

CHAPTER 1: DIVERSITY OF LIFE PROTISTA

Purpose:

1. To see a small sample of the variety of living organisms and to be aware of the problems facing biologists in dealing with this variety.
2. To learn how to make a dichotomous key.
3. To learn proper use of the microscope.
4. To study different groups of single-celled organisms and their complexity.

Materials:

A series of colored photographs of various animals and plants from the different regions of the earth. Microscopes and slides. Pasta. Living cultures of *Amoeba*, *Euglena*, *Paramecium*, *Stentor*, and *Vorticella*. Prepared slides of radiolarians and foraminiferans. Materials for experiments with *Euglena* and *Paramecium*. Models of *Amoeba* and *Paramecium*.

Introduction:

The living world is filled with an incredibly complex array of organisms adapted to various habitats and ways of life. Biologists try to make some kind of order out of the apparent chaos through the process of classification and the theory of evolution. The method of biological classification is aimed at producing a system that reflects the similarities and differences between organisms based on observed characteristics which are believed to be inherited. Some of the techniques modern biologists use to determine evolutionary history, common ancestry and therefore true evolutionary relationships are:

1. Basic similarities in morphology supported by embryological development.
2. Fossil history.
3. Similarity in biochemistry and physiology of organisms.
4. Comparison of chromosomes (for example, karyology and allozyme analysis).
5. Studies of distribution (biogeography).
6. Comparison of behavior patterns.
7. Clues from newly discovered organisms.

This information is used to classify organisms into categories from the general to the specific --

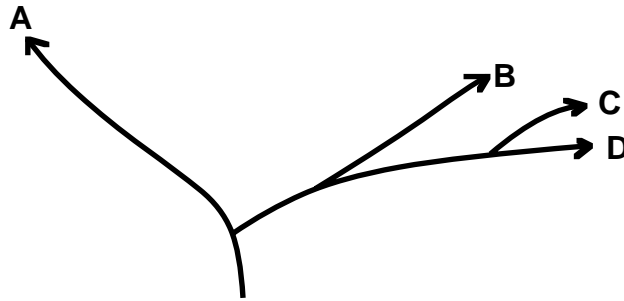
Kingdom
 Phylum (or Division)
 Class
 Order
 Family
 Genus
 Species

Some relationships are not fully understood and classifications may change or may have different interpretations by different biologists. Be sure to review classification in your textbook. We will follow the criteria quite closely during the semester and you should continually refer to it.

Phylogeny

The phylogenetic tree (commonly known as a family tree) is an important tool in classification and is a graphic representation of the evolutionary relationships between groups of organisms. Phylogeny refers to the pattern of historical development of the group of species being considered. In constructing a phylogenetic tree, it is important to carefully choose those characteristics upon which the organisms are compared. The organisms are then grouped according to those characteristics.

Example:



In this example, A diverged early in evolutionary history to become distinct from the other line. Taxon B is more closely related to C and D than to A. In this example C and D are most closely related to each other.

Procedure:

The purpose of this lab is to show you how a variety of organisms can be classified and identified -- some ordinary, some bizarre (you aren't required to recognize and know about the organisms that you see.) They are arranged as photographs of scientific expeditions with appropriate titles - H.M.S. Basset, *etc.* Each expedition has a series of organisms labelled A, B, C, *etc.* Work as a group with your collection.

Name of your expedition _____

Use letters or make up names for your creatures if you choose.

These are animals _____

These are plants _____

Which are most closely related to each other? Construct a phylogenetic tree.

Can you make a guess as to where in the world your expedition is?

Once organism groups are classifiable, individuals must then be identifiable so that classification assignments can be made. One method of identification is based on a taxonomic or diagnostic key which is a technique to organize species characteristics in a form to make a proper identification possible. The most widely used type of key is the **dichotomous key** which is based on a set of paired choices. For example, does the individual fly or not fly? Does it make its own food or feed on others?

Example:

A dichotomous key could be constructed using the following denominations of U.S. currency: a five dollar bill; a one dollar bill; a quarter; a dime; a nickel; and a penny.

- | | | |
|----|--|------------------|
| 1. | a. Composed of paper. | go to 2 |
| | b. Composed of metal. | go to 3 |
| 2. | a. Abraham Lincoln is pictured | Five Dollar Bill |
| | b. George Washington is pictured. | One Dollar Bill |
| 3. | a. The edges have ridges | go to 4 |
| | b. The edges are smooth. | go to 5 |
| 4. | a. Smaller than 20 mm in diameter. | Dime |
| | b. Larger than 20 mm in diameter. | Quarter |
| 5. | a. Silver in color. | Nickel |
| | b. Copper in color | Penny |

Practice making a key to pasta using spaghetti, macaroni, fettucini and rigatoni and then make one for your group of organisms (from the expedition above). Exchange photographs and keys to see if you can use someone else's key. Remember, there is no one way of constructing a dichotomous key.

PASTA KEY

ORGANISM KEY

PROTISTA SINGLE-CELLED ORGANISMS

Classification:

Kingdom Protista

Phylum Rhizopoda
Phylum Actinopoda
Phylum Foraminifera
Phylum Ciliophora
Phylum Euglenophyta

Phylum Myxomycota
Phylum Acrasiomycota
Phylum Oomycota

Phylum Dinoflagellata
Phylum Bacillariophyta
Phylum Chrysophyta
Phylum Phaeophyta
Phylum Rhodophyta
Phylum Chlorophyta

Introduction:

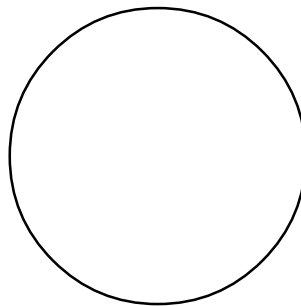
The protists are all eukaryotic and most are unicellular. Besides these two basic characteristics, the protists are extremely diverse, a result of millions of years of evolution. The various organisms represented in this group are mainly distinguished from each other by their mode of acquiring nutrition: the protozoan phyla are primarily heterotrophic, although some are parasitic (and will be discussed in a later lab); the fungus-like protist phyla, which include slime and water molds, feed by absorption; and the photosynthetic autotrophic alga phyla. In fact, very closely related forms may feed in totally different ways, especially among the flagellates. Some protists, particularly ciliates, are so sophisticated that the term acellular is often used in place of unicellular. There is a great range of size in these organisms; *Stentor* is one of the largest and can be seen with the naked eye. The size of each type of organism is in parenthesis after the name.

Procedure:

The Protozoa

Phylum Rhizopoda

1. This group contains the amoebas and other similar organisms. Prepare a wet mount of the culture *Amoeba proteus* (600µm). The best way to obtain an amoeba is to take your drop from the culture near the rice grain on the bottom. The amoeba will first be rolled up in a ball but will eventually start to move. It will push out lobes called **pseudopodia** and essentially pour itself into the lobes; this kind of movement is termed **amoeboid motion**. Does your amoeba have a specified anterior end? What happens when it meets an obstacle? Will it attempt to move in several different directions at once? Use high power on the microscope and look at one of the pseudopodia in detail. Try to distinguish the inner, fluid layer with numerous particles called the **endoplasm** from the rigid, clearer **ectoplasm**. Food vacuoles are usually present in the endoplasm and the nucleus is usually recognizable as a greyish rounded structure. Within the endoplasm you may notice that one of the vacuoles appears to be growing and then suddenly shrink. This is the **contractile vacuole** and is used for excretion of excess water. Would the contractile vacuole of a freshwater organism work as fast (or perhaps faster than) that of a marine organism?



Amoeba
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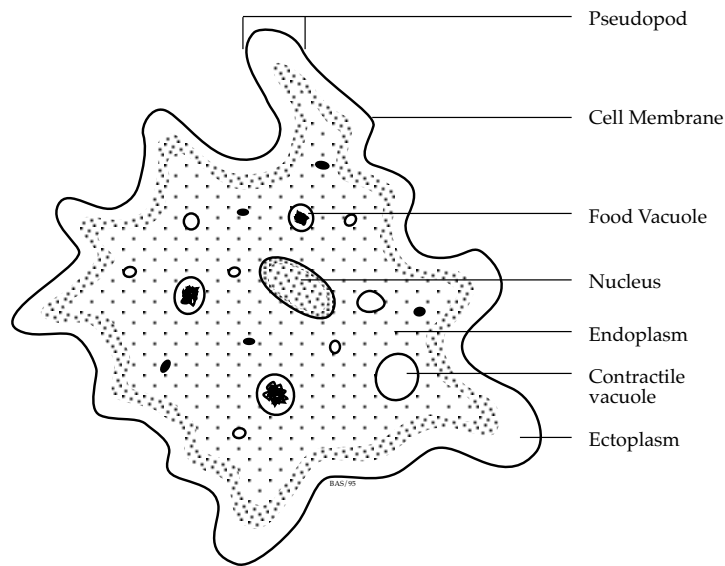
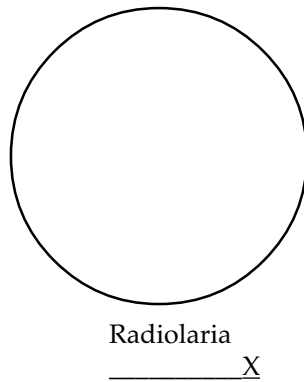


Figure 1.1. *Amoeba proteus*.

Phylum Actinopoda (Heliozoans and Radiozoans)

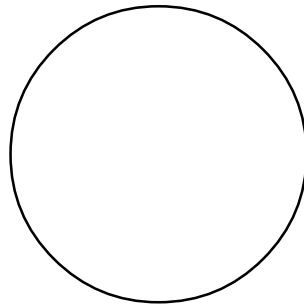
Heliozoans, commonly known as sun animals, are primarily found in fresh water. The bodies of heliozoans, like that of the genus *Actinosphaerium*, are composed of needle-like axopodia which radiate from the surface of the body. The radiozoans are entirely marine, and like the heliozoans, are usually spherical. Radiolarians are relatively large protozoa, reaching diameters of several millimeters. Some forms of radiolaria are colonial and can reach a length of up to 20 cm.

1. Obtain one of the prepared slides demonstrating the radiolaria. The name radiolarian indicates the overall appearance of this group. Most members of the subclass Radiolaria have a skeleton composed of silica; the siliceous spicules in the skeleton accumulate in marine sediments and will, when consolidated, form rocks such as flint. Radiolarians are said to be among the oldest known fossils, having been found in Pre-Cambrian rocks.



Phylum Foraminifera (Forams)

1. Obtain a prepared slide of the shells of the foraminifera. These marine organisms are really amoeboid-like, but the pseudopodia are very fine, nearly thread-like and penetrate through a series of fine pores (foramina) in the thick shell that covers each organism. The shells may be single-chambered, but some may be multi-chambered; the addition of new chambers as the organism grows usually follows a symmetrical pattern. The shells are made of calcium carbonate, and therefore the forams have an extensive fossil record, having first appeared in the Cambrian period. Extensive accumulations in marine sediments during the Mesozoic and early Cenozoic eras eventually led to the formation of limestone deposits and carbonate rocks such as chalk.



Foraminifera
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Phylum Euglenophyta

1. The flagellates are usually considered the most primitive group of protists; most biologists will derive all other protozoans as well as the multicellular plants and animals from them. We will look at a member of the genus *Peranema* (20-70 μ m). Prepare a wet mount by placing a drop of the culture on a depression slide, adding a drop of methyl cellulose to slow down the organisms for viewing. Carefully cover the sample with a cover slip. Look first for the flagella; at what end of the organism (in terms of its motion) are the flagella; is there more than one flagellum? Notice that at the anterior (head) end is large and drop-shaped; this is the **cytopharynx** where food is taken in. Near the cytopharynx is the **rod-organ** which is used to grasp food. Further back in the body you will recognize the greyish-looking nucleus and several **food vacuoles**. Is the shape of this organism fixed? Does it react to the presence of obstacles? How?

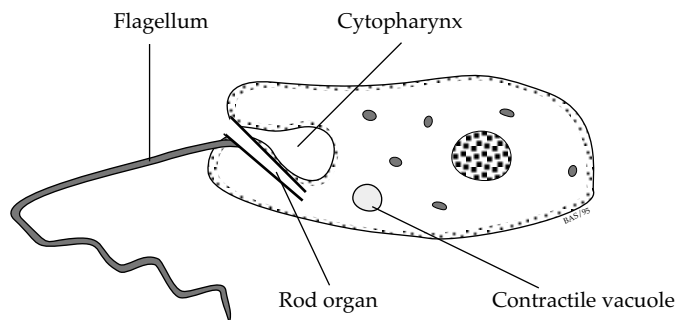


Figure 1.2. *Peranema*.

2. *Euglena* (40 -50 μ m) is another flagellate similar to *Peranema* except that this organism is autotrophic (it contains chlorophyll and can make its food). Prepare a wet mount of living *Euglena*. How does its internal and external structures compare with those of the *Peranema* you just observed?

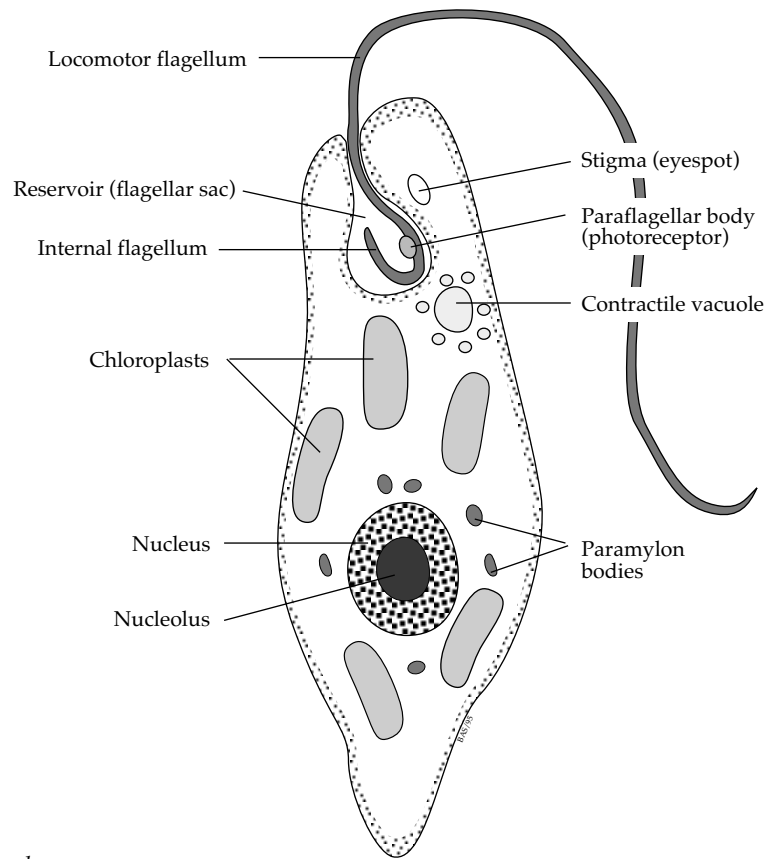


Figure 1.3. *Euglena*.

Phylum Ciliophora

This is the largest group of protozoans; ciliates are found nearly everywhere as free-living, commensal and parasitic forms. They are by far the most complex of the protozoans and have a whole series of different organelles.

1. Prepare a wet mount of *Paramecium* (180 - 300 μ m); you may have to use methyl cellulose to slow the individuals down so that you can study them. Notice first that the individuals appear to have a fixed shape which is due to the presence of a **pellicle** that is not visible with ordinary microscopes. Stop down the light carefully and the cilia that cover the body become more visible. The arrangement of the cilia can be seen by carefully focusing up and down. Do the ciliates have any specialized regions or are they evenly covered with cilia?

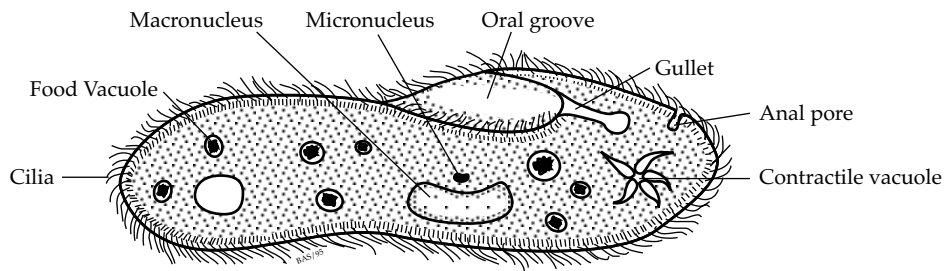


Figure 1.4. *Paramecium caudatum*.

Notice especially the cytopharynx on one side; are the cilia associated with this structure the same as in other regions? The bottom of the conical pit is called the **cytostome** and is used to swallow the food particles caught in the cytopharynx. Look for food vacuoles in the interior of the ciliate. Are there any contractile vacuoles? More than one? Use a piece of absorbent paper and draw off most of the fluid under the edge of the cover slip. In the flattened ciliate the **macronucleus** is visible as a greyish rounded area; this nucleus is responsible for the normal processes of the cell. The smaller **micronucleus** (or nuclei) may be more difficult to recognize; these organelles are responsible for the sexual reproductive activities and exchange haploid nuclei during the sexual phase called **conjugation**.

Do paramecia have a nervous system? Are they capable of detecting and responding to changes in their environment? Try the following experiment:

2. Obtain a depression slide. Add 2 drops of *Paramecium* culture to the trough. Observe the paramecia under the dissecting scope using the black side of the stage or directlight from above the specimen (depending on which type of dissecting scope you are using). Does their movement seem random?
 - a. Dampen one end of a toothpick with **one** small crystal of potassium permanganate (K_2MnO_4). Continue watching the paramecia while pushing the crystal into the culture. What happens when a paramecium approaches the purple area of K_2MnO_4 ? Is its response to this salt positive or negative? Were any paramecia killed? Enter your observations in Table 1.1. Is their response of survival value?
 - b. Clean and dry your depression slide and start over by adding 2 drops of *Paramecium* culture. Soak the large end of your toothpick in red-colored 5% acetic acid for about 30 seconds. Watch again what happens when you dip the acid-soaked toothpick into one end of the culture drop. Is the response of the paramecia positive or negative? Enter your observations in Table 1.1. After 4 minutes where are all the paramecia? Their response to acid is normally of survival value. How can this be?
 - c. To observe the lysosomic activity in *Paramecium*, mix approximately equal volumes of culture and yeast/ neutral red preparation. Observe the feeding process. Enter your observations in Table 1.1. Periodically examine the protozoans for changes in the color of the food vacuoles.

Table 1.1 *Paramecium* response to various stimuli

	+ response (moved toward stimulus)	- response (moved away from stimulus)	Died
Potassium permanganate (K_2MnO_4)			
5% Acetic Acid			
Yeast with Neutral red			

The Slime Molds and Water Molds

The slime molds and water molds were once regarded as fungi but are now considered part of the Protista due to: 1) the presence of flagellae, lacking in the Fungi; 2) the presence of male and female gametes in some groups, also lacking in the Fungi; 3) the lack of a dikaryotic stage, present in the Fungi; and 4) either a cellulose-based or no cell wall, whereas the Fungi primarily have a chitinous cell wall.

The two groups of slime molds, the plasmodial (acellular) slime molds of the phylum Myxomycota (450 species), and the cellular slime molds of the phylum Acrasiomycota (65 species), are motile, ingest particulate food by endocytosis, and form spores on erect fruiting bodies. Slime molds prefer the cool, moist habitats of the forest and range from colorless to brilliant yellows and oranges. The phylum Öomycota (580 species) contain the aquatic water molds and also include white rusts and the terrestrial downy mildews.

Phylum Myxomycota

During most of its life, a myxomycete exists as a wall-less mass of cytoplasm with numerous diploid nuclei called a **plasmodium**. The plasmodium is an example of a **coenocyte**, a cell in which many nuclei are enclosed in a single plasma membrane. The outer cytoplasm of the plasmodium is thicker than that of the interior and provides some measure of rigidity like the ectoplasm of the amoebas. As the plasmodium spreads over its substrate it engulfs bacteria, yeasts, spores of fungi, and decaying animal and plant matter for its food source. As food and water sources disappear, or the temperature drops below a tolerable range, one of two things can happen. The plasmodium can temporarily form a **sclerotium**, a resistant, irregular mass of hardened cell-like components that re-form the plasmodium when favorable conditions return, or the plasmodium can transform itself into sporangiophores, which are stalked, or branched fruiting bodies. **Sporangia** form at the ends of the stalks; the nuclei within the sporangia undergo meiosis producing haploid spores which are released and germinate to form wall-less, flagellated, haploid swarm cells. Upon the return of favorable conditions, the swarm cells can act as gametes and fuse to form a diploid zygote, dividing by mitosis but without wall formation to produce a new coenocytic plasmodium.

Phylum Acrasiomycota

Cellular slime molds exist as individual amoeboid cells with a single haploid nucleus. These cells reproduce by mitosis or fission and form swarms of independent, isolated cells called **myxamoebas** which engulf bacteria and other food particles as long as the environment is favorable. When conditions become unfavorable, the myxamoebas aggregate to form a **pseudoplasmodium** which is not a coenocyte since each cell retains its own plasma membrane. The pseudoplasmodium ultimately forms a stalked fruiting body; cells at the top of the stalk (**sporangium**) form thick-walled haploid spores. These spores are released, and when conditions again become favorable, these spores germinate into free-living haploid myxamoebas. This mode of the acrasiomycete life cycle is asexual; a sexual phase exists in which two myxamoebas of possibly different mating types fuse, forming a spherical structure that ultimately germinates and releases new myxamoebas.

Phylum Öomycota

All of the members of this phylum are either parasitic or **saprobic** (feed on dead organic matter). Some of the terrestrial forms are responsible for such plant diseases as the late blight of potatoes which caused the Irish potato famine of 1845 - 1847 during which 400,000 people died of starvation or diseases complicated by starvation. These protists are coenocytic; they have **hyphae** (thin filaments) without cross walls to divide them into individual cells. The cytoplasm is continuous and there is no single structural unit containing a single nucleus except in certain reproductive stages. The Öomycota are diploid throughout most of their life cycle and have cellulose in their cell walls. The öomycetes are distinguished from other protists by their **zoospores**, motile spores which have two unequal flagellae facing in opposite directions. These zoospores are produced asexually in a sporangium and germinate to form the diploid hyphae. Sexual reproduction involves meiosis in a female **ögonium** that produces haploid eggs and the male **antheridium** that produces haploid male nuclei. The contents of the antheridium flow into the ögonium and one or more pairs of male nuclei fuse with an egg. The resulting zygote forms a thick-walled cell called an **öospore**. The öospores germinate to form the diploid hyphae of the öomycete.

The Algae

The algae are photosynthetic autotrophs and are responsible for approximately 50 - 60 percent of all the photosynthesis on the planet, with the Kingdom Plantae, cyanobacteria, and other photosynthetic bacteria accounting for the rest. The algae differ from plants in that the zygote of an alga is not protected by the parent whereas a plant zygote grows into a multicellular embryo that is encased in parental tissue. The six algal phyla can be placed into three groups according to the photosynthetic pigments they possess:

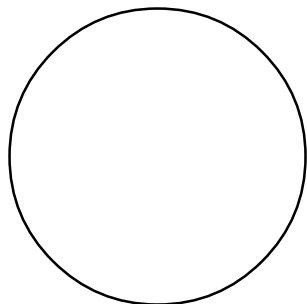
- 1) the multicellular brown algae, phylum Phaeophyta, along with the unicellular phyla Dinoflagellata (the dinoflagellates) and Bacillariophyta (the diatoms) as well as the Chrysophyta, (the golden algae), have chloroplasts which contain the pigments chlorophylls *a* and *c*, carotenoids, and xanthophylls;
- 2) the red algae, phylum Rhodophyta, have chloroplasts which contain chlorophyll *a*, carotenoids, and phycobilins; and
- 3) the green algae, phylum Chlorophyta, have chloroplasts which contain the pigments chlorophylls *a* and *b*, and carotenoids. This phylum is assigned to the Kingdom Protista even though the green algae include the ancestors to plants.

Phylum Chlorophyta

Due to space and time limitations of the laboratory, we will limit the discussion of algae to the Chlorophyta. Green algae were among the first organisms to discover sex and in so doing they also invented death. Asexual reproduction by fission is a kind of immortality since the protoplasm of the original individual is contained in the 2 daughter cells. In evolutionary terms, there are definite advantages to sexual reproduction. What are some of these? Green algae have several types of sexual reproduction that may suggest an evolutionary pattern.

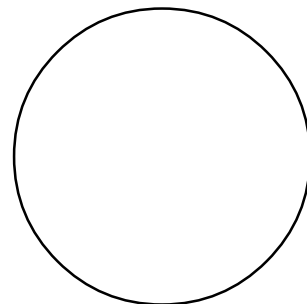
A primitive type of sexual reproduction is illustrated by *Chlamydomonas*, a unicellular flagellated alga. *Chlamydomonas* is normally **haploid** and commonly reproduces asexually by dividing to form from 2 - 8 **zoospores** which rupture from the parental cell wall and swim away as independent haploid individuals. Occasionally, the parent cells divide and form gametes. These resemble zoospores and adults, but are smaller. The two gametes fuse and form a **diploid zygote**. The zygote forms a thick cell wall and is capable of enduring unfavorable environmental conditions, such as a cold winter or the drying up of a pond. When the environment is favorable again the zygote undergoes **meiosis** and liberates four haploid cells that become independent. *Chlamydomonas* is **isogamous** -- male and female gametes are the same size and shape.

- 1) Examine living cultures and prepared slides of *Chlamydomonas* under high power. Can you locate the nucleus and flagella? Look for different stages in the life cycle.



Chlamydomonas

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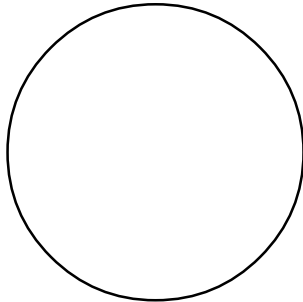


Chlamydomonas

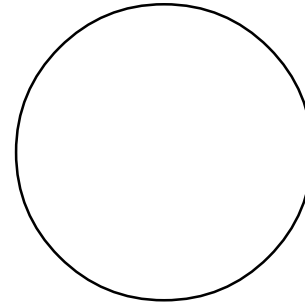
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The next step might be represented by *Spirogyra*, the common pond "scum". It consists of long filaments made up of a series of cells, which feel silky to the touch. This plant is haploid and can reproduce asexually. The sexual phase usually occurs in the fall when one filament comes to lie next to another filament and a connection is formed between the two adjoining cells. One nucleus oozes through the connection and joins the other nucleus. The nuclei fuse and a diploid zygote results. This process is called conjugation. This zygote develops a thick wall, also enabling it to withstand cold winters. In the spring it divides by meiosis to form four haploid cells. There are no gametes with flagellae and *Spirogyra* is also isogamous. A specialization in *Spirogyra* is that certain filaments supply gametes and certain filaments receive gametes.

2. Examine living *Spirogyra* filaments and prepared slides of *Spirogyra* conjugating. Look for individual cells. Each contains a green coiled ribbon which is the **chloroplast**. The "buttons" on the ribbon are called **pyrenoids**, collecting points for starch molecules. Locate the nucleus in the stained slides.



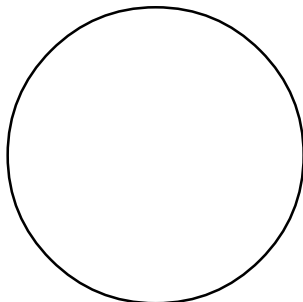
Spirogyra
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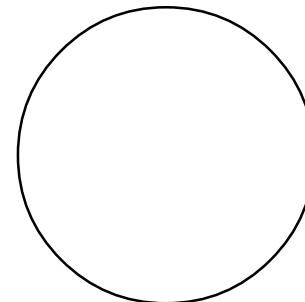
Spirogyra
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Another possible step in the evolution of sexual reproduction is demonstrated by the filamentous alga *Oedogonium*. This step is **heterogamy** or the fusion of dissimilar gametes. The female gamete is a larger food-laden egg and the male is a small motile sperm. Certain cells form female gametes and others form male gametes. **Öogamy** is the specific type of heterogamy describing the large non-motile eggs and the smaller free-swimming sperm.

3. Study the living examples and prepared slides of *Oedogonium*. Look for dark-colored swollen cells in the filaments. These are **öogonia** containing a single egg. After fertilization the egg becomes a diploid zygote. If the mass inside an öogonium is not surrounded by a definite cell wall, it is unfertilized. A fertilized egg has a thick independent wall surrounding it. Look for a portion of the filament which has divided transversely into four or eight small cells. These are antheridia and each produces two motile sperm. The zygotes undergo meiosis as in the previously studied algae. *Oedogonium* reproduces sexually more often than asexually.



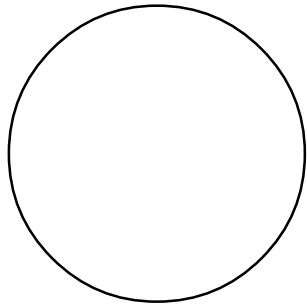
Oedogonium
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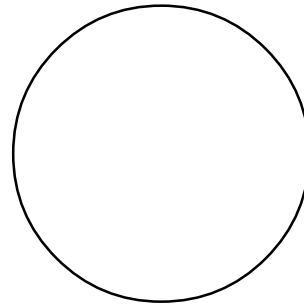
Oedogonium
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Volvox is a colonial alga consisting of a hollow ball of cells loosely connected to each other. Each individual cell has two flagellae. Some colonies are very large, containing up to 50,000 cells. Most of these cells are exclusively vegetative. Certain cells in the colony (**öogonia**) are specialized to produce eggs and others (**antheridia**) to produce the smaller motile sperm. The sperm are released and swim to the egg forming a diploid zygote with a thick cell wall. During germination meiosis occurs and haploid cells are formed. These cells, after many mitotic divisions, form a new colony. This alga is a good illustration of sex differentiation in which certain parts of the organism are specialized for sexual reproduction. *Volvox* also reproduces asexually by producing daughter colonies inside the original colony. When the daughter colonies become large enough, they turn inside out, leave the mother colony, and begin an independent life.

4. Examine living and prepared slides of *Volvox*, looking for zygotes, sex cells, and daughter colonies.



Volvox
_____ X



Volvox
_____ X

The organisms studied do not, of course, reflect an actual evolutionary sequence. None of these organisms evolved from another, however, the types of specializations suggest the evolutionary trends. Review the life cycle of each alga, remembering which part is haploid and which part is diploid, as well as the particular specializations each has. Under what kind of conditions does the sexual phase occur?

