BIOL 1005
Concepts in Biology

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Chapter 12: Wildcam Gorongosa materials used with permission from the Howard Hughes Medical Institute.

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Note: WildCam Gorongosa materials are copyrighted by HHMI and are therefore not included in this lab manual. For materials, visit https://www.hhmi.org/biointeractive/wildcam-gorongosa.

Lab Safety Guidelines

1. Tell your TA if you are taking immunosuppressive drugs, are colorblind, are pregnant, have any allergies (including penicillin), or have any other medical conditions that may require precautions in lab.
2. Wear closed-toed shoes in the laboratory – you don't want nasty, corrosive stuff splashing onto your feet.
3. Cover any open cuts with bandages before coming to lab – you don't want bacteria and nasty chemicals entering your bloodstream through an open wound.
4. Bring something to tie your hair back with, and don't wear long/dangly jewelry, oversized sleeves, etc. that may get in the way as you work – you don't want your hair, jewelry, or clothes to catch on fire or get dragged in nasty bacteria and chemicals.
5. Put your backpacks and other items in the cubbies built into the lab desks. If there's no room there, put them on the floor in the back of the room where no one will trip on them.
6. Don't eat or drink anything during lab – you really don't want to ingest bacteria and nasty chemicals.
7. Make sure you know where the fire extinguisher, chemical shower, first aid kit, and eyewash stations are. You might think you're so careful that you'll never need them, but your lab mate might be a klutz. You can be the one to help out in an emergency.
8. If there is a fire alarm, leave your belongings in lab and walk down the nearest safe stairway (probably the one on the north side of the building, right next to the lab room). Wait with your classmates a safe distance from the building. Do not wander off, especially without telling someone; your TA may need to account for your safety.
9. While conducting experiments, wear either glasses or the provided goggles when your TA says it's necessary – you don't want bacteria and nasty chemicals splashing into your eyes.
10. Wear gloves when your TA tells you to (we have latex-free gloves if you need them). If you come into direct contact with chemicals or microorganisms, wash your skin thoroughly.
11. Don't leave alcohol burners unattended, and keep flammable liquids such as ethanol at a safe distance from them. If you do start an alcohol fire in a beaker, don't panic. Just cover it with a glass dish and it will go out.
12. Don't let liquid get near the electrical outlets on your lab benchtop. Ditto for tablets, phones, and laptops.
13. Report any spills, breakages, or equipment that doesn't work to your TA immediately.

14. Clean up any broken glass with a broom and dustpan, or ask your TA for help. Don't touch broken glass with your hands.
15. Some materials cannot go into the general trash. Make sure you pay attention to your TA for special disposal instructions.
    a. Dispose of glass in the broken glass bin, not in the regular trash.
    b. You may need to deposit some specimens into biohazard bags.
    c. Soil from plants may need to be bagged separately from the general trash, as large quantities are too heavy for custodians to handle.
16. Don't perform any unauthorized experiments or use lab equipment without permission.
17. Wash your hands before leaving lab.

Don't Be a Jerk.

1. When your TA is trying to get the class's attention, help out –if you notice that your TA needs to say something to the whole class, stop talking and ask others to do the same.
2. Don't mess with your phone, goof around with your lab mates, or work on assignments for other classes while you are in lab. Yes, it's a free country, but your "side projects" are a distraction to your classmates. Stay focused on the lessons that the professor and TA have planned for you. Teaching a class is a lot harder when students are distracted.
3. If you're done with an activity before everyone else is, quietly review course material on your own, answer the review questions, or see what you can do to help your classmates or your TA. Don't do things that distract other students or that take your focus away from what you're supposed to be learning.
4. Don't write on or carve anything into the lab benchtops. You might have an important message to deliver to future generations, but you can find another way to express it.
5. Do not torture any of the live animals you'll observe in the lab, not even the worms and other invertebrates. Do not intentionally pour chemicals on them, shine a direct light on them until they dry out, etc. Even small animals experience pain, and it's unkind to take pleasure in another individual's pain.
6. Clean up after yourself. Throw away your trash, push in your chair, etc. It takes only a little time for you to tidy up your own space, but it takes your TA a long time to do it for the whole class. Respect your TA's time.

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The Process and Tools of Science

Mariëlle Hoefnagels and Sarah Greenwood

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Introduction

During this semester, you will have many opportunities to come up with your own hypotheses, then design and carry out your own experiments to test those hypotheses. To do that, you have to learn how to set experiments and collect some data. And that requires that you learn how to use some tools. We'll teach you some of those tools – the more sophisticated ones – as we go along. But today you'll learn some of the basic tools that you'll use over and over again this semester.

This lab begins with lessons on the scientific method and on the fundamentals of scientific notation and the metric system. Then you'll get used to putting your data in graphs that others can interpret. Finally, you'll practice with some ways to measure in metric units. In coming weeks you'll be writing short lab reports (abstracts) in which you'll have to use metric measurements to set up your experiments and collect your data, and then you'll have to graph

your data correctly. (For abstract-writing guidelines, and a sample abstract, see the appendix of this manual). The purpose of this lab, then, is to make sure everyone is up to speed on these fundamentally important skills.

Activity 1: Science, Hypotheses, and Experimental Design

Objectives

1. Know the steps of the process of science and understand why scientists use this process.
2. Understand the necessary parts of experiments like hypothesis, variables, etc. and recognize them in experiments.
3. Write an IF … AND … THEN statement to express a hypothesis, proposed experiment, and prediction.

You should have already purchased a textbook; please read the portion of chapter 1 that describes the process of science and experimental design. This lab manual will not repeat this material; instead, we will only provide a brief summary.

Figure 1 shows one way to summarize the overall process of science:

1. Observations
2. Questions
3. Hypothesis
4. Predictions
5. Experimental test

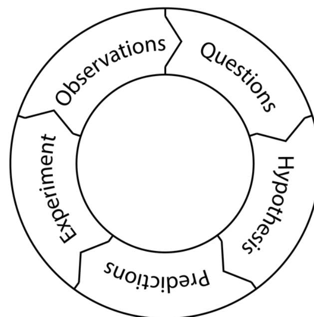

Figure 1: The process of science.

__Observations__ about the world may come from your own experience or from previously published work. The observations may raise **__questions__** about how or why the world works the way it does. In its simplest form, a **__hypothesis__** is a tentative explanation for how or why the world works the way it does. A hypothesis must be testable, and there must be a way to collect information that could lead you to reject the hypothesis. For that reason, "People become mentally ill because they are possessed by demons" is not testable because no experiment can prove it false, whereas "People become mentally ill because of chemical imbalances in the brain" is testable.

A hypothesis should lead directly to one or more testable **__predictions,__** which express what we should observe if the hypothesis is correct. These predictions may be linked to one or more **__experiments__** that are carefully designed to test the hypothesis in laboratory or field conditions.

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In fact, the hypothesis, test, and predictions are often combined into one **"If … and … then" statement.** For example, we might say that:

[Hypothesis = Explanation]: *IF* vitamin E is essential because it helps support the immune system …

[Test = Experiment]: … *AND* we alter the amount of vitamin E in the diets of mice while exposing them to bacteria that cause pneumonia, …

[Prediction = Result that would support hypothesis]: … *THEN* mice with higher doses of vitamin E should have a lower incidence of pneumonia than mice with less or no vitamin E.

Successful experimental design means identifying a few **variables,** which are factors that may change over the course of the experiment. Here are the most important variables:

1. **Independent variable:** The factor (or factors) that the investigator *directly* changes or manipulates. In the example above, the independent variable would be the amount of vitamin E that the mice receive. *Pro tip:* The independent variable often goes on the X axis of a graph. If you have trouble deciding how to phrase the independent variable, try thinking of the title you would give the X axis of a graph depicting the experiment's data.
2. **Dependent variable:** Any variable that the investigator thinks might change as a result of changes in the independent variable. In other words, this is the experimental "output" that investigator measures. In the example above, the dependent variable would be the incidence of pneumonia in the mice.
3. **Standardized variables:** Variables that the investigator holds equal for **all** subjects in the experiment, **including the control subjects** (see below). These are important because they help the investigator reduce the effects of other factors that may affect the dependent variable. For example, other dietary ingredients besides vitamin E should be standardized because they may affect immune function in mice. That is, all mice should receive diets that are identical in all respects (standardized variables) except for the vitamin E content (independent variable). Age is another important standardized variable because older mice are most susceptible to pneumonia. Finally, all mice should receive the same exposure to the bacteria that cause pneumonia.

In designing an experiment, the investigator often begins by deciding on the **treatment levels,** which are the values of the independent variable. In experiments that compare a new situation (e.g., vitamin E addition) to an old way of doing things (e.g., no vitamin E), the **control group** forms the basis of comparison to the other experimental treatments. In our example, the four

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treatment levels are the high, medium, low, and zero doses of vitamin E. Subjects that receive that zero level (no vitamin E) make up the control group.

Some types of experimental designs, however, do not require a control group. For example, instead of studying vitamin E levels, the investigators may compare the incidence of pneumonia (dependent variable) in mice receiving different brands of mouse chow (independent variable). In that case, there would not be a control because a "no-chow" treatment would not make sense.

One final important consideration is the **sample size,** which is the number of subjects that receive each treatment level. That is, suppose our investigator purchases 100 identical mice and randomly divides them into four equal-sized groups, and each group is randomly assigned to one treatment (high, medium, low, control). In that case, the sample size is 25. In a perfect world, the sample size for every experiment would be enormous, because more subjects mean more reliable results. But in the real world, sample sizes are much smaller because of limitations on time, space, money, supplies, or the availability of suitable subjects.

With this background in mind, your TA will lead you through an activity designed to help you practice with these concepts of experimental design.

Fill these out for the Pellagra video:

- Independent variable: the factor that YOU change or manipulate

  e.g. in the Goldberger experiment: ____

- Dependent variable: what you measure

  e.g. in the Goldberger experiment: ____

- Control: basis for comparison

  e.g. in the Goldberger experiment: ____

- Standardized variables: any factors held constant because they might affect dep. var.

  e.g. in the Goldberger experiment: ____

- Write an IF … AND … THEN statement that Goldberger might have used to guide his experiment.

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Activity 2: Scientific Notation and the Metric System

Objectives

1. Know that the metric system is the standard for science around the globe.
2. Understand and be able to use scientific notation.

The **metric system** gives scientists around the world a common language of weights and measures. (The United States is one of the few countries that is not all-metric, but even U.S. scientists use metric measures so they can easily communicate with their peers worldwide). Just in case you're not comfortable with scientific notation and metric units of measurement, here's a brief review.

First, remember **scientific notation**? This is a simple way to express very large or very small numbers. If you want to express the number of cells in your body, is it clearer to write 1,000,000,000,000 or just to write 1×10^{12}? Many numbers are much larger than that, such as the estimated number of bacterial cells on earth:

$$5{,}000{,}000{,}000{,}000{,}000{,}000{,}000{,}000{,}000{,}000, \text{ or } 5 \times 10^{30}$$

You will probably agree that the scientific notation is somewhat easier to read than the "normal" way of writing numbers.

OK so what is scientific notation? It's just a way of expression numbers based on powers of ten. First of all, let's recall exponents:

10,000	=	10^4
1,000	=	10^3
100	=	10^2
10	=	10^1
1	=	10^0
0.1	=	10^{-1}
0.01	=	10^{-2}
0.001	=	10^{-3}
0.0001	=	10^{-4} (etc.)

You can express any number as a power of 10. For example, it's easy to see that the number 20, for example, is 2×10^1, and 30 is 3×10^1, and so on. But what about 15? Well, it's 1.5×10^1. What about 1500? Same idea, multiplied by 1000: it's 1.5×10^3. For this class, you don't have to

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worry about the rules for adding, multiplying, dividing, and subtracting exponents because you won't have to do much of that in this lab.

But you will have to convert numbers (which may be in conventional or scientific notation) from one type of unit, say centimeters, into another type of unit, say meters. The reason scientists love the metric system (and the reason we will use it exclusively in this class) is that the metric system is very simple and sensible:

length is based on meters (m)
mass is based on grams (g)
volume is based on liters (l)

If you live far away from your grandma, you express that distance as thousands of meters (kilometers), since the basic metric unit of length is the meter. The good news is that the prefixes we use to describe just how many multiple-meters you live away from grandma (or multiple liters of Coke you drink per day) are standardized for all metric measurements. For example, since "kilo" always means 1000, a kilometer is 1000 meters, a kilogram is 1000 grams, and a kiloliter is 1000 liters. The only hard part about it is that you have to know (yes, memorize) the prefixes. **Table 1** shows the ones you need to know for this class:

Table 1: Metric system prefixes.

Prefix	Meaning	Common example(s)
kilo-	10^3, or 1000	kilometer (km) = 1000 m; kilogram (kg) = 1000 g
centi-	10^{-2}, or 1/100	centimeter (cm) = 10^{-2} m
milli-	10^{-3}, or 1/1000	millimeter (mm) = 10^{-3} m; milligram (mg) = 10^{-3} g; milliliter (ml) = 10^{-3} l
micro-	10^{-6}, or 1/1,000,000	micrometer (µm) = 10^{-6} m; microgram (µg) = 10^{-6} g; microliter (µl) = 10^{-6} l
nano-	10^{-9}, or 1/1,000,000,000	nanometer (nm) = 10^{-9} m; nanogram (ng) = 10^{-9} g

To convert from one to the other, all you have to do is multiply by the right conversion factor. So if you want to convert a distance of 2 meters into centimeters, just multiply your 2 meters by 100 cm (since there are 100 cm in a meter) → 2 meters = 200 cm. If you want to know how many millimeters that is, just multiply your 2 meters by 1000 mm instead (since there are 1000 mm in a meter).

Table 2 has some problems for you to practice on (before lab if you wish); if you're not sure you're doing them right, check with your TA during lab.

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Table 2: Metric system conversions.

Item	**Size**	**Convert to…**
Length of your esophagus	2.4×10^{-1} m	= ________ cm
Diameter of your eyeball	2.5×10^{-2} m	= ________ cm
Length of *Salmonella* bacterium	1×10^{-6} m	= _______ µm
Diameter of one of your cheek cells	2×10^{-5} m	= _______ nm
Sack of potatoes	4.5 kg	= _______ g
Raisin	1 g	= _______ mg
Amount of sodium in 2 granola bars	160 mg	= _______ g
Amount of Coke in a sip	1×10^{-2} l	= _______ ml
Amount of urine you produce per day	1.5 l	= _______ µl
Volume of a drop of water	0.05 ml	= _______ µl

Make sure you know how to do these types of conversions. In doing the problems, you should always do a mental check to see if your numbers make sense. Does it make sense that there should be 200 cm in 2 meters? Yes, because centimeters are smaller than meters, so there should be lots of them in 2 meters. Does it make sense that there should be 2000 liters in 2 milliliters? No!!! Just think about it to ensure that you did your conversion in the right direction.

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Activity 3: Standard Metric Units of Measure

Objectives

1. Understand the metric system and recognize common units for each kind of measurement: length, mass, volume, and temperature.
2. Use instruments, like pipettes, graduated cylinders, and thermometers, to accurately measure things in metric units.

Length: The standard metric unit for length is the meter, which is slightly shorter than a yard. In lab you're more likely to use the centimeter, which is 1/100th of a meter, or a little less than half an inch. The tool we'll use to measure length is a **ruler** (**Figure 2**); it is probably safe to say that most of you have used one before!

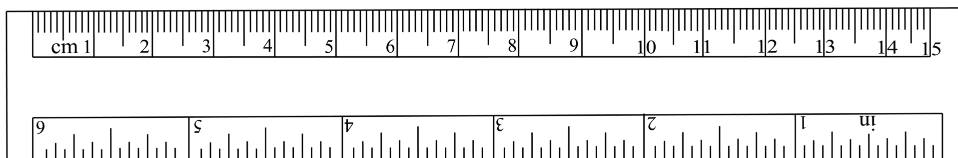
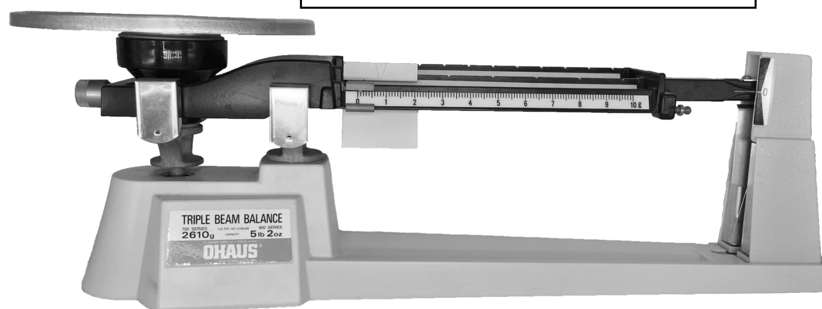


Figure 2: Ruler with centimeters and inches.

Mass: The standard metric unit for mass is the gram, which is about as much as the mass of a paper clip. The tool we'll use to measure mass is an **electronic scale** or a **triple-beam balance** (**Figure 3a**). Like the ruler, it doesn't take a genius to operate either piece of equipment. But it is good for this lab if you get some intuition about the approximate mass of common objects. **Figures 3b-3d** have some examples.

Figure 3a: Triple-Beam Balance



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Volume: The standard metric unit for volume is the **liter**. Probably the easiest way to visualize a liter is to picture a 2-liter bottle of Coke. However, we will mostly be working with much smaller volumes in this lab, usually on the scale of a milliliter (ml). For reference, there are about 20 drops of liquid in a milliliter.

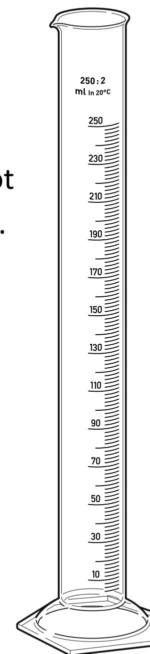
Figure 3b: A common highlighter weighs about 10 g.

Figure 3c: A plastic spice container weighs about 100 g.

Figure 3d: A large book, like a dictionary, weighs about 1 kg.

There are several ways to measure out a particular volume of a liquid. Some are much more precise than others. In lab this semester, you will use two of these tools: graduated cylinders (for volumes greater than 10 ml or so) and pipettes (for smaller volumes). By the way, you will notice that many glass items we use this semester, like beakers and flasks, have volume markings, but these are approximate volumes only. You should never use beakers or flasks to measure volumes unless you really don't care if the measurement is off by a lot.

Figure 4: Graduated cylinder.



A **graduated cylinder** (**Figure 4**) is a clear glass or plastic tube with volume markings on it. Used correctly, the graduated cylinder should yield a pretty accurate volume measurement. A graduated cylinder is not hard to use; you just fill it up to whatever mark you need and off you go. The only thing to remember is that it is standard practice to use the bottom of the liquid's **meniscus** (**Figure 5**) as your reference point when measuring volume in a graduated cylinder. So what's the meniscus? It's the very top of the fluid, and seen from the side (through a graduated cylinder) it may look sort of like a "skin" on top of the fluid. Depending on the properties of the graduated cylinder, the "skin" can creep up the sides of the cylinder a bit; that's why it's important to always use the bottom of the meniscus as your reference point, like this:

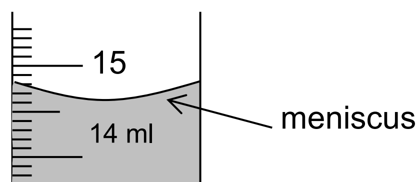


Figure 5: Meniscus. What is the volume of the liquid in this cylinder?

Your lab bench will be provided with some graduated cylinders and water with food coloring so you can practice using a graduated cylinder.

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Another way to measure volume is to use a **pipette.** As you can see from **Figures 6-8**, a pipette is sort of like a glass or plastic straw, and it often has volume markings on it. Pipettes are used for precisely measuring fairly small volumes of liquid (from less than 1 ml to about 10 ml). You really need to get good at using pipettes, because you'll start using them for real (lots of them) in subsequent labs.

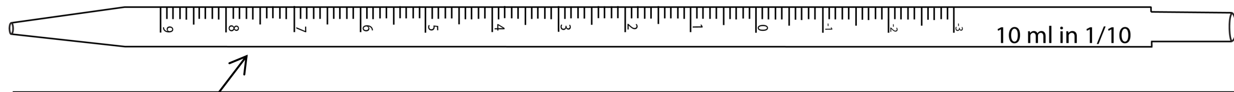

Figure 6: These **graduated pipettes** are used for larger amounts of liquid, typically 1 or more milliliters; this one measures 10 ml. Graduated pipettes are used with a pipette pump; see **Figure 9**.

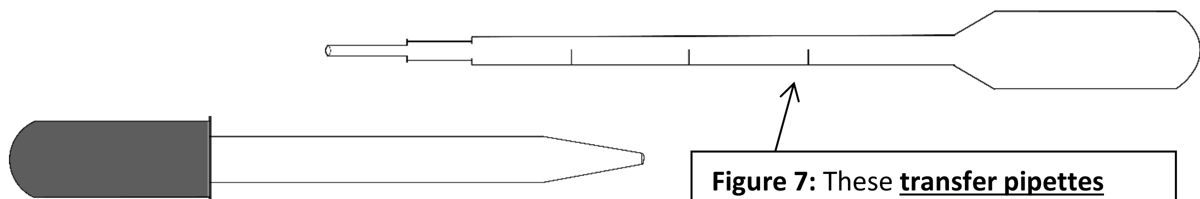

Figure 7: These **transfer pipettes** have a built-in bulb, and are used for small amounts of liquid. They are made of plastic and often measure 1 ml. The marks along the pipette show approximate volumes.

Figure 8: These **Pasteur pipettes** (also called droppers) are made of glass and have a separate rubber bulb. They are meant for dispensing small, inexact amounts of liquid.

Using the graduated pipette: Transfer pipettes and Pasteur pipettes are really easy to use, but you'll need a little training to use a graduated pipette. The first thing you need to do when you use a graduated pipette is to look at the top of it, where it says the total volume of the pipette. For this class, the volume will usually be 1 ml, 5 ml, or 10 ml. It's surprising how many experiments are blown because the investigator was using a 1-ml pipette when he or she thought it was a 10-ml pipette—of course the results will be bad. We want you to learn approximately how big 1 ml is (and we want you to learn to always look at the top of the graduated pipette before you begin) so that you can avoid mistakes like that.

As mentioned above, a graduated pipette looks sort of like a fancy glass or plastic straw with volume markings on it. If it's a 1-ml pipette, the major markings will indicate portions of 0.1 ml; if it's a 5-ml or 10-ml pipette, the major markings will delineate portions of 1 ml. When measuring liquid, the volume in the tip of the pipette counts. For example, in the graduated pipette illustration, the first ml of liquid would go from the tip to the "9" mark. Again, always look at the volume before you pipette, and notice whether the demarcations are numbered from the bottom up or from the top down. **Pipettes are not all created alike!**

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A graduated pipette works just like a straw, except you are NOT allowed to suck up the liquid with your mouth in this class! No, no, no! In this class, we will ALWAYS use pipette pumps to suck the liquid up the graduated pipette, no matter how harmless the liquid. After all, you never know where a pipette has been before you started handling it! A **pipette pump** (see **Figure 9**) fits over the top of the graduated pipette, and you turn the little wheel to suck the fluid in. Then you press the plunger down to release the fluid into the receiving container. It's a lot safer and easier than most any other method of filling a graduated pipette.

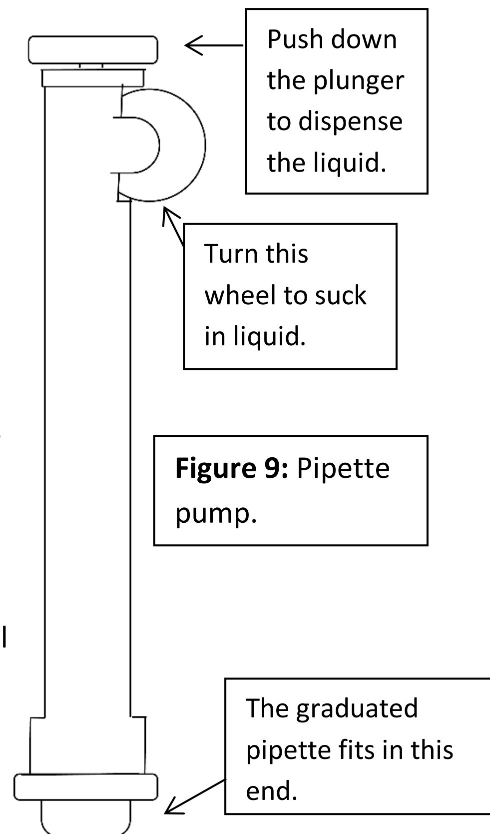

Figure 9: Pipette pump.

Different size pipette pumps fit different size pipettes. During this lab, practice using the appropriate pump to fill each size pipette provided. **Table 3** includes "target" volumes for you to use; practice pipetting each volume until you can do it well and with confidence (in next week's quiz you may be asked to demonstrate your pipetting skills). Remember, the same rule applies here as for the graduated cylinder: the bottom of the fluid's meniscus is your target for measuring the volume.

Table 3: Pipetting practice.

Target volume to pipette	Which pipette should you use? (1 ml, 5 ml, or 10 ml)	Check here when you're good at pipetting this exact volume
0.2 ml		
1.1 ml		
8.2 ml		

Temperature: In the metric system, the unit for measuring temperature is the degree Celsius (or centigrade), abbreviated C. The system is very simple: 0^oC is the temperature at which water freezes and 100^oC is the temperature at which it boils. As another reference point, normal human body temperature, so familiar to you as 98.6^oF (Fahrenheit), is about 37^oC.

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As you probably already know, the **thermometer** (**Figure 10**) is the tool we use to measure temperature. You probably already know how to use a thermometer; the point of this exercise is to get you familiar with temperatures measured in degrees Celsius. Therefore, your instructor will have some items of various temperatures available for you to measure; record the data in **Table 4**.

Figure 10: Thermometer.

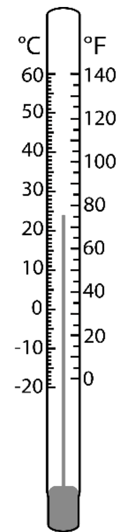

Table 4: Thermometer practice.

Object	Temperature (°C)

Activity 4: Data Presentation

Objectives

1. Understand the difference between continuous and discrete independent variables and what kind of graph works best for showing them.
2. Recognize the necessary parts of a graph.
3. Analyze the data a graph presents and explain what it means for the experiment.

This exercise is really critical because you will have to be able to present your data in a table and a graph for each of the abstracts you'll write (see the appendix for guidelines that will help you write your abstracts).

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Tables are pretty straightforward, so we won't bother with them here (but see the appendix if you want more information on tables). Graphs have a few more rules and regulations than tables, and we will work a bit here on constructing and interpreting graphs.

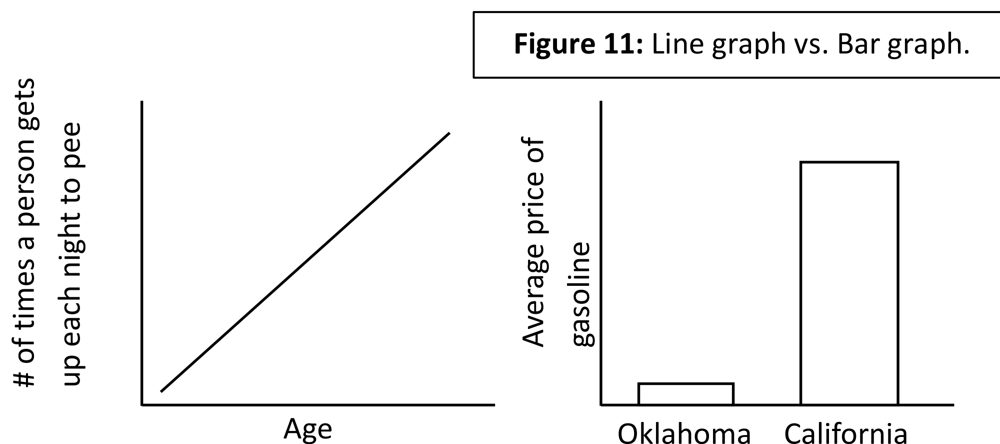
Scientists follow some conventions when constructing graphs. The first is that the independent variable (what you manipulate in your experiment) almost always goes on the X axis, and the dependent variable (what you measure as your experiment's "output") almost always goes on the Y axis. So let's say you did an experiment to see if dietary garlic makes turtles live longer.

What values would go on the X axis of your graph?

What values would go on the Y axis?

Another convention has to do with the type of graph you construct. Basically, there are two types of graphs: line graphs and bar graphs. **Figure 11** shows an example of each type (the line graph is on the left, and the bar graph is on the right):

Figure 11: Line graph vs. Bar graph.



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How do you know when to use each type of graph? To answer, you have to know that independent variables may be continuous or discrete. **Continuous independent variables** are those that can take any value within a range. (Huh?) Look at the X axis for the graph on the left. A person's age can be 25 or 26, or 25 ½, or 25 ¼, and so on. If you give me two points on the X axis, I can always name a point between them; that's what continuous means. **Discrete independent variables** have only a limited number of possible values. There is no such thing as "Oklahoma + ½" or "California + ¼". You can be in one category or the other; there is no in between. Here's the rule of thumb: **Use a line graph when the X axis depicts a continuous independent variable and a bar graph when the X axis depicts a discrete independent variable.**

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Yet another convention is that each axis of a graph must be labeled with the variable that is being measured and the units used to measure it (e.g. "Temperature, °C" or "Volume, ml"). You should also mark each axis with intervals. For example, if you were graphing how many Cokes the OU students from various countries drink each day, you might mark your axis (which one?) with the numbers 1-10 (or whatever the maximum and minimum numbers of Cokes are), and some logical number of intervals in between. The good news is that when you make a graph on a computer, as you must do for this class, the computer kindly marks logical intervals on your axes for you.

Now look once more at the sample graphs in figure 11. In light of the conventions listed above, what essential elements are missing from each of the graphs?

Now that you know how to dissect a graph, you can look at some pre-fabricated graphs and answer some questions about them. **Figure 12** is a graph you have seen before:

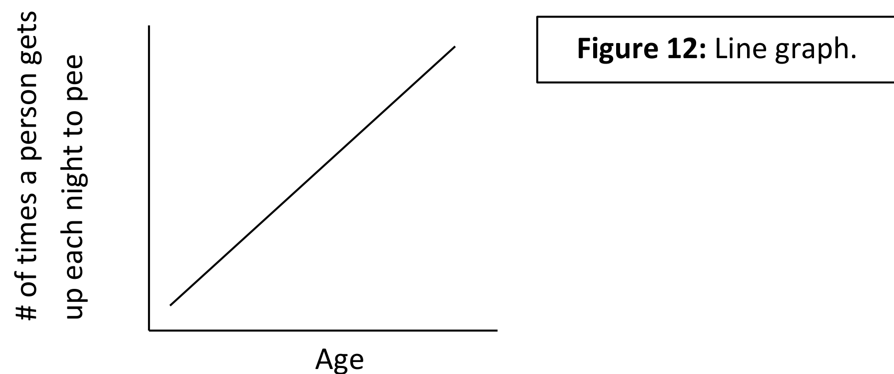

Figure 12: Line graph.

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- Describe what the graph shows.

- What is the dependent variable in this experiment?

- What is the independent variable in this experiment?

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- What other information would you need if you wanted to fully interpret this graph?

Figure 13 shows another graph. In this hypothetical experiment, researchers wanted to learn about the frequency that dogs bark at strangers, by both breed and age. They tested chihuahuas and golden retrievers of different ages by putting them in a standardized room with a window and having 10 people walk by the room over 2 hours. They recorded the number of times each dog barked and computed the average for each breed and age:

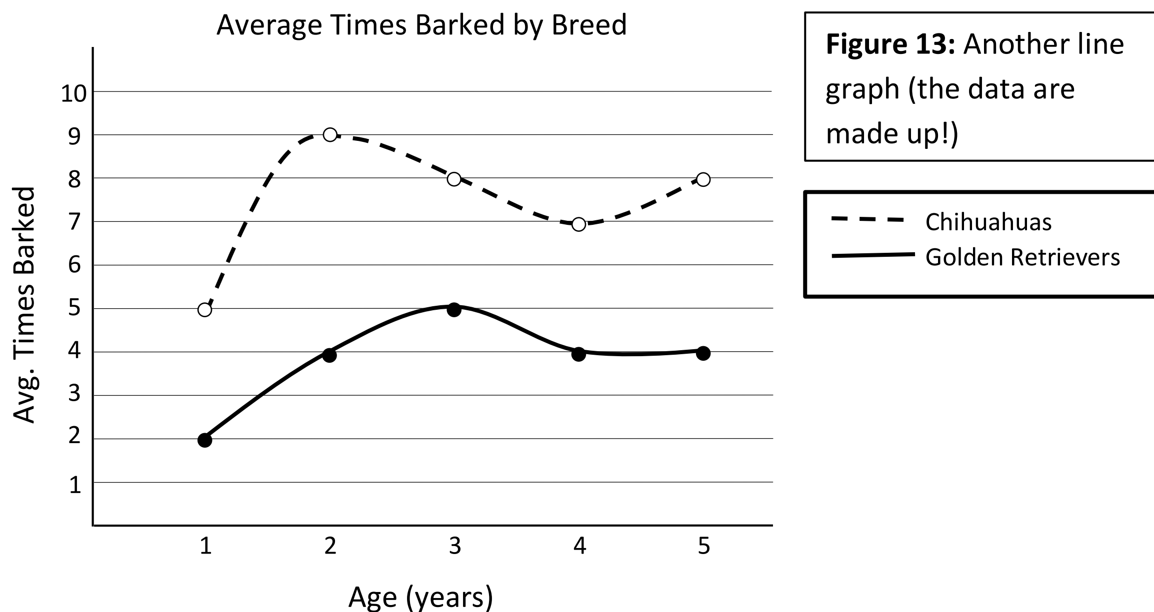

Figure 13: Another line graph (the data are made up!)

- In your own words, describe what the graph shows.

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- How many dependent variables are in this experiment? What is it/what are they?
- How many independent variables are in this experiment? What is it/what are they?
- How many times did the dogs bark when they were 3 years old?
- At what age did the dogs bark the most?
- If you prefer dogs that do not bark very much, which breed should you adopt? (Remember, the data are made up, so don't rely on this graph if you're actually getting a dog!)
- Devise a testable hypothesis that might explain the data presented in the graph above.

Activity 5: Putting It All Together (With Condoms)

Objectives

1. Design an experiment using measurement tools to test the properties of condoms.
2. Accurately measure quantities using the metric system.
3. Create your own hypothesis and conduct an experiment to test it.
4. Analyze the data you collect and use it to make graphs and tables.
5. Draw conclusions based on the data you collect.

Your TA will provide several different brands and types of condoms. Your group's job is to design an experiment to answer (almost) any question about the condoms, as long as the question can be answered using the scientific equipment provided in lab.

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In designing your experiments, keep the following guidelines in mind:

- You must use metric units of measure.
- You must use **at least two different measurement tools**, i.e.:
  - Length (m) – use a ruler to measure in centimeters or meters
  - Mass (g) – use a balance to measure in grams
  - Volume (l) – use a graduated cylinder and/or pipette
- Spend some time brainstorming questions with your group. Think about rumors you might have heard about factors that boost or lower a condom's strength. Also, check the labels for patterns in brand, thickness, texture, size, country of origin, or other useful information; the patterns you notice might inspire questions. Write at least five possible questions in the space below:

    1.

    2.

    3.

    4.

    5.

- After brainstorming and discussion, what question did your group decide on?

- Write an IF … AND … THEN statement that summarizes your hypothesis, test, and prediction. Remember, the hypothesis part should focus on a tentative **explanation**; it should not simply state your prediction.

    [Hypothesis = Explanation]: *IF* …

    [Test = Experiment]: … *AND* …

    [Prediction = Result that would support hypothesis]: … *THEN* …

- Which condoms will you use? Make sure there's enough of each condom for you to repeat your test as well.

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- Give a brief, very general description of your methods.
- What is your independent variable? What are the treatment levels?
- What are your dependent variables?
- What are your standardized variables?
- What is your control, if any?

Before you actually carry out your experiment, create one or more tables that will hold the data that you will collect:

Finally, before you actually carry out your experiment, draw one or more graphs of your *predicted* results (what you think is going to happen). Graphs should have axis labels with metric units (as needed), and treatments should have meaningful labels. (E.g., not “Treatment 1” vs. “Treatment 2” but “Ribbed texture” vs. “Non-ribbed texture.”) In addition, you will need to decide if a line graph or a bar graph is appropriate for the data you will collect.

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Your TA must approve your group's question, IF … AND … THEN statement, and experimental design before you move on! **TA, make your mark here: ________**

Once your plan is approved, use the table(s) you created above and the space below to record your group's actual methods and observations.

When all groups are finished, your group will make a brief presentation to the class. Your presentation must include the following elements:

- Title of presentation and names of group members.
- The question you are trying to answer.
- IF … AND … THEN statement with hypothesis (explanation), test, and prediction.
- Experimental design, including independent variable, dependent variables, standardized variable, and control (if any).
- One or more graphs showing your **predicted** results.
    - You should select the graph format (line or bar graph) to fit your independent variable.
    - Graphs should have axis labels with metric units (as needed).
    - Treatments should have meaningful labels. (E.g., not "Treatment 1" vs. "Treatment 2" but "Ribbed texture" vs. "Non-ribbed texture.")
    - During your presentation, you should walk the audience through your graph, explaining what is on each of the axes.
- A description of your methods, including photos and at least one video.
- One or more graphs showing your **actual** results. See criteria above.
- Conclusions.

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Acknowledgments and Attributions

The condoms activity was adapted from Poli, Dorothybelle. August 2011. Sex and the Scientific Method: Using Condoms to Engage College Students. The American Biology Teacher, p. 348.

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Using the Microscope

Mariëlle Hoefnagels and Sarah Greenwood

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Introduction

Microscopes are vital for biologists. Most cells, the smallest component of life, are not visible to the unaided human eye. Biologists therefore use a variety of microscopes to study life at its smallest scale. Throughout this lab, you'll be using microscopes to study a diverse selection of specimens—from simple printed text to samples of plant and animal tissues.

This lab will introduce you to the two microscopes you'll use in this class: the compound light microscope and the dissecting microscope. First, you'll learn to use the compound microscope, which has a more powerful magnification and allows you to look at smaller specimens. You'll learn the parts of the microscope and practice the correct techniques for using it. Then, you'll use the compound microscope to examine different kinds of living cells, including some of your own! You'll also apply your microscopy skills to investigate different adaptations in plant leaves and animal skin. Then, you may learn to use the dissecting microscope, which is used for larger specimens and small living organisms. By the end of the lab, you'll understand the correct

methods of using and handling microscopes, and you'll understand why they're so important for biologists.

Activity 1: The Compound Microscope

Objectives

1. Know the magnifications of each objective lens on the microscopes we have in lab.
2. Calculate the total magnification for each objective we have in lab.
3. Identify the parts of the compound microscope.
4. Use the correct procedure to focus the microscope at different magnifications.
5. Measure your field of view at each magnification in micrometers.
6. Understand how depth of focus changes as the magnification increases.
7. Change the plane of focus at high magnifications by using the fine focus knob.

During this lab, you will be most frequently using the compound microscope. **Compound microscopes** work by combining the magnifying power of two lenses: one on the eyepiece, and the other on the **objective lenses** (see **Figure 1)**. You can find the total magnification by multiplying the power of the two lenses. The eyepiece magnification is fixed at 10X, but the objective magnification changes depending on which objective you have in place. On the microscopes we have in lab, the objective magnifications are 4X (low-power, or scanning), 10X (medium-power), 40X (high-power), and 100X (oil immersion). We will not be using the oil immersion objectives in this lab. Calculate the total magnification you will see when you are using each objective:

Low-power (scanning) objective: ________ (eyepiece) x ________ (objective) = _______

Medium-power objective: ________ (eyepiece) x ________ (objective) = ________

High-power objective: ________ (eyepiece) x ________ (objective) = ________

Part 1: The Parts of the Compound Microscope

Here's a breakdown of the parts of the microscopes we have in lab (see **Figure 1**):

Eyepieces

The eyepieces hold magnification lenses at the top of the microscope. On our microscopes, they magnify at 10X. We have binocular (rather than monocular) microscopes, which means they have two eyepieces. The space between the two eyepieces can be adjusted to fit your preference. Additionally, a diopter adjustment ring on the left eyepiece allows you to adjust for the differences in eyesight between your eyes. This will come in handy if you notice one

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eyepiece is in focus but the other one is blurry. In that case, focus first for the right eyepiece (**without** the adjustment ring), then use the adjustment ring to focus the left eyepiece.

Objectives

The objectives hold the other magnifying lenses. On our microscopes, they magnify at 4X, 10X, 40X, and 100X. To change which objective is in place, grasp and turn the ridged ring, called the **revolving nosepiece**, at the top of the objectives, as opposed to the objective lenses

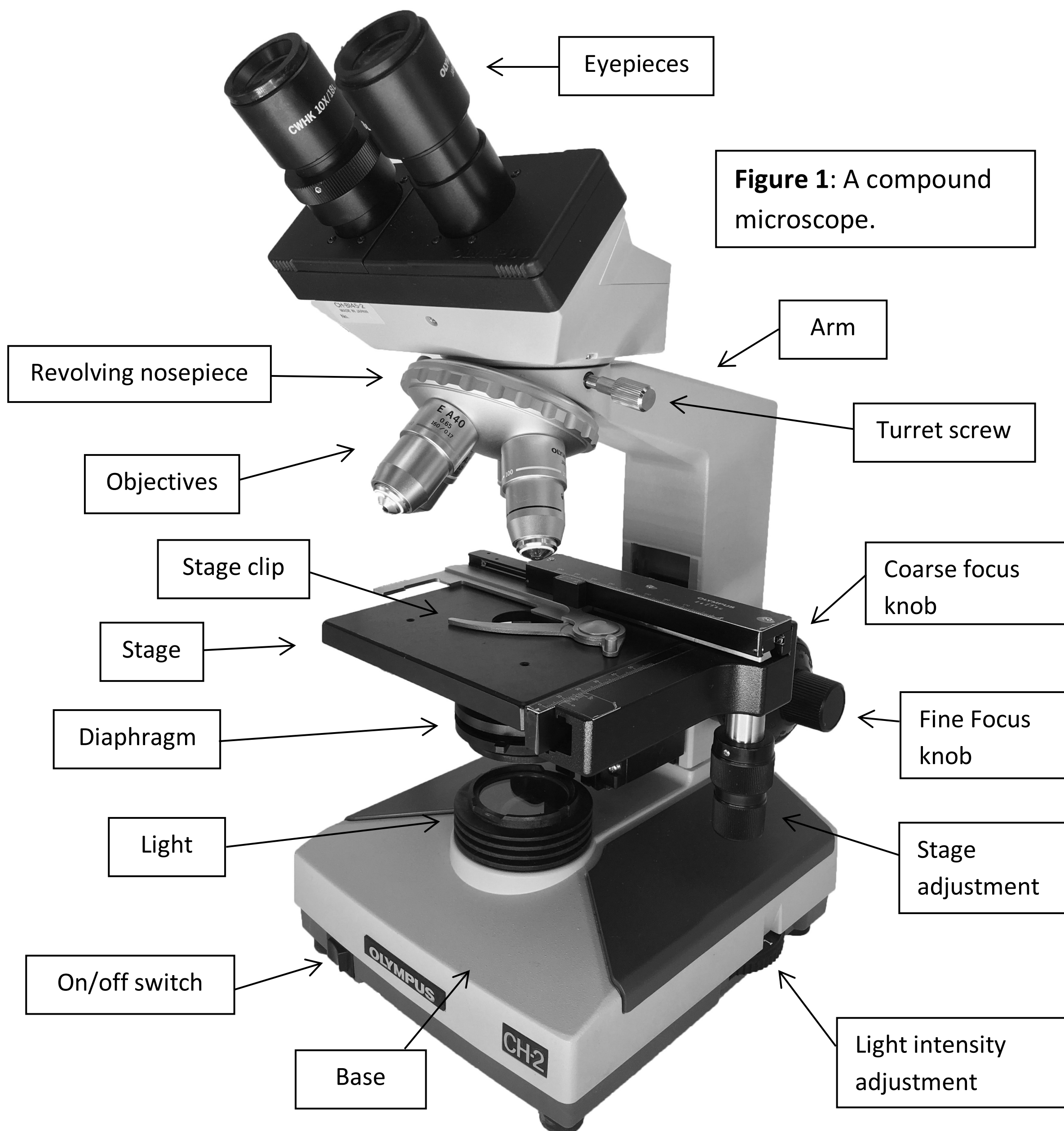

Figure 1: A compound microscope.

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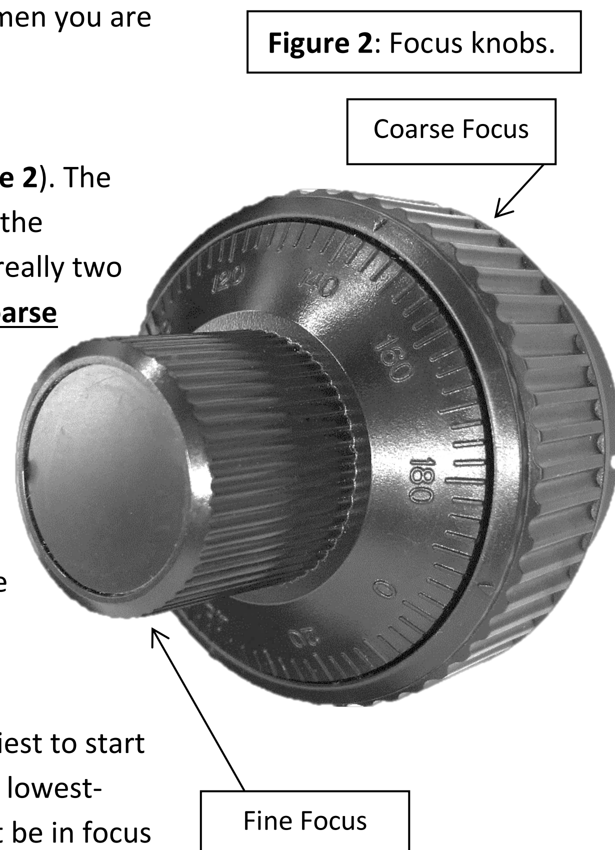
themselves, until the next objective clicks into place. When focusing on a specimen, always start from the lowest-power objective and work your way up. You must focus at each objective before moving to the next.

As mentioned earlier, there are lenses both in the eyepieces and the objectives. Sometimes, theses get dirty. To clean them, your TA can provide you with lens tissue and lens cleaner. Spray a bit of the lens cleaner on a lens tissue (NOT on the lens itself) and wipe the lens off. Never use a paper towel, water, or anything other than lens tissue and lens cleaner to clean the lenses, as anything else can damage them.

Stage

The stage holds the slide on which the specimen is mounted. The slide is placed on the stage and held with a clip. Light travels through the hole in the center of the stage, illuminating the specimen – so one of the first things you should do when you put a slide on the stage is **to make sure that light is passing through the specimen**, not another part of the slide. On the right of the stage are two small knobs that control the position of the slide. One knob moves the slide side-to-side, and the other moves it either toward or away from you. By moving the slide, you can control which part of the specimen you are focusing on.

Figure 2: Focus knobs.



Coarse Focus Knob

The microscopes have two focus knobs (**Figure 2**). The knobs move the stage up and down, bringing the specimen into and out of focus. Each knob is really two knobs in one. The outermost portion is the **coarse focus knob**. This knob quickly moves the stage, and it is used **only with the low-power (scanning) objective**. If you use the coarse focus with higher-power objectives, you may bash the slide into an objective and damage the lens. But the low-power objective doesn't reach that far down, so it's easy (and relaxing) to use the focus knob at that power.

To get your initial view of a specimen, it's easiest to start with the stage at the highest position and the lowest-power objective in place. The specimen won't be in focus at first. But if you use the coarse focus knob to move the stage slowly downward from there, while looking through the eyepieces, the specimen will gradually come into focus.

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Fine Focus Knob

The fine focus knob is the smaller knob sticking out of the side of the coarse focus knob. It moves the stage up and down by very small increments. The fine focus knob isn't used for the scanning power objective. However, it is the **only knob used for the medium- and high-power objectives.** This lab has **parfocal** microscopes, which means that once the microscope is focused correctly at one objective, it will only need very small adjustments to be focused at a higher-power objective. After achieving sharp focus at the scanning power, you should therefore only need to make adjustments using the fine focus knob at higher powers.

Light

A light source occupies the base of the microscope. The light goes through a **diaphragm**, which controls the size of the beam that goes through. There is a lever on the diaphragm that you can use to control the width of this beam. Sometimes, it will be too dark when you look through the eyepieces. You can change the light intensity by moving the wheel on the right side of the base. By increasing the light intensity, the specimen becomes more illuminated. It should always be set on "1" when you turn on the microscope, as the lightbulb may burst if it's too high. You can increase the light intensity as needed while focusing.

Turret Screw

By loosening the turret screw, you can rotate the top of the microscope. This can make it easier to switch between group members who want to look through the microscope. We'd prefer you didn't use it, however, because loosening the screw may allow the top of the microscope to fall off and cause some very expensive damage. Instead, we prefer your group simply take turns standing where the microscope is currently placed to look at the specimen. However, if you do use it, **make sure you tighten the screw when you're done rotating the top**.

Support Structure

The support structure on the microscope consists of parts like the **arm**, which holds the eyepieces and lenses, and the **base**, which holds up the rest of the microscope. When carrying the microscope, use two hands—one holding the base, and the other holding the arm. This strategy minimizes the chance of dropping the microscope while also minimizing the pressure put on the arm. When the microscope is on a lab bench and you need to move it, pick up the microscope and put it back down where you need it. Do not scoot or drag it across the table.

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Part 2: Using the Compound Microscope

First, you will learn to focus the microscope on a specimen. You have slides that say "Univ. of Okla. Botany Dept." on them. You will use this slide to learn to focus on a simple object.

Procedure: Focusing the microscope

> ***Please read this:*** *To a beginner, it will seem like it's an awful lot of trouble to go from the low-power objective (4X) through the medium-power objective (10X) to get to the high-power (40X) objective. If we want to magnify something a lot, why not go straight to the high-power one? The reason is that at the high-power objective, you are looking at an extremely thin slice of a specimen. At that power, if the stage is moved slightly up or slightly down from the optimal position, you may not be able to see anything at all. It is therefore nearly impossible to start with the high-power objective and focus on your specimen straight away, and that strategy carries with it a high risk of damaging a slide or an objective. However, it is very easy and safe to start at low power, get everything centered and sharp, then move to medium power, get it centered and sharp again, and then move to high power.*

1. Make sure the low-power (scanning, or 4X) objective is in place and that the stage is at its lowest position.
2. Place the slide on the stage and use the clip to hold it in place.
3. Before you turn on the light, make sure the light intensity is at the lowest setting (if not, the bulb may burst).
4. Turn on the microscope using the switch on the left front of the base. Once the light is on and the intensity is adjusted to a reasonable level (maybe 5 or 6), make sure the beam is shining through the "Univ. of Okla. Botany Dept." letters on the slide.
5. Use the coarse focus knob to bring the stage all the way to the top position. **Assuming the low-power (scanning) objective is in place**, you cannot bash the slide into the objective, even if the stage is at its highest point.
6. Look through the eyepieces, adjusting the distance between them to your preference.
7. Use the coarse focus knob to move the stage down until the specimen is in focus.
8. You may need to move the stage to the side or forwards/backwards to make sure the letters are in your **field of view**, or the area that you see when looking through the microscope. Move the stage until one letter is in the center of your field of view.
9. Focus on a letter until you can see its edges in sharp focus. Note how the letter is positioned. Does it look different from how it is oriented on the stage? If so, how?

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Where does the letter appear to move when you move the stage to the right? How about when you move the stage towards you?

10. Slide the diaphragm adjustment lever so the diaphragm is open as far as it can go. Now use the lever to slowly close the diaphragm, while looking through the eyepiece at the specimen, and see how it changes the illumination. Set the aperture to a place that is comfortable for you and make sure one of the letters is in the middle of the field of view.
11. Change the light intensity until the specimen is comfortably bright. In general, the light intensity may need to be higher as you focus using higher-power objectives.
12. Is the letter in sharp focus? It can't be "kind of OK" but still blurry – it needs to be sharp, or the next steps won't work. If you aren't sure, ask a TA for help.
13. Once the letter is centered and in sharp focus, use the revolving nosepiece (ridged ring) above the objectives to swing the medium-power objective (10X) into place. It will click into place when it is positioned correctly. DO NOT MOVE THE STAGE DOWN BEFORE MOVING THIS OBJECTIVE INTO PLACE. Yes, we know you're worried about breaking something, but I promise you with all of my heart that the objective will not hit the slide if you have focused correctly.
14. Look through the eyepiece. Because we have parfocal microscopes, the letters should almost be in focus.
15. Use the **fine focus knob** (NOT the coarse focus) to bring the specimen into sharp focus. As before, "kind of" in focus will not do the job. Use the fine focus knob to carefully move the stage up and down until you cannot make the image any sharper.
16. Make sure the letter is centered in your field of view. Now bring the high-power objective (40x) into place. Again, do not move the stage down at this point; the objective will not hit the slide as long as you have focused correctly (although it will be very, very, very, very close).
17. Look through the eyepieces again, and use the fine focus knob to bring the letter into focus. Describe how it looks now, compared to how it looked at step 9.

Compare the field of view as it is now to how it was at the lowest-power and medium-power objectives.

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18. To remove the slide when you're done, bring the scanning power objective back into place. Only after bringing this objective into place, move the stage down and remove the slide.
19. **Important**: When you are done using the microscope for the day (not now), there is also a correct procedure to follow. Turn off the microscope, set the light intensity to "1", put the low-power objective in place, remove the slide you were looking at, bring the stage to the lowest position, and put the cover over the microscope (if available).

Part 3: Field of View

Now, you will get some practice thinking about and measuring the field of view at different magnifications.

Procedure

1. Put the clear plastic ruler on the stage. You won't be able to clip it like you would a slide, so just make sure it's secure.
2. Focus on the ruler at the scanning objective. It won't be completely clear, but do it to the best of your ability. Make sure you are focusing on the metric side of the ruler.
3. Move the stage so the edge of one of the millimeter markings is on the edge of the field of view. Use the markings to estimate the diameter of the field of view (see **Figure 3**).

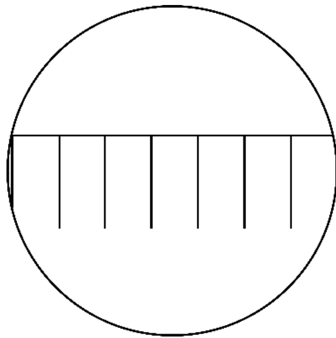

Figure 3: This is how you should line the ruler up to measure the field of view. In this hypothetical example, the diameter of the field of view would be about 7 units. If you know how big the units are, you can calculate the diameter of the field of view.

Total magnification (eyepiece X objective): _______________

Diameter of field of view (include units): _______________

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4. Take out your **cell cam mount**, a tool we have that lets you take pictures of what you see through the microscope, and put it on one of the eyepieces as shown in **Figure 4**. The screw at the top controls the part of the cam mount that fits on the eyepiece, so adjust it until the cam mount fits nicely. It can fit on either the left or right eyepiece.
5. Adjust the width of the cell cam mount by turning the screw on the left side. Place your phone in when it is at a good width.
6. Align your phone's camera with the eyepiece. This may require you to adjust the position of your phone itself by angling it or sliding it up or down in the cam mount, or adjusting the cam mount by rotating it or using the screw on the back to slide the part that holds your phone up and down. The black piece at the top of the mount can help adjust for glare.
7. Use the cell cam mount and your phone's camera to take a picture of the ruler lined up in the field of view.
8. Use the knowledge that there are 1000 micrometers (μm) in every millimeter to convert your field of view diameter to micrometers. Write the answer in **Table 1**. For a refresher on how to convert between units of length in the metric system, check Activity 2 in the Process and Tools of Science lab.
9. As we move to the higher-power objectives, the rulers measuring millimeters will be too large. Lower the stage, remove the ruler, and put the **stage micrometer** slide in place. These are very expensive small rulers, so be careful with them!

Figure 4: Cell cam mount. This contraption allows you to easily take pictures with your cell phone.

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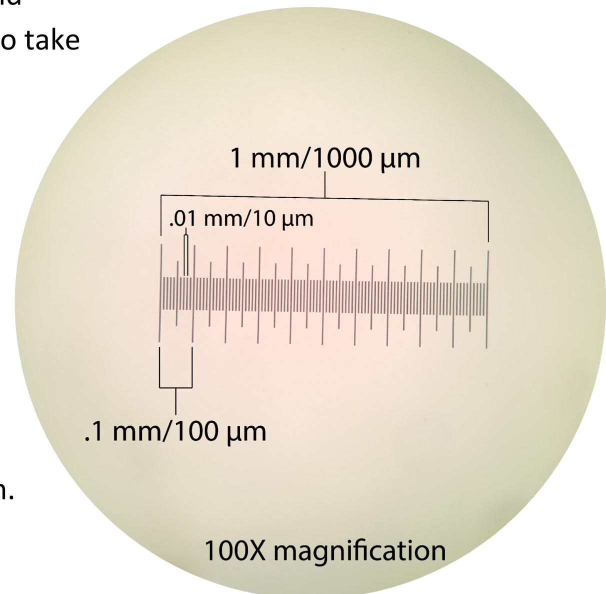
10. Focus on the stage micrometer at the scanning power objective and make sure it is centered, then move to the medium-power objective. Focus again using the fine focus knob.
11. Move the stage until the edge of one of the measurement lines is on the side of the field of view, and then use the cell cam mount to take another picture. Use the picture to help estimate the diameter of the field of view. Write the diameter in **Table 1.**
    Note: On our stage micrometers, each division is 0.01 millimeter, so each of the long lines is equal to 0.1 millimeter, and the entire length of the "ruler" is 1 mm. Since there are 1000 micrometers (µm) in each millimeter and each division is .01 millimeters, each division is equal to 10 µm. See **Figure 5.**

Figure 5: Stage micrometer. Brackets indicate the length of the divisions on our stage micrometers.



12. Repeat again with the high-power objective, making sure to take another picture.
13. Return to the scanning power objective, lower the stage, and remove the stage micrometer.

Table 1: Field of view diameter.

	Objective magnification	Total magnification	Diameter (µm)
Low-power			
Medium-power			
High-power			

To use the diameter of the field of view to estimate the size of an object, first estimate the proportion of the diameter that the object occupies. If an object occupies about 50% of the field of view in your microscope at 40X (total magnification), about how big is that object?

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If the object occupies about 10% of the field of view in your microscope at 100X, about how big is that object?

If an object occupies about 25% of the field of view in your microscope at 400X, about how big is that object?

Part 4: Depth of Focus

Specimens on a slide may seem, at first, like they are two-dimensional. However, they are actually slices of objects, and they have a certain thickness. When you are using the microscope to look at the object, you are looking at just one layer (or plane) within that slice. How thick is that layer? It depends on the magnification. In this exercise, you will investigate how this works.

The thickness of the layer in focus is called the **depth of focus** (see **Figure 6a**). With thicker specimen, there is often different things to observe at each layer; for example, you may only be able to see one layer of cells when there are multiple layers of cells in the sample. You can change which layer the microscope is currently focused on, the **plane of focus** (see **Figure 6b**), by making small adjustments using the fine focus knob. This allows you to see different layers of the specimen. You will be using slides that have colored threads placed on top of one another to investigate depth of focus.

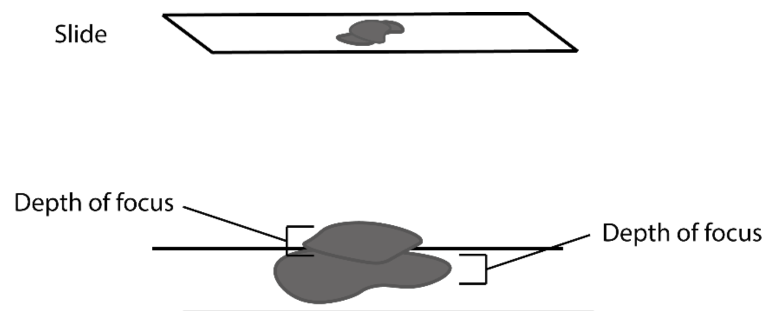

Figure 6a: Depth of focus. Two organisms are stacked on this slide, but the depth of focus at the current magnification may mean that you can focus on only one at a time.

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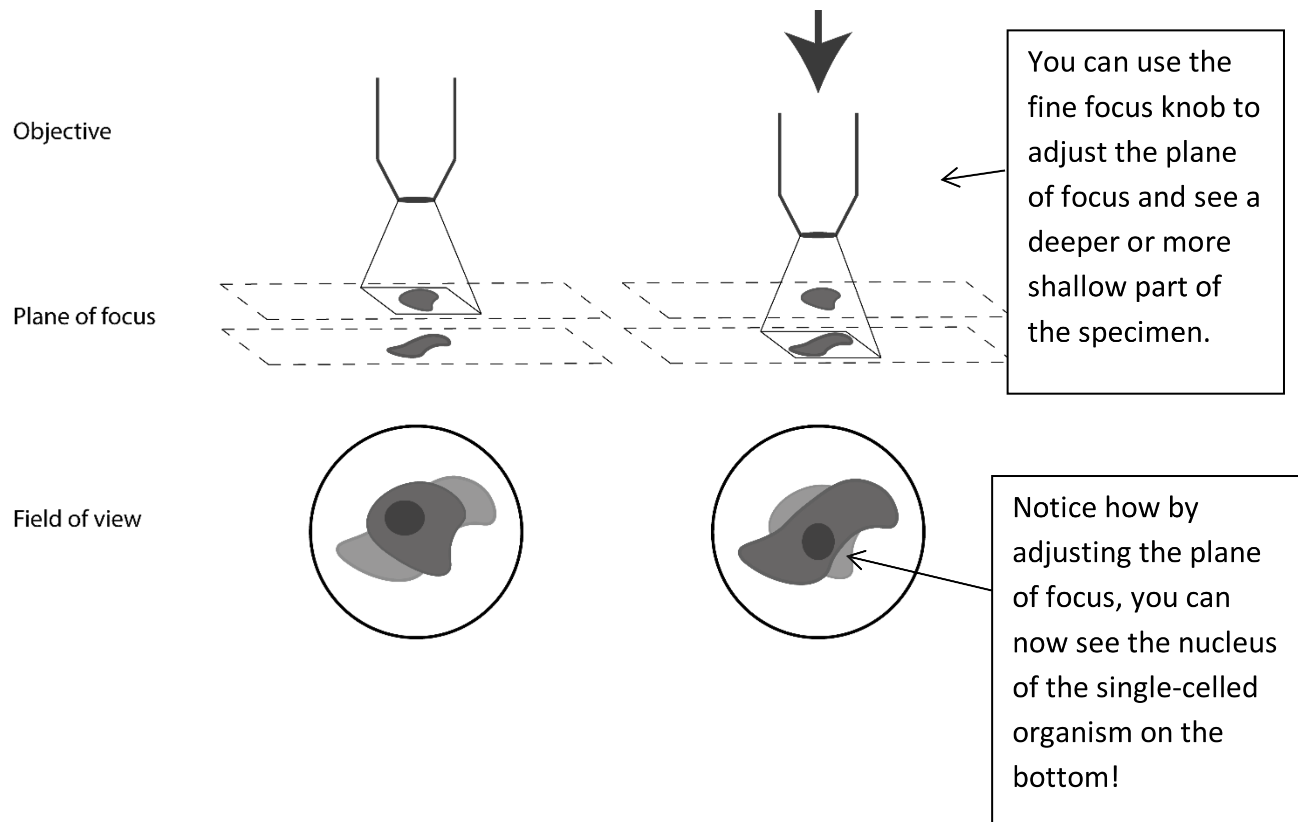
Figure 6b: Plane of focus. When there is a shallow depth of field, you cannot see all of the specimen at once. Adjusting the plane of focus is useful for specimen that are thick or have layers such as the multiple organisms, such as the one in this example.



Procedure

1. Place the slide with silk threads on the stage, and clip it in.
2. Focus on the strings using the scanning power (4X) objective. Change the light intensity setting to meet your preference. How many of the strings are in focus?

   Which color thread is on the bottom, middle, and top?

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3. Go to the medium-power (10X) objective. Focus on the strings using the fine focus. You may need to adjust the light intensity. Again, how many of the strings are in focus? How does the view change as you move the fine focus?

4. Move to the high-power (40X) objective, and focus using the fine focus knob. You may need to adjust the aperture setting again. How much of the string is in focus? Can you see more than one string? Is the depth of focus thinner than it was on the other settings? Can you change which part of a single string is in focus by moving the fine focus?

   Focus on the intersection between the top and bottom string. Use the fine focus knob to change the plane of focus from the top string to the bottom string and back. Can you see anything about the string on the bottom that you couldn't see at a less deep plane of focus? Explain:

   What is the relationship between the focus knobs and the three-dimensional nature of the specimens you are looking at?

   As the magnification increases, does the depth of field – the thickness of the plane that's in focus – become thicker or thinner?

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We have been teaching you to focus first on an object at low power, then medium, then high. Keeping in mind that the depth of field lessens as the magnification increases, why do we teach you to focus at each objective instead of just starting at high power?

Activity 2: An Intimate Look at Living Cells

Objectives

1. Stain living cheek cells so they're easily visible using the compound microscope.
2. Identify the parts of your cells that indicate they're animal cells.
3. Explain how depth of focus changes as you increase the magnification.
4. Focus on plant cells at different planes of focus.
5. Identify the parts of *Elodea* cells that indicate they're plant cells.

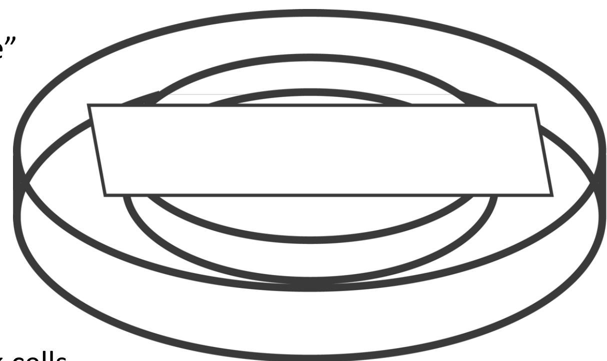
Part 1: Animal Cells – Your Own!

Animal cells are too small to see with the human eye, but they are easily visible using a compound microscope. The cell contains many types of organelles, most of which are invisible without special stains that attach pigments to certain structures; the pigments then reveal each organelle's location. You'll be investigating both the basic structure of animal cells and the staining process using your own cells.

Procedure

1. Use a toothpick to gently scrape some cheek cells from the inside of your mouth.
2. Spread the cells on in the middle of a clean slide, and then wait for them to dry.
3. Place a small dish inside a petri dish, and then place the slide with your cheek cells on top of the smaller dish (see **Figure 7**).
4. A small glass bottle labeled "methylene blue" is provided at your station. Use the dropper to put a few drops of methylene blue over your cells, and then wait two minutes for the stain to work.
5. Use the water squirt bottle at your station to gently wash the excess stain off the slide. Be careful that you don't wash off the cheek cells themselves.
6. Use a paper towel to gently dry the bottom of the slide. Don't wipe the top because you may wipe off the cheek cells.

Figure 7: Staining platform.



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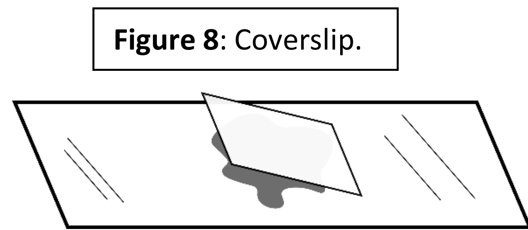
7. Add one drop of water to the top of the cheek scrapings to make the coverslip stay, and then place the coverslip on top. When placing the coverslip, put one of the edges down first and then lower the rest down slowly to minimize air bubbles (See **Figure 8**).

Figure 8: Coverslip.



8. Place the slide on the microscope stage, and follow the correct procedures to focus the microscope at the low and medium-power objectives.
9. While the medium-power objective is in place, use the fine focus to change the plane of focus. How does what you see change? Can you see everything in one plane of focus or is the depth of focus too shallow?

10. Focus using the high-power objective and sketch your cheek cells as they look at 400X. List the features that indicate they are animal cells.

11. Focus on one of your cells, and then use the fine focus to change the plane of focus. How does what you see change? Is the depth of focus more shallow than it was at 100X? How can you tell?

Part 2: Plant Cells – An Aquatic Plant Called *Elodea*

Plant cells are structured differently from animal cells, and these differences are visible under the microscope. Plant cells have rigid cell walls which are visible and cause the cells to form neat rows. Also visible are chloroplasts, the organelles that carry out photosynthesis in the cell. The nuclei of plant cells are often not visible at the magnifications we can look at in lab. You will use cuttings of *Elodea* to observe plant cells.

Procedure

1. Place a small *Elodea* leaf on a clean slide with a drop of water.
2. Place a coverslip on top of the slide, making sure to start with one edge to minimize air bubbles.

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3. Place the slide on the stage and follow the correct procedure to focus the microscope using different objectives, going from scanning power (4X), to medium-power (10X), to high-power (40X).
4. Use the fine focus knob to look through different depths of the specimen. Remember, a cell is a three-dimensional object. Focusing up and down on the entire depth of a cell should help you to visualize the cells more like shoeboxes than like flat rectangles. (This effect is less apparent with cheek cells, which have a flattened shape.)
5. Can you see the chloroplasts move? They are being swept along tiny tracks (too small for you to see) as the cytoplasm moves inside the cell.
6. Sketch the *Elodea* cells. You will definitely be able to label the cell walls and the chloroplasts. You may also see the central vacuole, which looks like a large empty space in the cell.

7. List the features that indicate that you are looking at plant cells.

Activity 3: Evolution Under the Microscope

Objectives:

1. Learn to use microscopes and measure diameter of field of view.
2. Make predictions about thickness of cuticle/epidermis in plants and the epidermis of animals from different habitats.
3. Practice measuring cuticle/epidermis.
4. Collect data to test predictions.

All organisms need water. Animals and plants that live on land have adaptations that limit the evaporation of water from the body surface. In animals, the epidermis consists of many layers of cells, and its total thickness determines in part how quickly water evaporates. In plants, the epidermis is typically one cell layer thick, but the epidermis may be covered by a waxy layer called a cuticle that reduces water loss from the leaves and stem (**Figure 9**).

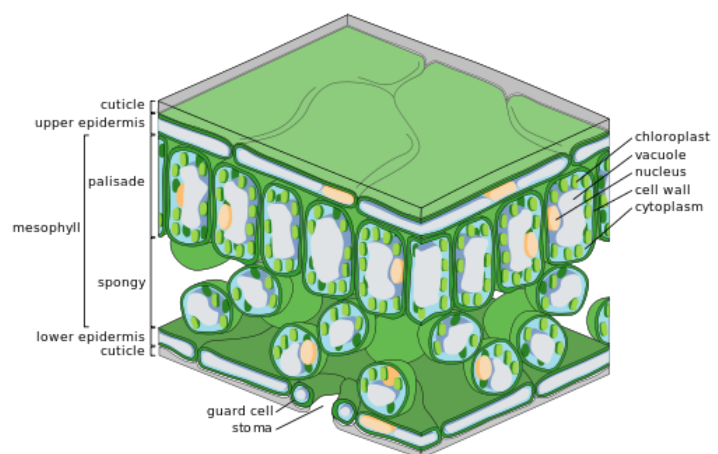

Figure 9: Leaf cross section, including cuticle.

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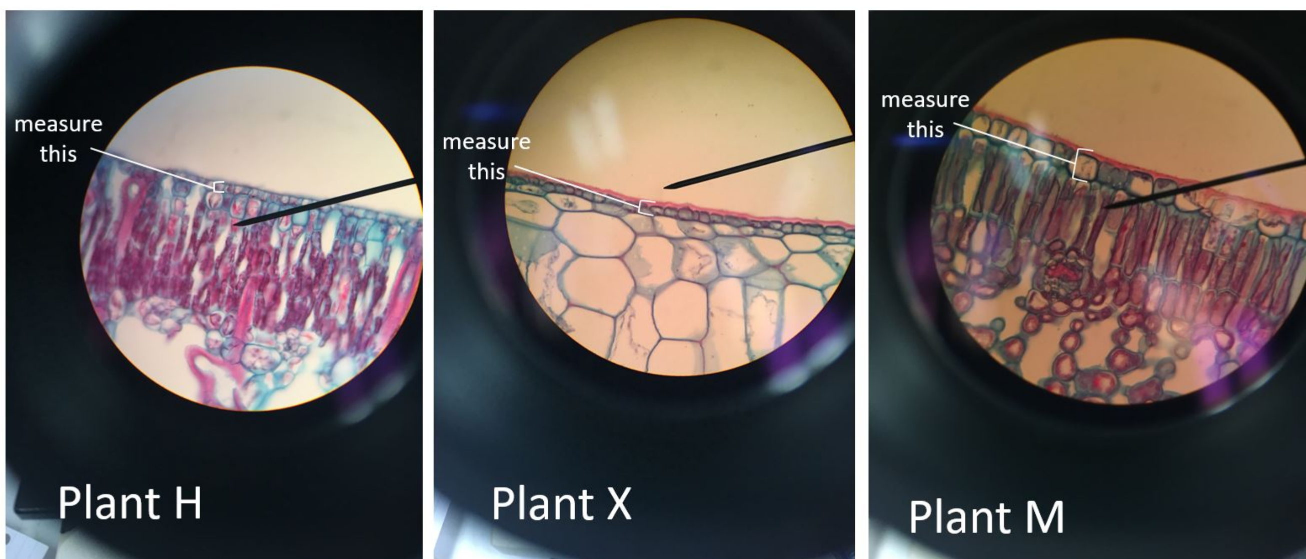
Before continuing, consider exactly how these coverings might affect evaporative water loss. Does a thicker outer layer (epidermis or cuticle) mean faster or slower water loss?

Procedure

1. Examine the three leaf cross sections (H, X, and M) under the compound microscope at 400X. They are all mounted on one slide, so scan the slip from top to bottom. Using the known diameter of the field of view at 400X (go back to **Table 1** and look at the pictures you took in Activity 1 Part 3 for a refresher), estimate the thickness of each leaf's cuticle and epidermis. **Figure 10** shows pictures of each cross section so you can see what you are supposed to measure. As you go, fill in **Table 2** with your measurements, and indicate whether a cuticle is present.

Note: You might find it useful to look back at the stage micrometer periodically to refresh your memory about the diameter of the field of view.

Figure 10. Leaf cross sections. Brackets show what to measure on each type of leaf.



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2. Examine the cross section from Animal A under the compound microscope at 400X. **Figure 11** shows pictures of each animal skin cross section so you can see what you are supposed to measure. Using the known diameter of the field of view at 400X, estimate the thickness of the epidermis for Animal A. Again, this is easier when you use the pictures you took earlier as well as **Table 1**. Record your measurement in **Table 2.**

Figure 11: Animal skin cross sections. Brackets show what to measure for each type of animal.

3. Examine the cross section from Animal B under the compound microscope at 400X, looking at **Figure 11** again to make sure you're measuring the correct thing. Using the known diameter of the field of view at 400X, estimate the thickness of the epidermis for Animal B. Record your measurement in **Table 2.**

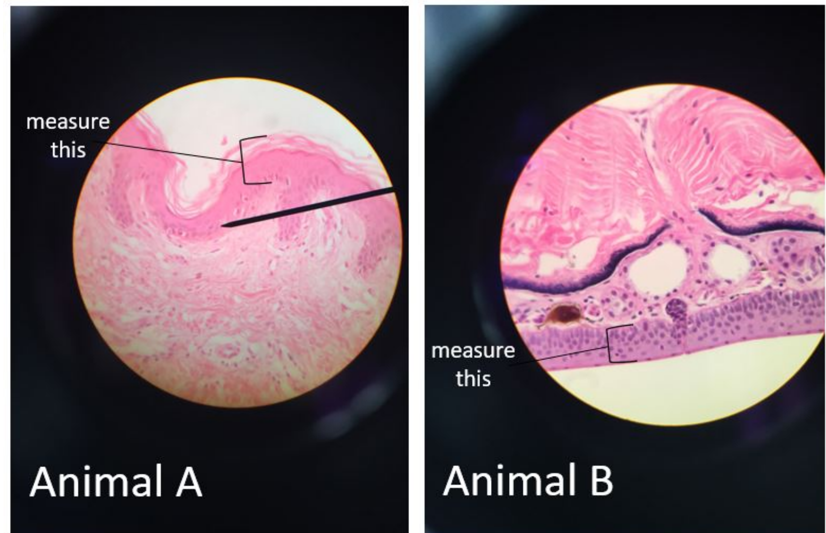

Table 2: Data collection table for estimated thickness of body covering.

	Estimated thickness of body covering(µm)	Cuticle present? (for plants only)
Plant H		
Plant X		
Plant M		
Animal A		N/A
Animal B		N/A

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4. Each leaf cross section is from plant originating in a dry, temperate, or wet climate. One animal is from a wet climate, and the other is from a temperate climate. Use your measurements to predict which habitat each organism is from, and record your justification in **Table 3**.

Table 3: Habitat prediction table

Specimen	Habitat	Justification
Plant H		
Plant X		
Plant M		
Animal A		
Animal B		

5. Report your group's data from **Table 2** in the class table. As part of your homework, you will use Google Sheets (or a similar spreadsheet program) to create a graph for the class's combined data. Your TA will give you further instructions for the homework.

   Important: *When you are done using the microscope, make sure to turn it off, switch the light intensity back to "1", switch to the low-power objective, put the stage in the lowest position, and place the cover back on the microscope.*

Questions for Review

- Why do we tell you to focus with the low-power objective first, and then with the medium-power, and then with the high-power (instead of just starting with the high-power objective)?
- Which focus knob do you use with each objective, and why? What are the possible consequences of using the wrong focus knob?
- Say you've tried to focus your microscope but it's still blurry. What are some potential causes of the blurriness?

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- Say you are focusing on an object using the low-power objective, but it isn't centered in the field of view. What happens when you move to the medium and high-power objectives?

Activity 4: The Dissecting Microscope

Objectives

1. Calculate the total magnifications for each objective on the dissecting scopes in lab.
2. Identify the parts of the dissecting microscope.

Stereoscopic dissecting microscopes are also compound microscopes because they combine the magnification of two lenses. However, the microscopes we already learned how to use depend on the specimen being very small and thin—thin enough for light to travel through it. Dissecting scopes, however, are different. The light source is on top of the specimen, light reflects off the surface of the specimen instead of passing through it, and the lenses are lower-power. This makes dissecting microscopes perfect for examining the surface characteristics of larger specimens, like small animals or whole flowers.

Part 1: The Parts of the Dissecting Microscope

Eyepieces

See **Figure 12** for a labeled diagram. The eyepieces serve the same function as in the compound light microscope. Also like the other microscope, the distance between the eyepieces can be adjusted, and there is a diopter adjustment ring on the left eyepiece. The eyepieces on these dissecting scopes magnify at 10X.

Changing the Magnification

The objectives are inside the dissecting microscope, so you cannot see them from the outside. Rather, knobs below the eyepieces allow you to switch between the objectives. For the microscopes in this lab, the objectives magnify at 1X and 3X. Based on this information, what are the total magnifications for the dissecting scope?

Low-power objective: ________ (eyepieces) x ________ (objective) = ________ (total)

High-power objective: ________ (eyepieces) x ________ (objective) = ________ (total)

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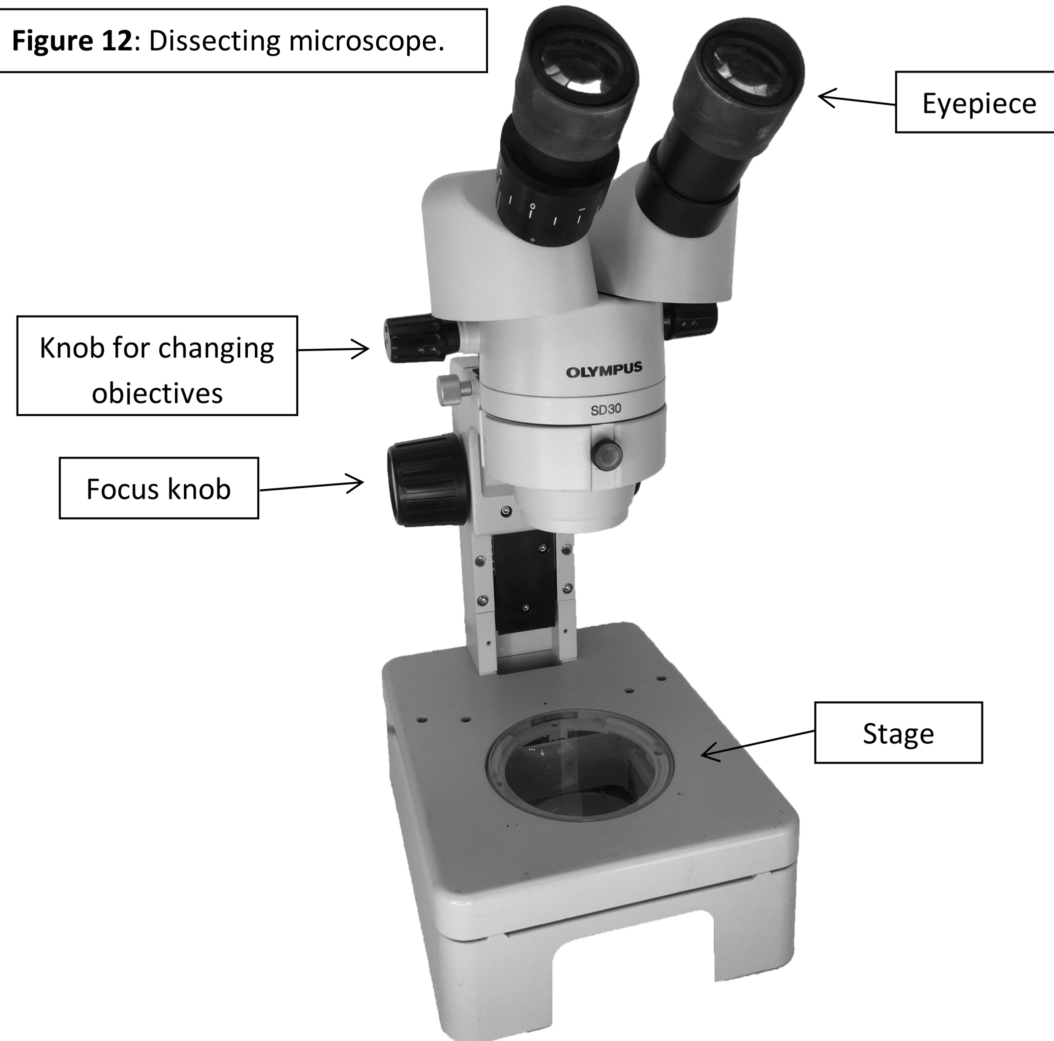

Figure 12: Dissecting microscope.

Stage

The stage on the dissecting scope does not move. Rather, you must manually move the container holding the specimen to the correct position, centered under the light.

Focus Knob

The large knobs near the stage focus the dissecting microscope. Rather than moving the stage up and down, the knobs move the arm of the microscope up and down to bring the specimen into focus.

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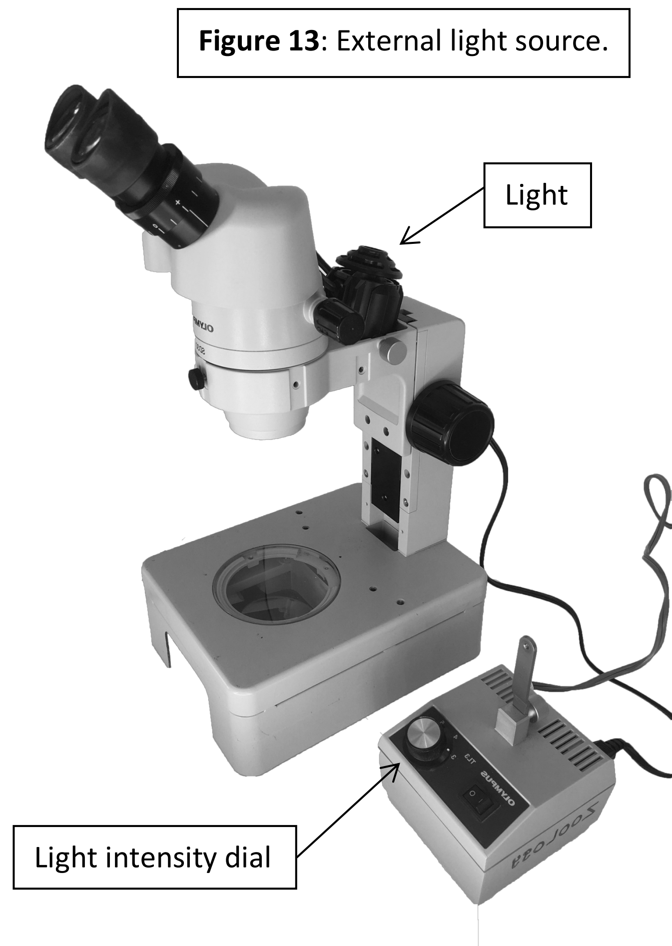
Light

For the dissecting scope you use in this lab, the light source is external (See **Figure 13**). It should already be plugged in and attached to the microscope when you get to lab. The light intensity is controlled by the dial on the light source stand.

Figure 13: External light source.



Support Structure

The support structure is similar to the compound light microscope. It should be handled in the same way. Carry the microscope with two hands, and do not drag it across the lab bench. Rather, pick it up and place it back down where it needs to be. Better yet, move your body to the microscope instead of moving the microscope to you.

Part 2: Using the Dissecting Scope

Your TA will give you pond water to look at under the dissecting scope.

Procedure

1. Use a pipette to put some water in a petri dish. Place the petri dish on the stage of the dissecting scope.
2. Use the focus knob to bring the specimen in focus. You may need to adjust the position of the petri dish on the stage.
3. Use the knob to change to the high-power objective.
   What do you see? Plant material? Small living organisms? Sketch anything interesting here.

   How does what you see change when you change the objective that's in place?

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As you can tell, the dissecting microscope can't magnify as much as the compound microscopes you used earlier. Why would they still be useful? Is there anything you see in the pond water under the dissecting scope that may not work with the compound microscope?

Acknowledgments and Attributions

Activities 1, 2, and 4 are adapted from: Jean Dickey. 2003. Using the Microscope. *Laboratory Investigations for Biology, 2nd Edition.*

Activity 3 was adapted from: Troy R. Nash, Suann Yang, John C. Inman. 2015. Growing a Thicker Skin: An Exercise for Measuring Organismal Adaptations to Terrestrial Habitats. The American Biology Teacher, Vol. 77 No. 6, August 2015; (pp. 426-431) DOI: 10.1525/abt.2015.77.6.426

Figure 1: Compound Microscope c Sarah Greenwood used under a CC BY 4.0 license
https://commons.wikimedia.org/wiki/File:Compound_Microscope.png

Figure 8: Coverslip Graphic c Sarah Greenwood used under a CC BY 4.0 license
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Figure 9: Anatomy of a eudicot leaf c Zephyris used under a CC BY-SA 3.0 license
https://en.wikipedia.org/wiki/Plant_cuticle#/media/File:Leaf_Tissue_Structure.svg

Figure 12: Dissecting Microscope © Sarah Greenwood used under a CC BY-SA 4.0 license
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Figure 13: Dissecting Microscope and Light Source © Sarah Greenwood used under a CC BY-SA 4.0 license
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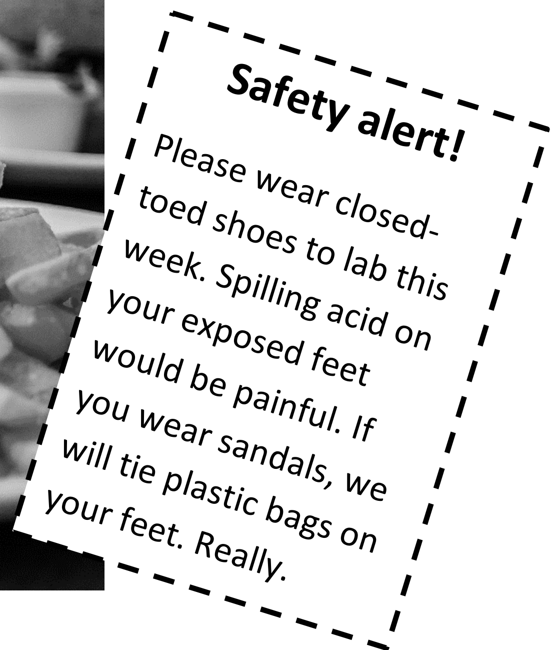
Digestion

Mariëlle Hoefnagels and Sarah Greenwood



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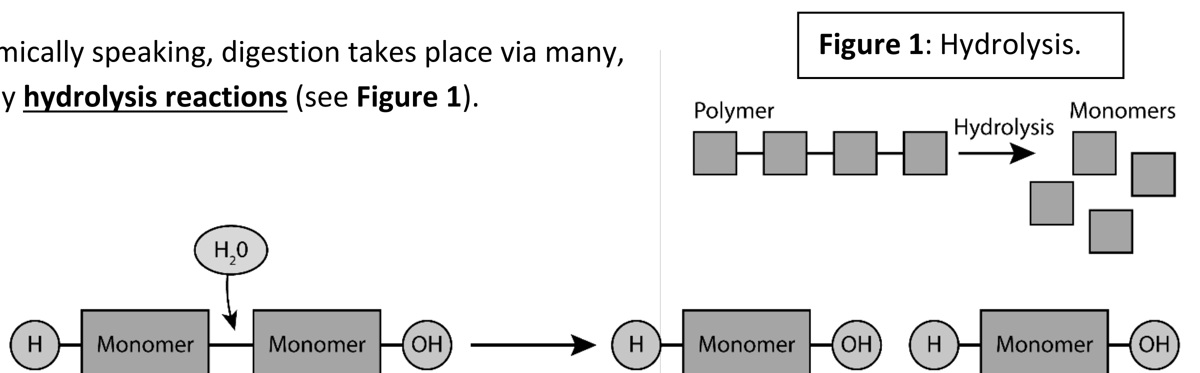
Introduction

You probably already know that you – and other animals – rely on digestion, that digestion has something to do with food, and that it happens somewhere inside your "guts." Swallowed food travels from your mouth to your stomach to your small intestine, a long tube where most digestion happens (see your "digestion man" handout from lecture). In **digestion,** enzymes break down large food molecules (e.g., polymers) into small pieces (e.g., monomers) that can be absorbed from the small intestine into the blood, distributed to the body's cells, and used for energy or as raw materials for building new molecules.

Chemically speaking, digestion takes place via many, many **hydrolysis reactions** (see **Figure 1**).

Figure 1: Hydrolysis.



In hydrolysis, enzymes use water molecules to break the bonds that hold large molecules together, separating polymers into monomers that your cells can use.

So that's all well and good, and it won't surprise you to know that all animals – not just people – use some version of this process to get energy and raw materials from their food. But did you know that all other organisms, including microbes and plants, use hydrolysis reactions too? See **Figures 2a-2b** for examples.

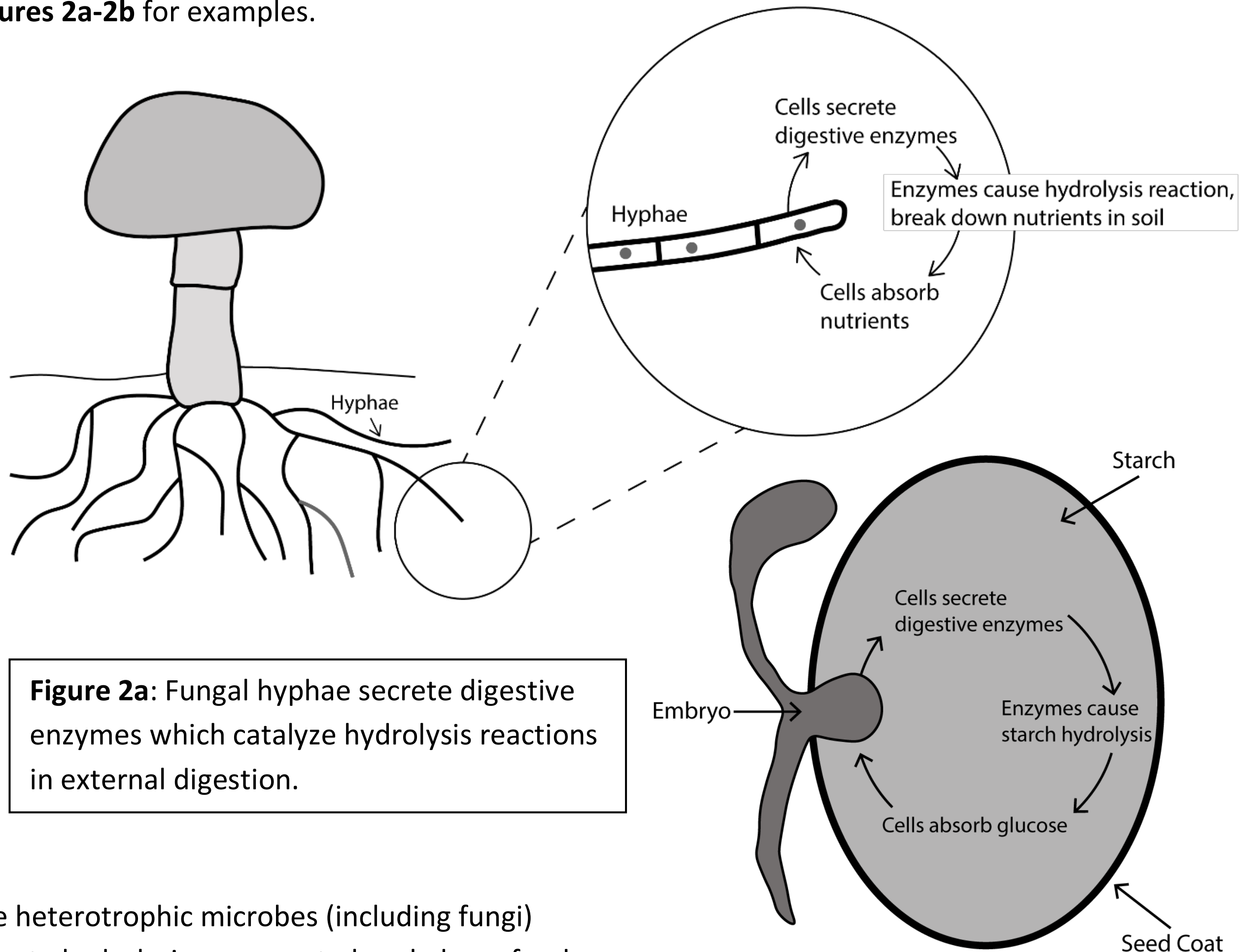


Figure 2a: Fungal hyphae secrete digestive enzymes which catalyze hydrolysis reactions in external digestion.

Figure2b: A plant embryo uses hydrolysis reactions to break down starch stored in the seed, then uses the resulting glucose for growth.

The heterotrophic microbes (including fungi) secrete hydrolysis enzymes to break down food outside their bodies. The autotrophs (including plants) store away large amounts of starch, protein, and lipids and when they need to use their stored resources, they use hydrolysis to break those large storage molecules into small, useful pieces.

In this lab, you will conduct experiments in which you observe digestion as it occurs and learn about what is required for digestion to happen. As you may remember, digestive enzymes are proteins, which are molecules, which means they are too small to see with the naked eye. That

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means that you cannot "watch" digestion *directly*; this lab therefore will teach you some *indirect* ways to measure whether enzymes have digested the food molecules we provide.

Each of the activities in this lab follows the same pattern. You will set up a series of test tubes that you will fill with a liquid food molecule (like a starch solution or vegetable oil) and a solution containing digestive enzymes. You will also add chemicals that will help you know whether the enzymes have digested the food. And some tubes will contain "extra" materials that are necessary to make the reaction go, that will help you understand some of the factors that affect enzyme activity, or that will serve as experimental controls.

Your TA will begin by showing you an example of protein digestion. Then, you will conduct pre-planned experiment on lipid digestion and starch digestion. Once you have those tools in hand, you will design, carry out, and interpret your own experiment investigating other factors that can influence digestion.

First, watch your TA for a demonstration of protein digestion. This demonstration does not use test tubes or the same digestive enzyme solution that you will use in your other experiments.

When proteins are digested, the product of digestion is: ________________________________

What is the protein source in this demonstration? ____________________________________

What is the enzyme source in this demonstration? ____________________________________

What is the no-enzyme control in this demonstration? _________________________________

What is the independent variable in this demonstration? _______________________________

What is the dependent variable in this demonstration? _________________________________

What are two examples of standardized variables in this demonstration?

What has happened to the no-enzyme control to "take away" its active enzymes?

In the treatment with active enzymes, how did you know that digestion happened?

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Activity 1: Lipid Digestion

Objectives

1. Understand the purpose of lipid digestion.
2. Recognize why lipid digestion requires emulsifiers.
3. Recognize that lipase is the enzyme involved in lipid digestion.
4. Explain the role of each component of the experiment.
5. Explain how phenolphthalein (the pH indicator) allows you to interpret whether digestion has occurred.

Essential Background Information

Lipids are energy-rich molecules, which explains why we eagerly seek them out in our diets. Plants store lots of lipids in their seeds (usually in the form of oil), and mammals sock away lipids to store for the future and to help retain heat in our bodies (usually in the form of fat reserves).

You may remember that lipids are insoluble in water. In elementary school, many students learn about this by putting oil and colored water in a bottle. No matter how much the student shakes or rotates the bottle, the two liquids will separate out. This is the same phenomenon that causes the olive oil to separate from vinegar in Italian salad dressings.

In the experiments in today's lab, all of the digestive enzymes are in a solution called **pancreatic extract**. It originates in a cow's pancreas, which is the organ that secretes digestive enzymes into the cow's small intestine (your pancreas does the same thing). The pancreatic extract is dissolved in water, but the lipid source – vegetable oil – is hydrophobic and therefore will not mix with the pancreatic extract. If the enzymes in the pancreatic extract can't reach the oil, no digestion will occur.

To study lipid digestion, then, we have to get the oil to mix with the pancreatic extract. How does that occur? We will shake up the oil and pancreatic extract together with **bile**, a chemical that causes the oil to form tiny droplets that remain suspended throughout the watery pancreatic extract; the resulting mixture is called an **emulsion**. (Bile is therefore called an emulsifying agent, or emulsifier.) In the emulsion, the digestive enzymes can easily access the lipid molecules. **Figure 3** shows how bile will work in this experiment.

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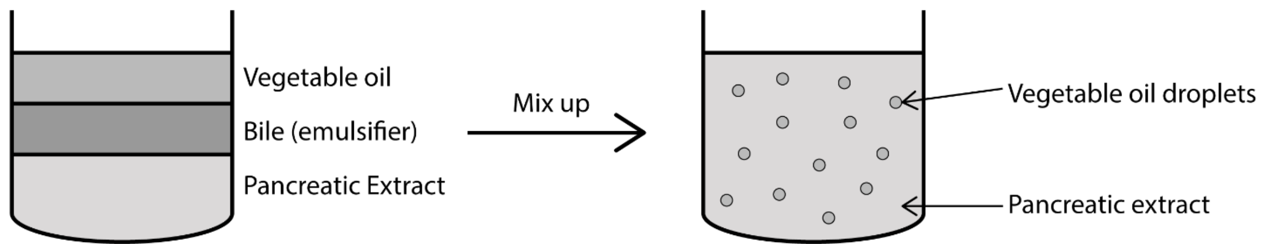

Figure 3: Emulsion. For this experiment, bile works as an emulsifier to separate the vegetable oil into tiny droplets and suspend them in the pancreatic extract.

Lipase is the fat-digesting enzyme that is in pancreatic extract (among many other enzymes). Lipase breaks down the fat (in vegetable oil) into glycerol plus three fatty acids. As digestion occurs, the fatty acids are detached from the molecule, causing the pH of the solution to decrease as it becomes more acidic (see **Figure 4** below).

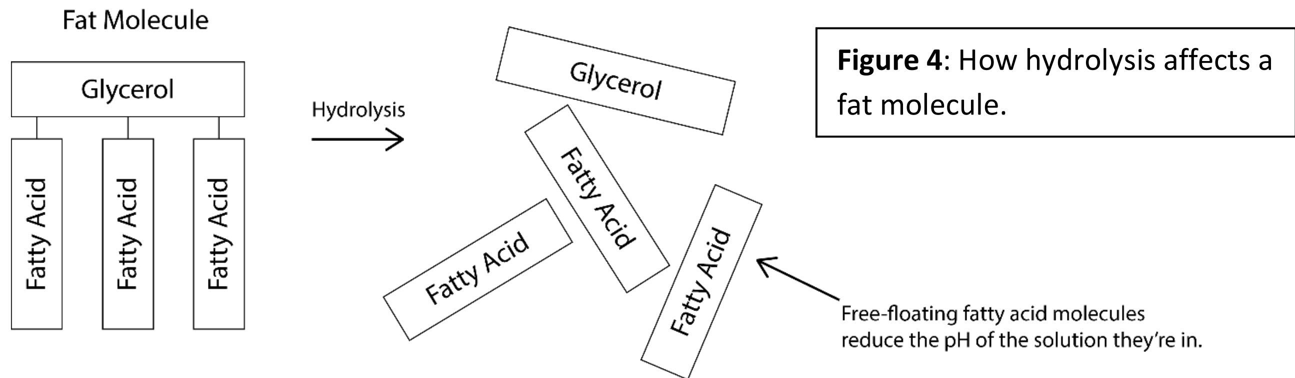

Figure 4: How hydrolysis affects a fat molecule.

Overall Experimental Design

You will conduct small-scale fat digestion reactions using the materials on your bench:

- **Vegetable oil:** Fat source to be digested
- **Pancreatic extract:** Source of lipase
- **Bile:** Emulsifier
- **Phenolphthalein:** pH indicator. At pH above ~8, phenolphthalein is pink. As the solution is acidified below ~8, however, phenolphthalein becomes colorless. This color change is our indirect measure of the progress of digestion.
- **Sodium hydroxide (NaOH):** This chemical (a base) will bring the initial pH of the solution in your test tubes above 8.
- **Water:** Participates in hydrolysis but also equalizes the volume in each tube.

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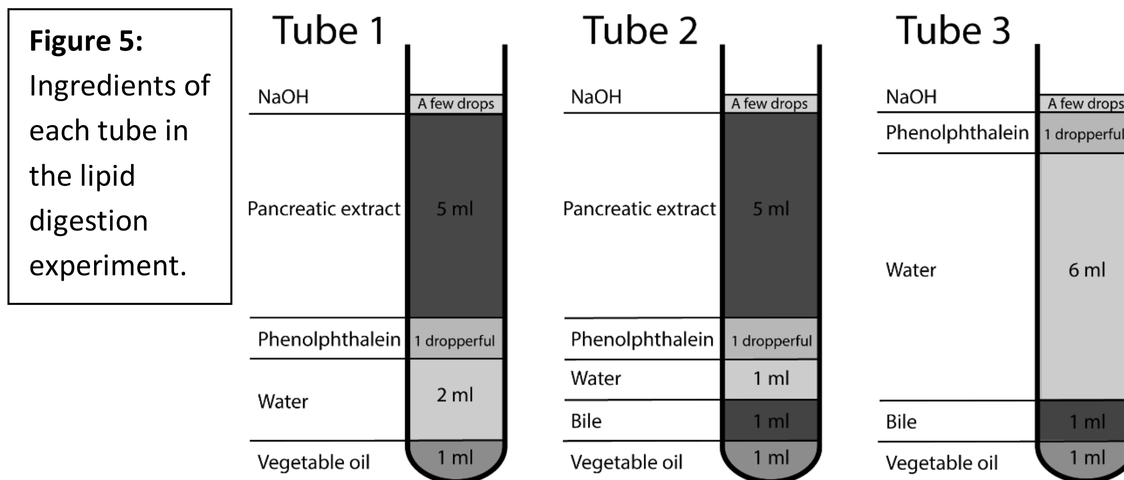
Figure 5 and **Table 1** show the amounts of each ingredient you'll add to each test tube. Don't start filling your test tubes, however, until you've answered the questions that follow figure 5.

Figure 5: Ingredients of each tube in the lipid digestion experiment.



Carefully compare the volumes of each ingredient in each tube (see figure 5 and table 1). What TWO questions is this experiment specifically designed to answer?

1.

2.

- What are the two independent variables in the experiment?
  1.

  2.

- What is the dependent variable in the experiment?

- If the NaOH brings the initial pH above 8, what color will the phenolphthalein be at the start of the experiment?

- As digestion happens, what do you predict will happen to the color of the pH indicator?

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- What is the relationship between that color change and the activity of lipase?
- In which tube do you predict the enzyme will work fastest?
- If you wanted to know what would happen in the absence of bile, which tube would you compare the no-bile tube to?
- If you wanted to know what would happen in the absence of digestive enzymes (pancreatic extract), which tube would you compare the no-enzymes tube to?
- What other factors or conditions might affect lipase activity?

Procedure

Note: Add the ingredients in the order listed.

1. Take three clean test tubes and label them 1, 2, and 3.
2. Use a clean graduated pipette (with pump) to put 1 ml of vegetable oil into each tube.
3. Use a clean graduated pipette to put 1 ml of bile into tubes 2 and 3 only.
4. Use a clean graduated pipette to put water into each tube (ml amounts on **Table 1**).
5. Add a dropperful of phenolphthalein to each tube.
6. Use a clean graduated pipette to add 5 ml of pancreatic extract to tubes 1 and 2 only.

Table 1: Ingredients.

	Tube 1	Tube 2	Tube 3
Vegetable oil (ml)	1	1	1
Bile (ml)	0	1	1
Water (ml)	2	1	6
Phenolphthalein (dropperful)	1	1	1
Pancreatic extract (ml)	5	5	0
NaOH (drops, not droppersful)	Until pink	Until pink	Until pink

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7. Cover each tube with Parafilm, place your thumb over the top of the tube, and shake each tube 10 times.
8. As soon as possible after you complete step 7, remove the Parafilm and put 2-3 drops (NOT dropperfuls, just drops) of NaOH into each tube. Put the Parafilm back on and shake. Add more drops of NaOH until the solution stays pink.
9. Write down the color of the solution in **Table 2** at time 0.
10. Record the color every minute for five minutes, then again after 20 minutes and 30 minutes. You might want to take pictures as you go along, too.

Table 2: Record the color of each tube at each time interval. Express the color as a number on a "degree of pinkness" scale, where 10 = deepest pink and 0 = no trace of pink.

Time (min)	Tube 1- Oil, Lipase	Tube 2-Oil, Bile, Lipase	Tube 3-Oil, Bile
0			
1			
2			
3			
4			
5			
20			
30			

- In which tube did the lipid digestion reaction occur most rapidly? How do you know?

- Why was the reaction slower in each of the other two tubes? (Each tube will have a different reason, so list each of the other tubes and explain why the reaction was slow.)

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Activity 2: Starch Digestion

Objectives

1. Understand the purpose of starch digestion.
2. Recognize amylase, the enzyme involved in starch digestion.
3. Explain the role of each component of the experiment.
4. Explain how I_2KI (the starch indicator) allows you to interpret whether digestion has occurred.

Essential Background Information

Digestive enzymes break down large carbohydrates into monosaccharides (**Figure 6**). The basic reaction is the same for something as simple as table sugar (a disaccharide) and as complex as starch (a polysaccharide). Another kind of polysaccharide, cellulose, makes up plant cell walls. However, the carb-digesting enzymes in animal bodies cannot break down cellulose; instead, cellulose passes intact through our digestive system. This is why vegetables that are high in fiber contribute fewer calories to our bodies than do starchy vegetables of similar size.

In this activity, you will observe digestion by the enzyme **amylase**, the starch-digesting enzyme that occurs in your saliva (as well as in the pancreas, so it is also present in pancreatic extract). Amylase breaks down carbohydrate molecules into monosaccharides that can be used as energy or (in animals) raw materials for building glycogen.

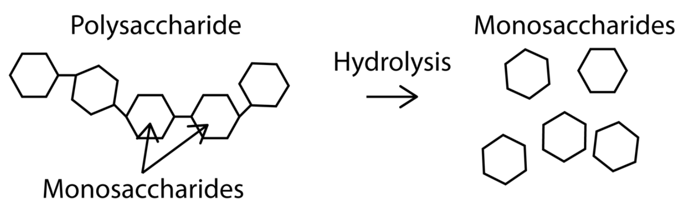

Figure 6: How hydrolysis affects a polysaccharide molecule.

In the lipid digestion experiment, you watched the pH indicator change color as fatty acids were released. In the starch digestion experiment, you will measure enzyme activity in a different way: You will periodically take samples from your reaction tubes and determine whether any starch is left in each one.

Overall Experimental Design

Here are the starch digestion materials on your bench:

- **Starch:** Carbohydrate to be digested
- **Pancreatic extract:** Source of amylase
- **I_2KI (iodine):** Starch indicator. In the presence of starch, I_2KI turns deep blue; in the absence of starch, it is brownish or yellowish. **I_2KI will STOP the digestion reaction, so it**

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should never go directly into your reaction tubes! Stay tuned to learn how you will use it to test samples that you will withdraw from your reaction tubes.

- **Hydrochloric acid (HCl):** This chemical (an acid) will reduce the pH of the solution in one of the test tubes to see whether low pH affects amylase activity.
- **Water:** Equalizes the volume in each tube.

Figure 7 and **Table 3** show the specific amounts of each ingredient you'll add to each test tube. Don't start filling your test tubes, however, until you've answered the questions below.

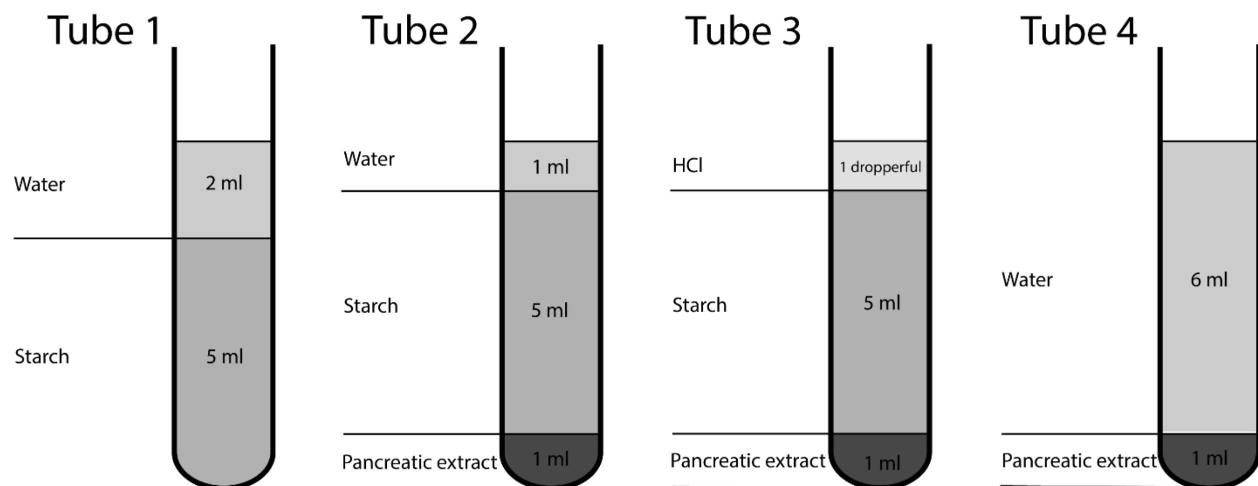

Figure 7: Ingredients in each tube in the starch digestion experiment.

- Carefully compare the volumes of each ingredient in each tube (see figure 7 and table 3). What THREE questions is this experiment specifically designed to answer?
  1.
  2.
  3.
- What are the three independent variables in the experiment?
  1.
  2.
  3.
- What is the dependent variable in the experiment?

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- If you were to withdraw fluid from each test tube and test it for starch with I_2KI at the start of the experiment, what color would the I_2KI be?

  Tube 1: Tube 2: Tube 3: Tube 4:

- If starch digestion happens, what do you predict will happen to the color of the I_2KI?

- What is the relationship between that color change and the activity of amylase?

- In which tube do you predict the amylase enzyme will work fastest?

- If you wanted to know what would happen in the absence of starch, which two tubes would you compare?

- If you wanted to know what would happen in the absence of digestive enzymes, which two tubes would you compare?

- If you wanted to know what would happen in the presence of HCl, which two tubes would you compare?

Important: To test the amount of starch still present at different times during the experiment, you will remove some liquid from the experiment's tubes and put it into separate tubes to be tested with I_2KI. Do not put I_2KI into the original tubes or it will stop digestion. See **Figure 8** for an example of what this will look like.

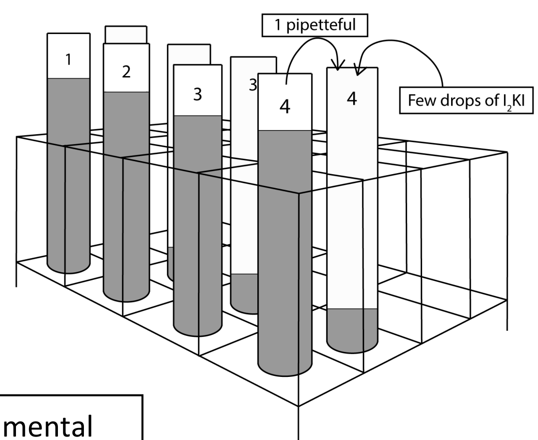

Figure 8: Testing starch digestion. Follow the experimental procedure carefully. Remove liquid from the original tubes and add I_2KI to the new tubes to see if any starch is present.

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Procedure

Note: Add the ingredients in the order listed.

1. Get out four clean test tubes. Label them 1, 2, 3, and 4.

Table 3: Reactants

	Tube 1	Tube 2	Tube 3	Tube 4
Starch (ml)	5	5	5	0
HCL (dropperful)	0	0	1	0
Water (ml)	2	1	0	6
Pancreatic extract (ml)	0	1	1	1

2. Use a clean graduated pipette (with pump) to transfer 5 ml of starch solution into Tubes 1, 2, and 3.
3. Put 1 dropperful (NOT drop, but dropperful) of 0.5% HCl into Tube 3.
4. Use a clean graduated pipette to transfer water into each tube as labeled in **Table 3**.
5. Use a clean graduated pipette to transfer 1 ml of pancreatic extract into Tubes 2, 3, and 4.
6. Cover each tube with Parafilm and invert once to mix everything together.
7. Label a second set of test tubes with numbers 1, 2, 3, and 4. These will be used to hold samples to test for your starting level of starch.
8. As soon as you can after you have completed steps 6 and 7, use a transfer pipette to transfer one pipetteful from each the first set of tubes to the second set of tubes. **Use a clean pipette for each transfer**, so you don't cross-contaminate the subsamples from multiple tubes. Look back at **Figure 8** for reference. Set a timer for 5 minutes; while the timer runs, proceed to step 9.
9. Put a few drops of I_2KI into each of the **second set** of tubes (DON'T put it into the tubes where the digestion is happening – you will have to start over). Use the "Initial" row in **Table 4** to record the color of the I_2KI and whether starch is present.
10. While the 5-minute timer is still running, label a third set of test tubes 1, 2, 3, and 4. These will be used to hold your final samples to test for starch.
11. Once the 5 minutes have elapsed, use clean transfer pipettes to transfer one pipetteful from each the first set of tubes to the third set of tubes.
12. Put a few drops of I_2KI into each of the third set of tubes. Use the "After 5 minutes" row in **Table 4** to record whether starch is present.
13. If starch digestion has not proceeded as planned, you may need additional rows in the table to test additional samples after 10 or 20 minutes. Your TA will help guide you.
14. You might want to take pictures as you go along, as you may have done for lipid digestion.

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Table 4: Record the color of I_2KI and whether starch was present in each tube. Express the color as a number on a "degree of blueness" scale, where 10 = deepest blue and 0 = no trace of blue.

Time	Tube 1-Starch	Tube 2-Starch, Amylase	Tube 3-Starch, Amylase, HCl	Tube 4-Amylase
Initial				
After 5 minutes				
After __ minutes				
After __ minutes				

For all four digestion tubes, compare the color of the I_2KI in the subsamples taken at the start of the reaction and after 5 (or more) minutes. In which tube did the starch digestion reaction occur most rapidly? How do you know?

Why was the reaction slower in each of the other three tubes? (Each tube will have a different reason, so list each of the other tubes and explain why the reaction was slow.)

Activity 3: Conducting a Digestion Experiment

Objectives

1. Given an IF … AND … THEN statement, design an experiment that includes all necessary variables, a control, and repetition.
2. Conduct the experiment you designed.
3. Understand the effect certain factors can have on the rate of digestion.

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4. Collect data and create visual aids, like charts and graphs, that can help you interpret your results.
5. Interpret your results and compare them to initial predictions.

So far, you've seen real-time examples of lipid and starch digestion. Now, you will conduct your own experiment based on an IF … AND … THEN statement that your TA will assign to your group. Each IF … AND … THEN statement will be different, but all groups will investigate how environmental factors can affect digestion rates. You will use your data to write a mini lab report (called an abstract), which will be due in a few days after lab; you can learn more about abstracts in the appendix of this lab manual.

Part 1: Experimental Design

After you get your IF … AND … THEN statement from your TA, design an experiment to test the hypothesis and fill out the following prompts accordingly. You must get your experimental design checked by your TA before moving to Part 2.

- Hypothesis:

- Description of your dependent variable:

- Description of your independent variable, including levels:

- Description of control:

- Replication (number of tubes for each treatment):

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- Explanation of experimental design:

- Prediction:

- Methods (as part of your methods, you may want to construct a table like **Table 1** or **Table 3** to keep track of what each tube should contain):

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- Design a table in which you will record your group's data:

Your TA must approve everything before you move on! TA, make your mark here: ______

Part 2: Conducting the Experiment

Conduct your experiment! Make sure everyone in the group participates and takes useful notes so writing the abstract is easier. Additionally, take pictures of your test tubes at different phases of the experiment so you can use them in your abstract (check the appendix for help writing the abstract). The more detailed your notes and responses are, the easier the abstract will be.

1. Summarize the results of your experiment in a few sentences.

2. Do your results support or reject your hypothesis? Explain how data from your experiment helps you decide the answer to this question.

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3. Do your results match with your prediction? If not, why not?

4. Did you make any mistakes or notice anything that may have influenced the results of the experiment?

5. What conclusions can you draw from your results?

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Questions for Review

1. Suppose that you and your friends bought some chicken alfredo pizza for a late-night biology study session, and you decide to use the pizza to help you study for possible exam questions about digestion. For each part of the pizza, explain the following:
    a. what kind of organic molecule(s) the part is composed of;
    b. how it's digested; and
    c. in what form it enters your bloodstream. Don't forget what you learned about cellulose.

   Crust:

   Cheese/Alfredo sauce:

   Chicken:

   Spinach:

2. You are reviewing a poorly explained description of a digestion-related experiment. The student wrote that the group combined pancreatic extract with liquid containing different types of organic molecules, but forgot to write exactly what organic molecules were included. They tested digestion initially and then after 30 minutes. The test with phenolphthalein was red at the beginning, but it turned colorless by the end of the 30 minutes. The test with I_2KI is initially blue, but then is yellow after 30 minutes.

    a. What are some of the organic molecules present before digestion, and how do you know?

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b. What molecules would be in the solution after the 30 minutes had passed, and how do you know?

c. Is it possible that other types of organic molecules were present in the original solution that you can't guess from the experiment write-up? Why?

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Acknowledgments and Attributions

Procedures for Activities 1,2, and 3 adapted from: Dickey, Jean. 2003. *Laboratory Investigations for Biology,* 2nd edition. Pearson Education, Inc.

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Figure 2a: Fungi External Digestion © Sarah Greenwood used under a CC BY-SA 4.0 license
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Figure 4: Simple Triglyceride Hydrolysis © Sarah Greenwood used under a CC BY-SA 4.0 license
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Figure 6: Simple Polysaccharide Hydrolysis © Sarah Greenwood used under a CC BY-SA 4.0 license
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Food Microbiology I

Mariëlle Hoefnagels and Sarah Greenwood

Allergic to penicillin?

Ask your T.A. about special precautions you should take during this week's lab and the next!!

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Introduction

Many boxed foods in grocery stores are heavily processed. Often, labels tout a lack of artificial preservatives as a way of marketing the products as "natural." For other foods, the labels explain which preservatives they contain. Regardless of how you feel about artificial preservatives in your own diet, they do have benefits—after all, preservatives may help decrease food waste and make it more practical to transport food to hard-to-reach or impoverished areas. In this series of two labs, you will investigate how different kinds of bread hold up to mold growth and see how this relates to the brands' claims about preservatives.

For these labs, you will pretend that you and your group mates are business partners who have decided to open a bakery that will specialize in fresh bread. First, you need to decide on your future bakery's identity. As mentioned earlier, some people advocate the use of artificial preservatives to extend shelf life, minimizing food spoilage and reducing the amount of food that gets thrown away. Others advocate an "all-natural" approach, staying away from artificial preservatives. If you were opening a bakery based on what you know now, what would be your opinion on the issue of preservatives?

Do all of your group members have the same opinion as you?

To learn more about food preservation, your team decides to conduct an experiment comparing mold growth on bread with and without preservatives. In this week's lab, you will design and carry out your experiment. In next week's lab, you will collect and interpret your data.

After the second lab, you will turn in a short report (abstract) to your shareholders, describing your experiment, summarizing your findings, and issuing a recommendation on the issue of preservatives. Even though you collected the data as a group, you must write your own report and develop your own tables and graphs! The second lab contains some questions to guide your efforts, but the basic format and "rules" for your report are in the instructions for writing abstracts in the appendix of this lab manual.

Background Information

Common Preservatives

Food manufacturers – including independent bakeries – can add multiple types of preservatives to bread to keep it from spoiling rapidly. Some common cooking ingredients, such as garlic and honey, can act as natural preservatives. Lecithin is a compound found in egg yolks and soy that is also used in baking bread and acts as a preservative. Vitamin C (ascorbic acid) is also often used as a natural preservative. Many artificial preservatives also make their way into bread, including calcium propionate. Some brands that use calcium propionate market their bread as having "extra calcium" although its actual function is to act as a preservative.

Use the preceding list of preservatives to guess what ingredients in the bread you test may make it more or less resistant to mold growth. Note that you won't be able to make claims about the efficacy of individual preservatives through this experiment, but you can use it as a way to get ideas for your experimental design.

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Handling Mold Spore Suspension/Aseptic Technique

About a week ago, your instructors inoculated petri dishes with a culture of *Penicillium notatum*, a type of mold. Once the cultures produced spores, your instructors flooded the petri dishes with sterile water and gently scraped the surfaces of the cultures with a sterile glass "hockey stick" (a bent glass rod). They poured the resulting fluid, which contained spores and small fragments of fungal filaments (hyphae) into a squirt bottle that had been previously sterilized with 20% bleach and rinsed with sterile water. After repeating this flooding-scraping-pouring process two additional times, they added enough sterile water to fill the squirt bottle. They also prepared sterile squirt bottles containing only sterile water.

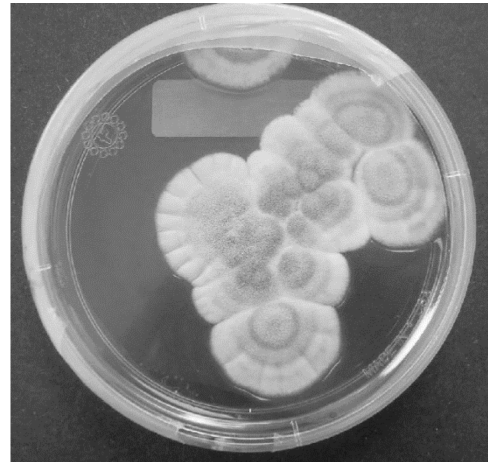

Figure 1: *Penicillium notatum.*

Items that are **sterile** are free of any kind of life, including bacteria and fungi. When you do your experiment, you don't want to expose your bread to any "intruder" microbes. To prevent this, you will use a set of procedures that are collectively called **aseptic technique**. Aseptic literally means "germ-free," so aseptic technique means not contaminating your bread, any sterile implement, or anything else with unwanted microorganisms.

Aseptic technique means starting with sterile materials. In this lab, for example, you'll begin with sterile plastic baggies and bagged bread – the bread may not be 100% free of mold spores, but it's pretty "clean." Aseptic technique also means not contaminating these sterile materials with microorganisms. In other words, in this lab you are interested in counting mold colonies that you inoculate onto bread, so you don't want to contaminate your bread with mold spores from your skin or the lab room's air.

Here are some simple rules to follow when using aseptic, or sterile, technique:

- Wash your hands before you begin and after you finish.
- Wipe down your bench with Lysol before you begin and after you finish.
- Only open sterile containers such as plastic baggies for a few moments at a time, and only when they are being used.
- Sterile surfaces should never touch any other surface, such as the lab bench or your fingers. You shouldn't breathe on any sterile surface either.

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- You will cut your bread into four equal pieces (your replicates). Right before you cut the bread, you will need to wipe your lab bench, your cutting board, and your knife with Lysol (and allow them to dry so the Lysol doesn't soak into the bread).
- Put on gloves before you handle your bread, and spritz Lysol onto the gloves once they're on. Rub in the Lysol until your gloves are pretty much dry. Don't touch anything other than the bread and other sterile surfaces once you have sterilized your gloves.
- Do not take out your bread until you are ready to cut it, and immediately place it into sterile plastic baggies after you have cut it.
- You will be spraying sterile water or spore solution on your bread, depending on the treatment. Handle all treatments involving sterile water before you apply any treatments involving spores.
- Whether you are spraying sterile water or spores, keep the bread inside the baggie and open the baggie only long enough to squirt the spray bottle. Keep the baggie's opening off to the side as you spray, minimizing the chance that mold spores will drop in on your bread from above. Seal the baggie immediately after spraying and set it aside.
- Once they contain a piece of bread, do not stack the baggies on top of each other. (That's not really about sterile technique, but it seems like a good idea to mention it. You don't want some bread at the bottom of the stack to get squished.)

These precautions might seem extreme, but mold spores are truly everywhere. You want to minimize their opportunities to land on your bread and confound your results.

Read this for safety: You will handle mold spores in this lab. During the first week's lab, they will be confined to a squirt bottle, so you are unlikely to inhale them. During the second week's lab, however, at least some of your bread should be moldy. Please do all you can to keep your bags closed, minimizing everyone's exposure to spores. Also, make sure you wash your hands and disinfect your work space after you have completed the lab activities during both weeks.

And take note: Your TA will throw you out if your behavior in this lab endangers your fellow students in any way.

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Activity 1: Design and Carry Out an Experiment

Objectives

1. Design an experiment that includes all necessary variables, a control, and repetition.
2. Conduct the experiment you designed.
3. Understand the role of chemical preservatives in food.

Now that you have some background, you are ready to compare mold growth on bread with and without preservatives. But first, you need to design the experiment. You may have already proposed an experimental design online, or your T.A. may ask your group to complete an Experimental Design Worksheet during the lab. Work together to decide on ONE hypothesis to test as a group, then think through every element of the experiment as carefully as you can.

Write out the details of your final experimental design below. Your TA will check it over before you begin, and you will also need this information when you write your abstract after next week's lab.

- Title of experiment:
- Hypothesis (remember, this should contain an **explanation**, not a prediction):
- Independent variable:

  Describe your treatments:

  Describe your control(s):

  Why is it important to include that control (or those controls)?

- Sample size (the number of replicates for each treatment or control):

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- Dependent variable:

  Describe the data you plan to collect next week:

- Think about what you know about factors that might affect mold growth, then list the most important variables to standardize:

- Describe your methods:

You now have enough information to summarize your ideas in an IF … AND … THEN statement. Remember the IF … AND … THEN statement from the Process and Tools of Science lab and from the Digestion lab? Here's an example:

- **[Hypothesis = Explanation]:** IF vitamin E is essential because it helps support the immune system …
- **[Test = Experiment]:** … AND we alter the amount of vitamin E in the diets of mice while exposing them to bacteria that cause pneumonia, …
- **[Prediction = Result that would support hypothesis]:** … THEN mice with higher doses of vitamin E should have a lower incidence of pneumonia than mice with less or no vitamin E.

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- Write an IF … AND … THEN statement that corresponds to your group's experiment:

  [Hypothesis = Explanation]: *IF…*

  [Test = Experiment]: … *AND…*

  [Prediction = Result that would support hypothesis]: … *THEN…*

Sketch a graph of your predicted results:

Your TA must approve this before you move on! TA, make your mark here: _______

Once your TA approves your hypothesis, methods, and predictions, **carry out your experiment.**

- Use a sharpie to label each baggie with the treatment, the name of your group or the names of your group members, and the date.
- As you carry out your experiment, take careful notes on the next page about what your group did, who did what, how they did it, and in what order. In addition, describe anything that goes wrong (or anything you think MIGHT have gone wrong – don't worry, you wouldn't be the first to make a mistake, so don't be afraid to document it). I promise you, with my hand on my heart, that you will not remember the details if you don't write them down. No detail is too small; come next week, you may need to refer to these detailed notes to interpret your results.

When you are done, tidy up, wash your hands, and disinfect your work space before you leave.

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Notes from your experiment:

Acknowledgments and Attributions

Procedures were adapted from Handelsman, J.; Houser, B.; Kriegel, H. 1997. Experiment 3: Bread, mold, and environment: a lesson in biology and the environment. Pages 39-51 in Biology brought to life: a guide to teaching students to think like scientists. Times Mirror Higher Education Group, Dubuque, IA.

Figure 1: Penicillium notatum © Crulina 98 used under a CC BY-SA 3.0 license
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Plant Diary:
Prep Work for the Plant Lab

Mariëlle Hoefnagels

Instructions for Planting your Corn and Bean Seeds

Write the date you planted your seeds: ___________________________

1. Label one pot "corn" and one pot "bean."
2. Divide the potting mix between the two pots.
3. Moisten the potting mix thoroughly.
4. Bury the corn seeds about 1 inch deep and a couple of inches apart from each other in one pot. Do the same with the bean seeds in the other pot. If you plant them too deep, the seedlings might run out of energy before they reach light, and then they'll die. Sad!
5. If you like, you can cover the pots with plastic wrap as soon as you have planted your seeds. Do not add more water until the seeds have germinated, or the seeds may rot. Once the seeds have germinated, remove the plastic from the pots.
6. If you don't see seedlings within 7-10 days of planting your seeds, ask your TA for a new kit.
7. Once the seeds have germinated, place them near a window and water them whenever the surface of the potting mix dries out. (These plants need very little care, but light and water are absolute requirements -- it's your responsibility to provide them!)
8. To get the largest possible plants, you will have to thin your seedlings to one per pot once they have developed their first true leaves. If the idea of doing that breaks your heart, you can let them all grow, but your plants will be smaller.
9. As described below, you'll be documenting the growth of these plants until the Flowering Plants lab in 5-6 weeks. Be sure to make arrangements for care and data collection if you leave town.

If your plants die, save the pots!
It'll be worth a pop quiz to bring them to lab during the Flowering Plants lab.

Keeping your Corn and Bean Photo/Data Diary

Once your seeds germinate, it's time to start your photo and data diary. At least **three times a week,** do the following (no matter what the condition of your plants):

- Take a picture of both pots "posing" with something unique to you (your student ID, something with your name or your initials on it, or any other unique object).
- In each pot, measure the tallest plant's height (in cm) and record the data in the table provided.
- In each pot, count the leaves on the tallest plant and record the data in the table.
- Note your observations (e.g. plants wilted, leaves fell off, flowers/fruits forming …)

At the end of the data collection table, you will find a list of **what to turn in at the end of the plant-growing period.** Better have a look now, so you'll know what to do!

Date	Days after planting	Bean or corn?	Height (cm)	Number of leaves	Other observations

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Date	Days after planting	Bean or corn?	Height (cm)	Number of leaves	Other observations

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Date	Days after planting	Bean or corn?	Height (cm)	Number of leaves	Other observations

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Date	Days after planting	Bean or corn?	Height (cm)	Number of leaves	Other observations

During the week of the Flowering Plants lab, you will be required to turn your diary in as a single document (file size: 20 MB or less), including:

- All of your photos, labeled with the date and arranged chronologically. You may need to compress each photo to keep your total file size down (Google it if you don't know how).
- A typed table (Word or Excel is fine) with all measurements and observations.
- Two graphs, each comparing data for your corn and bean plants in the same graph:
  - One with "Days after planting" on the X axis and "Height (cm)" on the Y axis.
  - One with "Days after planting" on the X axis and "Number of leaves" on the Y axis.

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Food Microbiology II

Mariëlle Hoefnagels and Sarah Greenwood

Allergic to penicillin?

Ask your T.A. about special precautions you should take during this week's lab!!

Baker © Thomas Berg used under a CC BY-SA 2.0 license https://www.flickr.com/photos/decafinata/290467645

Introduction

In this series of two labs, you are pretending that you and your group mates are business partners who are opening a bakery that will specialize in fresh bread. Recall that one task is to decide on your future bakery's identity. Some people advocate the use of artificial preservatives to extend shelf life, minimizing food spoilage and reducing the amount of food that gets thrown away. Others advocate an "all-natural" approach.

Last week, you carried out an experiment designed to compare mold growth on bread with and without artificial preservatives. For the past week, your baggies with bread have been kept at room temperature to give the fungi time to grow.

In today's lab, you will figure out a way to quantify the extent of mold growth on your bread samples. You will use the data you collect in your final report (abstract) to your shareholders, describing your experiment, summarizing your findings, and issuing a recommendation on the issue of preservatives. Even though you collected the data as a group, you must write your own report and develop your own tables and graphs! Your report should follow the instructions for writing abstracts in the appendix of this lab manual.

Activity 1: Quantifying Mold Growth

Objectives

1. Design a way to quantify mold growth on bread.
2. Collect data from your experiment.

One of the challenges of this experiment is measuring the extent of mold growth on your bread samples. It is not enough to eyeball it and say, “Eh, I think that’s about 25% moldy.” Your group will be given a transparency with a 1-cm grid marked on it, plus a variety of water-soluble markers. You will need to use these tools to design a way to scientifically quantify the amount of mold on each piece of bread. In your final report, you will need to describe (in words) what you did, in enough detail that someone else could repeat your method.

Describe the method your group is using:

For each individual replicate (piece of bread), record your data here:

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Activity 2: Possible Error

Objectives

1. Reflect on your experiment to determine possible sources of error.

Note that several types of mistakes can mess with the results in experiments such as this one. Think about your experimental design and methods and determine if there were possible errors that would affect your results. There may also be errors that you can't control. Use the following for a few examples:

- One of your pieces of bread was already contaminated with mold spores before you began the experiment.
- The squirt bottle containing sterile water got contaminated with mold spores.
- You squirted more of the mold spore solution or sterile water onto one of your pieces of bread than on others.
- You forgot to shake the squirt bottle of mold spores before squirting on your baked goods.

Is it possible that any of these happened to your group? Are there any other errors? If so, what happened, and how might that affect your interpretation of your results?

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Activity 3: Graph Your Data

Objectives

1. Create visual aids that can help you interpret your results.
2. Interpret your results and compare them to initial predictions.
3. Understand the role of preservatives in food.

Procedure

1. In Google Sheets, create a spreadsheet for your experiment. Write all of your data in this spreadsheet.
2. For each treatment, calculate the average amount of mold. To do this in Google Sheets, click and drag over the values you'd like to average to highlight those cells. Then, click Insert → Function → Average. Note that this won't work if your units are included in each cell – that is, cells must contain numbers only. Include units in the column/row titles instead.
3. If your TA says you need to add error bars, you will be given instructions for calculating the variation within each treatment (standard error or standard deviation) and for adding the error bars to your graph. Note: If the error bars for one treatment overlap those of another treatment, the treatment averages are probably not significantly different; in other words, any differences you see are probably due to chance. If the error bars do not overlap, however, the treatment averages are likely to be significantly different. (Note that a real statistical test would include a lot more sophisticated analysis than we did here; this is just a quick way to see whether any differences between your average are likely to be "real" or just due to chance.)
4. Use the ingredient labels provided to look for specific ingredients that may act as preservatives. If you don't know what an ingredient is for, look it up online to see if it's a preservative or not. Get in the habit of checking food labels and you'll see how common artificial preservatives are in commercial foods!

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Activity 4: Start Your Report

Objectives

1. Reflect on your experiment to make conclusions about your results.
2. Write an abstract that follows the rubric outlined on Canvas and follows the guidelines in the appendix.

Once you have graphed your results, answer the following questions before you write your report to your shareholders:

- Look back at your hypothesis and prediction from last week. Look at the graph of your data. Do your results support your hypothesis or lead you to reject it? Explain your answer, using your data for support.

- If your results did not correspond to the prediction you made, explain how your results are different from your expectations and why this might have occurred.

- Describe how your data are supported by information from other sources (e.g. textbooks, online information, or other lab teams working on the same problem).

- If you had any problems with the procedure or questionable results, explain how they might have influenced your conclusion.

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- Summarize the conclusion you have drawn from your results.

- Now that you have seen your results, what is your opinion on the issue of preservatives? Do you think you should include preservatives in your new bakery's breads? Briefly explain your reasoning.

Once you've answered the questions, you're ready to write your report as outlined in the appendix.

Acknowledgments and Attributions

Procedures were adapted from Handelsman, J.; Houser, B.; Kriegel, H. 1997. Experiment 3: Bread, mold, and environment: a lesson in biology and the environment. Pages 39-51 in Biology brought to life: a guide to teaching students to think like scientists. Times Mirror Higher Education Group, Dubuque, IA.

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Exposing Your Petri Dish: Prep Work for the Bacteria and Disease lab

Mariëlle Hoefnagels and Sarah Greenwood

Do this one week before the Bacteria and Disease lab

You should have been given a petri dish containing nutrient agar. Many—but by no means all—types of bacteria and fungi will grow on this medium, and your task is to expose your plate in almost any way you choose to sample the bacteria and/or fungi that may lurk in your environment. DON'T WAIT!! **Expose your plate as soon as you can after you receive it**, so you will have well-developed, disgusting colonies to show off during next week's lab.

Please **DO NOT** sample your mouth, ears, or throat, because your bugs might make others sick. Other than that, you may expose your agar to almost anything you like. We don't want to put a damper on your creativity, but here are some suggested methods:

- To sample the **air** in a particular place, open your dish for a few minutes, then close it.
- If you want to sample a **liquid**, dip a clean Q-tip into the liquid and lightly streak a Z-shape onto your dish; then close the dish. Alternatively, you can spray the liquid lightly on the surface of the agar.
- If you want to sample a **solid object**, place the object briefly on the surface of the agar, then remove it. Press lightly, because if you press too hard the agar will tear and it will be harder to see the organisms that will grow there.

No matter how you expose your plate:

- close it immediately after the exposure is finished, then …
- tape it shut, and …
- store it **UPSIDE-DOWN** at room temperature (to prevent water from condensing on the lid and running back down on the agar surface). If you leave the lid off, or store it in too hot a location, the agar will dry out and nothing good will grow.

Finally, remember to **bring your plate to class next week;** you will need it for lab. Have fun!

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Bacteria and Disease

Mariëlle Hoefnagels and Sarah Greenwood

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Introduction

Bacteria are everywhere. They're in soil, in water, on nearly every surface, and inside most other living organisms. For humans, the vast majority of bacteria inside and around us are non-threatening. Many are even necessary to stay healthy. However, some are **pathogenic**, meaning they cause disease.

In this lab, you will learn about the basic features of bacteria and some of the ways bacteria differ from each other and from eukaryotic cells. You will learn about how antibiotics function and about antibiotic sensitivity and resistance. Lastly, you will conduct a class-wide experiment that simulates disease transmission.

Activity 1: Bacteria Basics

Objectives:

1. Know basic features of bacteria and how they differ from eukaryotes.
2. Identify the three common bacterial shapes: cocci, bacilli, and spirilla.
3. Understand how bacterial colonies form.

In the microscopy lab, you got to see first-hand some of the markers of **eukaryotic cells**, including organelles like nuclei and chloroplasts. Bacteria, however, are prokaryotes. (So are archaea, but you will not study them in this lab.) Prokaryotic cells have a different structure from eukaryotic cells. The most obvious difference are that **prokaryotic cells** are much smaller than eukaryotic cells, and they lack membrane-bounded organelles. For example, prokaryotic cells don't have a nucleus; instead, the DNA congregates in the middle of a cell without a membrane surrounding it. Prokaryotic cells also lack mitochondria and chloroplasts. They do, however, have ribosomes for protein production, and most bacteria have cell walls. **Figure 1** shows the evolutionary relationship between Prokaryotes and Eukaryotes. In this activity, you will investigate the general features of bacterial cells.

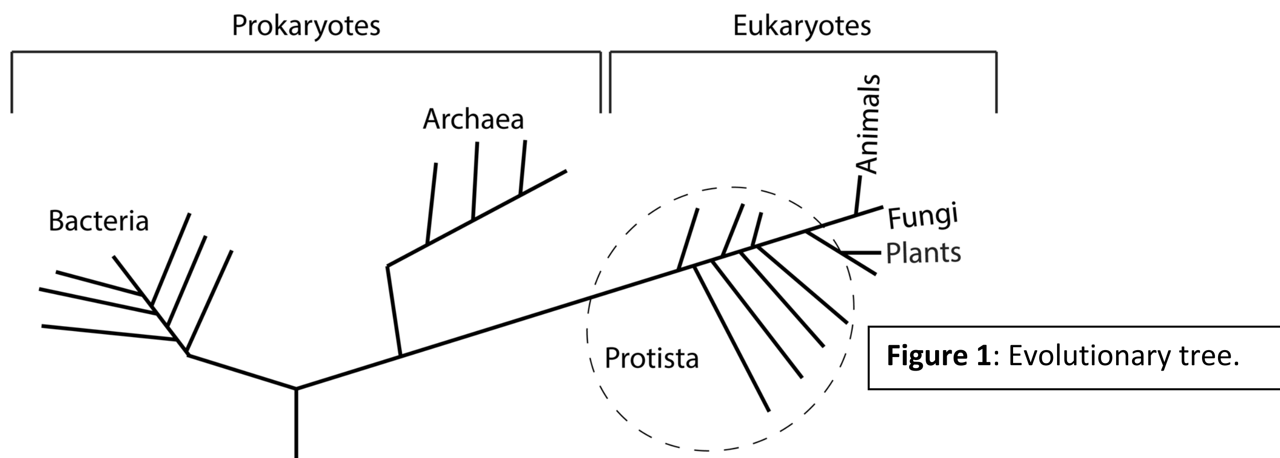

Figure 1: Evolutionary tree.

Part 1: Bacterial Shapes

As mentioned, most bacteria have cell walls. These cell walls cause most bacteria to be one of three shapes: **coccus** (spherical), **bacillus** (cylindrical), or **spirillum** (spiral). See **Figure 2** for examples of each. In this part of the activity, you will use the microscope to look at slides with bacteria of each of the three shapes. Make sure you follow the correct procedures for using the microscope; consult the *Using the Microscope* chapter of this lab manual for a refresher.

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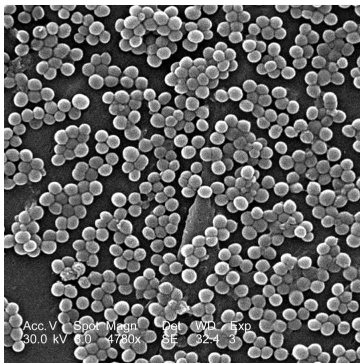
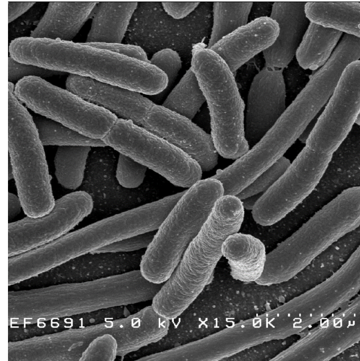
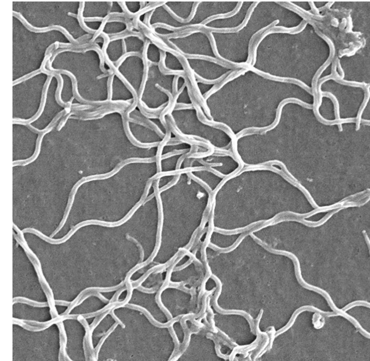
Figure 2: Examples of common bacteria.



a. Cocci. *Staphylococcus aureus.*

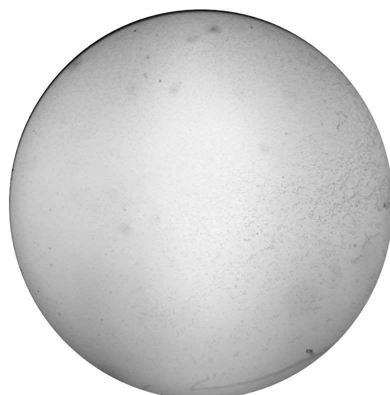


b. Bacilli. *E.coli.*

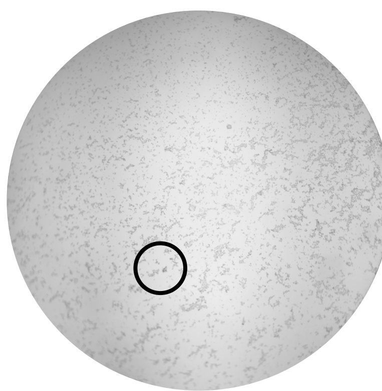


c. Spirilla. *Borrelia burgdorferi.*

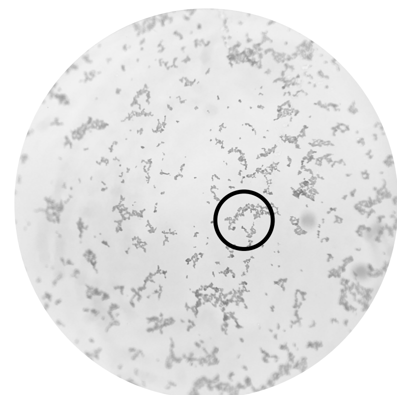
The photos in **Figure 2** were taken with an electron microscope, which allows for much greater magnification than the compound microscopes we use in lab. That means that the bacteria on your prepared slides will appear in much less detail. See **Figure 3** for reference on what some of your bacteria may look like. All of the bacteria in **Figure 3** are cocci. At 40X, the bacteria look like tiny pink dots. At 100X, you begin to more clearly see their spherical structure. At 400X, you can more clearly see individual bacteria. It's easy to accidentally focus on a small bit of dirt on the slide rather than on the bacteria, so check with your TA if you need help focusing or aren't sure if you are looking at the right thing.



40X magnification



100X magnification



400X magnification

Figure 3: This is what your field of view might look like when looking at cocci. In the right two images, a few bacteria are circled in black to help you see what you are trying to focus on. They're especially hard to make out at scanning power, but should be easier to see in real life since they're dyed pink.

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Procedure

1. Focus on the bacteria using the low-power (4X) objective. What is the total magnification when you use this objective? ___________
2. Move to the medium-power (10X) objective and use the fine focus knob to produce a sharp image. Repeat using the high-power (40X) objective. You may need to move the stage during this step to center on the bacteria. (Hint: sometimes, the bacteria are difficult to locate. Ask the TA if you aren't sure what you're looking at.)
3. Draw what you see below, and then repeat with the slides demonstrating the other bacterial shapes. **Note**: the spirilla can be particularly difficult to make out, so don't hesitate to ask your TA for help focusing on the right thing.

   Coccus Bacillus Spirillum

4. Some bacteria have a **capsule**, which is made of a gelatinous material and can affect whether the bacteria is pathogenic. For example, the bacteria that cause pneumonia only actually cause the disease if they have capsules. Put your slide of bacteria capsules on the stage and focus up to high power. Draw what you see below.

   Bacteria with Capsule

Can you see any of the bacteria's internal structures under the microscope? What can you see/not see?

Thinking back to the *Using the Microscope* lab, what structures could you see if you were looking at a eukaryotic cell?

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Part 2: Bacterial Colonies

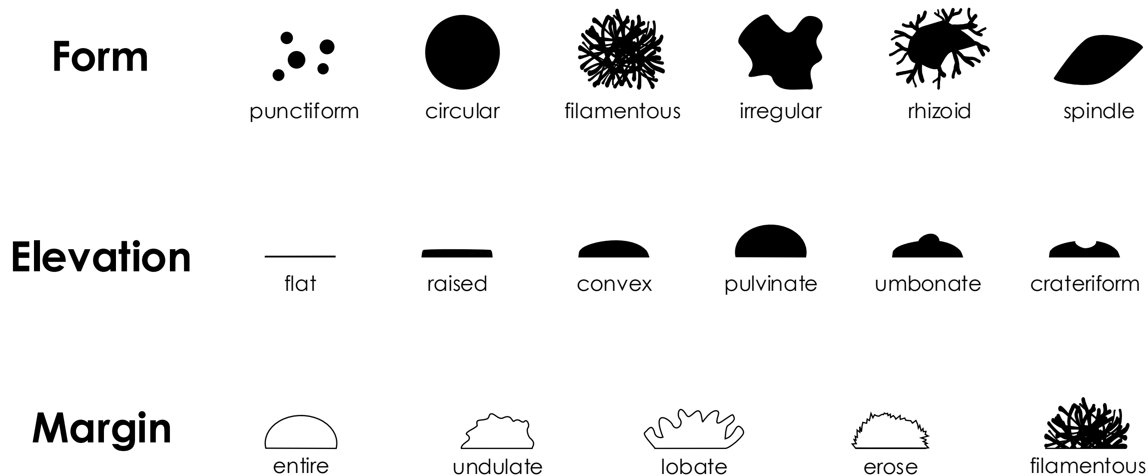
Bacteria reproduce very quickly, and under the right conditions they form colonies. A **colony** is a cluster of thousands (or millions) of essentially identical bacterial cells, each descended from one original cell that divided countless times. A colony may eventually become large enough to see with the unaided eye, even though each individual cell is microscopic.

Last week, you were given a petri dish with **agar**, which provides food and moisture for many bacteria to grow. Hopefully, you exposed it to the outside world and can now see colonies of bacteria (and perhaps other microorganisms like fungi). You will use your petri dishes to investigate the diversity of microorganisms in different places. While completing this section of the lab, **do not** open your plate, because it may contain pathogenic bacteria or fungi.

Procedure

1. Pick one of your group member's plates. Count the different types of colonies. Use **Figure 4** to help describe the shape, elevation, and margin of different colonies; sometimes colonies have unique colors, too. In some cases, it may help you to look at the colonies through your dissecting microscope.

Figure 4: Common colony types.



2. Record where the plate was exposed and the number of different types of colonies in **Table 1.**

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3. Repeat with a plate contributed by another group member and continue filling out the table.

Table 1: Colony diversity.

Where plate was exposed	Number of types of colonies

Which plates in your group had the least diversity, and which had the most diversity? What could explain this difference?

Did any plates have similar types of bacteria? Why do you think that is?

Was any bacteria type on all of the plates in your group? Why might that be?

Activity 2: Bacteria and Antibiotics

Objectives

1. Understand how scientists use antibiotics to inhibit or kill bacteria.
2. Explain how targeting bacterial ribosomes affects bacteria.
3. Understand the difference between gram-positive and gram-negative bacteria and conduct your own Gram stain.
4. Understand how and why antibiotic sensitivity tests are used.
5. Explain how bacteria become antibiotic-resistant.
6. Understand why taking a full course of antibiotics is so important.

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Antibiotic drugs are compounds that inhibit or kill bacteria, and they are important tools in the fight against bacterial infections. Developing antibiotics that are safe and effective requires scientists to understand the differences between bacterial cells and eukaryotes. After all, if you take an antibiotic drug to kill off bacteria, you don't want that drug to damage your own cells. Some antibiotics, for example, block bacterial ribosomes (which differ from eukaryotic ribosomes). Other antibiotics block the bacterium's ability to build its cell wall.

If a drug blocks bacterial ribosomes, what process in the bacterial cell will stop, and why should that kill the bacteria?

Why would antibiotics not work against fungal infections, like yeast infections or athlete's foot?

Why would antibiotics not work against viral infections, like flu or the common cold?

However, not all bacteria are alike, and no single antibiotic can stop all bacterial infections. And even the best antibiotics can become less useful over time as they select for bacteria with mutations that confer resistance.

In this exercise, you will investigate some of the differences between bacteria, learn how to measure a bacterial population's sensitivity to an antibiotic, and model the process by which antibiotics select for antibiotic-resistant bacteria.

Part 1: Gram Staining

A process called **Gram staining** allows scientists to differentiate between the cell walls of two major categories of bacteria: **gram-positive bacteria** and **gram-negative bacteria**. These two kinds of bacteria respond to antibiotics differently; although there are antibiotics designed specifically for gram-negative bacteria, gram-positive bacteria are often much more susceptible to antibiotics. You will perform your own Gram stain to see what kinds of bacteria are currently in your mouth.

Procedure

1. Use your china marker to draw a small circle in the middle of a clean slide. The circle should be about the size of a dime or nickel. This circle will help designate the area where you will prepare your Gram stain.

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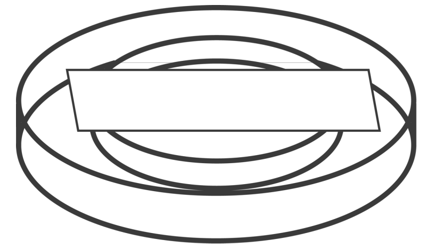
2. The china marker will interfere with the stain, so flip the slide over so your circle is on the underside of the slide.
3. Have one person in your group use a toothpick to gently scrape dental plaque from their teeth (the teeth in the back may work best, at or near the gums) and spread it in a very thin layer around the center of the circle. Let the slide dry.
4. Light your alcohol lamp.
5. Use a clothespin to pick up the slide and pass it quickly over the flame to heat fix the bacteria to the slide. Make sure you don't overheat the bacteria.
6. Place the slide on the staining platform (See **Figure 5**) and cover it in with crystal violet stain. Leave the stain on for one minute.
7. Tilt the slide to drain off some of the stain, and then use a wash bottle to gently rinse it with water.
8. Put several drops of Gram's iodine on the slide and leave it on the slide for one minute.
9. Tilt the slide again and use your wash bottle to gently rinse it with water.
10. Tilt the slide and apply drops of 95% ethanol until it runs off clear. Do it drop by drop, and don't rush.
11. Use the wash bottle to gently rinse the slide with water again.
12. The gram-positive bacteria should be stained blue or purple now. To stain the gram-negative bacteria, you will use a counterstain. Flood the slide with safranin for thirty seconds.
13. Tilt the slide and gently rinse it with your wash bottle. Let as much of the water drain off as possible, and then let the slide dry. Now, the gram-negative bacteria should be stained red.
14. Look at your slide under the microscope. You don't need to add a coverslip.
15. Use your cell cam mount to take a picture of what you see on your phone.
    Note: Look back at Activity 2/Part 1 of the *Using the Microscope* lab to see the drawing you made of cheek cells. You will probably see some here as well. The bacteria should be much, much smaller than your cheek cells; focus on these small bacteria for this activity. (Hint: there's a gram-positive species of bacteria called *Streptococcus* that causes tooth decay. They are cocci linked in chains. Do you see any?)
16. On the picture you took, circle the bacteria and note whether they are gram-positive or gram-negative by adding + or – signs. Then, note whether the bacteria are cocci (c), bacilli (b), or spirilla (s) and whether they are single bacteria, clusters, or chains. Your TA may ask you to turn this in as part of an assignment.

Figure 5: Staining platform.



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17. If you like, use **Table 2** to sketch and describe the bacteria you see.

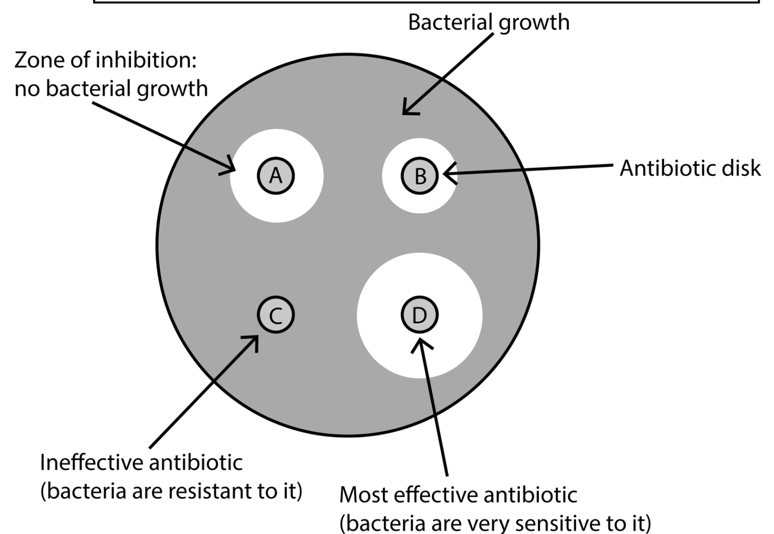
Table 2: What bacteria do you see?

Bacteria sketch	Gram – or +?	Coccus/Bacillus/ Spirillum?	Single, clusters, chains?

Part 2: Antibiotic Sensitivity

The invention of antibiotics has completely changed the way humans handle disease. Once-untreatable and devastating diseases like cholera and syphilis can now be treated easily by antibiotics and the right care. However, not all bacteria respond the same way to antibiotics. In this exercise, you will observe the effects of different antibiotics on gram-negative and gram-positive bacteria.

Figure 6: Zones of inhibition.
A, B, C, and D represent paper disks that have each been soaked in a different antibiotic.



You will be given two large petri dishes: one with colonies of gram-positive bacteria (such as *Staphylococcus epidermidis*) and the other with colonies of gram-negative bacteria (such as *Escherichia coli*). Paper disks that have been soaked in different kinds of antibiotics have been placed on

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each plate. If the bacteria are **resistant** to the antibiotic, they will be able to grow right up against the margins of the paper disk. If the bacteria are **sensitive** to a specific antibiotic, they will not be able to reproduce near the antibiotic, and you will see a **zone of inhibition** around the disk. You can see the zones of inhibition as clear spaces around the antibiotics (see **Figure 6**).

Use a ruler to measure each zone of inhibition on your plates; record the results in **Table 3.**

Table 3: Zones of inhibition.

	Zone of inhibition (mm)	
Antibiotic	Gram-negative (*E. coli*)	Gram-positive (*S. epidermidis*)

Which antibiotics are most and least effective against gram-negative bacteria?

Most: Least:

Which antibiotics are most and least effective against gram-positive bacteria?

Most: Least:

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With this information in mind, why do physicians need to learn what sort of infection you have before prescribing antibiotics?

Do you see any bacterial colonies growing within any mostly-clear zones of inhibition? If so, what does the presence of these colonies suggest?

Part 3: What Happens to Bacteria when Patients Are Careless?

While antibiotics have drastically improved humanity's ability to fight disease, they aren't a perfect solution. Through naturally occurring random mutations, bacteria may acquire resistance to antibiotics. The drugs, combined with our own behaviors, can select for the resistant bacteria. Over time, the drugs therefore become less effective.

When a doctor prescribes antibiotics, the patient instruction leaflet often says something like, "Do not skip any doses, or stop taking it, even if you begin to feel better, until you finish your prescribed treatment." Some patients, however, stop taking them early because they don't feel symptoms anymore. Others forget to take a few pills during their prescription period. How do these practices select for bacteria that are resistant to antibiotics?

Not taking a full course of antibiotics, or forgetting to take some in the middle, can kill off the weaker bacteria. At that point, the surviving bacteria are the most resistant ones, and they give rise to the next generation of bacteria. This process is repeated every time the bacteria reproduce, which can occur as often as every 20 minutes under ideal conditions. Ignoring the leaflet instructions means more and more resistant bacteria in the body, which in turn means a longer period to recover from the infection. The infection may even require treatment with a different antibiotic drug. In the worst-case scenario, a new strain of the bacteria may develop that doesn't respond to any current antibacterial medications, leading to an untreatable infection. This is currently happening with tuberculosis, which scientists and medical professionals once thought was on the brink of eradication. Instead, a resistant strain of TB bacteria developed and recent years have seen a resurgence of TB.

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This activity simulates what happens as you take antibiotics to fight an infection. You will use dice to determine whether you remembered to take your antibiotics, and you will use colored beads to represent bacteria that vary in their resistance to the antibiotics:

- Green beads: least resistant (they die first upon exposure to antibiotics)
- Yellow beads: resistant
- Red beads: most resistant (they die last upon exposure to antibiotics)

Procedure – work in pairs (not groups of 3+)

1. You should have a cup or dish with 20 green, 15 yellow, and 15 red beads in it. Put 13 green beads, 6 yellow beads, and 1 red bead in a separate petri dish, which represents your body and the bacteria currently infecting it. You are taking antibiotics to fight this infection.
2. Roll your die, then record the number you rolled in **table 4:**
   a. **If you rolled a 1, 3, 5, or 6,** you remembered to take your antibiotics. Remove a total of 5 bacteria from your dish. *Remember, the least resistant bacteria die first.* So if you have 5 or more green ones, remove 5 of them. Once there are no more green beads, remove yellow beads. Once there are no more yellow beads, remove red beads. Don't take more than 5 total in any given round.
   b. **If you rolled a 2 or 4,** you forgot to take your antibiotics. In that case, do nothing and move to step 3.
3. **IMPORTANT!** Whatever number you rolled, after you complete step 2, add one more of bead of each color that is still in your petri dish—the surviving bacteria will continue replicating as you take medication, after all. That is, if you have any green ones left, add one more green one. If you have any yellow ones left, add one more yellow one. And if you have any red ones left, add one more red one.
4. Describe your actions from steps 2 and 3 in **Table 4**, and record the number of beads remaining in your petri dish.
5. Repeat steps 2-4 until you have no more bacteria in your dish.

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Table 4: Antibiotic resistance activity.

Round	Roll of die	Took or forgot dose?	Action taken (# of bacteria of each color removed and added)	# of least resistant bacteria (green)	# of resistant bacteria (yellow)	# of most resistant bacteria (red)	Total # of bacteria in your body
Initial	N/A	N/A	N/A	13	6	1	20
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
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17							
18							
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22							
23							
24							
25							
26							
27							
28							

When you're done, answer these questions:

1. How many rounds total did it take until all your bacteria were gone? ___________
2. How many times did you "forget" to take your antibiotics? _______________
3. How many rounds did it take until …
    a. all of the green bacteria were gone? _______________
    b. all of the yellow bacteria were gone? _______________
    c. all of the red bacteria were gone? _______________
4. How do you think the results would be different if you never forgot to take your antibiotics?

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5. How do you think the results would be different if you forgot to take your antibiotics more often than you actually did in the simulation?

6. Describe how this simulation relates to the instructions to finish your entire course of antibiotics, even if you feel better.

7. Look at the numbers and types of bacteria that remained when you were halfway through the simulation. If you had stopped taking your antibiotics at that point, what would have happened?

8. What is the relationship between the bead colors in this simulation and the genetic diversity in a population of real bacteria?

9. Given your answer to question 8, how does this activity simulate the events of natural selection?

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Activity 3: Disease Transmission

Objectives

1. List ways in which bacterial infections can spread from person to person.
2. Understand how bacterial infections spread quickly.

Although bacteria are everywhere, pathogenic bacteria tend to live close to humans, where they can more easily infect people and spread to others. From there, bacterial infections can spread in a variety of ways. They can pass via sexual contact, via contaminated food and water, via the exchange of bodily fluids such as blood or saliva, via the air from sneezes or coughs, via handshakes, etc. Pathogens thrive where many potential hosts live in close quarters. This is the reason that epidemics often affect large cities; for example, think of tuberculosis, caused by the bacteria *Mycobacterium tuberculosis*. TB killed many in big cities before the advent of vaccines, and continues to be a problem because of a drug-resistant strain of the causal bacteria. Famously, Edgar Allen Poe lost both his mother and his cousin/wife to tuberculosis.

Scientists study disease transmission to help lower the chance of a disease becoming an epidemic. They study how the disease is transmitted, what paths it takes (ever heard of the "patient zero" of a disease?), and possible means of prevention. In this part of the lab, you will simulate disease transmission with your classmates and trace the path it took.

You will each receive a numbered test tube with liquid in it. One student's test tube has been "infected" with an invisible chemical that represents a bacterial infection. The exercise requires careful record-keeping, so your TA will lead you through the exercise. **Do not begin any exchange until your TA tells you to.**

Procedure

1. Get a test tube and transfer pipette.
2. Find another student in the class, and withdraw a dropperful of your solution from your tube. Your partner should withdraw a similar amount of fluid from their own tube, at the same time.
3. Once both of you have withdrawn your dropperful, squirt your dropperful into your partner's tube. Your partner should do the same into your test tube. Use **Table 5** to record your partner's name and test tube number in the row marked "Contact 1."

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Table 5: Disease transmission activity contact history.

	Name	Test Tube #
Contact 1		
Contact 2		
Contact 3		

4. Find a different person in the class, and repeat the process – draw fluid into your pipettes at the same time, then transfer to the partner's tube. Write their name and test tube number in the row marked "Contact 2."
5. Repeat the exchange process once again with a third person, and record their name and test tube number ("Contact 3").
6. Once the entire class has gone through three exchanges, your TA will put phenol red, a pH indicator, in each of the test tubes. If yours turns red, you are "infected." If it turns yellow, you haven't been infected.
7. Your TA will make a chart listing all of the people those who were infected, along with their contacts. Copy it into **Table 6**. From there, your class can figure out in which tube the infection started.

Table 6: Use this table to trace the infection's path.

Infected Person	1st Contact	2nd Contact	3rd Contact

How many people are in your lab, and how many were infected?

people in lab: # infected:

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What was the maximum number of people that could have been infected at each stage of the activity?

After the first exchange:

After the second exchange:

After the third exchange:

Why is it possible that fewer people are infected than the maximum?

If you had done four rounds, what is the maximum number of people would could have been infected?

This exercise presents transmission in a very controlled environment. In the “real” world, you come into contact with many people each day, and you don’t carefully record who you interact with and in what order. How do you think the results of the exercise would have been different if you had been allowed to transfer solution with as many people as you wanted in 5 minutes, without keeping track of your exchanges?

While we can often tell when someone is sick just by looking, this isn’t always the case. Can you think of any invisible infections like the one in the exercise?

Can you think of any ways of getting exposed to a bacterial infection without directly coming into contact with someone at all?

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Wrap-Up

What are some of the structures that differentiate eukaryotic cells and prokaryotic cells?

How do bacterial colonies form? Do any special conditions need to be met? If bacteria are everywhere, why don't colonies form on tables or bathroom counters?

What are antibiotics, and how do they kill bacteria without killing our own cells?

For antibiotics that specifically bind to bacterial ribosomes, how do they affect bacteria?

How can disease be transmitted? List several examples.

What is one way scientists can trace an epidemic to its source? What are some of the limitations of this method?

Why could gram staining be useful for science and medicine?

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Why could testing antibacterial sensitivity be useful for science and medicine?

Acknowledgments and Attributions

Antibiotic resistance activity adapted by Sarah Greenwood and Mariëlle Hoefnagels from the following source: Spreading Disease – It's Contagious! Using a Model & Simulations to Understand How Antibiotics Work. Eva M. Ogens, Richard Langheim. *The American Biology Teacher*, Vol. 78 No. 7, September 2016; (pp. 568-574) DOI: 10.1525/abt.2016.78.7.568

Figure 1: Simple Phylogenetic Tree of Life © Sarah Greenwood used under a CC BY-SA 4.0 license https://commons.wikimedia.org/wiki/File:Simple_Phylogenetic_Tree_of_LIfe.png

Figure 2a: Janice Carr used under a CC0 license https://pixnio.com/science/microscopy-images/staphylococcus-aureus/methicillin-resistant-staphylococcus-aureus-infections

Figure 2b: EscherichiaColi NIAID c Rocky Mountain Laboratories, NIAID, NIH used under a CC0 license https://commons.wikimedia.org/wiki/File:EscherichiaColi_NIAID.jpg

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Figure 4: Colony morphology.svg c Macedo used under a CC BY-SA 4.0 license https://commons.wikimedia.org/wiki/File:Colony_morphology.svg

Figure 6: Zones of Inhibition © Sarah Greenwood used under a CC BY-SA 4.0 license https://commons.wikimedia.org/wiki/Special:UploadWizard

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Fun with Genetics

Mariëlle Hoefnagels and Sarah Greenwood

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Introduction

Every organism relies on DNA, which encodes the proteins that are necessary to carry out everyday cellular functions. The proteins are produced through **protein synthesis**, in which DNA is first **transcribed** into RNA and then the RNA is **translated** into proteins (chains of amino acids). On a macro level, DNA and protein synthesis – together with environmental influences – are largely responsible for each organism's anatomy, behaviors, and phenotypes.

Before cells divide, they must replicate the DNA that encodes their proteins. Most eukaryotic cells divide by **mitosis**, a process that produces nearly identical daughter cells used for asexual reproduction, growth, and tissue repair. In the ovaries and testes, however, meiosis is another kind of cell division necessary for sexual reproduction. Only **germ cells** in the ovaries and testes undergo meiosis. **Meiosis** produces genetically variable **gametes**, or sex cells. Gametes from two parents combine at fertilization to form a zygote, which then grows into an adult through mitosis. The DNA of the parents determines many of the traits the offspring will express.

In this week's lab we will practice with the fundamentals of genetics, including how DNA encodes proteins (Activity 1), the stages of mitosis (Activity 2), and the connection between meiosis and patterns of inheritance (Activity 3).

Activity 1: Protein Synthesis

Objectives

1. Recognize the molecules that play important roles in protein synthesis.
2. Understand how the RNA polymerase enzyme transcribes DNA to RNA.
3. Become comfortable with the complementary base pairs in DNA and RNA.
4. Understand how ribosomes, mRNA, and tRNA interact to produce proteins.

In this activity, you will play a game to approximate the process of protein synthesis. Every organism has cells containing DNA, the molecule that specifies the "recipes" for the proteins that the cell needs to live. Before you begin, you should already be familiar with the double-helix shape of DNA and the overall process of protein synthesis. Review it in your textbook if you need to.

Form teams of four players. *Remember, as you play this game, whenever ANY team yells "STOP!!!!" all teams should stop. You will be told when to resume after a team yells stop. No cheating allowed!*

Part 1: Transcription

See **Figure 1** for an example of transcription. You will receive a template strand of DNA cards, which you will transcribe into a sequence of mRNA codon cards. *Please do not write on these cards.* In this part of the game, all players are RNA polymerase enzymes. You will be told when to begin; when you finish, yell STOP!!! An instructor will check your sequence. If you have made a mistake, your RNA strand will be destroyed after the point of the error, then all teams will resume transcription. The teams will be rewarded in the order in they finish.

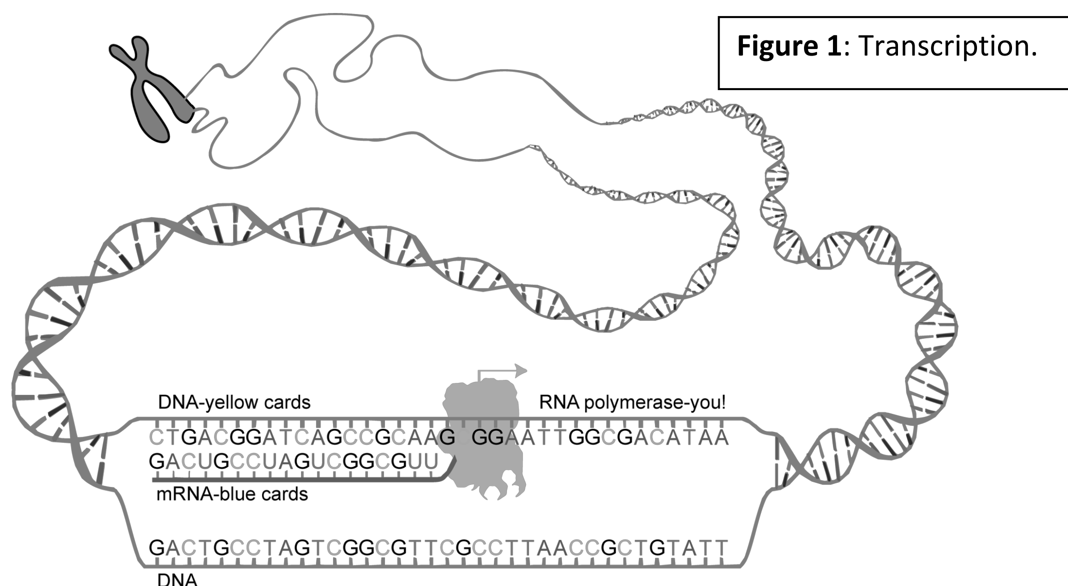

Figure 1: Transcription.

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Part 2: Translation

Choose a player to be in charge of the ribosome, which consists of two chairs side by side (representing “slots” for two tRNA molecules; see **Figure 2**). All other players are tRNAs, who will carry the amino acids (beads) that are to be linked together into a protein. Note that only one tRNA from each ribosome may be at the metabolic pool at a time, and each tRNA may only take one amino acid at a time.

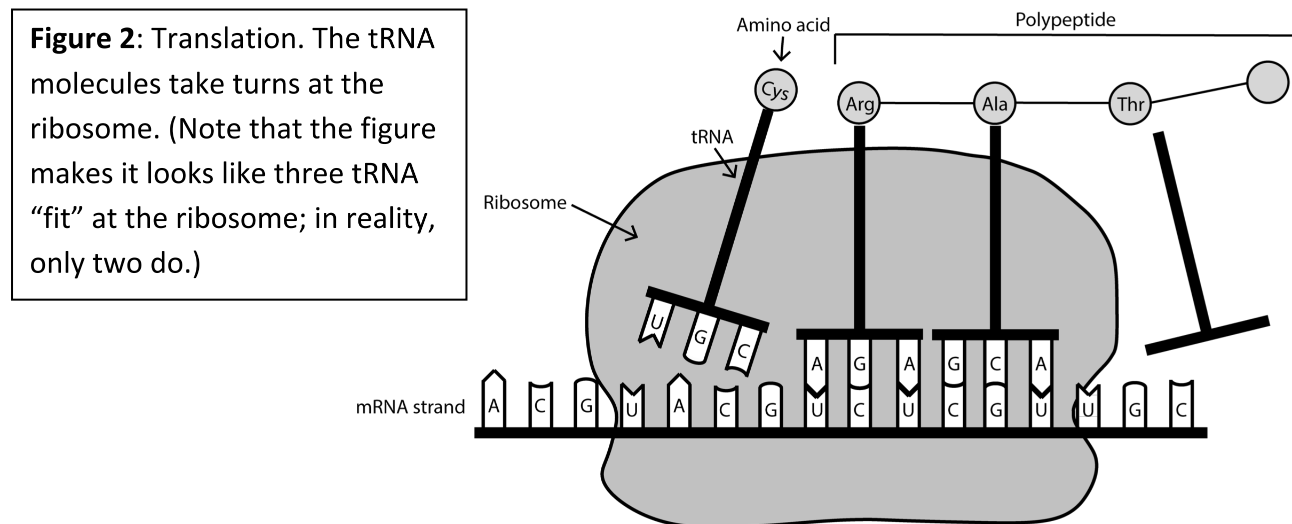

Figure 2: Translation. The tRNA molecules take turns at the ribosome. (Note that the figure makes it looks like three tRNA “fit” at the ribosome; in reality, only two do.)

The ribosome will use **Figure 3** to translate each RNA codon to an amino acid. When you are told to begin, the ribosome player instructs a tRNA player which amino acid to pick up from the active metabolic pool, which is accessible to all teams. The tRNA finds the correct bead, returns to the ribosome, sits in one chair, and adds the bead to the peptide backbone (the string). Another tRNA is sent to gather the second amino acid and should sit in the second chair upon returning. After the second amino acid has been added to the chain, the first tRNA can leave, and the second tRNA should scoot over to the first chair. The vacant chair is now ready for the tRNA carrying the third amino acid. See **Figure 2** to understand why you’ll be switching chairs like this; there is only room for a certain number of tRNA molecules at the ribosome during translation. Continue building the protein in this way until all amino acids have been added to the chain.

When you finish, yell STOP!!! An instructor will check your sequence. If you make a mistake, a protein-destroying enzyme (i.e. an instructor) will remove amino acids after the point of error, and all teams can continue translating. Again, you will be rewarded in the order you finish.

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After this first version of the game is complete, your instructor may introduce modifications, including a way to simulate mutations. Mutations are important because they create new alleles (versions of genes). A new allele may encode a new protein sequence, which may change the organism's phenotype and improve or reduce its reproductive success. What's more, any allele may be passed on to the next generation, according to the rules of genetics illustrated in activity 3.

Figure 3: Dictionary of the genetic code.

First Base	Second Base: U	Second Base: C	Second Base: A	Second Base: G	Third Base
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
U	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
U	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
U	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
C	CUC Leu	CCC Pro	CAC His	CGC Arg	C
C	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
C	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
A	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
A	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
A	AUG Met or Start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
G	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
G	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
G	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

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Activity 2: Mitosis and Meiosis

Objectives

1. Know the stages of mitosis, and model each stage using pipe cleaners.
2. Know the stages of meiosis.
3. Understand the differences between mitosis and meiosis.

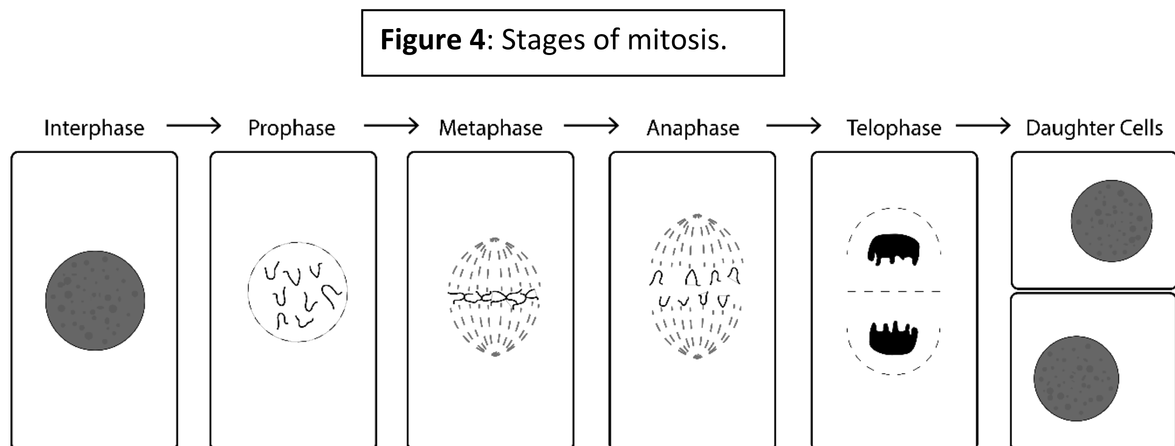
Part 1: Mitosis

Before you begin, recall that eukaryotic cells (such as those in plants and animals) copy themselves by mitosis. This process occurs throughout life as the organism grows and repairs damage. The key to understanding mitosis is knowing what happens to the chromosomes. You may remember that chromosomes are just tightly coiled-up DNA – the same stuff you were practicing with in activity 1.

Procedure

1. Review the stages of mitosis in **Figure 4** and in the “Plant Histology” and “Animal Histology” apps on the lab iPads. Pay attention to the chromosomes, which are the squiggly things inside each cell.

Figure 4: Stages of mitosis.



2. Take one of the pipe cleaners at your bench and put a pony bead around it so the bead is in the center of the pipe cleaner. This is an unreplicated chromosome, and the pony bead is a centromere.
3. Make an “unreplicated chromosome” using one of each size/type of pipe cleaner and arrange them in a “cell” – a.k.a., just put them all near each other. The pipe cleaners of different sizes represent different chromosomes, and the pipe cleaners of different

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colors but the same size are homologous chromosomes, meaning they encode different versions of the same gene.

With this in mind, does your “cell” have pairs of homologous chromosomes or not? In other words, is it a diploid cell or a haploid cell?

4. Now, “replicate” your chromosomes by putting another of the same color/size pipe cleaner in the pony bead with each chromosome. This replicated chromosome is composed of two identical sister chromatids. What stage of mitosis would this occur in?

5. Next, line up the chromosomes lengthwise at the center of the cell. What stage of mitosis does this represent?

6. Finally, separate the sister chromatids and separate the chromosomes with one chromatid going to each side. What stage of mitosis does this represent?

7. Now, you should have two identical daughter cells. What stage of mitosis does this represent? Are the resulting daughter cells haploid or diploid?

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Part 2: The Differences Between Mitosis and Meiosis

Refer to **Figure 5** for an illustration of meiosis. Remember, the purpose of meiosis is for one diploid cell (containing two sets of chromosomes) to divide in such a way that it produces haploid gametes (each containing one set of chromosomes) for reproduction. In this activity, you will directly compare the stages of mitosis and meiosis.

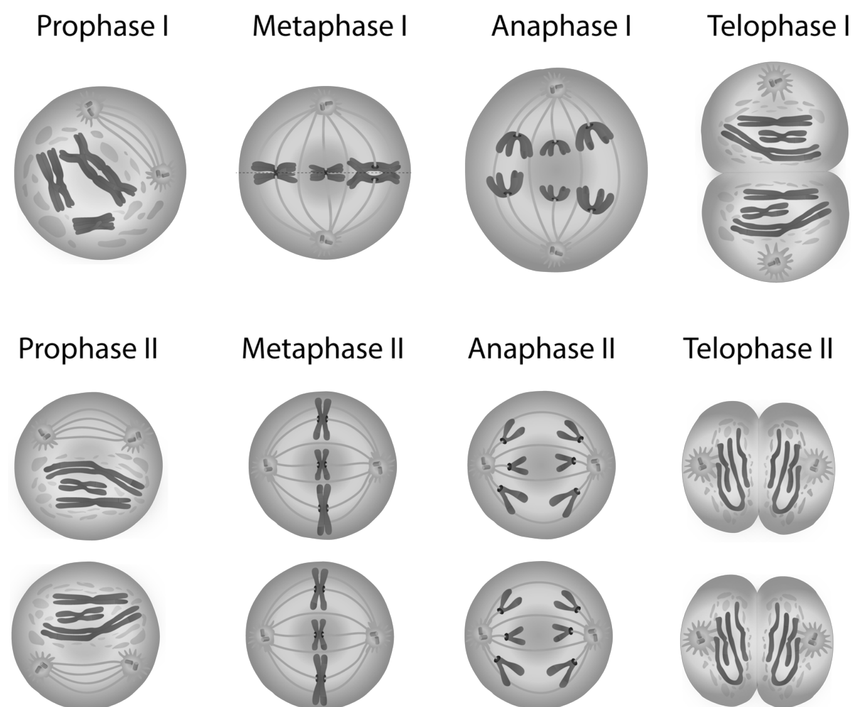

Figure 5: Stages of meiosis.

Procedure

1. Your TA will give you an envelope with small slips of paper that list the events of mitosis and meiosis. Four colors have slips with regular type on some and bold type on others. Find those and use **Table 1** to compare and contrast the contents of the regular type vs. the bold type.

Table 1: Compare and contrast the text on colored slips.

Color of paper	How are the regular and bold phrases similar?	What is unique to the-regular type?	What is unique to the bold type?
Yellow			
Blue			
Pink			
Green			

2. Read all of the slips of paper.

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3. You will have a large sheet of paper with headings reading “Mitosis” and “Meiosis.” Start arranging the slips of paper under the correct heading, in the correct order that events occur.
4. When you have everything in the right order, tape the slips of paper down.
5. On the side of your taped-down columns of paper, write which stage the events on each paper represent. Draw brackets to notate which steps are in meiosis 1 and which are in meiosis 2.
6. Check your order with that of those around you. Your TA will go over the correct answers.

Activity 3: Meiosis, Gametes, and Inheritance (a.k.a., the Reptilobird)

Objectives

1. Understand the stages of meiosis.
2. Carry out meiosis in a sample organism, keeping track of what each stage represents in terms of meiosis in a real organism.
3. Understand the relationship between meiosis and inheritance.
4. Understand different kinds of gene expression: complete dominance, codominance, and incomplete dominance.
5. Explain the relationship between meiosis and genetic diversity.

This activity should help you understand not only the events of meiosis but also the relationship between meiosis and the inheritance of alleles from one generation to the next.

As stated earlier, the purpose of meiosis is for one diploid cell (containing two sets of chromosomes) to divide in such a way that it produces haploid gametes (each containing one set of chromosomes). The resulting gametes – sperm or egg cells – unite in fertilization to form the first diploid cell of the new generation. In short, **the events of meiosis in a mother and a father determine which alleles their baby will inherit.**

In this activity we will model meiosis using the chromosomes of an imaginary “reptilobird,” which we will call *Chromoseratops meiosus*. This reptilobird has six chromosomes: two pairs of autosomes (one long chromosome and one short chromosome) and one pair of sex chromosomes. As is the case in many animals, female *C. meiosus* reptilobirds bear two X chromosomes, while males bear one X chromosome and one Y chromosome.

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Only germ cells in the testes or ovaries undergo meiosis. You will receive two sheets of paper representing the chromosomes inside a germ cell from either a male or female reptilobird. Each sheet contains a set of duplicated chromosomes (sister chromatids; represented by a table with letters). The two sheets represent the chromosomes that your reptilobird inherited from its own dad (in blue) and its mom (in yellow).

Before you begin working with your chromosomes, answer the following questions:

1. At which stage of meiosis did duplication of the chromosomes occur in the germ cell?

2. Should your duplicated chromatids be identical? _______ Are they? _________

3. What is the sex of your reptilobird? How can you tell?

4. How many chromosomes does your reptilobird have? _____

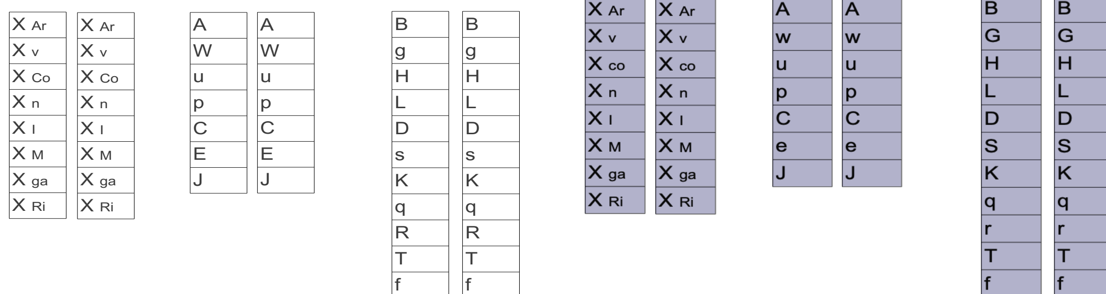
5. How many chromosomes did it inherit from its dad? _____ From its mom?_____

6. How many chromosomes are in a somatic (body) cell of *C. meiosus*? _______

7. How many chromosomes are in a gamete of *C. meiosus*? _______

Cut out each sister chromatid and arrange them such that each will be next to the other, forming a duplicated chromosome. **Figure 6** shows an example (below, blue is shown as white, and yellow is shown as light gray):

Figure 6: Duplicated chromosomes.



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Next, as in **Figure 7,** place each duplicated chromosome that your bird inherited from its mom next to its homolog (inherited from its dad):

Figure 7: Duplicated chromosomes, arranged in homologous pairs.

X Ar	X Ar	X Ar	X Ar
X v	X v	X v	X v
X Co	X Co	X co	X co
X n	X n	X n	X n
X I	X I	X I	X I
X M	X M	X M	X M
X ga	X ga	X ga	X ga
X Ri	X Ri	X Ri	X Ri

A	A	A	A
w	w	W	W
u	u	u	u
p	p	p	p
C	C	C	C
e	e	E	E
J	J	J	J

B	B	B	B
g	g	G	G
H	H	H	H
L	L	L	L
D	D	D	D
s	s	S	S
K	K	K	K
q	q	q	q
R	R	r	r
T	T	T	T
f	f	f	f

The different letters on each chromatid represent different genes, each of which encodes a certain trait. Did you notice that some letters in homologous chromosomes are in lower case while others are in upper case? Each letter represents a gene conferring a trait (ex: eye color), and the upper and lower case represent different alleles (ex: G = green eyes; g = red eyes). Right now it is not important to know what character each letter represents; we will get back to it later.

8. How many genes does your reptilobird have on each of the following chromosomes?

   Chromosome 1 (the shorter one): ______

   Chromosome 2 (the longer one): ______

   X-chromosome: ______

Part 1: Modeling Meiosis

9. In what cells in your reptilobird's body does meiosis occur? ___________________

Prophase I

During prophase I, the homologous chromosomes align themselves next to each other in preparation for crossing over (the exchange of genetic material between homologous chromatids; see **Figure 8**). Note that crossing over occurs between ***non-sister*** chromatids. Only the inner chromatids, the ones that are next to the other homologous chromosome, exchange genetic material in crossing over. The other chromatids do not change at all.

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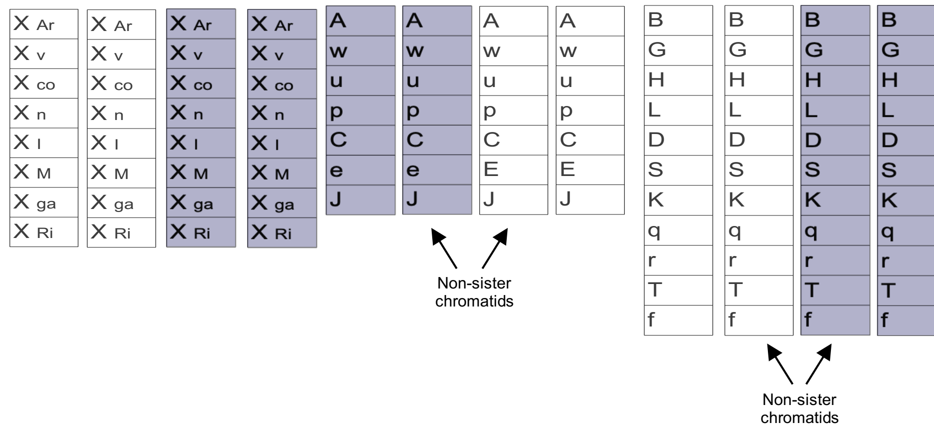
Figure 8: Make sure crossing over only occurs between non-sister chromatids.

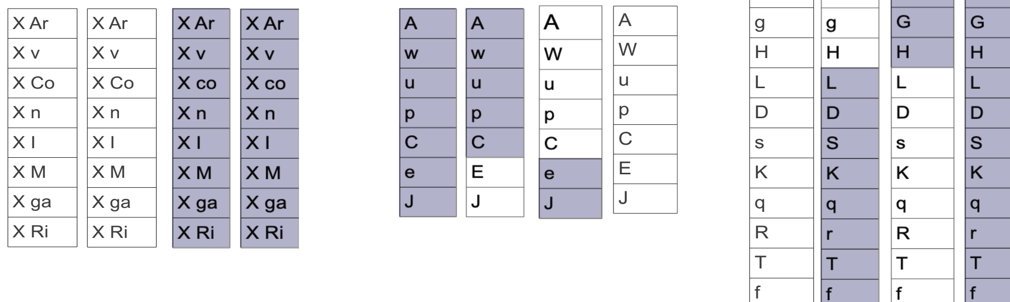


10. What would happen if crossing over occurred between sister chromatids?

Simulate crossing over in chromosomes 1 and 2 by cutting the inner chromatids of each homologous chromosome pair on **one of the lines** using scissors. **Do not cut through a letter!** Make sure you cut at the same line in both chromatids that display crossing-over, and tape the pieces at the right spot (see **Figure 9**).

By the way, you can choose which line to cut on in the **autosomes**. In sex chromosomes, crossing over is a little complicated and is ignored here for the sake of keeping the exercise simple.

Figure 9: Crossing over.



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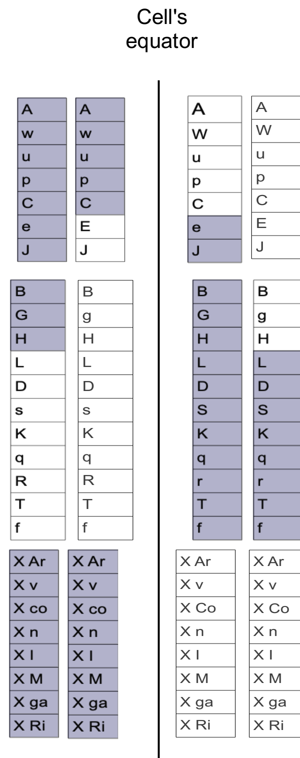
Metaphase I

During metaphase I, paired homologous chromosomes line up at the cell's equator (**Figure 10**). Go ahead and arrange your paired homologous chromosomes at the midline of your cell (chromosomes can go in any order, as long as homologous pairs stay together). Note that not all chromosomes from Dad (gray in the example below) have to be on the same side of the equator.

Figure 10: Metaphase I.



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Anaphase I

Homologous chromosomes now split apart from each other, as in **Figure 11.**

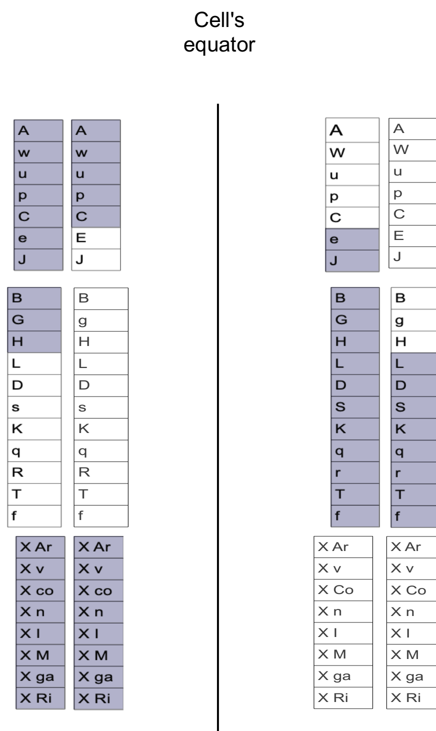
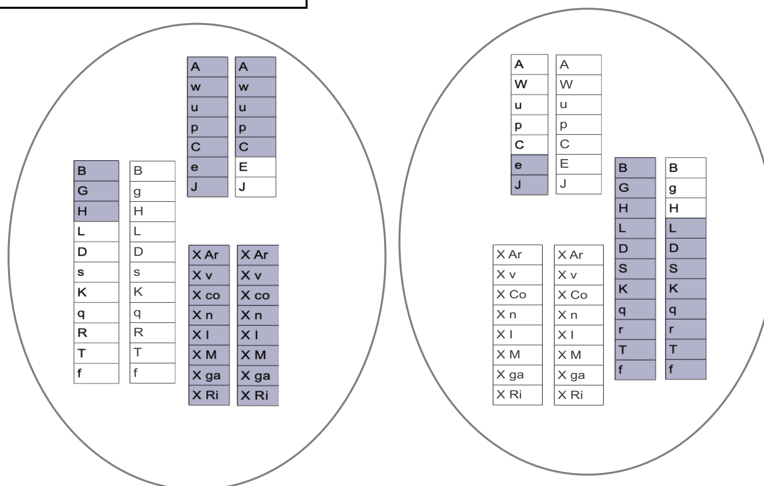


Figure 11: Anaphase I.

Telophase I and Cytokinesis

Your products of meiosis I should look something like **Figure 12.**

Figure 12: Telophase I/cytokinesis.



11. How many cells do you have at this stage? _________

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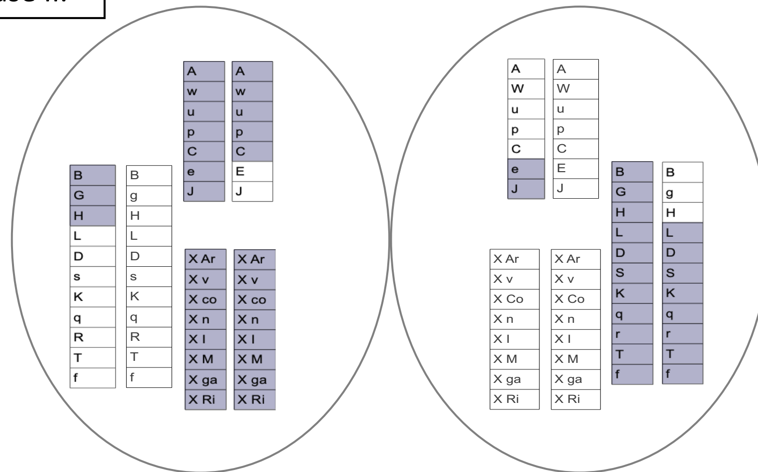
12. How many chromatids does each cell have? ________

13. Did the chromosomes in each cell come from the same parent? ________

Prophase II

Start with the products of meiosis I, shown in **Figure 13.**

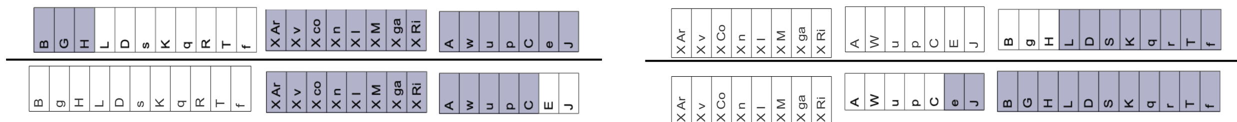
Figure 13: Prophase II.



Metaphase II

Sister chromatids now align at the equator of each cell, as in **Figure 14.**

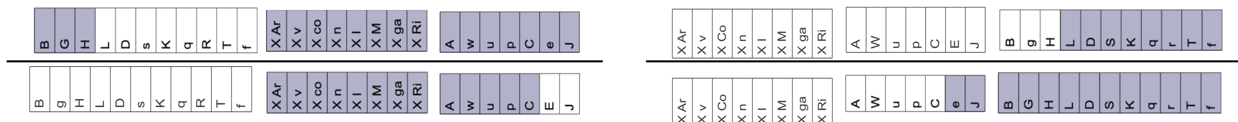
Figure 14: Metaphase II.



Anaphase II

Next, the sister chromatids in each cell separate (the centromeres split), as in **Figure 15.**

Figure 15: Anaphase II.



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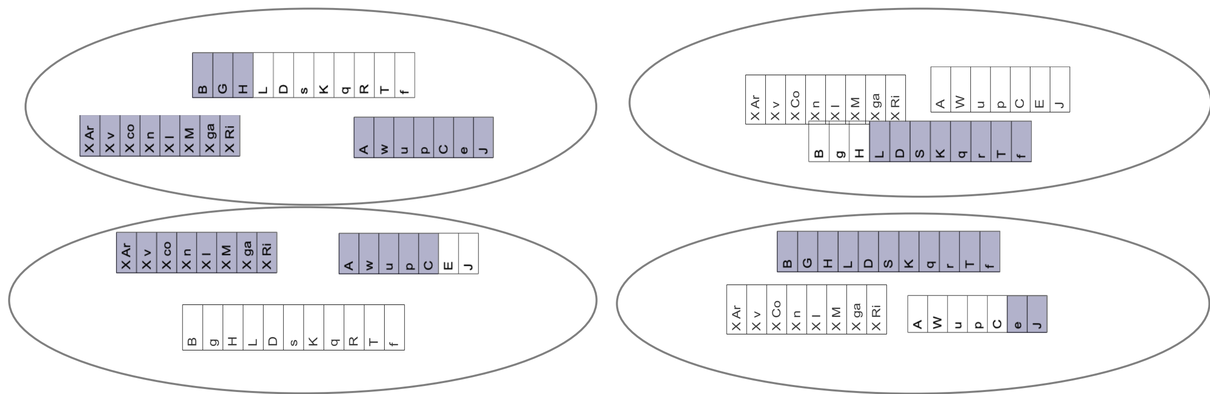
Telophase II and Cytokinesis

One telophase II and cytokinesis are complete, you should have four daughter cells, as in **Figure 16.**

Figure 16: Daughter cells.



14. How many daughter cells did you get? ________

15. Are they all genetically identical? ________

16. Is the number of chromosomes in each <u>daughter cell</u> equal to the number of chromosomes in the <u>germ cell</u>? ________
    If they aren't equal, describe how they are different.

Now get four coin envelopes—one for each daughter cell—and place the chromosomes of each daughter cell into an envelope.

17. Are your four daughter cells egg cells or sperm cells? ________________

    How do you know?

Label each coin envelope as an egg cell or a sperm cell (or draw an egg or sperm cell on it). Get ready for part 2!

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Part 2: From Gamete to Phenotype

While modeling meiosis (part 1), you created gametes of the reptilobird *Chromoseratops meiosus*. In this exercise (part 2), you will fertilize an egg cell with a sperm cell and create a zygote that will develop into a baby reptilobird. You will determine the phenotype of the baby reptilobird based on its genotype.

Randomly choose one of your gametes: Line the coin envelopes with chromosomes up on your desk, and throw a die. Choose the envelope that corresponds to the number on the die (1 for the first one from the left, and 4 for the fourth from the left; if you roll a 5 or 6, try again until you get a 1, 2, 3, or 4).

If your reptilobird produced sperm cells, you will need an egg cell from one of your neighbors. If your bird produced egg cells, you will need to get a sperm cell from one of your neighbors.

As a "mating pair," remove the chromosomes from the chosen egg and sperm envelopes, and place the homologous chromosomes next to each other. This is the zygote that will develop into your baby reptilobird. The chromosomes may look like something like **Figure 17.**

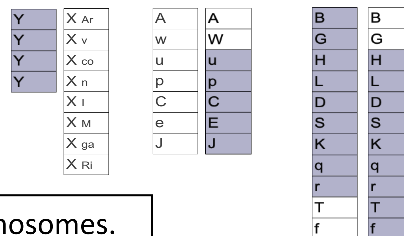

Figure 17: Zygote chromosomes.

18. When the zygote begins to divide and develop into a baby, will its cells be dividing by mitosis or meiosis? ______________ *(Note that your TA may give you extra chromosomes to simulate this cell division.)*

19. What is the sex of your baby reptilobird? __________ How do you know?

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20. **Table 2** is a list of characters and the genes that code for them. For each gene, circle the genotype and phenotype of your baby reptilobird.

Table 2: Reptilobird genotypes/phenotypes.

Gene	Trait	Genotypes and Phenotypes
G	Eye and tail color	*GG* or *Gg* – green *gg* – red
H	Leg thickness	*HH* – thick *Hh* – medium *hh* – thin
D	Wing length	*DD* or *Dd* – short *dd* – long
T	Number of tail feathers	*TT* – 3 *Tt* – 2 *tt* – 1
A	Eye pigment deposition	*AA* or *Aa* – pigment deposited *aa* – no pigment (eye color: white)
B	Beak shape	*BB* – straight *Bb* – curved *bb* – forked
X^{Ec}	Beak color	X^{Ec} – blue (dominant) X^{ec} – colorless

21. Gene *D* is an example of:
    a) codominance.
    b) complete dominance.
    c) incomplete dominance.
22. Genes *H* and *T* are examples of:
    a) complete dominance.
    b) incomplete dominance.
23. Genes *A* and *G* interact with each other. Describe this interaction.

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How does your answer to this question relate to the common misconception that each trait is controlled by exactly one gene?

24. The gene conferring beak color is ___________-linked.

25. Does your baby reptilobird have any chromosomes with recombinations (mixtures of its grandparents' genetic information) due to crossing-over? ___________

    How do you know?

Assemble your baby reptilobird's features according to your its genotype. Cut out the appropriate legs (gene *H*), wings (gene *D*), tail feathers (gene *T*), and beak shape (gene *B*) from the selection of "bird parts" provided. Color in the bird's eyes (be sure to check both genes *A* and *G*), tail (gene *A*), and beak (gene X^{Ec}) as appropriate, then tape the body parts to your bird model.

When you are done, tape your reptilobird on the chalkboard or wall. Looking at your reptilobird and the others, answer the following questions:

26. What brought about this variation in the offspring?

27. Can such variation occur in asexual reproduction? Why or why not?

28. What are the sources of variation in asexual reproduction?

29. What are the advantages and disadvantages of sexual reproduction compared to asexual reproduction?

30. What is the relationship between genetic variation and evolution?

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Acknowledgments and Attributions

Protein synthesis game adapted from Clements, L. and Jackson, K. 1998. *American Biology Teacher* 60: 427-429.

Pipe cleaner and mitosis/meiosis comparison exercises adapted from a presentation at the 2018 ABLE conference by Valerie Schawaroch, Baruch College, New York, NY.

Meiosis exercise adapted by Mariëlle Hoefnagels and Doug Gaffin from Eliyahu, Dorit. 2014. "*Chromoseratops meiosus*": A simple, two-phase exercise to represent the connection between meiosis and increased genetic diversity. *The American Biology Teacher* 76 (1): 53-56.

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DNA and Evolutionary Trees

Mariëlle Hoefnagels, Krystal Gayler, and Sarah Greenwood

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Introduction

Until this point in the semester, we have thought about DNA only as the molecule of genetic inheritance. In this lab, however, we will expand that view to think about how DNA sequences can be used to test hypotheses about evolutionary relationships. **Phylogenetics** is the discipline of science that studies and evaluates these relationships.

Before DNA sequencing technology existed, biologists compared physical characteristics (**morphological** traits) to make hypotheses about the evolutionary relationships among organisms. Now, however, it is easy and cheap to sequence the DNA of one organism and compare that to the sequence of another; the more similar the sequences, the more closely related the two organisms. Today's biologists combine morphological and DNA-based approaches to propose increasingly accurate lineages.

Activity 1: Reading an Evolutionary Tree

Objectives

1. Understand how phylogenetic trees represent evolutionary relationships.
2. Recognize nodes, clades, and branches on phylogenetic trees.

Evolutionary relationships can be spatially represented using **phylogenetic trees**, which consist of nodes and branches (**Figure 1**). Each **node** represents the last common ancestor shared by the members of a branch. All of these common ancestors existed in the past; they no longer exist. At each node the tree splits into two **branches**, which may lead to yet another node or to an existing organism. Existing organisms (species, genera, phyla, etc.) occupy the tips of the branches. A **clade** is a common ancestor (node) and all of its descendants. **Figure 1** uses dotted lines to enclose three of the clades in the figure.

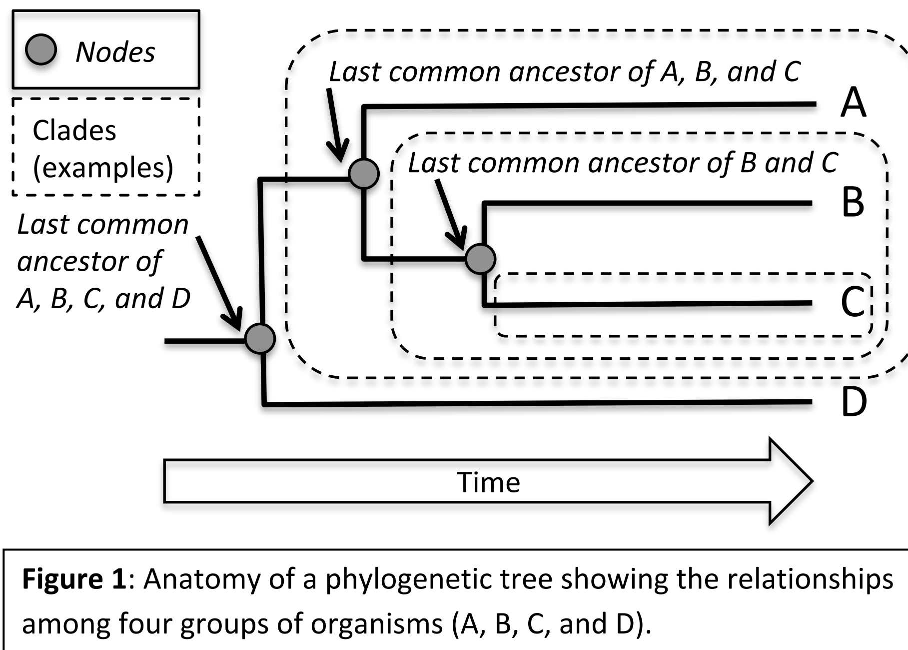

Figure 1: Anatomy of a phylogenetic tree showing the relationships among four groups of organisms (A, B, C, and D).

Note that nodes can be rotated without changing the information in a phylogenetic tree. See, for example, the three trees in **Figure 2**. They all convey the same information, but node 3 is rotated in the second tree, and both nodes 1 and 3 are rotated in the third one. Even though the species order along the right edge of each tree has changed, the clades and the

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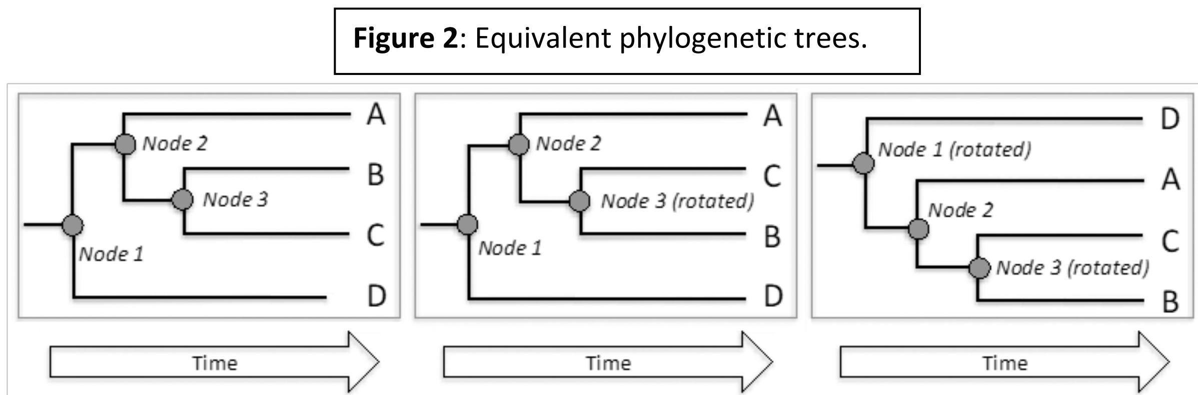
evolutionary relationships among them have not, so all three trees are equivalent to each other.

Figure 2: Equivalent phylogenetic trees.



So far we have focused on the anatomy of a tree. One last thing to know is that phylogenetic trees are often annotated with a brief description of the characters that describe each branch (**Figure 3**). You can use these characters to show which characters are common to an entire clade and which are unique to only some branches.

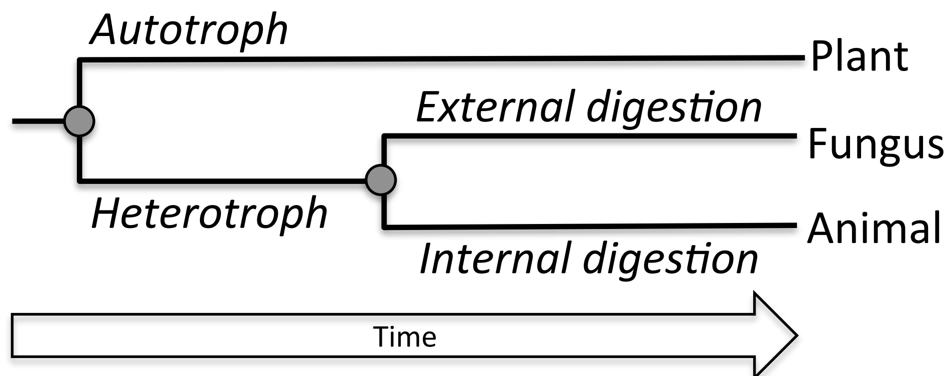


Figure 3: Sample tree with characters labeled.

With this background in mind, answer the following questions (answers are at the end of this lab):

1. How many nodes are in figure 3?
2. How many clades are in figure 3?
3. In the past, biologists thought fungi were simple plants. Does figure 3 support this hypothesis? Why or why not?

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4. In figure 2, is it possible to create a fourth equivalent tree in which A and D form a clade that excludes B and C?

5. In figure 2, which organism is most closely related to C?

6. In figure 2, how many characters do B and C share?

7. In figure 2, is D more closely related to A than it is to C?

Activity 2: Nails and Screws

Objectives

1. Hypothesize what the "evolutionary relationships" between objects might be based on their physical characteristics.
2. Create a phylogenetic tree that accurately portrays your proposed evolutionary relationships.

The objective of this exercise is to learn how to make observations and to construct a simple "phylogenetic tree." Note that the objects in this tree are not the products of biological evolution, so the term "phylogenetic tree" does not really apply. Nevertheless, we can still use them to illustrate tree-making principles.

In your lab kit you will find a small bag of screws and nails. Together with the other members of your group, observe the similarities and differences among these items, then categorize them into a hypothesized "evolutionary tree." As you make your tree, keep the following in mind:

- Your tree should **include a picture of each screw and nail** in your kit.
- Each screw or nail is an existing organism, so each picture must occupy the tip of its own branch (figure 2). (Remember, organisms at nodes are common ancestors that occurred in the past; they are now extinct.)
- Each branch should be labeled with a brief description of the character(s) unique to the branch (e.g. "threaded" or "smooth").
- You might find it helpful to start by organizing the screws and nails into pairs with similar characteristics, then organizing those pairs into increasingly larger clades.

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Procedure

1. Take pictures of your screws. Alternatively, you can take one giant picture of all of them in the correct order.
2. Open a new Google Slides presentation and "Share" it with your group members. Name your presentation how your TA prefers, and make sure each of the group members' names are on the title slide.
3. On a blank slide, use the "Insert" button (represented as a "+" on the iPads) to insert the photos you took of your screws.
4. If you took individual pictures of your screws, line them up in the correct order. You can crop the photos to make them take up less space. On the iPads, you can bring a picture to front/send it to the back by double tapping on the picture.
5. Use the line tool to create the lines in the tree. On the iPads, the line tool is under the "+" button.
6. Use the text tool to create labels for each branch. On the iPads, this is (you guessed it) located under the "+" button.

Activity 3: Plant Phylogenetic Trees

Objectives

1. Observe characteristics of plants and hypothesize on their evolutionary relationships based on their morphology.
2. Create a properly-labeled morphological tree based on your hypothesized evolutionary relationships.
3. Compare and contrast your tree with a molecular phylogenic tree of the plants.

Now that you are comfortable making a phylogenetic tree, you will apply your skills to propose a hypothesis about the evolutionary relationships among plants. (Unlike screws and nails, plants really are the products of biological evolution.) You will construct this tree using the morphological traits of the plants located around the room. Later, you will use DNA sequences to create a second phylogenetic tree using the same species. You will need both trees to complete your homework this week.

Part 1: Morphological Tree

To construct your hypothesized morphological tree, everyone in your group should begin by closely examining the plants around the room – touch them if you want! (For now these plants are numbered; you will be able to download a key to the plants from the Canvas dropbox where you turn in your homework.) Take one or more pictures of each plant, being sure to

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include the number of the plant in your picture. Then start looking at the characteristics that might be helpful in building your tree. (Remember, however, that every trait you can see is not necessarily informative, and some informative traits may not be visible 100% of the time.) Use the questions below to guide you, and just do the best with the information you have.

- Which plants live in water, and which live on land?
- Which plants produce flowers?
- Which plants produce cones or spores?
- Which plants have veins in their leaves? Veins indicate vascular tissue, which transports water and materials within the plant and also adds physical strength. In general, plants with vascular tissue can grow taller than plants without it.
- Which plants have needle-like leaves, and which have broad leaves?

As you did with the screws and nails, create a new slide in your Google Slides file and propose a tree based on your observations. You might find it helpful to start by organizing the plants into pairs with similar characteristics, then organizing those pairs into increasingly larger clades. As before, each branch should be labeled with a brief description of the character(s) unique to the branch. Once you have a tentative tree, look it over and make sure the labels apply to every organism in each branch.

Do your best, and keep in mind that this tree doesn't have to be "right"; we're doing this so you can start to think as scientists might have before DNA sequencing technologies emerged.

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Part 2: Molecular (DNA-based) Tree

To construct your molecular phylogenetic tree, you will use sequences for a gene known as ribulose-bisphosphate carboxylase (*rbcL*). This gene occurs in the chloroplast DNA of plant cells. The *rbcL* gene encodes an enzyme used in photosynthesis; it is thought to be one of the most abundant enzymes on earth, and it occurs in all plants. You will use *rbcL* sequences for the same plant species that you used to create your morphological tree.

The screenshot at right shows a portion of the molecular data. Note that each plant species is associated with a unique sequence of A, C, G, and T. It would not be fun to manually compare the *rbcL* gene sequences for all of our plant species. Luckily, computers can do this work for us. In this part of the lab, you will direct a website to analyze the DNA sequences and use them to assemble a molecular tree.

```
>Chara
atgtcaccacagacagaaactaaaacaagtgctggatttaaagcaggggtaaaagattac
agattaacttactatactcctgagtataaaactaaagatactgacattttagctgcattt
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aaggccgatgctatgatattgagcctgttgctggagaagaaaatcaatatattgcttatg
```

Procedure

1. Navigate to the following website: https://www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/.
2. In the Canvas files for this class, point to the file called “rbcL_seq_align.fasta”. Download the file. If you’re using an iPad, download the file either onto the Drive or Pages app (under “My iPad”).
3. On the Simple Phylogeny website, click Browse to upload the file (see screenshot on next page).

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Simple Phylogeny

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Tools > Phylogeny > Simple Phylogeny

Simple Phylogeny

This tool provides access to phylogenetic tree generation methods from the ClustalW2 package. Please note this is NOT a multiple sequence alignment tool. To perform a multiple sequence alignment please use one of our MSA tools.

STEP 1 - Enter your multiple sequence alignment

Enter or paste a multiple sequence alignment in any supported format:

Or, upload a file: Browse... le selected. See example inputs

4. Upload the file. Don't change the options in STEP 2. Go to STEP 3 and click SUBMIT. You don't have to click the box to be notified by email, as your results should be available on the website shortly.
5. After you click SUBMIT, the program will think for a few minutes. It will ask you to be patient, it will show some mysterious text, and at the end of that process it will produce a little phylogenetic tree that shows which species are most closely related to which – at least according to the sequence of that one *rbcL* gene.

STEP 1 - Enter your multiple sequence alignment

Enter or paste a multiple sequence alignment in any supported format:

Or, upload a file: Browse... rbcL_seq_align.fasta See example inputs

STEP 2 - Set your Phylogeny options

TREE FORMAT	DISTANCE	EXCLUDE GAPS	CLUSTERING METHOD	P.I.M.
Default	off	off	Neighbour-joining	off

STEP 3 - Submit your job

Be notified by k this box if you want to be notified by email when the results are available)

Submit

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6. Make a screenshot of your molecular tree and paste it in a new slide in the same Google Slides file with your morphological trees.

Homework

At this point, you have been working in groups, and your Google Slides document should contain: (1) your group's morphological tree for nails/screws; (2) your group's proposed morphological tree for the plants; (3) your group's molecular tree for the plants.

Make sure that your group's Google Slides document is copied to each member's individual Google Drive, because you will have to submit a homework assignment at your own Canvas account.

Once you have it on your own Google drive, add a new slide with (4) a paragraph explaining the differences and similarities between the morphological and molecular trees for the plants. In writing this paragraph, think about what you have learned about morphological characteristics, DNA relationships, and how phylogenetic trees illustrate those relationships. Answering the following questions should help.

- What is the relationship between a plant's visible traits and its DNA sequence?
- Is *rbcL* the only gene that the plants in this exercise are likely to share? Why or why not?
- What are some reasons that a tree derived from morphological traits could be different from a tree derived using similarities in *rbcL* DNA?
- What could you do to make your morphological tree more accurate?
- What could you do to make your molecular tree more accurate?

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Answers to questions in Activity 1: 1) Two nodes. 2) Five clades. 3) No. Fungi are more closely related to animals than to plants. 4) No. 5) B. 6) B and C share three characters (one for each shared clade). 7) No. A, C, and B are all equally related to D.

Acknowledgments and Attributions

This lab was inspired by an activity (*Molecular Phylogeny of Plants*) appearing in Investigating Biology Laboratory Manual, Sixth Edition, by Judith G. Morgan and M. Eloise Brown Carter (2008) and published by Pearson Education, Inc. Screenshot of *Chara* rbcl sequence is also from this lab. All sequences of *rbcL* came from resources associated with the Morgan and Carter lab and are also available in GenBank.Krystal Gayler and Marielle Hoefnagels wrote new introductory material, instructions, illustrations, and questions; added the "screws and nails" activity; and incorporated the use of iPads. Sarah Greenwood clarified and added to the instructions.

Dr. Ben Holt helped with the selection of the EMBL-EBI Simple Phylogeny site and formatted the files for class use.

Screenshots from the Simple Phylogeny Website: https://www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/.

rbcL sequences were downloaded from NCBI Reference Sequence Database (http://www.ncbi.nlm.nih.gov/refseq/).

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Flowering Plants: Structures and Sex

Mariëlle Hoefnagels and Sarah Greenwood

Wild Flowers in Meadow used under a CC0 license https://www.publicdomainpictures.net/en/view-image.php?image=36787&picture=wild-flowers-in-meadow

Introduction

Angiosperms, or flowering plants, are the most diverse and abundant plants. Nearly all of the plants you see around you on campus are angiosperms, from grasses to tulips to oak trees. They also have some easily recognizable adaptations (stems, leaves, flowers, fruits, and seeds). You might think you hate plants or that they're boring, but give them a chance. In this lab, you will learn about a flowering plant's basic parts, how they grow, and how they have sex and make babies **(Figure 1).**

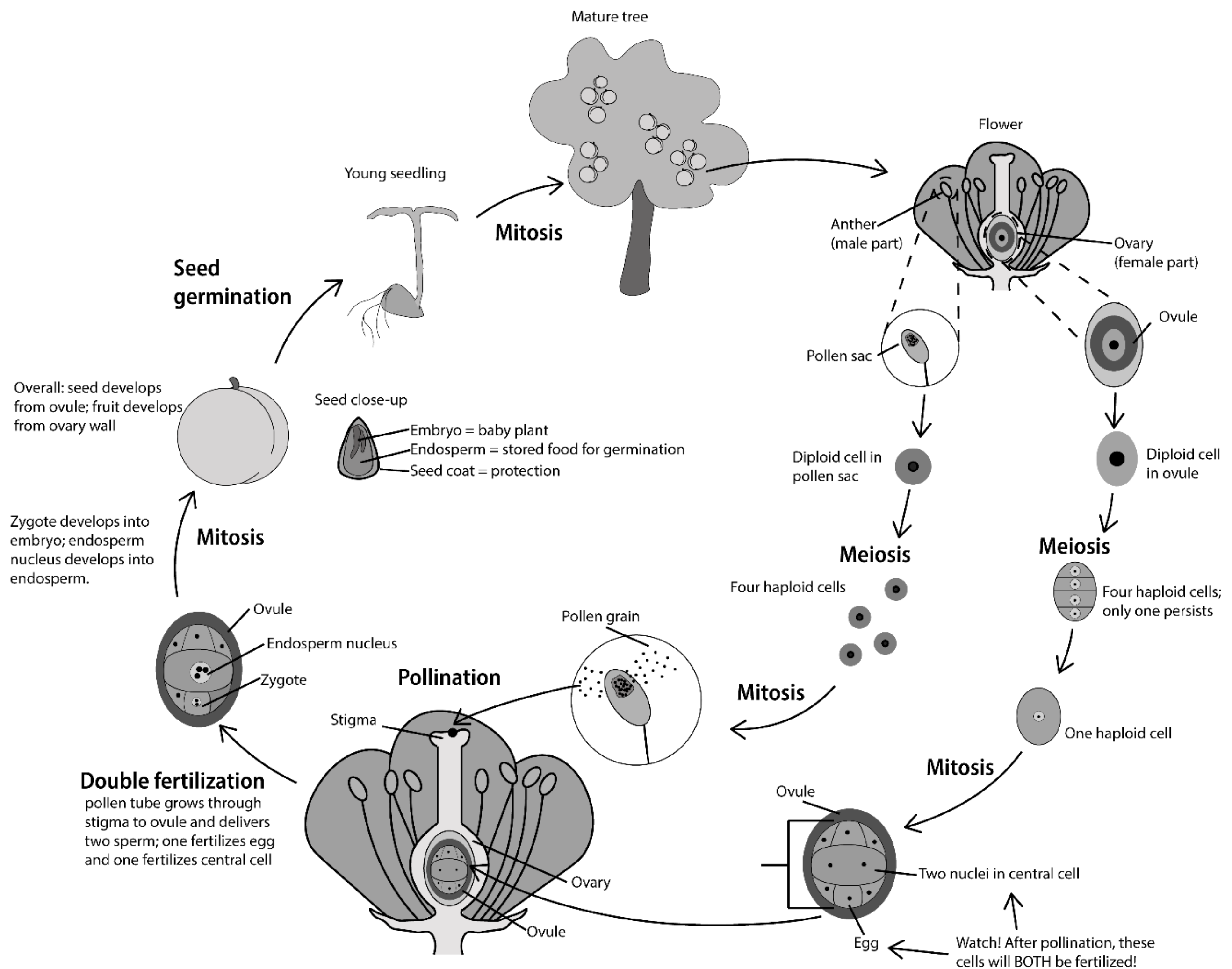

Figure 1: Angiosperm life cycle. Refer to this figure throughout the lab to help you understand how each part works together!

Activity 1: Stems, Leaves, and Roots

Objectives

1. Recognize and point out the parts and functions of stems and leaves.
2. Recognize and point out the parts and functions of roots.
3. Understand how the stems, leaves, and roots of a plant interact.
4. Understand the structural differences between monocots and eudicots/dicots.

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Plants need three basic resources from the environment: sunlight, water, and nutrients. The main adaptations that have evolved in plants over millions of years to access these resources include an extensive **root system** to reach water and nutrients, a **shoot system** consisting of stems and leaves to reach the sunlight, and **vascular tissue** (similar to our circulatory system) which helps transport materials throughout the plant (**Figure 2**).

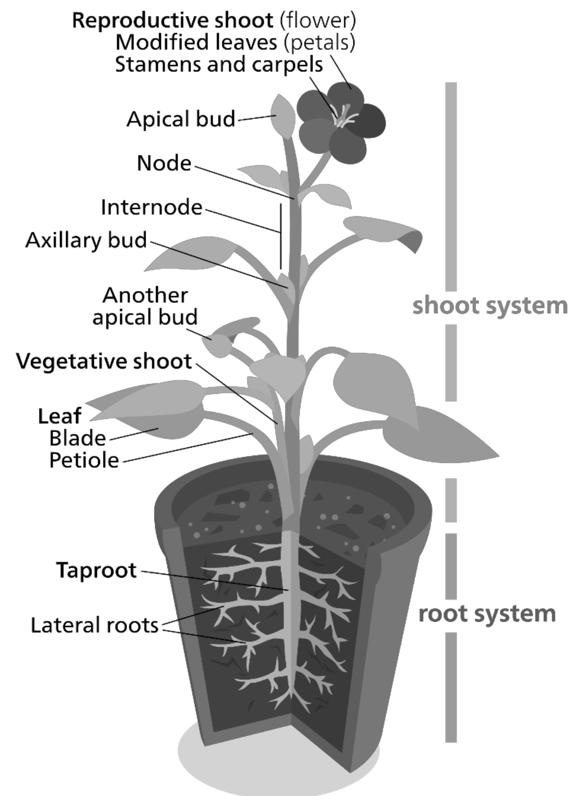

Figure 2: Root and shoot system diagram, showing the important structures of each.

Plants have two different types of vascular tissue: **xylem**, which carries water and minerals absorbed from soil upwards (from the roots to the leaves); and **phloem**, which carries sugars from the leaves to any plant part that cannot photosynthesize (e.g., flowers, roots, etc.). The shoot system includes everything above ground. New parts of the shoot grow at the **apical** and **axillary buds**. **Nodes** are points where leaves attach to the stem, and **internodes** are the spaces in between the nodes.

Leaves are made up of two main parts: The **blade** and the **petiole**. The blade is the flat part with veins of vascular tissue running through it; the blade primarily captures sunlight and does photosynthesis. The petiole attaches the blade to the stem. Reproductive parts, such as flowers, are also included in the shoot system.

The root system includes parts of the plant that are below ground, including (no surprise here) roots. Some plants have a central taproot with lateral roots that emerge from it. Other plants have fibrous root systems, which lack a taproot and have many slender roots that are organized more haphazardly.

Angiosperms are split into two groups: **monocots** and **eudicots** (sometimes called, simply, dicots). Monocots have parallel veins in their leaves and a fibrous root system, while eudicots have net-like branching veins in their leaves and a taproot system. You will be observing these structures in the corn and bean plants that you grew. Note that an understanding of plant

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adaptations, along with the basics of plant reproduction, will help you understand plant evolution, a topic that is also relevant to material in lecture.

Procedure

1. The time has come to sacrifice your beautiful, carefully tended bean and corn plants. Dig each plant up and dump the soil where your TA tells you to (not just in the regular trash). Shake off as much soil as you can from the roots, then lay out your group's plants on your paper towels—put the bean plants to one side, and the corn plants to the other.
2. Examine your bean and corn plants. Identify the following structures on both plants and sketch: shoot system, root system, stem, leaf, petiole, node, internode, apical bud, auxiliary bud, taproot, and lateral roots. (You may need to unearth the plant to examine the roots.) *If your plants have flowers or fruits, set those aside and examine them later.*

3. ***HOMEWORK ALERT!*** You may need a picture of a bean or corn plant with the major vegetative organs labeled (leaf, stem, root). Take it now, before it's too late!
4. Is the bean plant a eudicot or a monocot? What about the corn plant?

Complete **Table 1** by listing the parts of your plants that you can see, describing the function of each part, and explaining how the structures look different between the bean and corn plants.

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Table 1: Your plants' characteristics.

Part	Function	Appearance in Bean Plants	Appearance in Corn Plants

5. Answer the following questions:
   - Although we give the parts separate names, they all work together as part of the same organism. Choose any different pair of parts (e.g. roots and leaves, stems and flowers, or any other pair) and explain what each one does for the other.
   - Every plant needs soil (source of nutrients and water), air (source of O_2 and CO_2), and sunlight to grow. Compare the best plants in your group to the worst. What do you think the best ones got that the worst ones lacked?
6. Your TA may ask you to collect additional data from the class and use the data in a homework assignment. If so, you may want to note the data in the space below.

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Activity 2: "You Killed My Horse!" A Case Study

Objectives

1. Recognize the parts of angiosperm flowers.
2. Trace the events that occur in angiosperm sexual reproduction.
3. Brainstorm ways to prevent sexual reproduction in flowering plants that are pests.

This activity is a case study will help you understand angiosperm reproduction and appreciate why it is important. Before we begin, it will be useful to explain some background about plant reproduction.

Flowers are the sexual organs of angiosperms; their function is to produce pollen (male sex cells) and eggs. Once fertilization happens, seeds begin to form; a **seed** is an embryo packaged with a food supply inside a seed coat. Meanwhile, part of the flower develops into a **fruit** that surrounds the seeds, providing protection as well as a means for transport. Together, flowers, fruits, and seeds allow angiosperms to increase genetic diversity and spread their offspring over a large area – even though each individual plant cannot move.

See **Figure 3** for a labeled diagram of each of the flower parts. Flowers are arranged in concentric rings. Each ring includes different parts, all held together by a center structure called a **receptacle**.

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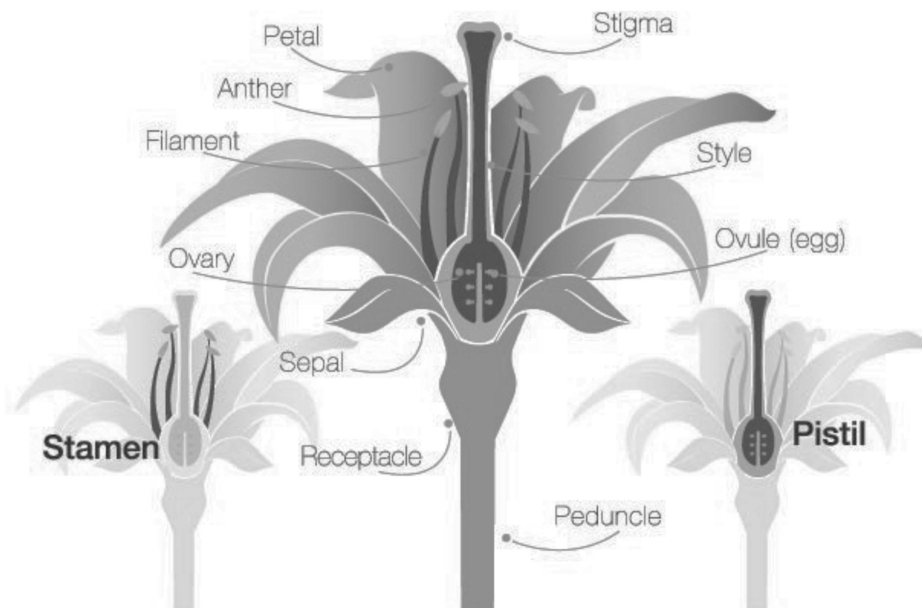
Figure 3: Parts of a flower.



The structures on the outermost ring are **sepals**. They are usually green and easily distinguishable from the rest of the flower. While the flower is a newly developing bud, the sepals protect it. As the flower blooms, though, the sepals protect the fertile parts of the flower. The next ring is made of **petals**, which usually have bright colors that attract pollinators.

The next ring is made of **stamens**, which are the male part of the flower. Stamens are composed of **filaments** and **anthers**. The filaments are stalks that hold up the anthers. Pollen grains are produced in the anthers. Pollen grains contain the flower's sperm cells.

The ring inside that is made of the female part of flowers, **carpels** (called the "pistil" in figure 3). At the top of each carpel is a **stigma**, which receives pollen. Below the stigma is the **ovary**, which houses one or more ovules. Each **ovule** produces an egg cell. If a sperm cell fertilizes the egg, the ovule becomes a seed, and the ovary around it becomes a fruit.

The case study is about a plant called the yellow star-thistle. **Figure 4** is a simplified flower diagram that you should find useful as you work your way through the case. Note that this is not exactly what a yellow star-thistle flower looks like, but the basic parts are the same. As the case study goes along, use the diagram trace how flowering plants produce seeds and fruits.

Hang onto it after lab, as it will help you study for exam 3.

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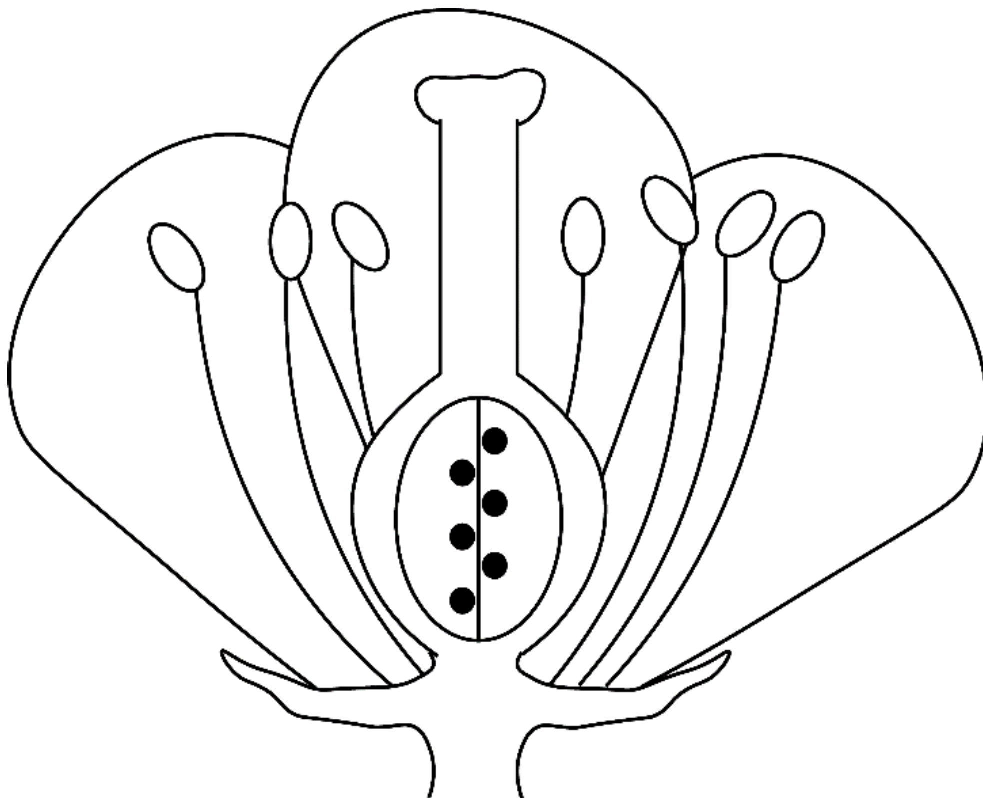
Figure 4: Basic Flower Illustration. Label this diagram and use it to trace the events of flowering plant reproduction as the case study goes along.



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Activity 3: Flower Diversity and Pollination

Objectives

1. Understand the difference in flower structure between monocots and eudicots/dicots.
2. Dissect a flower and recognize its parts.
3. Understand the dominant methods of pollination, and the common adaptations angiosperms have depending on how they're pollinated.

As we have already seen, flowers are made of sepals, petals, stamens, and carpels. Earlier we learned how to tell the difference between monocots and eudicots by looking at their stems and roots. However, you can also tell the difference by looking at the flowers. Monocots generally have floral organs (the petals, sepals, and reproductive parts) in threes and multiples of threes. Eudicots typically produce floral organs in fours or fives (and their multiples). However, species vary in the presentation of these four basic parts. **Complete flowers** have all four types of parts. **Incomplete flowers** are missing one or more of these parts.

Likewise, not every flower is the same. We said earlier that sepals are usually green, but that isn't always the case. Sometimes, sepals help attract pollinators and are only distinguishable from the petals because they are on the outer ring. Similarly, some flowers have modified leaves that look like petals, but actually aren't. For example, the large red "petals" on poinsettias aren't petals at all—rather, they're modified leaves. The actual flowers are the smaller structures in the center of the leaves.

Part 1: Flower Dissection

Procedure

1. Have someone in your group get a flower from your TA.
2. Look at the outer ring of **sepals** on your flower. Remove the sepals after you have noted where they are on your flower.
3. The next ring is made up of **petals**. Remove the petals once you've noted where they are on your flower.
4. The **stamens** are the male flower structure, and they produce pollen grains. The pollen is produced in the **anthers**, which are supported on a stalk. Remove the stamens when you've noted where they are on the flower.
5. The female flower structures are **carpels**. The stigma, which is at the top of a carpel, receives pollen. The ovary, which encloses the ovules, is at the base of the carpel.

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6. Cut across the ovary horizontally as shown in **Figure 5**.
    - How many ovaries do you see?
    - How many ovules do you see?
    - How many stigmas do you see? The stigmas receive pollen, and the stalk under each stigma directs the sperm to the egg. Therefore, there should be one stigma per ovule. Is that true?

Figure 5: This is how you should cut across the ovary.

7. Sketch the flower and label its parts.

8. ***HOMEWORK ALERT!*** You may need a picture of a flower with the major parts labeled (petal, sepal, stamen/anther, stigma/ovary, ovules), along wih labels for parts that will develop into a fruit wall and a part that will develop into a seed. Take it now, before it's too late!
9. If the corn or bean plant that you grew had flowers, feel free to dissect one now!

Part 2: Pollination

Flowers depend on pollination for reproduction. Pollination is the transfer of a pollen grain from an anther to a stigma, which then allows for fertilization. Pollination usually happens either because of wind or animals. In fact, some species of angiosperms have actually coevolved with a particular animal species. The flowers of that plant will have adaptations in size, shape, color, nectar production, etc. that attract that species of animal, and the animal with likewise have adaptations that enable it to pollinate that plant.

Procedure

1. Read the following descriptions of common pollinators, their abilities, and the adaptations of the plants they pollinate.

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Animal pollinators: insects

Beetles: *Beetles don't have very good vision, but they smell very well. Beetle-pollinated flowers therefore often have dull colors and very heavy scents. Beetles are not the most effective pollinators, as they may eat pollen (and sometimes the entire flower). Beetle-pollinated flowers often have adaptations that make them more difficult to eat—for example, the ovules are often deep in the flower and not easily accessible.*

Bees: *Bees are excellent pollinators, and the vast majority of bee species depend on flower nectar for food. They have really cool visual abilities that allow them to easily navigate between and recognize flowers. They can see a broad range of colors, including some in the ultraviolet spectrum, so angiosperms pollinated by bees often have flowers with brightly-colored petals. Sometimes, they have markings in the ultraviolet spectrum that are invisible to humans. Bees have long sucking mouthparts that allow them to access the nectar in flowers, and the flowers often have special petals that allow for easy landing.*

Flies: *Two kinds of flies pollinate flowers: long-tongued and short-tongued. The long-tongued flies have similar adaptations to bees, so they have tend to pollinate similar flowers. However, short-tongued flies are different. These flies don't have good eyesight, so flowers pollinated by short-tongued flies often have dull colors. Instead of offering nectar, the flowers attract pollinators with scents resembling the fly's food sources, such as rotting meat. Thus, short-tongued flies tend to pollinate foul-smelling flowers like the famed Rafflesia.*

Butterflies: *Butterflies get nectar from flowers. Like bees, they have long tongues (seriously, really long tongues). The flowers they pollinate sometimes have petals that form in long, thin tubes. The plants also often have adaptations that allow butterflies to easily land on the petals.*

Moths: *Moths have some similar adaptations to butterflies. They have long tongues that help them collect nectar. Moths, however, are nocturnal. Moth-pollinated flowers usually have bright colors that can be easily distinguished at night, like white. Some of them stay closed during the day and open at nighttime. Moths have a strong sense of smell, so moth-pollinated flowers also often have strong, sweet odors.*

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Animal pollinators: vertebrates

Birds: *Birds have a poor sense of smell, but they see colors well. Many bird-pollinated flowers are therefore colorful but odorless. These flowers produce more nectar than other flowers, an adaptation that attracts birds (which have higher metabolic rates and therefore require more food than insects). The flowers are typically shaped in such a way that the nectar is inaccessible to smaller animals (like insects).*

Bats: *Bat-pollinated flowers have similar adaptations as bird-pollinated flowers. They are often large and produce more nectar than other flowers. However, since bats are nocturnal, the flowers are often much less colorful than bird-pollinated flowers. Bats also have a good sense of smell, so bat-pollinated flowers often have strong odors.*

Wind

Wind-pollinated flowers evolved from insect-pollinated flowers, but they have many special adaptations. Wind-pollinated plants do not "waste" energy on adaptations that attract animal pollinators; the flowers are usually small, drab-colored, and odorless, and they do not produce nectar. The stamens and stigmas are usually exposed, enabling the plant to easily release and capture pollen. Often, they don't even have petals. (Grasses are wind-pollinated – do you remember ever noticing a grass plant's flowers?) However, wind pollination has a hidden cost: Much of the pollen that the flowers release ends up somewhere other than a flower of the same species. Wind-pollinated plants therefore typically produce a lot of pollen. **Table 2** compares animal-pollinated and wind-pollinated flowers.

Table 2: Insect Pollination vs. Wind Pollination.

Structure	Insect Pollination	Wind Pollination
Petals	Large and brightly colored	Very small or absent; dull colors
Nectaries	Present and scented	No scent or nectar; often absent
Stamens	Attached to short filaments inside flower	Attached to long filaments and hang outside the flower
Pollen	Small quantities; easily attaches to insect	Large quantities; smooth and very light for maximum wind dispersal
Stigmas	Sticky, small stigmas inside the flower	Large and feathery for catching pollen; extend outside flower

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2. Observe the flowers around the room. Look for the four major parts of each flower and determine if they're complete or incomplete; write this in **Table 3**. In addition, explain why you think each flower could or could not be pollinated by each of the pollinators listed in **Table 3**.

Table 3: Flower Observations.

Flower	Complete or Incomplete	Beetles	Bees	Butterflies	Birds	Wind	Other

How do you think the flower you dissected is pollinated?

As discussed in this section, angiosperms often coevolve with animals. Sometimes, the corresponding adaptations enable certain species of flowering plant to be pollinated exclusively by a certain species of animal. How might such exclusive relationships boost a plant's reproductive success?

Can you think of any disadvantages?

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Activity 4: Structure and Function of Fruits

Objectives

1. Understand the functions of fruits.
2. Recognize the parts of fruit.
3. Understand how fruits participate in seed dispersal.
4. Explain how seed dispersal improves a plant's reproductive success.

After pollen sticks to a flower's stigma, the pollen grain produces a pollen tube that grows toward an ovule. At the ovule, a unique process called **double fertilization** occurs. First, inside an ovule, the egg is fertilized by one of the two sperm cells in the pollen grain; the resulting zygote will mature into an **embryo**. The second sperm fertilizes a different cell in the ovule, which develops into the nutritious **endosperm** surrounding the embryo.

After double fertilization is complete, the ovule develops into a **seed,** which consists of three parts: a plant embryo, the endosperm, and a protective outer seed coat. As one or more seeds mature inside the ovary, the ovary wall develops into a **fruit** that surrounds the seed(s). **Figure 6** shows a familiar fruit – a peach – with a single seed. (You don't need to know the layers of the fruit wall, or that it is called the pericarp.)

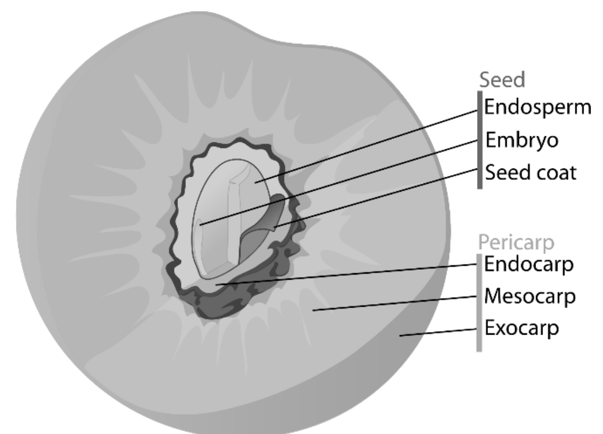

Figure 6: Parts of a fruit.

Fruits are unique to angiosperms. One function is to protect the seed(s) as they develop. Also, once the seeds are mature, the fruit helps with their dispersal.

Seed dispersal is the movement of the seeds away from their parent plant. Some fruits, like fleshy ones, are eaten by animals. The fruit's color and nutritional value attract the animal, which may carry the seeds for long distances in its gut. The animal digests the flesh of the fruit, but the seed remains undigested and may be deposited some distance from the parent plant. (In fact, some seeds need to go through the digestive system of an animal before they can germinate.) Many animals, such as squirrels, bury lots of fruits and retrieve them later, but they die or forget some before eating them. Other fruits have burrs that stick to an animal's fur or feathers, and still others are carried away from the parent plant by wind.

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Clearly, plants invest a lot of energy in seed dispersal. How does this investment boost the plant's reproductive success? To answer this question, consider what would happen if a plant produced 100 seeds inside 100 fruits, all of which dropped at the base of the plant. What would happen to the young plants and the parent? They would all compete with each other for the same space, water, and nutrients; all of them would be harmed.

Procedure

1. Observe the fruits and seeds on display and think about how they may be dispersed. Write the dispersal method in **Table 4**. In the "Characteristics" column, write the fruit or seed's characteristics which make you think they were dispersed that way.

Table 4: Seed Dispersal.

Plant	Dispersal Method	Characteristics

2. ***HOMEWORK ALERT!*** You may need a picture of a cut fruit with the fruit wall and seed(s) labeled, along with labels for parts that developed from the ovary wall and from an ovule. Take it now, before it's too late!
3. Have you ever tasted an unripe apple or tomato? The fruit is hard, green, and not at all sweet or delicious. Why do you think many fruits are hard and green when they are immature, then become soft, red, and tasty when they are ripe? How might this transition boost a plant's reproductive success?

4. If the corn or bean plant that you grew had fruits, feel free to dissect one now!

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Activity 5: Seeds, Seed Germination, and Seedling Development

Objectives

1. Recognize the parts of seeds.
2. Understand how plants develop from seeds.

Part 1: Seed Structure

Remember that each ovule produces an egg cell. If a sperm cell fertilizes the egg, an embryo begins to develop. At the same time, the endosperm forms; the starch and/or oil stored in the endosperm will be the initial food source for the embryo once the seed germinates. The embryo and endosperm are surrounded by a protective seed coat.

A seed's embryo may include one or two cotyledons, depending if the plant is a monocot (one) or eudicot (two). The word **cotyledon** is confusing because it literally means "seed leaf," but the cotyledons are not true foliage leaves in the familiar sense of the word "leaf." The **first foliage leaves,** which you can recognize by their flat shape and veins, develop later. The cotyledons may take many forms. In some species, the cotyledons remain separate from the endosperm, and the endosperm is the embryo's food source for germination. In other species, the cotyledons absorb the endosperm and are the direct food source for germination. Once the seed has germinated, the cotyledons may emerge from the soil with the stem and carry out photosynthesis for a while. Or they may not; it depends on the species.

In this activity, you'll investigate these parts of the seed yourself. *IF YOU HAVE A PEANUT ALLERGY, PLEASE LET YOUR TA KNOW!*

Procedure

1. Get a peanut from your TA. For peanuts, the tough, fibrous outer shell is the fruit wall, and it contains one to three seeds.
2. Crack open the peanut fruit; it should easily split lengthwise. Remove the seed(s) from the fruit.
3. Remove the super-thin, papery seed coat covering a seed.
4. The two halves of the peanut seed are the two cotyledons. In peanuts, the cotyledons absorb the endosperm during seed development and are the direct food source for the embryo as the seed germinates.
5. Separate the two halves of the peanut, being careful not to damage the seed. Look closely at the embryo; you should be able to see a short root/shoot axis crowned with two tiny leaves. Had the seed germinated (rather than being roasted), the seedling

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would have grown at both the root and shoot tip, and the two tiny leaves would have become the first true foliage leaves.

6. Sketch the peanut and label all of the parts of the seed.

7. ***HOMEWORK ALERT!*** You may need a picture of a cracked-open peanut seed, with the embryo and its food supply labeled. Take it now, before it's too late!

Part 2: Seed Germination and Seedling Development

If a seed remains dry, it can remain dormant for a very long time – thousands of years, in some cases! During dormancy, the embryo does not grow or respire, and it does not use the food stored in the endosperm. If the seed absorbs water through its seed coat, though, it may germinate. During **seed germination,** water absorbed through the seed coat activates enzymes, which convert the endosperm's starch or oil into simple molecules that the embryo can use in respiration. The root and stem of the embryo begin to grow, forming a seedling. The seedling is in a race against time, because food supplies from the endosperm are limited. If the seedling does not reach sunlight by the time the stored food is gone, it will die. If it reaches sunlight on time, however, the seedling can start making its own food via photosynthesis.

Procedure

1. Get a bean seed, a corn seed, and a cup with germinated corn and bead seeds from your TA.
2. Remove the seed coat from the bean seed and separate the cotyledons to see the embryo inside, just as you did for the peanut. Is the bean a monocot or eudicot? Explain.

3. Sketch the seed and label its parts: seed coat, cotyledons, and embryo. Also, look for the first foliage leaves, which are at the top of the embryo's stem.

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4. Sketch the germinated bean seedling, and label the cotyledons and first foliage leaves.

   The bean cotyledons may be a bit shriveled after germination. Why are they not as full and fleshy as they were in the seed stage?

5. In your sketch of the bean seedling, label the **primary root**. This is the largest root, and it will eventually grow straight downwards and become a taproot. You can also see lateral roots beginning to form by along the sides of the taproot. Lateral roots increase the surface area for water and mineral absorption.

   If you were to watch germination in time lapse, you would see that the root emerges first, then the shoot. Why might this be? It will help to think of the root functions.

6. Cut open your corn seed longitudinally. The fruit wall in corn (and other grains) is hard, and it is fused with the seed coat. You won't be able to distinguish between the two. Compare your seed to the diagram in **Figure 7**. Can you find all of the structures?

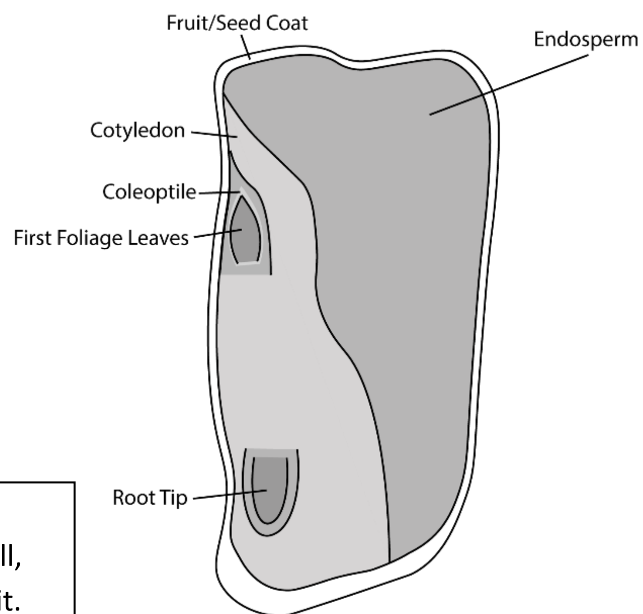

Figure 7: Corn seed. In grains, the seed coat is fused to the ovary wall, so the "seed" is actually also a fruit.

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7. Sketch and label a corn seedling below. Make sure to label the first foliage leaf. Also, look for the **coleoptile** (a thin layer that encases the first foliage leaves in grass species, like corn and other grains). Don't forget to include the roots in your drawing.

In monocots, the primary root only lasts for a little while, and the majority of the roots grow from the base of the stem instead. Because they don't have a taproot, the roots in monocots are more shallow than those of eudicots, and they spread across a larger area. With this in mind, do you think it would be easier to transplant monocots or eudicots? Why?

Basil plants have tiny seeds; when you plant them, you should cover them with less than ¼ inch of soil. Bean plants have much larger seeds and can be covered with an inch or more of soil. What do you think determines the depth at which a plant should be planted? What would happen if you covered a seed with too much soil?

Activity 6: Plants, Artificial Selection, and the Foods We Eat

Objectives

1. Apply your knowledge of plant structure and function to interpret the plant parts of common foods from the grocery store.

Humans have used artificial selection (selective breeding) to modify many plant species to improve their food value. Leaves, stems, flowers, and other structures can be greatly

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exaggerated, or they may be practically absent. One prime example of this is the wild mustard plant. Over many years, artificial selection has shaped this one species into kale, broccoli, kohlrabi, Brussels sprouts, cabbage, cauliflower, and more.

All of these mustard relatives are considered vegetables, of course. But have you ever heard someone say that a tomato is actually a fruit, but you thought it was a vegetable? It turns out that unlike the word "fruit," the word "vegetable" does not have a botanical definition. In general, though, vegetables are plant parts that are not sweet. Fruits can therefore be vegetables, but not all vegetables are fruits. Clear as mud? The exercise below may help.

Procedure

1. Obtain an assortment of produce from your instructor.
2. Examine each item carefully.
3. Decide individually what part of the plant you think each one is.
    - Options: **stem, leaves, flower buds, axillary bud, apical bud, flower buds and stem, fruit, seed, taproot, petiole, tuber (underground stem)**
4. Discuss your answers as a group and decide on your final answer. Record your answers in **Table 5.**
5. Check your answers with your TA at the front.

Table 5: Plants from the Produce Aisle.

Name of Food	What Plant Part Is It, and Why Do You Think That?

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Acknowledgments and Attributions

Activities 1 and 6 written by Katherine Hooker and Mariëlle Hoefnagels.

Activity 3, Activity 4 Part 2, and Activity 5 adapted from: Jean Dickey. 2003. Fruits, Flowers, and Seeds. *Laboratory Investigations for Biology, 2nd Edition.*

Figure 1: Angiosperm Life Cycle © Sarah Greenwood used under a CC BY-SA 4.0 license https://commons.wikimedia.org/wiki/File:Angiosperm_LIfe_Cycle.png

Figure 2: A diagram representing a "typical" eudicot. © Kelvinsong used under a CC BY-SA 3.0 license https://en.wikipedia.org/wiki/Plant_morphology#/media/File:Plant.svg

Figure 3: Flower Anatomy © ProFlowers used under a CC BY 2.0 license https://www.flickr.com/photos/proflowers/15099656846

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Figure 6: Drupe fruit diagram-en © LadyofHats used under a CC0 license https://commons.wikimedia.org/wiki/File:Drupe_fruit_diagram-en.svg

Figure 7: Labelled Corn Seed Illustration © Sarah Greenwood used under a CC BY-SA 4.0 license https://commons.wikimedia.org/wiki/File:Labelled_Corn_Seed_Illustration.png

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Animal Diversity I: Invertebrates

Mariëlle Hoefnagels and Sarah Greenwood

Christmas Tree Worm (*Spirobranchus giganteus*) © Kris-Mikael Krister used uder a CC BY 2.0 license
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Introduction

In class and in lab, you have learned about the domains and kingdoms of life. The taxonomic category right under kingdom is called a **phylum** (plural—phyla); in other words, each kingdom is divided into multiple phyla. This lab focuses on some of the phyla in kingdom Animalia. There are over 30 phyla of animals, covering everything from sea sponges, to beetles, to humans. Some of the animals don't even look like animals! For example, the animal in the photo above is a *Spirobranchus giganteus*—a member of the phylum Annelida (a segmented worm). Learning about the phyla can help you gain a deeper understanding of the evolutionary relationships among animals. They aren't always intuitive!

In this lab, we'll focus on eight phyla (**Figure 1**): Porifera (sponges), Cnidaria, Platyhelminthes (flatworms), Mollusca (mollusks), Annelida (segmented worms), Nematoda (roundworms), Arthropoda, and Echinodermata. You will cover Chordata, another phylum, in a later lab.

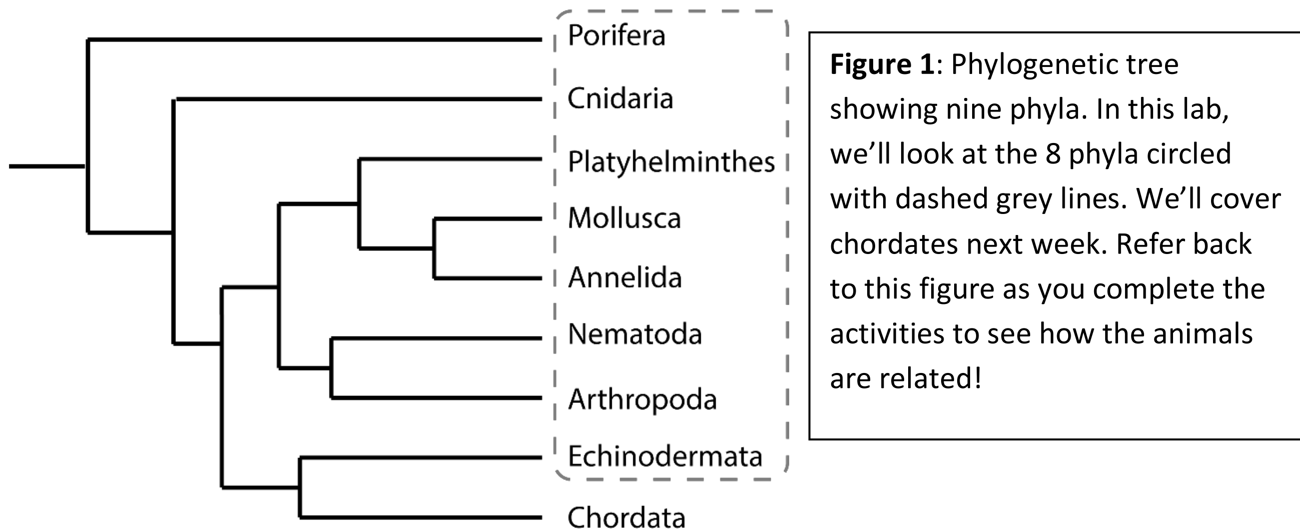

Figure 1: Phylogenetic tree showing nine phyla. In this lab, we'll look at the 8 phyla circled with dashed grey lines. We'll cover chordates next week. Refer back to this figure as you complete the activities to see how the animals are related!

All animals have certain features in common. They are multicellular, eukaryotic, and heterotrophic. Their cells lack cell walls (though of course they have a cell membrane, as all organisms do). They take food into their bodies, digest it, and release the undigestible bits into the environment. And they have a type of embryonic development (involving a blastula and, in most cases, a gastrula) that is unique to animals.

Within that definition of animals, there is a lot of variety. Throughout the lab, you'll evaluate certain characteristics of each phylum:

Habitat

An animal's **habitat** is the place it lives. Some common descriptors for habitat are **terrestrial**, meaning the animal lives on land, and **aquatic**, meaning it lives in water. Some animals live in water and on land. Aquatic animals can be further classified into **marine** animals, meaning they live in the ocean, and **freshwater** animals, which live in lakes or rivers.

Feeding

Animals have different feeding habits and digestive systems. In the lab, you may see that animals in a phylum are **carnivorous**, meaning they eat animals; **herbivorous**, meaning they eat only plant matter; or **omnivorous**, meaning they eat plants and animals. **Filter feeders** are aquatic animals that filter yummy organic particles out of the water, and **parasites** get their

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food from living host animals. Additionally, you will see that some animals have different kinds of digestive systems. Animals with an **incomplete digestive system** have one hole through which food comes in and waste goes out. Animals with a **complete digestive system** have two holes: one for food to go in (the mouth) and one for waste to come out of (the anus).

Mobility

Though you may not think of them as animals, some animals like sponges are **sessile**, meaning they are fixed in one place. Most animals that you could think of are **motile**, meaning they can move around freely. Some animals are both sessile and motile, transitioning between the two at different stages of their lives.

Symmetry

Not all animals have symmetry. Many sponges, for example, have no symmetry. Animals are **symmetrical** when they can be divided into equivalent halves. Most animals you would think of have **bilateral symmetry**, meaning they are symmetrical in only one direction (by halving them in a plane that divides the head and torso). Humans, beetles, dogs, and fish have bilateral symmetry. **Radial symmetry** is when animals are arranged around an axis point in the center of their body, like a pizza, and are therefore are symmetrical in many directions as long as the lines pass through the center point. Jellyfish have radial symmetry.

Activity 1: Phylum Porifera (Sponges)

Objectives

1. Understand the main characteristics of animals in the phylum Porifera.

The phylum Porifera is made up of the sponges. Sponges are aquatic animals, and the vast majority of them are marine. They are the simplest animals; their bodies have a few cell types, but the cells do not “cooperate” to form true tissues and organs. Sponges have asymmetrical body plans. Adult sponges are sessile, but sponge larvae are motile. There are between 5,000 and 10,000 currently known species of sponges.

Figure 2: Sponges.

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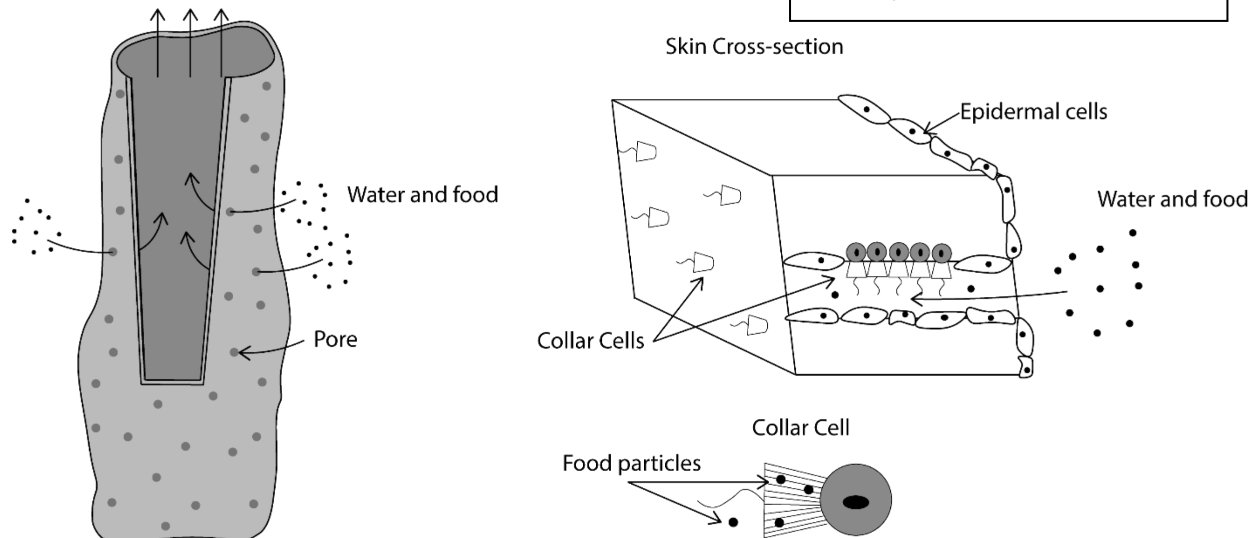
See **Figure 3** for a typical sponge. The diagram at the left shows an entire sponge, where the body wall contains a multitude of pores. There is a large hole at the top.

Figure 3: A typical sponge with a closeup of the layers that make up its body, plus a closeup of a collar cell.



If you take a cross section of the body wall, you'll see an outer epidermal layer, a middle layer, and an inner layer consisting of **collar cells** that have flagella on the end. Water carrying food (like bacteria, algae, and other assorted organic particles) travels through the pores, into the central cavity of their body, and out through the hole at the top. The collar cells trap food and nutrients. In the middle layer of the body wall, amoeba-like cells carry out other functions, like the digestion of the food that the collar cells trap.

Procedure

1. Your TA will provide a specimen of *Grantia* (formerly called *Scypha*), a small marine sponge. Use your forceps to place it in the provided dish, and then use your razor to cut the sponge open longitudinally (through the hole at the top).
2. Place the dish with *Grantia* in it on the stage of your dissecting scope. Look at the sponge, and sketch it below. Can you see the features from **Figure 3**?

3. Dispose of your sponge as directed by your TA.
4. There will be a prepared slide of *Grantia* at your lab bench. Use your compound microscope to focus on it. Draw and label what you see on the next page.

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Drawing of *Grantia*:

Activity 2: Phylum Cnidaria

Objectives

1. Recognize the common features of cnidarians.
2. Know the difference between polyp and medusa forms.
3. Understand radial symmetry and explain why it may be advantageous.

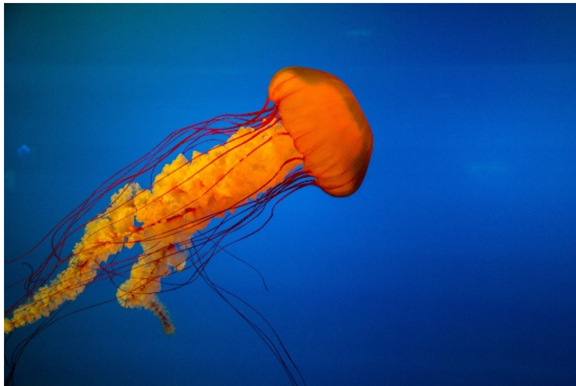

Figure 4a: A jellyfish.

Figure 4b: A sea anemone.

Phylum Cnidaria contains some easily recognizable animals, including jellyfish, sea anemones, and corals. The defining feature of animals in this phylum is that they all have true tissues, radial symmetry, and stinging cells. They also have tentacles surrounding a central mouth, which both takes in food and expels waste. The digestive system is therefore incomplete.

Like sponges, all cnidarians are aquatic, and most are marine. Cnidarians have two body forms: a motile medusa and a sessile polyp (see **Figures 4a and 4b**). For example, jellyfish have the medusa form, and both sea anemones and corals have the polyp body form. Some cnidarians take on both of these forms at different life stages, including some jellyfish.

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Cnidarians have a more complex means of digestion than sponges. Cnidarians are carnivores, and their tentacles have special cells that sting their prey (or any animal that threatens them, as you can attest if you have ever been stung by a jellyfish). They take their prey through their mouth holes and into the digestive cavity (the "gut" in see **Figure 5**). Cells lining the digestive

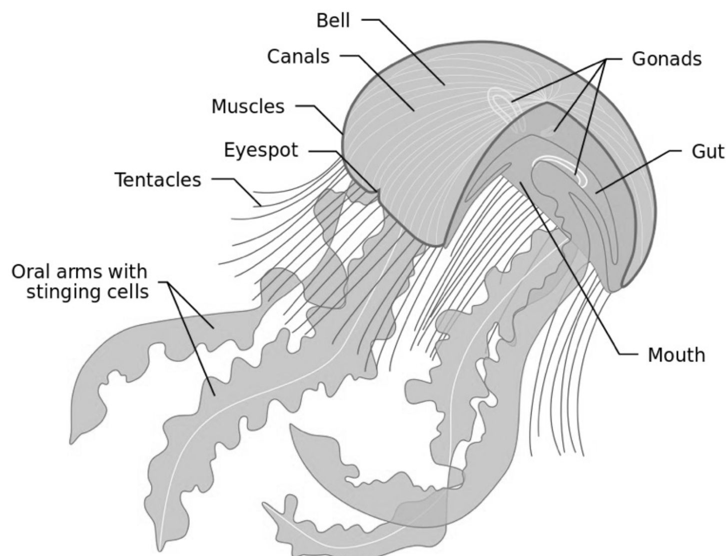

Figure 5: Jellyfish anatomy. Pay attention to the stinging cells and the location of the mouth!

cavity secrete digestive enzymes into the cavity and absorb the digested nutrients, which are then distributed throughout the rest of the body.

Procedure

1. Get a dish with hydra, a small freshwater cnidarian with a polyp body form. (A polyp is something like an upside down jellyfish, with the tentacles sticking up instead of hanging down.)
2. Put the dish on the stage of your dissecting scope. To a hydra, the lamp from the dissecting scope is really hot, so don't let yours get singed. Also note that the hydra are tiny and colorless, so it may take a while to confirm you're focusing on one. Ask your TA if you are having trouble.
3. Once you locate it, sketch your hydra below.

4. Use a Pasteur pipette to squirt some brine shrimp into your dish, as near to the hydra as you can without touching it, as they are easily stressed.

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5. Try to observe the feeding behavior of the hydra. Note that since they are sessile, they can't move to find food. The food must be within reach of the hydra's tentacles.

   Did your hydra eat? If so, what did you observe?

   Why are incomplete digestive systems inefficient?

   Why might it be advantageous to have a polyp and medusa form at different stages of life?

   Why is radial symmetry advantageous for aquatic sessile animals like hydra?

Activity 3: Phylum Platyhelminthes (Flatworms)

Objectives

1. Understand the digestive system in flatworms.
2. Know the difference between radial and bilateral symmetry and explain why bilateral symmetry is adaptive in some animals.

Figure 6: A marine flatworm.

There are around 20,000 species of flatworms (see **Figure 6** for an example). As the name suggests, their bodies are always flat. They are motile, and they have bilateral symmetry. Free-living flatworms may roam fresh water or the oceans. Other flatworms are parasites – think tapeworms – living in the moist environment of an animal host's body. (Yuck.)

Like cnidarians, flatworms have an incomplete digestive tract. Food enters and is digested, and then undigested wastes exit the body through the same hole.

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In this lab, you'll be observing planaria, a small, freshwater flatworm. It's surprisingly cute.

Procedure

1. Have someone in your team get a planarian in a dish.
2. Place the dish on the stage of your dissecting scope and focus on the planarian.
3. Sketch the planarian below.

   The planarian has two eye spots, which sense light. Don't forget to draw those too!
4. Put a bit of bloodworm, provided by your TA, in with the planarian. How does the planarian's behavior change?
5. Pay close attention to how the planarian feeds. Where do you think its mouth is, and what makes you think it's there?
6. Outline the planarian's digestive tract on your sketch above.
7. Compare the lifestyle of a free-living flatworm to that of a sessile sea anemone. Why might bilateral symmetry be adaptive to a motile animal like a flatworm?
8. Your TA will have preserved specimens of flatworms on display in lab. Go look at them!

Activity 4: Phylum Mollusca

Objectives

1. Understand the defining characteristics of mollusks.
2. Recognize the differences between gastropods, bivalves, and cephalopods.

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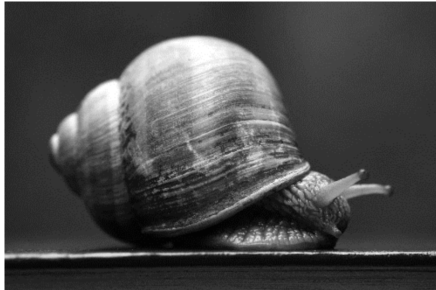

Figure 7a: Snail (a gastropod).

Figure 7b: A bunch of clams (bivalves).

Figure 7c: Nautilus (a cephalopod).

Phylum Mollusca is another very diverse phylum; mollusks include easily recognizable animals such as clams, snails, oysters, scallops, slugs, and octopuses, and squids. Individuals can be very small or very large, be filter feeds or active hunters, and be dim-witted or highly intelligent. The majority of mollusks are marine species, but some live in freshwater habitats. However, snails are a group of mollusks that can live on land.

Mollusks commonly have a body that consist of a **foot**, which is muscular and helps them move, and a **visceral mass**, which holds the animal's organs. They have a complete digestive tract, with a mouth and anus. Mollusks have **gills** that allow them to get oxygen from the water; some filter feeders also use their gills to get food. Many, but not all, mollusks have shells.

Mollusks are categorized into different classes based on their body forms:

> **Gastropods** (**Figure 7a**), like snails and slugs, have a muscular foot at the bottom of the body, where you'd expect the stomach of a crawling animal to be ("gastropod" means "stomach-foot"). Most gastropods are herbivores, but some are carnivores. Most have shells, but some (like slugs) don't. Most of them have **radula**, which is a unique, rasping, tongue-like organ that they use to scrape off food.
>
> **Bivalves** (**Figure 7b**) are what you may think of when you imagine a mollusk: oysters, clams, and scallops. They can open their shell to stick out the muscular foot, which they use to burrow into sand. Most bivalves are filter feeders: They extend their gills into the water, then yummy organic particles stick to the mucus on the gills. The food is transported to the mouth, and from there to the rest of the digestive tract.

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Cephalopods (**Figure 7c**) include animals like squids, octopi, and nautili. Their feet have been modified into arms or tentacles, and they are the most motile of the cephalopods. While nautili look similar to other recognizable mollusks because of their shell, squids and octopi may seem out of place. Cephalopods other than nautili have reduced shells when compared to other mollusks, and octopi don't have shells at all.

Whereas bivalves don't have strong senses and are closed off by their thick shells, octopi and other cephalopods are known for their sensory abilities and have reduced shells. Why could these traits be adaptive for members of each group?

If live snails are available, sketch them below. Make sure to label the foot. (Your TA may have you complete other snail-related activities as well.)

There will be preserved specimens of mollusks in the lab; look at some. Find one example of a bivalve and one example of a cephalopod. Sketch them below. Label any of the organs you can see.

Activity 5: Phylum Annelida (Segmented Worms)

Objectives

1. Recognize the main characteristics of annelids.
2. Understand how annelid movement differs from other worms.

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Annelids are also a diverse group of animals. They may live on land, in the ocean, or in fresh water; the Christmas tree worm on the first page of this lab is a marine annelid. Like with other worms, some are parasites – for example, most leeches are parasites that feed on the blood of other animals.

Figure 8: Earthworm.

Annelids have bilateral symmetry, but unlike the bodies of the other types of worms, annelid bodies are segmented. Like round worms, annelids have a complete digestive system. However, annelid digestive systems are more complex and include more organs.

The most recognizable annelids are earthworms **(Figure 8),** which feed on decaying organic matter in soil. Leeches are also annelids, and while they are famously parasitic, some leeches are predators that eat other invertebrates.

Annelids have a different way of moving than other kinds of worms, thanks to more complex muscle systems and bristles on their bodies that help them move.

Procedure

1. Take an earthworm and put it on a paper towel at your lab bench. Sketch it below.

2. Pay attention to how it moves. How is this different from the movement of the nematodes you observed earlier?

3. Touch the underside of the worm so you can feel the bristles that aid in the earthworm's movement.

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Activity 6: Phylum Nematoda (Roundworms)

Objectives

1. Understand the main characteristics of nematodes.
2. Observe the movement of vinegar eels.

Figure 9: Nematode.

Nematodes are everywhere. They live in diverse habitats—terrestrial and aquatic, marine and freshwater, and at nearly every temperature. They could be found in any handful of dirt and even on the ocean floor. Some nematodes, such as hookworms, are parasitic.

Nematodes have bilateral symmetry **(Figure 9),** and most are small (although some can be large). They eat other nematodes, microorganisms, or decaying matter.

Like annelids, nematodes have a complete digestive system – they take food into the digestive tract at the mouth, digest it in the gut, and expel undigested waste through the anus. Unlike annelids, however, nematodes are unsegmented.

In this exercise, you'll observe vinegar eels, a kind of nematode that eats the bacteria used in the production of vinegar. (Yes, they can live in vinegar, which is a type of mild acid. The vinegar we buy in the U.S. has been filtered to remove the nematodes, so don't worry.)

Procedure

1. Get a drop of the culture with vinegar eels in it from your TA and put it on a clean microscope slide at your lab bench. Cover it with a coverslip.
2. Use your compound microscope to observe the vinegar eels. Draw one below.

How do the vinegar eels move?

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Compare the digestive tract of a flatworm to that of a nematode. What are the potential benefits of a complete digestive tract?

Activity 7: Arthropoda

Objectives

1. Recognize the basic features of arthropods.
2. Know that arachnids, insects, and crustaceans are all arthropods.
3. Look at and sketch a daphnia.

Arthropods are an incredibly diverse phylum and make up over 80% of all of the animals on earth. The most easily recognizable groups of arthropods are insects, crustaceans, and arachnids.

Arthropods, like annelids, have segmented bodies. They also have bilateral symmetry. Arthropods are characterized in part by their jointed appendages. While some of these appendages are used for movement or in feeding, others work as sensory organs, contribute to defense, or participate in reproduction. Arthropods also have hard exoskeletons made in part of chitin, a super-tough polysaccharide that protects the animal while staying flexible where needed to allow free movement. This exoskeleton doesn't grow; instead, arthropods have to molt it and grow another, bigger exoskeleton when necessary.

Figure 10: Tick.

Arachnids (**Figure 10**) are a group of arthropods that includes spiders, scorpions, ticks, and mites. Almost all arachnids have eight appendages for movement, but in some species, two of them are modified into other appendages that help them with other functions. Most arachnids are carnivores and have adaptations like claws, stingers, or venom that help them to capture prey.

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Insects (such as the beetle in **Figure 11**) are the largest group of arthropods and the most diverse class of animal. They have a diverse range of feeding behaviors; they can be herbivores, carnivores, or parasites. They all have a three-part body consisting of a head, thorax, and abdomen. Six legs and (often) two pairs of wings are attached to the thorax. Insects fill a wide variety of niches and therefore affect humans in different ways. Some are regarded as pests, like termites. Others, like mosquitoes, can transmit diseases. As you learned earlier, many insects pollinate angiosperms. Insects such as honeybees and silkworms produce materials that are commercially valuable to humans. (Watch out, folks – cricket flour is probably already in a store near you!)

Figure 11: Beetle.

Crustaceans are arthropods such as crawfish, lobsters, shrimp, crabs (**Figure 12**), barnacles, and pill bugs. Most crustaceans are aquatic, but some (like pill bugs) are terrestrial. Most crustaceans have two-part appendages for movement and sensations. You will look at *Daphnia*, a small crustacean commonly known as a water flea.

Procedure

1. Get a sample of water with daphnia in it from your TA.
2. Place the sample on the stage of your dissecting microscope.
3. Look at the daphnia, making sure to notice the segmented appendages.
4. Sketch the daphnia below.

Figure 12: Crab.

5. Your TA will have preserved specimens of different insects, arachnids, and crustaceans out. Look at some!

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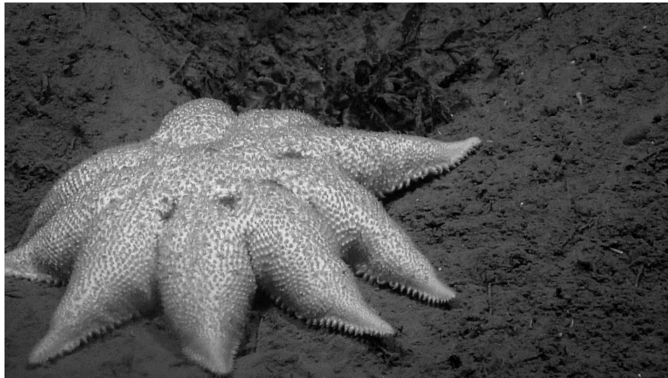
Activity 8: Echinodermata

Objectives

1. Understand the basic characteristics of echinoderms.

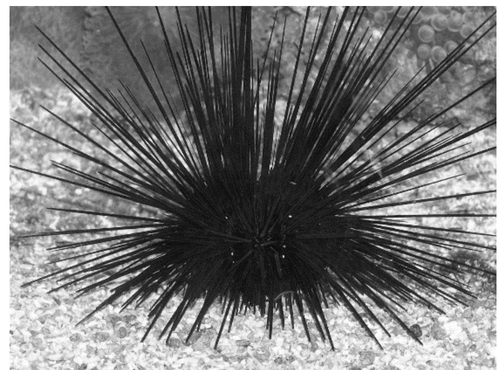
The last phylum we are covering today is Echinodermata **(Figures 13 and 14).** Echinoderms include animals like sea stars, sea urchins, sea cucumbers, and sand dollars. Echinoderms have radial symmetry, and all of them are marine animals. It would seem logical that echinoderms should be most closely related to cnidarians, but if you refer back to **Figure 1**, you'll see they evolved from animals that had bilateral symmetry.

Figure 13: Sea star.



Echinoderms have complete digestive systems, and sea stars are often carnivorous. Echinoderms move using their **tube feet**, which are controlled by varying internal water pressure. These tube feet can also be used to grab onto rocks or catch prey. Echinoderms also have a unique skeletal structure which includes spines that stick out from their skin. The spherical echinoderms, like sand dollars and sea urchins (**Figure 14**), look different from sea stars. It may help to picture a sea star with its arms curved upward to form a sphere – that is how the body forms are related. Some echinoderms, like sea cucumbers, don't have spines or arms. They are filter feeders, but can still move using their tube feet and water vascular system.

Figure 14: Sea urchin.



Look at some of the echinoderms stationed around the room and sketch them below.

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Activity 9: Put It All Together

When learning about animals in different phyla, it's easy to perceive some as more "evolved" than others. This is, however, not a good way to think about the relationship between the animals. After all, all of the animals discussed in this lab do well in their environments. For each of the following pairs, describe why each of the characteristics could be adaptive for the animals that have them:

- Bilateral vs. radial symmetry
- Sessile vs. motile
- Incomplete digestive tract vs. complete digestive tract
- Filter feeding vs. other means of getting food

Your TA may have some other activities to help you put information about the eight phyla together. Filling out **Table 1** on the next page will help you master the differences/similarities between the phyla.

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Table 1: Phyla Differences

	Porifera	Cnidaria	Platyhel-minthes	Mollusca	Annelida	Nematoda	Arthro-poda	Echino-dermata
Habitat(s)								
Feeding								
Mobility								
Symmetry								
Digestive tract								
Segment-ation								

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Acknowledgments and Attributions

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Figure 2: Blue Tube Sponges © Patrick Briggs used under a CC BY 2.0 license https://www.flickr.com/photos/87241965@N00/371542695

Figure 4a: Used under a CC0 license https://pixabay.com/en/jellyfish-underwater-marine-exotic-918694/

Figure 4b: A bright pink sea anemone – panoramio © Jiaqian AirplaneFan used under a CC BY 3.0 license https://commons.wikimedia.org/wiki/File:A_bright_pink_sea_anemone_-_panoramio.jpg

Figure 5: Anatomy of a jellyfish © Whidou used under a CC BY-SA 4.0 license https://commons.wikimedia.org/wiki/File:Anatomy_of_a_jellyfish.svg

Figure 6: Pseudoceros bifurcus - Blue Pseudoceros Flatworm © Stephen Childs used under a CC BY 2.0 license https://commons.wikimedia.org/wiki/File:Pseudoceros_bifurcus_-_Blue_Pseudoceros_Flatworm.jpg

Figure 7a: © Ulleo used under a CC0 license https://pixabay.com/en/snail-shell-mollusk-nature-reptile-2308761/

Figure 7b: Clams © Marlith used under a CC BY-SA 3.0 license https://commons.wikimedia.org/wiki/File:Clams.JPG

Figure 7c: Nautilus Palau © Manuae used under a CC BY-SA 3.0 license https://nv.wikipedia.org/wiki/E%CA%BCelyaa%C3%ADg%C3%AD%C3%AD:Nautilus_Palau.JPG

Figure 8: In the public domain https://www.nps.gov/grsm/learn/nature/dff-earthworms10.htm

Figure 9: Colorized electron micrograph of soybean cyst nematode (Heterodera sp.) and egg used under a CC0 license https://commons.wikimedia.org/wiki/File:Soybean_cyst_nematode_and_egg_SEM.jpg

Figure 10: Tick © John Tann used under a CC BY 2.0 license https://www.flickr.com/photos/31031835@N08/6368335425

Figure 11: Beetle-Bessbug © Lokal Profil used under a CC BY-SA 3.0 license https://en.wikipedia.org/wiki/File:Beetle-Bessbug.jpg

Figure 12: Spider crab at manila ocean park © Charles Laigo used under a CC BY-SA 3.0 license https://commons.wikimedia.org/wiki/File:Spider_crab_at_manila_ocean_park.jpg

Figure 13: Sea Star © NOAA Ocean Exploration & Research used under a CC BY-SA 2.0 license https://www.flickr.com/photos/oceanexplorergov/42477161985/

Figure 14: Sea urchin © Filip Maljković used under a CC BY-SA 2.0 license https://www.flickr.com/photos/dungodung/217110954

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Animal Diversity II: Vertebrates

Mariëlle Hoefnagels and Sarah Greenwood

Indian elephant bull in musth* in Bandipur National Park © Yathin Krishnappa used under a CC BY-SA 3.0 license https://en.wikipedia.org/wiki/Asian_elephant#/media/File:Elephas_maximus_(Bandipur).jpg

Introduction

Last week, you explored eight of the animal phyla: Porifera, Cnidaria, Platyhelminthes, Mollusca, Annelida, Nematoda, Arthropoda, and Echinodermata. These animals were all **invertebrates** – they don't have a backbone or a skeleton made of bone. This week, several guest speakers will come to show you even more diversity of animals. The speakers vary from semester to semester, but usually we have speakers to talk about arthropods, amphibians, reptiles, and mammals. With the exception of arthropods, all of these animals come from the phylum Chordata. Most chordates are vertebrates, meaning they have a backbone and a skeleton made of cartilage or bone. See **Figure 1** for the phylogenetic tree representing the evolutionary relationship between all of these phyla.

**According to Wikipedia, "musth" in bull elephants is associated with aggression and high levels of testosterone.*

Objectives

1. Understand the characteristics of arthropods.
2. Understand the characteristics of chordates, including fishes, amphibians, reptiles, birds, and mammals.
3. Learn about the animals the guest speakers bring to the lab.
4. Be respectful during the presentations!

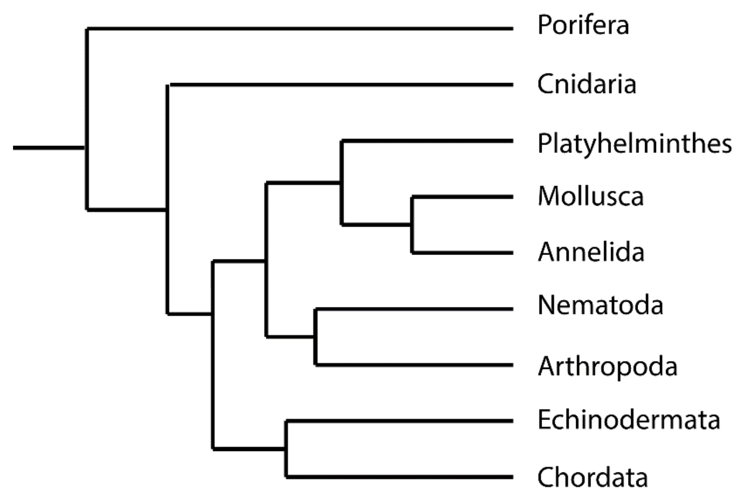

Figure 1: Phylogenetic tree with each of the nine phyla you've studied. Today, you'll be seeing live examples of arthropods and chordates.

Phylum Arthropoda

As a refresher, here's some information about arthropods (despite the title of this lab, arthropods are not vertebrates!). With the possible exception of nematodes, which are usually microscopic, arthropods are probably the most numerous and diverse animals on earth. Many people equate arthropods with "bugs" but in reality, arthropods are so diverse that it is hard to generalize too much about them. Some live on land, and some live in water. Some are herbivores and some are carnivores. Some are important human food sources (can you name an example?), others are important pests (can you name an example?). Many arthropods are beneficial to humans (can you name an example?).

"Arthropoda" literally means "jointed legs", but the walking legs aren't the only appendages that may be jointed in arthropods. Besides locomotion, the jointed appendages may be modified for food handling, defense, taste, touch, or reproduction. Hopefully you will get to see some examples in class; if not, ask the guest speaker with the arthropods about some interesting uses for those jointed appendages! Three of the major classifications of arthropods are insects, arachnids, and crustaceans.

Phylum Chordata

Although some chordates are invertebrates (the aquatic animals called lancelets and tunicates, or sea squirts, are invertebrates), probably all of the chordates you've ever seen are vertebrates. Vertebrates are animals that have a backbone made of segments called vertebrae.

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Phylum Chordata contains five groups of vertebrate animals: fishes, amphibians, reptiles, birds, and mammals. Here's some information about each:

Fishes

The earliest fishes had no jaws and survived as scavengers or parasites. Most familiar fishes, however, do have jaws, and they form two major groupings: the cartilaginous fishes (e.g. sharks, skates, and rays) and bony fishes. Bony fishes have a bony skeleton and a swim bladder that makes them buoyant. All fishes are aquatic, using gills to extract dissolved oxygen from water, although a few also have lungs and can survive for short periods away from water. Surprisingly, the bony fishes comprise the largest and most diverse class of vertebrates! As land animals, this fact may be hard for most of us to appreciate.

Amphibians

This group includes such animals as frogs, toads, and salamanders. The word "amphibian" means "double life", which refers to their life in terrestrial and aquatic habitats. Although most amphibians have legs and other adaptations for life on land, all amphibians require free water for reproduction. Their eggs are surrounded only by a single membrane, so they are very vulnerable to drying out. Also, their larvae have an aquatic "tadpole" stage, strongly resembling a fish, through which they develop before they metamorphose into the mature, adult forms. Amphibians also have thin, moist skin through which they breathe.

Reptiles

Snakes, lizards, turtles, and dinosaurs are (or were!) reptiles. These animals were the first to become completely independent of the aquatic habitat. First, their eggs are protected by a shell that contains food and moisture for the developing embryo. Thus they can reproduce away from water. Second, their skeletons are stronger and better adapted to support the animals in air than those of amphibians. Finally, reptiles have dry, scaly skin that protects them from drying out. This works because their lungs are efficient so, unlike amphibians, reptiles do not have to breathe through their skin.

Birds

All birds have wings and hindlimbs (legs) for perching, walking, grasping food, etc. They also all have beaks, and like reptiles they all lay waterproof, self-contained eggs. They also have feathers, a feature found in no other animals. Feathers are essential for flight and insulation. They evolved from reptilian scales, and birds still have scales on some parts of their bodies, especially their feet. Most birds are adapted for flight, with hollow, thin bones and adaptations for a high rate of ATP production – because flight takes a lot of ATP!

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Mammals

This group includes animals as diverse as tiny shrews, bats, cows, humans, dolphins, and whales. Mammals can fly, swim, climb trees, burrow underground, and walk upright. What unites these different animals? All have fur or hair, and all nourish their young with milk. The young of most mammals develop inside the mother in the uterus (although spiny anteaters and the duck-billed platypus are monotremes, which are mammals that lay eggs). In marsupials such as kangaroos, the young develop for a short time in the uterus, but the embryos soon crawl from the uterus into a pouch on the mother's body. Humans and most other mammals are placental, meaning that the entire development of the embryo occurs inside the mother.

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Appendix

Mariëlle Hoefnagels and Sarah Greenwood

For some labs in this course, you will need to turn in a writing assignment called an abstract. Technically, an abstract is a condensed version of a scientific paper – but for our purposes, an abstract is a mini-lab report. It should tell the essential information about your experiment, including the following:

1. **A concise introductory statement of the objective, hypothesis, and predictions**: Include 1-2 sentences that tell what you were trying to find out, what you THOUGHT would happen, and why you thought so.
2. **Experimental design and methods**: Boil down your experimental design and methods to a few sentences that tell the essential features. Write it in the past tense ("We placed our tubes in a 37°C water bath)" not in command format ("Place the tubes in a 37°C water bath"). Don't just copy the instructions word-for-word from the lab manual – instead, summarize your methods in your own words. Also, don't get too bogged down in details (for example, you don't have to write, "We recorded our data in our lab books"). Just give enough information so that a reasonably skilled reader could understand and repeat the experiment based on your description. (Feel free to add a photo if it helps you to explain your work; if you include a photo, it must have a figure number and caption and be referenced in the text, just as you would for other figures and tables.)
3. **Results**: Describe your results in your own words. Don't restate the exact numbers from your table/figure, but indicate the trends you observed (if any). Be sure to reference your tables and/or figures in conjunction with their appropriate results statement. For example, you could say, "The growth rate increased as the temperature was raised (Figure 1)." See below for guidelines for presenting data in tables and figures. (Feel free to add a photo if it helps you to explain your work, but photos are no substitute for tables and figures.)
4. **Conclusions**: In one or two sentences, state what you can take away from the results. Did the experiment turn out the way you expected? Did you support or refute your hypothesis? If your results did not support your hypothesis, what do you think happened? Regardless of the outcome, what limitations of the experiment might affect your conclusions? How would you change the experiment if you were to do it again?
5. **References** (if any): Note any references you used (besides the lab manual) to help you interpret your results or arrive at your conclusions.

Note: As you write your abstracts, use the sample abstract and graph at the end of this appendix as a model. Pay particular attention to the organization of the sample abstract and the presence of all of the essential components**.**

Each abstract you turn in for this class should follow the format described above (and shown in the sample at the end of this appendix. Each abstract must also:

- Be typed, double-spaced, and carefully proofread.
- Include your name and the names of your lab partners.
- Include your raw data in a table.
- Include a graph of your data, made in Excel or a similar spreadsheet program (no hand-drawn graphs).
- **Be your own work**! This includes the **text** of your abstract and your **graphs**. Yes, you worked together to design the experiment and collect the data, but your abstract and graph must be your very own, identical to no others in the class. Your TAs will be looking for unique features that should reflect independent work, such as the title, axis labels, graph design, etc.

Grades will be based on the following criteria:

- Objectives, hypothesis, and predictions are clearly explained.
- Methods are clearly explained in sufficient but not excessive detail.
- Experimental design is valid.
- Results are clearly and accurately explained.
- Tables/figures have in-text references (callouts), are formatted correctly, and have complete captions.
- Conclusions are clearly explained and connect the results to the stated purpose/hypothesis.
- Caveats and limitations are clearly explained.
- Grammar/spelling are correct.
- Presentation is neat and organized.

Remember, we are here to help you. Talk to one of your instructors if you are having trouble with your abstract. Also, the O.U. Writing Center is an excellent resource for helping you to improve your writing.

A sample abstract appears on the next page. Read it, then answer the questions that follow.

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Sample Abstract

(Your name)

(Your lab partners' names)

Pulse rates of self-reported athletes and non-athletes in an introductory biology class.

Introduction and objectives	The lungs acquire oxygen, and the heart delivers oxygen-rich blood to the muscles; to excel in most sports, athletes must therefore expand the capacity of their heart and lungs. To investigate whether this trend holds for self-reported athletes in biology class,
Hypothesis and predictions	our group tested the hypothesis that athletes undergo sustained conditioning and therefore should have better cardiovascular fitness than non-athletes. One measure of cardiovascular conditioning is a person's pulse rate before and after he or she exercises. We predicted that the pulse rates of athletes would be lower both before and after exercise than those of non-athletes.
Methods	The class selected six people (three athletes and three non-athletes) to participate in the experiment. Athletes were defined as those who exercised for at least 30 minutes three times a week. The resting pulse rate of each subject was measured by counting the number of beats in a 15-sec interval, then each subject stepped on a uniform (20-cm) platform at a constant rate for one minute. We then measured the post-exercise pulse rate of each subject.
Results and conclusions	As we predicted, athletes recorded substantially lower average resting and post-exercise pulse rates than non-athletes (Table 1; Figure 1). These results support our hypothesis that athletes have better cardiovascular fitness than non-athletes.
Caveats/ limitations	Our experiment did not, however, control for variables other than exercise that can affect cardiovascular fitness such as gender, age, weight, etc. If we were to repeat this experiment, we would use more subjects and screen them for such factors.

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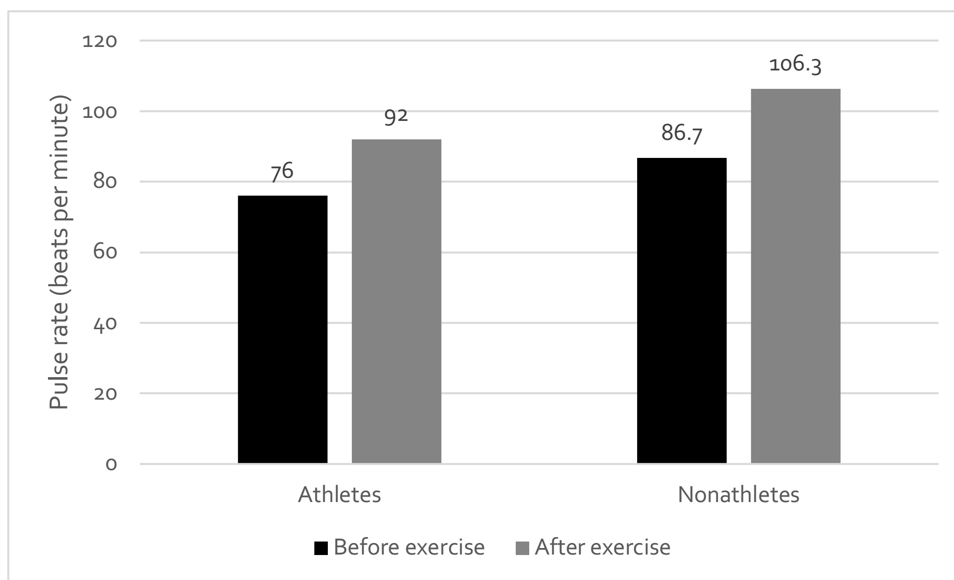
Table 1. Average pulse rates (beats per minute) before and immediately after one-minute exercise session for athletes and non-athletes.

	Resting pulse rate	**Pulse rate after exercise**	**Change**
Athlete #1	76	100	24
Athlete #2	76	100	24
Athlete #3	76	76	0
Athlete average	**76**	**92**	**16**
Nonathlete #1	84	104	20
Nonathlete #2	92	112	20
Nonathlete #3	84	103	19
Non-athlete average	**86.7**	**106.3**	**19.7**

Figure 1. Average pulse rates (beats per minute) before and immediately after one-minute exercise session for athletes and non-athletes.



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Sample Abstract: Analysis

You can use the sample abstract to help you build your own abstracts for this class. Answer the questions in the following worksheet, and use your answers to determine how you will word similar statements in your own abstracts.

Sample abstract	Your own experiment
1. How does the sample abstract word the objectives or purpose of the experiment?	1. How will you word the objectives or purpose of your experiment?
2. How does the sample abstract word the hypothesis being tested?	2. How will you word the hypothesis being tested?
3. How does the sample abstract word the predicted outcome of the experiment?	3. How will you word the predicted outcome of your experiment?
4. In how much detail does the sample abstract explain the experimental methods? Could you reproduce the experiment if you wanted to?	4. How will you word your experimental methods, striking a balance between excessive and insufficient detail?
5. How does the sample abstract word the results of the experiment?	5. How will you word the results of your experiment?

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6. How were the figures/captions in the sample abstract designed? Do they conform to the requirements explained in this appendix?	6. How will you design the figure(s)/captions in your abstract? Do they conform to the requirements explained in this appendix?
7. How were the tables/captions in the sample abstract designed? Do they conform to the requirements explained in this appendix?	7. How will you design the table(s)/captions in your abstract? Do they conform to the requirements explained in this appendix?
8. How does the sample abstract "call out" the figures/tables in the body of the abstract?	8. How will you "call out" the figures/tables in your abstract?
9. How does the sample abstract declare whether the results supported the hypothesis and prediction?	9. How will you declare whether the results supported the hypothesis and prediction in your own abstract?
10. How does the sample abstract describe potential caveats and limitations to the experiment?	10. How will you describe potential caveats and limitations to the experiment in your own abstract?

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Tables and Illustrations: A Few Rules, Guidelines, and Examples

Tables

Each table should be numbered and include an informative caption that briefly describes the content of the table. Below is an example:

Table 1: Average number of times barked at passerby by age and breed

	Age				
Breed	1	2	3	4	5
Chihuahuas	5	9	8	7	8
Golden Retrievers	2	4	5	4	4

Graphs

In drawing graphs, use a scale that fits your data best; experiment a little to find the best increments. Two basic forms of graphs are line graphs and bar graphs.

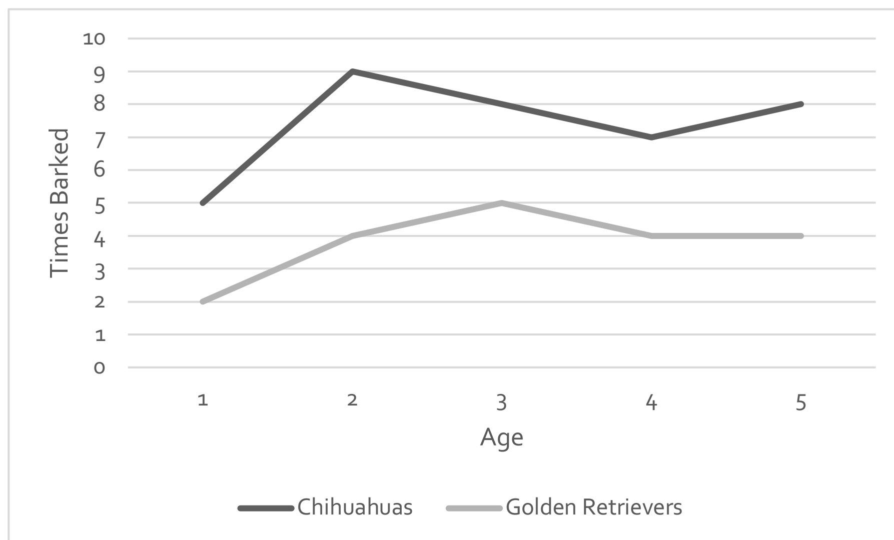

Figure 1: Average number of times barked at passerby for Chihuahuas and Golden Retrievers over two hours. Ten passerby walked by in total.

- **Line graphs** are appropriate for presenting continuous data. The independent variable is typically placed on the horizontal (x) axis; the dependent variable is typically placed on the vertical (y) axis. A line graph can contain two or more lines; if you use more than one line, use broken, colored, or dotted lines to represent each class of data. See **Figure 1** for an example of a line graph.
- **Bar graphs** are appropriate for presenting data that are in discrete groups. See **Figure 2** for an example of a bar graph.

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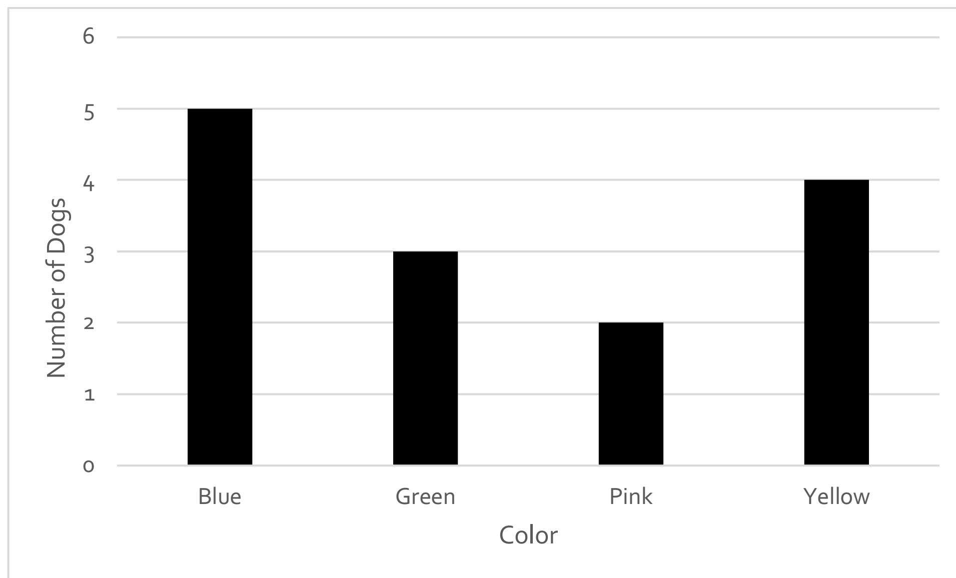

Figure 2: Number of dogs (out of fourteen) that walked to each color paper, when given a choice of blue, green, pink, and yellow paper equally spaced from the dogs.

Regardless of the type of graph you choose, each figure should be numbered and include an informative caption that summarizes the contents of the graph. Also, both axes should be labeled with the parameter and units of measure (e.g. "length, cm"). Each numerical axis should begin with zero and include equally spaced intervals (e.g. 0, 2, 4, 6 …). Refer to figures 1 and 2, and to the sample abstract, for examples.

Got photos?

Your abstract may include one or two carefully selected photos, if they help you tell your story; do not clog up your abstract with a large number of them. Each photo should have a figure number and an informative caption that summarizes what the photo shows.

Acknowledgments and Attributions

Sample Abstract Analysis table was inspired by the MATE ("Most Awesome Tool Ever") Tool developed by Patricia Goodson and Katherine L. Wright, as presented to M. Hoefnagels in a workshop at the 2018 Text and Academic Authors Association conference.

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