, *Rhizopus stolonifer*, grows on many foods as well as bread. In this lab study, you will observe the structure of this species to see many general fungi characteristics. Fungi are made up of threadlike individual filaments, called **hyphae,** which are organized into the body of the fungus, called the **mycelium.** This filamentous mass secretes enzymes into the substrate and digests food that will then be absorbed into its cells. Cells of fungi have cell walls made of **chitin** combined with other complex carbohydrates, including cellulose. You may recall that chitin is the main component of insect exoskeletons.

Rhizopus stolonifer

Rhizopus reproduces both sexually and asexually. In the Zygomycota, cells of the hyphae are haploid. Hyphae grow over a substrate, for example, a slice of bread, giving the bread a fuzzy appearance. In asexual reproduction, certain hyphae grow upright and develop **sporangia,** round structures, on their tips. Haploid spores develop in the sporangia following mitosis, and when they are mature, they are dispersed through the air. If they fall on a suitable medium, they will absorb water and germinate, growing a new mycelium.

Rhizopus also reproduces sexually when compatible mating types designated as (+) and (–) grow side by side. In this case, (+) and (–) hyphae fuse, and, ultimately, nuclei from opposite strains fuse to form $2n$ zygote nuclei that develop in a thick-walled **zygospore.** Following meiosis, haploid spores are produced in sporangia borne on filaments that emerge from the zygospore.

Pilobolus crystallinus

Pilobolus crystallinus (also called the *fungus gun,* or *shotgun fungus*) is another member of the phylum Zygomycota. This fungus is called a **coprophilous** fungus because it grows on dung. It displays many unusual behaviors, one of which is that it is positively phototropic. Perhaps you can investigate this behavior in Exercise 3. Bold et al. (1980) describe asexual reproduction in *Pilobolus*. This species has sporangia as does *Rhizopus*, but rather than similarly dispersing single spores, in *Pilobolus* the sporangium is forcibly discharged as a unit; the dispersion is tied to moisture and diurnal cycles. In nature, in the early evening the sporangia form; shortly after midnight, a swelling appears below the sporangium. Late the following morning, turgor pressure causes the swelling to explode, propelling the sporangium as far as 2 meters. The sticky sporangium will adhere to grass leaves and subsequently may be eaten by an animal—horse, cow, or rabbit. The intact sporangia pass through the animal's digestive tract and are excreted, and the spores germinate in the fresh dung.

In this lab study you will investigate *Rhizopus* and observe *Pilobolus* on demonstration.

Procedure

1. Obtain a culture of *Rhizopus* and carry it to your lab station.
2. Examine it using the stereoscopic microscope.
3. Identify the **mycelia, hyphae,** and **sporangia.**

4. Review the life cycle of *Rhizopus* (Figure 8). Locate the structures in this figure that are visible in your culture. Circle the structures involved in asexual reproduction.
5. Using forceps and aseptic technique, remove a small portion of the mycelium with several sporangia and make a wet mount.
6. Examine the hyphae and sporangia using the compound microscope. Are spores visible? How have the spores been produced?

   How do the spores compare with the hyphal cells genetically?

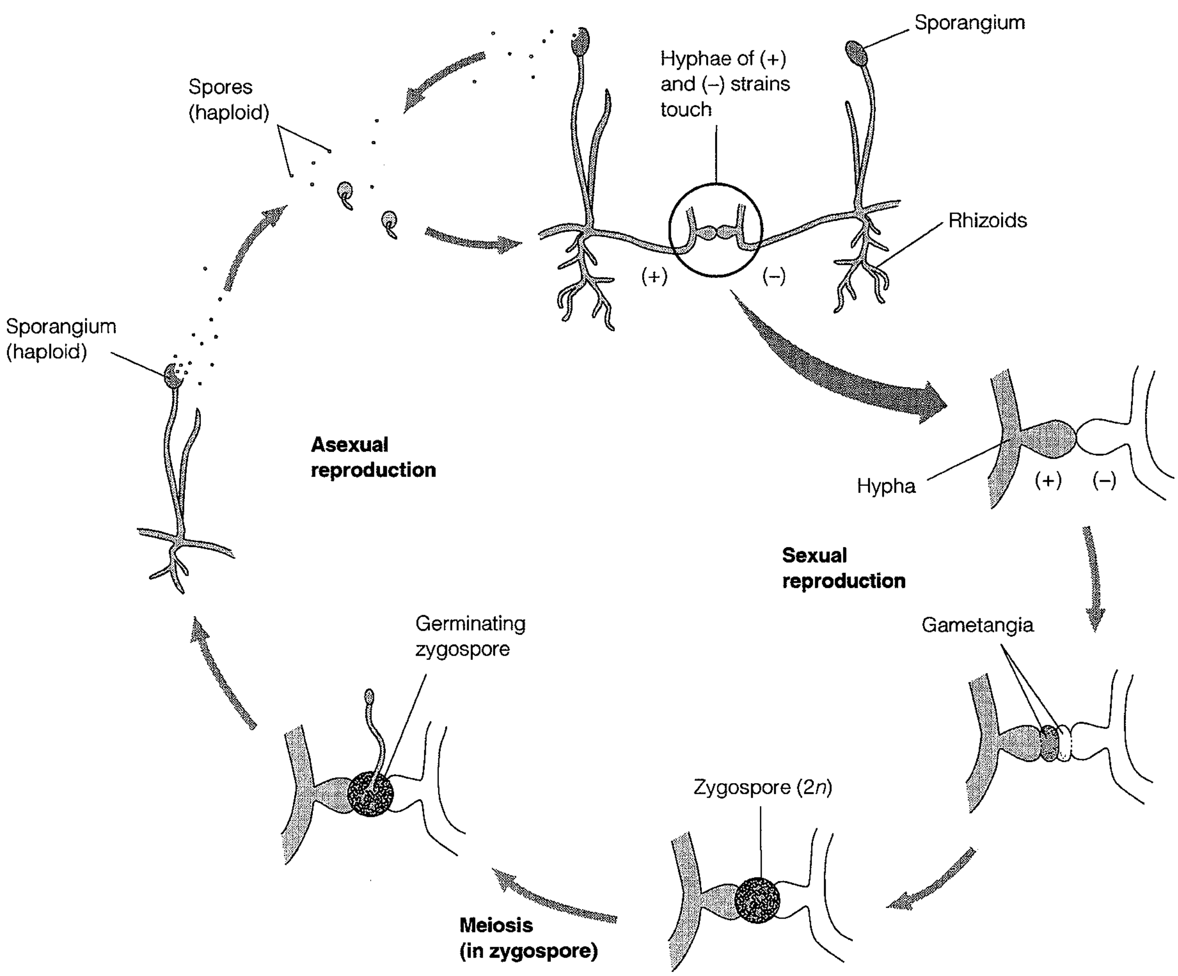


Figure 8.
Rhizopus stolonifer. *Rhizopus* reproduces both sexually by zygospores and asexually by sporangia producing asexual spores. In sexual reproduction, (+) and (–) mating types fuse and a $2n$ zygospore results.

How would spores produced by sexual reproduction differ from spores produced asexually?

7. Observe the cultures of *Pilobolus* (Figure 9) growing on rabbit dung agar that are on demonstration.
8. Identify the **sporangia**, **mycelia**, and **hyphae.** What color are the sporangia and spores?

Results

1. Review the life cycle of *Rhizopus* and the structures observed in the living culture and compare with Figure 8.
2. Review the structures observed in *Pilobolus* and compare with Figure 9.

Discussion

1. The body form of most fungi, including *Rhizopus,* is a mycelium composed of filamentous hyphae. Using your observations as a basis for your thinking, state why this body form is well adapted to the fungus mode of nutrition.

2. Refer back to the description of *Pilobolus*. Speculate about the adaptive advantage of having a system to propel sporangia, as seen in *Pilobolus.*

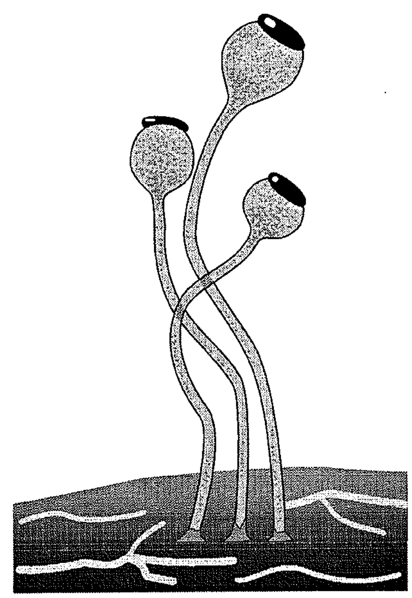


Figure 9.
Pilobolus crystallinus.

Lab Study B. Sac Fungi—Ascomycota

Materials

compound microscope
stereoscopic microscope
dried or preserved *Peziza* specimen
prepared slide of *Peziza* ascocarp
preserved or fresh morels
plastic mounts of ergot in rye or wheat

Introduction

Fungi in the phylum Ascomycota are called *sac fungi,* or ascopore-producing fungi. This division includes edible fungi, morels, and truffles, but it also includes several deadly plant and animal parasites. For example, chestnut blight and the Dutch elm disease have devastated native populations of chestnut and

American elm trees. The fungi causing these diseases were introduced into the United States from Asia and Europe.

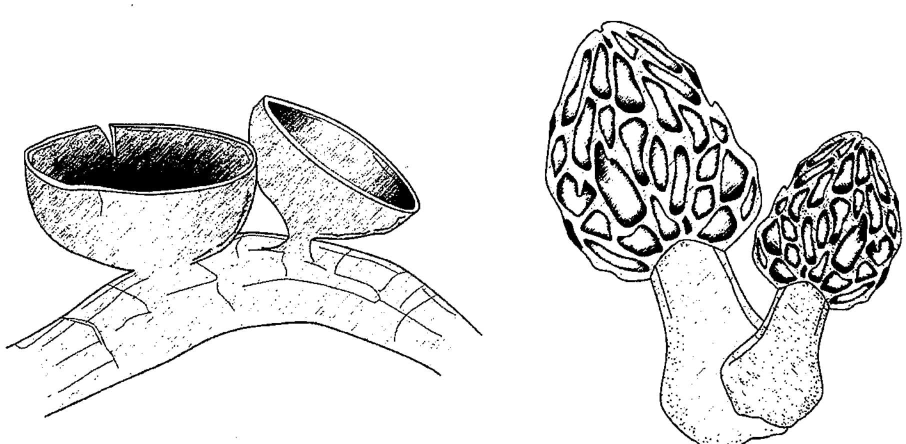
Sexual reproduction in the ascomycota fungi produces either four or eight haploid **ascospores** after meiosis in an **ascus.** Spores in *Sordaria* form after meiosis within asci (Figure 7). Asci form within a structure called an **ascocarp.** In *Sordaria* the ascocarp, called a *perithecium,* is a closed, spherical structure that develops a pore at the top for spore dispersal. In some species of sac fungi, the asci are borne on open cup-shaped ascocarps called *apothecia* (sing., *apothecium*). In asexual reproduction, spores are produced, but rather than being enclosed within a sporangium as in zygote fungi, the spores, called **conidia,** are produced on the surface of special reproductive hyphae.

Other features of sac fungi also vary. For example, yeasts are ascomycetes, yet they are single-celled organisms. Yeasts most frequently reproduce asexually by **budding,** a process in which small cells form by pinching off the parent cell. When they reproduce sexually, however, they produce asci, each of which produces four or eight spores.

In this lab study, you will examine a slide of the sac fungi *Peziza* and will observe demonstrations of additional examples of Ascomycota.

Procedure

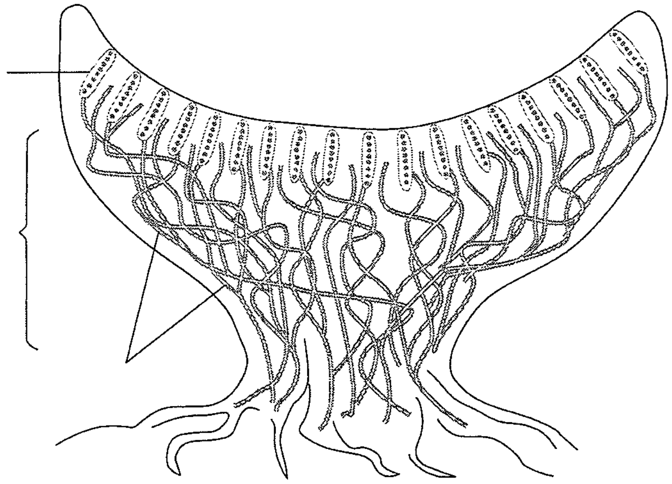
1. Obtain a dried or preserved specimen of *Peziza* (Figure 10a). Notice the open, cup-shaped apothecium, the **ascocarp,** that bears asci within the cup (not visible with the naked eye). Fungi with ascocarps shaped in this fashion are called **cup fungi.** The cup may be supported by a stalk.
2. Examine a prepared slide of *Peziza* using low and intermediate magnifications on the compound microscope. This slide is a section through the ascocarp. Identify **asci.** How many spores are present per ascus? Are they diploid or haploid?



a. b.

Figure 10.
Examples of sac fungi, phylum Ascomycota. (a) *Peziza* has a cup-shaped ascocarp with asci within the cup. (b) Morels are cup fungi that resemble mushrooms.

3. Complete the sketch of the ascocarp section below, labeling **asci, spores, hyphae**, and **mycelium.**



4. Observe the preserved **morels** that are on demonstration (Figure 14.10b). These fungi resemble mushrooms, but the "cap" is convoluted. Asci are located inside the ridges.
5. Observe demonstrations of the mature inflorescence of wheat or rye grass infected with the ascomycete *Claviceps purpurea*, the **ergot** fungus. The large black structures seen among the grains are the ergot.

Results

Review the structures observed in *Peziza*, morels, and ergot. Modify Figures 10a and 10b to reflect features of your examples not included in these figures. Sketch ergot examples in the margin of your lab manual.

Discussion

What characteristics are common to all sac fungi?

Lab Study C. Club Fungi—Basidiomycota

Materials

compound microscope
stereoscopic microscope
fresh, ripe mushroom basidiocarps
prepared slides of *Coprinus* pileus sections

Introduction

The Basidiomycota phylum (club fungi, or basidiospore-producing fungi) includes the fungi that cause the plant diseases wheat rust and corn smut as well as the more familiar puffballs, shelf fungi, and edible and nonedible mushrooms (the latter often called *toadstools*). A mushroom is actually a reproductive structure that grows upward from an underground mycelial mass and produces spores by meiosis. In asexual reproduction, conidia form by mitosis. In this lab study, you will study mushrooms and learn some features of their life cycle.

Procedure

1. Obtain a fresh mushroom, a **basidiocarp,** and identify its parts: The stalk is the **stipe**; the cap is the **pileus.** Look under the cap and identify **gills.** Spores form on the surface of the gills. Examine the gills with the stereoscopic microscope. Do you see spores? Children often make spore prints in scouts or in elementary school by placing a ripe mushroom pileus with the gill side down on a piece of white paper for several hours, allowing the spores to drop to the paper. Scientists use similar spore prints to accurately identify mushrooms.
2. Label the parts of the mushrooms in Figure 11a.
3. Obtain a prepared slide of a section through the pileus of *Coprinus* or another mushroom. Observe it on the compound microscope using low

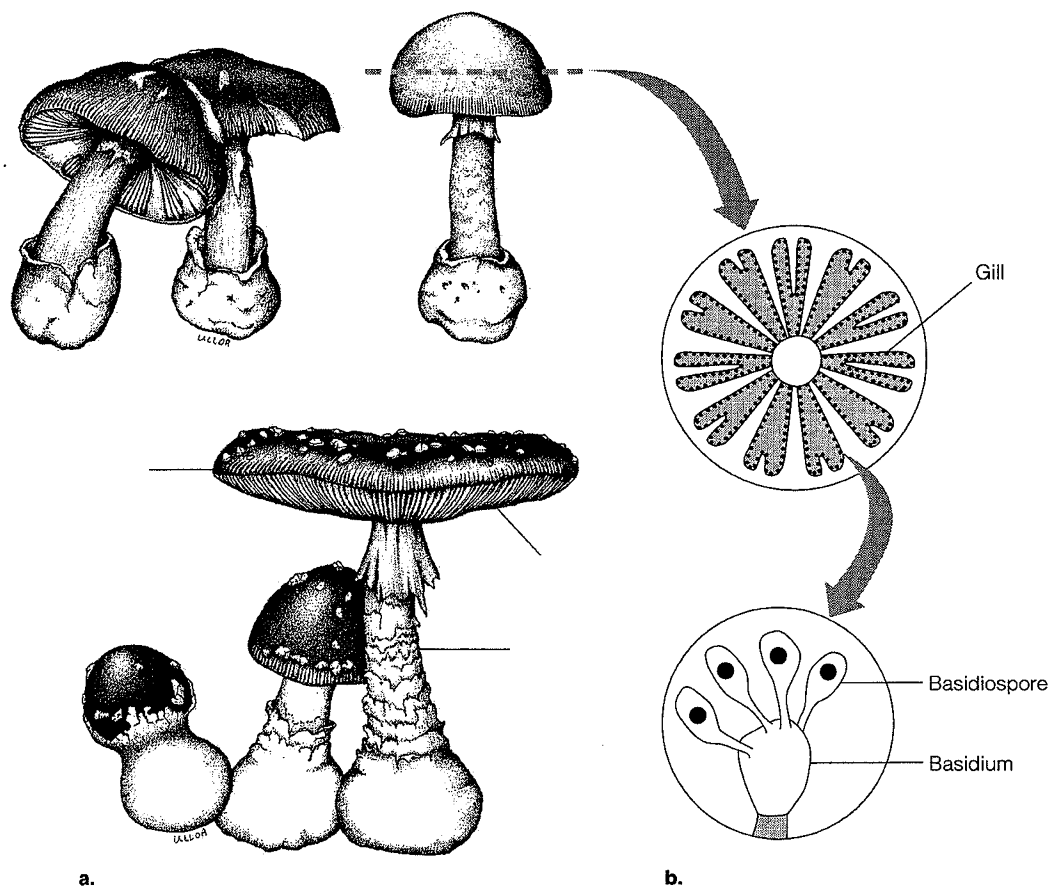


Figure 11.
Club fungi, phylum Basidiomycota. (a) Mushrooms, or basidiocarps, each consisting of a cap, the pileus; and a stalk, the stipe. (b) A section through the gills on a whole basidiocarp reveals basidia and basidiospores.

and then intermediate powers. Is your slide a cross section or a longitudinal section through the pileus? Make a sketch in the lab manual margin indicating the plane of your section through the basidiocarp. Compare your section with the fresh mushroom you have just studied and with Figure 11b.

4. Using the prepared slide, observe the surface of several gills using high power. Spores are produced at the tips of small club-shaped structures called **basidia.** Locate a basidium and focus carefully on its end. Here you may see four knoblike protuberances. Each protuberance has a haploid nucleus that formed following meiosis, and each becomes a **basidiospore.** When the spores are mature, they are discharged from the basidium and are dispersed by the wind.

Results

Review the structures observed and label Figure 11a. Modify the figure to include features observed in your materials that differ from the figure.

Discussion

State the characteristics shared by all Basidiomycota.

Lab Study D. Imperfect Fungi—Deuteromycota

Materials

cultures of *Penicillium* on demonstration
Roquefort cheese on demonstration

Introduction

Most fungi are classified based on their sexual reproductive structures; however, many fungi (as far as is known) reproduce only vegetatively. Because the sexual reproductive stages of these fungi do not exist or have not been found, they are called **asexual,** or **imperfect fungi** (following the botanical use of "imperfect" to indicate a flower lacking one reproductive part). This group is of interest because several human diseases—athlete's foot, ringworm, and candida "yeast" infections—are caused by species of imperfect fungi. Also in this group are several beneficial species—for example, one species of *Penicillium* that produces the antibiotic penicillin and another that is used to make Roquefort and blue cheeses.

Procedure

1. Observe the *Penicillium* on demonstration. You may have observed something similar growing on oranges or other foods in your refrigerator.
2. Describe the texture and the color of the mycelium.

Results

Sketch your observations of *Penicillium* in the margin of your lab manual. Note any features that may be important in distinguishing this organism.

Discussion

Compare the appearance of *Penicillium* with that of *Rhizopus*.

Lab Study E. Lichens

Materials

examples of foliose, crustose, and fruticose lichens on demonstration

Introduction

Lichens are symbiotic associations between fungi and usually algae or cyanobacteria forming a body that can be consistently recognized. The fungal component is usually a sac fungus or a club fungus. The lichen body, called a **thallus,** varies in shape and colors, depending on the species of the components. Reproductive structures can be bright red or pink or green. Photosynthesis in the algae provides nutrients for the fungus, and the fungus provides a moist environment for the algae or cyanobacterium. Because lichens can survive extremely harsh environments, they are often the first organisms to colonize a newly exposed environment such as volcanic flow or rock outcrops, and they play a role in soil formation.

Procedure

Observe the demonstrations of different lichen types: those with a leafy thallus (**foliose**), a crustlike thallus (**crustose**), or a branching, cylindrical thallus (**fruticose**) (Figure 12). Look for cup-shaped or clublike reproductive structures.

Results

1. Sketch the lichens on demonstration in the margins of your lab manual.
2. Identify and label each according to lichen type.

Discussion

Imagine that you are the first scientist to observe a lichen microscopically. What observations would lead you to conclude that the lichen is composed of a fungus and an alga?

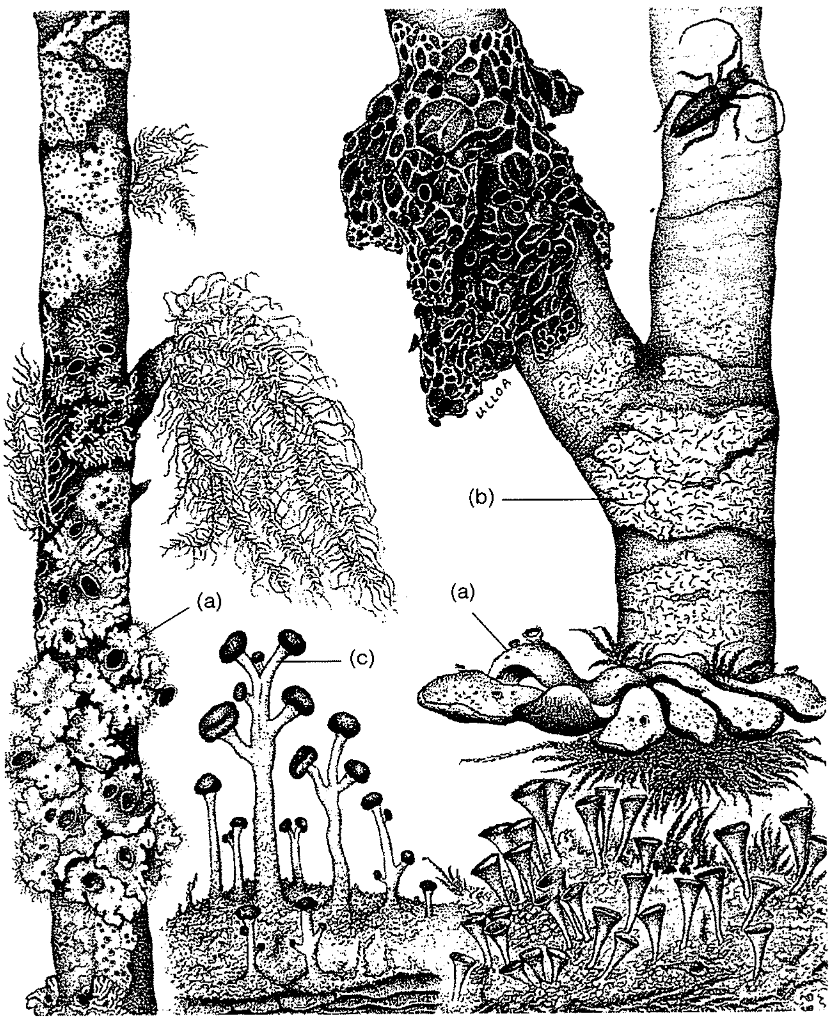


Figure 12.
Lichen types. Lichens may have (a) a leafy thallus (foliose), (b) a crust-like thallus (crustose), or (c) a cylindrical thallus (fruticose).

EXERCISE 3

Designing Your Independent Investigation

Introduction

In this exercise, you will choose one of the organisms observed in this lab topic and design a simple experiment answering a question about its behavior, growth patterns, or interactions with other species.

Be ready to assign tasks to members of your lab team. Be sure that everyone understands the techniques that will be used. Your experiment will be successful only if you plan carefully, cooperate with your team members, perform lab techniques accurately and systematically, and record and report data accurately.

Materials

protozoa and algae cultures
cultures of slime molds *Physarum, Didymium, Dictyostelium*
cultures of *Pilobolus crystallinus, Rhizopus, Penicillium*
sterile agar plates to grow each species
sterile agar with oat flakes
sterile agar with sugar
sterile agar with albumin
sterile agar with pH 6, 7, or 8
aluminum foil
various breads from the health food store—wheat, rye, corn, potato, rice
bread with preservatives
sterilized dung from various animals

Procedure

1. Choose a question from this list to investigate or choose a question from your own observations. *Write your question in the margin of your lab manual.*
   a. Will varying the molarity of the culture medium change the rate of contractile vacuole formation in paramecia?
   b. Do plasmodia of the same species of slime mold unite when growing on the same agar plate? How about different species of slime mold?
   c. Do slime mold plasmodia demonstrate chemotaxis (response to chemical stimuli such as food molecules) or phototaxis (response to light)?
   d. What happens to slime molds if grown in different temperatures?
   e. Do the same fungi grow on different varieties of bread?
   f. How effective are preservatives in preventing fungal growth on foods?
   g. Is *Pilobolus* phototaxic? What about other fungi?
   h. Does succession take place in dung cultures of fungi?
2. Formulate a testable hypothesis.
   Hypothesis:
3. Summarize the experiment. (Use separate paper.)
4. Predict the results of your experiment based on your hypothesis.
   Prediction: (If/then)
5. Outline the procedures used in the experiment.

Queso Fresco - WSU Creamery

Start with clean equipment. Something is going to grow in the cheese milk. Ideally it will be the cultures that are intentionally added!

Into a large cheese making pot add 1 gallon of pasteurized milk and 1 quart of cultured buttermilk. If the buttermilk comes in a half gallon, it is ok to add it all. The butter milk is the starter culture and the source of acid for the cheese.

Heat the cheese milk to 90° F. While the milk is heating, soak one Junket rennet tablet in ¼ cup of water. Put a little bit of milk into the soaking water ***before the tablet is added*** to react with any residual Chlorine in the city water system. (If using Chymosin, use .045ml/pound milk. For 10.75 lbs of milk, add .48 ml.)

Ensure Junket tablet is completely dissolved, and then add to cheese milk. Stir for only long enough to mix in the tablet. Let the cheese milk set ***very*** still; do not bump. Avoid vibration. Vibration may interfere with the coagulation process. Check after 20 – 30 minutes. (If you are using Chymosin it might be as fast as 5 – 10 min.)
Check "set" by cutting with a small spatula – should be thicker than yogurt, not as solid as Jello.

Once the cheese is "set", cut it into ½ inch cubes. A set of wire curd knifes would be ideal, but a knife or spatula would work. Let curd sit for 10 minutes to "heal". (For a quick demonstration this time can be shortened at the cost of yield and quality)

Turn on the heat and slowly raise the temperature to 120°. (Ideally it would take 30 minutes to raise the temperature, but for a quick demonstration faster is great.)

(If you are going to weigh the salt, use .5 pounds/100 pounds of milk. 5 qts = 10.75 pounds, so weigh out 0.054 pounds = .86 ounces of salt)

Pour off 2/3 of the whey though a colander (or screen) covered with a cheese cloth.
Return any captured curd back to the cheese making pot.
Add 1/3 of the salt (.5 tablespoon/ 5 qts) and stir for 5 min.

Pour off the rest of the whey, don't press it or try to get every last drop.
Add 1/3 of the salt (.5 tablespoon/ 5 qts) and stir for 5 min.

Pour off the last of the whey, don't press it or try to get every last drop.
Add the last 1/3 of the salt (.5 tablespoon/ 5 qts) and stir for 5 min.

Ladle curd onto cheese cloths (12 inch x 12 inch or bigger) and wring into balls. (If you are using plastic disposable cheese cloths, smooth side up; bumps down away from curd.) After wringing, tie into balls to hold shape.

Refrigerate after making. Eat within one week.