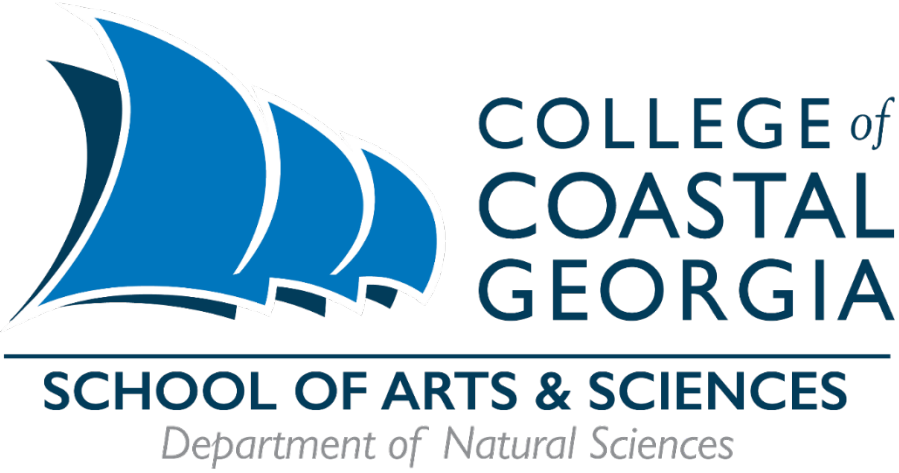
# BIOL 1108L
# Principles of Biology II
# Laboratory Manual




Created by: Joshua Clark, Kelly Clark, Jennifer Hatchel, C. Tate Holbrook, Traesha Robertson, and David Stasek

# Table of Contents

# Scientific Reasoning

## Objectives

- Define science as a process of discovery about the natural world based on empirical evidence and scientific reasoning.
- Apply scientific reasoning ("the scientific method") to biological research.

## Introduction

What is *science*? It is not simply a collection of facts to be memorized, although that is often required to establish a foundation for scientists to build upon. Science is best understood as a *process* of discovery about the natural world. More specifically, science is a way of knowing based on **empirical (observable) evidence** and **scientific reasoning**, a kind of logic that is commonly portrayed as **"the scientific method"**.

The scientific method is not a rigid prescription or recipe that always follows same path; scientific research actually involves a lot of creativity and sometimes even serendipity. Nonetheless, the scientific method provides a helpful way to identify the basic components of the scientific process. Throughout this course, you will apply this process to biology, the scientific study of life. As you will see, conducting scientific research doesn't require an advanced degree; anyone can *do* science!

## The Scientific Method

The scientific process is summarized in the following steps, which are elaborated below:

1. Make observations
2. Ask a question
3. Generate a hypothesis(-es)
4. Conduct a test (observational study or experiment, with prediction)
5. Collect and analyze results (data)
6. Draw a conclusion (compare results to prediction to either support or reject hypothesis)
7. Ask new questions or generate/test alternative hypotheses…

Science starts by **observing** nature. Scientists pay close attention to the world around them, using all their senses (be careful when tasting!), which can be extended by using modern scientific instruments such as microscopes. Further insight into natural phenomena can be attained through background reading and knowledge.

Puzzling observations lead scientists to ask **questions** to be answered, or in more applied areas of science, to identify problems to be solved. Scientists are naturally curious, always asking questions, many of which do not (yet) have answers. The best questions to inspire scientific research are open-ended, *causal* questions (i.e., *How? Why? What causes?*), rather than simple yes/no questions, which can usually be reframed as hypotheses.

Next, scientists generate a **hypothesis** or, better yet, multiple alternative hypotheses, which are possible explanations or answers to their question. A scientific hypothesis is not really a "guess"; it should be informed by previous knowledge and observations that allow the scientist to develop a sound *rationale or justification* for the hypothesis.

Scientific hypotheses must be *testable* and *falsifiable* (i.e., they can be rejected by empirical evidence). Scientists use different approaches to **test** hypotheses, including but not limited to observational studies and experiments. **Observational studies** test for predicted patterns or *correlations* between naturally occurring variables, with minimal interference by the investigator. Only **experiments**, however, can demonstrate *cause-and-effect* relationships between variables. In an experiment the investigator actively *manipulates* (i.e., intentionally alters) one or more factors that is known as the **independent variable(s)**. The independent variable(s) is related to the hypothesis being tested. The investigator then measures some outcome called the **dependent variable(s)** or response variable(s). If the hypothesis is correct, the dependent variable(s) will *depend on* or *respond to* the manipulation of the independent variable(s). In a "controlled" experiment, other factors sometimes referred to as **standardized variables** are "controlled for" or held constant so they do not affect the outcome of the experiment. Another important feature of experimental design is **replication**, or applying each experimental manipulation or treatment to multiple, independent units/subjects known as *replicates*. Replication reduces the likelihood that experimental results are due to chance or to unknown variables that were not controlled.

Whether the scientist is conducting an observational study or a manipulative experiment, they should make a clear **prediction** before collecting any data. A prediction is an expected pattern or outcome of a test, *if* the hypothesis is correct. Then, once the study or experiment has been conducted and the **data** have been collected and analyzed (using statistics – to be covered elsewhere), the observed **results** can be compared to the predicted results to draw a **conclusion**. If the actual results match the prediction, then the hypothesis is *supported*. If the actual results are inconsistent with the prediction, then the hypothesis is *rejected*. Note that hypotheses can never be *proved* absolutely true; scientific conclusions are tentative and can change if contradicting evidence is later observed.

The scientific process does not end there, but is *cyclical:* the results of one research project lead scientists to ask new follow-up questions and to generate alternative hypotheses, which can then be tested through future research. Scientists share or disseminate their findings through presentations and publications that are rigorously peer-reviewed by fellow scientists. Thus, collectively, science builds toward finding better explanations of natural phenomena that are supported by stronger evidence. Once a hypothesis or set of related hypotheses has been thoroughly tested and overwhelmingly supported by an extensive body of evidence, it becomes recognized as a **scientific theory** (e.g., atomic theory, cell theory, gravity, evolution).

## Example: Interactions between Native and Invasive Anole Lizards

Next let's apply the logic of science to a specific example that illustrates how biologists (in this case, ecologists) think about and investigate the living world.

Suppose that while walking around campus, you **observe** lots of small lizards scurrying around or basking in the sun on trees, walls, and other structures. Some appear to be doing pushups and extending a colorful, semicircular flap of skin (dewlap) from their throat region. Upon closer inspection, you distinguish two different body forms that are similar in size and shape but vary in color: some lizards are lime green, while others are darker brown/grey with a zig-zag pattern on their back. You consult a field guide to reptiles (or do a quick internet search) and identify

that the lizards are anoles belonging to two different species: the green anole *Anolis carolinensis* and the brown anole *Anolis sagrei*. You also read that the green anole *A. carolinensis* is native to the southeastern U.S. while the brown anole *A. sagrei* is an invasive species that recently colonized coastal Georgia from the Caribbean, by way of Florida. Based on your observations, it seems like the brown anole is more common than the green anole on campus.

Given your sense of scientific curiosity, these observations inspire a flurry of **questions**. How did the brown anole get here? Why did it only recently colonize coastal Georgia? What caused its invasion? Why does the brown anole seem to be more abundant than the green anole? How do the two species of anoles interact? Has the spread of the brown anole harmed the green anole, causing its populations to decline? Can the green anole persist alongside the brown anole, or does it face local extinction? Is the green anole evolving in response to the brown anole? How is the brown anole affecting ecosystems more broadly along its introduced range? How can brown anole populations be managed or controlled?

These questions could fuel an entire Ph.D. dissertation or even a scientific career's worth of research. Let's start small by addressing the question: *How* do the green anole and brown anole interact? You **hypothesize** that the brown anole negatively impacts the green anole by competing with it for limited resources. More specifically, you propose that the two species compete for food. You justify this hypothesis by referring to your background reading that the two species have similar diets of insects, spiders, and other small invertebrates. (You might also consider alternative hypotheses such as competition for space, or another type of species interaction such as predation. You could also state a null hypothesis that there is no interaction between the species.)

To **test** the hypothesis that the green anole and brown anole compete for food, you might start with an **observational study** conducted under natural conditions. For example, perhaps you could measure the abundance of green anoles and their spider prey in local forest sites where (a) only green anoles occur (i.e., brown anoles are naturally absent) compared to areas where (b) both brown anoles and green anoles co-occur. If your hypothesis is correct, then you would **predict** that green anoles (and spiders) should be less abundant in areas colonized by brown anoles than in areas where green anoles occur on their own. If your actual **results** matched your prediction, then you could **conclude** that the hypothesis was *supported:* competition with brown anoles for food apparently limits the local abundance of green anoles. If, on the other hand, you found no difference in the abundance of green anoles between sites with and without brown anoles, then your hypothesis would be *rejected*.

The observational study described above could demonstrate a *correlation* between the presence of brown anoles and lower abundance of green anoles, which is consistent with the hypothesis that the two species compete for food. However, because additional factors such as habitat quality, temperature, etc. were not controlled for, there are other possible explanations for the observed difference in green anole abundance between sites (e.g., maybe the two species prefer different forest types). To directly **test** whether competition with brown anoles *causes* green anole populations to decline, you must conduct an **experiment** in which you manipulate the presence/absence of brown anoles (*independent variable)* and measure the abundance of green anoles (*dependent or response variable)* while holding other factors constant

(*standardized variables;* e.g., density of trees, availability of prey, etc.). For example, you could try to capture and remove all the brown anoles from a subset of forest plots, leaving only green anoles. Or alternatively, with permission, you could introduce anoles to marsh hammocks (small islands) lacking prior populations of either species; some islands would receive only green anoles, while other islands would receive both green and brown anoles. Note that to minimize bias and the effect of chance, you would need to *replicate* each condition (brown anoles present/absent) across multiple islands or forest plots; it would be insufficient to compare just two sites, which might vary in other ways too. Regardless of the details of your experimental design, your hypothesis that the two species compete for food would lead you to **predict** that, over time, green anoles should be more abundant at sites where brown anoles are absent. If the data you collected showed that pattern, as expected, then the **results** would *support* your hypothesis and you could **conclude** that the brown anole *caused* the green anole to decline. If green anoles instead persisted at high numbers even when brown anoles were present, then you would *reject* the hypothesis that the two species compete. (Note that if you wished to more specifically test the hypothesis that competition *for food* is the leading cause of decline for green anoles, you could also manipulate the availability of food as a second independent variable. You might predict that providing supplementary food will reduce the strength of competition and allow more green anoles to persist alongside brown anoles.)

Depending on your findings, you might then proceed to generate and test alternative hypotheses that address the same question (*How* do the green anole and brown anole interact?), or move on to investigate other question(s) inspired by your observations and initial research (Master's degree?). And hopefully you would present your work at an on-campus undergraduate research symposium and/or at a regional scientific conference, and possibly even write a manuscript for publication so other scientists can learn from and build upon your research!

Your instructor may now lead you through a case study to give you more practice applying the process of science ("the scientific method"). Don't worry, this class and others will give you lots of opportunities to develop skills in scientific reasoning and research so you can *do* biology with confidence!

# Natural Selection: Faux Finches

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Simulate the process of natural selection and explain how selection acts on phenotypic variation to drive adaptation.
- Observe how changes in the frequency of traits and alleles within a population over several generations provide evidence for evolution.

## Introduction

**Evolution** is a concept that most of us are familiar with, at least in the context that species change over time. It is a unifying theme of biology, tying together fields like microbiology, genetics, and paleontology. Evolution is an old concept – even Greek philosophers supported the notion that species changed over time. However, it was the mechanism behind evolution, **natural selection**, that explains how evolution occurs. Charles Darwin and Alfred Russell Wallace first described the process of natural selection in the 1850s. Darwin outlined four requirements necessary for natural selection to occur, including:

1. *Variation: Individuals within a population have variable observable traits (phenotypes)*
2. *Inheritance: Some of this variation is passed from parent to offspring*
3. *Overproduction: More young are born than can survive in an environment*
4. *Fitness differences: Individuals that possess traits that give them an advantage in their environment are more likely to survive and/or reproduce*

If all these conditions are present, natural selection is occurring, and the population is said to be evolving. Recall that a **population** is a group of organisms of one species that interbreed and live in the same place at the same time. Evolution is said to occur at the level of populations (Figure 1).


Figure 1. Evolution in a population of mice. Images of the mouse and falcon are attributed to Vecteezy CC-BY 4.0.

The reproductive success (i.e., number of viable offspring) of an individual relative to others in the population is known as the individual's biological **fitness**. Any *heritable trait* that increases the fitness of an individual is known as an **adaptation**. Individuals that are best adapted to their environment have higher fitness and thus produce more offspring than others in the population. In the next generation, the helpful trait will become more common (more "frequent") and, barring a change in the environment, it will continue getting more common with each generation. In this manner, the entire population evolves and becomes better adapted to the environment. Therefore, **evolution** can be defined as a change in the frequency of heritable traits in a population over time Figure 2).

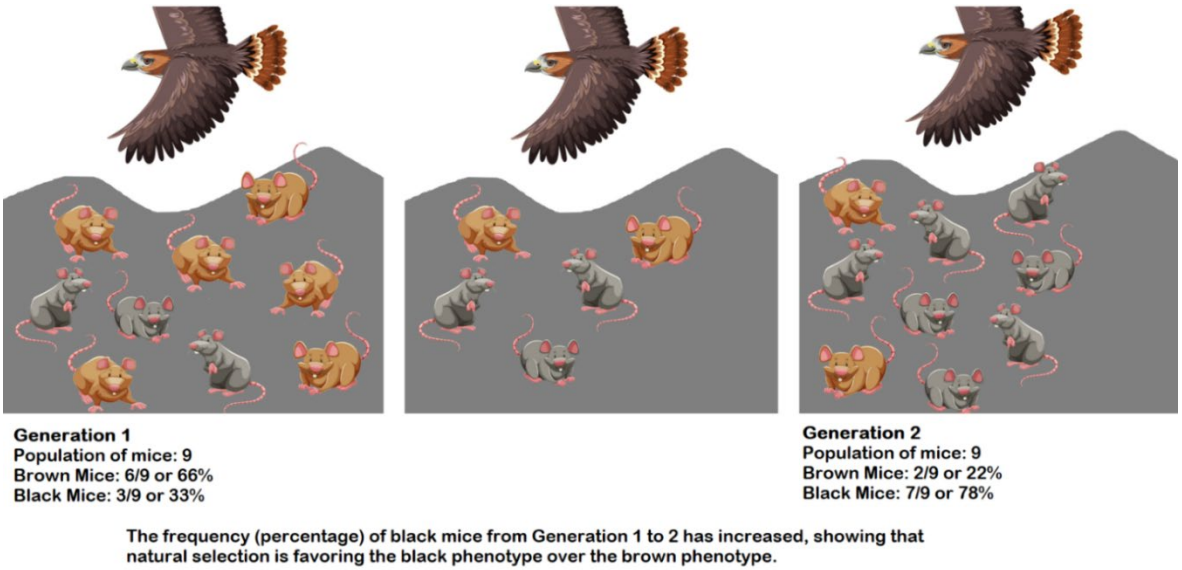



Figure 2. Change in frequency of heritable traits (fur color) in a population of mice.

Some adaptations allow organisms to exploit resources, like food. One of the best examples of evolution by natural selection are the so called "Darwin's finches" (Figure 3). Thirteen species of finch inhabit the Galapagos islands. Each finch species possesses a unique beak adaptation allowing them to specialize on a different food type. Darwin hypothesized that these birds descended from a single common ancestor. Variations in beak size within the ancestral population may have made it possible for individuals to survive by exploiting specific food types.

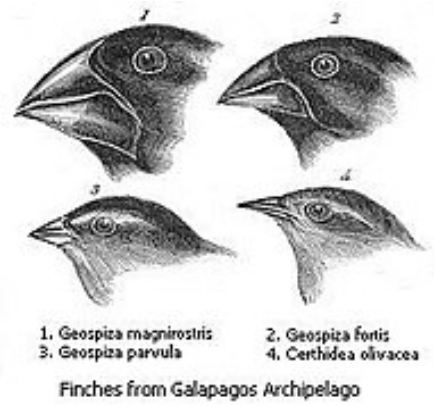



Figure 3. Darwin's Finches. Image credited to John Gould (14.Sep.1804 - 3.Feb.1881), Public domain, via Wikimedia Commons.

# Methods

In this lab, we will simulate the process of natural selection and track the frequency of certain heritable traits in predator and prey populations over several generations.

You and your fellow students will represent members of a population of a single predatory species (finches). Beans will represent a population of a prey species. Both the predator and prey populations have heritable variation. In the predator population, variation consists of different utensils that can be used to "capture" prey, starting with a fork or forceps. The prey population varies in size, shape, and color of bean: black beans, lima beans, or lentils.

Your instructor will divide the class into three groups with approximately eight students each. Each group will receive a tray in which to place their prey items; this represents the "environment."

The simulation will consist of three rounds of prey capture, in which the predators will attempt to capture as many prey as they can in 1 minute. The number of prey captured will be used to determine each predator's fitness (reproductive success), which will determine the makeup of the next generation of predators. Before beginning the simulation, and following each round, each group will calculate the frequency of each trait in their predator and prey populations.

# Round 1

1. Setting Up the Simulation: Each member of the group will be given a utensil for capturing prey. Start by giving half of the students in your group forks and half forceps. This means that your beak phenotypes are split evenly to start:
   - Fork phenotypic frequency – 50% or 0.5
   - Forceps phenotypic frequency – 50% or 0.5

2. Record these phenotypic frequencies under Generation 0 in Table 1.
3. Collect 100 beans of each type (300 total), and put them each in their own container, bringing them back to your station. Each bean will represent the same species of prey, with three phenotypes.
4. In the initial prey population, there are 100 of each type of bean present. Therefore, the frequency for each phenotype of prey would be:
    - Lima beans – 100/300 = 0.33
    - Black beans – 100/300 = 0.33
    - Lentils – 100/300 = 0.33
5. Record these prey phenotypic frequencies under Generation 0 in Table 4.
6. Obtain a rubber tray, and scatter all of your beans <u>evenly</u> throughout it.

- Before getting started, as a group, <u>hypothesize which predators and which prey you think will be the most successful and explain why</u>.

7. Before you begin, follow these guidelines:
    - To perform the simulation, each group member will attempt to capture as many prey items (beans) as possible in 60 seconds, using the following rules:
    - You can only use your utensil to capture prey.
    - Each student will be given a cup to collect their captured prey, and the cup <u>must be kept on the table at all times</u>.
    - <u>You can only capture one prey item at a time</u> and each prey item must be successfully placed in your cup to be considered a successful capture. <u>Start a timer for 60 seconds and perform the first round of prey capture!</u>
8. When the 60 seconds of prey capture is complete, all of the predators with forks will add up how many prey they captured in total and record in Table 1 under Round 1. Likewise, the predators with forceps will add up how many prey they captured in total and record in Table 1 under Round 1. Then, calculate the total of all prey captured for that round in the same column.
9. The success of the predators will be determined by how many prey were captured by that beak phenotype. (See Table 1 for Prey Captured).
    - *For example, if the Forks caught a combined 75 prey out of 120 total captured in Round 1, this would be 62.5% or 0.625.*

    This will represent the success of a predator's phenotype, and therefore their fitness. We will use it to reflect a change in phenotypic frequency within the predator's population. Calculate the phenotypic frequencies for both Forks and Forceps under Generation 1 in Table 2.
10. Based on the predator's phenotypic frequencies you calculated for Round 1, redistribute the forks and forceps among the group.
    - *For example, if the Fork's phenotypic frequency for Round 1 was 0.625 or 62%, then five people would now receive forks. (8 group members x 0.625 = 5 forks). The remaining three group members would receive forceps. You may need to*

*round your numbers. Just try and approximate the percentages as closely as possible.*

11. Next, determine the number of prey items of each type that remain on the table. The easiest way to do this is to tally the total number of each type of prey that were captured and subtract it from the number you started with.
    - *For example, if 65 black beans were captured, then there should be 35 remaining on the table (100 black beans in Generation 0 – 65 captured = 35 remaining).*
12. Record the prey for each phenotype surviving in Round 1 on Table 3.
13. Each prey will produce offspring for the next generation. Multiply each surviving prey type by two and record the appropriate amount on Table 3 under Round 1. This is what we will start the next round with.
    - *If as in our previous example, 35 black beans survived Round 1, then have offspring: 35 survivors x 2 = 70.*
14. Add the offspring of each prey type to the tray.
    - *For example, if 35 black beans survived – add 70 more to have 105 total black beans on the tray. You would do the same for the limas and lentils.*
15. Now calculate the new prey phenotype frequencies under Generation 1 in Table 4.
    - *For example, if the offspring for the black beans in Round 1 were 105, and there were a total of 225 offspring produced across all beans, then the frequency of black beans for Generation 1 would be: 105/225 = 0.466 or 46%.*

# Round 2

1. Forks and forceps should be redistributed as in the instructions in Round 1, based on the beak phenotypic frequencies in Table 2 (Generation 1). Likewise, prey should be scattered randomly on the rubber tray, based on the prey offspring for Round 1 in Table 3.
2. Repeat the simulation as Round 1. Use the same rules and time limit (60 seconds).
3. Record your results as you did before, in Round 1:
    a. Prey captured for both beak phenotypes in Table 1.
    b. Beak phenotypic frequencies calculated in Table 2.
    c. Prey survivors calculated in Table 3.
    d. Prey phenotypic frequencies calculated in Table 4.
4. Redistribute beak types for the predators, as well as prey offspring on the tray, according to your results.

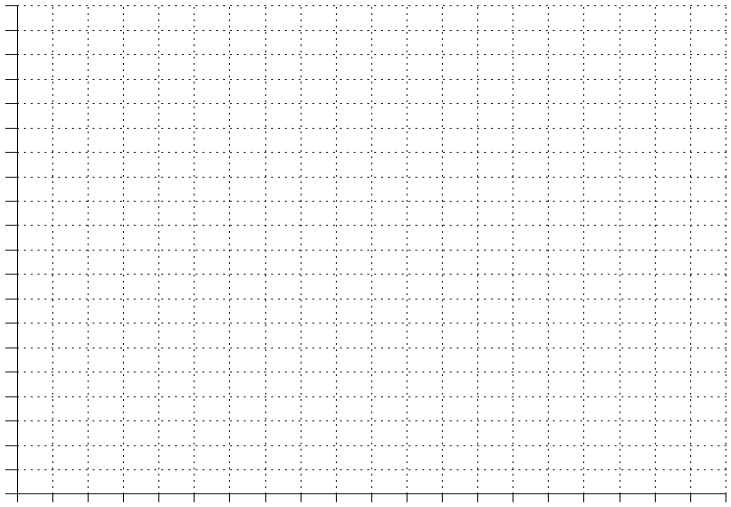
# Round 3

1. Once the forks and forceps are redistributed based on your results from Round 2, and the prey replenished on the tray, repeat the same steps as in the previous round.
2. Record your results as you did before, as in Rounds 1 and 2:
    a. Prey captured for both beak phenotypes in Table 1.
    b. Beak phenotypic frequencies calculated in Table 2.
    c. Prey survivors calculated in Table 3.

d. Prey phenotypic frequencies calculated in Table 4.

3. Each student should create two line graphs showing their observed changes in beak phenotypic frequencies (Graph 1) and prey phenotypic frequencies (Graph 2) from Generation 0 to Generation 3. For each graph, *Generation* is the independent variable on the x axis and *Phenotypic frequency* is the dependent variable on the y axis. Graph a separate line (set of connected points) for each phenotype and include a key to distinguish the lines.

Graph 1: Beak Phenotypic Frequencies



Graph 2: Prey Phenotypic Frequencies

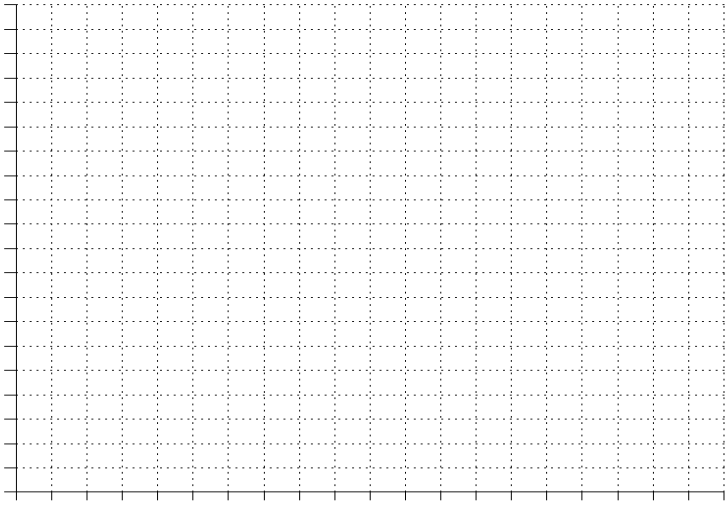


Table 1. Prey Captured. Input the number of prey captured each round.

| | **Round 1** | **Round 2** | **Round 3** |
|---|---|---|---|
| Forks | | | |
| Forceps | | | |
| Total Captured | | | |

Table 2. Beak Phenotypic Frequencies. Calculate the phenotypic frequencies of predators using their success at catching prey (data in Table 1) for each round.

| | **Generation 0** | **Generation 1** | **Generation 2** | **Generation 3** |
|---|---|---|---|---|
| Forks | | | | |
| Forceps | | | | |
| Total | 1.00 | 1.00 | 1.00 | 1.00 |

Table 3. Prey Offspring. Calculate the offspring of the surviving prey for each round (multiply the survivors by two, then total all of the offspring).

| | **Round 1** | | **Round 2** | | **Round 3** | |
|---|---|---|---|---|---|---|
| | **Survivors** | **Offspring (x2)** | **Survivors** | **Offspring (x2)** | **Survivors** | **Offspring (x2)** |
| Lima | | | | | | |
| Black Bean | | | | | | |
| Lentil | | | | | | |
| **Total** | | | | | | |

Table 4. Prey Phenotypic Frequencies. Use the offspring in Table 3 to calculate the phenotypic frequency for each generation. For example if there were 150 lima offspring out of a total of 300, that would be a frequency of 0.50 (150/300).

| | **Generation 0** | **Generation 1** | **Generation 2** | **Generation 3** |
|---|---|---|---|---|
| Lima | 0.33 | | | |
| Black Bean | 0.33 | | | |
| Lentil | 0.33 | | | |
| Total | 1.00 | 1.00 | 1.00 | 1.00 |

# Round 4 (Instructor's Choice)

1. Start a fresh round, with 100 of each bean type. However, this time, you are to be adding a new beak phenotype to the exercise. This will simulate a mutation, or the addition of a brand-new allele into the population of predators. Two of your group members will use spoons, while the remaining group members will be evenly divided based on forks and forceps.
    a. Calculate the starting break phenotypic frequencies and record under Generation 0 in Table 5.
    b. Calculate the starting prey phenotypic frequencies and record under Generation 0 in Table 6.
2. When the round is over, as before, determine the success of each predator type and record in Table 5. Use their success to calculate frequency of each beak phenotype for Generation 1, also in Table 5.
3. Record the surviving prey in Table 6, using this data to calculate the frequency of each prey phenotype under Generation 1.
4. If you have time, you could simulate another 1-2 generations with the additional beak phenotype, following the same procedure as in Rounds 2-3.

Table 5. Bonus Round 4 - Predators

| | **Generation 0** | **Prey Captured** | **Generation 1** |
|---|---|---|---|
| Forks | | | |
| Forceps | | | |
| Spoons | | | |
| Total | 1.00 | | 1.00 |

Table 6. Bonus Round 4 – Prey

| | **Prey Offspring** | | **Phenotypic Frequencies** | |
|---|---|---|---|---|
| | **Survivors** | **Offspring (x3)** | **Generation 0** | **Generation 1** |
| Lima | | | 0.33 | |
| Black Bean | | | 0.33 | |
| Lentil | | | 0.33 | |
| **Total** | | | 1.00 | 1.00 |

# Extension: Population Genetics

While Darwin and Wallace were developing their theory of evolution by natural selection, Gregor Mendel was quietly discovering that the gene is the unit of inheritance. Mendel's work was later rediscovered and incorporated into the "Modern Synthesis" of evolutionary theory and genetics, which informs our current understanding of how evolution works. Before proceeding, you may need to review some basic terms and concepts in Mendelian genetics:

- **gene**: a section of DNA that codes for a protein or trait
- **alleles:** alternate versions of a gene
- **genotype:** the combination of alleles an individual receives from its parents
    - In a diploid organism with two copies of each gene, an individual's genotype for a given gene may either be *homozygous* (two copies of the same alleles) or *heterozygous* (two different alleles).
- **phenotype**: an observable trait of an individual (i.e., the expression of its genotype)

Biologists now recognize that natural selection causes changes not only in phenotypic frequencies, but also in allele frequencies. Following Darwin's logic (see above), the alleles that code for the traits that are favored by natural selection will increase in frequency (become more common) from one generation to the next. In fact, evolution can be defined as a change in allele frequencies in a population over time.

- You will explore more detailed population genetics models of evolution in lecture. Here, we will apply an extremely simplified genetic framework to give you practice calculating allele frequencies. Referring to the simulation above, let's suppose that prey (bean) size is controlled by a single gene with two alleles, represented by the genotypes below: Lima beans (big) are homozygous for the *B* allele (genotype *BB*)
- Black beans (medium-size) are heterozygous (genotype *Bb*)
- Lentils (small) are homozygous for the *b* allele (genotype *bb*)

(*Note that dominant-recessive allele interactions do not apply in this case. Heterozygotes express an intermediate phenotype. This does not match the procedure for prey reproduction used above – just ignore that.)*

**Allele frequencies** are calculated much like phenotype frequencies above, except you must account for the fact that each diploid individual has two alleles for a given gene; therefore, the total number of alleles in a population is twice the number of individuals. The first step is to add up all the copies of each allele in a population, across all genotypes. The frequency of a specific allele is then calculated by dividing the number of copies of that allele by the total number of alleles summed across all individuals in the population.

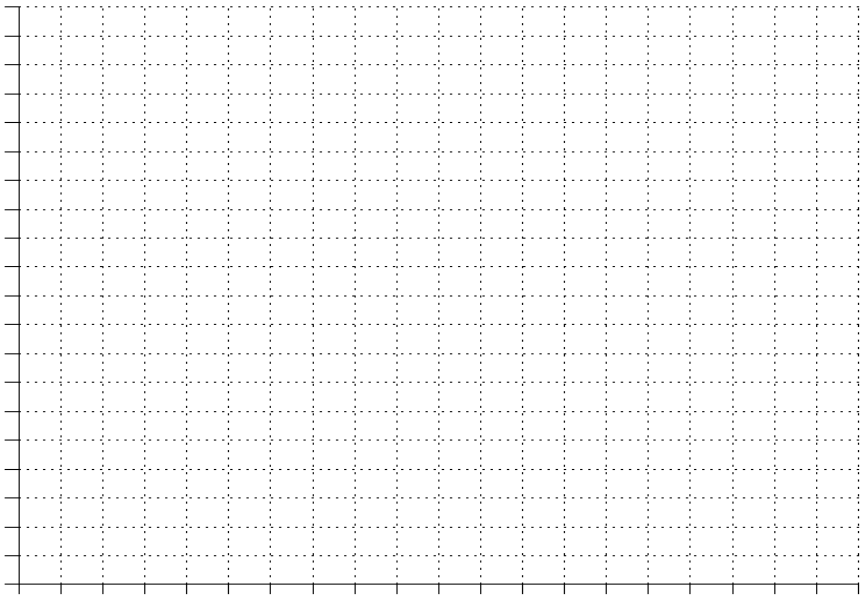
- For example, in Generation 0 above, you started with 100 lima beans, 100 black beans, and 100 lentils (= 300 beans total). Each of the 100 lima beans (genotype *BB*) has two copies of the *B* allele, representing 200 *B* alleles all together. Each of the 100 black beans (genotype *Bb*) has one copy of the *B* allele (= 100 copies total). The lentil beans (genotype *bb*) do not have any copies of the *B* allele. Adding across all individuals, there are 300 copies of the *B* allele in the population, out of 600 alleles total (300 individuals × 2 alleles each). Therefore, the frequency of the *B* allele is 300/600 = 0.5 or 50%.

# Task 1 - Calculate Allele Frequencies

1. Use the prey (bean) offspring numbers that you entered in Table 3 to calculate the underlying allele frequencies for Generations 1-3. Record your answers in Table 7. The starting allele frequencies from Generation 0 have already been filled in. "Number" refers to the total number of copies of each allele summed across all individuals, which you will use to calculate frequencies.
2. Create a line graph showing the observed changes in prey allele frequencies from Generation 0 to Generation 3. *Generation* is the independent variable on the x axis and *Allele frequency* is the dependent variable on the y axis. Graph a separate line (set of connected points) for each allele (*B, b)* and include a key to distinguish the lines.

Table 7. Prey allele frequencies observed across Generations 0-3 of the original simulation with only fork and forceps predators (Rounds 1-3).

| | **Generation 0** | | **Generation 1** | | **Generation 2** | | **Generation 3** | |
|---|---|---|---|---|---|---|---|---|
| **Allele** | **Number** | **Freq.** | **Number** | **Freq.** | **Number** | **Freq.** | **Number** | **Freq.** |
| *B* | 300 | 0.5 | | | | | | |
| *b* | 300 | 0.5 | | | | | | |
| Sum | 600 | 1.0 | | 1.0 | | 1.0 | | 1.0 |



## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Describe the changes in *phenotypic frequency* (predator beaks and prey type) that you observed between Generations 0-3. Reference your line graphs and include them with your submission. Did the simulation results support or reject your initial hypothesis? Explain what happened, using specific terminology from the lab introduction (natural selection, population, variation, fitness, etc.).

2. How did the introduction of a new beak phenotype (spoon) in Round 4 affect the process of natural selection? Did it alter which beak and/or prey phenotype was most successful? Did it result in changes to phenotypic frequencies across generations?

3. Suppose we wished to simulate a change in the environment (tray) that might influence natural selection on predator beaks and/or prey traits. Describe how you would alter the environment and predict the outcome of evolution over time.

4. Describe the change in *allele frequencies* shown in your third line graph (include the graph with your submission) and explain how the pattern is related to the change in prey *phenotype frequencies* observed above.

5. Suppose the *B* allele for big prey size is completely dominant over the recessive *b* allele for small prey size, so that any individual with at least one copy of the *B* allele (genotypes *BB* or *Bb*) is big, whereas only individuals with the *bb* genotype are small. (There is no intermediate size in this new scenario.) How do you think this new mode of inheritance of body size might alter the course of evolution compared to the change in allele frequencies described in the previous question? Explain.

# Reconstructing Phylogenies

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Apply the concept of **phylogeny** to infer patterns of evolutionary relatedness among organisms.
- Be able to construct and interpret phylogenetic trees using morphological characteristics.

## Introduction

A phylogeny (i.e., phylogenetic tree or cladogram) is a way that biologists can construct and interpret relatedness among organisms and trace lines of evolutionary descent. A phylogeny is constructed by tracing a population over time – called a **lineage** – and how each population diverges over time. If you picture a phylogeny as a tree, the base of the tree, called the **root**, represents the common ancestor to all organisms on the tree. Then, if we follow each lineage, we may see that they branch, over time, into their own separate lineages over time. Each branching point represents a time where one lineage (population) becomes two separate lineages. These branching points are called **nodes**. A branch, which includes the ancestor and all of its descendants, is called a **clade** (i.e., **monophyletic group**). If you picture the end of a branch as a "leaf," the leaf at the tip of the branch represents a distinct **taxon**, or group of organisms (Figure 1).

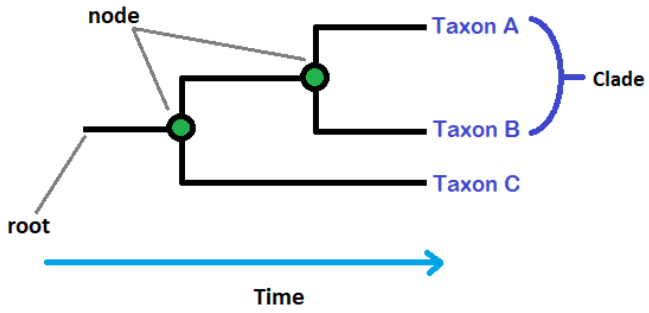



Figure 1. We can see that Taxa A, B, and C all share common ancestry by the root of the tree. However, Taxon A shares a more recent common ancestor with B than it does with C. Another way to look at it is that Taxa A & B shared a common lineage before recently branching into two separate lineages. Therefore, Taxa A & B form their own clade.

Common ancestry among organisms is revealed to biologists using shared characteristics, such as morphology, developmental patterns, or molecular similarities such as gene or protein sequences. Using anatomical or physical features, like wings, to determine relationships is the study of morphology. For example, we can tell that humans are closely related to other vertebrates, such as humans, dogs, birds, and whales, by comparing the anatomy of their bones (Figure 2).

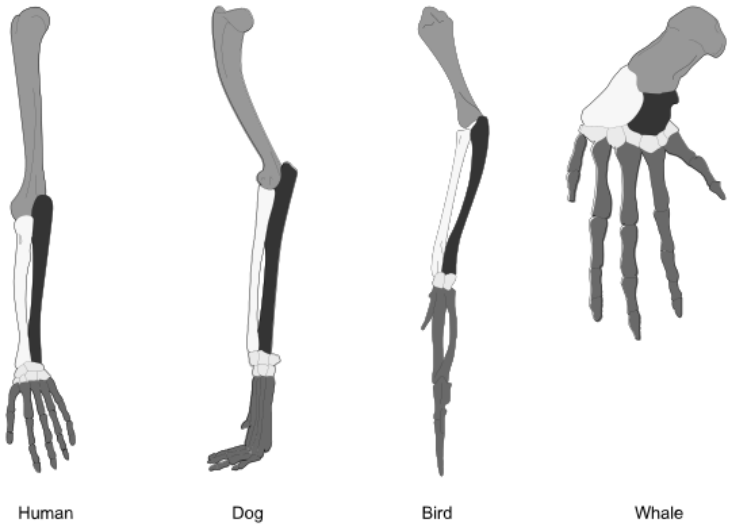



Figure 2. A comparison of the arm bone anatomy among vertebrates.[2]

The similarity in arm bones shown in Figure 2 is an example of **homology** (i.e., a *homologous* trait), which refers to a trait is shared by members of multiple taxa because it was inherited from a common ancestor; in this case, the ancestor of all terrestrial vertebrates (back-boned animals that live on land) had those same arm bones, although the shapes of the bones have since been modified for different uses.

Note that homology is not the only source of similarity across taxa. **Homoplasy** refers to traits that are shared for reasons other than common ancestry. Homoplasy is often caused by **convergent evolution**, which occurs when natural selection favors similar solutions to similar environmental pressures in distantly related taxa. For example, the streamlined bodies of dolphins (marine mammals), ichthyosaurs (extinct marine reptiles), and sharks (fish) evolved separately and independently to help those taxa swim more efficiently.

Figure 3. The presence of scales is a homology among reptiles. That is, the ancestral reptile possessed scales, so it is a morphological trait passed down to all of its descendants. However, some reptiles have secondarily modified this trait – as in the bird, which has modified them into structures known as *feathers*.[3,4]

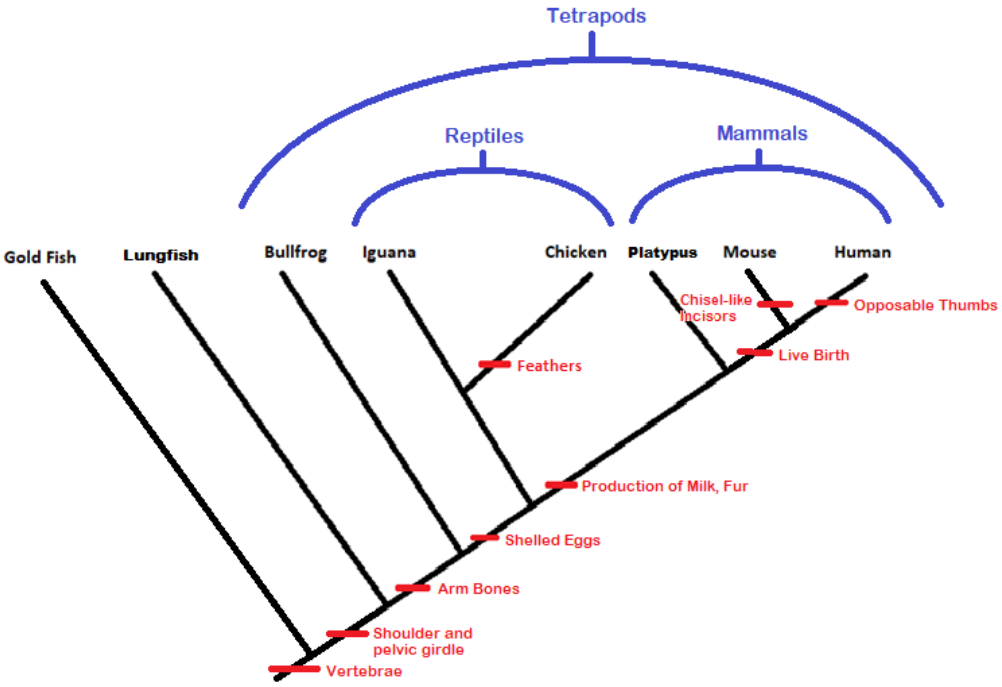



Figure 4: A phylogeny of vertebrates, which includes evolutionary traits marked at key branching points, or nodes. Some lineages are grouped together into clades, such as reptiles and mammals.

The tree in Figure 4 above mainly focuses on the group of vertebrates known as tetrapods, which includes all animals that have (at least ancestrally) four limbs. Most of the animals in this tree (except the gold fish and lungfish) are tetrapods. Note the origin of arm bones marked on the tree at the common ancestor of all tetrapods, before the various descendant groups diversified.

You may also notice that in Figure 4, a number of the taxa or clades have unique morphological characteristics that are not shared among all of the organisms of the tree. For example, the clade that includes the mammals has the unique character of fur. These traits – arm bones and fur – are examples of *synapomorphies*. A synapomorphy is a shared *derived* character, or one that is found in two or more taxa and was present in their most recent common ancestor but was modified from the ancestral character state found in more distant ancestors. Biologists use synapomorphies to distinguish clades or monophyletic groups. Arm bones is a synapomorphy that distinguishes tetrapods from other vertebrates such as fish. Fur is a synapomorphy that distinguishes mammals from other tetrapods such as reptiles. Note that synapomorphies must be homologies that reflect common ancestry; similarities resulting from convergent evolution (homoplasy) are not useful for reconstructing phylogenies and can actually be misleading.

In addition, the lineage or taxon most closely related to our group of interest is called the sister group (or sister clade). A sister group shares a recent common ancestor with the group of interest. You could say that they are the closest relatives of that group. For example, lungfish are the sister group to the clade known as tetrapods.

When forming phylogenies, ancestral character states (found in distant ancestors) are distinguished from derived character states (modified in descendants; may be used as synapomorphies) by the principle of outgroup comparison. An outgroup is a group that is distantly related to the group under study (in this case, tetrapods), but lacks a trait found in the most recent common ancestor of all other taxa on the tree. The outgroup may be thought of as "the next branch out" on the evolutionary tree or cladogram. Thus, goldfish could serve as an outgroup for the study of phylogenetic relationships within the clade known as Tetrapods, as they lack arm bones.

*Note that all phylogenetic trees represent hypotheses that are estimated from the best available data, which may include morphology, developmental patterns, the fossil record, and/or molecular similarities (e.g., DNA sequences). Hypothesized phylogenies can then be tested using independent data sources.*

- What is a synapomorphy for chickens?

- What is a synapomorphy that unites reptiles and mammals into a distinct clade?

- If we were to recognize a clade which included reptiles and mammals (called Amniotes), what taxon would be the outgroup?

# Methods

*Specifying the outgroup* - Using outgroups to form phylogenetic trees is called "outgroup analysis." As mentioned above, outgroups can be used to determine which character traits are ancestral for your group of organisms, and which are derived (which makes them useful as indicators of relationship). For example, suppose you were reconstructing a phylogeny of a group of organisms, where some of the organisms have teeth and others don't. If your outgroup has teeth, we can infer that being toothless would be a derived characteristic and an indicator of a close relationship. Does that make sense?

*Generating a data matrix* – You will be using variation among organisms to generate a hypothesis of how they are related. As described above, you will need to choose characters and determine which species exhibit which character states. For example, you may have the character "claws" and the character states "present" or "absent." Tabulating this type of information for all of your characters would give you a data matrix like the one below.

Table 1. Data Matrix

| Taxon/Character | Claws | Skull Length | Teeth | Etc. |
|---|---|---|---|---|
| Outgroup | absent | short | Present | |
| Species 1 | absent | long | Present | |
| Species 2 | present | long | Present | |
| Species 3 | present | long | Absent | |
| Species 4 | present | long | Absent | |
| Etc. | Etc. | Etc. | Etc. | |

As an example, we will examine egg-laying mammals, such as the platypus. Egg-laying among mammals is an ancestral feature in mammals (shared with many other vertebrates such as birds, reptiles, and the ancestors of live-birthing mammals - see Figure 2). It is not a feature that would indicate modern egg-laying mammals, like the platypus, have a unique ancestor in which it evolved.

Table 2. Note that, in the matrix below, a "1" indicates the presence of the character, whereas a "0" indicates an alternate state or absence of the character.

| Character | Outgroup (Chicken) | Platypus | Mouse | Human |
|---|---|---|---|---|
| Milk Production | 0 | 1 | 1 | 1 |

The distribution of characters in the table above suggests that platypus, mice, and humans are more closely related to each other than any are to the chicken. Why? Because they share a feature that evolved fairly recently (i.e., it is not in the outgroup). No single character is going to give you all the information you need to construct a phylogeny, and some characters can give contradictory information. In phylogenetic reconstruction, we attempt to find the tree that best explains the data – i.e., requires the fewest number of independent evolutions of shared features. For example, we may infer that the chicken and platypus are more closely related to each other because both lay eggs. But that would require that platypus evolved milk production independently from the mouse and human, the other two mammals. It takes fewer evolutionary steps to explain that platypus, mice, and humans are each other's closest relatives. Making inferences in this way is called **parsimony**.

- To illustrate the concept of parsimony in constructing phylogenetic trees, consider the following three possible phylogenetic trees for 4 species of butterfly (Figure 5). Which tree is the most parsimonious? A, B, or C? How many evolutionary changes are present in each cladogram?

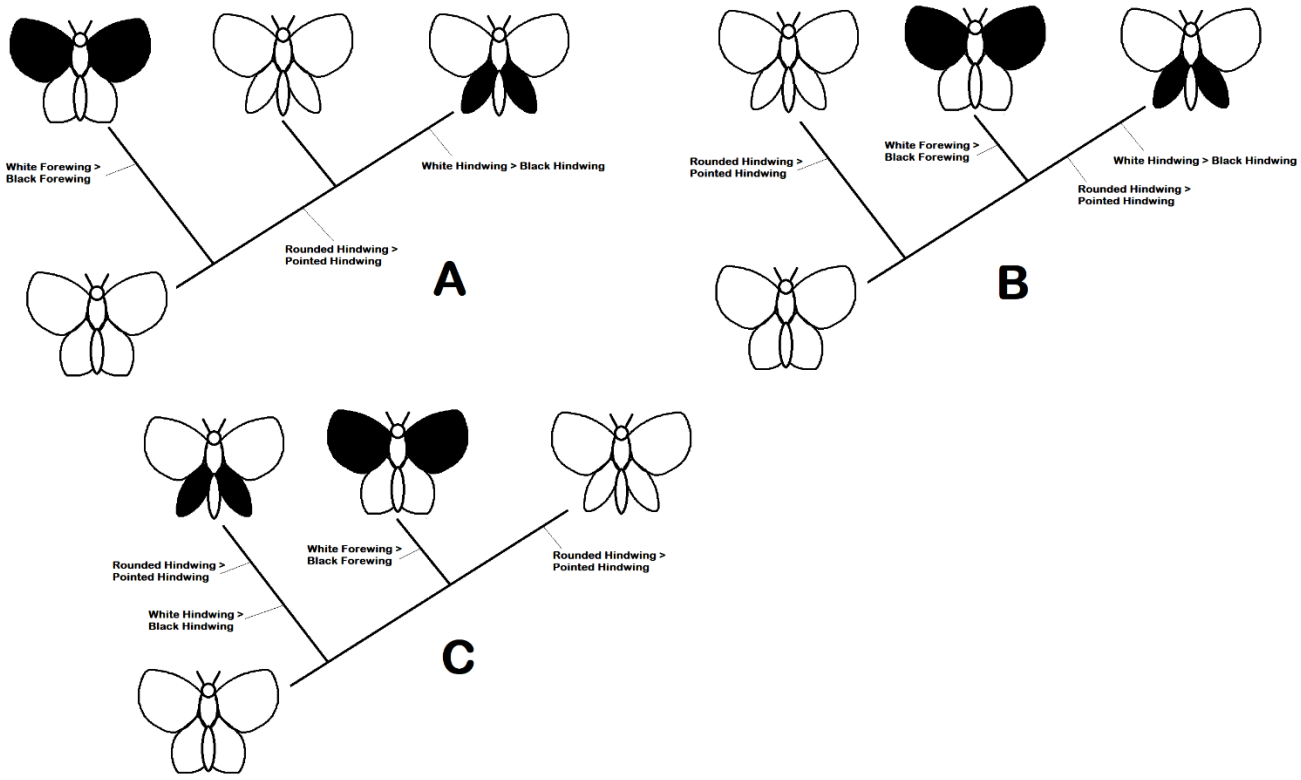



Figure 5. Hypothetical phylogenetic trees for butterflies.

- Now let's expand the data matrix to illustrate this reasoning.

Table 3. Note that, in this data matrix, the chicken acts as the outgroup, as it lacks not only the ancestral characteristic that unites mammals (milk production) but also the derived characters**.**

| Character | Milk Production | Fur | Live Birth | Chisel-like Incisors | Opposable Thumbs |
|---|---|---|---|---|---|
| Chicken (Outgroup) | 0 | 0 | 0 | 0 | 0 |
| Platypus | 1 | 1 | 0 | 0 | 0 |
| Mouse | 1 | 1 | 1 | 1 | 0 |
| Human | 1 | 1 | 1 | 0 | 1 |

- You will propose a hypothetical phylogenetic tree, called a cladogram, based on a set of characters you have chosen. For reasons discussed above, the shortest cladogram (the one requiring the fewest number of evolutionary steps) is preferred.

Now that we have created sample data matrix using morphological information, we can use this information to create a phylogenetic tree. As we do so, remember that ancestral similarity (symplesiomorphy) is not an indication of close relationship, and that we can distinguish ancestral similarity from derived similarity (synapomorphy) using outgroup analysis. In outgroup analysis, the trait found in the outgroup is determined to be the state found in the common ancestor of the group under study. Therefore, if two species share an ancestral similarity, the

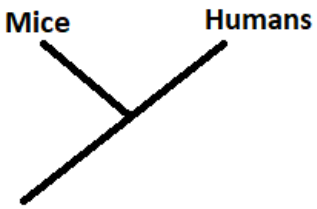
shared trait was probably inherited from a common ancestor, rather than indicating close relatedness.

- We can construct a phylogeny by hand if we cluster together the species that share the greatest number of derived features (indicated by a "1" in Table 3). Let's make a matrix of shared derived characters (synapomorphies) for our group under study, mammals.

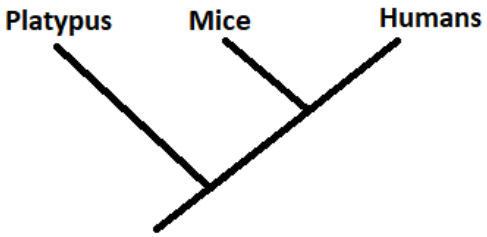
Table 4. Matrix of shared derived features of vertebrates

| | Chicken | Platypus | Mouse | Human |
|---|---|---|---|---|
| Chicken | | 0 | 0 | 0 |
| Platypus | | | 2 | 2 |
| Mouse | | | | 3 |
| Human | | | | |

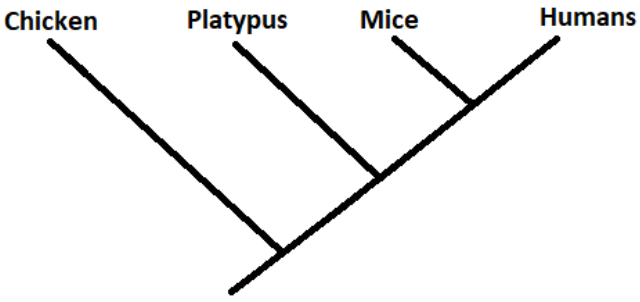
- If we link the two species that have the greatest number of derived features (Humans and Mice), we get:




- Platypus share two derived traits with both humans and mice so we add them next. The outgroup is the last one added:




- The outgroup is the last one added:



# Task 1 – Phylogeny of Canimalcules

As the first biologist to arrive at the recently discovered island of Camin, you are excited to investigate its biological wonders. Arriving at an inland valley, you are delighted to find delightful animals cavorting along the valley floor (Figure 6). You quickly make a collection of species A, B, C, and D. Fascinated by these small animals, called Caminalcules, you decide to investigate their evolution. You begin, quite reasonably, by recalling that in order to reconstruct their phylogeny, you need to undertake the following steps:

1. Select an outgroup.
2. Generate a data matrix.
3. Find the shortest tree.

With that in mind, we can work through the steps to generate a tree:

- *Selection of an outgroup* - A Caminalcule found on an adjacent island is thought to represent the ancestral form, and will serve as your **outgroup**. This Caminalcule is labeled **OG**.

- *Generation of a data matrix* - Examine the Caminalcules carefully (Figure 6). What characters vary among these species? What are the character states? For this exercise, the characters and states are provided in Table 5. You will find it helpful to refer to Figure 6 (below) when referring to anatomical features of the Caminalcules. Use Table 6 below to compile your data. For convenience, code the character states with numbers and assign the number 0 to the state found in the outgroup and 1 for the different state found in the ingroup. If there are multiple derived states in the ingroup you can give them different numbers (e.g. 1 vs. 2 vs. 3 etc.)

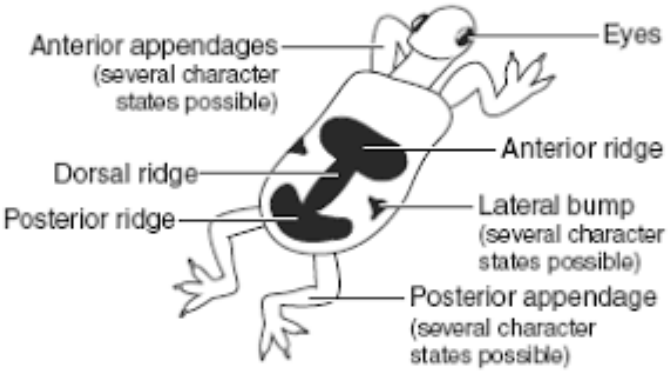



Figure 6. Anatomy of a Representative Canimalcule.

Table 5. Caminalcule characters and states.

| Character | State 0 | State 1 |
|---|---|---|
| Posterior Ridge (PosRid) | Absent | Present |
| Anterior Ridge (AntRid) | Absent | Present |
| Posterior Appendages (PosApp) | Absent | Present |
| Dorsal Ridge (DorRid) | Absent | Present |
| Lateral Bump (LatBmp) | Circular | Triangular |

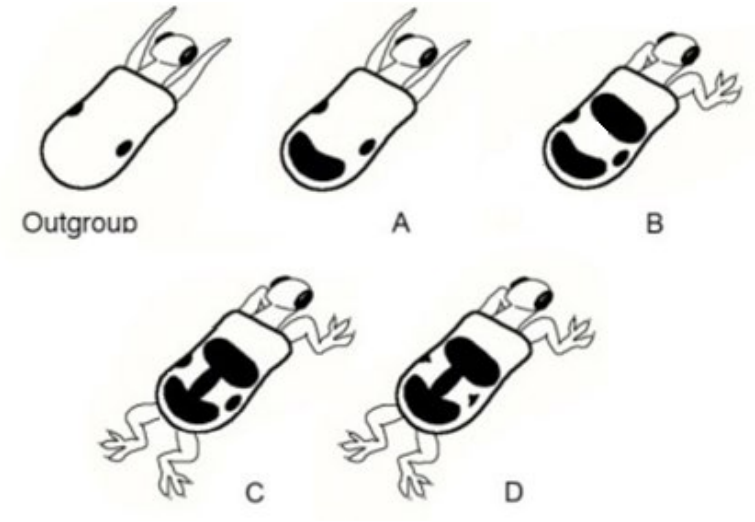



Figure 7. Different Caminalcules.

Table 6. Data Matrix for Caminalcules.

| Taxon/Char | PosRid | AntRid | PosApp | DorRid | LatBmp |
|---|---|---|---|---|---|
| Outgroup | | | | | |
| A | | | | | |
| B | | | | | |
| C | | | | | |
| D | | | | | |

Note that the character states have been abbreviated. For the Caminalcules, we have chosen an outgroup for you, Species OG, to represent the ancestral set of traits for your ingroup (species A, B, C, and D). For all the traits you investigate it will have "State 0". The other species will have State 0 for some traits (indicating that they retained the ancestral trait found in the outgroup) or state 1 (a derived trait not found in the outgroup).

- Now, construct a matrix in Table 7 using the data from Table 6. Remember that this matrix is based on shared derived traits (synapomorphies), so you need to enter the total number of traits in which the two species share derived character states (i.e., coded with a "1").

Table 7. Matrix of shared derived features of Caminalcules.

| | OG | A | B | C | D |
|---|---|---|---|---|---|
| OG | | | | | |
| A | | | | | |
| B | | | | | |
| C | | | | | |
| D | | | | | |

- Creating a cladogram - Now cluster your Caminalcule species into a tree using the matrix above.
  1. Start by finding the species that have the greatest number of shared derived features and join them. This amounts to making the hypothesis that they are each other's' closest relatives. Look back at the mammal example if you need to.
  2. Then find the species with the next greatest number of shared derived features
  3. If neither taxon belongs to a previously constructed group, place the new pair as nearest relatives.
  4. If either member or both are part of a previous group, join the two groups or the previously unplaced taxon within the group.
  5. Continue in this way until all of the taxa have been placed.
  6. The last group that you should add is the outgroup.
  7. Draw out the whole cladogram. Create your tree in the space below.
  8. On your cladogram, mark and label a synapomorphy that distinguishes each clade. See Figure 4 (vertebrates) for an example.

# Task 2 – Phylogeny of Fossil Sharks

You will be provided with four (4) fossil shark teeth, and an outgroup species, in which their phylogenetic relationships are unknown. Follow the steps of the hypothetical caminalcule exercise that you have just completed to derive the most parsimonious hypothesis of phylogenetic relationship. In this exercise, there are five characters that are variable among the ingroup species. You are provided with a list of characters and their character states (Table 8), but you must create a data matrix (Table 9), a matrix of shared derived characters (Table 10), and finally, a labeled cladogram with synapomorphies included.

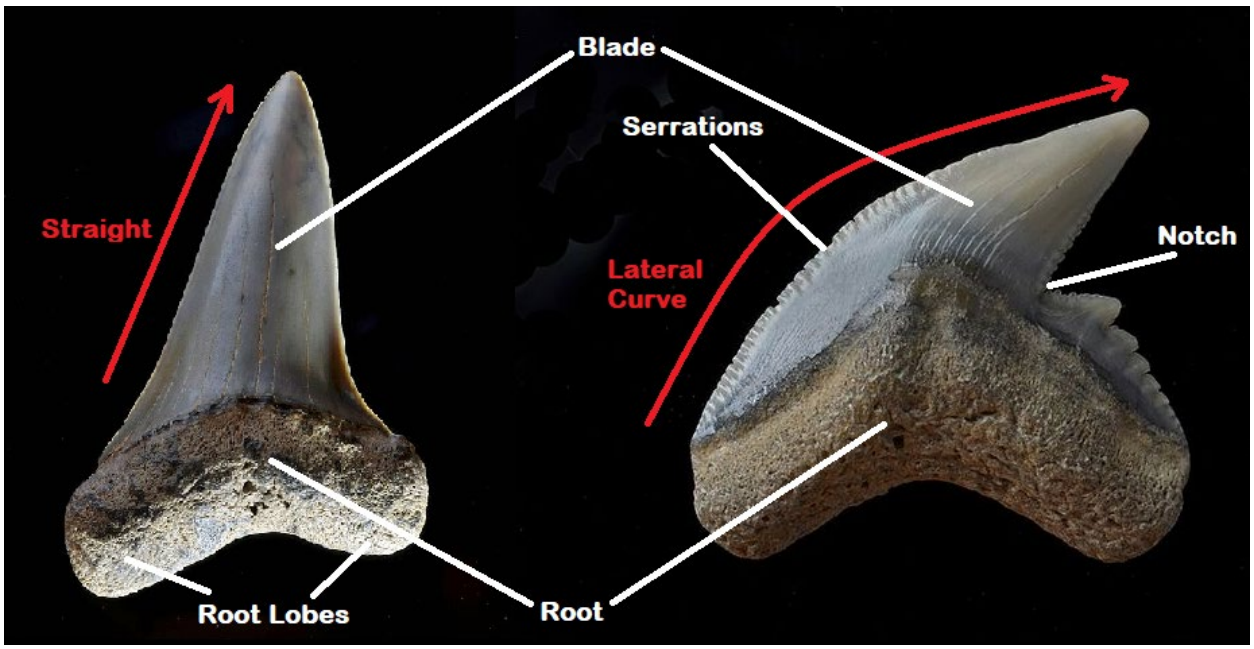



Figure 8. Terminology associated with shark tooth morphology.[5]

Table 8. Fossil Shark characters and states.

| Character | State 0 | State 1 |
|---|---|---|
| Presence of blade (BL) | Absent | Present |
| Blade shape (BLSHP) | Laterally Curved | Straight |
| Presence of serrations (SER) | Absent | Present |
| Serration Type (SERTYP) | Fine | Coarse |
| Notch in blade of tooth (NOTCH | Extend past blade | Meet edge of blade |

Table 9. Data Matrix for Shark Fossils

| Taxon/Char | BL | BLSHP | SER | SERTYP | NOTCH |
|---|---|---|---|---|---|
| Stingray | | | | | |
| Lemon Shark | | | | | |
| Whaler Shark | | | | | |
| Snaggletooth Shark | | | | | |
| Tiger Shark | | | | | |

Table 10. Matrix of shared derived features of Shark Fossils.

| | Stingray | Lemon | Whaler | Snaggletooth | Tiger |
|---|---|---|---|---|---|
| Stingray | | | | | |
| Lemon Shark | | | | | |
| Whaler Shark | | | | | |
| Snaggletooth Shark | | | | | |
| Tiger Shark | | | | | |

Sketch your fossil shark phylogeny here. Label a synapomorphy for each clade.

## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Based on the phylogeny you constructed for Caminalcules, is species C more closely related to species B or to species D? How can you tell?

2. If Caminalcule species A and B were grouped together in a named taxon (e.g., family Nodorsalridgae), would that taxon represent a clade (i.e., monophyletic group)? Explain.

3. Based on the phylogeny you constructed for the fossil sharks, are serrations (SER) ancestral or derived for the Tiger Shark? Explain.

4. Which lineage in your shark phylogeny is a sister group to tiger sharks? How can you tell?

5. If RNA sequencing suggested that tiger sharks were most closely related to lemon sharks, how would that change our interpretation of tooth morphology, such as tooth shape and serration? Use specific terms from the lab introduction, such as homology vs. homoplasy.

# References


1. Lemke, Hans, and Jeffrey Jensen. 2012. Exploring Systematics and Phylogenetic Reconstruction Using Biological Models. Proceedings of the Association for Biology Laboratory Education 33: 123-144.
2. Image sourced from Volkov Vladislav Petrovich, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons
3. Modified from Haplochromis. Leigh, Bedford, Dmitry, Bogdanov, Kevmin, Nobu, Tamura, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons
4. Image Sourced from Reptilia, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via Wikimedia Commons
5. Image modified from dw_ross, CC BY 2.0 <https://creativecommons.org/licenses/by/2.0>, via Wikimedia Commons

# Microscope Use and Safety

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Identify the parts of a compound light microscope and a dissecting (stereoscopic) microscope and explain their functions.
- Properly carry, maintain, and operate compound and dissecting microscopes.
- Use compound and dissecting microscopes to examine biological specimens.
- Prepare a wet mount.

## The Compound Light Microscope

Microscopes are vital tools that allow modern biologists to view organisms, cells, and other structures that are too small to see with the naked eye. A **compound light microscope** utilizes a light source and two sets of lenses to **magnify** or enlarge specimens for observation. It also enhances **resolution**, which allows the viewer to distinguish between two points that are close together.

### Parts of the Compound Light Microscope

This list begins at the top of the microscope and moves down the instrument. Follow along with Figure 1 and label the parts A-K of the microscope being described.

A. Attached to the head of the microscope are the **ocular lenses** (also called the eyepieces), which allow the user to view the specimen. The microscopes used in our laboratories are binocular microscopes because they have two ocular lenses (microscopes with only one are called monocular). Each ocular lens magnifies the image 10X (i.e., makes objects appear 10 times larger than their actual size).
B. The **arm** connects the binocular head to the rest of the microscope. This is one location to place a hand when carrying the microscope from one location to another (the other hand goes below the base).
C. A revolving **nosepiece** holds the objective lenses. When observing a specimen, the user can rotate this nosepiece to change the total magnification of the image.
D. Attached to the nosepiece are the **objective lenses**. Our microscopes are equipped with four different objective lenses with varying degrees of magnification. Total magnification of a specimen is calculated by multiplying the magnification of the ocular lens times the magnification of the objective lens being used. The four objective lenses of our compound microscopes are:
    i. **Scanning objective (4X)**: This lens has a red band around it and magnifies an image 4X. This lens is to be used when centering a specimen in the field of view. Total magnification when using this lens is (10X)(4X) = 40X.

ii. **Low-power objective (10X)**: This lens has a yellow band and magnifies an image 10X. Total magnification is (10X)(10X) = 100X.
iii. **High-power objective (40X)**: This lens has a blue band and magnifies the specimen 40X. Total magnification is (10X)(40X) = 400X.
iv. **Oil-immersion objective (100X)**: This lens has a silver or white band and magnifies the specimen 100X. Total magnification is (10X)(100X) = 1000X. ***This lens can only be clicked into place once immersion oil has been placed on the slide. Do not attempt this without further instruction.***

Figure 1. Label the major parts of a compound light microscope.

E. The **stage** is the location where a slide containing the specimen will be placed for viewing. The stage is equipped with a **stage clip** to ensure the slide stays in place when moving the stage to center the image in the field of view. There are two adjustment knobs attached to the stage called **stage controls**. The top control adjusts the stage forward/backwards while the bottom control moves the stage clip (and slide) left/right. These microscopes are **parcentered**, meaning the specimen will stay mostly in the center between objective lenses (though some slight adjustments may need to be made after increasing the magnification).
F. Underneath the stage is the **condenser**, which functions to collect the light beams being emitted from the light source and concentrate them on the specimen through the lens in the middle of the stage.
G. Attached to the condenser is the **iris diaphragm**. This adjustable lever allows the user to adjust the diameter of light being focused on the specimen at one time.

H. Two adjustment knobs are imbedded in one another and serve to raise or lower the stage to bring the specimen closer to or farther from the objective lens. These knobs have a set limit with how far they can adjust. *Never force knobs* to move as this could damage the microscope and/or the specimen.
    i. The **coarse adjustment knob** is the outermost knob and will move the stage up or down in large increments. ***The coarse adjustment knob is to only be used when the scanning objective (4X) is in place. Never use the coarse adjustment with the low- or high-power or oil-immersion objectives.***
    ii. The **fine adjustment knob** is the innermost knob and moves the stage in very small increments. This knob is used to refocus the specimen after the next objective lens has been clicked into place. These microscopes are **parfocal**, meaning they will remain mostly in focus once the next objective lens is clicked into place.

I. The **light source** is found in the base of the instrument and will shine light upward to illuminate the specimen on the stage.
J. Below the light source is the **light adjustment knob**. This is used to control the brightness of the light coming out of the light source. It is recommended to have this at the lowest setting when using the scanning objective. The brightness will need to be adjusted as the viewer moves up in magnification.
K. The **base** of the microscope is the bottommost section of the instrument. Place one hand under the base when carrying the microscope.

## Proper Handling of the Compound Light Microscope

The compound light microscope is a precision instrument that must be well cared for and properly maintained. Appropriate measures must be taken when transporting the microscope to ensure the user does not drop the microscope, which has the potential to cause harm to both the microscope and the individual carrying it. When holding the microscope, two hands are to be used at all times: one hand must be firmly wrapped around the neck of the microscope with the other hand placed under the base for support.

## Cleaning and Care of the Compound Light Microscope

Our microscopes are for communal use, meaning they are utilized by multiple students daily. To avoid contamination and degradation of microscope quality, the instruments must be properly cleaned after each use. We will now cover how to properly clean and store the microscopes. When the microscope is no longer needed, lower the brightness and switch the microscope off. Unplug the microscope and wind the power cable appropriately. Your instructor will demonstrate the following.

An alcohol prep pad should be used on the rubber pieces surrounding the ocular lenses. This removes particles from the eyepieces and ensures they are cleared prior to the next individual using the instrument. The glass portion of the oculars are then wiped with lens paper dampened with a special lens cleaning solution to remove any dust from the lenses.

The objective lenses are also wiped with lens paper dampened with lens cleaning solutions. ***It is imperative that only lens paper is used***, rather than a paper towel or a Kim wipe, as these are harsher materials and have the potential to scratch the lenses. When preparing to store the microscope, ***the scanning objective lens should be clicked into place.***

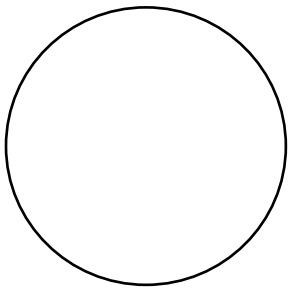
Specimen/slides are to be removed from the stage area and the stage must be free of any liquids or other materials. ***The stage must be lowered as far as it can go*** prior to storage (do not force knobs to go further than designed). After confirming the stage has been lowered and the scanning objective is in place (i.e., the **"safe position"**), cover the microscope with its dust jacket. Place the microscope in the cabinet or on the designated bench top per instructions from the lab instructor (be sure to handle the microscope appropriately).

# Task 1 – Use a Compound Microscope (letter e slide)

1. Collect the microscope, remove the dust jacket, and plug it in. Use a clean piece of lens paper to gently wipe the ocular lenses, the objective lenses, the condenser and light source to remove dust. Ensure the scanning objective (4X) is currently in place and the stage is all the way lowered, then power on the microscope.

2. While looking through the ocular lenses, adjust the distance between the occulars to match the distance between your pupils. You should see a single unified circle of light through both eyes.

3. Collect one prepared slide of the letter *e*. In the circle below, draw how this appears on the slide *without using the microscope*.



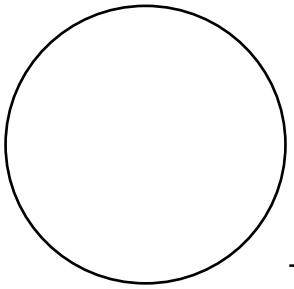
4. Place the microscope slide on the stage, securing it in place with the stage clip. Adjust the position of the slide using the stage controls to ensure the "specimen" (the letter *e*) is positioned over the condenser in the middle of the stage. While looking through the ocular lenses, raise the stage using the coarse adjustment knob, bringing the microscope slide physically closer to the objective lens. Only use the coarse adjustment knob when the scanning objective (4X) is in place. Otherwise, you could crack the slide or the objective lens.

5. Once the specimen has become visible, use the fine adjustment knob to bring it into focus. (If you do not have 20/20 vision and your eyes have different prescriptions, you should first focus using only your right eye, then adjust the left ocular to your vision by

rotating the dial on the eyepiece. Otherwise, the left ocular should be set to zero.)

6. Adjust the amount of light using the light intensity knob, the diaphragm, and/or the condenser until you can clearly see the letter *e*. Brighter is not always better! Depending on the specimen and magnification, reducing the light intensity may improve contrast.

7. In the circle below, draw how the letter *e* appears through the microscope (scanning objective).



Total Magnification: _____

8. Answer the following questions based on your observations.
    a. Does the letter *e* appear different when observed through the microscope? If so, how?

    b. While looking through the ocular lenses, use the stage controls to move the stage away from you. Which direction did this move the letter *e* in your field of view?

    c. What happens when you move the slide to the right?

9. On the microscope, rotate the nosepiece to click the low-power objective (10X) into place. Use the fine focus adjustment knob to bring the letter *e* back into focus. Do not use the coarse adjustment, or you could crack the slide or the objective lens. Adjust the lighting as needed.

10. Rotate the nosepiece to click the high-power objective (40X) into place. Use the fine adjustment knob to bring the letter *e* back into focus. Do not use the coarse adjustment, or you could crack the slide or the objective lens. Adjust the lighting as needed.

    a. Are you able to bring the entire letter *e* into clear focus at this magnification, or is the center of the image in focus while the outside edge is unclear?

11. When you are done viewing the slide, rotate the nosepiece back to the scanning objective (4X)*. Do not pass through the oil-immersion objective (100x)*, which requires proper use of immersion oil to avoid damaging the slide and microscope. Lower the stage all the way using the coarse adjustment knob. Once the microscope is in the "safe position", you may remove the slide.

## Task 2 – Determine the Depth of Field (colored threads slide)

Before we observe biological specimens, this task will demonstrate another important feature of microscopes. Depth of field refers to the thickness of an object that is in clear focus. Depth of field varies between different objectives and magnifications.

Examine a prepared slide of three colored threads mounted on top of each other. As always, begin in the safe position with the scanning objective (4X).

1. Focus up and down to determine the order of the threads from top to bottom. (The order may vary between slides.) This technique can help you discern the three-dimensional structure of objects, including biological specimens.

2. Now examine the threads using the high-power objective (40X).

3. Answer the following question based on your observations.
    a. How does the depth of field change when you switch from lower magnification (scanning objective) to higher magnification? In other words, when can all three threads be viewed in focus at the same time (= greater depth of field)?

*Your instructor may provide additional prepared slides for further practice.*

## Preparing a Wet Mount of a Biological Specimen

Wet mounts are often used to view microscopic living organisms through a compound microscope (vs. prepared slides of preserved specimens). The following steps are to be taken when performing a wet mount preparation.

1. Obtain a clean glass slide and coverslip. Note: the coverslips are very thin and made of glass. They can break easily and cut through gloves and skin. Handle these with extreme caution.

2. Use a pipet to gather a small amount of liquid from a culture containing live specimens and place a **single drop** onto the slide. (If you are viewing a specimen that is not already suspended in a liquid, place the specimen on the slide first, then add a drop of liquid.)

3. Hold the coverslip by the edges and place one edge on the slide, so you are holding the coverslip at an angle. Slowly bring the edge of the coverslip into contact with the liquid until you can visibly see the water coating that part of the coverslip. Carefully lower the coverslip down over the sample. Your instructor will demonstrate.

4. If prepared correctly, minimal air bubbles should be present under the coverslip. If any large bubbles are visible, carefully push on the coverslip with an eraser to guide them out of the coverslip.

5. The single drop of liquid placed on the slide is sufficient to keep the coverslip in place. If there is too much liquid, the coverslip will move around the slide. If too much liquid was used, gently touch the corner of a paper towel to the side of the coverslip to draw up some of the excess liquid.

6. View the wet mount slide using a compound microscope (ensure the bottom of the slide and the top of the coverslip are dry prior to placing it on the stage).

## Task 3 – Prepare a Wet Mount of *Daphnia*

1. Prepare a wet mount of a living *Daphnia* as directed above.

2. Focus on the animal with the scanning objective (4X). Keep the light intensity low so you do not overheat the animal and kill it.

3. Locate as many of the following structures as possible (**Fig. 2**): eye, brain, antennae, exoskeleton, thoracic appendages (legs), gills, mouth, beak, stomach, intestine, heart, brood chamber and eggs (embryos). You will have to alter your depth of field (using the fine adjustment knob) to see all of the structures.

4. When you are finished, clean and prepare your microscope for storage as described above.

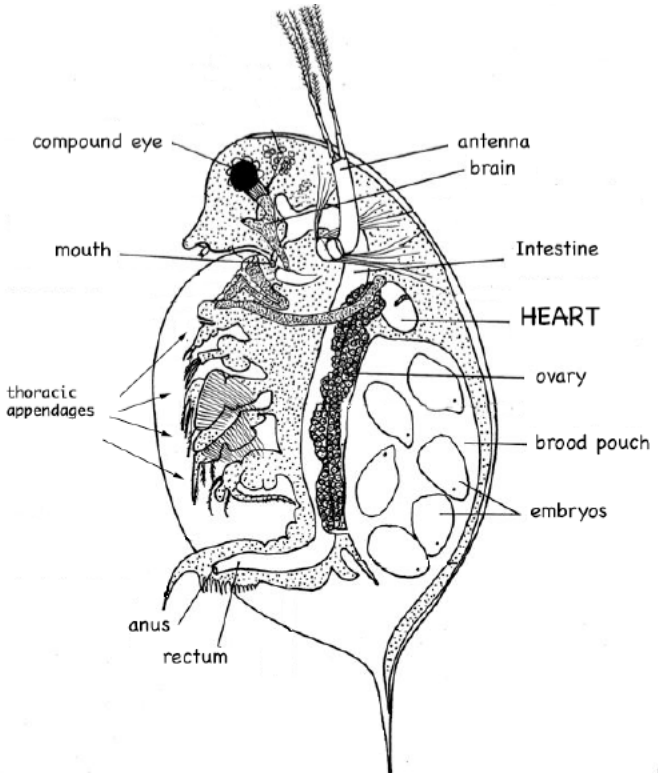



Figure 2. The crustacean *Daphnia magna.*

# The Dissecting (Stereoscopic) Microscope

Compound light microscopes produce high magnification and resolution, but their use is limited to slides with thin specimens that light can pass through. In contrast, a **dissecting (stereoscopic) microscope** can be used to view larger, opaque specimens. The dissecting microscope also provides a larger working distance between the objective lens and the specimen, allowing the user to manipulate the specimen while observing it. The binocular design of a dissecting microscope produces a three-dimensional image with a greater depth of field but lower magnification and resolution than a compound microscope.

## Parts of the Dissecting (Stereoscopic) Microscope

Dissecting microscopes have fewer parts and are easier to operate than compound microscopes. Label the major parts on Figure 3 below.

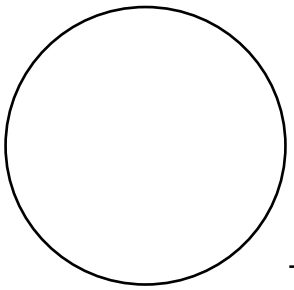
A. Next to the ocular lenses is a magnification adjustment that is used to adjust the magnification.
B. On the **arm** is a **focus adjustment** that is used to focus the image.
C. There is a single **objective lens**.
D. There are at least two light sources that can be used individually or in combination. On some models, the intensity of each light source can be adjusted.
    i. The **reflected light source(s)** shines down on the specimen from above. Some models include multiple reflected light sources that provide a variety of lighting options.
    ii. The **transmitted light source** illuminates the specimen from the **stage** below.
E. When carrying the microscope, place one hand on the **arm** and the other hand under the **base**.

Figure 3. Label the major parts of a dissecting (stereoscopic) microscope.

## Task 4 – Use a Dissecting Microscope

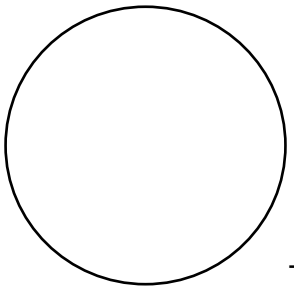
1. Collect a dissecting microscope and, if needed, clean the lenses using lens paper.

2. Use the dissecting microscope to view a specimen provided by your instructor. Start with the lowest magnification and use the focus adjustment to bring the image into focus. Adjust the reflected and transmitted light sources to improve contrast. Sketch the specimen at the lowest magnification.



Total Magnification: _____

3. While looking through the microscope, gradually increase the magnification and adjust the focus and lighting as needed. Sketch the specimen at the highest magnification.



Total Magnification: _____

4. While looking through the microscope, move the specimen toward and away from you. Move it to the left and right.
    a. How does the image seen through a dissecting microscope move when the specimen is moved?

*Your instructor may provide additional specimens for further practice.*

## Discussion/Post-Lab Questions

After completion of the lab activities, answer the following questions and submit your responses to your instructor as directed.

1. Describe the "safe position" on a compound light microscope.

2. With which objective lens(es) is the *coarse adjustment* knob to be used?

3. How many times is an image magnified with viewed through 10X ocular lenses and the high-power (40X) objective lens? Show your work.

4. Describe three differences between a compound light microscope and a dissecting (stereoscopic) microscope. Give an example of when to use each type of microscope.

# Exploring Microbial Diversity

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Practice using compound and dissecting microscopes to observe and characterize bacterial cells and colonies, respectively.
- Utilize microbiology lab techniques to culture bacteria, test for antimicrobial susceptibility, and quantify abundance and species diversity.
- Investigate patterns of microbial diversity in relation to environmental factors.

## Introduction

**Bacteria** are among the most ancient, abundant, and impactful life forms on Earth. Individually, they are tiny, unicellular organisms, but collectively they reach astounding population densities in virtually every environment. That includes the human body, where our own human cells are outnumbered by the cells of our bacterial guests. Bacteria were traditionally grouped with archaea as "prokaryotes" because their cells (= prokaryotic) share common features, most notably lacking nuclei and typical membrane-bound organelles, such as mitochondria and chloroplasts, found in the eukaryotic cells of plants, animals, fungi, and protists. Modern genetic evidence, however, supports three distinct **domains** of organisms: Bacteria, Archaea, and Eukarya. Unlike archaea and eukaryotes, bacterial cells contain a structural component called **peptidoglycan** in their cell walls. Broadly speaking, "microbes" (adj. microbial) refers to microscopic organisms, which may include bacteria, archaea, single-celled eukaryotes, and even viruses.

Bacteria are incredibly diverse. They live in a variety of habitats and serve a wide range of ecological roles, sometimes helping other species (e.g., via photosynthesis, decomposition, or nitrogen fixation) and sometimes harming them (e.g., as parasites or pathogens that cause disease). Bacterial species also vary in shape, cellular organization, and metabolism. Three basic shapes of bacterial cells are shown in Figure 1: **bacillus** (rod-shaped), **coccus** (spherical), and **spirillum** (spiral or corkscrew). Depending on the species, bacterial cells may live singly or form chains or clusters of cells. Most bacteria are **heterotrophic**, meaning they derive energy from other organisms, while others are **autotrophic**, obtaining energy from either sunlight or inorganic molecules.

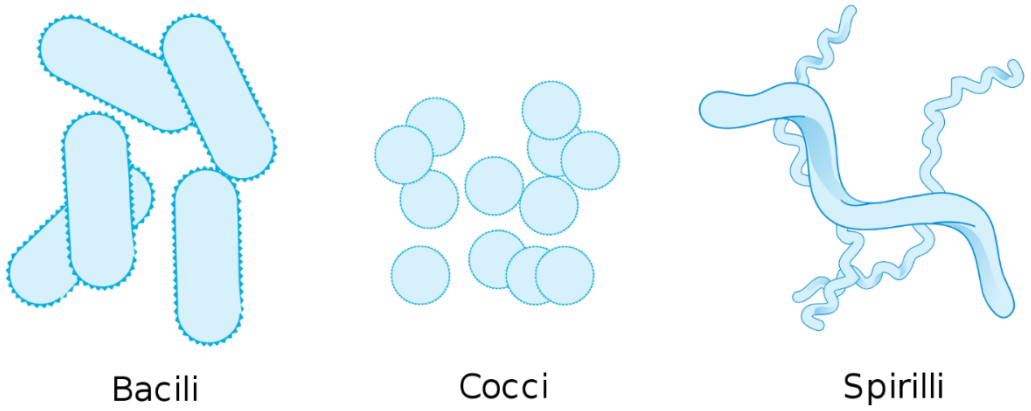



Figure 1. Three basic shapes of bacterial cells: bacilli (singular: bacillus), cocci (singular: coccus), and spirilli (singular: spirillum). This work has been released into the public domain by its author, LadyofHats.

In this two-week lab, you will explore a small slice of Earth's microbial diversity by observing and characterizing bacterial cells and colonies, testing for antimicrobial susceptibility, and investigating how environmental factors affect the distribution of bacteria.

## Week 1. How Do Environmental Factors Affect Microbial Diversity?

Biological diversity, or **biodiversity**, can be described and analyzed at multiple levels—species, genetic, ecological—and in a variety of organisms and environments. For this task, you will explore patterns of species diversity in microbial communities, particularly those bacteria that live in human-associated environments or habitats. Like all organisms, each bacterial species requires a suitable habitat with a tolerable range of environmental conditions that allow individuals to survive and reproduce (i.e., the **ecological niche** of a species). Different bacterial species vary in their resource requirements, including sources of energy and carbon, and in the habitats/niches they occupy. A bacterial **community** consists of all the individual bacterial cells belonging to all the species that co-occur in a particular location. The broader microbial community may also contain archaea, fungi, etc. in addition to bacteria.

- Think about where different types of bacteria can be found and what specific resources they require (e.g., consider factors such as temperature, moisture, energy, nutrients, species interactions, etc.). Then hypothesize which environmental conditions will favor a high **abundance** of bacteria (i.e., the total number of individuals or cells). Also hypothesize which environmental conditions will favor a high **species diversity** of bacteria (i.e., the number of different species that co-occur; a.k.a. species richness).

- Explain your rationale for each hypothesis, based on the resources and other conditions that bacteria need to survive and reproduce. (Note that patterns of abundance and diversity may or may not be correlated with one another. For example, a corn field has a high abundance but a low diversity of plant species.)

- Based on your hypotheses above, identify several locations/surfaces either inside the building or on your body/belongings where you expect to find abundant and/or diverse bacterial communities.

Your instructor will survey the class for potential locations/habitats and list them on the board. *As a class*, you will then decide on three habitats where you will compare bacterial abundance and diversity.

- Rank the three habitats from high to low in order of their relative (a) abundance and (b) species diversity of bacteria that you predict to find. Again, these **predictions** should be based on your **hypotheses** above. What environmental factors differ between these locations, and how do you expect them to affect the bacterial communities living there?

- Do you expect to culture **heterotrophic** or **autotrophic** bacteria at each location? Explain.

Use the following procedure to sample and culture bacteria from each of the three habitats selected by the class. Coordinate within your group to collect one sample (replicate) per habitat per group.

1. Obtain a sterile cotton swab and a closed petri dish containing nutrient agar.

2. Label the bottom of the dish (agar side) with your initials and the habitat.

3. Open the sterile swab and wipe the top across the surface you are sampling, rotating the swab to ensure full coverage.

4. Open the dish and drag the exposed swab over the surface of the agar in the manner demonstrated by your instructor and illustrated below (Fig. 4). Be careful not to push too hard on the swab. Do this quickly without breathing on the plate or touching it.

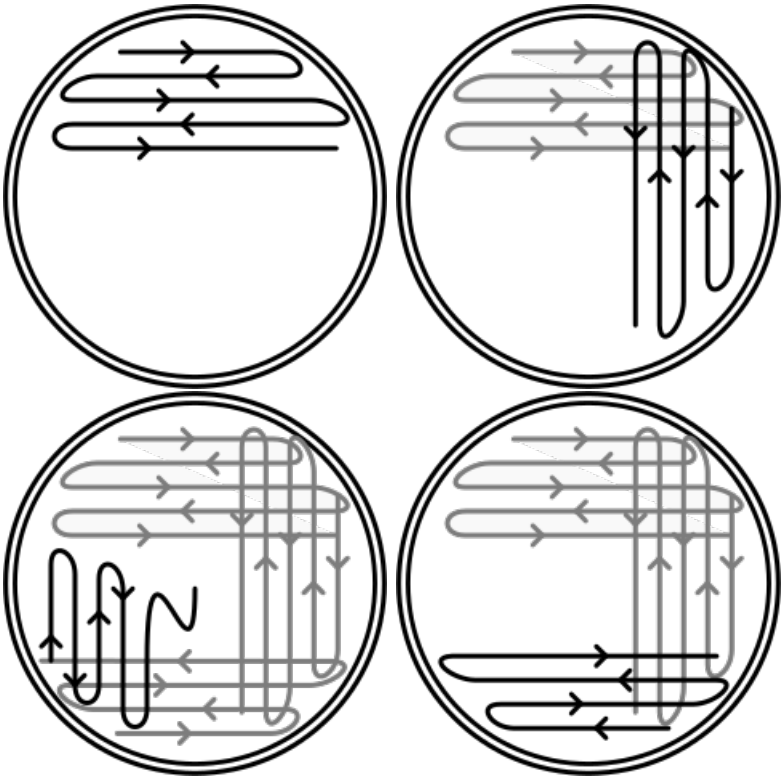


Figure 4. Bacterial streak plate technique, clockwise from top left. This work has been released into the public domain by its author, Reytan at the Wikipedia project.

5. Close the lid and tape it shut.

6. Turn the dish upside down and place it in the incubator rack.

7. Put the used swab in the biohazard container.

8. Repeat for each habitat (one agar plate per habitat per group).

9. Is a **control group** necessary? How would you set it up?

10. When you are done, clean your lab bench and wash your hands.

11. After lab, the agar plates will be transferred to an incubator at 37°C for 24-48 hours, then refrigerated until next week, when you will examine them for bacterial growth.

Answer the following questions about your study design.

- What is the **independent variable(s)**?

- What is the **dependent variable(s)**?

- What **standardized variable(s)** did you control for (hold constant)?

# Week 2. Microbial Diversity Continued

## Task 1. Observing Bacterial Cells and Gram Stain

**Gram staining** is a technique commonly used to distinguish between different types of bacteria. Gram-positive bacteria (e.g., *Staphylococcus*) have a thicker peptidoglycan cell wall layer that retains a purple dye called crystal violet. Gram-negative bacteria (e.g., *E. coli*) have a thinner peptidoglycan cell wall layer that does not retain the crystal violet dye as well. A subsequent counterstain gives Gram-negative bacterial cells a red color that contrasts with the purple Gram-positive bacterial cells under a compound light microscope (Fig. 2). Gram staining is the first of many steps/tests that can be used to identify bacterial species.

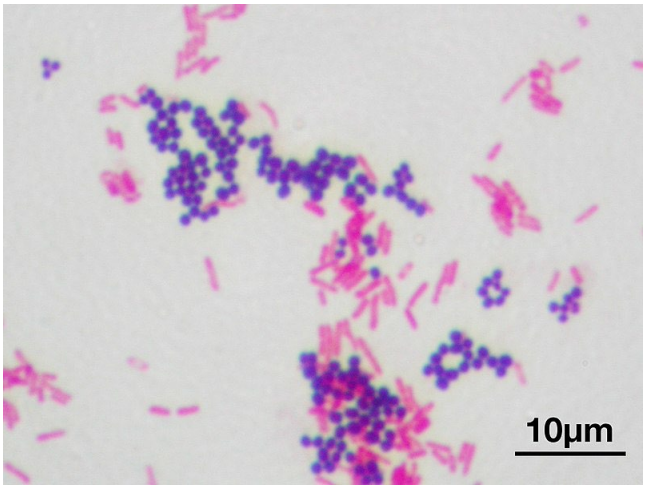



**Figure 2.** Microscopic image of a Gram stain of mixed Gram-positive cocci (*Staphylococcus aureus*, purple) and Gram-negative bacilli (*Escherichia coli* ATCC, red). Magnification:1,000. Gram stain by Y tambe is licensed under CC BY-SA 4.0.

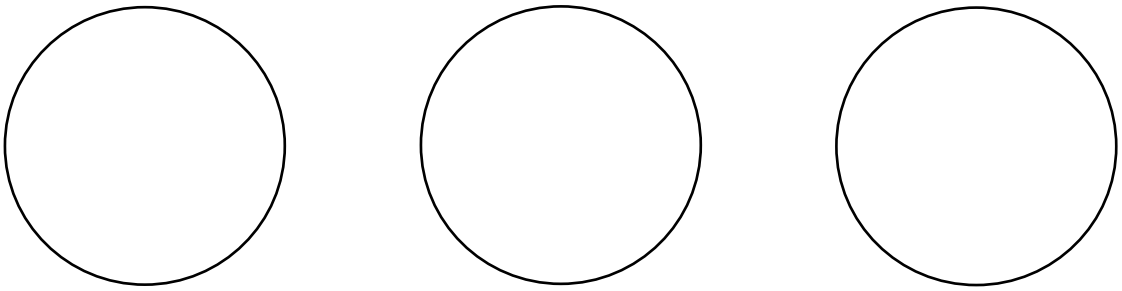
For this task, you will practice using a compound light microscope to examine bacterial cells and identify their shape and Gram stain. Because bacterial cells are so small (typically 1-10 micrometers in diameter), it helps to observe them through a high-magnification (100X) oil-immersion objective lens, which requires a special technique that you will learn in later classes. To avoid damaging the microscope and/or slide, *never use the 100X oil-immersion objective without following the proper technique!*

Three demonstration microscopes have been set up for you to use. Each microscope displays a different species of bacteria that was previously Gram-stained. The oil-immersion objective is already in position, with oil. *Do not move the objective lens.*

1. Review the parts and operation of a **compound microscope** (Microscope Use and Safety lab).

2. Observe the bacteria shown on each demonstration microscope. *Slowly rotate the fine-adjustment knob* back and forth until the bacterial cells are in focus. *Do not use the coarse-adjustment knob or move the slide.*

3. Sketch representative cells of each bacteria below. Identify the cell **shape** (bacillus, coccus, or spirillum; Fig. 1) and the **Gram-stain** (positive or negative; Fig. 2).



Shape: _____ Shape: _____ Shape: _____
Gram-stain: _____ Gram-stain: _____ Gram-stain: _____

## Task 2. Describing Bacterial Colony Morphology

To identify and study bacteria in the laboratory or clinic, they can be cultured either in a liquid broth of nutrients or on a plate of jellylike agar (a polysaccharide derived from a red alga) that has been mixed with nutrients. You will start by examining several bacterial cultures that have been grown on agar plates. Each plate consists of one isolated bacterial species. Remember that bacterial cells are incredibly small, requiring high magnification to see; the visible dots, clumps, or streaks on the agar plates are bacterial **colonies**, each consisting of thousands to millions of bacterial cells. Different bacterial species have characteristic colony **morphologies**, including features such as growth form, elevation, margin, color, and size (Fig. 3). An initial step in identifying an unknown bacterium in culture is to observe its colony morphology.

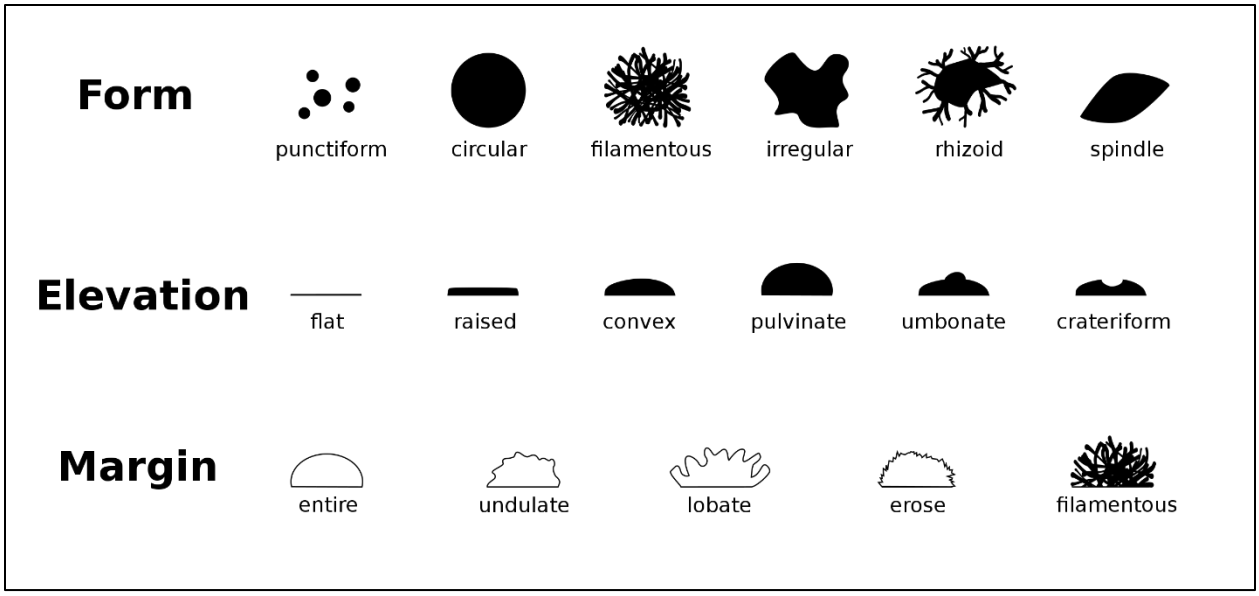



Figure 3. Morphological features used to classify bacterial colonies.
Colony morphology of bacteria by Macedo is licensed under CC BY-SA 4.0.

1. Obtain a **dissecting microscope** and review its parts and operation (Microscope Use and Safety lab).

2. Examine each prepared bacterial culture (agar plate) under a dissecting microscope and describe its **colony morphology** using size (diameter), color, and features illustrated in Figure 3. Record your observations in **Table 1** below. These characteristics will help you distinguish bacterial species in your own cultures (Task 3).

Table 1. Description of bacterial colony morphology.

| Plate ID | Species | Colony Diameter (mm) | Color | Form | Elevation | Margin |
|---|---|---|---|---|---|---|
| A | | | | | | |
| B | | | | | | |
| C | | | | | | |

## Task 3. Evaluate Results from Week 1. How Do Environmental Factors Affect Microbial Diversity?

1. Acquire your group's agar plate(s) from Week 1. *Keep bacterial cultures closed at all times!*
2. To measure **abundance** of bacteria, place a transparent sampling grid over each plate. Use a dry-erase marker to trace the outline of all the bacteria colonies. Then count (estimate) the total number of squares that are completely filled by bacteria of any species. You can round to the nearest 0.25 grid square if needed. Your instructor will demonstrate. Keep in mind that each visible bacterial colony contains thousands to millions+ of microscopic bacterial cells. Record your group's data in **Table 2** *and* on the board. Copy the class-wide results across all replicates.

Table 2. Abundance of bacteria sampled from different habitats.

| | Total number of grid squares filled by bacteria | | |
|---|---|---|---|
| Replicate | Habitat 1: | Habitat 2: | Habitat 3: |
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| Average | | | |

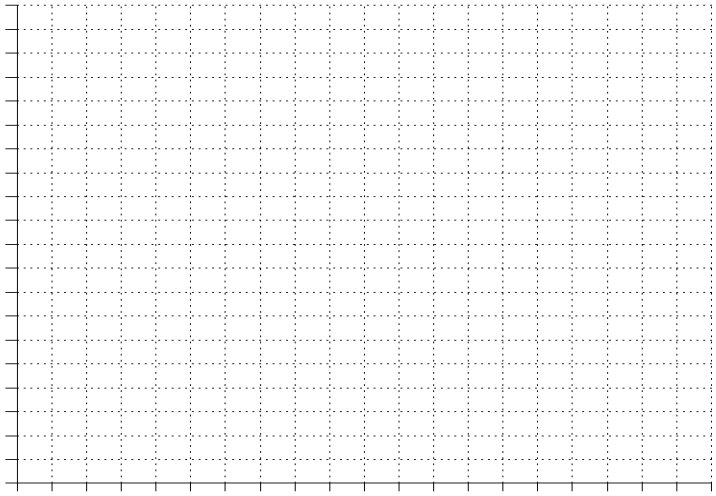
The replicate numbers are arbitrary. They may correspond to group numbers, or you may simply record your own data in the row for Replicate 1 and fill in the other rows with data from other groups.

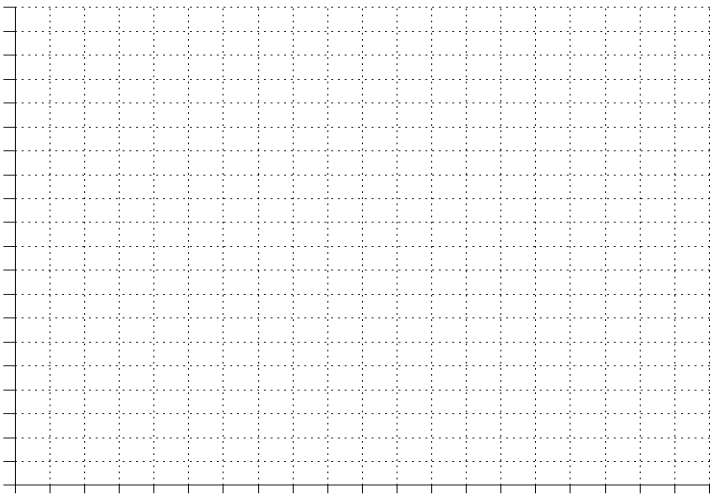
3. To measure **species diversity** (richness) of bacteria, examine each agar plate under a dissecting microscope. Count (estimate) the number of distinct "morphospecies" based on colony characteristics such as size, color, shape, texture, and margins (see Task 2 above). Record your group's data in **Table 3** *and* on the board. Copy the class-wide results across all replicates.

Table 3. Species diversity (richness) of bacteria sampled from different habitats.

| | Number of unique bacteria morphospecies | | |
|---|---|---|---|
| Replicate | Habitat 1: | Habitat 2: | Habitat 3: |
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| Average | | | |

4. Once everyone in your group has observed the agar plates and recorded the data, put the used plates in the biohazard container, clean your lab bench, and wash your hands.
5. Create two separate **bar graphs** (figures), one for abundance and one for diversity. Each bar should represent the *average* value observed for a particular habitat, calculated across all class replicates. Be sure to label your *x* and *y* axes with the independent and dependent variable, respectively. Number each figure (Figure 1, Figure 2) and provide a descriptive figure legend (i.e., a detailed title with context) below each figure.





## Task 4. Testing for Antimicrobial Susceptibility

**Antimicrobial** agents—antibiotics, antiseptics, and disinfectants—are used to inhibit the growth of bacteria inside the body, on the skin, or on other surfaces. Some groups of bacteria are more **susceptible** to particular antimicrobial inhibitors than are other groups of bacteria. This is why it is important for a health care provider to identify the source of a bacterial infection so they can prescribe an appropriate antibiotic that will be effective in inhibiting bacterial growth. Gram staining often correlates with differences in antimicrobial susceptibility; i.e., Gram-positive and Gram-negative bacteria may be inhibited by different types of antimicrobial agents.

One way to test for antimicrobial susceptibility is by using a **sensitivity plate**. A sensitivity plate is a petri dish with a growth medium, often Mueller Hinton Agar, that has been inoculated on its entire surface with a bacterium (e.g., a sample from an infected patient). Small paper discs are then soaked in different antimicrobial agents and spaced out evenly on the culture surface. Each paper disc is labeled with the abbreviated name of the antimicrobial and the amount that it contains (Fig. 4). After allowing the bacteria to grow in the presence of the paper discs, the sensitivity plate will be examined to see if **zones of inhibition** have formed around the paper discs. The zone of inhibition is the area around the disc where no bacteria have grown, and it is measured with a ruler from one side of the zone to the other to determine the diameter in mm (Fig. 5). If an antimicrobial agent is very effective against a bacterium (i.e., the bacterium is very susceptible), the zone of inhibition will be relatively large. If an antimicrobial agent is ineffective (i.e., the bacterium is not susceptible, or it is resistant), there will be no zone of inhibition – the bacteria will grow right up to the disc (Fig. 6).

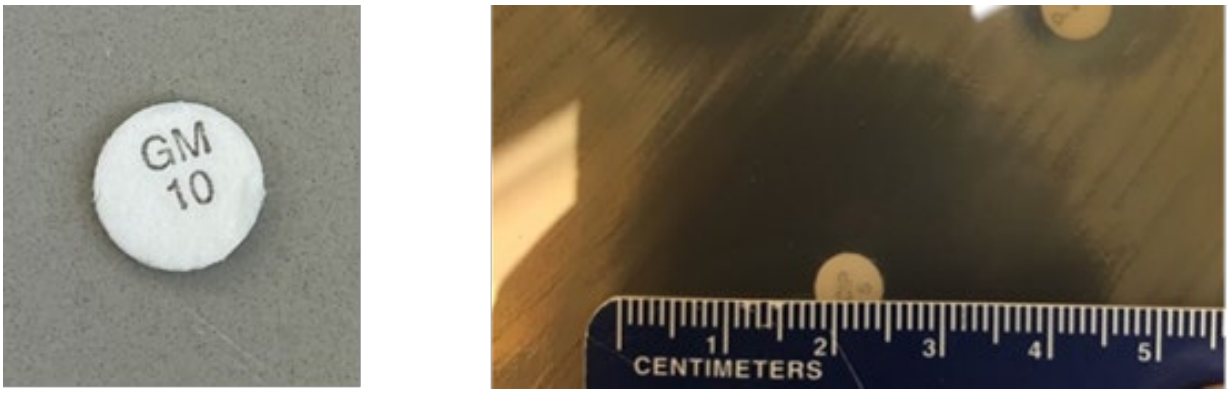



Figure 5. Using a sensitivity plate to test for antimicrobial susceptibility. On the left: A paper disc containing 10 ug of the antibiotic gentamicin (GM). If an antimicrobial agent is effective in inhibiting the growth of a bacterium, then a zone of inhibition with no bacterial growth will be visible surrounding the paper disc (looks like a clear halo around the disc). On the right: the zone of inhibition measures approximately 45 mm in diameter.

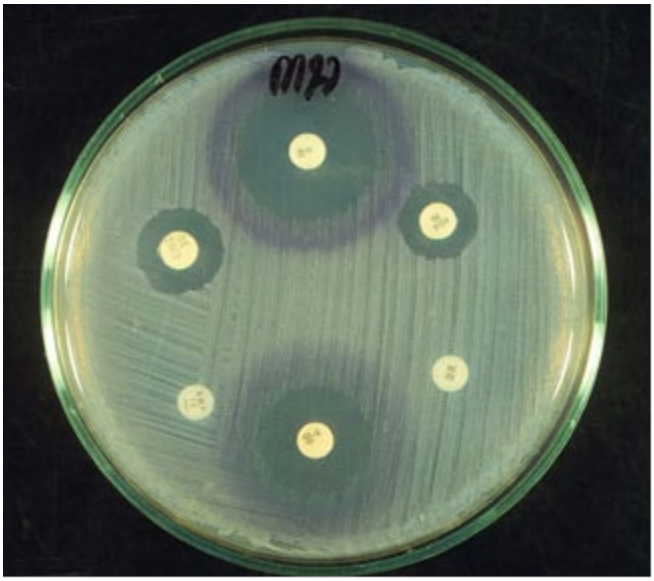


Figure 6. Interpreting sensitivity plate results. The paper discs with large zones of inhibition (e.g., top middle) contain antimicrobial agents that are very effective at inhibiting the growth of the bacterium on the plate. The paper discs with no zone of inhibition (e.g., bottom left and bottom right) contain antimicrobials that are not effective against this particular bacterium.

1. Obtain two sensitivity plates that have been inoculated with two different species of bacteria and exposed to the same antimicrobial agents. *Keep bacterial cultures closed at all times!*

2. Examine each plate and measure the zones of inhibition (diameter in mm). Record your results in **Table 4** below. If there is no visible zone of inhibition for a particular antimicrobial-bacterium combination, record "No inhibition".

Table 4. Sensitivity plate results – inhibition of two bacterial species by antimicrobial agents.

| | Plate 1 | Plate 2 |
|---|---|---|
| Antimicrobial Agent (abbr) | Zone of Inhibition Diameter (mm) | Zone of Inhibition Diameter (mm) |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

- For each bacterial species (Plate 1 vs. Plate 2), rank the antimicrobial agents in order from most effective (most susceptible) to least effective (least susceptible). For reference, your instructor may also provide a standardized chart that is used to measure antibiotic sensitivity in a clinical lab.

- Are there any antimicrobials that are effective against one bacterium but not the other?

# Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

### Tasks 1-2. Bacterial Cells and Colonies

1. Conduct a Google Images search(-es) for bacterial shapes and Gram-stain slides. Do the examples online look exactly like the ones you observed in lab? If not, how do they vary?

2. Which colony characteristics were the most useful in distinguishing between the bacterial species in Task 2? Which were most difficult to discern? Explain.

3. Why did you use a compound (light) microscope for Task 1 and a dissecting microscope for Task 2?

### Task 3. How Do Environmental Factors Affect Microbial Diversity?

4. Briefly summarize the **results** of the class-wide study. Describe the overall patterns of abundance and species diversity illustrated by your two figures (bar graphs). Which habitat(s) harbored the most/least abundant bacteria? Which habitat(s) harbored the most/least diverse bacteria? Include your **figures** with your submission.

5. Were the class-wide results consistent with your predictions? Discuss two possible **explanations** for the observed patterns – at least one explanation should refer to environmental factors that affect bacteria, while the other may acknowledge a potential source of error.

6. If you were to build upon this pilot study by conducting a follow-up study or experiment, what might you do and why?

### Task 4. Testing for Antimicrobial Susceptibility

7. One of the sensitivity plates was inoculated with a **Gram-positive** bacterium, while the other was inoculated with a **Gram-negative** bacterium. Based on the differences in antimicrobial susceptibility that you observed, and using outside internet resources, which plate do you think is Gram-positive and which plate do you think is Gram-negative? Explain your answer. Also explain why Gram-positive and Gram-negative bacteria tend to differ in antimicrobial susceptibility.

8. Suppose that surfaces in the lab are regularly cleaned with disinfectants that kill some but not all bacteria. Explain how bacterial populations in the lab room might **evolve resistance** to commonly used cleaning products over time. *Be specific* about the underlying process (i.e., the mechanism of evolution).

## References

Waggoner CM, Keller K, McArthur J. 2003. Exploring biodiversity. pp. 1-16, in Tested studies for laboratory teaching, Vol. 24. (MA O'Donnell, Editor). Proceedings of the 24th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 334 pp.

# The "Protists"

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Become familiar with the diversity of several kingdoms of mostly unicellular eukaryotes – specifically, those that are not plants, animals or fungi.
- Practice using compound microscopes to observe and characterize protists.
- Learn about the ecological importance of these microscopic organisms and their roles in biological communities.

## Introduction

Found within the Domain Eukarya, the protists are a diverse and **paraphyletic** group. That is, they are all eukaryotes, but lumping them together as a group is informal, because it excludes the multicellular plants, fungi, and animals. Many of the protists are plant-like, with some even sharing a common origin with plants (i.e. Chlorophytes). Some are more animal-like in their ecology, acting as parasites or predators. In fact, it is more appropriate to say that a "protist" is a eukaryote that is neither a plant, fungus, or animal (Figure 1).

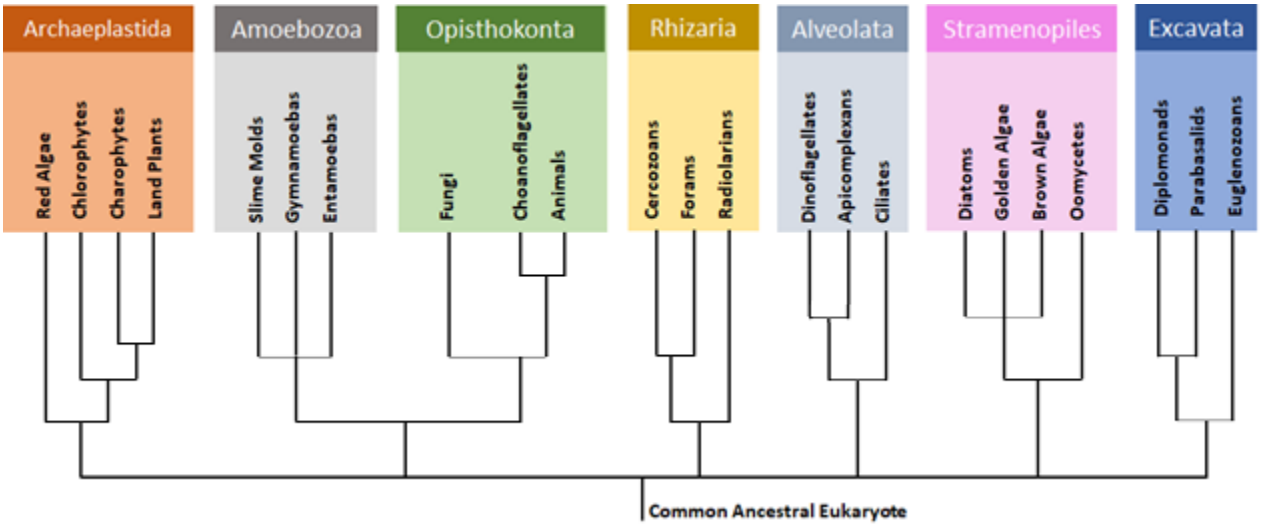



Figure 1: A phylogenetic tree of eukaryotic life. Note that Land Plants (Kingdom Plantae) is included with several organisms that are considered protists, such as the red algae and chlorophytes (green algae). Likewise, Kingdom Fungi and Animalia share a clade with the protists known as choanoflagellates. Additionally, many protists that one might assume are related, such as amoebas, are organized into several different clades – e.g., gymnamoebas and cercozoans.

In addition, while many are microscopic and unicellular, such as *Paramecium,* others, like giant brown kelp, are multicellular and may reach almost 150ft in length! Our focus in today's lab will mostly be the unicellular varieties. As we will see, they are organized into many diverse clades and phyla, based on morphological features and shared common ancestry. We will start with the plant-like protists, the Algae.

# Part I. The Algae

The algae are a **polyphyletic** group of about 10 phyla that are predominantly photosynthetic. In fact, many of these phyla are distinguished, in part, by what photosynthetic pigments they use (i.e., green algae, red algae, brown algae). And like all protists, they vary considerably. Some are unicellular, some are colonial, and some, like true plants, are multicellular. Some even grow like plants, such as the kelps, attaching to the seafloor.

In addition, algae are very important to the world's aquatic ecosystems. They are one of the primary oxygen producers, along with plants and cyanobacteria. They are primary energy producers, supporting the complex food web of the ocean. Being chemically unique, algae also hold pharmaceutical and economical importance.

*Note: It is important to remember that the term "algae" is often used loosely to refer to any photosynthetic microorganism. Here, it specifically refers to photosynthetic eukaryotes that are not plants.*

## Phylum Chlorophyta

Chlorophytes belong to the clade Archaeplastida along with land plants. They can have many cellular arrangements, with species being single-celled, colonial, or multicellular. They possess the same photosynthetic pigments as plants, **chlorophyll *a*** and ***b***. They also store the sugar they produce through photosynthesis in the same form that plants do, as **starch**. They also have double-membraned **chloroplasts**, as plants do. Therefore, they share a close affinity with land plants, being grouped in the clade Viridiplantae.

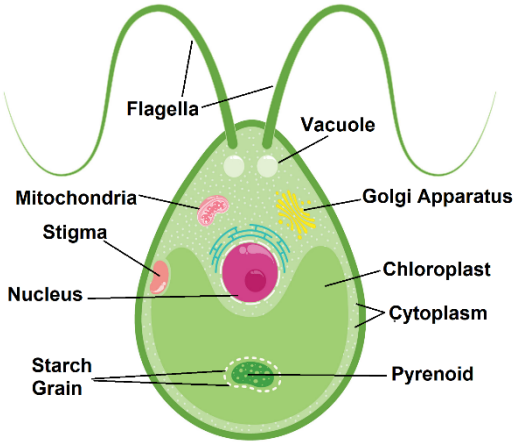



Figure 1. The anatomy of *Chlamydomonas* – credited to Vecteezy (CC-BY 4.0)

## Task 1 – *Chlamydomonas* – Unicellular Green Algae

1. Use the plastic pipet to add one drop of *Chlamydomonas* culture to a blank slide. You may add equal parts Protoslo (one drop) to the slide, before applying a cover slip.
2. View the specimen at high power using a compound light microscope.
3. Carefully observe the locomotion of the *Chlamydomonas.* Look for the whip-like **flagella** in action.
4. Also note the presence of a reddish eyespot called a **stigma,** which can only sense light and dark.
5. Each Chlamydomonas should have a single, large chloroplast, which contains a compartment called a **pyrenoid**. This is where carbon fixation occurs – note the presence of starch granules around it.

- Why might a **stigma** be useful to an organism such as *Chlamydomonas*?

## Task 2 – *Volvox* – Colonial Green Algae

1. Prepare a wet mount of the colonial green algae, *Volvox*. You will only need a single drop of culture, and no Protoslo is required for this specimen.
2. View the *Volvox* specimen at high power using the compound light microscope.
3. The outer layer of *Volvox* is composed of individual cells which look strikingly similar to *Chlamydomonas* – they even use flagella for locomotion. These cells are **vegetative cells**. Observe the surface of the Volvox carefully to see this.
4. On the interior of *Volvox* are smaller daughter colonies that are eventually released from the parent colony as they mature. These colonies are called **gonidia** (sing. gonidium).

- How many gonidia did you see within your *Volvox* colony?

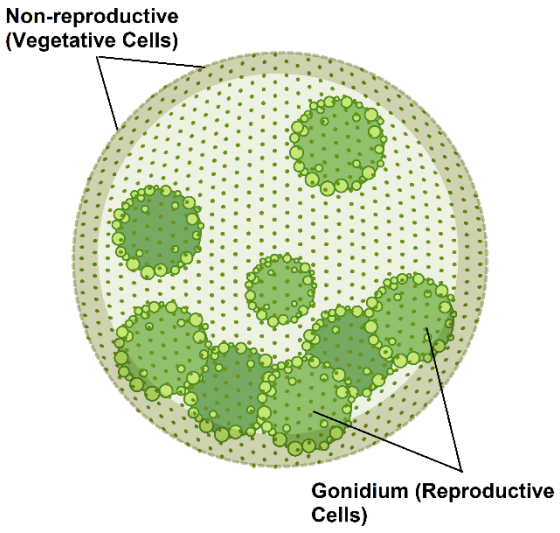



Figure 2. A colony of *Volvox* – credited to Vecteezy (CC-BY 4.0)

## Task 3 – *Ulva* – Multicellular Green Algae

1. View some of the multicellular green algae, *Ulva*, which has been set out for display on the side bench.
2. The main body of multicellular algae is called a **thallus**. It may often appear plant-like but remember that algae are not plants.

- What makes *Ulva* plant-like? How is it noticeably different from a plant?

# Phylum Phaeophyta

Phaeophytes are more commonly known as brown algae, or kelp. The brown coloration of these algae is due to a difference in the primary pigment they use, compared to the Chlorophytes. They use a pigment called **fucoxanthin**, rather than chlorophyll. In addition, their products of photosynthesis are oils, rather than starches. These brown algae look superficially like plants, but they are unrelated, and entirely marine in their distribution – whereas plants are terrestrial. They often have a large, branching **thallus**, ending in air bladders for buoyancy, and attach to substrate on the seafloor using a sticky structure called a **holdfast**.

Phaeophytes include species such as: *Fucus,* an intertidal seaweed, see Figure 3; *Sargassum*, a seaweed that is found in the region of the Sargasso Sea; and the Giant Kelp, *Macrocystis*, which can reach sizes up to 175 feet long!

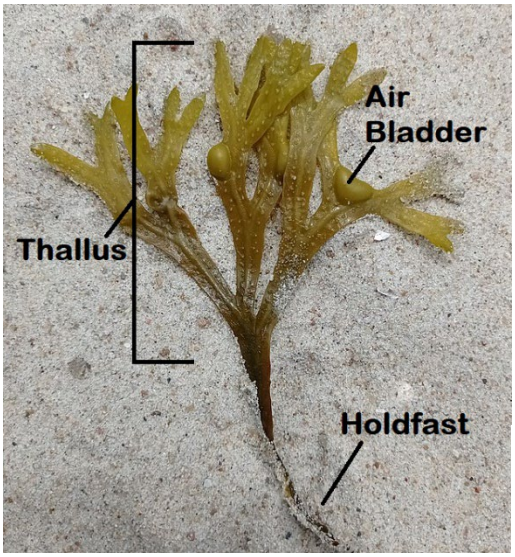



Figure 3. A thallus of *Fucus*, also known as Rockweed. Modified from Adrian Tync, licensed under CC BY-SA 4.0

## Task 4 – *Fucus* – Rockweed

1. View the specimen of rockweed, *Fucus*, that has been set out on the side bench.
2. Identify the **air bladders**, **thallus**, and **holdfast**.
3. Note that some of the ends of the blade-like thallus are swollen. These are reproductive structures known as **conceptacles**. They contain egg and sperm, depending on the specimen.

- How do you think the roots of a plant differ from the holdfast in *Fucus*?

# Diatoms

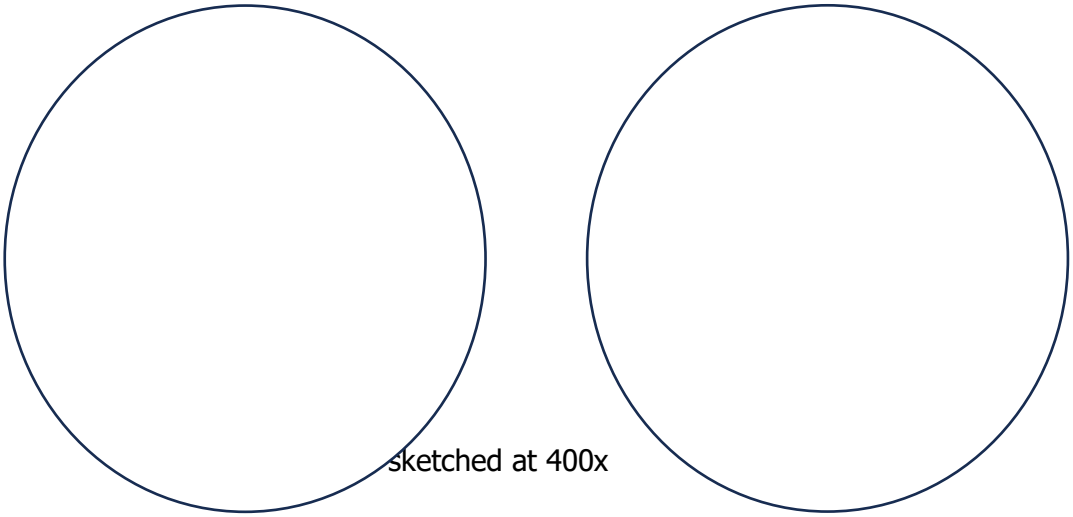
Diatoms are a close relative of the brown algae, belonging to the clade known as the **Stramenopiles**. They also possess the same pigment as brown algae, fucoxanthin, as well as chlorophyll *a* and *c*. This gives them a brownish-gold pigmentation. However, unlike the brown algae, they are entirely unicellular – though some may take on filamentous forms. The most striking difference are the **tests** (shells) of diatoms, which are constructed from **silica** – essentially glass! Their shells are composed of two halves, which fit together somewhat like a petri dish.

These organisms are significant in marine and freshwater ecosystems as both plankton and major producers. We often refer to organisms like this as **phytoplankton**. Along with the dinoflagellates you will observe later, they produce the bulk of energy that goes into marine ecosystems.

Generally, diatoms are found in the upper levels of the water column, where sunlight penetrates, but when they die, their shells settle to the ocean floor. Over time, this produces sediment composed primarily of their tests – we call this **diatomaceous earth**.

## Task 5 – Diatomaceous Earth

1. Place a small amount of diatomaceous earth on a blank slide, and add a drop of water.
2. Observe the sample under the compound light microscope at high power.
3. Sketch some of the diatoms you see under the microscope in the space below.



sketched at 400x

- Do a bit of online research. What are some of the household uses of diatomaceous earth?

## Dinoflagellates

Dinoflagellates are, just like the diatoms mentioned above, a group that is considered to be an important producer in aquatic environments. And, like diatoms, they are all unicellular and are covered by a test. However, they belong to a different clade, the **Alveolata** – which they share with some parasitic and ciliated protists. In addition, their tests are composed of **cellulose**, rather than silica.

As their name implies, they move using whip-like flagella. Each of these two flagella sit in grooves on their test – one longitudinal, and the other transverse (Figure 4).

Some other unique characteristics include the ability of some species to be bioluminescent. That is, they can produce light when disturbed. Some species are also not only photosynthetic but can be parasitic. One such species, *Pfiesteria piscicida*, is a species that becomes abundant during algal blooms, causing what is known as **red tides**. These toxic tides can be devastating to marine life and shut down fishing operations in coastal areas.

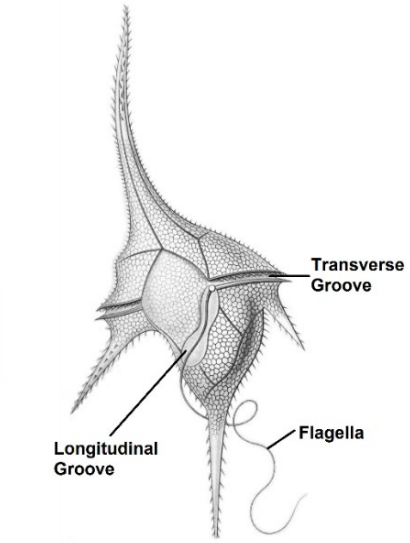
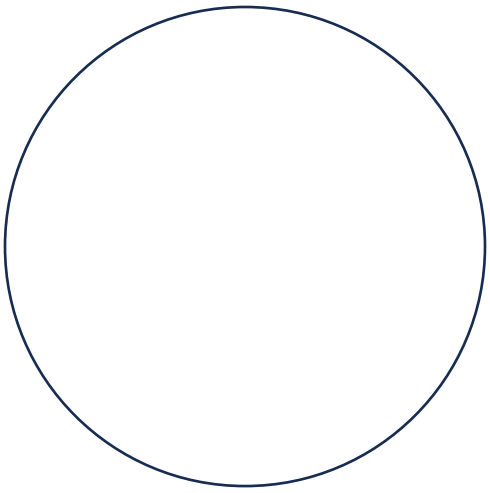



Figure 4. The dinoflagellate *Ceratium hirudenella*. Modified from Ernst Haeckel, Public domain, via Wikimedia Commons

## Task 6 – Freshwater Dinoflagellates

1. Prepare a wet mount using a single drop of the freshwater dinoflagellate culture on the side bench. Do not add a drop of Protoslo.
2. View the specimens at high power under the compound light microscope.
3. Attempt to observe the locomotion of the dinoflagellates, particularly looking for the motion of their whiplike flagella. Specimens should be brownish in coloration.
4. Sketch one of the dinoflagellates you see under the microscope in the space below.



Dinoflagellate sketched at 400x

## Phylum Euglenozoa

Members of this phylum are a diverse group, as they contain unicellular protists that are both free-living and parasitic. We will cover the parasitic varieties later on. However, of particular interest is the free-living variety known as the **Euglenids**. Euglenids are both **autotrophic** and **heterotrophic**. However, it is important to note (as you will read in your textbook), that euglenids are secondarily photosynthetic. This means that ancestrally, they were primarily heterotrophs, feeding on other microscopic organisms. Over time, they obtained chloroplasts through **secondary endosymbiosis**, gaining the ability to photosynthesize. Being secondarily photosynthetic, we will discuss them here with the algae.

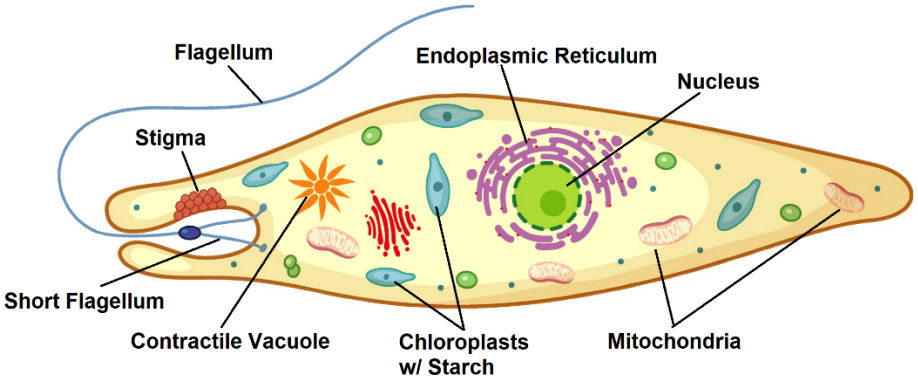



Figure 5. The anatomy of *Euglena gracilis*. Image credited to Vecteezy (CC-BY 4.0)

## Task 7 – *Euglena*

1. Obtain and prepare a wet mount using *Euglena* culture. Apply a single drop of Protoslo to the wet mount before applying a cover slip.
2. Observe *Euglena* at high power under the compound light microscope.
3. Euglenids are flagellated protists. They have two unequal flagella, one being very long and used for locomotion. As such, they can move very quickly in search of prey. Try to observe the motion of this flagella.
4. Try to identify the reddish eyespot (stigma) for detecting light.
5. Also note the greenish coloration of their chloroplasts.
6. The surface of *Euglena* is covered in a **pellicle**, a tough but flexible covering used to maintain the streamlined shape of *Euglena*.

- Describe the movement of *Euglena* as you observe it under the microscope. What structure is it using to locomote?

# Part II. The Protozoans

Protozoan means "first animal," which is a name that distinguishes them from the more plant-like protists, the algae. They are indeed more animal-like, in their heterotrophic ecology - being active consumers. Again, much like the algae, the protozoan group is a mixed grab-bag of organisms. Most are unicellular but exhibit a variety of ecologies. Some are free-living, some **symbiotic**, and some are **parasitic**. The one thing they do have in common is that they are all heterotrophic- they must consume organic matter to survive.

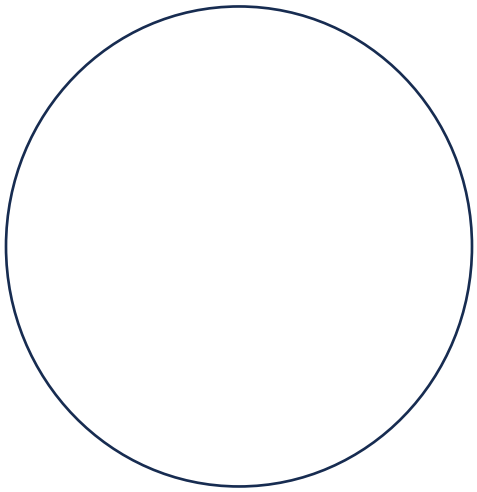
Do note, as you continue through this section, that protozoans do not belong to a single clade. For example, the Trypanosomes we are about to discuss are parasites, but belong to the same clade as Euglenids (Euglenozoa) which may be referred to as an algae. Likewise, Dinoflagellates share an origin with the parasitic Apicomplexans.

## The Trypanosomes

The protists known as trypanosomes, as mentioned above, are related to the euglenids. They share the same elongated body plan and locomote using flagella. However, unlike the euglenids, their flagella are internal, causing them to move in an undulating fashion. The biggest contrast is their purely parasitic ecology. All trypanosomes are obligate parasites, being spread to their hosts by biting insect **vectors** – particularly in tropical areas where these insects are abundant. For example, the parasite *Trypanosoma brucei* is known to cause the disease African Sleeping Sickness. Their vector is the Tsetse fly. When a Tsetse fly bites a host, such as a human or livestock, the trypanosome moves from the bite wound, where it replicates by **binary fission** in infected cells. It then bursts out of these cells and moves into the blood stream – where another insect may pick it up during a blood meal.

## Task 8 - Trypanosomes

1. Obtain a slide of a *Trypanosoma* blood smear.
2. View the specimen at high power using the compound light microscope.
3. Find some of these parasitic protists among the blood cells on the slide and sketch them in the circle below.



*Trypanosoma* sketched at 400x

- *Trypanosoma cruzi* is a close relative of *T. brucei.* What disease is it responsible for? Do some research.

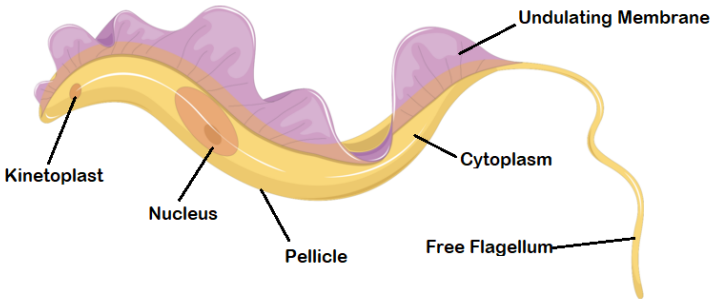



Figure 6. The anatomy of *Trypanosoma brucei.* Modified from Servier Medical Art, CC BY 2.0

# The Ciliates

As their name implies, these protists are covered in short, hair-like structures called **cilia**. These cilia allow them to locomote, or sweep food into their funnel-like **oral grooves**. Some of these ciliates, like *Paramecium*, are free-swimming predators or scavengers. Some species are sessile, with *Stentor* capturing large prey, and *Vorticella* acting as a suspension feeder.

Ciliates like *Paramecium* have a unique cellular structure among protists. They have two types of nuclei, a large **macronucleus** and smaller **micronucleus**. Using the micronuclei, they are able to practice a form of "sex," called **conjugation** – Figure 7. In this process, (2) two

paramecia line up, side by side, and (3) divide their micronuclei through meiosis. This produces four **haploid** micronuclei. Then, all but one of these micronuclei break down. The remaining micronucleus is replicated using mitosis, leaving two haploid micronuclei each (4). Each *Paramecium* then exchanges one of the haploid micronuclei (5). Each Paramecium will continue the process individually from here (6). The haploid nuclei are then fused into a new **diploid** nucleus (7). This act is similar to the fusion of a sperm and an egg in animals. Then, the new diploid micronucleus is replicated through mitosis, producing four micronuclei (8). The old macronucleus degenerates, and will be replaced by a newly replicated micronucleus (9). Now, both the macronucleus and micronucleus are composed of new, genetically varied material (10).

Note that the *Paramecium* does not produce any new cells during conjugation. Therefore, it is not considered a form of reproduction – though it exchange genetic material, making it a form of sex. However, if a Paramecium does want to reproduce, it clones itself through a process called **binary fission**. Here, the ciliate copies the genetic material in both the macronucleus and micronucleus and exchanges it between two new daughter cells, which are attached end-to-end before dividing (11).

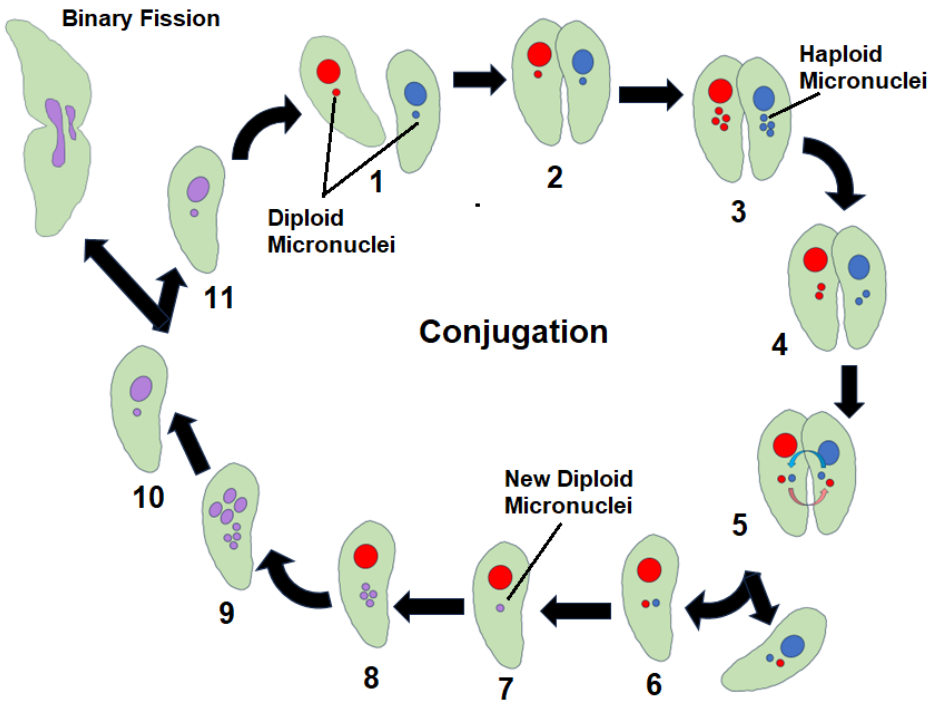



Figure 7. The process of conjugation in *Paramecium caudatum.* Note that during conjugation, the *Paramecium* are side-by-side. During binary fission, they are attached end-to-end.

## Task 9 – Paramecium

1. Prepare a wet mount using the live *Paramecium* stock culture. Make sure to add one drop of Protoslo for one drop of culture.
2. View the live *Paramecium* at high power under the compound light microscope. Observe the movement of these protists and answer the question below.
3. Next obtain a prepared slide of *Paramecium* Conjugation and view it at high power under the compound light microscope.
4. Finally, view the prepared slide of *Paramecium* Fission (binary fission) at high power under the compound light microscope.

## Task 10 – Vorticella

1. Prepare a wet mount of the live ciliate *Vorticella*. Observe the use of cilia during the process of filter feeding. Tap on the side of the microscope stage to disturb the specimen.

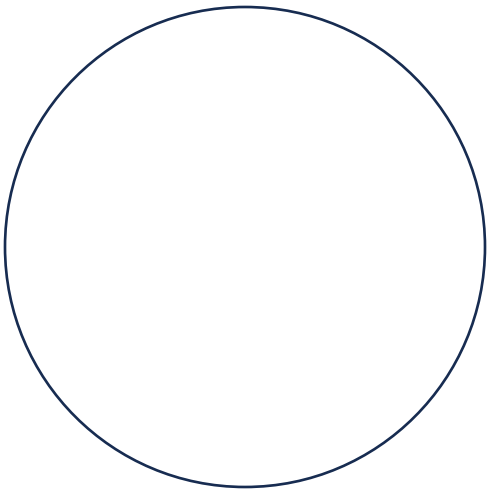
- Describe the movement of *Paramecium*. What structures are they using to locomote?

# The Apicomplexans

A close relative of the ciliates are the parasitic protists known as apicomplexans. They are so named because of the cluster of organelles in the anterior end of the protist, called the **apical complex**. This complex is used to burrow into a host's cells. The most well-known of these is the apicomplexan *Plasmodium falciparum*, which is the parasite responsible for **malaria**. This disease is restricted to tropical and subtropical zones, and is spread by an insect vector – an *Anopheles* mosquito. In this way, it is like the trypanosomes, but unlike them, *Plasmodium* burrows into red blood cells to reproduce. Eventually, it causes the red blood cells to rupture and release offspring, which can cause numerous symptoms like anemia, fever, flu, chills, muscle aches, headaches, and eventually (if treatment is not received) possibly death.

## Task 11 – *Plasmodium*

1. View the prepared slide of a blood smear, containing *Plasmodium*.
2. Sketch a few infected and uninfected red blood cells in the circle space below.



Blood cells sketched at 400x

- *Toxoplasmosa gondii* is a close relative of *Plasmodium*. What is the primary host of this parasite? Do some online research.

## The Amoeba-like Protists

Amoebas are protists that move using extensions of cytoplasm called **pseudopodia**. In addition, they also engulf entire food particles by using a process called **phagocytosis**. This is the act of surrounding food with the plasma membrane, drawing it into the cell, and surrounding it in a food vacuole. There are several protists that can be considered to move and feed in an amoeba-like way, though not all are closely related. We will observe them here.

## Gymnamoebas

Gymnamoebas are shell-less, amorphous protists that are what we typically think of when we refer to amoebas. They contain the well-known freshwater *Amoeba proteus*, which engulfs and feeds on smaller protists, bacteria, and algae. They move using lobe-shaped pseudopods, extending them forward and anchoring them with protein filaments, then dragging themselves forward.

### Task 12 – Amoeba proteus

1. Prepare a wet mount using the stock culture of *Amoeba proteus*. Try to collect a drop of culture along the bottom of the container, close to the food pellet.
2. Amoeba are difficult to find, so beginning at low power under the compound light microscope, carefully look for a web-like mass of pseudopodia, which is slowly extending.
3. View the specimen closer, first at medium power then at high power. You will know it is an amoeba as you watch the cytoplasm flow into the pseudopodia and watch them extend.

4. There may also be ciliates present. Observe any interaction between *Amoeba* and it's prey.

- Describe the movement of the living amoeba. What structures is it using to locomote?

## Foraminifera

Foraminiferans, or "forams", are also considered to be amoeba-like, as they use pseudopods. But they are not closely related to shell-less amoebas and differ in several ways. First, they do have a shell, which is composed of **calcium carbonate**. Second, they use **reticulose** pseudopodia, which are long and thin. They are benthic marine protists, which drift along with their pseudopods extending out of their shells. They use these thin pseudopods to snare bits of organic material, which they draw into their shell and plasma membrane using phagocytosis.

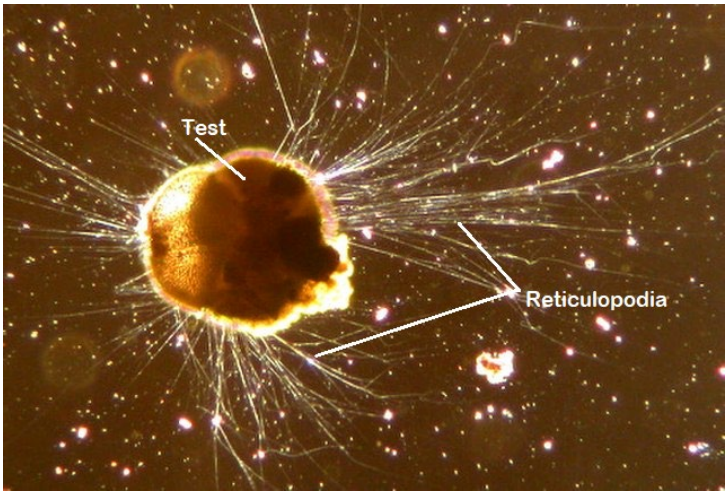
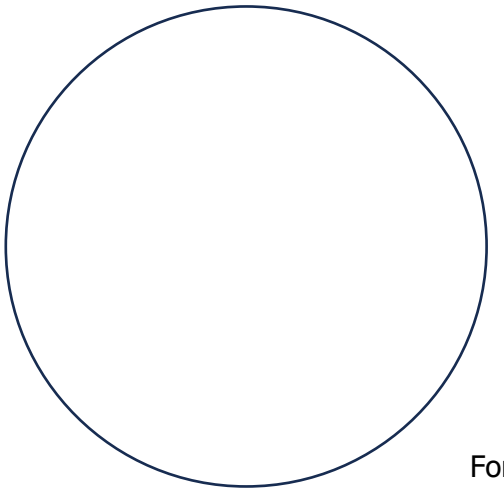



Figure 8. The anatomy of a foraminifera. Image credited to Scott Fay, CC BY 2.5

Forams are so abundant in marine ecosystems that when they die, their shells accumulate on the ocean floor, producing meters upon meters of calcium carbonate deposits. Over millions of years, these shells accumulated into the chalk deposits we use today. The white cliffs of Dover in England, for example, are made up primarily of such deposits!

## Task 13 - Foraminiferans

1. View the prepared slide of Foraminifera tests at high power under the compound light microscope. Note that these are only shells, no pseudopodia will be visible.
2. Sketch an example of one of these tests in the circle space on the right.



Foraminifera test sketched at 400x

## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. All algae are photosynthetic. However, not all algae are closely related. What are some characteristics we can use to distinguish these phyla from one another, such as the Chlorophytes and Diatoms, for example?

2. Even though green algae are related to plants, *Ulva* cannot support itself on land. How are plants able to do this? *You may research your answer.*

3. Compare and contrast the methods of locomotion used between the following protists:
    a. Euglena

    b. Paramecium

    c. Amoeba

4. Why is **conjugation** in *Paramecium* considered sex, but not reproduction? What process does *Paramecium* use to reproduce?

5. Explain why we couldn't simply use **heterotrophy** and **autotrophy** to determine relatedness among protists.

# Plant Evolution: Life Cycles & Adaptations

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Understand the generalized plant life cycle alternation of generations and become familiar with the following terms: gametophyte generation, sporophyte generation, fertilization, meiosis, sporophyte, gametophyte, gametangia, gametes, spores, & sporangium
- Be able to recognize the differences among the four plant groups: bryophytes (mosses), monilophytes (seedless vascular plants), gymnosperms (cone bearing plants), and angiosperms (flowering plants)
- Understand evolutionary significance of the cuticle, gametangia/sporangia, pollen, seeds, and flowers in plant evolution
- Analyze the adaptative strategies for water loss in thallus or leaf structures for green algae, bryophytes, monilophytes, gymnosperms, and angiosperms

## Introduction

Plants have evolved many adaptations throughout geological time. Plants originally evolved from a green algae ancestor within the phylum Chlorophyta. Algae are protists and have been around for at least 2.1 billion years. Members of the Chlorophyta are the organisms the most similar to plants. They have similar photosynthetic pigments (chlorophyll a and b, carotenes, and xanthophylls) and their main food storage reserve is starch. Some have a cell wall similar to plant cells walls (composed of cellulose and pectins).

Another feature plants obtained from their algae ancestors is the **alternation of generation life cycle** (Figure 1). This life cycle consists of a series of events, with the duration of each event varying for each plant group. For plant sexual reproduction, there is an alternation between a diploid (2n) **sporophyte** phase (sporophyte generation) and a haploid (n) **gametophyte** phase (gametophyte generation). A gametophyte is a multicellular haploid form that grew from a spore and will eventually produce the haploid **gametes**. Any cell in the gametophyte generation is haploid. A sporophyte is a multicellular diploid form resulting from the union of gametes (fertilization) and will eventually produce the **sporangium**, a diploid organ in which meiosis occurs & haploid **spores** develop. Any cell in the sporophyte generation is diploid.

The basic alternation of generations consists of two processes (meiosis and fertilization) and four parts (gametophyte, gametes, sporophyte, and spores). These six steps are found within all plant life cycles, only the duration of each step varies. The two processes are especially important by allowing the preservation of an individual's chromosome number from one generation to the next. **Meiosis** is the process of nuclear division in which the chromosome number is reduced by half leading into the gametophyte generation, while **fertilization**

involves the fusion of two gametes, forming a zygote and leading into the sporophyte generation (Figure 1).

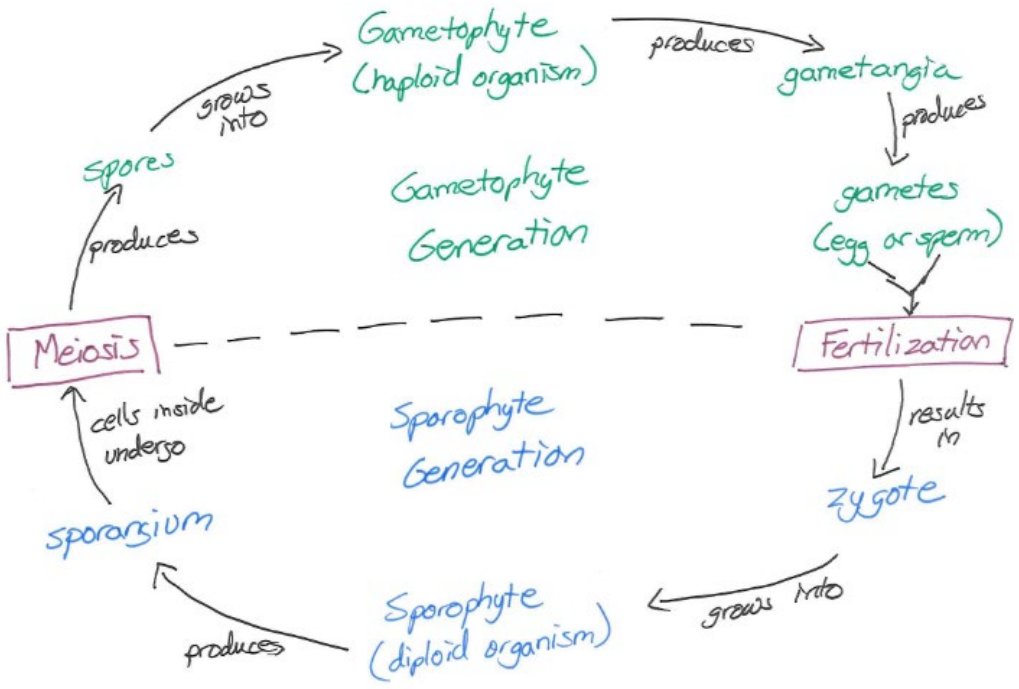



Figure 1: This is the general alternation of generation life cycle. The steps can vary depending on the algal group or plant group. Drawing Credit: TR Robertson, CCGA DNS. 2023.

There were four major events in plant evolution, leading to the development of four major plant groups. The first major event resulted in the development of the cuticle, gametangia, and sporangia. The **cuticle** is a protective waxy outer covering to prevent water loss in order to survive on land. **Gametangia** are specialized structures that protect the formation of gametes (the *archegonium* forms the eggs and the *antheridium* forms the sperm), while **sporangia** are specialized structures to that protect the formation of spores. Both of these structures led to the evolution of non-vascular plants, also known as **Bryophytes** (e.g. mosses).

The next major event of plant evolution was the development of vascular tissues. **Vascular tissues** are specialized tissues for transporting sugars from photosynthesis and water. Bryophytes can only transport sap and water from cell to cell via diffusion, which keeps these plants very small and needing to grow near water sources. Vascular tissues are more efficient in sap and water transport, allowing plants to grow taller and develop true roots, stems, and leaves. This led to the evolution of the vascular seedless plants, **Monilophytes** (e.g. ferns, horsetails, and whisk ferns). Being able to grow taller allows for greater access to sunlight, but these plants still need water for reproduction due to having swimming sperm. Therefore, they still need to grow near water sources. The development of **heterospory** – the production of two types of spores in two different kinds of sporangia – is also seen for the first time. This allows the separation of sexes at different points in the life cycle and promotes outcrossing among plants.

The third major event of plant evolution was the development of pollen and ovules. **Pollen** are specialized structures that protect and carry the sperm, so water is no longer required for reproduction. Pollination is the act of bringing pollen close to an ovule. **Ovules** are specialized structures that protect the eggs and, after fertilization, will develop into a **seed** that will protect the embryo inside. Seeds not only provide protection to a developing embryo, but are also a food source and aid in dispersal of the contained embryo. This led to the evolution of the first group of vascular seed plants, the **Gymnosperms**. Most gymnosperms produce pollen and seed-bearing cones and rely mainly on wind for pollination. The most diverse division within the gymnosperms are the Coniferophyta, which are the needle and scale-leaved conifers (e.g. pines, spruces, cedars, etc.).

The second group of vascular seed plants are the **Angiosperms**. They evolved during the fourth major event of plant evolution – the development of the flower. **Flowers** are actually modified leaves that help protect the reproductive structures as well as attract pollinators for more efficient pollination. After pollination and following fertilization, a **fruit** develops from the flower ovary. Fruits provide additional protection for the developing seeds and serve as dispersal vessels for seeds. How they are dispersed depends on their shape, appearance, and other modifications. Angiosperms belong to the Division Magnoliophyta (formerly Anthophyta). They compromise a number of evolutionary lines, with most belonging to two general groups: **monocots** (Lilopsida) and **eudicots** (Magnoliopsida).

## Methods

You will be given live specimens and slides to compare and contrast the features and how each specimen has evolved to adapt to dealing with the challenge of water loss in terrestrial environments. You will start with the aquatic green algae, muskgrass (*Chara* species) so you can observe what characteristics and adaptation an aquatic protist has before observing the terrestrial plants. Then you review a moss (either *Polytrichum* or *Sphagnum* sp.) and determine how it has adapted to a terrestrial environment compared to the green algae. You will follow this with a fern (*Polypodium* sp.), a gymnosperm (*Pinus* sp.), and two angiosperms (eudicot species and monocot species). Make sure you record all your observations in the results section and answer the associated questions.

## Task 1 – Green Algae: Muskgrass (*Chara* sp.)

Muskgrass (*Chara* sp.) is a freshwater green alga that can be found submerged in shallow or slow running water habitats. This protist belongs to the Class Charophyceae of the Phylum Chlorophyta. They are multicellular and resemble land plants, but they are still very distinct due to lacking the tissue complexity seen in land plants.

These organisms spend the majority of their lives as a gametophyte (Figure 2). Their sporophyte generation is very short, with the zygote quickly performing meiosis to start the gametophyte generation.

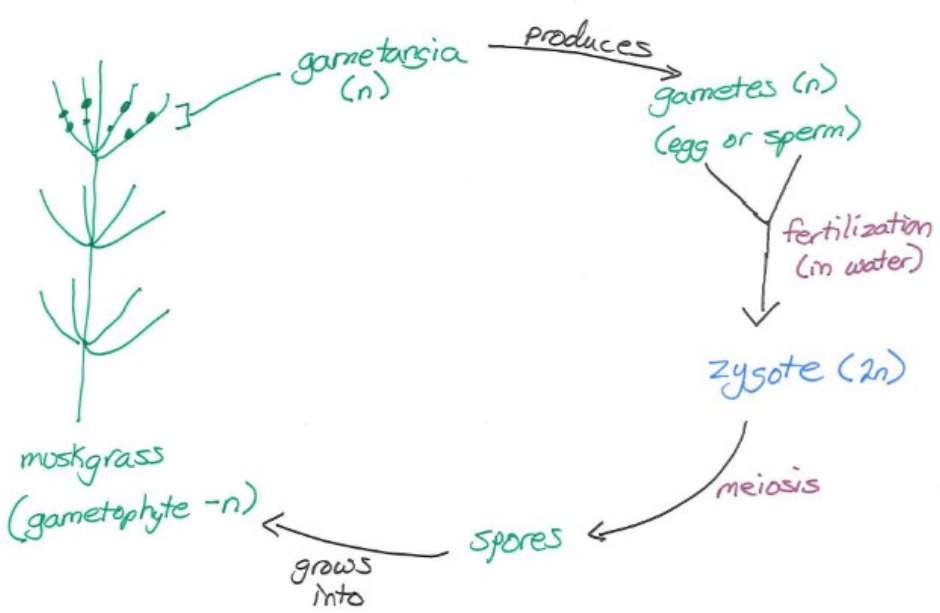



Figure 2: This is the alternation of generations life cycle of a muskgrass algae. Haploid (n) refers to components of the gametophyte generation while diploid (2n) refers to components of the sporophyte generation. Drawing Credit: TR Robertson, CCGA DNS. 2023.

As you examine the live specimen and associated slide, consider how this algae's structures allow it to survive in an aquatic environment.

1. Provide a rough sketch of the live specimen and include a brief description of its overall characteristics.

2. Now provide a rough sketch of the associated slide and include a brief description of what you observed of its overall characteristics.

Consider the following questions as you interpret your results:

- How would this organism transport water throughout its body?

- What features do you think allow this organism to survive in aquatic environments? Why do you think this?

- What do you think would happen if this alga was placed in a terrestrial environment? Why do you think this?

- Aquatic alga has to release their sperm into the water for fertilization. What could be a possible advantage and disadvantage with this reproductive strategy?

# Task 2 – Bryophytes: Moss (*Polytrichum or Sphagnum* sp.)

Mosses (e.g. *Polytrichum* or *Sphagnum* sp.) are low-growing non-vascular plants commonly found in warm, moist environments. There are three divisions of non-vascular plants, but the largest division is Bryophyta, also known as the true mosses. These plants have more specialized features to control water loss. However, they are still restricted due to their flagellate sperm needing water for fertilization. They also spend the majority of their lives as a gametophyte, a haploid organism, which is a trait they retained from their algal ancestors (Figure 3).

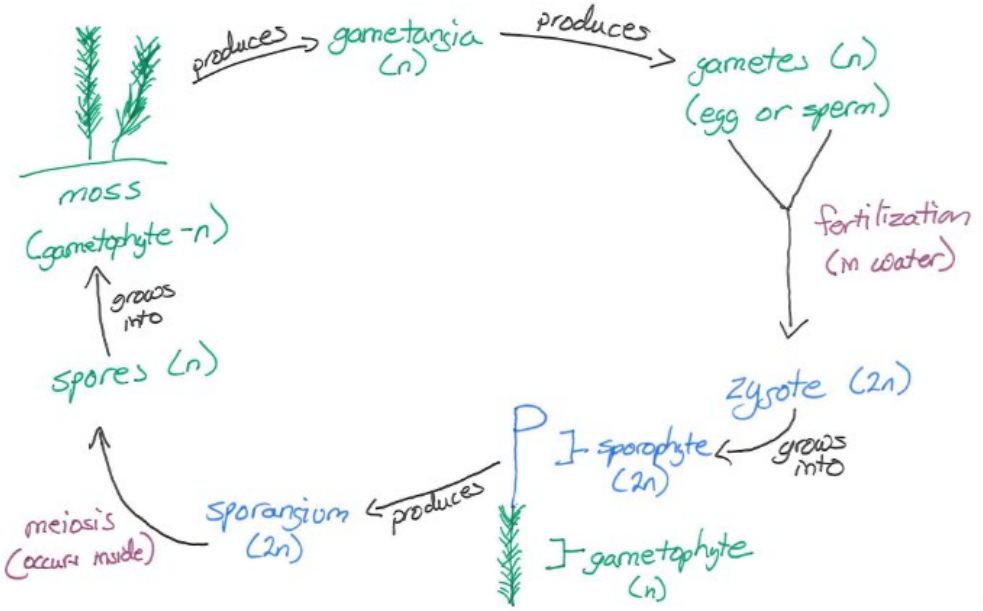



Figure 3: This is the alternation of generations life cycle of a general moss. Haploid (n) refers to components of the gametophyte generation while diploid (2n) refers to components of the sporophyte generation. Drawing Credit: TR Robertson, CCGA DNS. 2023.

As you examine the live specimen, consider how this moss's structures allow it to survive in on land compared to the green algae, yet still need to remain in a moist environment. Then look at the thallus 'leaf' cross section slide. Keep in mind that mosses do not produce true leaves, stems, or roots due to the simplicity of the tissues and not having any vascular tissues to transport water and nutrients throughout the plant. As you examine the slide, make sure you can locate the epidermis and cuticle, as well as the chain-like lamella which is involved in photosynthetic cells and gas exchange.

1. Provide a rough sketch of the live specimen and include a brief description of its overall characteristics.

2. Now provide a rough sketch of the associated thallus slide and include a brief description of what you observed of its overall characteristics. You may need to really zoom in so you can label the epidermis, cuticle, and lamella.

Consider the following questions as you interpret your results:

- How would this organism transport water throughout its body?
- What features do you think allow this organism to survive in terrestrial environments? Why do you think this?
- What do you think would happen if this moss was placed in a terrestrial arid environment? Why do you think this?
- Like their algal ancestors, terrestrial bryophytes must release their sperm into water for fertilization. Suggest a possible advantage and disadvantage of this reproductive strategy.

## Task 3 – Monilophytes: Ferns (*Polypodium* sp.)

Monilophytes or seedless vascular plants (e.g. ferns) are the first terrestrial plants to produce vascular tissues. They also produce true roots, stems, and leaves, and they are able to grow quite large. However, they are still restricted to moist habitats due to needing water for fertilization for their flagellate sperm. Monilophytes, or seedless vascular plants, belong to the Division Monilophyta, with the largest group being the ferns (Class Polypodiopsida).

Another advantage monilophytes have is they spend the majority of their lives as a sporophyte in the sporophyte generation (Figure 4). They greatly reduced their gametophyte generation to the size of a half dollar coin, which is much smaller than mosses, and typically only lasts for a few days. There are many advantages to switching to a sporophyte dominant generation, but the general view is by having two sets of chromosomes (diploid), this provides more potential for evolutionary adaptations to evolve.

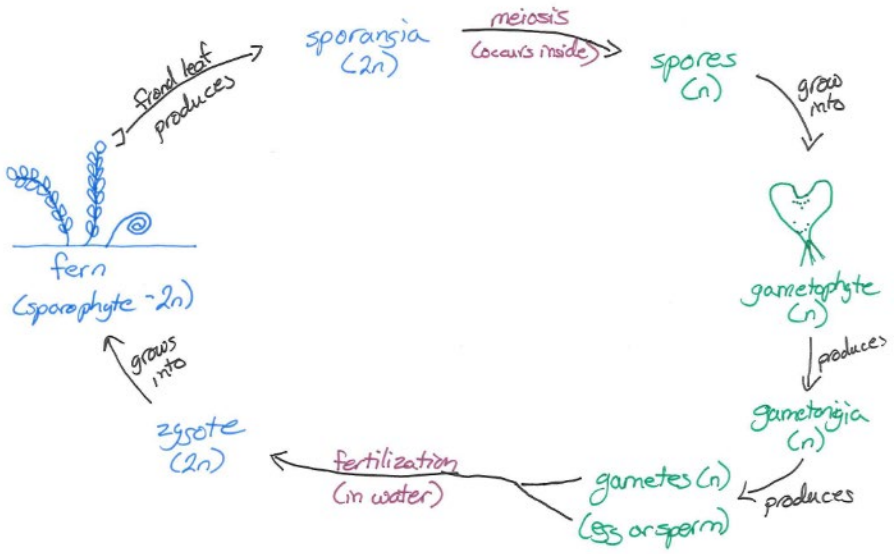



Figure 4: This is the alternation of generations life cycle of a general fern. Haploid (n) refers to components of the gametophyte generation while diploid (2n) refers to components of the

sporophyte generation. Drawing Credit: TR Robertson, CCGA DNS. 2023.

The development of heterospory in this division starts to be seen, although ferns are primarily homosporous (Figure 5). **Homospory** is when the sporophyte produces a single type of sporangium, which in turn produces a single type of spores. All spores thus produced are capable of growing into mature, multicellular gametophytes. **Heterospory** is when a sporophyte produces two types of sporangia: microsporangia and megasporangia. The microsporangia will produce male spores, known as microspores. Microspores grow into microgametophytes which will produce sperm. Megasporangia will produce female spores, megaspores. Megaspores grow into megagametophytes which will produce the eggs. This allowed the separation of sexes at different points in the life cycle and promotes outcrossing among plants.

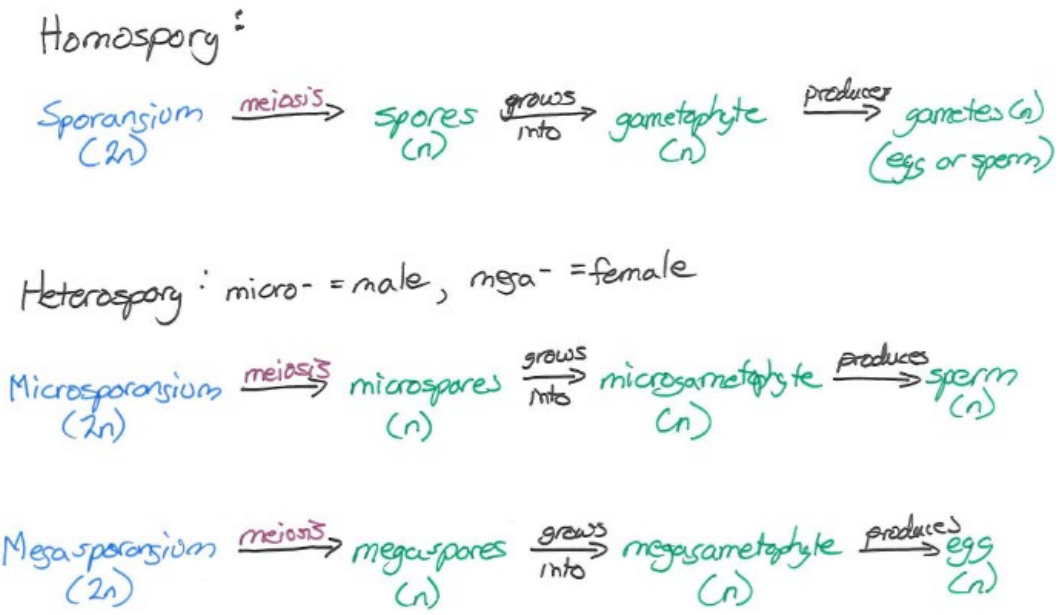



Figure 5: This is a general comparison of homospory versus heterospory in the alternation of generations life cycle. Haploid (n) refers to components of the gametophyte generation while diploid (2n) refers to components of the sporophyte generation. Drawing Credit: TR Robertson, CCGA DNS. 2023.

As you examine the live specimen, consider how this fern's structures allow it to survive on land compared to the mosses, yet still need to remain in a moist environment. Then look at the leaf cross section slide. You will also see more complexity of tissues, and you see a large midvein and smaller veins. These contain the vascular tissues, which transport sugars and water throughout the leaf. The rest of the tissue between the two epidermis is called mesophyll. Mesophyll cells contact chloroplast so is where photosynthesis occurs. As you examine this slide, make sure you can locate the epidermis, cuticle, mesophyll, and vascular tissues. You may also see a sorus, which is a cluster of sporangia.

Finally, you will examine a slide showing the gametophyte (a haploid organism) with the sporophyte (a diploid organism) growing out of it. Remember, the gametophyte produces gametangia (archegonium for egg production and antheridium for sperm production). Although the gametophyte releases its sperm into water, it doesn't release the eggs so fertilization occurs on the individual. As the zygote grows into an embryo and finally into an adult sporophyte, it utilizes the energy from the gametophyte until the gametophyte dies. As you examine this slide, make sure you can recognize the gametophyte from the sporophyte.

1. Provide a rough sketch of the live specimen and include a brief description of its overall characteristics.

2. Now provide a rough sketch of the associated leaf slide and include a brief description of what you observed of its overall characteristics. You will need to label the epidermis, cuticle, mesophyll, and vascular tissues.

3. Finally, provide a rough sketch of the gametophyte and sporophyte and include a brief description of what you observed.

Consider the following questions as you interpret your results:

- Why was producing vascular tissue such an evolutionary advantage in plant evolution?

- How are monilophytes (seedless vascular plants) similar to and different from bryophytes (mosses)?

- What advantages do you think the fern leaf structure has compared to the moss thallus? Why do you think this?

- Monilophytes spend the majority of their lives in the sporophyte generation. What advantages do you think this shift from the dominant gametophyte generation of mosses to the sporophyte generation provided these seedless vascular plants? Why do you think this?

## Task 4 – Gymnosperms: Conifers (*Pinus* sp.)

One of the most dramatic innovations to arise during vascular plant evolution was the seed. Seeds not only provide protection to a developing embryo, but also provide a food source and aid in dispersal of the contained embryo. Seeds have allowed the seed plants (gymnosperms and angiosperms) to dominate the globe. Gymnosperms first appeared in the late Paleozoic (~275 million years ago) with four divisions still alive today. All gymnosperms are heterosporous and have further reduced their gametophyte generation to several cells (Figure 6). By doing this, it allowed more energy to be used for pollen production which protects and transports the sperm so that water is no longer required for fertilization. It has also allowed more energy for seed production. Both adaptations have allowed gymnosperms to spread beyond moist environments.

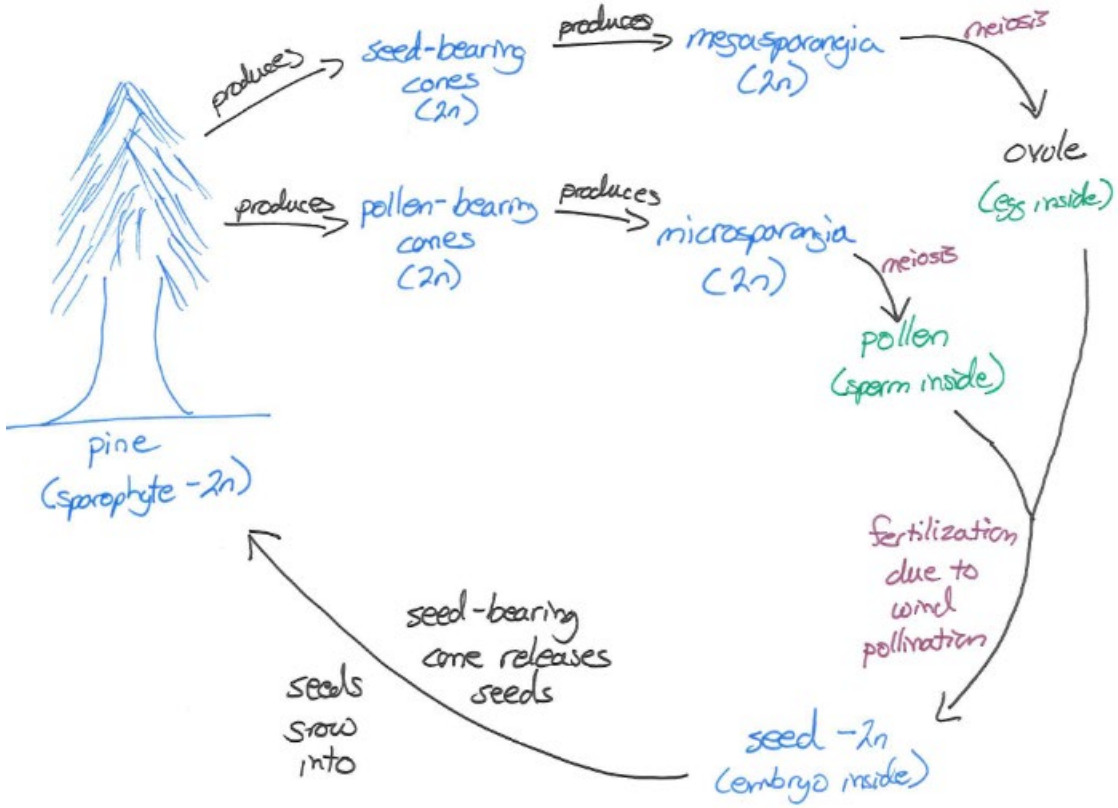



Figure 6: This is the alternation of generations life cycle of a general pine. Haploid (n) refers to components of the gametophyte generation while diploid (2n) refers to components of the sporophyte generation. Drawing Credit: TR Robertson, CCGA DNS. 2023.

The largest division of gymnosperms is the Coniferophyta, which include many different types of conifers such as pines, spruces, and junipers. Conifers (e.g. pines – *Pinus* sp.) produce pollen and seed-bearing cones, and mainly rely on wind for pollination. These plants have many adaptations for cold, arid environments. They also produce woody growth which allows survival through many environmental conditions (e.g. drought, fire, cold, etc.). Many conifers grow slowly, but some are able to live hundreds to even thousands of years.

As you examine the live specimen, consider how this pine's structures allow it to survive in drier environments compared to the ferns and mosses. Then look at the leaf cross section slide. Conifer leaves tend to be needle or scale like, which work well in cold, arid environments. You will also even more complexity of tissues, and 2-3 larger vascular bundles. You will may see resin ducts. These secrete a resin which assist in deterring herbivory and give the conifers their classic scent. As you examine the slide, make sure you can locate the epidermis, a thicker cuticle, mesophyll, vascular tissues, and resin ducts.

Finally, you will examine a slide showing gymnosperm pollen. Remember, pollen are specialized structures that protect and carry the sperm, so water is no longer required for reproduction. As you examine this slide, make sure you notice the bladder-shaped wings. This shape is useful for wind pollination.

1. Provide a rough sketch of the live specimen and include a brief description of its overall characteristics.

2. Now provide a rough sketch of the associated leaf slide and include a brief description of what you observed of its overall characteristics. You will need to label the epidermis, cuticle, vascular tissues, mesophyll, and resin ducts.

3. Finally, provide a rough sketch of the gymnosperm pollen and include a brief description of what you observed.

Consider the following questions as you interpret your results:

- Why was producing seeds such an evolutionary advantage in plant evolution?

- How are gymnosperms similar to and different from monilophytes?

- What advantages do you think the pine leaf structure has compared to the fern leaf? Why do you think this?

- Because gymnosperms produce pollen, they no longer require water for fertilization. What could be a possible advantage and disadvantage with this reproductive strategy?

# Task 5 – Angiosperms: Flowering Plants (eudicot species & monocot species)

Angiosperms also produce pollen and seeds, but they are the only plant group to produce flowers and fruits. They are the largest plant group, have the greatest diversity, and can be found world-wide. Angiosperms are also vital to human civilization and provide the majority of our food, clothing, and medicines.

Flowers allowed angiosperms to attract their pollinators, which provided more precise pollination than wind pollination. Flowers are often brightly colored and have strong scents to attract specific pollinators, such as bees, hummingbirds, bats, and even rodents. After pollination and fertilization, as the seeds develop, the ovary of the flower will grow into a fruit. The fruit not only protects the seeds, but it also aids in transporting the seeds away from the parent plant. Animals will consume the fruit and seeds, and defecate the seeds away from the parent plant, limiting competition between the offspring and the parent.

Angiosperms are in the Division Magnoliophyta. All are heterosporous and have even further reduced their gametophyte generation to a few cells so there is no longer a need to produce complex gametangia (Figure 7). This freed up even more energy for flower and fruit production.

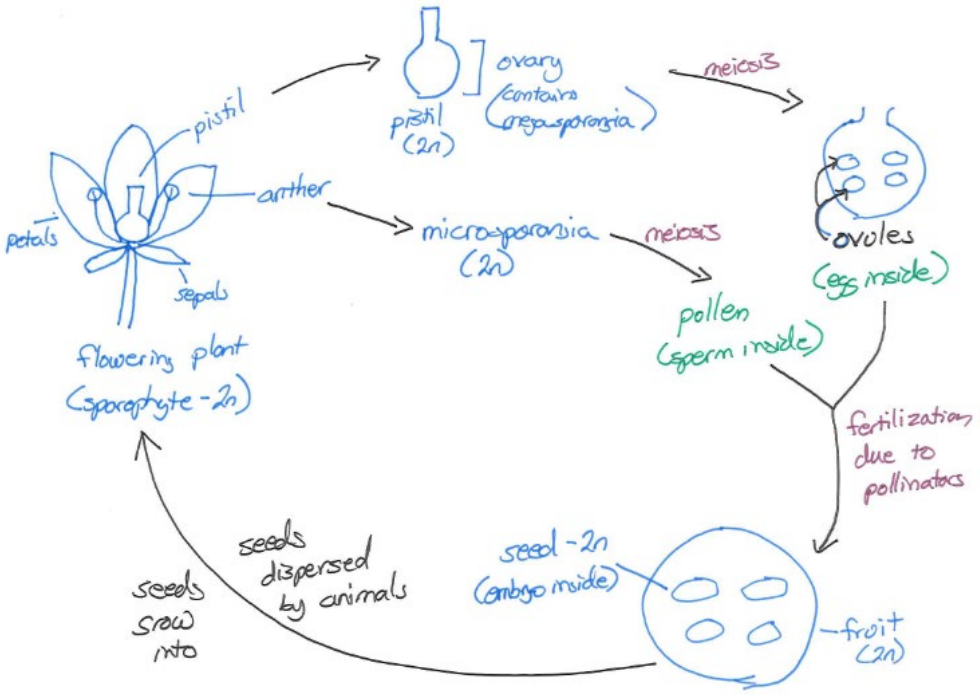



Figure 7: This is the alternation of generations life cycle of a flowering plant. Haploid (n) refers to components of the gametophyte generation while diploid (2n) refers to components of the sporophyte generation. Drawing Credit: TR Robertson, CCGA DNS. 2023.

Angiosperms are the largest group of plants alive today. They compromise a number of evolutionary lines, with most belonging to two general groups: monocots (Lilopsida) and eudicots (Magnoliopsida). Monocots, such as grasses, lilies, and irises, generally have a fibrous root system, narrow leaves with veins parallel each other, and flowers with 3 petals. Many of our grain crops (e.g. barley, rice, oats, and corn) come from this group. Eudicots, such as roses, cotton, beans, and maple, generally have a taproot system, broader leaves with veins that branch to form net-like patterns, and flowers with 4-5 petals. Some eudicots can also produce woody growth, such as oaks and maples. Most of our food and clothing come from this group.

You will be provided a live specimen and leaf slide representative from the monocot and eudicot groups. As you examine the live specimen, consider how each group's structures allow it to survive multiple environments compared to the other groups. Then look at the two leaf cross sections. You will see even more complexity of in the mesophyll and vascular tissues. The eudicot has two different types of mesophyll, which allow for greater light capture. You may also see bulliform cells in the monocot. These help the leaves to roll up during drought conditions. As you examine the slides, make sure you can locate the epidermis, a thicker cuticle, mesophyll variation, vascular tissues, and bulliform cells.

Finally, you will examine a slide showing a cross section of a corn seed (grain). Remember, seeds are specialized structures that contain the embryo and a food source (known as endosperm in angiosperms). Seeds form after the sperm from pollen fertilizes the egg contained inside an ovule. Both gymnosperms and angiosperms produce seeds; however,

angiosperm seeds develop inside a fruit while gymnosperm seeds develop inside a cone. As you examine this slide, make sure you locate the embryo and endosperm. You may also see the outer seed coat.

1. Provide a rough sketch of the monocot and eudicot live specimens and include a brief description of each's overall characteristics.

2. Now provide a rough sketch of the associated leaf slide and include a brief description of what you observed of its overall characteristics for monocots and eudicots. You will need to label the epidermis, a thicker cuticle, mesophyll variation, vascular tissues, and bulliform cells.

3. Finally, provide a rough sketch of the angiosperm seed and include a brief description of what you observed.

Consider the following questions as you interpret your results:

- Why was producing flowers and fruit such an evolutionary advantage in plant evolution?

- How are angiosperms similar to and different from gymnosperms?

- What advantages do you think the angiosperm leaf structure has compared to the gymnosperm leaf? Why do you think this?

- Although both gymnosperms and angiosperms produce seeds, angiosperm seeds have a more nutritious food source (endosperm) inside. What could be a possible advantage and disadvantage with this reproductive strategy?

## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Provide two different structures or features bryophytes and monilophytes have that may have contributed to their success in terrestrial environments compared to algae? Explain why each one would be an advantage.

2. What advantages do pollen and seeds give gymnosperms and angiosperms over bryophytes and monilophytes? Provide two advantages and explain why for each one.

3. What advantages do flowers give angiosperms over gymnosperms? Provide two advantages and explain why for each one.

4. Create a table, flowchart, or diagram outlining the differences among algae, bryophytes, monilophytes, gymnosperms, and angiosperms.

# Plant Ecology: Invasive Species

Students must be prepared for fieldwork, including closed-toe shoes and other clothing to protect you from sharp plants, insects, and weather (sun, rain, etc.).

## Objectives

- Understand the concept of invasive species as well as the impact they have on biological communities – primarily in relation to other plants.
- Observe the biodiversity of plant life in coastal Georgia and understand the importance of plant diversity in terrestrial habitats.

## Introduction

Every organism is native to a particular habitat. However, either by accident or with intention species may be transported to a new environment. Oftentimes, these non-native species may be beneficial to us in some way or another. Much of the United States' agriculture is invested in non-native crops, like corn, soybeans, and oats; in fact, it is easier to list the few species of crops that are native to the U.S. than those that are not: pecans, sunflowers, cranberries, and a few legumes, for example. In addition, there are many plants grown in horticulture that are non-native, such as lilacs, willow trees, and irises. Non-native livestock includes cattle, pigs, sheep, horses, and chickens. Most domestic animals, including our pets, are considered non-native. The honeybees we depend on to produce honey and pollinate our crops come from Europe! While we benefit from many of these non-native species, issues often arise when some of them overpopulate and cause ecological issues.

**What are native species?** Every species has specific area in the world where it has existed and adapted for thousands of years. This area is referred to as the **native range** of the species. The Eastern red cedar (*Juniperus virginiana*), for example, is a coniferous tree native to North America, whereas the salt cedar (*Tamarix gallica*) is native to the Mediterranean.

**What are exotic species?** A **non-native** or **exotic** species is one that has been introduced into an area from somewhere else. Not all of these introduced species are harmful; in fact, some exotic species may be beneficial. Some plants are introduced as ornamentals, such as the mimosa tree (*Albiza julibrissin*) and chrysanthemum (*Chrysanthemum*). Of course, as mentioned earlier, some supply us with food such as oats, peaches, almonds and even animals like European honeybees (*Apis mellifera).* They are not native to the United States but pose little or no threat to our natural ecosystems. In cases like this, these species were introduced intentionally and their spread outside their native range has been managed. However, if a species spreads out of control and it begins to outcompete natives or negatively impact the environment, it becomes **invasive**.

**What are invasive species?** An **invasive species** is one that is not native to the ecosystem that it has been introduced to AND causes either economic or environmental harm to native species or human health. Consider that these species are introduced outside of their native ranges and how they are now escaping the selective pressures that would otherwise restrict their spread or population growth. For example, native predators may not know how or even

may not be able to prey on invasive species. Diseases in their native ranges may have also controlled their populations, but not in the area they are introduced to. In addition, native species may not be adapted to compete with the invasive species. Therefore, they can pose a significant threat to the biodiversity of the new area and negatively impact both agriculture and forestry. A famous example is kudzu (*Pueraria lobata)*, the "vine that ate the South," which was introduced in 1876. It was planted with good intention, to control erosion, but it has no predators outside of its native range in Japan. It now covers 150,000 acres annually and controlling it costs $6 billion dollars each year.

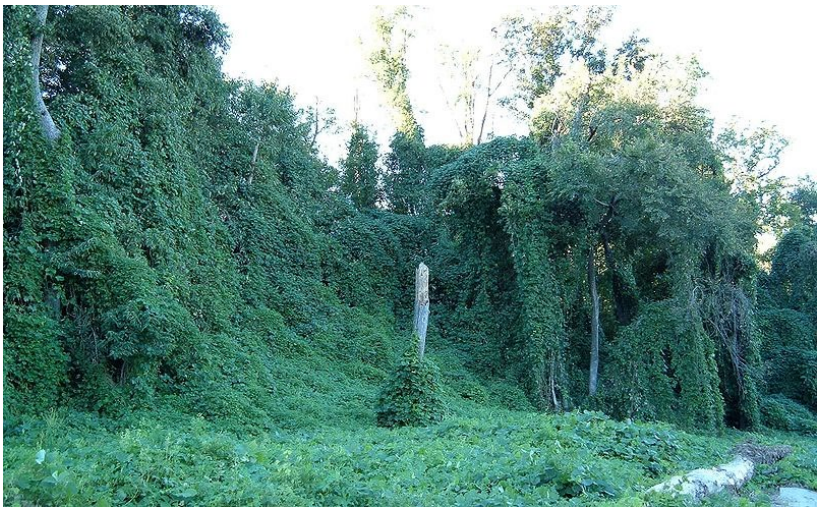


Figure 1. Kudzu – *Pueraria lobata* – in Atlanta Georgia. Photo by Scott Ehardt, Public domain, via Wikimedia Commons.

With the introduction of an invasive species, many native species are unable to compete and they are pushed out of their native ranges (eventually even being driven to extinction, in some cases). Some biologists believe the threat posed by invasive species is second only to loss of habitat as the primary reason for the loss of the Earth biodiversity in recent years. A number of species here in Georgia are threatened by invasive species. For example, the red bay ambrosia beetle was brought from Asia to Georgia in wooden packing material, such as wooden shipping pallets in 2008. Native red bay trees and their relatives, such as bay laurels, sassafras, spicebush, and even the introduced avocado trees in the South are vulnerable to the disease spread by these beetles – a disease called *Laurel Wilt.*

# Task 1 – Field Survey of Invasive vs. Native Plants

- We will begin with a field survey of invasive vs. native plants at a particular location on campus (specified by your instructor).
- Because this a field lab, you are expected to dress appropriately (for warm or cold weather depending on the semester). Bring water and outdoor clothes (those you may not mind getting dirty or sweaty). In addition, you may want to bring your own bug spray and sunscreen, as we may be outdoors for two or more hours.
- As we survey, we will collect the following information, and record in the table below.

Location of Survey: _____ Time:_____ Date:_____

| Common Name | Species Name | Habitat | Invasive? (Y/N) | No. Observed |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | **Total:** | |

Consider the following questions as you interpret your results:

- What percentage of plants were invasive in this habitat? What percentage of plants were native?

- Why was one percentage higher than the other? What advantages do you think this group has over the other group in this habitat?

- Note the environmental conditions for this habitat. How do you think this differs from the environmental conditions deeper into the forest?

# Task 2 – Herbivory Impacts on Invasive vs. Native Plants

- With your student group, find two invasive plants and two native plants that have at least 20 leaves on each plant (select plants within our area of survey).

  Invasive plants:

  Native plants:

- Randomly select five leaves from each plant. Place the leaves in separate, labeled bags (invasive 1, invasive 2, native 1, and native 2). Estimate the amount of **herbivory** (the consumption of living plant tissue by animals) of each leaf by using the plastic overlay with gridlines. Count the number of total squares that show any evidence of predation, disease (such as fungus), or parasitism.

| Leaves from Invasive Plant #1 | # Squares with Herbivory | Leaves from Invasive Plant #2 | # Squares with Herbivory | Leaves from Native Plant #1 | # Squares with Herbivory | Leaves from Native Plant #2 | # Squares with Herbivory |
|---|---|---|---|---|---|---|---|
| 1 | | 1 | | 1 | | 1 | |
| 2 | | 2 | | 2 | | 2 | |
| 3 | | 3 | | 3 | | 3 | |
| 4 | | 4 | | 4 | | 4 | |
| 5 | | 5 | | 5 | | 5 | |
| Average | | Average | | Average | | Average | |

Consider the following questions as you interpret your results:

- How does the amount of herbivory compare between invasive plant leaves and native plant leaves?

- Why was the amount of herbivory in one group of plants higher than the other? What defenses do you think this group has over the other group in fighting herbivory?

- What other factors could possibly be influencing the amount of herbivory for each group? Consider environmental conditions and natural control (predation, disease, etc.) to assist in answering this question.

- Based on the data you collected, would you say that natural control (predation, disease, etc.) of the non-native plants is comparable to that of native plants? Why or why not?

# Task 3 – Plant Diversity

As you learned in lecture, plants are divided among those that are vascular (have vessels for transporting sap and water) and those that are non-vascular, which have no vascular tissues and thus no true roots, stems or leaves. Among the non-vascular plants, the largest and most diverse are the **Bryophytes** – the so called "true mosses." These plants are low-growing, sprawling, and most often found near water sources. More advanced, but often found near sources of water, are the vascular **Monilophytes**, which include ferns, horsetails, and whisk ferns. These plants are more advanced than the bryophytes because they have true roots, stems, and leaves – and unlike the mosses, can grow tree-sized in some cases – but they still require water to reproduce, as they have swimming sperm. Though, unlike other vascular plants, they do not produce seeds.

The vascular seed plants are the most advanced plants today, and include two major groups, the **Gymnosperms** and **Angiosperms**. The gymnosperms reproduce primarily using pollen and seed-bearing cones. They rely mainly on wind for **pollination** (the act of bringing pollen close to an ovule). The most diverse division within the gymnosperms are the **Coniferophyta**, which are the needle and scale-leaved conifers, such as pines, spruces, cedars, etc.

The Angiosperms are mostly categorized within the division **Magnoliophyta** and are most well-known for their possession of both flowers and fruit. As such, they rely primarily on animal pollinators, such as bees, hummingbirds, and even rodents to bring pollen close to their ovule within the flower. As a result, flowers often are brightly colored and have strong scents associated with them. After pollination and following fertilization, a fruit develops from the ovary. Each fruit contains seeds with developing embryos, which are protected within the seed until germination.

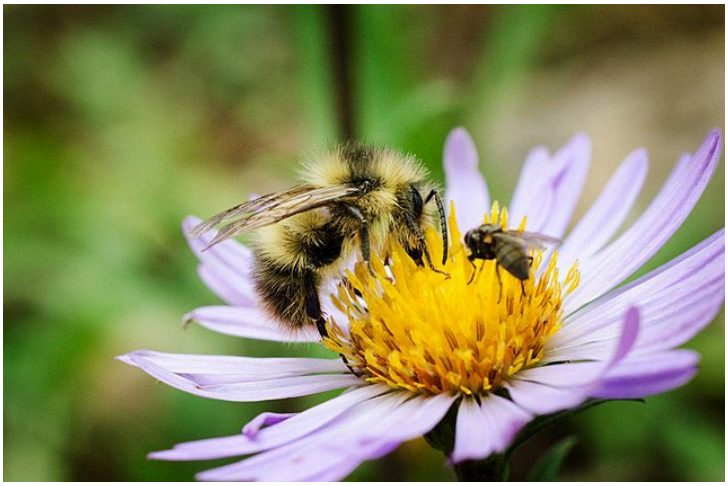


Figure 2. Bumblebee and fly acting as pollinators. Photo by Jakub Fryš, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons.

- Use your remaining time in lab to find and identify a plant representative for each of the groups mentioned above. Provide a rough sketch of each plant and a brief description (of its habitat and the plant's characteristics) in the spaces provided below.

| #1 - Bryophyte | #2 - Monilophyte |
|---|---|
| #3 - Gymnosperm | #4 - Angiosperm |

Consider the following questions as you interpret your results:

- What type of habitat conditions did you find for each group? Why do you think some groups were more restricted than the other groups?

- Which group had the tallest plants? What would be the advantage of growing so tall in this habitat?

- Which of the four plant groups (bryophytes, monilophytes, gymnosperms, or angiosperms) represented the bulk of diversity in today's survey? Why do you think this is?

## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Do you think the number of invasive species would have been higher or lower if we had surveyed the undisturbed area of the forest interior, rather than the disturbed forest edge? Explain your reasoning.

2. How do you think herbivory (or lack thereof) will affect the distribution and spread of these non-native plants? Do you think we need to introduce a method of control for these non-native plants in our area? Explain your reasoning.

3. Most invasive plants are angiosperms. Considering what you know about angiosperms, what characteristics do you think invasive flowering plants utilize that allow them to be more successful against native flowering plants?

# Diversity and Ecology of Fungi

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Become familiar with three major phyla of fungi.
- Understand the vital role fungi play in decomposition (spoilage), food production, and mutualistic relationships.

## Introduction

The eukaryotic kingdom Fungi includes some of the most recognizable organisms, such as mushrooms and bread mold. Yet their relationship to other organisms was for many years in debate. Superficially, they resemble plants, with branching, root-like systems, stems-like structures, and cell walls. But this is where their similarity ends. Evolutionarily, they share more features with the Animalia than they do plants, specifically motile zoospores and heterotrophic feeding behavior.

Relationships aside, this group is unique in its own right. The structures that most people are familiar with, such as the "fuzz" of mold and the mushroom found in the woods, are reproductive structures that produce the haploid spores necessary to reproduce. The main body of a fungus is called the **mycelium**, which usually exists underground (or in the substrate) and appears as a root-like system of **hyphae**. These hyphae are composed of interconnected cells with a common cytoplasm.

Like plants, they have a haplodiplontic life cycle. However, the diploid stage in most fungal phyla is very short. For most of a mature fungus' life, it either exists as a haploid (n) organism, or a **dikaryon** (n + n) organism.

In today's lab activity, we will review the characteristics and make general observations about the following three (3) phyla of fungi:

- Zygomycota
- Ascomycota
- Basidiomycota

## Phylum Zygomycota

The Phylum Zygomycota includes many recognizable species of mold, such as the common black bread mold, *Rhizopus stolonifera*. These molds readily cultivate on rotting food, such as breads, vegetables, and fruit. Therefore, most of these species are important decomposers in their environments. While many species are **saprobes** (feeding off decaying material), a few species are parasitic. While some Zygomycetes impact the economy in a negative way by spoiling produce and food, some are important commercially in the synthesis of semi-synthetic steroid hormones.

The hyphae of Zygomycete molds are **coenocytic**, meaning that there are no divisions between their cells. This allows for rapid transport of nutrients among the mycelium, which explains why many of these molds seem to grow rapidly overnight. Zygomycetes also asexually reproduce quickly, using structures called **sporangia** (Figure 1). The black tips of such a bread mold are the swollen sporangia packed with black spores. When these spores fall onto a new source of food, such as a piece of bread left on a counter, a new mycelium begins to grow from each spore (Figure 1).

Sexual reproduction in Zygomycetes begin when conditions are favorable (plentiful food, high humidity). The haploid mycelia of two mating types (+ and -) begin secreting chemical signals to attract one another. They begin growing toward one another and where they touch, the cells on the end of the hyphae fuse in an act called **plasmogamy**. At this point, the two fused cells contain two distinct nuclei from separate individuals – a **dikaryotic** cell. When the time is right, the nuclei within the cell fuse in an act called **karyogamy** (very much like fertilization), and a diploid cell is formed. A swollen sporangium containing sexually-produced spores, called the **zygosporangium**, forms. It is from this sexually-produced structure that the Zygomycota get their name.

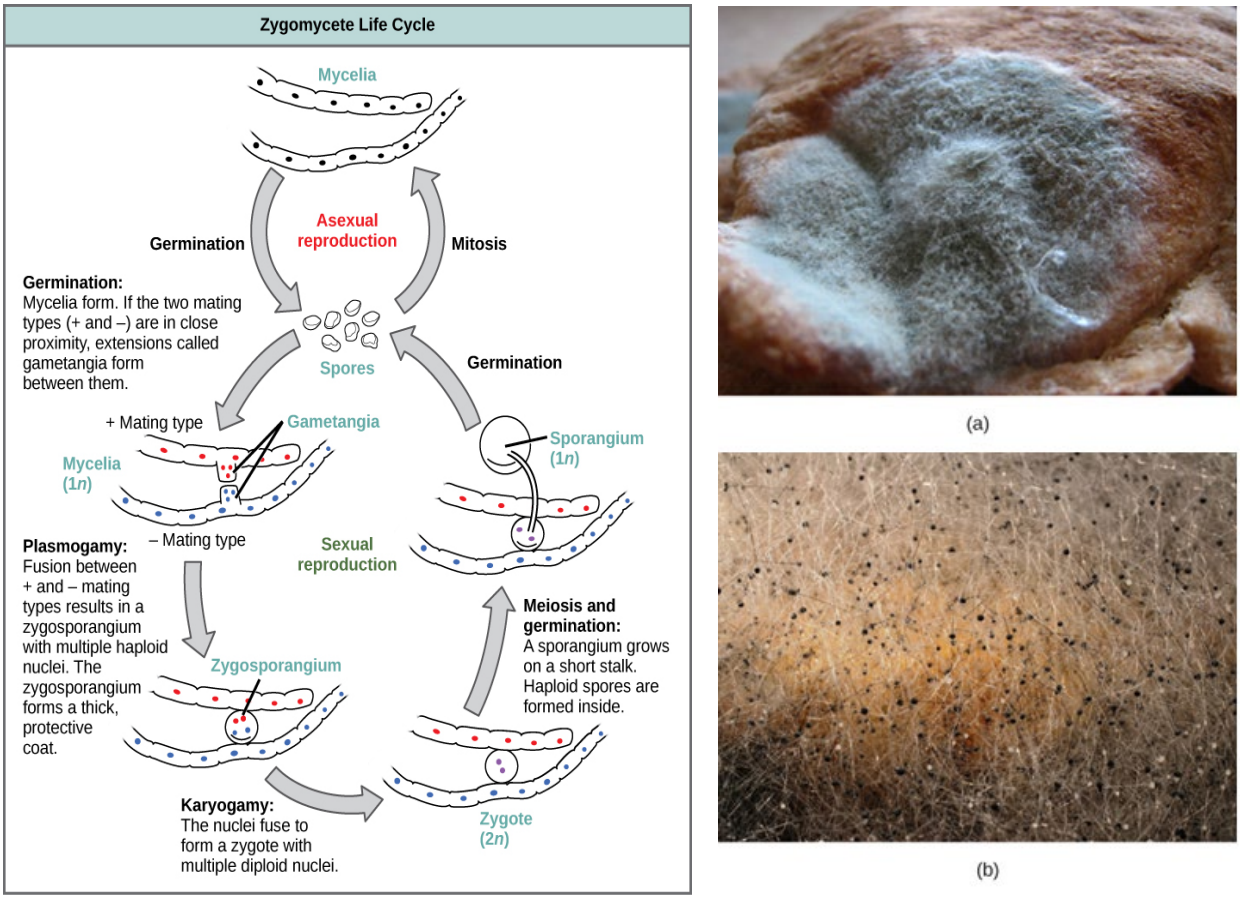


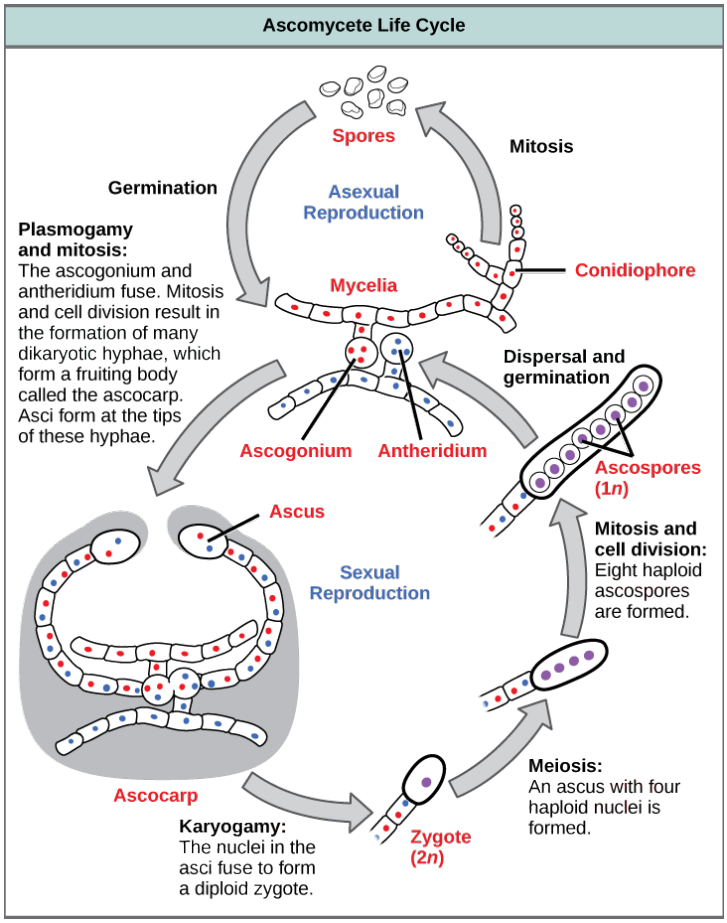

Figure 1. Zygomycetes have sexual and asexual life cycles. In the sexual life cycle, hyphae from two mating types fuse to create a zygosporangium. The white fuzz (a) of a common bread mold is actually the tips of the spore-producing sporangia. The black bread mold, *Rhizopus*, produces sporangia with black spores (b).[1]

# Phylum Ascomycota

The vast majority of fungal species belong to the Phylum Ascomycota. These fungi include many common edible forest fungi, such as truffles and morels. Other species play a vital role in food production, especially the single-celled Ascomycetes known as **yeasts**. Yeasts are used in baking, brewing, and wine fermentation. One of the most well-known yeast species is baker's or brewer's yeast, *Saccharomyces cerevisiae.* Some Ascomycetes are molds, such as *Aspergillus oryzae* is used in the fermentation of rice to produce sake. Still others are known to cause disease, such as a variety that causes Ringworm or Jock Itch, *Epidermophyton*.

Multicellular ascomycetes produce hyphae which have partial walls between cells, called **septa** (unlike the bread molds). These partial walls also allow for rapid growth and nutrient transport.

Asexual reproduction in Ascomycetes is best seen in the mold and yeast forms. In yeasts, they commonly produce clones through budding. The mold forms produce stalks with cloned haploid spores called **conidiophores** (Figure 2). Sexual reproduction is more complex, involving two haploid mating types of hyphae, one called an ascogonium and the other an antheridium to fuse through plasmogamy. Once fused, they form a sac called an **ascus**, which initially is dikaryotic. Eventually, when conditions are right, they fuse their haploid nuclei within the ascus and produce eight sexually-produced **ascospores**. When mature, these eight spores are jettisoned into the air by the ascus. It is from this ascus that Ascomycota get their name.



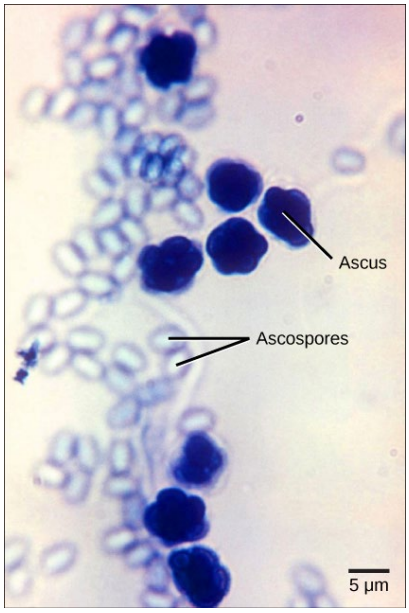




Figure 2. Left: The asexual stage of an Ascomycete is characterized by the conidiophore. The sexual stage, if present, is characterized by the eight-spore sac called the ascus. Above: A bright field light micrograph that shows ascospores being released by the yeast *Talaromyces flavus*.[1]

# Phylum Basidiomycota

The fungi within the Phylum Basidiomycota are some of the most recognizable fungi, such as those that you purchase from supermarket shelves. The edible button mushroom, *Agaricus bisporus*, and the shiitake*, Lentinula edodes*, are two well-known examples. Some, like shelf and bracken fungus, are commonly found on rotting logs. Basidiomycetes are among the principle decomposers of such trees, being able to break down the main components of wood, cellulose and lignin. While many of these fungi are beneficial, with some being edible, it is also important to note that some are extremely toxic. The toadstool known as the eastern destroying angel, *Amanita bisporigera*, produces a deadly toxin.

The Basidiomycete fungi are sometimes referred to as "gill fungi" because of the presence of gill-like structures on the underside of the mushroom cap. The "gills" and mushroom cap are actually compacted hyphae on which spore-producing structures are borne. These fungi are only known to sexually produce spores, in an act reminiscent of the Ascomycetes. As with such fungi, they fuse the hyphae of two mating types through plasmogamy. At the edge of each "gill," a swelling of the two hyphae produces a club-like structure called a **basidium.** This basidium then produces four haploid spores- called basidiospores (Figure 3). The basidia hang from the underside of the mushroom cap, and are generally dispersed by wind. A single mushroom cap may release trillions of such spores. It is from the basidium that Basidiomycetes get their name.

It is important to note that the haploid stage of a Basidiomycete fungus is very brief and consists of two haploid secondary mycelia. Once fused, they produce a dikaryotic primary mycelium. This primary mycelium produces the reproductive mushroom above ground, where karyogamy and spore production takes place (Figure 3).

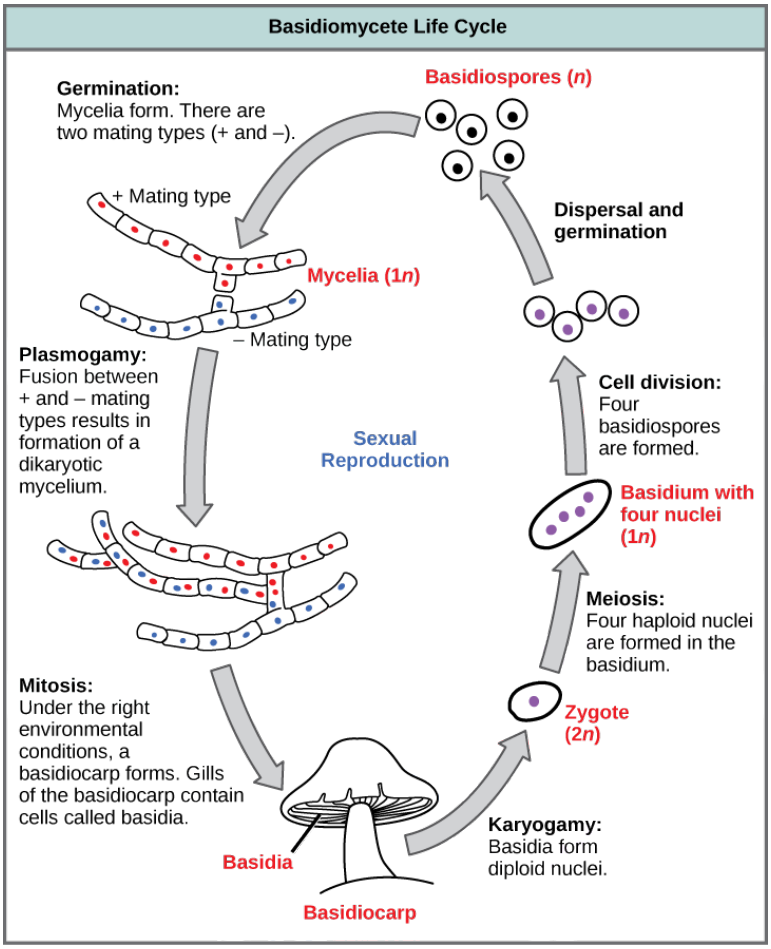



Figure 3. Left: A button mushroom, *Agaricus bisporus.*[2] Right: The lifecycle of a basidiomycete which displays the dikaryotic primary mycelium, along with formation of a basidium and mushroom cap.[1]

# Molds

Molds are a name we give fungi that live on the surface of plant and animal matter, decomposing it. Molds belong to either the phylum Zygomycota or Ascomycota. Molds are partly responsible for food spoilage, along with bacteria, and are visible due to the surface sporangia they use to asexually reproduce. Black bread mold (*Rhizopus stolonifer*), for example, has very large, black-colored sporangia, which are clearly visible as a dark, fuzzy patch on the surface of spoiled food. However, remember that the main body of a fungus is below the surface. The mycelium of a mold is growing throughout the spoiled food itself.

While many molds are the principal agents of decaying food, they can also be used to age or cure foods. Salami, for example, is aged using mold. That's what gives salami it's distinctive pale coloration on the surface (when it's not sliced). And of course, cheese is aged and often distinctly flavored using completely edible forms of mold. In this part of the laboratory exercise, we will review several different forms of mold and how each is responsible for both spoiling and producing food.

# Task 1 – Food spoilage

1. Your professor will provide you with bread that has been intentionally exposed to the environment, allowing it to mold. You will receive a small chunk of bread that has mold on the surface.
2. Obtain a dissecting microscope, and observe the surface of the chunk of bread, specifically noting the colorful colonies of mold on the surface. Make sure to increase objective magnification as much as possible, as mold structures are microscopic in nature. Answer Question #1 in the discussion section.
3. Take some time to observe the interior of the bread mold, specifically the interior spaces in the bread (the portion where air was trapped inside the bread). Note the presence of white filaments, or hyphae within the bread. Answer Question #2 in the discussion section.

Once you have sufficiently observed the chunk of bread and mold colonies, you will prepare a wet mount of the mold colony.

4. Set out your compound light microscope.
5. Obtain a blank microscope slide and place a single drop of Lactophenol cotton blue solution in the middle of the slide.
6. Take a small piece of clear scotch tape and press it against the surface of the bread where the mold colony is growing, being careful to not pick up any crumbs in the process.
7. Press the piece of tape, bread side down, over the drop of Lactophenol cotton blue and adhere the tape to the slide.
8. Mount your specimen under low power on your compound light microscope and increase magnification progressively until you can see entire hyphae, spores, and sporangia. Depending on the species of mold, you may have to adjust your objective magnification accordingly.

9. Use the provided mold I.D. keys to survey which species of mold you may have had growing on the surface of your chunk of bread. Answer Question #3 in the discussion section.

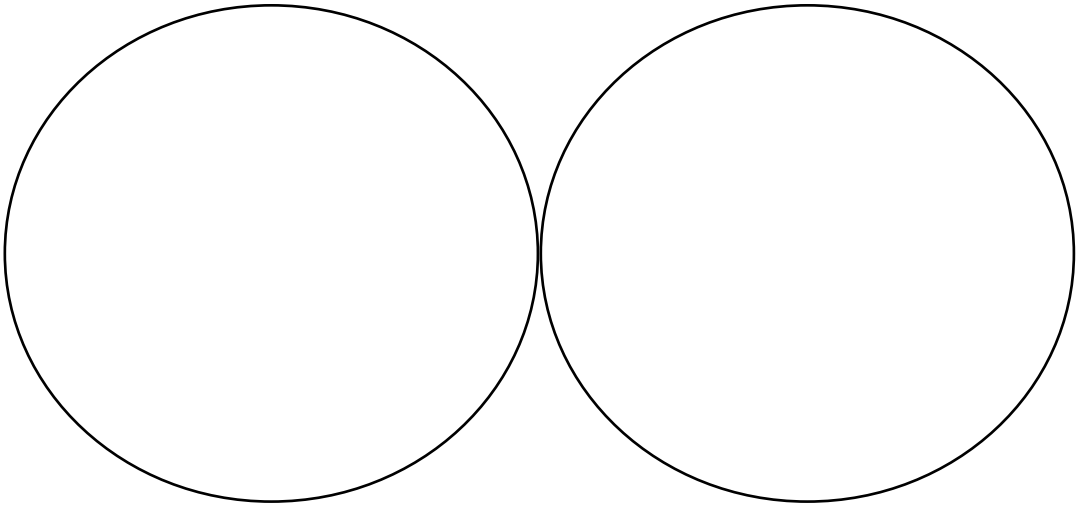
# Task 2 – Food Production

## Blue Cheese

1. Note the presence of both a slice of blue cheese (or Roquefort) and brie cheese on the side counter. Both have been cultured using a specific species of mold.
2. Obtain another blank slide, once again placing a drop of Lactophenol cotton blue solution in the center of it.
3. Using a clean toothpick, remove a small amount of the greenish mold from one of the crevices in the slice of blue cheese.
4. Swirl it around in the drop of lactophenol blue solution on the slide to dislodge it. Place a cover slip over it and return to your station.
5. Observe the specimen, working your way up to high power. Note the distinctive sporangia in the specimen and use the provided I.D. key to identify the species used to culture this cheese. Answer Question #4 in the discussion section.
6. Sketch your specimen at high power in the space provided below.

## Brie Cheese

1. Repeat steps 2-4 above to obtain a sample of brie cheese. However, obtain your sample by only wiping the surface (called the rind) of the cheese with the end of a clean toothpick. This should provide enough mold to prepare a wet mount.
2. As before, identify the mold used to culture this type of cheese using the provided I.D. key. Answer Question #5 and #6 in the discussion section.
3. Sketch your specimen at high power in the space provided below.



Blue Cheese Mold _____@400x  Brie Cheese Mold _____@400x

## Reproductive Structures

You've already seen how molds reproduce using spores and structures called sporangia. Sporangia can produce spores either through sexual or asexual reproduction. Larger fungi also have spore-bearing structures, though these are sexual structures. That is, they produce spores by fusing nuclei from two different individuals. These structures have different names, depending on which phylum of fungi we are studying. For example, fungi of the phylum Basidiomycota produce mushrooms – with the technical name **basidiocarp**. These include traditional mushrooms like button mushrooms, porcinis, and portabellas. These consist of a cap, stem, and gills. The gills contain club-shaped structures called **basidia**, each bearing four spores (see Figure 3 for their location). Ascomycota fungi often produce a cup structure, called an **ascocarp**, which contain spores within sacs call asci, rather than gills (see Figure 2). In this procedure, we will view the basidiocarp of the button mushroom, *Agaricus.*

## Task 3 – Basidiomycota Reproductive Structures

1. Get a mushroom from the side bench and place it on the paper towels in front of you.
2. Examine the mushroom closely. In the space provided, draw a diagram of the mushroom, **labeling the cap, stem** and **gills**. If the gills are not visible, remove the tissue (it's called a veil) protecting them gently with your forceps. Be careful not to touch the gills with the forceps.
3. Grasp the cap firmly with one hand and the stem with the other hand. Gently wiggle and/or twist the stem until it breaks away from the cap.
4. Pinch the stem between your fingers until it breaks into two or more long pieces. Gently pull the pieces apart. The thin, hair-like filaments you will see where you split the stem are the hyphae. Place the stem section under the dissecting microscope and examine the hyphae. Answer question #7 in the discussion section.
5. Place the stem pieces on a corner of your paper towel and turn your attention to the cap. Look at the underside of the cap to study the gills. Each gill is lined with thousands of small structures called **basidia**. Using your forceps, gently remove one gill from the cap. You will get better results if you GENTLY grasp the gill near where it attaches to the cap. Try to avoid touching the free edge, the one along the bottom of the gill, with your forceps. The basidia you want to see under the microscope are fragile and easily damaged if you aren't careful.
6. Place the gill on a microscope slide and use the standard procedure for preparing a wet mount.
7. Place the slide on the microscope and examine the gill under low power. Look at the edge of the gill that was not attached to the mushroom and look for the little finger-like projections. Switch the microscope to high power. Look at the finger- like projections under high power. These are the basidia. Draw & label the **gill** and **basidia** on this lab sheet. Answer question #8 in the discussion section.
8. After completing your observations and recording your data, clean off your slide and cover slip and place them as directed by your instructor.

Gill and Basidia _____@400x

## Lichens

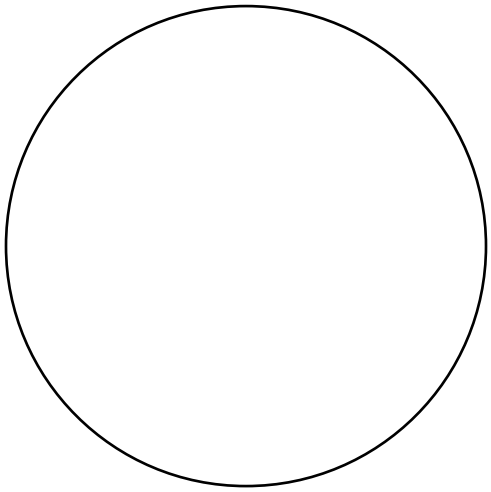
Lichens are not technically fungi, nor are they a specific phylum of organisms. In actuality, they are **mutualistic** symbiotic relationships of both an alga and a fungus. In most partnerships, the fungal portion is an ascomycete fungus, though new research suggests that many of these lichens contain both ascomycetes and basidiomycetes.[3] The other partner is most often a green alga or a cyanobacteria. The lichens are therefore two or more organisms, not one. In this case, the fungal hyphae create a watertight environment on the inside of the lichen, protecting the algae from desiccating or exposure to ultraviolet light, while the algae can carry out photosynthesis in safety. The algae therefore provide the host with a source of carbohydrates to feed on, while the fungi provide them a safe place to live.

There are three forms of lichen we recognize in today's lab (Figure 4):


Figure 4. Crustose lichen is nearly two dimensional and adhere directly to the substrate on which they grow. Foliose lichens are flattened as well, but somewhat leafy, like lettuce – you can clearly see an underside. Fruticose lichens are upright and shrubby in appearance.[3,4,5]

## Task 4 – Lichen Morphology

1. Note the sample of foliose lichen on the side bench. Using forceps, tear loose a small piece of lichen (a piece not much wider than the end of your forceps).
2. Obtain a blank microscope slide and place a single drop of distilled water in the center of the slide.
3. Holding the lichen with your forceps, hold it in the drop of water, then use a clean toothpick to rip it apart within the drop of water.
4. You should see small particles in the drop of water. Remove any larger particles, so you can place a coverslip on top of the specimen.
5. Observe your specimen under the compound light microscope, specifically to identify both the algae and the fungal hyphae. Sketch your specimen at high power in the space below.



Foliose lichen _____@400x

6. View specimens of lichen that have been set out on the side bench, noting the major differences between **crustose**, **fruticose**, and **foliose** lichens. Answer questions #9-10 in the discussion section.

## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. What color is the surface mold? Based on the color, how many types of mold do you observe?

2. What can you conclude about the contamination of food, when only surface mold has been scraped away?

3. List two or more species of mold that you identified in the contaminated bread here:

4. What species of mold is used to culture bleu cheese?

5. What species of mold is used to culture brie cheese?

6. *Research the following answer.* What are some other types of food, aside from bread, alcohol, salami, and cheese, that are prepared using molds?

7. Why do you suppose the hyphae that make up the structure of a basidiocarp are so densely packed, compared to the loose mycelium you might find underground?

8. What is the function of basidia?

9. Lichens and mosses often grow on the same substrates, such as tree trunks and rocks. How can you distinguish lichens from mosses when you come across them in the wild?

10. Lichens are particularly sensitive to air pollution and can thus be used as bioindicators of air quality. Why do you think that is?

# References


1. OER Commons. Biological Diversity. 18 July. 2023. Available from: https://oercommons.org/courseware/unit/8453
2. Jerzy Opioła, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via Wikimedia Commons
3. Rosa-Maria Rinkl, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons
4. Jan Czeczotka, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons
5. Rhododendrites, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons

# Animal Evolution & Diversity

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Become familiar with the characteristics of the Kingdom Animalia (Metazoa) as well as developmental characteristics that separate major clades within this Kingdom.
- Observe specimens of each major animal phyla and review their key characteristics.

## Introduction

All animals are united by the characteristics of being eukaryotic, multicellular, ingestive heterotrophs that are motile at some point during their life cycle. There are, however, several other features to consider when classifying animals. First, what kind of **symmetry** does the animal exhibit? The most primitive animals are the sponges (Phylum Porifera) which typically lack any form of symmetry. True symmetry may be observed in the form of **radial symmetry**, repeated body areas around a central axis, or **bilateral symmetry**, with identical right and left halves when divided from top to bottom (anterior to posterior). Bilateral symmetry is important among the animals as it gave animals a defined direction for movement, which led to the evolution of sensory structures on one end of the body (i.e., a head).

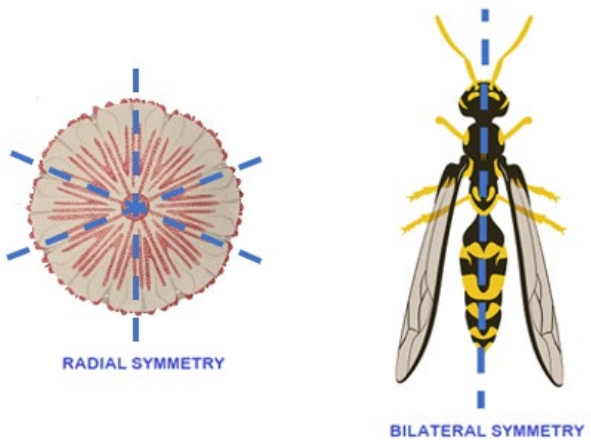



Figure 1. Animals which exhibit radial versus bilateral symmetry. Note that animals that display bilateral symmetry typically have a head and tail end. Animals with radial symmetry, such as the jellyfish above, do not have a head. The image of the jellyfish is public domain and the insect is credited to Vecteezy (CC-BY 4.0).

It is also important to consider the presence of tissues when comparing animals. Sponges, for example, are classified together by the lack of true tissues. In more well-developed animals with true tissues, there are three tissue types identified during early embryonic development that give rise to certain areas of the body. These embryonic tissues are the **ectoderm**, which

gives rise to outer body coverings and nervous system, the **mesoderm**, which gives rise to the muscles and skeleton, and the **endoderm**, which becomes the digestive tract. Animals which develop from only two germ layers are considered **diploblastic**, while those that develop all three are **triploblastic**.

Here we will review several animal phyla, based on morphological and developmental features, in order of increasing complexity:

- Phylum Porifera
- Phylum Cnidaria
- Phylum Platyhelminthes
- Phylum Nematoda
- Phylum Annelida
- Phylum Mollusca
- Phylum Arthropoda
- Phylum Echinodermata

# Part I. Phyla Porifera & Cnidaria

The sponges, members of the Phylum Porifera, and the sea jellies of the Phylum Cnidaria, are commonly considered among the most primitive animal groups. They both are found in marine and freshwater environments. Both groups lack complex behavioral patterns and sensory organs, but their groups have persisted unchanged for millions of years.

## Phylum Porifera (Sponges)

This phylum includes animals known as sponges. They have bodies full of pores (Porifera = "pore-bearer") and channels that allow water to circulate through them. Sponges lack organ systems such as nervous, digestive, and circulatory systems. Instead, most rely on maintaining a constant water flow through their bodies to obtain food and oxygen and remove waste. Water is pumped through the hollow interior of a sponge by the action of many individual cells called **collar cells**. Most sponges have no body symmetry (**asymmetrical**). Sponges also supplement their internal support with siliceous and calcareous **spicules** or collagenous **spongin**.

- After observing the sponges on display and observing Figure 2, how would you say the morphology of the sponges on display reflect their suspension-feeding lifestyle?

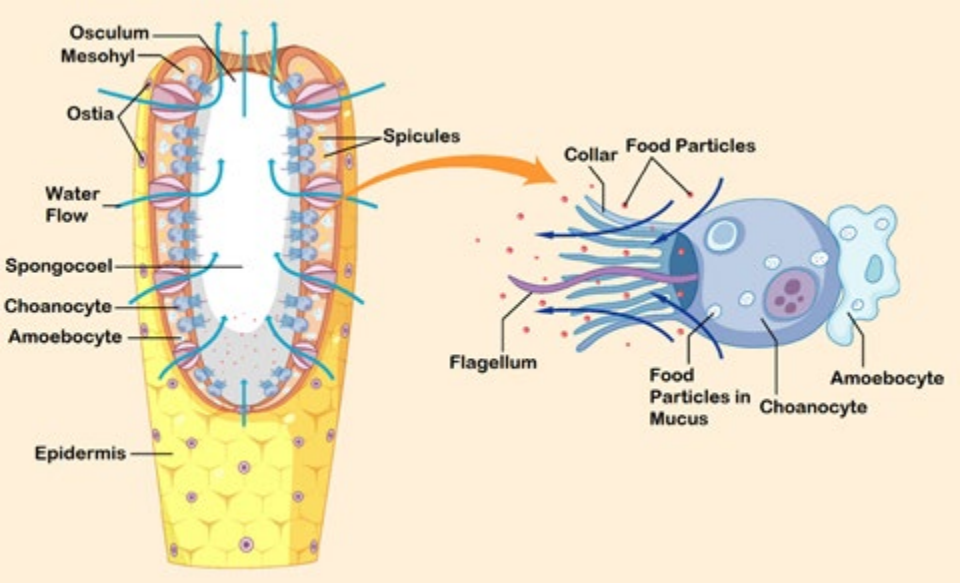



Figure 2. The anatomy of a sponge. Water flows in through openings called **ostia**, passing over the flagellated collar cells, also known as choanocytes. Collected food is moved to amoeba-like cells called **amoebocytes** for digestion. Water exits the sponge, flowing through the interior cavity (**spongocoel**) and out the top of th*e sponge, called the* ***osculum***. Image of the *sponge* modified from Vecteezy (CC-BY 4.0).

## Task 1

1. View the dried/preserved specimens on display along the side bench.
2. In particular, observe the specimen of *Euplectella*, a sponge which is almost entirely composed of glassy spicules.
3. Obtain a small piece of sponge on the side bench, place it on a slide and add a drop or two of diluted bleach.
4. Let it sit until bubbles cease to form. The bleach is oxidizing material to carbon dioxide and water but leaves the mineral spicules intact.
5. Add a coverslip, remove the excess bleach with a Chemwipe and examine the slide under the microscope at high power. Sketch the spicules you see in the space below.
    a. Alternative: Observe a prepared slide of sponge spicules and sketch several of them.

Sponge spicules _____@400x

# Phylum Cnidaria

This phylum includes corals, sea anemones, jellyfish, and hydras. Most live in salt water with a smaller number living in freshwater. Cnidarians have radial symmetry and develop true tissues. They form two layers of tissues; an external one called ectoderm, and an internal one called endoderm (also called **gastrodermis**). There are two body types of Cnidaria: a sessile **polyp** and a free-floating **medusa** (Figure 3). They have a blind gut with only a single opening (no separate mouth and anus) and a nerve net with no centralized control. Like sponges, cnidarians capture food from the water as it passes near them. Unlike sponges, cnidarians capture larger prey items by injecting them with toxins. Their stinging cells or **cnidocytes** produce those toxins. Cnidocytes eject **nematocysts**, which are harpoon-like structures that sting their prey and deliver the toxins. In a few species, these toxins can be extremely painful, even fatal, to humans.

Cnidarians include four major Classes: **Anthozoa**, which include corals and sea anemones; **Cubozoa**, the box jellyfish; **Scyphozoa**, the traditional jellyfish; and **Hydrozoa**, which include animals such as the freshwater hydra and Portuguese Man-O-War.

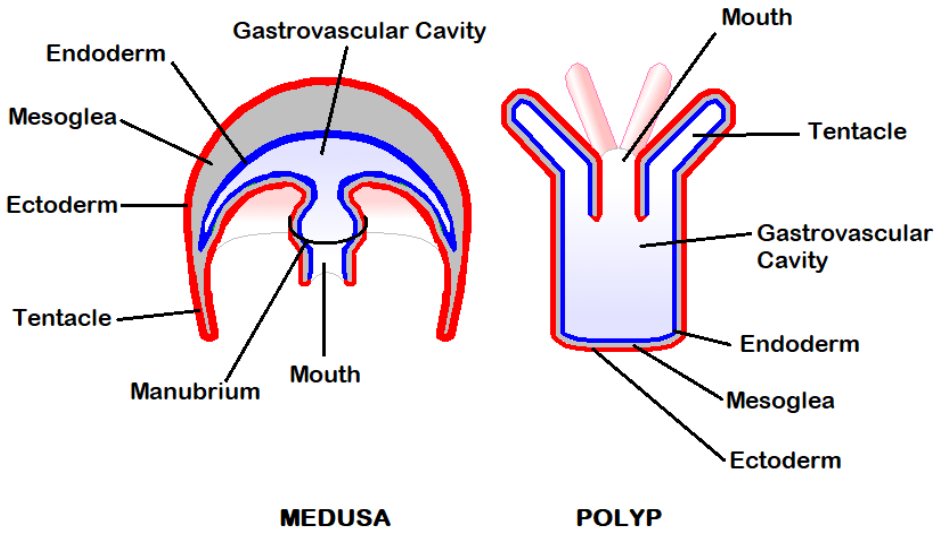



Figure 3. Corals and sea anemones are dominant in the polyp stage. Typical jellyfish, as well as the extremely venomous box jellyfish, are medusa-dominant. Some cnidarians, such as Portuguese Man-O-War, alternate between both stages in their life cycle. Image from *https://commons.wikimedia.org/wiki/File:Cnidaria_medusa_n_polyp.png*, Public domain, modified from Wikimedia Commons.

## Task 2

1. View the specimens of each Class presented on the side bench, including Anthozoa, Scyphozoa, and Hydrozoa.
2. Obtain a *Hydra* c.s. (cross-section) slide and view it at high power. *Hydra* is a freshwater member of the hydrozoans.

3. Study the histology of the body wall of *Hydra.* The body wall consists of two epithelia, the epidermis and gastrodermis, separated by the acellular tissue layer known as the **mesoglea.**
4. Obtain a *Hydra* l.s. slide, which is a whole mounted *Hydra*. View it at medium power and sketch the hydra in the space below, labeling it using Figure 3 as a reference.

*Hydra* c.s. _____@400x

*Hydra* l.s. _____@400x

- Observe Figure 4 on the next page. What layer replaces mesoglea in bilaterian animals?

# Part II. Bilaterians

All of the animals we will look at going forward are considered to exhibit bilateral symmetry. The most notable characteristic of these animals is that they possess **cephalization** – that is, a well-developed head. These animals are collectively called **Bilaterians**. They are further separated by the presence or absence of a body cavity, a large internal space which is typically either fluid or gas-filled and serves the role in some as a circulatory system or hydrostatic skeleton for support. Those animals that lack any sort of body cavity are considered **acoelomate** animals. Those that do exhibit a body cavity differ in how it is derived from tissue during early development. A **pseudocoelomate** animal has a "false" body cavity (**pseudocoelom**) which is found between tissues derived from mesoderm and endoderm tissue. A **coelomate** animal exhibits a body cavity (**coelom**) which develops entirely within mesoderm tissue alone.

Animals can reproduce both sexually and asexually. Asexual reproduction generally produces identical offspring (clones). Some notable examples are the budding in sea anemones and parthenogenesis of some reptiles. However, **sexual reproduction** is heavily favored in animals, primarily because it produces offspring which are genetically diverse – i.e. the product of genetically unique egg and sperm. Genetic diversity, as you learned during the discussion on natural selection, results in offspring which may survive environmental change.

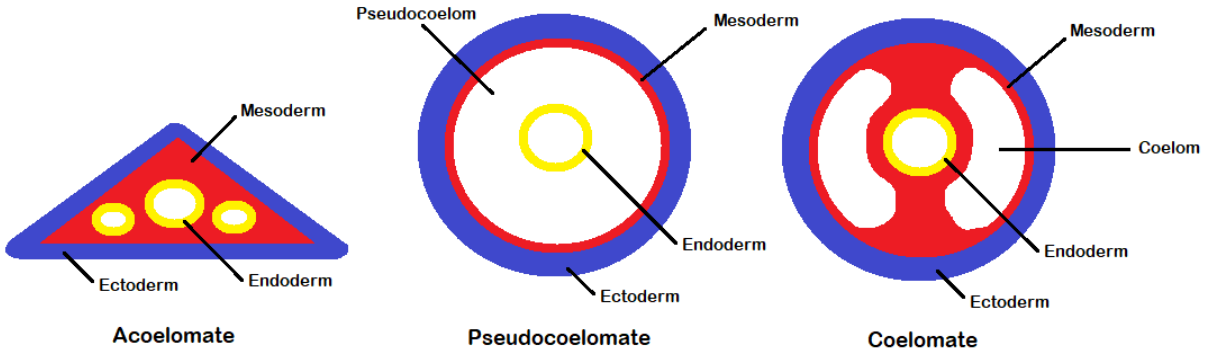



Figure 4. Acoelomate body plans do not have any body cavity, and are filled with a solid mass of tissue derived from mesoderm, called parenchyme. Flatworms are typical acoelomate animals. A pseudocoelomate body plan is typical of rotifers and roundworms, and has a fluid-filled space called the pseudocoel. Note, however, that a true body cavity, called a coelom, has tissue derived from the mesoderm that lines both the ectoderm and endoderm. A pseudocoel and coelom are generally fluid-filled and support invertebrate animals in circulation, support, and movement.

Bilaterian animals are divided into two major categories based on several key events that occur during their development from egg to embryo. The group known as **protostomes** which includes: flatworms, nematodes, arthropods, annelid worms, and mollusks. The remainder of bilaterians fall under the category of **deuterostomes**. This division includes echinoderms (sea stars, sea urchins, etc.) and the chordate animals, among others.

## Phylum Platyhelminthes

This phylum includes worms called "flatworms" such as planarians, tapeworms, and flukes. Flatworms may be free-living or parasitic, although most species are parasites, particularly of vertebrates. Free-living species are found in both saltwater and freshwater although some can be found in very moist habitats on land. Some small free-living flatworms have cephalization, with a head bearing chemoreceptor organs, two simple light-receptor organs, and a tiny brain. The digestive tract, if present, consists of a single opening into a blind sac called a **gastrovascular cavity**. This opening serves as both the "mouth" and the "anus." Some parasitic species, most notably tapeworms, have secondarily lost their digestive system (can you think of a reason why?). Characteristically, these animals do not have a body cavity, and thus are acoelomates. With no body cavity, they have no internal fluid support system – which causes them to be flattened in nature, as well as moving primarily using cilia and some muscles.

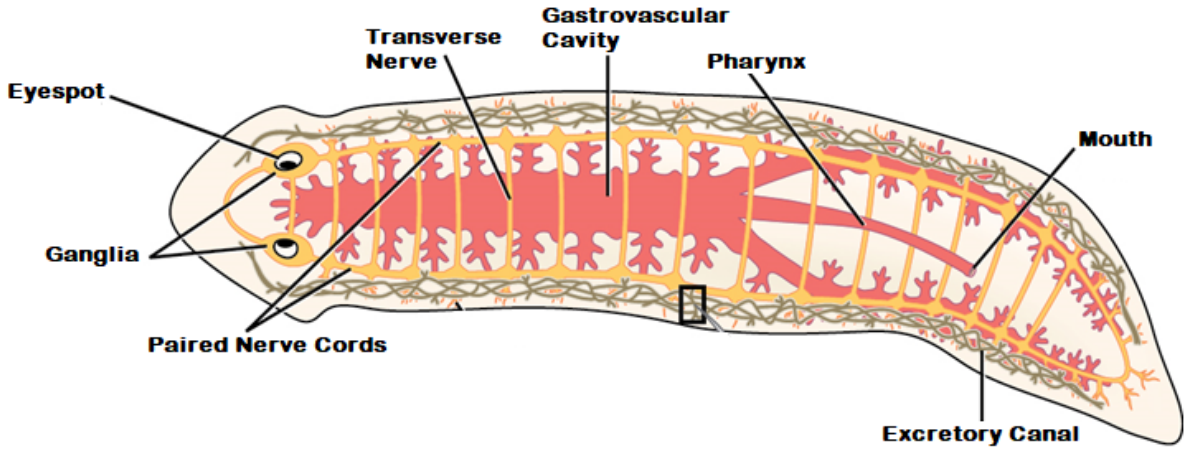



Figure 5. The anatomy of the free-living flatworm, *Dugesia*. The nervous system is ventral, and is composed of paired nerve cords, which pass signals to the **cerebral ganglia** (brain) at the head end of the worm. The digestive system of the flatworm is a gastrovascular cavity, with only a single opening, a tube-like pharynx.[1]

## Task 3

1. View the specimens available on the side bench, including the Classes Turbellaria, Trematoda, and Cestoda.
2. View the dish of live planarians at the side bench. These planarians have been provided with frozen blood worms (an annelid worm) for food. Observe the feeding method of these worms closely using the magnifying glasses provided.
3. Observe the locomotion of the planarian closely, and record your observations below:

- Describe the locomotion of the planarian here:

- If the planarian was observed feeding, describe this process:

# Phylum Nematoda

This phylum includes worms called "roundworms". Nematodes and their relatives are abundant and diverse and many are microscopic. The largest known nematode, which reaches a length of 9 meters (29.5 ft.!) is a parasite in the placentas of sperm whales. Nematodes are slender animals generally with a body that is round in cross section without many external features and tapered to a point at both ends. A tough **cuticle** composed of chitin covers the body of a nematode; therefore, these animals have to molt in order to grow in size. Nematodes may be parasites, predators, or herbivores. Many species are serious plant pests, others play an important role as decomposers in soil, and several species are important parasites of humans.

Nematodes have a body plan that is considered to be pseudocoelomate, meaning that they do not have a true body cavity. Instead, they have a **pseudocoelom**, which provides space for a hydrostatic skeleton and longitudinal muscle. This gives these worms the ability to flip back and

forth, relying on muscles to move, rather than cilia, as in the flatworms. Also, unlike the flatworms, they have a complete digestive tract. Also, unlike flatworms, they are most often **dioecious ("two houses")**, having dedicated male and female individuals, as in Figure 6.

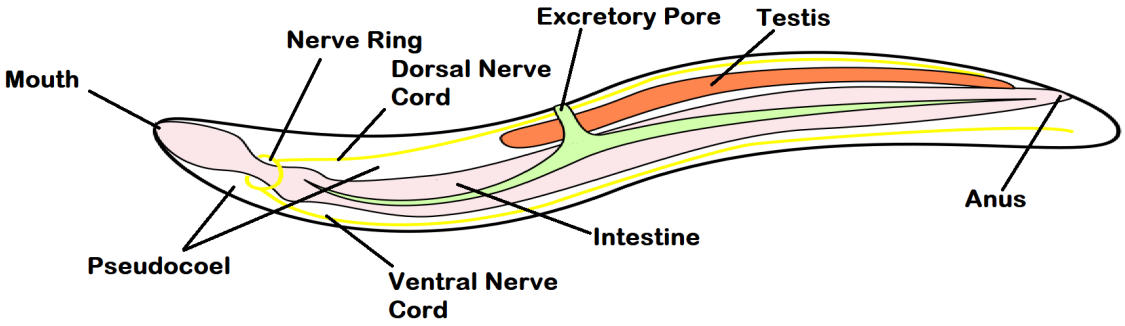



Figure 6. The anatomy of a plant-eating nematode. This specimen is a male, and possesses testis. Note the fluid-filled space known as the pseudocoelom. Image modified from https://commons.wikimedia.org/wiki/File:Nematoda-Anatomy.svg, Wikimedia Commons.

## Task 4

1. View the specimens of nematodes on the side bench.
2. Make a wet mount of the nematode called *Cephalobus*, present on the side bench, and view them at medium to high power.
3. Find relatively inactive worms to study on the slide. Try to identify both male and female worms in your sample. Describe this below.
4. Note the presence of the stylet on the head end of *Cephalobus*.
5. Observe the locomotion of these roundworms carefully and record this in the space provided below:

- Did you see any male or female roundworms in your sample? How did they differ in appearance?

- Describe the method of locomotion used by *Cephalobus*:

# Phylum Annelida

This phylum includes segmented worms such as earthworms, leeches, and polychaetes. **Segmentation** allows these animals to move different parts of their body independently of one another, giving them much better control of their movement. Compared to the other worms we have observed, they have a fully developed body cavity – or **coelom**. The interior of most annelid worms divides this coelom between each segment, using fleshy walls derived from the mesoderm, called **septa**. In addition, they have a complete digestive system (with separate openings for the mouth and anus), and an independent nerve center (called a ganglion) per segment, with a nerve cord that connects and coordinates the function of the ganglia. Unlike nematodes, annelids have both circular and longitudinal muscles that allow them to move more smoothly and quickly through their environment. This movement is called **peristalsis**. Finally,

some annelids, such as the polychates and earthworms possess bristles on each segment called **setae**.

Leeches and earthworms belong to the Class **Clitellata**, referring to a band of reproductive tissue called the clitellum. Members of the Class **Polychaeta** (meaning "many hairs") live in marine environments.

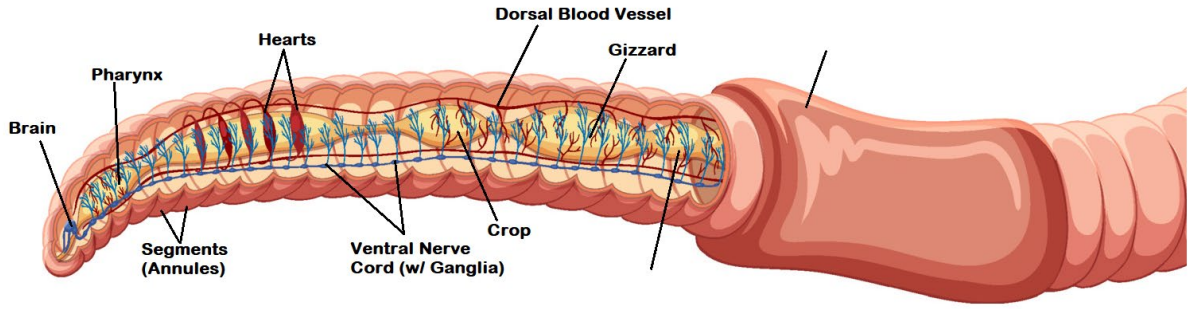



Figure 7. Anterior anatomy of the earthworm, *Lumbricus*. Image sourced from https://www.vecteezy.com/free-vector/earthworm.

## Task 5

1. View the specimens on the side bench, including examples of Clitellata and Polychaeta.
2. Obtain a live specimen of *Lumbricus* (a nightcrawler earthworm) and place it into a dissecting tray. You will not be dissecting, just observing.
3. Carefully, so as not to injure the worm, feel the anterior segments of the live earthworm with your hand (preferably un-gloved) and note the presence of the **setae**.
4. Note the movement of the live earthworm, in particular, comparing it to the movement of both the live flatworms and nematodes you observed previously.
5. Return the live earthworm back to the container you obtained it from, and wash out the dissecting tray – *keep the tray at your station, you will need it later.*

- Describe the method of locomotion used by *Lumbricus* here:

# Phylum Mollusca

This phylum includes clams, snails, squid and their relatives. Mollusks are a very diverse group, both with respect to the numbers of species and the environments they occupy. Mollusks have a muscular **foot** for locomotion (think of the soft, muscular portion that snails crawl around on). Most of their organs are concentrated in a centralized **visceral mass**. They also have a fold of tissue called a **mantle** that secretes the hard, calcareous **shell** that is typical in many molluscs. Finally, they may have a rasping, tongue-like structure used in feeding called a **radula**.

Mollusks include Class **Gastropoda**, known commonly as snails and slugs. Class **Bivalvia** includes clams, oysters, and scallops, which have hinged, two-part shells, and a foot modified for digging. Class **Cephalopoda** includes octopus, squid, cuttlefish, and nautili, which have modified their feet into tentacles. One of the most bizarre classes is **Polyplacophora**, or chitons, which have shells segmented into eight plates.

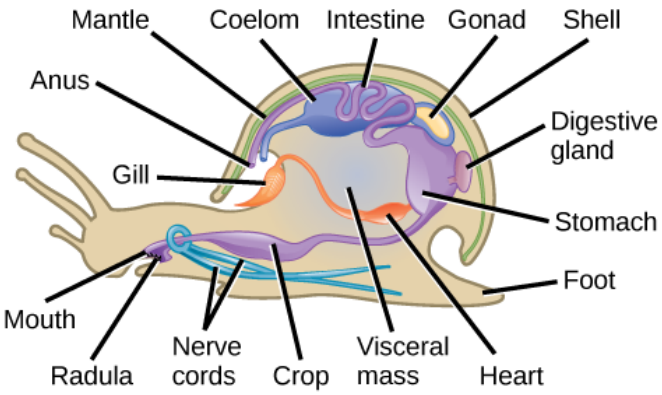



Figure 8. Anatomy of a gastropod, a typical mollusk, which displays the foot, mantle (and mantle cavity), visceral mass with the mantle cavity, and shell. Also visible is the rasping mouthparts known as the radula. Image attributed to https://www.nicepng.com/png/detail/285-2856987_mollusk-anatomy.png, free for non-commercial use.

## Task 6

1. View the specimens of mollusks on display and familiarize yourself with the characteristics of each class: Gastropoda, Bivalvia, Cephalopoda, and Polyplacophora.
2. You will be performing a dissection. Make sure you are wearing gloves and goggles. Obtain a preserved squid and place it in your dissection tray.
3. Observe the external anatomy, noting that a tough, muscularized **mantle** surrounds the animal.
4. Note that the arms have been modified into eight grasping **arms** and two longer **tentacles**.
5. Remove one of the tentacles and view it using a magnifying glass or dissecting microscope.
6. The mouth, which is located in the center of the mass of arms, contains a **radula** and jaws that look like a parrot's beak. Observe it carefully.
7. Note the presence of the well-developed eyes, which function in a similar way to those of vertebrates.
8. In addition, on the dorsal surface of the mantle cavity, you may note the presence of **chromatophores**, which are cells that contain color-changing pigments. This allows many cephalopods to rapidly camouflage themselves.
9. Turn squid onto its ventral side, and note the position of a **siphon**, just beneath the head. The siphon allows cephalopods to use jet propulsion as a means of locomotion.
10. The instructor may now ask you to perform an internal dissection.
11. Once finished, dispose of your squid and gloves in the biohazard bin. You may keep your tools, goggles, and tray for now, as you will need them again. But set them off to the side.

# Phylum Arthropoda

Arthropods are the most diverse group of animals with respect to number of species, and they are found in almost every environment on earth. Approximately 75% of the known animal species are arthropods, mostly insects. The name **Arthropoda** means "jointed limb" and is a reference to their segmented walking appendages. Arthropod bodies are divided into specialized segments called **tagmata**, with a rigid exoskeleton composed largely of a substance called **chitin**. Arthropods must molt in order to grow in size. This process is called **ecdysis**, and is a feature shared with the nematodes. Arthropods occupy an enormous variety of Earth's habitats.

Arthropods are divided into four major Subphyla, based on the number and type of appendages they have. **Chelicerates** include horseshoe crabs, spiders, scorpions, ticks, and mites, and are named after their mouthparts, called chelicerae. **Crustaceans** include crabs, shrimp, lobsters, crayfish, and even barnacles, and have exoskeletons reinforced with calcium carbonate. **Hexapods** are insects and allies. Insects are defined by the ability to fly, but also by having three tagmata with three pairs of walking appendages. Finally, the **Myriapods** are notable for having numerous body segments and many legs. They include centipedes and millipedes.

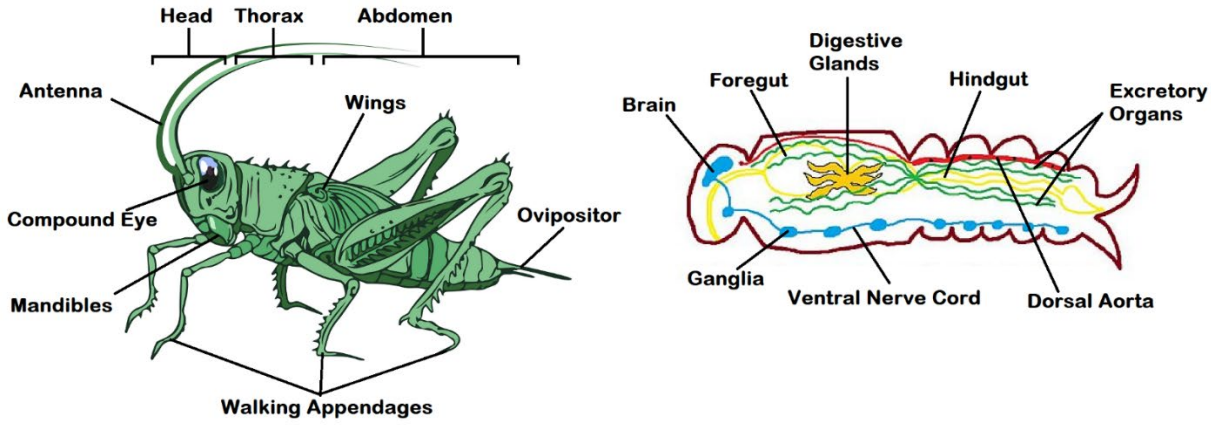



Figure 9. The anatomy of a grasshopper, an insect. Note that the body is divided into three tagmata, a **head**, **thorax**, and **abdomen**. Images from https://www.vecteezy.com/free-vector/grasshopper, Grasshopper Vectors by Vecteezy, and https://commons.wikimedia.org/wiki/File:Grasshopper_Organ_Systems.jpg, via Wikimedia Commons.

## Task 7

1. Observe the specimens on display, noting the characteristics that separate the Chelicerates, Crustaceans, Hexapods, and Myriapods.
2. Obtain two pinned insect specimens, and carefully observe each of them under the microscope. Sketch one of your insects in the space provided (on the next page), making sure the three tagmata are clear and labeled. Make sure to sketch and label all appendages, including the walking appendages, antenna, compound eyes, mandibles (or modified mouthparts), and wings. Use Figure 9 as a reference.
3. Use the dichotomous key found here https://www.insectidentification.org/insect-key.php to identify your insect specimens to Order.

Sketch and label your insect here

## Phylum Echinodermata

The final phylum that will be observed during this lab belongs to the division of bilaterians called **Deuterostomes.** You may recall that this division of animals differs from protostomes in their development, chiefly in regard to the formation of an anus first during development of the early digestive tract. Deuterostomes include phylum **Echinodermata**, Phylum **Hemichordata**, and Phylum **Chordata** – which is the phylum that the vertebrates, such as ourselves, belong to. You will observe vertebrates in the following lab.

The phylum Echinodermata includes animals such as sea stars, sea urchins, sea cucumbers, sand dollars, brittle stars, and sea lilies. These animals are all marine, primarily benthic, and obtain nutrition in a variety of ways, such as predation, herbivory, and suspension feeding. Unlike the other bilaterians, their anatomy is quite distinct. Echinoderms are secondarily radially symmetrical. That is, echinoderms have a bilaterally symmetrical larva but during their development they acquire **pentamerous** (5-point radial) **symmetry**. In addition, they move and circulate fluid using a **water vascular system** (Figure 10). This system powers structures called **tube feet**, on which they crawl around or capture prey. In addition, they have an endoskeleton composed of calcium carbonate plates called ossicles. These ossicles are often spiny, with the spines projecting through their thin skin. In fact, the name Echinodermata means "spiny skin."

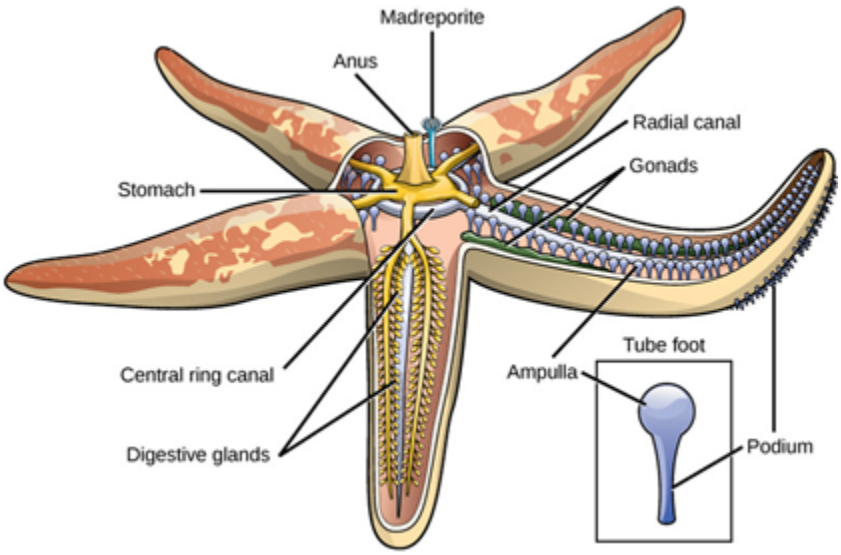



Figure 10. The anatomy of the sea star. Note the pentamerous symmetry, with organs and canals radiating into each of the five arms. The water vascular system of an echinoderm begins with water entering the **madreporite**, then flowing into the ring canal. The ring canal circulates this water into each of the radial canals. Each radial canal leads to bulbs called **ampulla**. The ampulla push water into the tube feet, almost like a system of hydraulics. Terminology like dorsal or ventral does not apply to echinoderms. Instead, the top side is called the **aboral** side and the underside (with the mouth) is called the **oral** side.[1]

## Task 8

1. Begin by observing the echinoderms on display and noting shared features of each, such as the pentamerous symmetry, tube feet, and the spiny/bumpy ossicles.
2. Obtain a preserved sea star and place in your dissecting tray.
3. Begin by observing the exterior anatomy of the sea star, noting the position of the **madreporite** on the aboral side. Then, turn the sea star over to its oral side, noting the **ambulacral grooves** on the underside of each arm. These grooves contain the **tube feet**.
4. Once you have observed this external anatomy, use scissors to carefully cut around the aboral part of the sea star's central disk, leaving the madreporite in place. The goal is to expose and remove the stomach, so you can observe the **ring canal**.
5. Then, cut the upper surface off of one of the arms, exposing the **digestive glands** and **gonads**. These are repetitive for each arm.
6. Remove these organs from the arm, then observe the **radial canal**, which is a hard, stony canal in the center of the arm. Note how bubble-like sacs run parallel to this canal on either side – these are the **ampullae**.
7. Observing the position of these ampullae, flip the sea star over and note how they line up with the tube feet on the underside.
8. Dispose of your sea star and gloves in the biohazard bin. Wash your dissecting tools and tray. Put away your goggles. Wipe down your lab bench.

## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. Sponges and cnidarians are among the most primitive animals. Describe two reasons why each phylum would be considered more primitive than the other animals you observed today:

2. What is the ecology of the nematode *Cephalobus*? *Research your answer.*

3. Compare and contrast the movement of all three types of worms we observed today: flatworm, nematode, and annelid worm. How do you think the type of body cavity, or lack thereof, influenced the movement of each type of worm?

4. What do you think is the evolutionary advantage of a cephalopod having well-developed vision, the arms/tentacles, the beak, and chromatophores?

5. Insects are the most diverse animals on the planet. What adaptations do you think account for their success?

6. How does a sea star utilize its tube feet to capture prey? *You may research your answer.*

## References

OER Commons. Biological Diversity. 18 July. 2023. Available from: https://oercommons.org/courseware/unit/8453

# Comparative Vertebrate Anatomy

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- Gain a better understanding of changes in vertebrate anatomy through evolution.
- Understand the variation of tissues, organs, and systems among three classes of vertebrates.

## Introduction

**Phylum Chordata** is one of the major divisions within the clade of animals known as Deuterostomes. Within this phylum is the **Subphylum Vertebrata**. Vertebrates are characterized by having a backbone, or **vertebral column**, that surrounds their dorsal nerve cord. They also have a bony **endoskeleton** throughout, which includes a **cranium** to protect a well-developed head and brain.

In this comparative anatomy lab, we will be comparing the anatomy of three classes of vertebrates: a bony fish (yellow perch), an amphibian (northern leopard frog), and a mammal (Norway rat). See the taxonomy of these three species below (Figure 1).

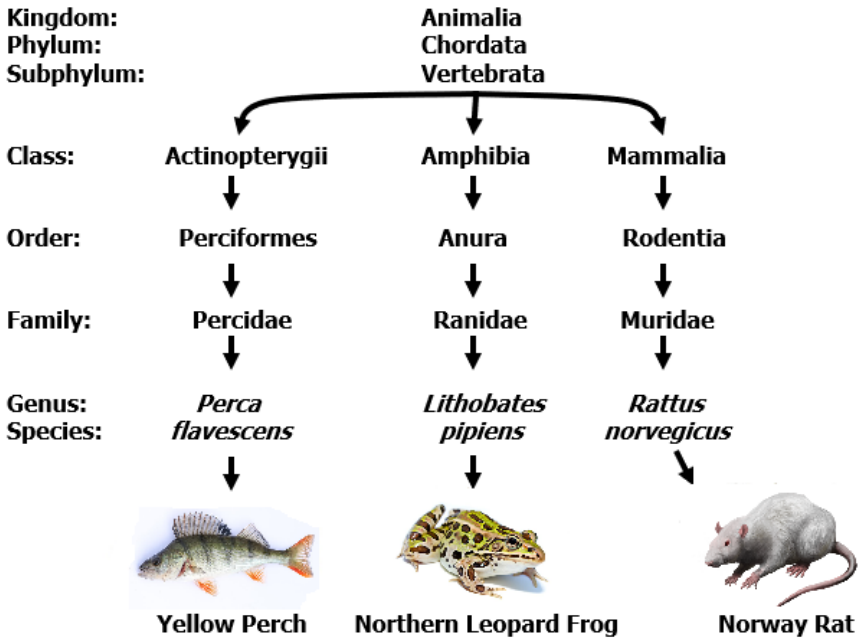



Figure 1. Taxonomy of three vertebrates.[1,6,8]

The anatomy of the yellow perch will demonstrate the organ systems of fishes, while also displaying many anatomical features derived for an aquatic lifestyle. The frog and the rat represent terrestrial vertebrates, also known as **tetrapods** (because they have four limbs). However, amphibians still have ties to the water, and this is indicated in both their internal and external anatomy. Likewise, many mammals, like rats and humans, are exclusively adapted for terrestrial life. Note these adaptations, as well as those characteristics that define their classes, Amphibia and Mammalia, respectively.

# Part I. Perch Anatomy

## Perch External Anatomy

1. Referring to the handouts provided in class, identify the external features of your perch. As you identify them, label them on the diagram below.

Figure 2. Label the external anatomy of the perch.[1]

2. Examine the **fins** of your fish. Observe their number and arrangement. Are they paired or unpaired appendages? Identify the bony rays that support the fins.
3. Note the location of the **operculum** (bony plate covering the gills). Trace the path of water as a fish respires.
4. Examine the mouth/head of the perch. Identify the **jaws** and feel the rough, needle-like teeth around the rim of the mouth. Locate the **nares**, or nostrils.
5. Locate the **cloaca** of the perch. The cloaca is the common opening for the intestinal, reproductive, and urinary tracts.
6. Locate the **lateral line** along the side of the fish.
    a. **Answer Question #1 in the discussion section.**

7. Most fish are covered with a layer of **scales**, giving them a somewhat rough texture. There are several types of scales, such as **cycloid scales** and **ctenoid scales**, which are composed of bone, flake off, and have an appearance like clear plastic.
    b. What type of scales do perch have, placoid or ctenoid?

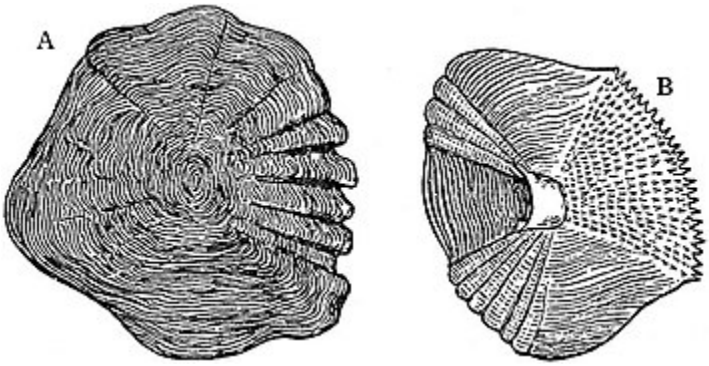



Figure 3. Two types of scales present in bony fish (A) Cycloid and (B) Ctenoid.[2]

## Perch Internal Anatomy – Dissection

1. Place the perch on a dissection tray with the head on your left. Using scissors, cut on one side from the corner of the mouth to the front lower edge of the gill cover (refer to figure below). Then remove the operculum. This will expose the sturdy **gill rakers** and finger-like **gill filaments**.

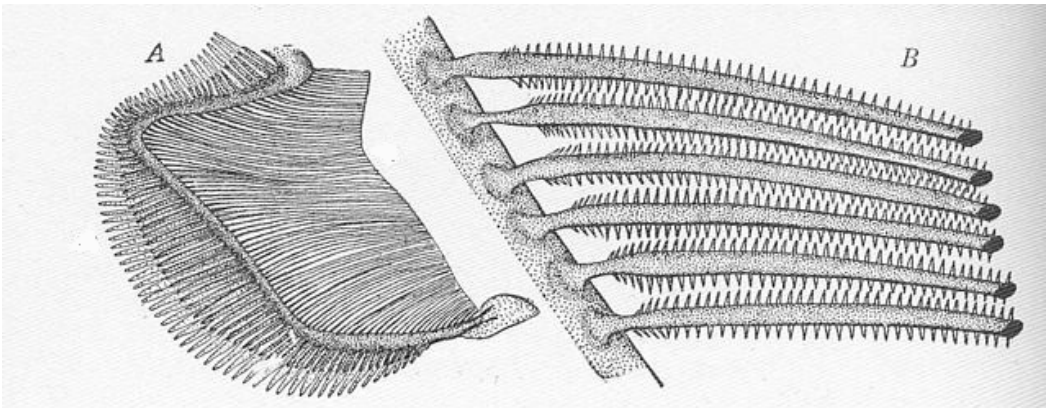



Figure 4. (A) The gill rakers of a bony fish. These will be located behind the operculum. When viewed up close, you can see the gill filaments (B) which extract oxygen from the water.[3]

2. With a scalpel or pair of scissors, continue your previous incision along the trunk of the fish. Refer to Figure 5 for assistance. Begin by making an incision through the body wall of the fish. Following Cut (A) below, cut along the belly of the fish from the front edge of the operculum through the trunk to the anus.

3. Next, create Cut (B) by cutting up along the side of the perch to the backbone.

4. Pin the perch on its side through the edge of the belly and the tail. Lift open the side of the fish, and follow Cut (C) by cutting forward through the ribs along the backbone to the tip of the operculum**.** Remove this portion of the trunk that you have cut away. This will expose the internal organs.

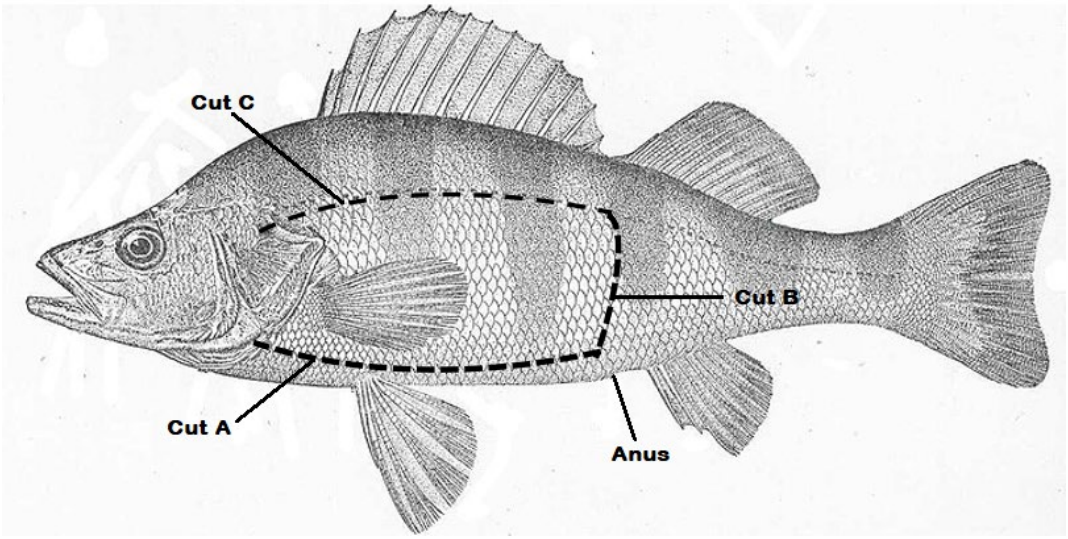



Figure 5. A dissection guide to the yellow perch.[4]

5. Identify the following internal organs: **gonad** (ovary or seminal vesicle), **stomach** and **pyloric caecum**, **intestine**, **liver**, **heart**, **swim bladder**, and **urinary bladder**.
6. Before you remove any internal organs, take a good look at the body cavity of the fish.
    a. **Is the body of the perch subdivided into different regions, as in humans?**
7. Determine if your fish is male or female based on the presence of either **ovaries** or **seminal vesicles**. Once you have identified them, you may remove them. Note that the perch has no external reproductive organs, as in mammals.
8. Examine the **swim bladder**. Note how it has its own supply of blood vessels.
    b. **Answer Question #2 in the discussion section.**
9. Locate and identify the **stomach**. It ends with the **pyloric caecum**, which a highly branched region of the small intestine.
10. Identify and remove the **liver** and **pancreas**.
11. Identify the **intestine** and **urinary bladder**. Follow their path to the cloaca. Note how they share this common opening with the gonads.
12. If you would like, you may remove the organs listed in steps 5-9 (if you haven't already). Do this to expose the circulatory system of the fish. Remember that in fish, the circulatory system is a single circuit. Locate the **heart**, which is subdivided into <u>two</u> chambers, the **atrium**, which receives blood from the body, and the **ventricle,** which pumps blood to the rest of the body. Blood flow out of the heart is controlled by a valve known as the **bulbus arteriosus**. See Figure 6 for reference.
    c. **Answer Question #3 in the discussion section.**
13. Follow the path of blood as it leaves the atrium, travels to the gills to be oxygenated, and flows through the rest of the body, then returning to the heart.

14. Dispose of your perch in the biohazard container.

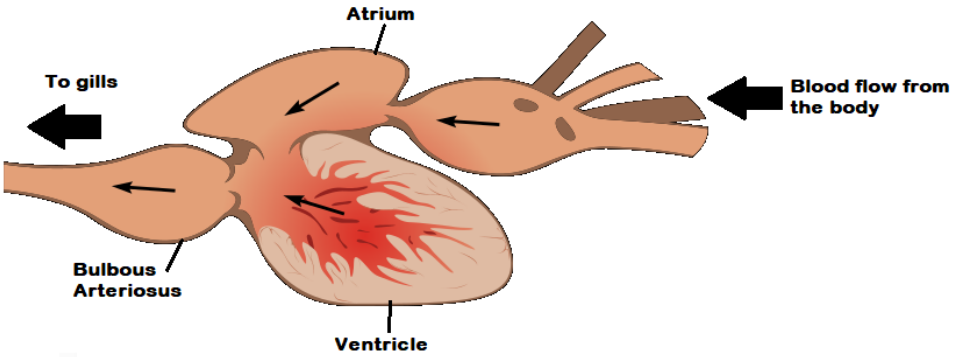



Figure 6. Blood flow and structure of a fish heart.[5]

# Part II. Frog Anatomy

## Frog External Anatomy

1. Place preserved frog (*Lithobates pipiens*) in a dissecting tray and remove from the bag.
2. Referring to the handouts provided in class, identify the external features of your frog. As you identify them, label them in the following diagram.

Figure 7. Label the external anatomy of the frog.[6]

3. Identify the bulging eyes, which have non-moveable upper and lower lids but can be covered by a **nictitating membrane**, which serves to moisten the eye.
4. Locate the **tympanum** behind each eye.
    a. **What do you think is the function of the tympanum?**
5. To determine the sex of your specimen, examine the size of the tympanum. If it is the same size as the eye, the specimen is a female. If it is larger than they eye, it is a male.
6. Examine the coloration of the dorsal and ventral surfaces of the frog.
    a. **Answer Question #4 in the discussion section.**
7. Examine the external **nares** (sing. *naris*: nostril). Insert a small probe into an external naris and observe that it protrudes from one of the paired small openings, the internal nares inside the mouth cavity.

8. Identify the paired limbs. The bones of the **fore-** and **hind limbs** are the same in all tetrapods in that the first bone articulates with a girdle and the limb ends in **phalanges**. The hind feet have five phalanges and the forefeet have four phalanges.

## Frog Internal Anatomy – Dissection

1. Open your frog's mouth very wide. Cut the mouth at each hinge joint to allow opening the mouth wider. You may ask for assistance from the instructor for this procedure.
2. Locate and describe the attachment of the tongue. Refer to Figure 8 for a reference of the frog's oral cavity.

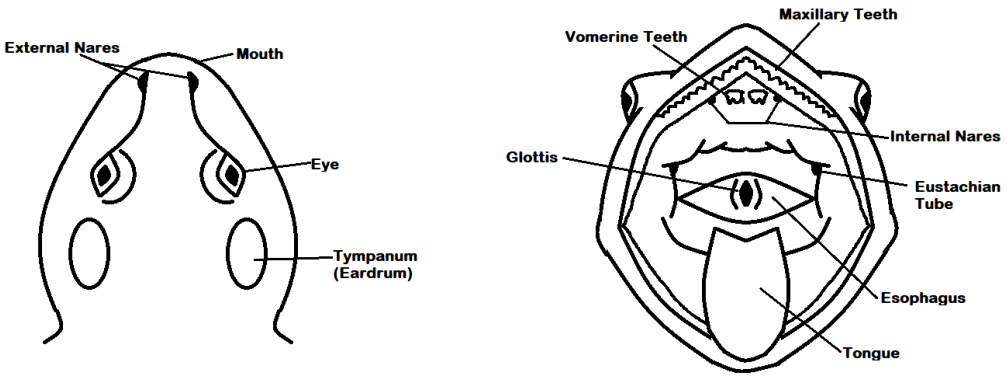



Figure 8. The anatomy of the frog head and oral cavity.

3. Examine the back of the mouth. Notice the two openings in the center. One is the elastic **gullet** and the other is the vertical slit **glottis**. Using your probe, examine each of these openings.
    a. **Where does each opening lead?**
4. Examine the upper area on each side of the jaw. Here you will find an opening on each side of the mouth. These are the **Eustachian tubes**. Using your probe, determine where they lead.
5. If your frog is already opened lift the flaps of the body wall and pin them back. If not, lay the frog on its dorsal side (ventral side up). Insert your scissors through the skin just above the anal opening and cut the skin, in a straight line, up to the tip of the lower jaw. Cut only skin, not muscle. Make four later cuts from the center cut outward to the sides of the body. This will produce two large flaps of skin that may be pinned back. Examine the pattern of underlying abdominal muscles. These muscles have a dual function: they support the internal organs (frogs lack ribs) and assist in locomotion.
6. Observe the dark line running down the center of the abdomen. This is the **abdominal vein**. You will be cutting through the abdominal wall alongside the vein. Do not damage it.
7. Repeat the same incisions made on the skin, making sure not to damage the underlying organs. Cut through the pectoral girdle and remove the muscles with the forceps. The body cavity may be filled with black and white eggs. If so, your specimen is female.
    a. **Answer Question #5 in the discussion section.**

8. Locate the large brownish colored liver anterior in the body cavity. Be careful when lifting the segments or lobes of the organ.
9. Located on the ventral side of the large lobe, locate the **gall bladder**. This organ may be hard to find since it may be collapsed.
10. Examine the large J-shaped **stomach** on the right side of the abdomen. Cut it out and open it with a vertical cut. Can you identify any undigested food?
11. Attached to the stomach is the **small intestine**. It is a highly coiled organ divided into three sections. Examine the coiled intestine carefully. It is held together by connective tissue called **mesentery**. Within this mesentery is a reddish organ called the spleen.
12. The small intestine connects to the smaller **large intestine**.
13. Carefully move back the alimentary canal and the liver. This will expose the underlying organs, such as the **kidneys**.
14. The reproductive organs should have also been exposed. Locate the **testes** if you have a male frog. Testes are yellow, oval organs attached to the anterior portion of the kidneys. Several small ducts, the Vasa efferentia, carry sperm into the kidney ducts that also carry urine from the kidneys. Fat bodies, which store fat, are attached to the testes.
15. Locate the **ovaries** if you have a female frog. The ovaries are attached to the dorsal body wall. Fat bodies are also attached to the ovaries. Notice the tightly coiled tubes on either side of the midline. These are the **oviducts**.
16. The **heart** is found in the center of the two front legs. It is covered by a thin membrane called the **pericardium**. Locate the right atrium, left atrium, and ventricle.
17. Locate the three large veins that join together beneath the heart to form the **sinus venosus**. Blood from the sinus venosus enters the right atrium from the body. The left atrium receives blood from the lungs.
    a. **Answer Question #6 in the discussion section.**
18. Referring to Figure 9 below, find the **conus arteriosus** (4), a single, wide arterial vessel leaving the ventricle and passing ventrally over the right atrium. Follow the conus arteriosus forward to where it divides into three branches on each side. The innermost branches (8) carry blood to the head. The branches next to those move blood to the rest of the body (7). The outer branches carry blood to the lung and skin to be oxygenated (6).
19. Examine the two small **lungs** on either side of the heart.
    a. **Answer Question #7 in the discussion section.**
20. Dispose of the frog in the biohazard bin.

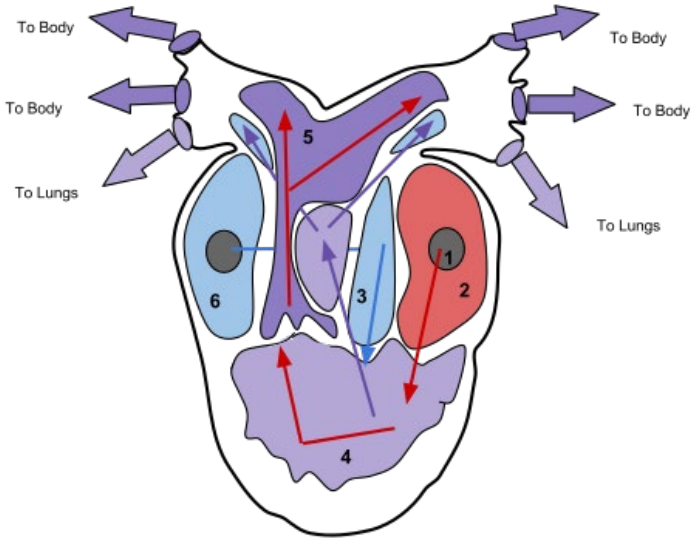



Figure 9. A cross section of an amphibian heart. The purple regions represent the oxygenated blood and the de-oxygenated blood interacting. 1. Pulmonary Vein, 2. Left Atrium, 3. Right Atrium, 4. Ventricle, 5. Conus arteriosus, 6. Sinus venosus.[7]

# Part III. Rat Anatomy

## Rat External Anatomy

1. Place the preserved Norway rat (*Rattus norvegicus*) in a dissecting tray.
2. Referring to the handouts provided in class, identify the external features of your rat. As you identify them, label them on the diagram below.

Figure 10. Label the External Anatomy of the rat.[8]

3. Note that the body is divided into a **head** and a **trunk**, separated by the neck region. Locate on the head the **external ears** or **pinna**, which are folds of skin supported by elastic cartilage. These direct sound waves to the **external auditory canal**.

4. Note that the eyes have an **upper** and **lower eyelid** and a reduced **nictitating membrane** at the medial corner of the eye.
5. The mouth is bordered by the **upper** and **lower lips**. Notice the long sensory hairs called **vibrissae** on either side of the cleft which runs between the mouth and the **external nares**.
    - **Answer Question #8 in the discussion section.**
6. The trunk of the rat consists of an anterior **thorax** and a posterior **abdomen** which are separated (internally) by the muscular **diaphragm**, which you will see later during the internal dissection.
7. Locate the following structures on the trunk of the rat: On the ventral surface of the trunk are two rows of **mammary papillae**, which extend from the axillary (armpit) region to the inguinal (groin) region on either side of the midline. In rats, there are usually 12 pairs which are most prominent in pregnant or lactating females. Compare this number of mammary papillae found on the female rat to that of humans.
8. Also present on the ventral surface is the **anus** just beneath the base of the tail. Slightly anterior to the anus are the **urogenital openings**.
9. In females there are two separate openings: the anterior **urethral orifice** leading from the urinary system and the more posterior **vaginal orifice** leading from the reproductive tract.
10. In males the urinary and the reproductive systems share a single opening at the tip of the **penis** which is hidden in a fold of skin (**prepuce**) located between the two prominent **scrotal sacs** which house the **testes**.
11. The **post-anal tail** is sparsely covered with hair and bears reptile-like **scales** of epidermal origin.
12. There are two appendages attached to the trunk, the **hindlimbs** arising from the **pelvic** (hip) region and the **forelimbs** arising from the **pectoral** (shoulder) region. Each of the **digits** on each foot bears a **claw**, a keratinized epidermal derivative.

## Rat Internal Anatomy – Dissection

1. Using scissors, make the incisions in the rat, following the guided numbers. Make sure to not cut too deeply and keep the point of the scissors facing upward. Once the body cavity is opened, pin down the flaps to the dissecting tray.
2. You should first notice the **diaphragm.** This is the thin layer of muscle that separates the thoracic from the abdominal cavity. Answer Question #9 in the discussion section.
3. Above the diaphragm, you should see the **heart.** This is located in the center of the cavity. Note the four chambers: 2 atria and 2 ventricles.
    a. **Answer Question #10 in the discussion section.**
4. Directly above the heart is the **thymus gland.** The thymus gland is involved in the development of T cells in the immune system. Locate the **trachea, bronchi,** and lungs. The trachea is a hard, ridged structure descending from the pharynx. The trachea will branch off in two tubes called bronchi, and then lead to the large soft tissue of the left and right lungs. Note that the only part of the digestive system found here is the **esophagus**.

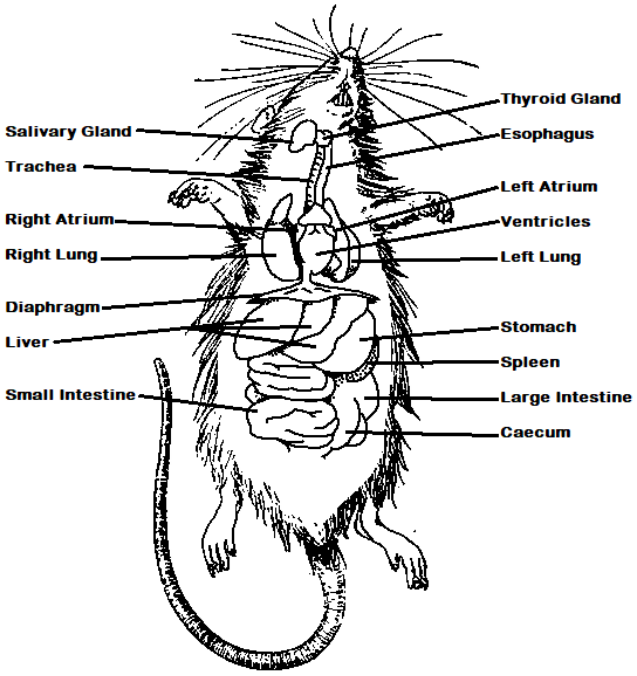



Figure 11. A general diagram of rat internal anatomy (for a more detail version, see the other handouts). Note how the internal organs are subdivided into two body cavities: the thoracic (upper half above the diaphragm), and the abdominal (below the diaphragm).[9]

5. Now observe the abdominal cavity. You will notice the organs are covered by a thin, clear membrane. This is called the visceral **peritoneum.** This membrane is continuous through connections made with the mesentery which supports the abdominal organs. After removing this membrane, locate the stomach.
6. Follow the stomach to the beginning of the **small intestine**, called the **duodenum.** Take notice of the accessory glands which contribute to digestion of food by providing secretions from the wall of the duodenum. The first accessory gland is the **pancreas**, which produces several enzymes and buffers, and the second is the **liver**, which produces bile salts for the emulsification of fats. The remainder of the small intestine is made up of the jejunum and ileum; these do most of the actual digestion and absorption. At the junction of the small intestine and large intestine, you can find a large, dead-end sac called the **cecum**, which houses bacteria that aid in digestion.
7. The remainder of the **large intestine** is made up of the ascending, transverse, and descending colon. These finally empty into the **rectum,** which serves as a storage area for feces until it can be removed.
8. If you move the intestines to one side, you will see one of the two **kidneys** embedded in the dorsal body wall. From these, ureters lead to the urinary **bladder.** The urinary bladder empties to the outside through the **urethra.** Be sure you can locate the urethral opening on both male and female rats.
9. *If the rat is female*: In the female the prominent **uterine horns** pass dorsally to the bladder and ureters. Where the horns join dorsal to the urethra, is the **vagina** which opens to the exterior through the **vaginal opening.** On the exterior, the anus is just

ventral to the tail, the vaginal opening is ventral to the anus, and the urethral opening is ventral to the vagina. At the anterior end of each uterine horn there is a short, convoluted oviduct which opens into a transparent pocket around the small, round ovary. Some female rats may be pregnant and have small embryos in the uterine horns. If yours is pregnant, you may cut longitudinally along the horns and observe the embryos.

    a. **Answer Question #11 in the discussion section.**

10. *If the rat is male*: Locate the male's scrotum and cut longitudinally through just the skin to locate the **testes**. Separate the skin from the testes and continue the cut up to the abdominal cavity. Notice the epididymis lying around each testis.
11. When you have finished with your rat, dispose of it in the biohazard bin. Wash and put away your tools and dissecting tray, making sure to wear gloves and goggles until you have finished.

## Discussion/Post-Lab Questions

Answer the following questions and submit your responses to your instructor as directed.

1. What is the function of a fish's lateral line?

2. What is the function of the swim bladder? How do both the swim bladder and the operculum help bony fish to conserve energy?

3. Why would the two chambered heart and single-circuit method of circulation be less efficient than that of terrestrial vertebrates?

4. How might the difference in frog coloration (dorsal-above and ventral-below) help a frog survive in its environment?

5. Using external and internal anatomy, describe whether your frog was male or female.

6. How is the division of the frog heart different from that of the perch? How does this affect circulation of the blood?

7. How are the frog's lungs similar to and different from the perch's gills?

8. What would be the purpose of the sensory vibrissae in a rat? Why do you think they are so large and numerous?

9. How does the diaphragm change the way that mammals breathe, compared to amphibians?

10. Compare the divisions of the heart in the rat to that of the frog. What is the major advantage of its structure?

11. The rat has a uterus that is divided into two horns (branches). The human uterus is undivided. What might account for this difference?

# References


1. Peter van der Sluijs, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons
2. John Graham Kerr, Public domain, via Wikimedia Commons
3. Hugh McCormick Smith, Public domain, via Wikimedia Commons
4. JonRichfield, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons
5. Ahnode, CC BY 3.0 <https://creativecommons.org/licenses/by/3.0>, via Wikimedia Commons
6. Brian Gratwicke, CC BY 2.0 <https://creativecommons.org/licenses/by/2.0>, via Wikimedia Commons
7. Cscanlon15, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons
8. DataBase Center for Life Science (DBCLS), CC BY 4.0 <https://creativecommons.org/licenses/by/4.0>, via Wikimedia Commons
9. Modified from Internet Archive Book Images, No restrictions, via Wikimedia Commons

# Investigating Animal Behavior

Students must wear proper lab attire and personal protective equipment at all times. Any safety violations may result in being prohibited to enter the lab, receiving a deduction in points, and/or dismissal from lab as outlined in the safety agreement.

## Objectives

- To observe and measure the locomotor behavior of terrestrial isopods in response to various stimuli.
- To utilize scientific reasoning in designing, conducting, and interpreting the results of an experiment.
- To communicate your research process and findings.

## Introduction

One of the basic characteristics of life is the ability to respond to environmental change. A specific change in an organism's environment, called a **stimulus**, may elicit a variety of responses that ultimately help the organism survive or reproduce. **Animal behavior** can be defined as the coordinated responses of whole animals to internal and/or external stimuli (i.e., changes inside or outside the body).

Biologists who study animal behavior (a.k.a. *ethologists*) apply different **levels of analysis** including the developmental origins of behavior, the physiological mechanisms that control behavior, the adaptive value of behavior (i.e., how it affects fitness), and the evolutionary origins of behavior. At each of these levels, alternative hypotheses can be tested via observational studies and experiments conducted either in the laboratory or in the animal's natural environment. By incorporating these complementary approaches to studying behavior, ethologists can develop a fuller understanding of *how* and *why* animals behave the way they do.

In this lab, you will investigate a form of behavior known as **locomotion**. Locomotion (i.e., locomotor behavior) involves movement from one place to another. Locomotor responses may be triggered by a variety of internal or external stimuli. Depending on the animal species and context, locomotor behaviors may either be **learned** (modified by experience) or **innate** (developmentally fixed; i.e., instinctual). Innate locomotor responses can often be classified as either taxis or kinesis, depending on the pattern of movement displayed.

**Taxis** is movement **directly toward or away from a stimulus**. A moth flying toward a light is a classic example of taxis (in this case, *positive phototaxis*). Taxis is said to be positive if the movement is toward the stimulus, or negative if the movement is away from the stimulus.

**Kinesis** is movement **caused by a stimulus but not oriented directly toward or away from the stimulus** (i.e., the direction of movement is random, but the cause is not). For example, exposure to light may cause a shrimp to start moving in a random direction that eventually leads the shrimp to darkness, where it stops moving. This behavior could be described as *negative photokinesis* because it indirectly results in the shrimp moving away from

the stimulus (light). A positive kinesis would indirectly result in movement toward a stimulus. As demonstrated above, a prefix is added to the terms taxis or kinesis to describe the nature of the stimulus. Some common stimuli include: *photo-* (light), *thermo-* (temperature), *hygro/hydro-* (moisture/water), *geo-* (gravity), *thigmo-* (touch).

It is often difficult to determine whether the response of an animal represents taxis or kinesis. In these cases you can simply state that the animal displayed a positive or negative locomotor response to the stimulus in question. For example, if it is unclear whether an animal moved directly or indirectly toward an area of high humidity, you could conservatively describe the behavior as a hygro-positive locomotor response.

In addition to studying the developmental origins of locomotion (e.g., learned or innate) and the physiological mechanisms that control locomotion (including how the stimulus is sensed and how the body responds), ethologists seek to explain the **evolution** of locomotion. Key questions include how does a particular locomotor behavior enhance the animal's **fitness** (i.e., its ability to survive and/or reproduce in its natural environment, and thereby pass on genes for the behavior)? And is the response an *ancestral* behavior that is shared by related species and their common ancestor, or is it a *derived* behavior that originated more recently in evolutionary history?

## Study System: Woodlice

Here you will explore the mechanisms and evolution of locomotion in the **sowbug *Porcellio scaber*** (Fig. 1). Sowbugs are related to pillbugs or roly-pollies, but they are unable to curl into a tight ball for defense. Sowbugs and pillbugs are both types of **terrestrial isopods or woodlice**. A more complete taxonomic classification of *P. scaber* is outlined below:

Domain Eukarya (eukaryotes)
Kingdom Animalia (animals)
Phylum Arthropoda (arthropods)
Class Crustacea (crustaceans)
Order Isopoda (isopods)
Suborder Oniscoidea (woodlice)
Family Porcellionidae (sowbugs)
Genus *Porcellio*
Species: *Porcellio scaber*

Figure 1. Sowbugs (*Porcellio scaber*). Common Rough Woodlouse by Alexis is licensed under CC BY 4.0.

Like all arthropods, woodlice are segmented animals with a rigid exoskeleton and jointed limbs. But notice they are crustaceans (along with shrimp, crabs, lobsters, crayfish, etc.), not insects. Woodlice have three body regions or groups of segments: head, thorax, and abdomen. The poorly developed compound eyes are located on the side of the head. Two large antennae

project from the head and function as sensory organs. The walking legs of the thorax are adapted for locomotion and burrowing. The highly modified legs of the abdominal segments function as **gills** and are not used for locomotion. The gills (called pleopods) serve as respiratory organs (for gas exchange). Oxygen from the air diffuses across the moist gill surfaces and into the blood. The oxygen is carried in the blood to the animal's cells where it is used in cellular respiration. Carbon dioxide produced as a waste-product of cellular respiration is transported in the blood back to the gills where it is released into the atmosphere. The gills must retain a covering film of moisture in order for gas exchange to take place.

Woodlice are thought to have colonized land directly from the sea about 50 million years ago and now occupy a wide range of habitats and display varying degrees of tolerance to desiccation. They lose a great deal of moisture by evaporation through the thin, ventral (bottom) surface of the exoskeleton. Much of this water is replaced by drinking and eating moist material (primarily decaying vegetation and small amounts of decaying animal material). Woodlice tend to be nocturnal and live under logs, stones, and other places where the environment is humid.

# Part I. Locomotor Response to Moisture

First, you will conduct a class-wide experiment to test how sowbugs respond to variation in moisture. You will work in groups of 2-3 students to conduct one experimental *replicate* as described below. We will then compile all the results across groups (replicates) and analyze them to draw a conclusion (review the Scientific Reasoning lab for an overview of the "scientific method"). Before we begin, answer the question below.

- Based on the biology of woodlice, **hypothesize** how sowbugs orient and move in response to variation in moisture. **Justify** your hypothesis by considering the adaptive value of the behavior (i.e., how it could enhance the individual's survival and/or reproduction in its natural environment).

## Methods

1. Obtain the following materials: 1 choice chamber, 2 filter paper discs, beaker with water, pipet, cup with 10 live sowbugs.

2. Place clean filter paper into each side of your choice chamber. Using a pipet, saturate the filter paper on one side of the chamber. Pour off any excess water so that it cannot run into the other side of the chamber and moisten the paper there.

    a. **Why** place filter paper on the dry side of the choice chamber instead of just leaving it empty?

3. Use your fingers or a sorting brush to carefully transfer 10 sowbugs to the center of the choice chamber. Try not to get soil in the chamber. Put on the lids. Do not move the dish, make loud noises, or subject the animals to bright light. The goal is to observe their behavior while influencing it as little as possible.

4. Count and record (in Table 1) the number of sowbugs on each side of the chamber every 30 seconds for 5 minutes. Continue to record, even if the sowbugs all move to one side or stop moving at all. If an individual is located near the center of the choice chamber but the majority of its body is past the midline toward either the moist side or the dry side, count it as being on that side (i.e., your total count at each time interval should always add up to 10).

5. Between collecting the quantitative data above, record qualitative behavioral observations such as how the sowbugs move (e.g., quickly, slowly, sporadically, continuously, etc.), how they interact with their environment, and social interactions, etc. Be mindful not to **anthropomorphize** or attribute human characteristics to non-human animals (e.g., we have no idea what sowbugs "like").

6. Return your sowbugs to the holding cup. You will use the same 10 individuals in Part 2 (Replicate 1).

7. Report your results from the 5-min mark to your instructor or enter the data on the board as directed.

8. Answer the following questions.

    a. What **independent variable** did you manipulate or alter in the experiment?

    b. What **dependent variable** did you measure or count?

    c. What **standardized variables** did you control for or hold constant?

    d. Based on your own observations (before analyzing the class-wide results), did the sowbugs appear to orient and move toward or away from the stimulus of moisture? **Describe** their locomotor response using specific terms from above (e.g., positive/negative, prefix-, taxis/kinesis).

## Results

**Table 1.** Number of sowbugs on each side of the choice chamber every 30 sec for 5 min.

| | Number of sowbugs at each time (min:sec) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0:30 | 1:00 | 1:30 | 2:00 | 2:30 | 3:00 | 3:30 | 4:00 | 4:30 | 5:00 |
| moist side | | | | | | | | | | |
| dry side | | | | | | | | | | |

Qualitative Observations:

# Data Analysis

Biologists use **statistics** to reach objective conclusions about whether their test results support or reject a hypothesis. There are many different statistical techniques used for different types of data. For this lab, we will use a **chi-square goodness-of-fit test** to analyze our results.

**Chi-square** is a statistical technique that is used when you are counting how many individuals fit into different categories. You must have an **expected** number of individuals in each category, and you can then test whether the **observed** numbers differ significantly from the expected values. We will use a chi-square test to determine whether the responses of sowbugs to moisture (and other environmental stimuli) differ significantly from what would be expected if their responses were random with respect to the stimulus.

For example, if I wished to know whether bees have a preference for white or pink flowers, my statistical **null hypothesis** would be that bees have **no preference** for flower color (i.e., they visit flowers randomly with respect to color). If I observed 50 flower visits by bees, the *expected* values would be those consistent with my null hypothesis, or 25 visits to white flowers and 25 visits to pink flowers (half and half, assuming white and pink flowers were equally available.). If I actually *observed* 18 visits to white flowers and 32 visits to pink flowers, the deviations from my expected values would be 7 and 7, respectively (25-18=7, 32-25=7). The **chi-square ($\chi^2$)** statistic is calculated by using both the observed and the expected values.

$$\chi^2 = \sum \left[ \frac{(observed - expected)^2}{expected} \right]$$

Chi-square equals the sum of each deviation squared divided by the expected value. So, in our example:

$$\chi^2 = \left[ \frac{(18-25)^2}{25} + \frac{(32-25)^2}{25} \right] = \left[ \frac{(7)^2}{25} + \frac{(7)^2}{25} \right] = \frac{98}{25} = 3.92$$

The next step is to compare the **calculated chi-square** (3.92 in this example) to a corresponding **critical value** that can be looked up in a table. The critical value depends on the number of categories and the "significance level" of your statistical test. In order to ***reject* the null hypothesis** (that bees have no preference for flower color), the calculated chi-square must be ***equal to or greater than*** the critical value. If your calculated chi-square is less than the critical value, then you fail to reject the null hypothesis. In this case and in our sowbug

experiment, you may use the **critical value of 3.84**, which corresponds to a dataset with two categories and a conventional 0.05 significance level[1].

In the example above, because our chi-square value (3.92) is *greater than* the critical value (3.84), we can *reject the null hypothesis* and conclude that bees exhibit a **significant** preference for pink flowers over white flowers. If our chi-square value was *less than* 3.84, we would *fail to reject the null hypothesis* and conclude that bees do not prefer either pink or white flowers. Note that the *statistical* null hypothesis is typically the opposite of your initial *biological* hypothesis.

Now let's analyze the class-wide experimental results: **Do sowbugs exhibit a significant locomotor response to moisture (i.e., a preference for either moist or dry environments)?**

1. State the **null hypothesis.**

2. Record the **observed** values from the board (*total* counts at 5 min, summed across all groups/replicates) and then calculate the **expected** number of sowbugs on each side of the choice chamber, *if the null hypothesis was true*.

| | Moist | Dry |
|---|---|---|
| Observed | | |
| Expected | | |

3. Calculate **chi-square ($\chi^2$)**. *Show your work.*

4. Compare your calculated chi-square statistic to the critical value (3.84). Can you reject the null hypothesis? Why or why not?

# Discussion/Post-Lab Questions (Part I)

Answer the following questions and submit your responses to your instructor as directed.

1. Based on the class-wide experimental results and your chi-square goodness-of-fit test, what **conclusion** can you can draw about the locomotor response of sowbugs to moisture? Was your initial biological hypothesis supported by the data? **Explain**.

2. Why was it important to include multiple **replicates** in the experimental design (i.e., independent trials using different sets of sowbugs)? Were the class-wide results consistent with your direct observations from your own choice chamber?

[1] This is an oversimplification; you will learn about the underlying theory and gain more experience with data analysis in other courses. An alternative approach to the critical value comparison involves using a computer program to calculate an exact *P* value, which represents the probability of obtaining a test statistic at least as extreme as the observed value, if the null hypothesis is true. Conventionally, you may reject the null hypothesis only when $P \leq 0.05$.

3. *Disclaimer*: For convenience, we are violating a critical assumption of the chi-square test – that all observations are independent of one another. How do you think the results of the experiment might be different if we were to test individual sowbugs one-by-one instead of in batches of 10?

# Part II. Design and Conduct Your Own Experiment

Next you will apply lessons learned above to investigate how sowbugs respond to another environmental stimulus of your choosing. During the remainder of Week 1, you will design an experiment and get approval from your instructor. Next week, you will conduct the experiment and analyze and interpret the results.

## Task 1 – Experimental Design

1. Brainstorm **environmental stimuli** *other than moisture* than might elicit a locomotor response from sowbugs. Consider factors that could vary in their natural environment, as well as the animals' requirements for growth, survival, and reproduction. You may browse materials around the lab for inspiration. If you would like to explore a stimulus that is not present, ask your instructor whether appropriate supplies might be available (or perhaps you can bring your own next week). Possible factors to test include but are not limited to temperature, light, pH, substrate, etc. *Be creative!*

2. With your group, choose **one stimulus** and form a **hypothesis** (or alternative hypotheses) about how you think sowbugs will respond. Provide a **rationale or justification** for your hypothesis(-es) that is relevant to the biology of woodlice and informed by your own observations.

3. **Design an experiment to test your hypothesis** during next week's lab period. When designing your experiment, be careful not to injure the isopods. Lamps placed too close to the organisms, for example, may generate dangerous heat.
    a. Follow the general procedure of the moisture experiment conducted above, except *manipulate* a different independent variable (ideally, only one) while *controlling for* moisture (i.e., hold constant across both sides of the chamber).

    b. Use only 10 sowbugs at a time, but conduct multiple replicates (3) with different individuals (i.e., test 30 sowbugs total).

4. Answer the following questions:
    a. What is your hypothesis and rationale/justification for your experiment?

    b. What **independent variable(s)** will you manipulate or alter in the experiment?

    c. What **dependent variable(s)** will you measure or count?

d. What **standardized variables** will you control for or hold constant?

e. Outline/sketch other components of your **experimental design**.

f. What specific results do you **predict**, if your hypothesis is correct?

5. **Present your hypothesis and experimental plan to your instructor and <u>get their approval before you leave lab</u>.** Come prepared to conduct your experiment *next week*.

# Week 2. Part II Continued

## Task 2 – Conduct Experiment

6. Review your hypothesis and experimental plan that were approved last week.

7. Gather your experimental supplies, including a cup with 10 live sowbugs.

8. Set up and conduct your experiment. Record your data in Table 2 below and note other observations. After the first replicate, return your sowbugs to the holding cup and trade with another group before conducting the second replicate with a different set of 10 individuals. Do the same before your third replicate (i.e., you should test 30 different sowbugs total).

9. When you are finished collecting data, clean up your lab space and return the sowbugs and other supplies to their original locations.

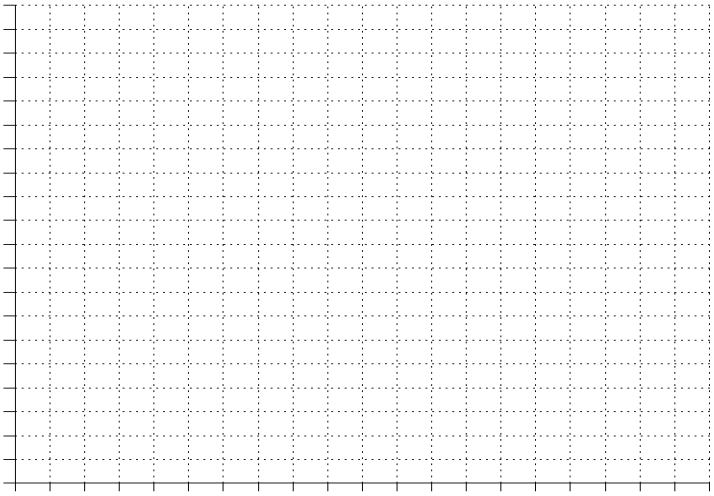
## Results

**Table 2.** Number of sowbugs on each side of the choice chamber every 30 sec for 5 min. Fill in the blanks for each side.

| | | Number of sowbugs at each time (min:sec) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0:30 | 1:00 | 1:30 | 2:00 | 2:30 | 3:00 | 3:30 | 4:00 | 4:30 | 5:00 |
| Replicate 1 | _____ side | | | | | | | | | | |
| | _____ side | | | | | | | | | | |
| Replicate 2 | _____ side | | | | | | | | | | |
| | _____ side | | | | | | | | | | |
| Replicate 3 | _____ side | | | | | | | | | | |
| | _____ side | | | | | | | | | | |
| Total (summed across all replicates) | _____ side | | | | | | | | | | |
| | _____ side | | | | | | | | | | |

Qualitative Observations:

1. **Graph your results below.** *At each 30-sec interval*, plot the *total numbers* of isopods (summed across all replicates) that were counted on each side of the choice chamber. Be sure to **label the x and y axes** and to **distinguish the two sides** of the chamber with different symbols/colors and a key (e.g., two separate lines on your graph, one for each side).



2. **Analyze the results of your experiment using a chi-square goodness-of-fit test**. Use the *total counts at 5:00 min*, summed across all replicates (i.e., only the two cells in the bottom right corner of Table 2).

## Discussion/Post-Lab Questions (Part II)

Answer the following questions and submit your responses to your instructor as directed. These questions will help you communicate your independent research process and findings, like you would in a more thorough scientific report. You are encouraged to discuss your project with classmates, but *answer the questions individually using your own words (and complete sentences).*

1. **Question:** What question was your experiment designed to answer? *Causal questions* (e.g., *How? Why? What causes?)* are better than yes/no questions.

2. **Hypothesis:** How did you expect the sowbugs to respond to the stimulus your group tested? What was the **rationale or justification** for your hypothesis, based on previous observations and/or the biology of woodlice?

3. **Methods:** Briefly describe your experimental design. What materials did you use and how did you use them to test your hypothesis? Clearly identify the **independent**, **dependent, and standardized (controlled) variables.**

4. **Results:** Briefly summarize your results. *Do not* report every single value; describe the *overall pattern* shown in your **graph** (include it with your submission). Also report the final results (*not* the detailed calculations) of your **chi-square test** and interpret the statistics (i.e., was the null hypothesis rejected? did the sowbugs exhibit a significant response to the stimulus?). Share other relevant observations to provide context about their behavior.

5. **Conclusion:** What conclusion can you draw from the results of your experiment? Was your initial biological hypothesis *supported* or *rejected?* Try to explain *why* the animals responded the way they did; think about how woodlice interact with their natural environment, and how particular behavioral responses might be adaptive (i.e., provide a fitness benefit).

## Acknowledgements

This lab was modified with permission from unpublished versions by William Glider and Kari Benson. Thanks!