BIOLOGY 101L LAB MANUAL



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Oxnard College

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Oxnard College

By Sharbani Banerjee, Casey Barr, Joe Frantz, Melissa Graham, Tyler Wilson

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TABLE OF CONTENTS

Licensing

1: Labs

1.1: Lab Safety

1.2: Scientific Method

1.3: Metric Measurements

1.4: Microscopy

1.5: Cell Biology

1.6: Diffusion and Osmosis

1.7: Enzyme Function

1.8: Cellular Respiration and Photosynthesis

1.9: Mitosis

1.10: Meiosis and Gametogenesis

1.11: Inheritance and Human Genetics

1.12: DNA Biology

1.13: Evolution

1.14: Ecology

1.14.1: Ecology (Alternate)

Index

Glossary

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1: LABS

1.1: Lab Safety

1.2: Scientific Method

1.3: Metric Measurements

1.4: Microscopy

1.5: Cell Biology

1.6: Diffusion and Osmosis

1.7: Enzyme Function

1.8: Cellular Respiration and Photosynthesis

1.9: Mitosis

1.10: Meiosis and Gametogenesis

1.11: Inheritance and Human Genetics

1.12: DNA Biology

1.13: Evolution

1.14: Ecology

1.14.1: Ecology (Alternate)

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1.1: Lab Safety

The following guidelines are for students working in the laboratory at Oxnard College. It is mandatory that students follow these regulations. Students are expected to read these regulations and sign the Safety Form electronically. This procedure must be completed before a student can begin any laboratory activity. The student should keep the regulations for future reference.

General

1. Read all laboratory instructions before coming to class.
2. Dress appropriately for lab: closed-toe shoes, long hair tied back, no dangling jewelry or loose clothing. Avoid wearing contact lenses.
3. Know the location and proper use of the following in your laboratory: first aid kit, fire extinguisher, eye wash, emergency telephone, fire blanket, closest fire alarm, chemical shower, emergency exits, emergency gas shut-off valves, evacuation site for your classroom. Keep the safety equipment and exits free of obstruction.
4. Behave in a professional manner.
5. Only registered students are allowed in the laboratory.
6. Students are not permitted access to prep rooms, laboratory cupboards, or instructor desks without prior approval from the instructor.
7. Students who have any medical conditions (e.g. diabetes, immunological defect, pregnancy, epilepsy) that may necessitate special precautions in the laboratory must inform the professor.
8. No food or drink (including water) allowed in the laboratory.
9. Keep hands away from face, eyes and clothing while using hazardous solutions or materials. Do not handle contact lenses or apply cosmetics during class.
10. Maintain a clean, open work area free of everything except material related to the exercise.
11. Use all equipment and materials with care and as authorized by instructor.
12. Report all spills or accidents to the instructor. Clean up spills immediately.
13. Wipe down work surfaces at the beginning and end of each lab.
14. Wash your hands at the beginning and end of class.
15. At the end of lab make sure all items are returned to their proper location. Push in chairs. Make sure sinks and your work space are clean and free of debris.

Disease Precautions

1. Perform a Self-Check for the following symptoms listed below prior to coming to lab. If any symptoms are present, do not come to lab.
2. Fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea.

Emergency Preparedness

1. During an earthquake stay in the room and immediately get under the desk. After the earthquake follow your instructor's directions.
2. During a fire or other emergencies requiring evacuation, meet at the designated evacuation location. Students are required to meet and remain at the evacuation site until dismissed by the instructor.
3. During a shelter-in-place emergency, students should remain in the classroom and follow instructions.

Handling Equipment and Materials

1. Properly label all glassware and slides.
2. Keep all equipment and materials away from the edge of the lab benches.
3. Use protective aids (e.g. test-tube clamps, hot pads) when handling hot glassware.
4. Light gas burners only as instructed. Be sure no volatile or flammable materials are being used nearby. Remember to have

long hair tied back, no dangling jewelry or loose clothing.
5. When heating a test tube do not look into the mouth of the tube or point it into the direction of any person.
6. Heat sources should only be on when in use. Do not leave heat sources unattended. When in doubt, students should assume a heat source is on or hot.
7. Operate electrical equipment in a dry area with dry hands.
8. When removing an electrical plug from its socket, pull the plug, not the cord.
9. Inform your instructor immediately if equipment or materials are broken or not working properly.
10. Never return reagents back into their original containers. Keep containers capped when not in use.

Handling Hazardous Material

1. Wear appropriate eye protection as directed by the instructor.
2. Wear disposable gloves when handling hazardous materials including chemicals, organisms, or bodily fluids.
3. Wear protective clothing when required by the instructor.
4. Follow correct disposal procedures as detailed in “Cleanup and Disposal.”
5. To prevent contamination by needle stick injuries, always use new needles and lancets.
6. Use the fume hood whenever noxious, corrosive or toxic fumes are produced or released.

Bacteria and Fungi

1. Never open Petri dishes containing bacterial or fungal growth unless directed to do so by the instructor.
2. Dispose of all discarded bacterial and fungal cultures as directed by the instructor.

Cleanup and Disposal

1. Follow correct disposal procedures – dispose of used materials promptly and in the appropriate disposal containers.
    1. Dispose of broken glass in labeled glass disposal containers.
    2. Dispose of needles and lancets in puncture-proof sharp containers.
    3. Dispose of used chemicals in labeled and capped waste containers as directed by your instructor.
    4. For disposal of items contaminated with organisms or bodily fluids, as directed by your instructor. In general,
        1. Decontaminate slides, spectrophotometer tubes, and other glassware in 10% bleach.
        2. Dispose of soft items (i.e. gauze or swabs) in labeled biohazard containers.
        3. Dispose of all animal tissues in labeled biohazard containers.
    5. Place micropipette tips, sample vials, and contaminated glassware in a labeled waste container or designated area.

Safety Pledge (Please sign the digital form)

I have read the Biology Laboratory Safety Guidelines and understand the rules and procedures of proper safety in the biology laboratory course listed below. I recognize my responsibility and pledge to observe all safety rules in the biology laboratory at all times.

I acknowledge that the instructor has covered all safety guidelines and equipment that we may need to use this semester.

Name of Student (please print)_____

Signature of Student _____

Date _____

Course _____ Section _____ Professor _____

Student copy

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1.2: Scientific Method

Purpose:

This lab provides hands-on experience with the scientific method by studying animal behavior. Students will explore how pill bugs respond to environmental stimuli, practice experimental design, and apply data analysis to understand their behavior.

In this laboratory students will:

1. Observe pill bug behavior in a neutral environment.
2. Develop a hypothesis and design an experiment to test pill bug preference for moist vs. dry environments.
3. Collect, record, and graph data from multiple trials.
4. Analyze results and draw conclusions about pill bug behavior.

Student Learning Objectives

- Develop a clear and logical hypothesis based on observations.
- Conduct a controlled experiment with reliable data collection methods.
- Create accurate, well-labeled graphs of your result.
- Explain the findings clearly and relate them to your hypothesis.

Lab Safety

Safety Guidelines for Handling Pill Bugs and Lab Equipment:

1. Handle pill bugs gently using a soft brush or plastic spoon to prevent injury to the animals.
2. Ensure behavior chambers are stable and securely assembled.
3. Avoid over-moistening the environment to prevent accidental spills.
4. Wash hands thoroughly after handling pill bugs or environmental materials.
5. Dispose of any biological waste in designated containers.

What is Science and how do we do science?

Science is how we gain knowledge about the natural world. Typically, it pertains only to what we can investigate or observe using our senses – or instruments that extend the ability of our senses. As a science, biology concerns itself with understanding the unity and diversity of living things – the 2,300,000, or so, described (and millions of undescribed) species with which we share the planet.

Ideally, the **Scientific Method** is a **process** that describes how scientists perform investigations to provide a systematic and rational approach to answering questions about the natural world. One goal is to be as objective as possible in what we study. Ideas that can't be tested, directly observed, or measured in some way should not be subjected to the scientific method. There are other ways to obtain knowledge (cultural, emotional, etc.), but they usually do not qualify as science because they do not follow the axioms of science. An axiom, in a general sense, is a truth that is accepted without proof. It might seem that science does not assume anything, but many assumptions are often ignored such as the relationship between cause and effect, and that our senses and measurements accurately represent reality.

The goal of today's lab is to familiarize you with the idealized steps of the scientific method but it's important to recognize that science rarely proceeds so linearly.

You will use these steps to determine the effects of different environmental conditions on the behavioral patterns of pill bugs (*Armadillidum vulgare*).



Figure 1: *Armadillidum vulgare*

Model organisms are non-human species used in research to investigate biological processes. Information learned in studies of model organisms can often be applied to other species, including humans. We use model organisms to learn about many different processes, including genetics, cellular mechanisms, and growth and development. Certain characteristics make a species an ideal model organism. For example, it must be easy to manipulate for study, inexpensive and easy to cultivate, and produce many offspring. The best model organism to use for a study depends upon the question being investigated. In this study, *Armadillidium* is a good model organism because of its simple behavioral patterns, which allows measurement of responses to a stimulus and gathering data on how this organism is affected by different environmental conditions.

Steps of the Scientific Method

The scientific method consists of the following steps:

1. Making an **observation**
2. **Asking a question** based on that observation
3. Forming a logical AND testable answer to that question, the **Hypothesis**
4. **Designing a controlled experiment** to see if the hypothesis is supported or rejected
5. **Collecting**, **analyzing**, and **interpreting the data** generated by the experiment
6. **Making a Conclusion**: Do the results support the hypothesis?

If the conclusion of an experiment does not support a hypothesis, another hypothesis must be developed and another experiment designed to test it. Many times, designing an experiment to test the hypothesis is one of the most difficult aspects of science. Ultimately, the results of experimentation are often published in peer-reviewed journals so that other researchers can replicate the experiment and build on that work.

An **idealized version of the scientific method** is demonstrated in Figure 2. It is considered “idealized” because chance sometimes play an important role. Sometimes, observations that result in important discoveries are stumbled upon by accident.

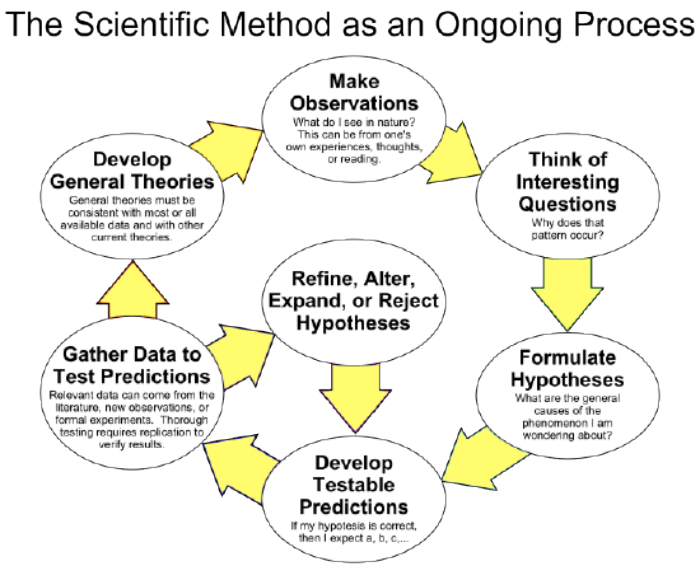


Figure 2: Idealized Steps of the Scientific Method (This image is an SVG adaptation of the original JPEG image by Whatiguana CC-BY)

Purpose

The purpose of this experiment is to provide hands-on experience with the scientific method by studying animal behavior. You will investigate how environmental factors affect pill bug habitat preference. This experiment will help you practice key scientific skills: observation, hypothesis formulation, experimental design, data collection, and data analysis. Moreover, you will develop your ability to reason scientifically about biological phenomena and connect experimental results to real-world behaviors in organisms.

You will design and carry out an experiment to study how pill bugs respond to different two different environments, one moist and the other dry.

Formulating a Question and Stating a Hypothesis

In science, observations often lead to the formulation of questions that generate hypotheses and predictions that are testable. In today's lab, we are considering the following question: "**Do pill bugs show a preference for a dry environment or a moist environment**"?

A **hypothesis** is a testable explanation of a set of observations based on available data. It is a tentative answer to the question you are asking based on knowledge about what you're observing and asking. This knowledge can be pre-existing or information from a published resource.

In this lab, you need to formulate a hypothesis about how you believe these two different environment conditions will affect the behavior of pill bugs based on your knowledge of pill bugs. After you formulate the hypothesis, you will test predictions based on these hypotheses. Hypotheses can be rephrased as predictions and can be written as "**If**...., **then**...." statements.

Formulate a hypothesis to describe what you predict will happen in your experiment (dry versus moist environments). Read through the procedure section of the lab before you define the variables.

Hypothesis: Pill bugs are more likely to spend time in a _____ environment because _____.

Prediction: If pill bugs can choose between a dry and a moist area, then more pill bugs will be found in the _____ area after 10 minutes.

An **independent variable** is the condition or event under study. It is the condition *the investigator* sets. Only one independent variable is tested at a time so that an observed response is a response to just that variable.

A **dependent variable** is what happens in response to the independent variable. The dependent variable provides your data. This is what you measure in your experiment.

Controlled variables are conditions kept the same during an experiment because they could affect the results. For example, both sides of the pill bug dish should be kept at the same temperature. In the spaces below, define the variables that will be considered in your experiment today:

What is the **independent variable**? _____

What is the **dependent variable**? Be specific with your answers. _____

What other **variables** should be **controlled**? List at least 2 controlled variables.

1. _____
2. _____

Importance of a Control Group:

Most well-planned experiments contain a **control group** in addition to an **experimental group**. The control group is not manipulated and is used for a comparison to the experimental group; it serves as a baseline against which the experimental group is compared. Not all experiments have a traditional control group.

MATERIALS AND METHODS

Materials and Supplies:

Materials

Equipment	Materials
Behavior chambers: Divided containers that allow separation of different environmental conditions (moist and dry sides)	**Pill bugs (*Armadillidium vulgare*):** 5-10 pill bugs (per group).
Soft brush or plastic spoon: To safely handle and transfer pill bugs between environments.	**Moist substrate:** Damp paper towels or moistened soil, used to create a humid environment on one side of the chamber.
Stopwatch or timer: To keep track of when to count the pill bugs on each side of the chamber (every 2 minutes).	**Dry substrate:** Dry paper towels or dry soil, used to create a dry environment on the other side of the chamber.
Data sheets: For recording observations, including the number of pill bugs on each side of the chamber at each time interval.	**Transfer pipette:** To add moisture to the substrate as needed throughout the experiment.

PROCEDURE

Part 1: Initial Observations and Drawing

Observation Period:

Place 5 pill bugs in a neutral environment (such as an empty Petri dish) and observe their behavior for 5-10 minutes. Record observations about their movements, responses to stimuli, and any distinctive behaviors below.

_____

_____

_____

Part 2: The Pill bug Enclosure

Each group will receive one Petri Dish Enclosure:

- The enclosure consists of two large petri dishes connected by a small opening in the side of the dish. This connection allows the pill bugs to travel from one dish to the other.
- The enclosure allows you to create two different environments, designed on the variables you plan to test, in this case a moist versus a dry environment.
- Prepare each dish by setting up your chosen environment in each. For example, one Petri dish might be lined with damp paper towels while the other is kept dry.

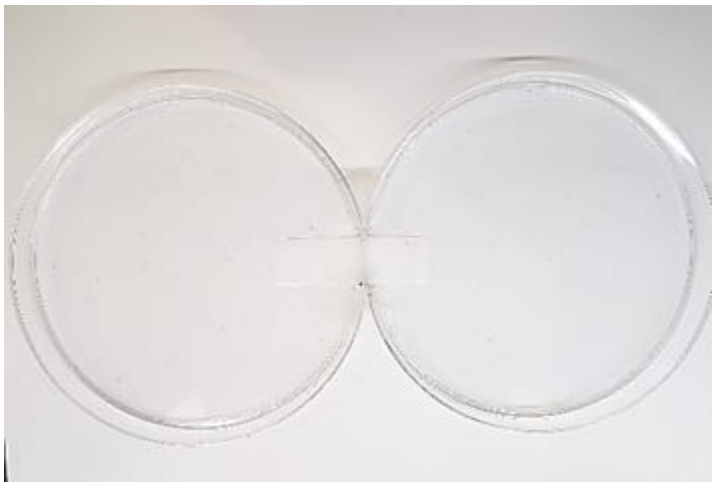


Figure 3: Environmental Chamber (By MG)

Part 3: Conducting the Experiment

Introduce Pill bugs to the Attached Dishes:

- Using a soft brush or plastic spoon, gently place 5 pill bugs into the dry dish of the connected Petri dishes, allowing them to explore both environments freely.
- Make sure that the pill bugs can easily move between the two dishes.

Observation and Data Collection:

- Start the timer and observe the pill bugs for 10 minutes. Every two minutes, record how many pill bugs are present in each environment.
- Use a data table like the one below to track your observations:

Table 1.2.1

Time (Minutes)	# of Pill bugs in Dry Environment	# of Pill bugs in Moist Environment	Notes on Behavior
0			
2			

Time (Minutes)	# of Pill bugs in Dry Environment	# of Pill bugs in Moist Environment	Notes on Behavior
4			
6			
8			
10			

Repeat the Experiment:

- After completing the first trial, return the pill bugs to their holding container.
- Repeat the experiment for **two** additional trials. This makes your data more reliable.

Time (Minutes) Trial Two	# of Pill bugs in Dry Environment	# of Pill bugs in Moist Environment	Notes on Behavior
0			
2			
4			
6			
8			
10			

Time (Minutes) Trial Three	# of Pill bugs in Dry Environment	# of Pill bugs in Moist Environment	Notes on Behavior
0			
2			
4			
6			
8			

Time (Minutes) Trial Three	# of Pill bugs in Dry Environment	# of Pill bugs in Moist Environment	Notes on Behavior
10			

Part 4: Analyzing and Graphing the Results

Average the Data:

- After completing all trials, calculate the average number of pill bugs in each environment at each time point. To calculate the average, add the number of pill bugs in each environment at each time point and divide by the number of trials (3) to find the average.
- Summarize the results in a table, showing the averages for both environments across time intervals.

Summary Table of Results

Time (Minutes)	# of Pill bugs in Environment A	# of Pill bugs in Environment B
0		
2		
4		
6		
8		
10		

Discuss any experimental limitations and any improvements for future experiments.

_____

_____

_____

Summarize your data in a graph.

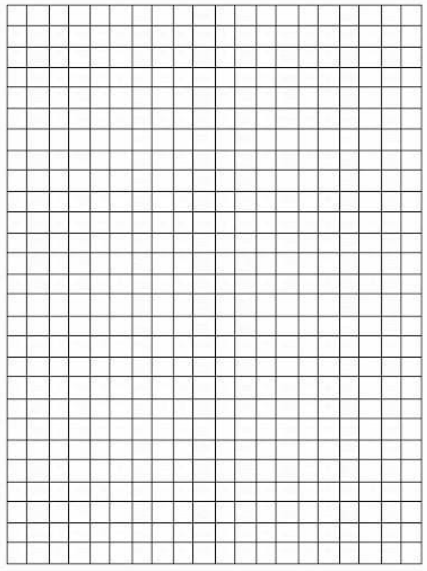
1. **Tables and graphs** help you organize data and see patterns in your results. A graph can show how one variable changes in relation to another. Usually, the independent variable goes on the horizontal (X) axis, and the dependent variable goes on the vertical (Y) axis. Each axis should be labeled properly with the name of the variable and the units of measurement.

2. **Use an even scale on each axis.** For example, if one mark represents 2 minutes, each following mark should represent another 2 minutes. Start at zero when it makes sense for the data.
3. All graphs should have a **title** to describe the information presented. There are no set rules for making the title, but I recommend capitalizing the important words in the title.
4. Choose a graph that best represents the type of data you collected:
    1. **Line graphs** show how a measured value changes over time. Points are plotted and connected with lines so you can see whether the value increases, decreases, or stays about the same. Since the pill bug counts are recorded every two minutes, this fits this exercise well.
    2. **Bar graphs** compare amounts in different groups or categories. Each bar represents one group, making it easy to see which groups have more or less. For example, a bar graph could compare the number of pill bugs on the moist and dry sides **at one selected time**.

Graphing Your Results

- Create a line graph to visualize the data. Plot time (minutes) on the X-axis and the average number of pill bugs on the Y-axis.
- Use separate symbols to represent the number of pill bugs in each environment, such as dots or triangles. Connect the symbols with lines, as shown in the sample graph below.

Use the grid below to graph your group's results:



Interpret Your Results:

Once you have collected your data and summarized it as a graph, the last step is to analyze and interpret your results. What do they tell you about your hypothesis? Was it **supported** or **not supported by your results?** _____. What evidence from your data supports your answer? _____

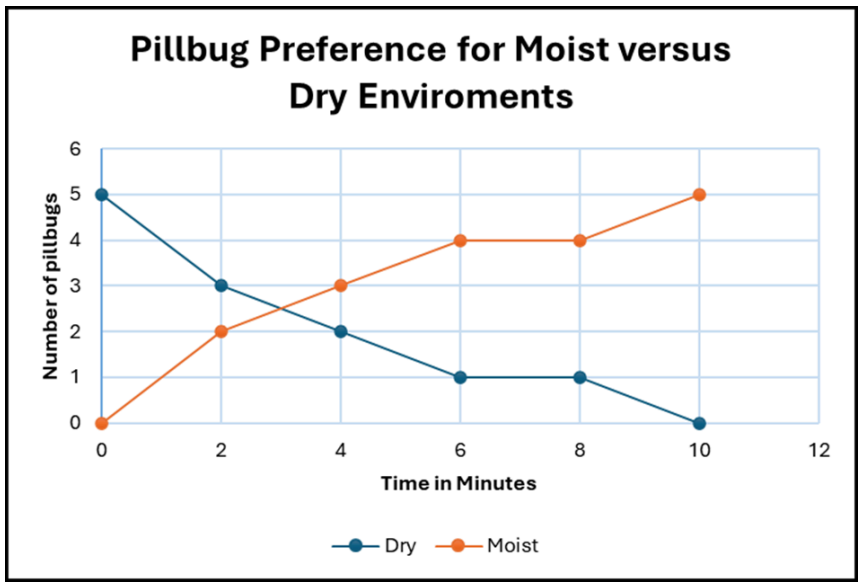
_____

Sample Data Table and Graph

Time (Minutes)	Average # of Pill bugs in Dry Environment	Average # of Pill bugs in Moist Environment

0	5	0
2	3	2
4	2	3
6	1	4
8	1	4
10	0	5

Sample Graph



Questions for Review

1. What is the difference between a control group and a controlled variable?
2. What are some types of questions science cannot answer?
3. Where would you find an independent variable on a graph?
4. List four features of a well-formatted graph. Use the graphing guidelines above to help you.

- _____
- _____
- _____

• _____

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1.3: Metric Measurements

How We Measure in Science

Learning Objectives

- 2.1 Metric System of Measurements and the base 10
  - Convert from one unit magnitude to another
- 2.2 Measuring Lengths
  - Determine which units to use
  - Practice measuring different lengths with different tools
- 2.3 Mass and Weight
  - Define mass
    - Understand why we use weight to find mass
  - Define weight
- 2.4 Volume and Density
  - Ways to measure volume
  - Define density
    - Be able to calculate/measure density
- 2.5 Temperature
  - Compare and contrast Fahrenheit and Celsius
    - Where might Fahrenheit be more appropriate in science
  - Understand the defining events for setting the scale of Celsius
  - Convert from Fahrenheit to Celsius and back

Introduction

Scientists needed a uniform set of units for which to measure with, leading to the creation of the Metric System. One of the major innovations of the metric system was to create units all related on a base 10 scale. Making it easy to convert 50 cm to .5 meters, or 30 cm^3 to .03 L. Making a standardized system of measurements made it easier for scientists to communicate, increasing science's ability to build on new discoveries.

Table 1 Metric conversions

Distance	.001 Kilometer (km)	1 Meters (m)	100 Centimeters (cm)	1000 Millimeters (mm)	10 (10^6) Micrometer (µm)	$10(0^9)$ Nanometer (nm)
Volume	.001 Kiloliter (kl)	1 Liter (L)	100 Centiliters (cl)	1000 Milliters (ml)	10 (10^6) Microliter (µl)	$10(0^9)$ Nanoliter (nl)
Mass	.001 Kilogram (kg)	1 Gram (g)	100 Centigrams (cg)	1000 Milligrams (mg)	10 (10^6) Microgram (µg)	$10(0^9)$ Nanogram (ng)

Weight	.001 Kilonewtons (kn)	1 Newton (N)	100 Centinewton (cn)	1000 Millinewton (mn)	10 (10^6) Micronewton (µn)	10(0^9) Nanonewton (nn)

Experimental procedure measuring length and conversions

Using small ruler measure some objects to the nearest mm, then convert them as directed.

1. Small wooden dowel is mm long and cm long and m long.
2. Small pebble (you can use calipers for this one, calipers can be helpful for measuring oddly shaped objects consistently) is mm long and µm long and nm long.
3. The metric system also makes it easy to convert from cm^3 to ml when measuring volume, as 1 cm^3 is equal to 1 ml. Get one of the small wooden blocks and measure its length, width, and height with a small ruler in mm. Then multiply all three of those numbers together to get the volume in mm^3 after that convert to_cm^3 then finally to ml.
4. Get one of the large meter sticks and measure one of the provided bones cm and convert it to_m and then finally to mm.

Mass and Weight

Mass is the measure of how much matter is present in object either living or not. It is fundamentally different than weight, which is the measure of how much force an object exerts on another object based on their gravitational attraction. On Earth we frequently these terms interchangeably, this is because the Earth pulls on all objects on or near its surface equally. In other countries where it is common to use the metric system when asked how much they weigh, the normal response is in kg the unit of mass. The easiest way to see the difference between these is if someone is 75 kg on Earth, they would still be 75 kg on Mars (a planet with a smaller gravitational pull). However someone who is 165 pounds on Earth would only weigh 62.2 pounds.

We use this interchangeably on Earth also because we calculate an objects mass with scales that measure an objects weight. This is due to any object on Earth that weighs 165.35 pounds will always be an object with 75 kg of mass.

Experimental procedure Mass

1. Using a scale measure the mass of several objects and record the results in the table below.
2. Then convert all of the mass to mg.

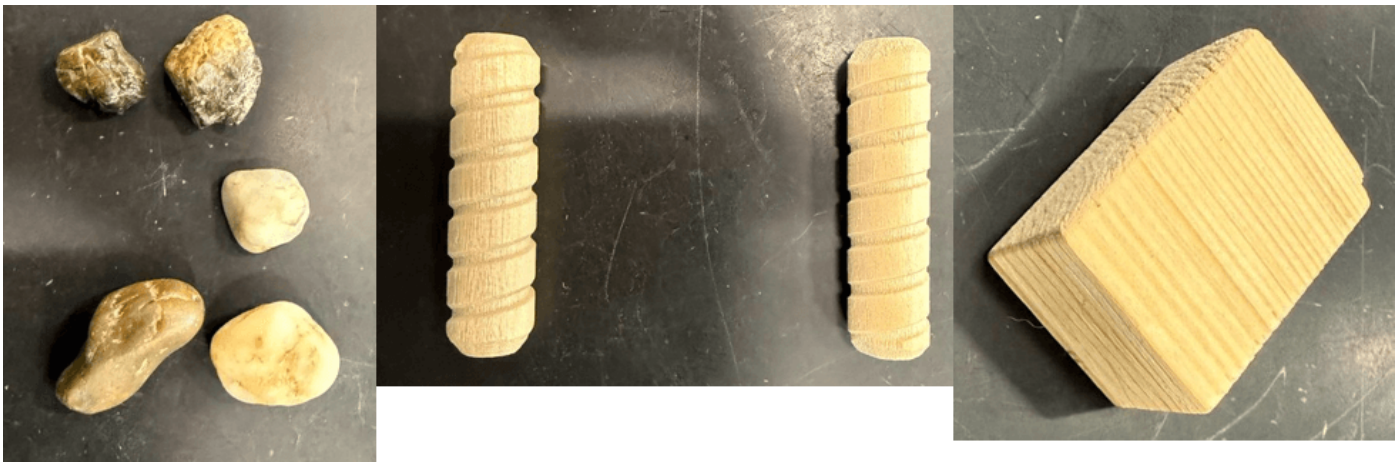


Figure 1: Possible objects to be weighed, from left to right: Pebbles, wooden dowels, and wooden block. The dowels and the block are geometric shapes with calculable surface areas and volumes if their dimensions are measured. The pebbles represent objects with non-calculable surface areas or volumes.

Table 2 Masses

Object	Pebble	Wooden dowel	Wooden block	Glass ball	Copper ball
Mass in (g)					
Mass in (mg)					

Follow-up questions

1. What units would you use to measure a person's mass?
2. What units would you use to measure a person's height?
3. What units would you use to measure the diameter of a cell?
4. What units would you use to measure the volume of femur bone?
5. Why wouldn't you use µm to measure a person's height?

Volume and Density

Volume is the measure of how much three dimensional space an object takes up. For basic rectangular object you can simply multiply an objects width X height X length to get its volume. In the metric system we can use both cubed measurements of distance such as cm^3 as well as conversions of liters. Switching between the two is also easily done as 1 cm^3 is the same as 1 ml. Cylinders and spheres are also easily measured for their volume using the formulas

$V=\pi r^2 h$ and $V=\pi r^3$

where π is 3.14, r is the radius of the cylinder or sphere, and h is the height of the cylinder.

For odd shaped objects it can difficult to measure their volume, instead we can use their displacement to calculate their volume. A graduated cylinder is a glass tube that has been graduated with measurements of volume, usually ml or l. When filled with water of a certain amount for example 50ml in a 100ml graduated cylinder, if we then drop an object such as a pebble into the water, then the displacement of the water in ml will be equal to the volume of the pebble.

Experimental procedure measuring and calculating volume

1. Using the same objects that we measured for mass, measure and calculate their volume. You should make sure to choose the appropriate method for finding their volume. Fill out the table of volumes below.

Table 3 Volumes

Object	Pebble	Wooden dowel	Wooden block	Glass ball	Copper ball
Volume in (cm^3)					
Volume in (ml)					

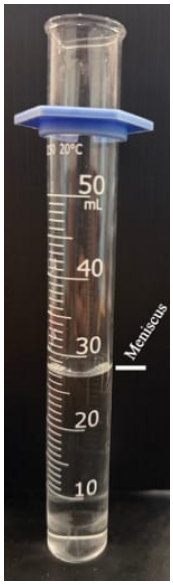


Figure 2: Example of a graduated cylinder for measuring liquid volume. Always measure at the bottom of the meniscus. Due to surface tension created by Hydrogen bonds in water, the surface of the water will form a depression rather than being flat.

Now that we have found the mass and the density of several objects we can also find their densities. Density is the relationship between the mass of an object and its volume. Density is represented as g/ml or g/cm3, or any conversion there of. Now that you have both the table of masses and table of volumes filled out, calculate the density of the objects measured. Record your results in the table of densities.

Table of Densities

Object	Pebble	Wooden dowel	Wooden block	Glass ball	Copper ball
Density in (g/cm^3)					
Volume in (g/ml)					

Follow-up questions

1. How did the densities compare to each other, and were there any that surprised you?
2. What units would you use to describe the density of lead?
3. If you have chunk of gold and you want to know how pure it is how would you go about doing that?

Temperature

In the metric system we use degrees Celsius (°C) most often, but in some cases we Kelvin (K) to measure temperature. There are some instances in science where degrees Fahrenheit (°F) are used instead of °C. Fundamentally temperature is a measure of the kinetic energy of molecules or how much the molecules are moving at the atomic level. The higher the temperature the more the molecules are moving, and is what gives us the primary states. Solids have molecules that barely move, liquids molecules move freely, gasses molecules move freely and to a much greater degree than liquids.

The degrees Celsius scale were modeled after the changes of state of matter of water. Freezing/melting point of water is at 0°C, and the boiling/condensing point of water is at 100°C. This compares to 32 °F and 212 °F in the degrees Fahrenheit scale. We can switch between the two scales by using the following formulas:

°C = (°F -32)/1.8 and back using °F = (1.8°C) + 32

The primary difference between Celsius and Fahrenheit is how fine of a scale they are. In °C between the temperature of freezing to boiling is just 100° while between the same points in °F there are 180°. This makes Fahrenheit a finer scale to use when measuring temperature, similar to how mm are a finer scale than m.

Experimental procedure

Using a thermometer find the temperatures of several items around the room in °C, and make sure to allow time for the thermometer to register the temperature.

Room temperature = °C. Surface of your skin = °C

Cold tap water = °C. Hot tap water = °C and ice water = °C

Follow-up questions

1. When would you expect using °F would be better suited for scientific measurements?
2. Did you notice the comparison between normal room temp and body temp in both °C and °F, what are the approximate equivalent temperatures for each?

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1.4: Microscopy

Introduction

Microscopes are among the most useful tools used by biologists. In this lab, you will learn how the microscope works, its limitations, and practice using it. There are different types of microscopes that magnify from as low as 10x to as much as 650,000x!

You will work with 2 types of microscopes: a compound light microscope and a stereoscopic dissecting microscope.

Activity A: Handling and Care of the Compound Light Microscope

In the lab, the Compound Light Microscopes (LM) are in the glass cabinets along the wall of the lab room. It is best to always use the same microscope, so note the number on it. Take good care of your microscope by following these guidelines:

- When retrieving your microscope from the cabinet, pick it up using two hands. Put one hand under the base and one around the arm. It is heavy, and you always use two hands!
- Clean the lenses before each use with lens paper that is on the lab tables and at the front of the class.
- If a lens accidentally touches a slide or gets wet, immediately dry and clean the lens with lens paper.
- When you move the microscope on the lab table, lift it rather than trying to slide it.

When you are done using the microscope, follow these steps:

- Remove the slide from the microscope and put it back in the appropriate tray.
- Using the revolving nosepiece, turn it back to the scanning (4x) objective lens - red.
- Lower the stage all the way using the coarse focus adjustment knob.
- Turn off the light and then unplug the microscope.
- Wrap the cord and tuck it in so that it won't come loose.
- Clean the lenses with lens paper.
- Using two hands, carry your microscope back and put it in the cabinet in the place where you picked it up. It will fit in sideways.

Activity B: The Compound Light Microscope

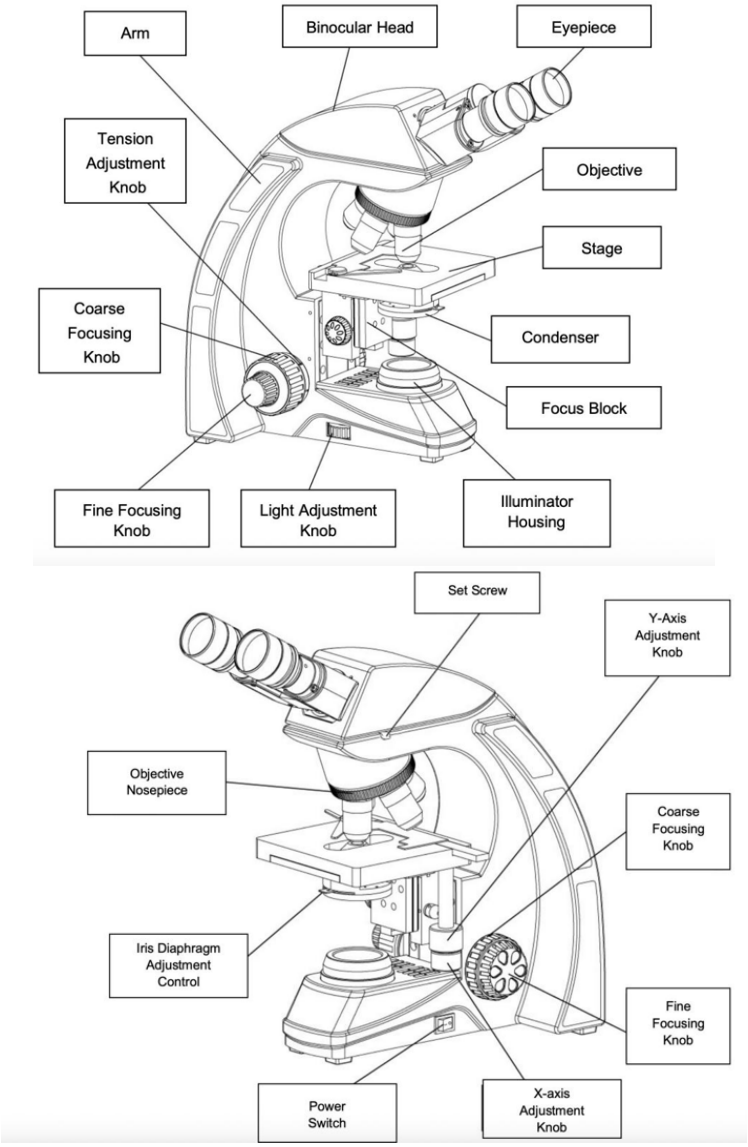


Figure 1: Oxnard College's Accuvision 3000 - LED Compound Light Microscope Accu-Scope 3000-LED Instruction Manual (Public Domain)

The **arm** holds the magnifying portions on the top and the illumination and focusing portions on the bottom.

Lenses on top are called **ocular lenses,** and they **magnify 10X.** There are 2 of them, making this a binocular microscope.

Lenses below are **objective lenses,** and they are in a **revolving nosepiece.** When you rotate the nosepiece, each one will click into position. Look at them, and you will see the magnification of each written on the side - **4X,10X, 40x, 100X**. Total magnification is the magnification of the ocular multiplied by the magnification of the objective. For example, with the 4X objective, total magnification would be **40X. Fill in Table 1** with the total magnifications for each lens and the name of each lens.

Field of view is the area of the slide that you can see. Higher magnification will give you less field of view, but you will see greater detail.

Light is directed through the condenser, the stage opening, the **slide** with the specimen, the **objective le**ns, the ocular **lens,** and then to the **retina of your eye.**

Light can be controlled with the **iris diaphragm adjustment control** on the condenser**,** which opens and closes a hole, allowing

more or less light through the condenser; this also affects contrast, and by the **light adjustment knob** on the left side of the base. Use medium to low amounts of light, since this affects color saturation, detail, and depth of field.

Place the slide that holds the specimen you are studying on the stage and hold it in position with the **stage clips** (not labeled in the diagram). You move the slide left/right with the **x-axis stage adjustment knob** and towards/away from you with the **y-axis stage adjustment knob.**

The **Coarse Focus knob** raises and lowers the stage to focus on the specimen. Always **start with the 4x objective** by raising the stage all the way with the **Coarse Focus,** but **do not use the Coarse Focus** with any of the other objective lenses**.** The **Fine Focus Knob** raises and lowers the stage very slightly with each turn. This is the knob that you use when looking through the microscope with the higher-powered **objectives - 10x, 40x and 100x.**

Activity C: Using a Prepared Slide - letter "e"

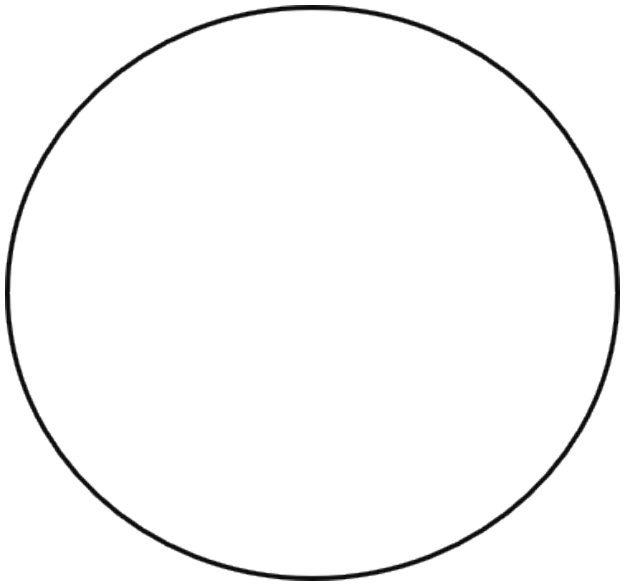
Follow these steps to get the letter “e” slide in focus with the scanning objective lens.

1. Get a slide from the front desk that has a letter “e” on it.
2. Put it on the stage and secure it with the stage clips.
3. Use the **x and y-axis adjustment knobs** to move the “e” so that it is **centered over the hole** in the middle of the stage.
4. **Turn the nosepiece** until the **scanning objective lens (4x)** colored red “clicks” into place.
5. Turn the coarse adjustment knob towards and away from you. This lowers and raises the stage. **Raise the stage all the way while** watching from the side as you do so.
6. **Look through the ocular lenses,** and you should see the “e” It will probably be out of focus and not centered.
7. Center it using the **Stage Adjustment Knobs** and **focus using the Fine Focus only**. **Do not use the Coarse Focus for this.**
8. Adjust the light using the **Condenser** and the **Light Adjustment Knob** to a level that is comfortable for your eyes, not too bright.



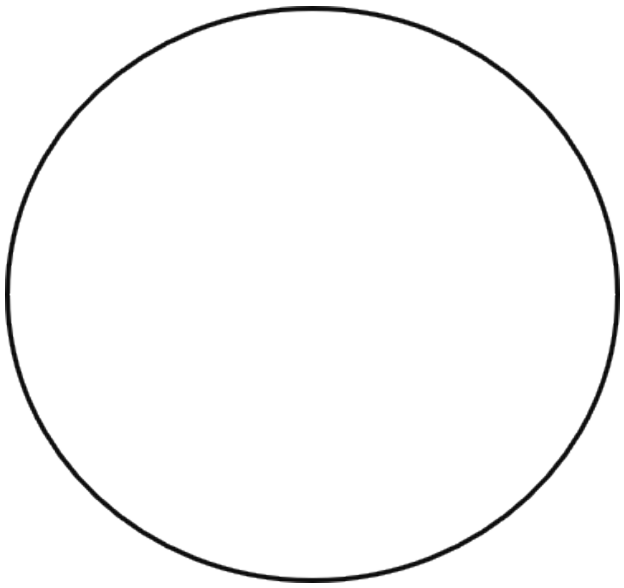
Figure 2: Close-up of the slide positioned correctly under the microscope. (Photo by Joe Frantz)

9. Draw what you see in the circle.



10. Describe what it is like. Is it right-side up or upside down (inverted)? _____
11. Is it correctly oriented, or is it backward (reversed)? _____
12. When you use the **Stage Adjustment Knobs** to move it up, does it move up or appear to move down? _____
13. When you use the **Stage Adjustment Knobs** to move it to the left, does it move the image to the left or does it appear to move to the right? _____
14. Now turn the scanning objective (4x) to the low power objective (10x). Make sure it clicks into position.
15. Look through the ocular. The "e" may be slightly out of focus. Focus with **Fine Focus only** by moving it towards you and away from you.
16. Use the **Stage Adjustment Knobs** to center it.

17. Draw what you see in the circle.



18. If you wish, you can repeat these steps to look at it under the 40x (high power) objective.

Activity D: Plant Cells - Elodea and Preparing a Wet Mount

Elodea is a plant that lives in freshwater. Plants are multicellular autotrophs - meaning that they carry out photosynthesis to produce their own energy.

Plants have 3 structures that animal cells do not have: cell walls, chloroplasts, and a central vacuole.

1. Obtain a small leaf of Elodea.
2. Place it on a microscope slide.
3. Add a drop or 2 of water.
4. Carefully place a coverslip over the leaf.
5. Focus and center with the scanning (4x) objective lens.

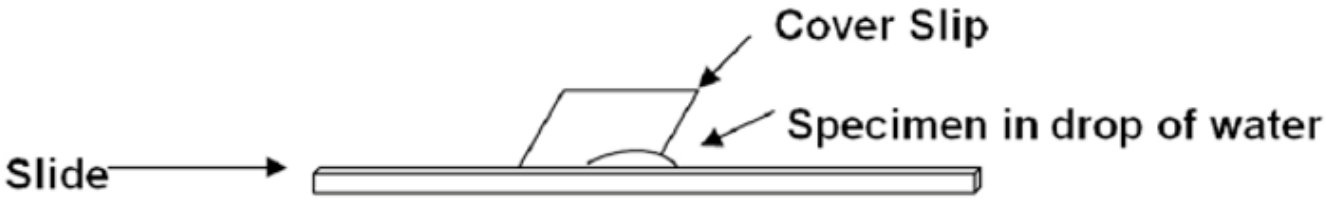
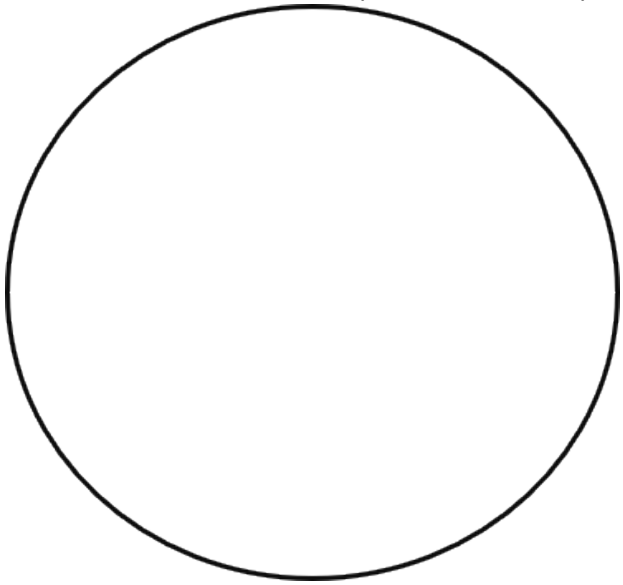


Figure 3: Making a wet mount (http://www.redwoods.edu)

6. Turn to the 10x, low power objective, focus and center.
7. Turn to the 40x, high power objective, focus and center
8. Draw and label - **cell wall, cell membrane, chloroplasts, vacuole, cytoplasm,** and, if visible, **nucleus.**

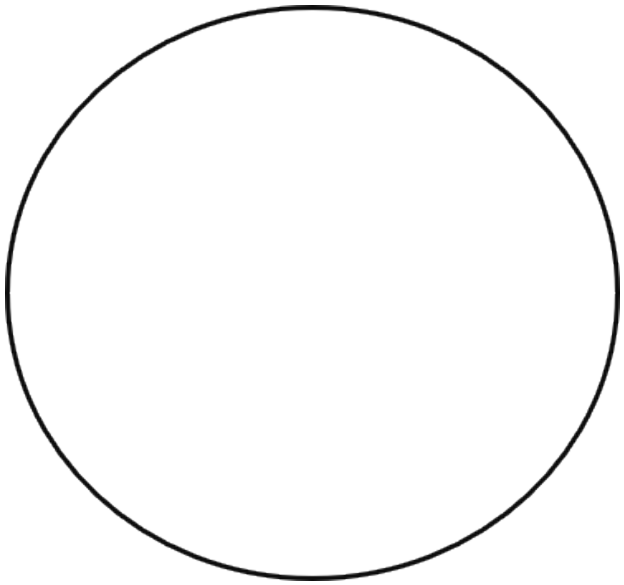


Activity E: Animal - cheek cells - squamous epithelium wet mount

Now you will make a wet mount of squamous cells that are found inside the mouth and in other locations. Animals are multicellular heterotrophs, meaning that they must consume energy from outside sources. They do not have chloroplasts, vacuoles, or cell walls. You will make a wet mount using the cells inside of your mouth (cheek)

1. Put 2 drops of water on a slide.
2. With a sterile cotton swab, carefully scrape a few cells from inside your mouth and smear into the water on the slide
3. Add a drop of methylene blue; it improves contrast and visibility.
4. Focus and center with the scanning (4x) objective lens.
5. Turn to the 10x, low power objective, focus and center.
6. Turn to the 40x, high power objective, focus and center.

7. Draw and label the **cell membrane, cytoplasm, and nucleus.**



Activity F: Depth of Field - crossed threads

Depth of field means the thickness of the specimen that appears in focus at one time. For example, if you are viewing an insect under the microscope, the top of the insect may be in focus while the legs underneath are blurry. As you adjust the fine focus knob, different layers come into focus.

Higher magnification will result in a shallower depth of field. Too much light will also have shallower depth of field.

So, when studying specimens to see all details, you will need to continuously change the focus using the fine focus knob.



Figure 4: Crossed threads. (http://www.redwoods.edu)

1. Obtain a slide labeled crossed threads.
2. Focus and center with the scanning (4x) objective lens.
3. Turn to the 10x, low power objective, focus and center.
4. Turn the fine focus toward you and away from you to ascertain the order of thread color from top to bottom.

In order from top to bottom, the colors are _____, _____, _____

Activity G: Stereoscopic Dissecting Microscope

The stereoscopic dissecting microscope is used to study specimens that are too large and/or thick to be studied with a compound

light microscope. They have a light source both above and below the specimen. They typically magnify to about 50x with a Zoom lens. Obtain an object such as a coin, rock, pencil, or even your finger and study it with the microscope. Draw what you see.

Label the parts of the stereoscopic dissecting microscope: **Ocular Lenses**, **Focus Knob**, **Zoom Magnification Knob**, and the **Upper** and **Lower Light Switches.**



Figure 5: The Stereoscopic Dissection Microscope. (Original Photo by Joe Frantz)

Additional Questions

Briefly describe how you will handle and care for your microscope each time you use it.

_____

Give the function of each of these and when you would use them.

Condenser

_____

Stage	Adjustment	Knobs
Coarse		Focus
Fine		Focus
Objective		Lenses

Define these terms:

Depth	of	Field
Field	of	View
Wet		mount

The Compound Light Microscope

Draw arrows to label the parts of the microscope. Label the Ocular Lenses, Revolving Nosepiece, Objective Lenses, Coarse Focus, Fine Focus, Condensor, Stage Adjustment Knobs.



Figure 6: The Compound Light Microscope. (Original Photo by Joe Frantz)

Table 1. Total magnification and Names of Objective Lenses

Name	Magnification	Ocular Magnification	Total Magnification
Scanning	4x	10x	

Low Power	10x	10x	
High Power	40x	10x	
Oil Immersion	100x	10x	

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This document, "Lab 4: Microscopy," serves as a comprehensive guide for students to understand and utilize various microscopes. It covers the fundamental principles of microscopy, practical handling and care instructions, and detailed activities for observing different types of cells and specimens.

Key Sections and Activities:

- **Introduction**: Provides an overview of microscopes, their magnification capabilities, and the types of microscopes to be used in the lab, specifically compound light microscopes and stereoscopic dissecting microscopes.
- **Activity A: Handling and Care of the Compound Light Microscope**: Outlines essential guidelines for retrieving, cleaning, moving, and storing the compound light microscope, emphasizing two-handed handling and proper lens cleaning techniques.
- **Activity B: The Compound Light Microscope**: Describes the various parts of a compound light microscope, including the arm, ocular lenses, objective lenses, revolving nosepiece, stage, condenser, and focus knobs. It also explains key concepts such as total magnification and field of view.
- **Activity C: Using a Prepared Slide - letter "e"**: Provides step-by-step instructions for focusing on a prepared "e" slide using different objective lenses and observing how the image is inverted and reversed. **Activity D: Plant Cells - Elodea and Preparing a Wet Mount**: Guides users through the process of preparing a wet mount of Elodea plant cells and identifying structures like cell walls, chloroplasts, and vacuoles.
- **Activity E: Animal - cheek cells - squamous epithelium wet mount**: Instructs on preparing a wet mount of human cheek cells and identifying the cell membrane, cytoplasm, and nucleus. 6 Page 10 Page
- **Activity F: Depth of Field - crossed threads**: Explains the concept of depth of field using a crossed threads slide and demonstrates how to determine the order of threads.
- **Activity G: Stereoscopic Dissecting Microscope**: Introduces the stereoscopic dissecting microscope for observing larger specimens and includes an activity for users to study an object and label the microscope's parts.

Additional Questions and Tables: Includes questions about microscope handling, the functions of various parts, definitions of key terms, and a table for calculating total magnification.

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1.5: Cell Biology

Introduction

The basic subunit of all living things is the **cell.**

Types of cells are divided into two categories - **prokaryotic cells** and **eukaryotic cells.**

Prokaryotic cells are simple cells found in Bacteria and Archaea.

Eukaryotic cells are complex cells found in Protists, Plants, Animals, and, though greatly modified, in Fungi.

Activity A: Prokaryotic Cells

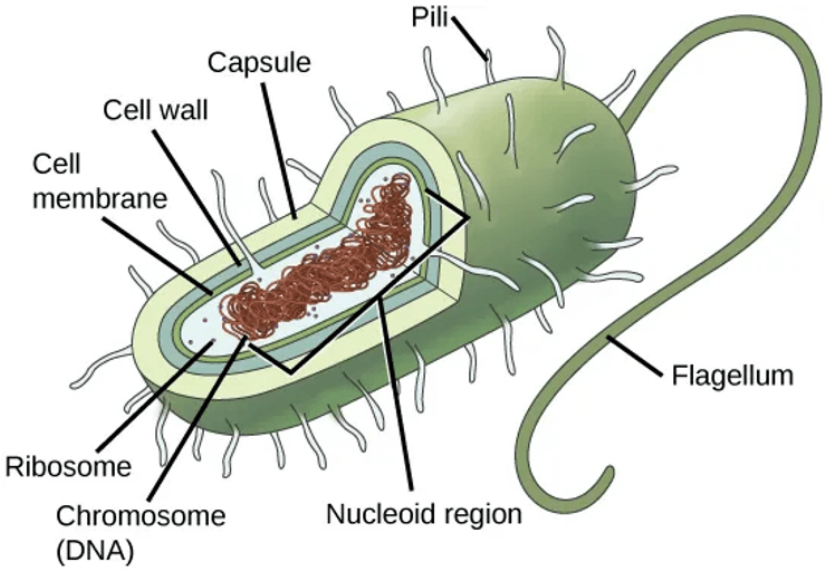


Figure 1: Structure of a Prokaryotic Cell (OpenStax.org)

- Prokaryotes are single-celled organisms.
- They have no membrane-bound organelles.
- Usually there is a single piece of circular, double-stranded DNA in the region called a Nulceoid.
- The cell wall is protective and gives the cell its shape.
- Many have a flagellum for locomotion.
- Pili enable the organism to attach to surfaces.
- Some have a capsule that makes pathogens more resistant to our immune responses.

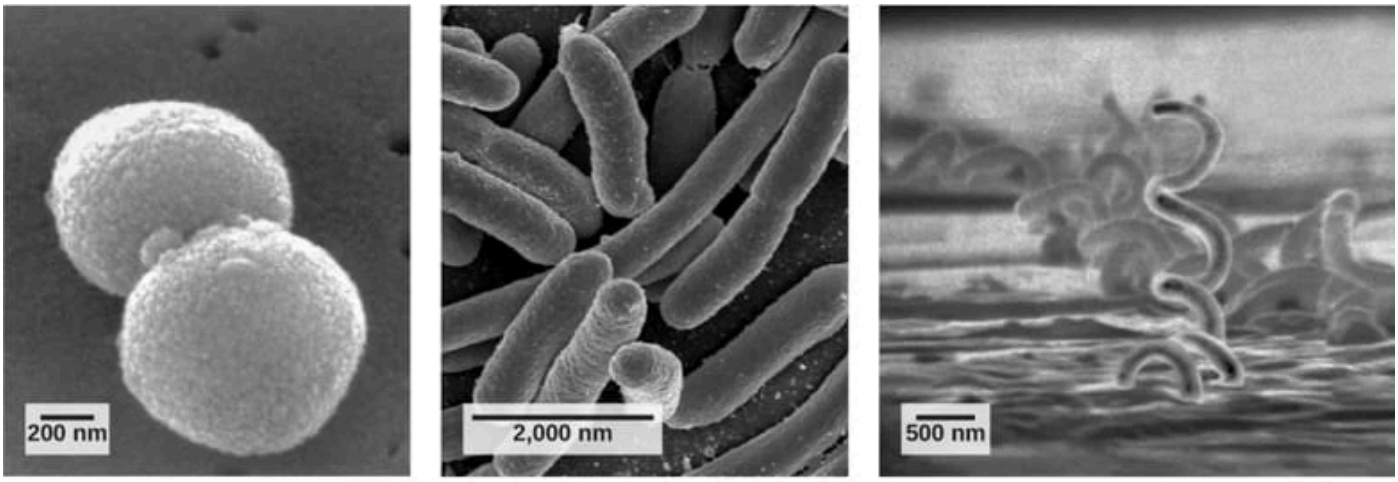
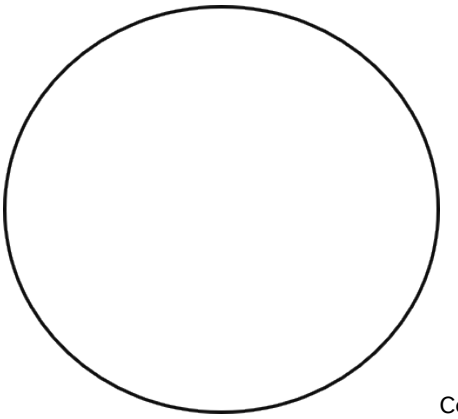


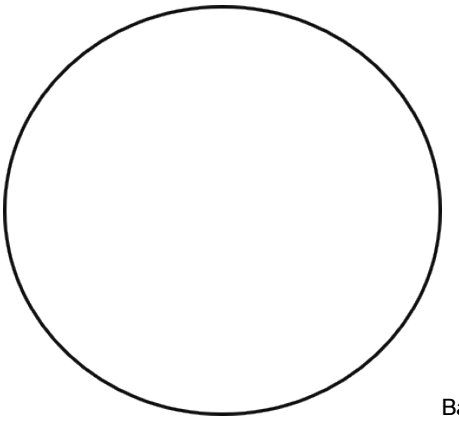
Figure 2: Scanning electron micrographs showing the shapes of Prokaryotic Cells - Coccus, Bacillus, Spirillum (https://openlab.citytech.cuny.edu/bio-oer)

1. Obtain slides of cocci, bacillus, and spirillum bacteria. Draw them below and list an example of each.



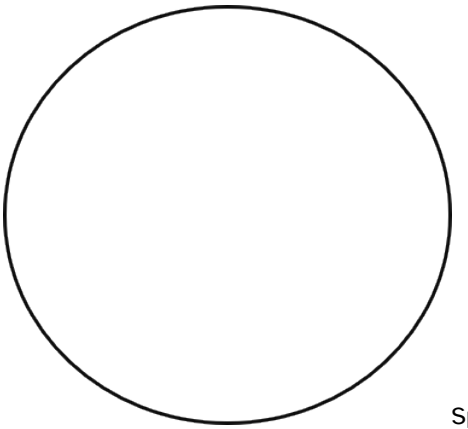
Cocci Bacteria

Example _____



Bacillus Bacteria

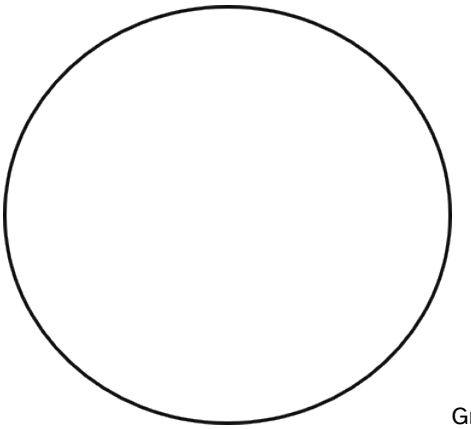
Example _____



Spirillum bacteria

Example _____

2. Look at a slide of Gram-negative and Gram-positive bacteria and draw what you see. Explain the difference between gram-negative and gram-positive bacteria.

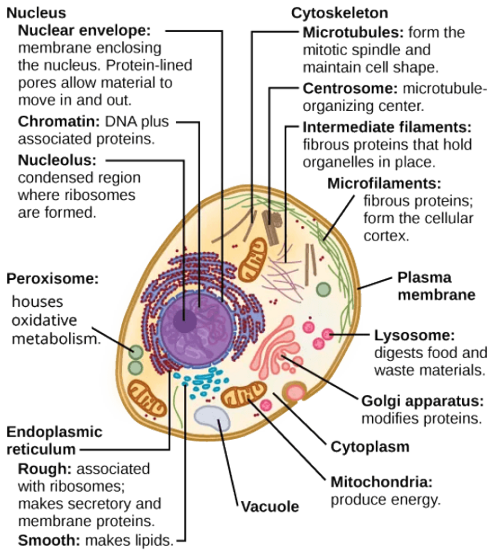


Gram-negative and Gram-positive bacteria

What is the difference between the two types in terms of human disease? _____

Activity B: Eukaryotic Cells

- Eukaryotic cells are those found in plants, animals, protists, and (though modified) in fungi.
- Eukaryotic cells can be found in both autotrophs, which carry out photosynthesis to produce their own energy, and heterotrophs, which depend upon other cells for energy.
- Eukaryotic cells have many internal membrane-bound organelles, such as mitochondria and chloroplasts, a nucleus surrounded by a membrane, and rod-shaped chromosomes.
- Recall that in the Microscope Lab you studied a plant cell (Elodea) and animal cells (squamous cells)
- Now we are going to look at more detail as revealed by the transmission electron microscope and scanning electron microscope.



(a)

Figure 3: Animal Cell showing details revealed by the electron microscope. (https://oercommons.org/authoring/566...ty-textbook-re)

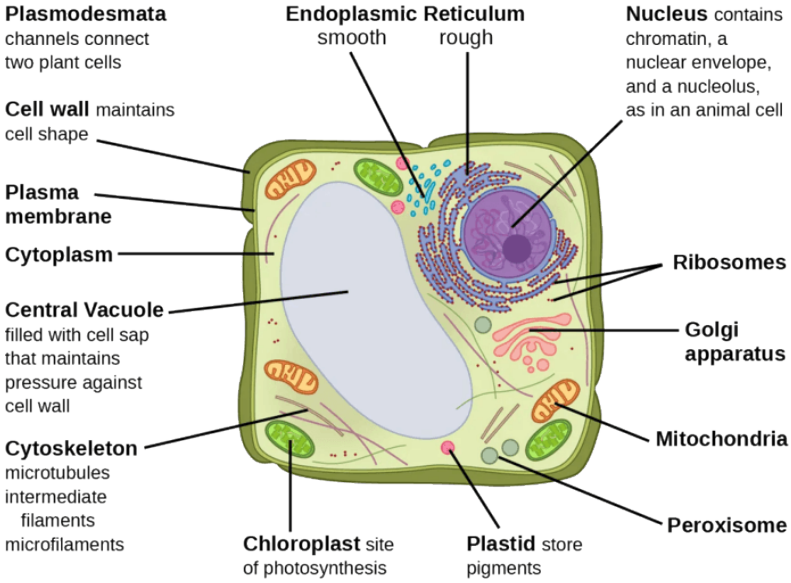


Figure 4: Plant Cell with details revealed by the electron microscope. (https://oercommons.org/authoring/566...ty-textbook-re)

Look at Figures 3 and 4 and answer these questions:

1. List 3 components that plant cells have that animal cells do not have.
   _____ _____ _____
2. List 2 components that animal cells have that plant cells do not have.
   _____ _____
3. What are the functions of the following structures?
   1. Rough endoplasmic reticulum
      _____
   2. Smooth endoplasmic reticulum
      _____
   3. Golgi apparatus
      _____
   4. Mitochondria
      _____
   5. Chloroplast
      _____

6. Lysosome
   _____
7. Cell wall
   _____
8. Central vacuole
   _____
9. Ribosome
   _____
10. Plasma membrane
    _____
11. Centrosome
    _____
12. Nucleus
    _____
13. Nucleolus
    _____

Activity C: Protista

- Protists are a diverse group of mostly single-celled eukaryotes.
- Protists are ideal examples of eukaryotic cells. Since they are single-celled, they contain all cellular components necessary for life, unlike multicellular organisms such as plants and animals, in which cells may be specialized and may not have all cellular components.
- Some are animal-like heterotrophs that move and eat other organisms. Examples include Amoeba and Paramecium
- Others are plant-like. Examples include Euglena and diatoms. They have chloroplasts that are used in photosynthesis.
- Most Protists have structures for locomotion. Common methods are cilia, flagella, and pseudopods. Humans have ciliated cells in the trachea that carry pathogens and other particles away from the lungs. Our white blood cells have pseudopodia.
- Protists live in freshwater, seawater, soil, and some are parasites.

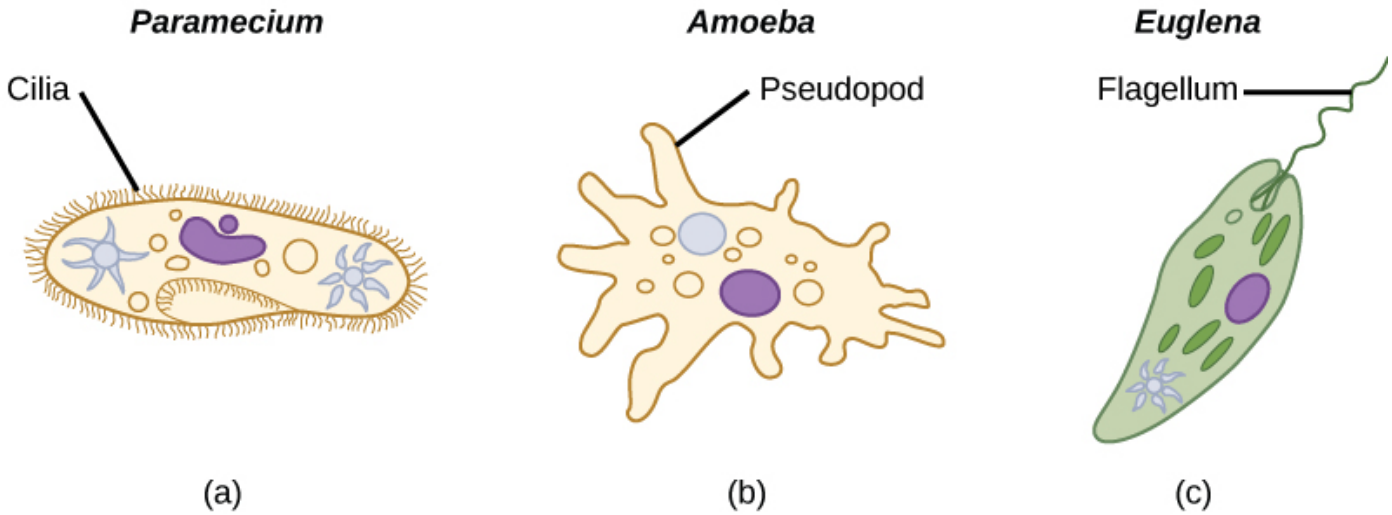


Figure 5: Drawings of 3 common protists: Paramecium, Amoeba, Euglena. (https://oercommons.org/authoring/566...ty-textbook-re)

(a) **Paramecium** lives in freshwater. It is a predator of bacteria and other small particles that it sweeps into an **oral groove** (mouth), It moves with hair-like structures called **cilia.** A **macronucleus** controls everyday cell activities. **Food vacuoles** contain ingested food. **Contractile vacuoles** pump excess water out of the cell.

(b) **Amoeba** is also a freshwater predator. It also lives in soil and other moist environments. It moves with **pseudopods** (false feet). It engulfs food such as bacteria and small protists by surrounding them with pseudopodia in a process called **phagocytosis**. You can see the **cell membrane**, **cytoplasm**, **nucleus**, **pseudopodia**, **food vacuoles**, contractile **vacuole**.

(c) **Euglena** lives in freshwater habitats. It has characteristics of both animals and plants. moves with a long whip-like **flagellum**. It can carry out photosynthesis using chloroplasts like a plant. When light is unavailable, it can ingest nutrients like an animal. It

has an **eye-spot** that detects light, helping it move toward brighter areas.

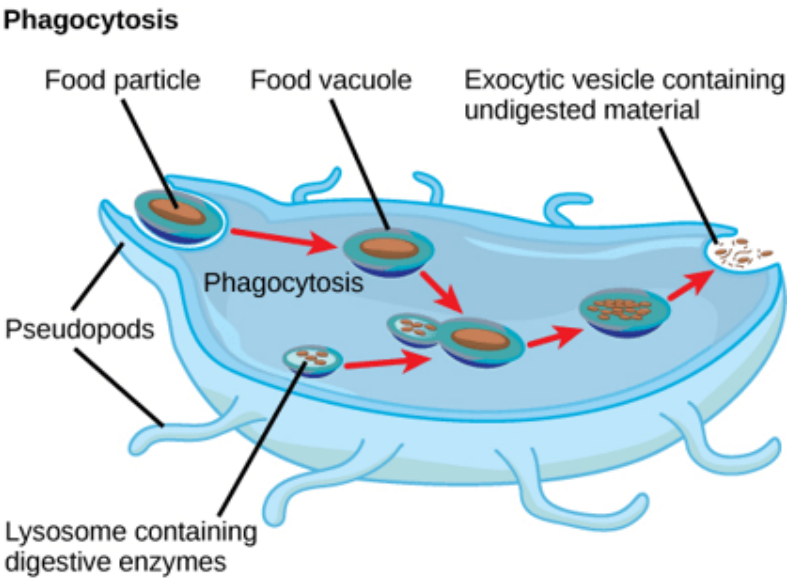


Figure 6: Process of Phagocytosis. (https://oercommons.org/authoring/566...ty-textbook-re)

The process of phagocytosis involves surrounding the food particle with pseudopodia, engulfing it in a food vacuole, digesting it with lysosomes, and then getting rid of the wastes by exocytosis. In humans, 4 of the 5 types of leukocytes (white blood cells) carry out phagocytosis of pathogens such as viruses and bacteria, as well as other agents.

Make wet mounts of each of these 3 protists. There may be others available.

If there are no live cultures, you may work from prepared slides.

After you have prepared your wet mount, begin with the scanning 4x objective, which magnifies 40x.

After centering and focusing on a specimen, turn to the low-power 10x objective, which magnifies 100x.

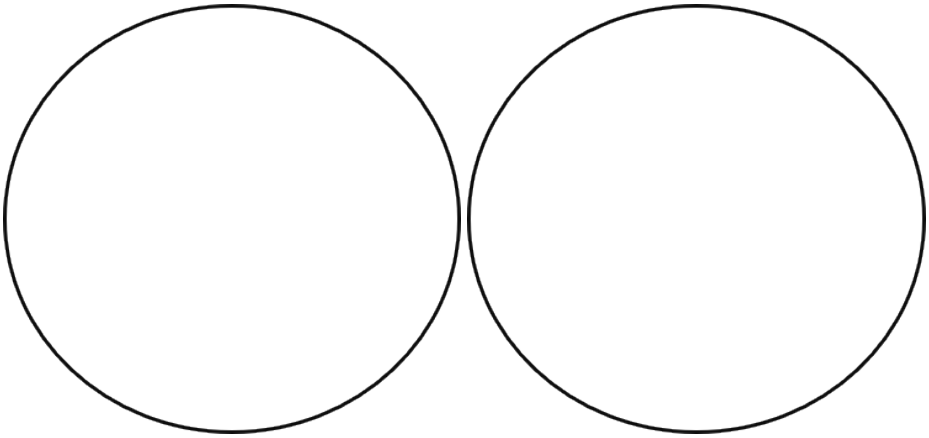
Center and focus again.

Finally, turn to the high-power 40x objective, which magnifies 400x. Make your drawings at high-power magnification.

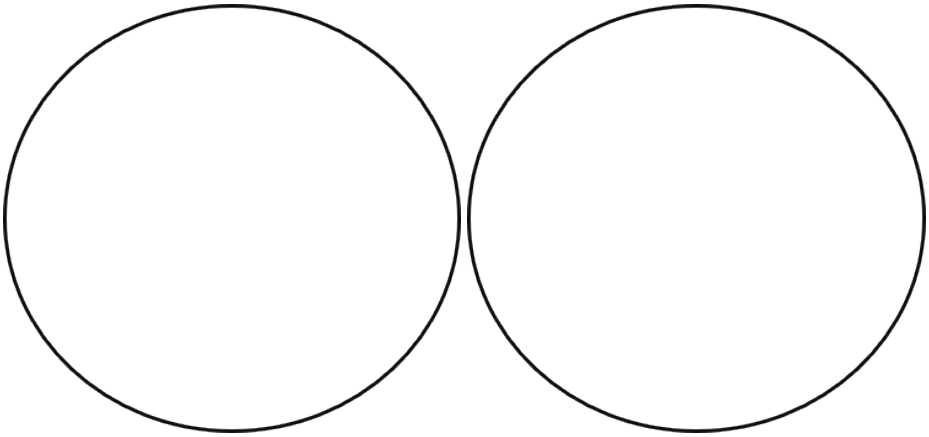
If your specimens move too fast for you to study, you can add a drop of methylcellulose (ProtoSlo). It will slow the movement without harming the organism.

Label as many structures as you can see.

Paramecium Amoeba



Euglena _____



Fill in:

Table 1. Comparison of Protists

Protist	Locomotion	Nutrition	Method of Feeding	Similar Cells in Humans
Paramecium				
Amoeba				
Euglena				

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This document, "Cell Biology," provides an introduction to the fundamental unit of life, the cell, and then delves into the characteristics and activities related to prokaryotic and eukaryotic cells, including a specific focus on protists.

Key Sections and Activities:

- **Introduction to Cells:**
  - Defines the cell as the basic subunit of all living things.
  - Categorizes cells into two main types: prokaryotic and eukaryotic.
  - Briefly describes prokaryotic cells as simple cells found in Bacteria and Archaea, and eukaryotic cells as complex cells found in Protists, Plants, Animals, and Fungi.
- **Activity A: Prokaryotic Cells**
  - Describes prokaryotes as single-celled organisms lacking membrane-bound organelles.
  - Highlights key structures such as a single circular DNA in the nucleoid, a protective cell wall, flagellum for locomotion, pili for attachment, and a capsule in some pathogens.
  - Includes an activity to draw and provide examples of different prokaryotic shapes (coccus, bacillus, spirillum).
  - Asks for an explanation of the difference between Gram-negative and Gram-positive bacteria and their implications for human disease.
- **Activity B: Eukaryotic Cells**
  - Reiterates that eukaryotic cells are found in plants, animals, protists, and fungi.
  - Explains that eukaryotic cells can be autotrophs or heterotrophs.
  - Details the presence of internal membrane-bound organelles (e.g., mitochondria, chloroplasts), a membrane-bound nucleus, and rod-shaped chromosomes.
  - Refers to previous microscope lab experience with plant and animal cells.
  - Presents figures of animal and plant cells and asks for a comparison of their components.
  - Requires listing the functions of various eukaryotic organelles (e.g., rough ER, smooth ER, Golgi apparatus, mitochondria, chloroplast, lysosome, cell wall, central vacuole, ribosome, plasma membrane, centrosome, nucleus, nucleolus).
- **Activity C: Protista**
  - Introduces protists as a diverse group of mostly single-celled eukaryotes, ideal for studying cellular components.
  - Classifies protists as animal-like heterotrophs (e.g., Amoeba, Paramecium) or plant-like autotrophs (e.g., Euglena, diatoms).
  - Discusses common methods of locomotion (cilia, flagella, pseudopods) and their human counterparts.
  - Describes the habitats of protists.
  - Provides detailed descriptions of Paramecium, Amoeba, and Euglena, including their structures and functions.
  - Explains the process of phagocytosis using Amoeba as an example and relates it to human immune responses.
  - Outlines a lab procedure for observing protists under a microscope.
  - Includes sections for drawing and labeling the observed protists.
  - Concludes with a table for comparing protists based on locomotion, nutrition, method of feeding, and similar cells in humans.

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1.6: Diffusion and Osmosis

Purpose:

This lab investigates the processes of diffusion and osmosis and focuses on the movement of molecules across selectively permeable membranes. Students will explore the effects of solute concentration (tonicity) on osmosis and understand how cells maintain homeostasis.

In this laboratory, students will:

- Conduct experiments to observe diffusion and osmosis using agar plates, diffusion in dialysis tubing, and osmosis in potato strips, Elodea cells, and red blood cells.
- Measure and analyze the effects of tonicity on cells.
- Use collected data to determine relationships between the properties of molecules and the rate of diffusion.

Student Learning Objectives:

- Accurately collect and record data for each experiment.
- Interpret experimental results to explain the behavior of cells in isotonic, hypertonic, and hypotonic solutions.
- Present findings in well-labeled tables and optional graphs.

Background

Cell theory states that all living things are composed of cells and that cells only arise from other cells. Some cells are simple in structure, while others are extremely complex. All cells share some common properties: the presence of DNA, intracellular proteins that enable the cell to perform its functions, and a plasma membrane. **Eukaryotic** cells also contain membrane-bound organelles that allow a more complex level of functioning.

Homeostasis is defined as the maintenance of a stable internal environment. To maintain homeostasis, cells continually transport substances in and out across their cell (plasma) membrane.

The cell membrane serves as a “gatekeeper” and is the cellular structure that regulates the transport of materials into and out of the cell. The phospholipid bilayer architecture of the cell membrane allows certain molecules to pass through while keeping others out, therefore the cell membrane is **selectively permeable (or semipermeable)**. Things that need to enter a cell for it to function properly include ions, nucleotides, sugars, oxygen, amino acids, water, vitamins, and some hormones. Cells also allow certain molecules like water, ions, and secreted proteins to leave. Additionally, cells must eliminate waste products like urea and carbon dioxide

In the following exercises, you will examine the semi-permeable nature of the cell membrane. You will also explore the concept of **tonicity**, which refers to the solute concentration of a solution, and its ability to influence the rate and direction of osmosis, or the movement of water into or out of the cell.

Diffusion and Osmosis

Diffusion is the movement of molecules from an area in which they are high in concentration to an area in which they are low in concentration. Molecules move down a **concentration gradient** until they are equally distributed, or **equilibrium** is reached (Fig.1). At equilibrium, there is no concentration gradient. Molecules still move once equilibrium is reached, but there is no **net movement** in any one direction.



Figure 1 - Diffusion of molecules from an area of high concentration to low concentration in coffee. Equilibrium is reached when the molecules are equally distributed. (image by iirliinnaa from Pixabay, CC-BY.)

Osmosis is a specific type of diffusion: the diffusion of **water** molecules across a semi-permeable membrane. Like other molecules, water molecules diffuse down a concentration gradient, from an area of higher free water concentration to an area of lower free water concentration. This means that water will move across a semipermeable membrane, like the cell membrane, in the direction of the higher solute concentration. (In solution, high solute concentration = low free water concentration; conversely, low solute concentration = high free water concentration.) You will observe this concept in **Part 2: Osmosis & Tonicity**.

Many substances surrounding a cell are dissolved in water. Substances dissolved in water are called **solutes**. The water is the **solvent**. For example, in salt water, salt is the solute and water is the solvent. The cell membrane is **selectively permeable**: it allows some substances to cross more easily than others.

Lab Safety

Safety Guidelines for Handling Solutions and Materials:

- Wear personal protective clothing as instructed by your instructor
- Handle glass slides with care to avoid injuries.
- Dispose of biological samples (e.g., blood, plant materials) as instructed.
- Wash hands thoroughly after completing the lab.

PART 1: DIFFUSION & OSMOSIS

Exercise 1: Diffusion Through a Semisolid Medium

The purpose of this lab is to demonstrate diffusion of a molecule through a semisolid medium. You will investigate the movement of an acid through agar gel media containing the dye bromothymol blue. This dye is blue under neutral pH conditions but changes to yellow when exposed to an acid which allows us to follow the extent of diffusion over time.

Materials:

- Petri dish of bromothymol blue agar
- Straw
- Vinegar

Procedure:

1. Obtain a petri dish of agar
2. Take the plastic straw and gently stick it down into the center of the agar without pushing the straw to the bottom of the plate. We want a layer of agar left on the bottom of the dish. Lift the straw to remove a small plug of agar.
3. Using the dropper bottle or a 1mL transfer pipet, place two drops of vinegar into the agar well you just created. (Fig. 2).

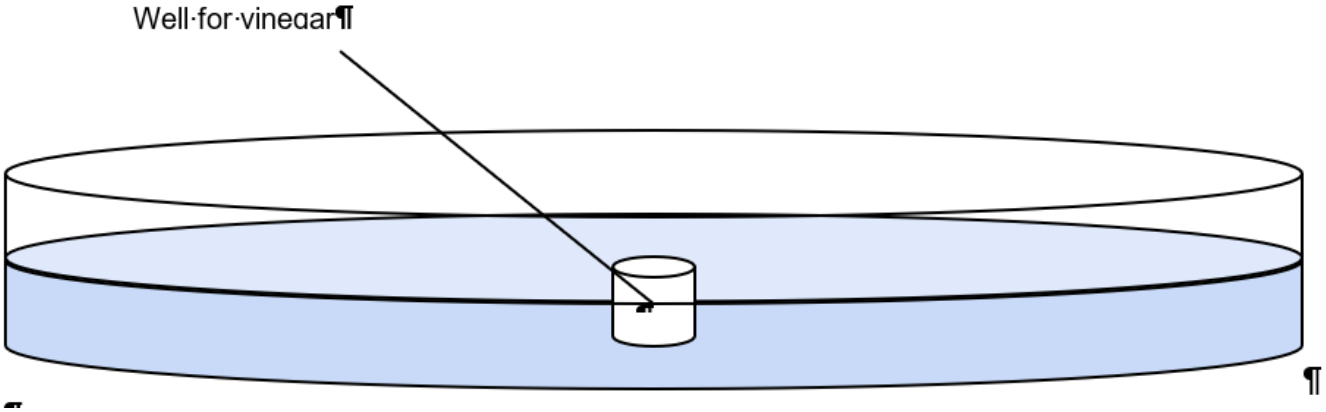


Figure 2 - Diffusion Experimental setup (by BB)

4. Place a small metric ruler underneath the Petri dish with the zero mark centered on the well to measure the distance (diameter) that the acid moves.

5. As the acid diffuses, the blue dye will turn yellow. This serves as an indication for the rate of diffusion. Record the distance traveled every 10 minutes in **Table 1**.

Table 1 - Diffusion results

Table 1 - Diffusion results

	Diameter after 10 min. (mm)	Diameter after 20 min. (mm)	Diameter after 30 min. (mm)	Diameter after 40 min. (mm)
Acetic acid (vinegar)				

Exercise 2: Diffusion Across a Membrane

In these experiments dialysis tubing is used to simulate the cell membrane. Dialysis tubing is semi-permeable because the tubing has tiny pores that allow some materials to enter and exit the tubing. You will perform experiments that ask if various molecules are able to enter or exit the tubing. Read about the experiments below and complete the following questions.

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment. _____
2. Record a **Hypothesis** for the question stated above. _____
3. Predict the results of the experiment based on your hypothesis (if/then). _____
4. Perform the experiment below and collect your data.

Materials:

Equipment	Materials
Dialysis tubing	15% Sucrose solution (MW sucrose = 342 g/mol)
Plastic clips or string	30% Sucrose solution (MW sucrose = 342 g/mol)
10mL graduated cylinder	30% Dextrose solution (MW dextrose = 180 g/mol)
4 x 600 mL beakers (or plastic cups)	15% starch solution (MW = variable)
Electronic balance	Iodine solution (MW = 166 g/mol)
Weigh boats	Benedict's reagent
Graduated cylinders (10 mL and 100 mL)	
Sharpie	
Hot plate or heat block	

Procedure:

1. Cut 4 pieces of dialysis membrane approximately 20 cm long. Soak the pieces in water until they are soft and pliable (3-5 minutes). *This step may already be done.*
2. Obtain 4, 600 ml beakers and label them #1 - 4. Fill each beaker with 400 mL of solution as shown below:
   - Beaker #1 – H2O
   - Beaker #2 – H2O
   - Beaker #3 – H2O
   - Beaker #4 - H2O and 1mL Iodine solution
3. Set beakers aside. Remove one piece of dialysis membrane from the soaking water and open it, forming a tube.
4. Close one end of the tube with dialysis tubing clips.
5. Fill the bag with 10 mL of H_2O using a pipet. Remove excess air and close the other end of the bag with a plastic clip. Set aside on a paper towel and ensure it doesn't leak. This is bag 1.



Figure 3: Dialysis tube "bag" (By MG)

6. Repeat steps 4 and 5 for the 4 remaining dialysis tubes, filling them with 10 mL of the solutions listed below as follows: Use a fresh or different pipet for each solution
   - Bag #2 – 10mL 15% sucrose
   - Bag #3 – 10mL 30% sucrose
   - Bag #4 –5 mL 30% dextrose solution and 5 mL 15% starch.

7. ◦



Figure 4: Completed bag and beaker setup (By MG)



Figure 5: Completed bag and beaker setup (By MG)

8. Rinse off the outside of the bags with water and carefully blot dry with paper towel.
9. Weigh bags #1 - 3 to the nearest 0.5g. Record the weights in **Table 1** below, in the column labeled "0 min." NOTE: We will be doing a slightly different experiment with bag 4 so weighing it isn't necessary. Go to step 15 for more about bag 4.
10. Place all 4 bags in their corresponding beakers (Bag #1 in Beaker #1, etc.). Make sure each bag is fully submerged in the solution.
11. Set a timer for 5 minutes.
12. At the end of 5 minutes, remove bags 1 - 3 from their beakers, blot excess fluid, and record the mass (in grams) in **Table 2**.
13. Return the bags to the appropriate beaker and wait another 5 minutes.
14. Repeat steps 11 - 13 every 5 minutes and record the weights in **Table 1**. You will only observe bag #4 once at the end of the 20-minute observation.

- *Table 2. Osmosis - mass (g) over time for dialysis bags*

Time (min.)		0	5	10	15	20
Beaker #1 (water)	Bag #1 (water)					
Beaker #2 (water)	Bag #2 (15 % sucrose)					
Beaker #3 (water)	Bag #3 (30% sucrose)					

15. Calculate the total weight change (weight change = final weight – initial weight) for each bag. Record the values in **Table 3**. Calculate the **rate** (g/min) of osmosis for each bag by dividing the weight change by the time change. Since all 3 bags were recorded for a total of 20 minutes, the time change for all 3 bags is 20 minutes. Record the rate of osmosis for all 3 bags in **Table 3**.

- *Table 3. Rate of osmosis*

	Weight Change (g)	Time (min)	Rate (g/min)
Bag #1 (water)			
Bag #2 (15% sucrose)			
Bag #3 (30% sucrose)			

16. Make observations about what you see in bag #4 and beaker #4 in **Table 3**.
17. Remove 2 mL of liquid from bag #4 and beaker #4 with a pipette and add the contents to separate test tubes. Be sure to label the test tubes.
18. Add 10 drops of Benedict's solution to each of the two test tubes and heat to 100 degrees Celsius in a boiling water bath or heat block for 2 - 5 minutes. Record the test results in **Table 4**. You are measuring the concentration of glucose in the beaker.
19. Alternatively, measure the concentration of glucose in the beaker using a Urine Test Strip. Dip the test strip in the beaker and remove. Determine the glucose concentration by comparing the color on the tip of the strip with the scale provided with the test strips.

Table 4. Selective Permeability

	Appearance of liquid after 20 minutes	Appearance of liquid after heating with Benedicts Solution Or after testing with a urine strip
Bag #4 (5 mL 30% dextrose solution and 5 mL 15% starch solution)		
Beaker 4 (H_2O and 1mL Iodine solution)		

Questions

1. Did the weight of each bag (#1 - #3) change significantly over 20 minutes? Explain why.
2. In which bag(s) did water move into the dialysis bag?

3. What can you conclude about the movement of Iodine, dextrose, and starch across the dialysis membrane based on your results in **Table 3**? Support your answers for each with the observation from bag #4 and beaker #4.
   - Iodine -
   - dextrose -
   - Starch -
4. In terms of **solvent** (water) concentration, water moved from the area of _____ concentration to the area of _____ concentration through the selectively permeable membrane. This is defined as _____.
5. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally the same as a cell membrane?

6. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally different from a cell membrane?

PART 2: OSMOSIS & TONICITY

Tonicity describes how a solution affects the net movement of water into and out of the cell.

An **isotonic solution** has the same concentration of solute (and therefore of free water) as the cell. When cells are placed in an isotonic solution, there is no net movement of water.

- A **hypertonic solution** has a higher solute (therefore, lower free water) concentration than the cell. When cells are placed in a hypertonic solution, water moves out of the cell into the lower free water solution.
- A **hypotonic solution** has a lower solute (therefore, higher free water) concentration than the cell. When cells are placed in a hypotonic solution, water moves into the cell from the higher free water solution.

Exercise 1: Observing Osmosis in Potato Strips

Employing Steps in the Scientific Method:

Read about this experiment and answer the following questions.

1. Record the **Question** that is being investigated in this experiment. _____

2. Record a **Hypothesis** for the question stated above. _____

3. Predict the results of the experiment based on your hypothesis (if/then). _____

4. Perform the experiment below and collect your data.

Materials:

Equipment	Materials
Cork borer	10% NaCl solution
Petri dish	0.9% NaCl solution
Sharpie	Potatoes
Metric ruler	
Forceps and scalpel	

Procedure:

1. Obtain a potato and use a cork borer to prepare 3 cylinders of potato. Push the borer through the length of the potato. When the borer is filled, use the flat end of a glass rod to gently push out the potato cylinder into the petri dish. Use the scalpel to cut each potato cylinder into a length of 5 cm.
2. With Sharpie, label 3 test tubes (1, 2, 3).
3. Using the metric ruler, mark each tube at the 10 ml (cm) level from the bottom of the tube.
4. Set up three test tubes with 10 ml of solutions as follows:
   - Tube #1 - distilled water
   - Tube #2 - 10% sodium chloride (NaCl)
   - Tube #3 - 0.9% NaCl
5. Place one potato cylinder into each test tube and allow them to soak for about 15 minutes in the solutions.
6. You can now move on to **Exercises 2 and 3** while your potatoes soak.
7. When time is up, remove the potato cylinders from the test tubes and make observations about relative limpness (water loss, flaccid) or stiffness (water gain, turgor).

Questions

1. Which tube contained the limp (flaccid) potato strip? Explain.

2. Which tube contained the stiff (turgid) potato strip? Explain.

3. In which solution did the potato strip change the least the? What does this result suggest?

A plant cell is surrounded by a rigid cell wall, so when the cell is placed in a hypotonic environment, the net flow of water is from the surrounding medium into the cell, and it simply expands to the cell wall and becomes **turgid**. When the same plant cell is placed in a hypertonic environment, water leaves the central vacuole and the cytoplasm shrinks. This causes the cell membrane to pull away from the cell wall. In this situation, the plant cell will undergo **plasmolysis** and die. Animal cells have no cell wall so when they are in a hypotonic environment they will expand and fill with water until they burst or lyse. **Figures 5 and 6** demonstrate these conditions.

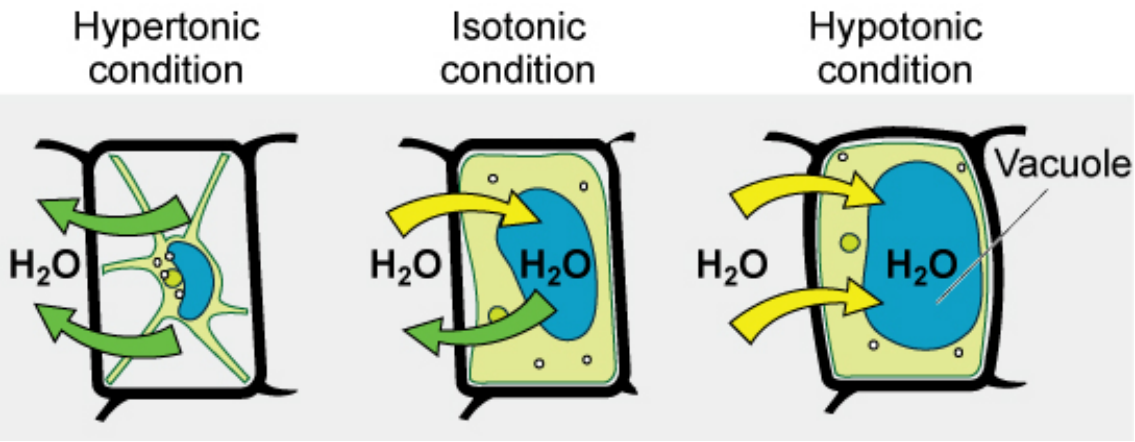


Figure 5 - Plant cell tonicity. The turgor pressure within a plant cell depends on the solution's tonicity in which it is bathed. (credit: modification of work by Mariana Ruiz Villareal CCBY)

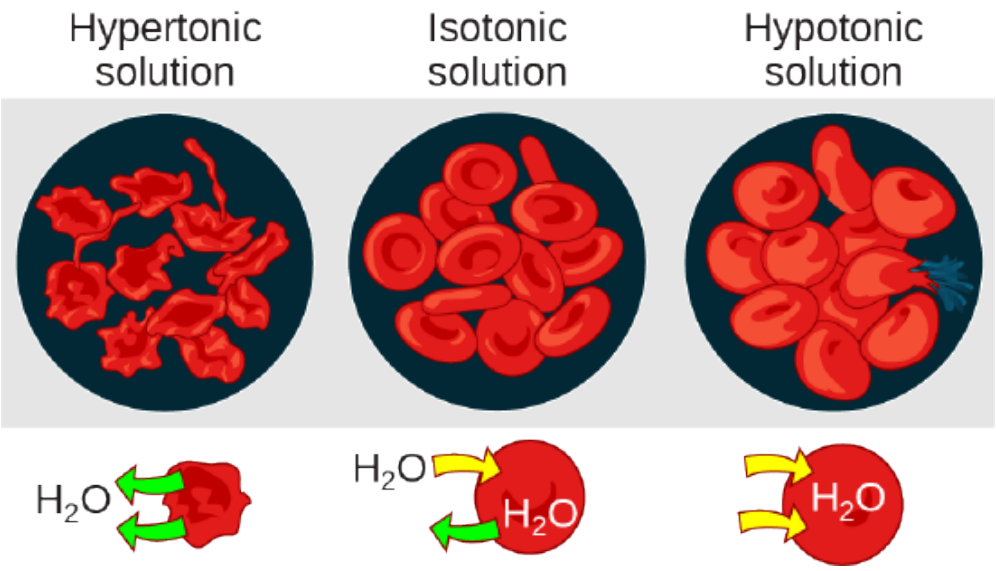


Figure 6 - Red blood cells in varied osmotic environments.(Diffusion and Osmosis. (2021, February 28). Retrieved April 1, 2021, from https://chem.libretexts.org/@go/page/34826 CCBY)

Exercise 2: Observing Osmosis in Elodea Cells

Employing Steps in the Scientific Method:

Read the experiment below and answer the following questions.

1. Record the **Question** that is being investigated in this experiment.
2. Record a **Hypothesis** for the question stated above.
3. Predict the results of the experiment based on your hypothesis (if/then).
4. Perform the experiment below and collect your data.

Materials

Equipment	Materials
Microscope	10% salt solution
Blank slides and coverslips	DI water
Forceps	Elodea leaf

Procedure

1. Place a drop of water onto a clean microscope slide. Gently tear off a small leaf from the *Elodea* plant. The greener, the better.
2. Prepare a wet mount by placing the *Elodea* leaf into the drop of water on your slide.
3. Place a coverslip onto the slide.
4. Use the **scanning** (4x) objective to bring the *Elodea* cells into focus. You may not be able to observe individual cells at this power.
5. Switch to the **low power** (10x) objective. The *Elodea* cell walls should be visible. They will look like dark green grid lines. Use the fine focus adjustment to focus the specimen.
6. Once you think you have located an *Elodea* cell, switch to the **high power** (40x) objective and refocus using the fine focus adjustment.
7. Next, add a drop of 10% salt (NaCl) solution to the edge of the coverslip to allow the salt to diffuse under the coverslip. Clean any excess water from around the coverslip with a Kimwipe or paper towel.
8. Observe what happens to the cells. Look for cells that have been visibly altered.
9. Record your observations in the following table. The cells in distilled water should look similar to the figure below.

Solution	Appearance of *Elodea* Cells
Distilled water (0% NaCl)	
10% NaCl	

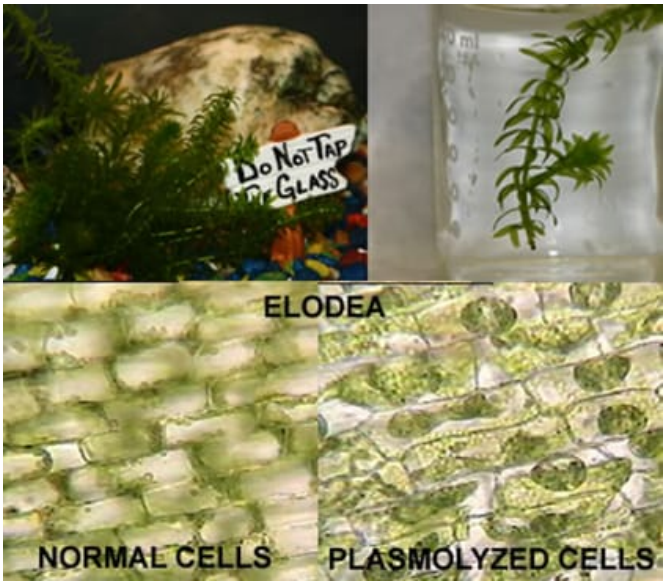


Figure 7: Photograph by Laurie Aiello and Tom Schuster, copyrighted 2005 from Cal State Northridge's secondary education program, used with permission from Norm Herr, Ph.D., Professor of Science Education.

Questions

1. Which solution is hypertonic to an Elodea cell? Use your observations to support your answer.
   _____
2. Would you expect pond water to be isotonic, hypotonic, or hypertonic to *Elodea* cells? Explain your answer.
   _____
3. Explain what happens to a plant cell that undergoes plasmolysis.
   _____

Exercise 3: Observing Osmosis in Red Blood Cells (Erythrocytes)

1. Predict the results of the observations of red blood cells in different solutions. (This experiment may be set up as a demonstration for observation and recording of data)

Materials

Equipment	Materials
Microscope	DI water
Blank slides and coverslips	10% NaCl solution
Sharpie	0.9% NaCl solution
	Fresh supply of red blood cells (Sheep RBCs)

Procedure

1. Obtain 3 test tubes and label them (1, 2, 3) with a Sharpie.
2. Set up the three test tubes with 5 mL of solutions as follows:
   - Tube 1 - Distilled water
   - Tube 2 - 10% NaCl
   - Tube 3 - 0.9% NaCl
3. Using a new transfer pipette, add 2 drops of blood to each tube and swirl gently to mix the contents.
4. Hold each test tube up to a sheet of paper with printed text. Attempt to read the print through each tube and record your results in the following table.

Test Tube / Solution	Appearance of Solution	Can you read print?
#1 - Distilled water		
#2 - 10% NaCl		
#3 - 0.9% NaCl		

5. Label 3 microscope slides (1, 2, 3) with a Sharpie.
6. Prepare wet mounts of each tube by placing a drop of the solution in each tube (1 - 3) on the appropriate microscope slide.
7. Add a coverslip to each slide.

8. View slide 1 through the microscope using the scanning (4x) objective first. Focus the image using the coarse adjustment. Then view the blood cells under low power and then high power. Only use the fine focus adjustment to focus the specimen.
9. Observe slide 2 and slide 3 in the same manner.
10. Record the appearance of the red blood cells in each solution in the following table.

Solution	Appearance of RBCs
#1 - Distilled water	
#2 - 10% NaCl	
#3 - 0.9% NaCl	

1. Which solution allowed you to read print through the solution? Explain.
   _____
2. Which solution is hypertonic to the RBCs? Use your observations to support your answer.
   _____
3. Which solution is hypotonic to the RBCs? How did you know? Use your observations to support your answer.
   _____
4. Which solution is isotonic to the RBCs? How do you know? Use your observations to support your answer.
   _____

Questions for Review

1. Define diffusion. What is the energy source for diffusion? Is diffusion considered an active or passive process? Explain.

2. Name a molecule that diffused through the artificial membrane (dialysis tubing) that we used in the laboratory. Can diffusion occur without a membrane? Give an example to support your answer.

3. What is osmosis? Is it an active or a passive process? Explain.

4. Fill in the blank in the following statements.
   1. A solution that has a lower solute concentration than another solution is said to be _____ when compared with the second solution.
   2. A solution that has the same solute concentration as another solution is said to be _____ when compared with the second solution.
   3. A solution that has a higher solute concentration than another solution is said to be _____ when compared with the second solution.

5. What does it mean when a membrane is selectively permeable?

Practical Challenge

6. Briefly explain what happens to a red blood cell when placed into the following solutions.
   a. Isotonic solution –
   b. Hypertonic solution –

c. Hypotonic solution -

7. Seawater contains a high concentration of dissolved salts. Using the concept of osmosis, explain why marine organisms are sometimes said to live in a desert environment.

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1.7: Enzyme Function

In every living cell, many chemical reactions are performed. In chemical reactions, the **reactants** are molecules that undergo a change, which results in the **products**. The arrow indicates the direction of the reaction as the reactants proceed to be transformed into the product(s). As you can see in the figure below, the number of reactants and products can vary, but the number of atoms is the same on both sides of the arrow. During synthesis reactions (Example 1), the reactants are joined to form a product. During decomposition reactions (Example 2), the reactant is broken down into products. During exchange reactions (Example 3) both decomposition and synthesis take place. The decomposition of two reactants is followed by an exchange and synthesis of two new compounds from them.

Example 1 (Synthesis Reaction): $\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{AB}$

$$2\,\mathrm{H_2} + \mathrm{O_2} \longrightarrow 2\,\mathrm{H_2O} \tag{1.7.1}$$

Example 2 (Decomposition Reaction): $\mathrm{AB} \longrightarrow \mathrm{A} + \mathrm{B}$

$$2\,\mathrm{H_2O} \longrightarrow 2\,\mathrm{H_2} + \mathrm{O_2} \tag{1.7.2}$$

Example 3 (Exchange Reaction): $\mathrm{A} + \mathrm{BC} \longrightarrow \mathrm{AC} + \mathrm{B}$

$$2\,\mathrm{Na} + 2\,\mathrm{H_2O} \longrightarrow 2\,\mathrm{NaOH} + \mathrm{H_2} \tag{1.7.3}$$

Enzymes are very efficient catalysts for biochemical reactions. These catalysts speed up reactions by providing an alternative reaction pathway that requires a lower activation energy needed for a reaction to get started. The lower the activation energy for a reaction, the faster the rate at which it will proceed. Enzymes only lower the activation energy, but do not change the difference in energy levels between the reactants and the products. They work by binding to the reactants and converting them to a different compound (the product). Enzymes are specific to a type of reactant, and therefore can catalyze only one type of reaction. This **enzyme specificity** is the result of the particular shape of the enzyme that only permits binding to one type of reactant, much like a key fits into a lock (Figure 2). The reactants in an enzymatic chemical reaction are called **substrates**. Notice how the shape of the enzyme fits its substrate. The location where the enzyme binds a substrate is called the **active site** because the reaction occurs here. At the end of the reaction, the product is released, and the enzyme can then bind to more substrate. Thus, an enzyme can perform the reaction over and over again, as long as the substrate is present.

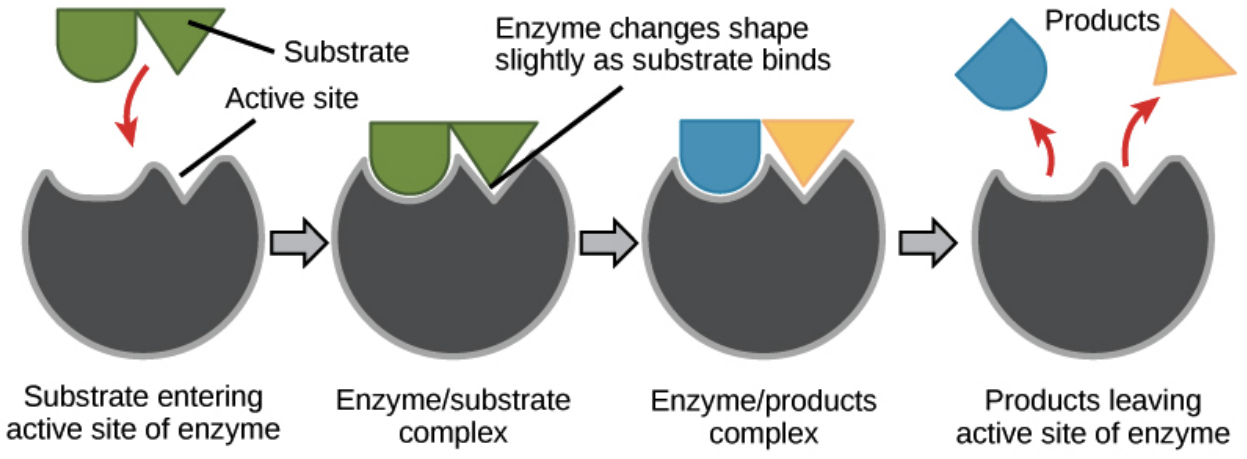


Figure 1: Enzymes bind a specific substrate and convert it into the products. The products are released, and the enzyme is unchanged. (CC By 4.0; OpenStax)

Most enzymes are complex proteins that function most efficiently within a specific range of temperature and pH. Extremes in temperature or pH will **denature** the enzyme by permanently altering its chemical structure. Even a small change in the protein's structure will change the enzyme's shape enough to prevent the substrate from binding, and thus keep the reaction from occurring. In this laboratory, you will demonstrate how altering the temperature, enzyme concentration, and pH will affect an

enzyme's ability to function. Specifically, you will be studying the actions of the enzyme **catalase**.

Exercise 1: Catalase Activity

Materials:

- Water (distilled or deionized)
- Disposable pipets
- Catalase (400 units/mL solution)
- Hydrogen peroxide (3% solution)
- Sucrose solution (5% solution)
- Small test tubes (10 ml test tubes) for Exercises 1, 2, and 4
- Large test tubes (20 ml test tubes) for Exercise 3
- Ruler
- Wax pencil / Sharpie
- Ice
- Boiling water bath
- Refrigerator
- Incubator (37o Celsius)
- Buffer pH 3.0
- Buffer pH 11.0
- Buffer pH 7.0
- Safety glasses
- Raw and boiled liver chunks on ice

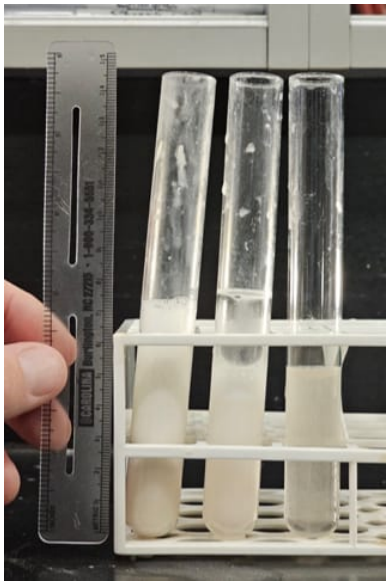


Figure 2: Generalized experimental set up for catalase experiments. Each experimental run will have different combinations of enzymes, substrates, etc. (By CB)

Catalase is an enzyme that speeds up the breakdown of hydrogen peroxide to water and oxygen:

Hydrogen peroxide → water + oxygen gas

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2 \tag{1.7.4}$$

- What is the **reactant** in this reaction? _____
- What is the **substrate** for catalase? _____
- What are the products for this reaction? _____

- Bubbling occurs as the reaction proceeds. Explain why. _____

Procedure:

1. With a wax pencil (or Sharpie), label three small test tubes (#1, #2, #3).
2. Using the ruler, mark each tube at the 1 cm (first mark) and 5 cm (second mark) levels from the bottom of the tube.
3. Fill tube #1 to the first mark with catalase. Then fill it to the second mark with hydrogen peroxide.
4. Swirl the contents to mix and wait at least 20 seconds for bubbles to develop.
5. Using the ruler, measure the height of the bubble column (in millimeters), and record your results in Table 1 below.
6. Fill tube #2 to the first mark with water. Then fill it to the second mark with hydrogen peroxide.
7. Swirl the contents to mix and wait at least 20 seconds for bubbles to develop.
8. Using the ruler, measure the height of the bubble column (in millimeters), and record your results in Table 1 below.
9. Fill tube #3 to the first mark with catalase. Then fill to the second mark with sucrose solution.
10. Swirl the contents to mix and wait at least 20 seconds for bubbles to develop.
11. Using the ruler, measure the height of the bubble column (in millimeters), and record your results in Table 1 below.

Table 1: Catalase Activity

Tube	Contents	Bubble Column Height (mm)
1	catalase, hydrogen peroxide	
2	water, hydrogen peroxide	
3	catalase, sucrose solution	

Graph your data: Be sure to label both the X and Y Axis

- Which tube showed the most bubbling? Explain why. _____

_____

- Which tube was the negative control? _____
- Which tube was the positive control? _____
- Describe what you observe in test tube #3. What was the purpose of this tube? _____

_____

Exercise 2: Effect of Temperature on Enzyme Activity

In general, cold temperatures slow chemical reactions, and warm temperatures speed up chemical reactions. Every enzyme, however, has an **optimal temperature** at which it works best. Some enzymes prefer cooler temperatures, and others prefer warm temperatures. However, extreme increases in temperature (i.e. boiling) will denature enzymes, making them inactive.

In the following exercise, you will test enzyme function at four different temperatures (on ice, in a refrigerator, in a warm incubator, and in a boiling water bath). Before setting up this experiment, formulate a hypothesis regarding the effect on enzyme activity. For example, do you think the enzyme will perform better at a warmer temperature? A cooler temperature? On ice? At boiling temperatures?

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment. _____
2. Record a **Hypothesis** for the question stated above. _____
3. Predict the results of the experiment based on your hypothesis (if/then). _____
4. Perform the experiment below and collect your data.

Procedure:

1. With a wax pencil (or Sharpie), label four small test tubes (#1, #2, #3, #4) and mark each at the 1 cm and 5 cm levels.
2. Fill each tube to the first mark with catalase.
3. Place tube #1 on ice, tube #2 at room temperature, tube #3 in a 37 degree Celsius incubator or warm water bath, and tube #4 in a boiling water bath or heat block. Wait 15 minutes.
4. While you are waiting, use a thermometer to measure the temperature of the environment for all four tubes. Record the values in Table 2 below.
5. After the 15-minute incubation, fill each tube to the second mark with hydrogen peroxide.
6. Swirl the contents to mix and wait 20 seconds.
7. Measure the height of the bubble column (in mm) for each tube and record your results in Table 2 below.

Table 2: Effect of Temperature on Enzyme Activity

Tube	Temperature (oC)	Bubble Column Height (mm)
1 - on ice		
2 - room temperature		
3 - warm water bath		
4 - boiling		

- Which tube showed the most enzyme activity? Explain. _____
- What is the **optimal temperature** for catalase activity? Explain _____
- Was your hypothesis supported? _____
- What is your conclusion concerning the effect of temperature on enzyme activity? _____
- What is the **optimal temperature** for enzymes in the human body? _____

- What effect could a fever have on enzymatic activity in the human body? _____

Extension Activity:

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 2.

2. What is the dependent variable? Which axis is used to graph this data? _____

3. What is your independent variable? Which axis is used to graph this data? _____

Measuring catalase activity in raw and cooked meat (liver)

Procedure:

1. With a wax pencil (or Sharpie), label two test tubes (#1, #2).
2. Mark tube #1 at the 1 cm and 5 cm levels.
3. Add 1 piece of raw liver to test tube 1 up to the 1st mark, add 1 piece of boiled liver to test tube 2 up to the first mark.
4. Fill each tube to the second mark with hydrogen peroxide.
5. Swirl the contents to mix and wait 10 seconds.
6. Measure the height of the bubble column (in mm) for each tube and record your results below.

Bubble column height (in mm) for raw liver _____

Bubble column height (in mm) for boiled liver _____

- Which tube showed the most enzyme activity? Explain. _____
- Based on this experiment, why do you think it is easier for humans to eat cooked meat versus raw meat? What happens during the cooking process and how do you think that can make it easier for us to digest the meat? _____

Exercise 3: Effect of Concentration on Enzyme Activity

In general, the amount of product produced in a given amount of time should increase if you increase the enzyme concentration.

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment. _____
2. Record a **Hypothesis** for the question stated above. _____
3. Predict the results of the experiment based on your hypothesis (if/then). _____
4. Perform the experiment below and collect your data.

Procedure:

1. With a wax pencil (or Sharpie), label three large test tubes (#1, #2, #3). Make sure to use the larger tubes provided by your instructor.
2. Mark tube #1 at the 1 cm and 5 cm levels.
3. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
4. Swirl the contents to mix and wait 10 seconds.
5. Measure the height of the bubble column (in mm) and record your results in Table 3 below.
6. Mark tube #2 at the 2 cm and 6 cm levels.
7. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
8. Swirl the contents to mix and wait 10 seconds.
9. Measure the height of the bubble column (in mm) and record your results in Table 3 below.
10. Mark tube #3 at the 3 cm and 7 cm levels.
11. Fill to the first mark with catalase and to the second mark with hydrogen peroxide.
12. Swirl the contents to mix and wait 10 seconds.
13. Measure the height of the bubble column (in mm) and record your results in Table 3.

Table 3: Effect of Concentration on Enzyme Activity

Tube	Amount of Enzyme	Bubble Column Height (in mm)
1	1 cm	
2	2 cm	
3	3 cm	

- The amount of bubbling corresponds to the degree of enzyme activity. Which tube showed the most activity. Explain. _____

_____

- If we waited for an unlimited amount of time, would the results be the same in all tubes? Explain. _____

_____

- In this experiment, was the amount of substrate the same in all three tubes? _____
- Generate a hypothesis about what you might observe if the amount of substrate concentration were increased while the enzyme concentration remained the same. Explain. _____

_____

Extension Activity:

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 3.

2. What is the dependent variable? Which axis is used to graph this data? _____
3. What is your independent variable? Which axis is used to graph this data? _____

Exercise 4: Effect of pH on Enzyme Activity

Each enzyme has an optimal pH, or level of acidity or alkalinity at which it functions best. A higher or lower pH affects hydrogen bonding and can alter the structure of the enzyme, leading to reduced activity.

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment. _____
2. Record a **Hypothesis** for the question stated above. _____
3. Predict the results of the experiment based on your hypothesis (if/then). _____
4. Perform the experiment below and collect your data.

Procedure:

1. With a wax pencil (or Sharpie), label three small test tubes (#1, #2, #3) and mark at the 1 cm, 2 cm, and 6 cm levels. Fill each

tube to the 1 cm level with catalase.

2. Fill tube #1 to the second mark with 0.1 M HCl.

3. Fill tube #2 to the second mark with buffer (pH 7.0). Fill tube #3 to the second mark with 0.1 M NaOH.
4. Carefully swirl to mix and wait 20 seconds.
5. Fill all three tubes to the third mark with hydrogen peroxide.
6. Carefully swirl to mix and wait 20 seconds.
7. Measure the height of the bubble column (in mm) for each tube and record your results in Table 4 below.

Table 4: Effect of pH on Enzyme Function

Tube	Acid / Buffer / Base	Bubble Column Height (in mm)
1	0.1 M HCl	
2	Buffer pH 7.0	
3	0.1 M NaOH	

- Which tube showed the most activity? _____
- What is the **optimal pH** for catalase? _____

Questions for Review

1. What is the function of an enzyme? What specific **monomers** (i.e. building blocks) make up enzymes?

2. What does it mean when an enzyme becomes **denatured**?

3. **Describe** at least **2** ways in which an enzyme can become denatured.
   1.
   2.
4. What types of bonds or interactions are broken when an enzyme becomes denatured (name at least 3)?

5. Explain the difference between **substrate** and **active site**.

6. If an enzyme functions well at a pH of 4, would you expect it to also work well at a pH of 7? Explain.

Practical Challenge

1. Eggs can contain bacteria such as *Salmonella*. Considering what you've learned in this laboratory exercise, explain how cooking eggs makes them safe to eat.
2. What can happen to an enzyme when the pH of the environment becomes too acidic or too alkaline? **Use your observations from the lab to support your answer**.
3. If you don't want a cut potato to get brown, what could you do to prevent it assuming that the 'browning' occurs because of an enzymatic reaction? **Explain** your answer.
4. What would happen to enzyme activity if the pH of your blood decreased (became more acidic) or increased (became more basic)? Explain.

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1.8: Cellular Respiration and Photosynthesis

Cellular Respiration

There are many ways to rebuild and recycle ATP, but not all methods are created equal nor are they free. Cells must bring in energy in order to rebuild ATP. They do this by bringing in and using the chemical energy stored in molecules such as glucose.

Cellular respiration is one process used to rebuild ATP by using the chemical energy stored in glucose. Glucose will go through a series of chemical reactions to move high energy electrons and hydrogen ions. Cellular respiration is *aerobic*, meaning the final electron acceptor is oxygen. This is why we rely on breathing in oxygen, as most of our ATP is produced by cellular respiration.

Cellular respiration is composed of three main stages: glycolysis, the citric acid cycle (or Krebs cycle), and the electron transport chain. Glycolysis occurs in the cytoplasm of the cell, while the citric acid cycle and electron transport chain both occur inside the mitochondria.

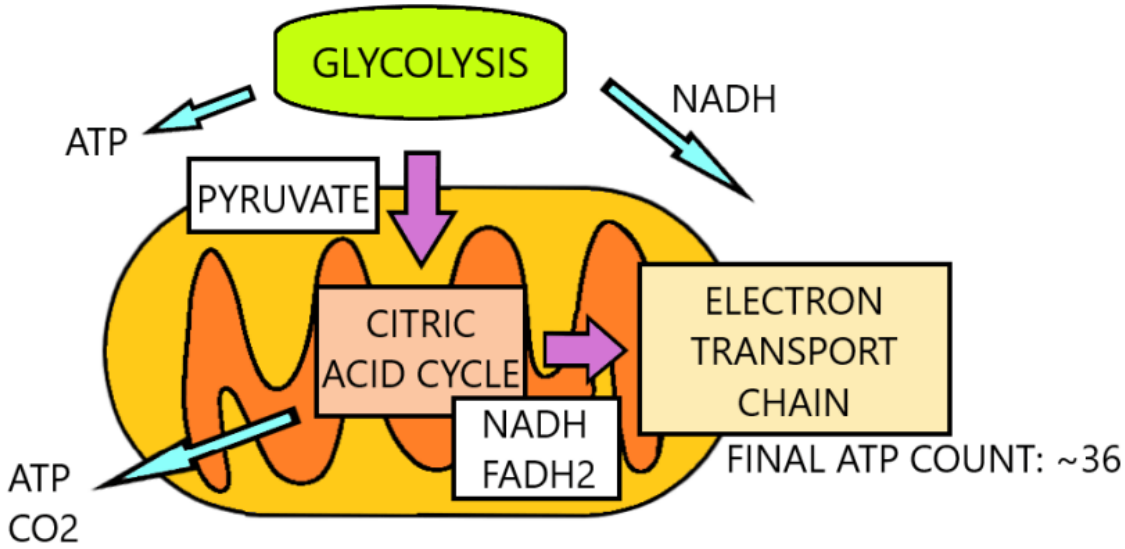


Figure 1: by Karen Marks, Reedley College is licensed under CC BY 4.0

For this activity, we will assess your ability to undergo cellular respiration. Remember that cellular respiration will use oxygen and produce carbon dioxide. Carbon dioxide forms an acid called carbonic acid when mixed with water. This acid can be easily detected with a pH indicator such as bromothymol blue. You will be performing an experiment to view how physical activity changes the rate of cellular respiration.

First let's formulate a hypothesis. In the table below, rank each activity from fastest (1) to slowest (3) rate of respiration.

Table 1 Cellular Respiration Hypothesis

Activity	Rank (1 = fastest, 3 = slowest)
Rest	
Slow Walk	

Activity	Rank (1 = fastest, 3 = slowest)
Fast Walk	

For the following procedure, you and your lab partner will both complete the following procedure. You may want to have one participant go first, and then have the second participant go after.

Materials:

- 6 test tubes, labeled (3 per person)
- 1 transfer pipette
- Bromothymol blue (may substitute with phenol red)
- 2 drinking straws (1 per person, different colors)

Make sure you only use your own straw for this exercise to prevent the spread of germs. Do not inhale, aspirate, or drink the bromothymol blue.

Procedure:

1. Label your test tubes according to the activities listed in Table 2
2. Add 1 mL of bromothymol blue to each tube.
3. Sit or stand still at your table for 30 seconds.
4. Place the straw in your “Rest” tube and prepare a timer.
5. Record how long it takes for the bromothymol blue to turn yellow while gently blowing into the straw.
6. Walk for 30 seconds. (You may want to do this in the hallway.)
7. Repeat steps 4-5 with your tube labeled “Slow Walk”.
8. Walk outside, and walk at a faster than normal pace around the building.
9. Repeat steps 4-5 with your tube labeled “Fast Walk”.

Table 2 Cellular Respiration Data and Results

Name of Each Participant	Time (s)		
	Rest	Slow Walk	Fast Walk

Now, let’s graph both your and your partner’s data in the graph below. Make a line graph using the data from Table 2. Make sure to color code yours and your partner’s data and label both the x and y axis.

Graph from Table 2

Title:_____

Was your hypothesis supported? If yes, how does your data support it? If not, justify your reasoning.

Fermentation and Substrate

Cellular respiration is not the only way to build ATP. Many organisms, such as eukaryotic yeast and many prokaryotes, use *fermentation* to produce ATP. As humans, we enjoy the benefits of this process and use it to make many food items like bread, kimchi, and beer.

Unlike cellular respiration, fermentation does not need oxygen as a final electron acceptor. Since oxygen is not required, fermentation is an *anaerobic* process. Instead of oxygen, fermentation uses organic molecules, such as acetaldehyde or pyruvate, as final electron acceptors. When pyruvate is used as the final electron acceptor, lactic acid is produced. When acetaldehyde is the final electron acceptor, ethanol is produced. Remember that regardless of the process, a fuel source, such as glucose, is required, otherwise the cell will not be able to use either cellular respiration or fermentation.

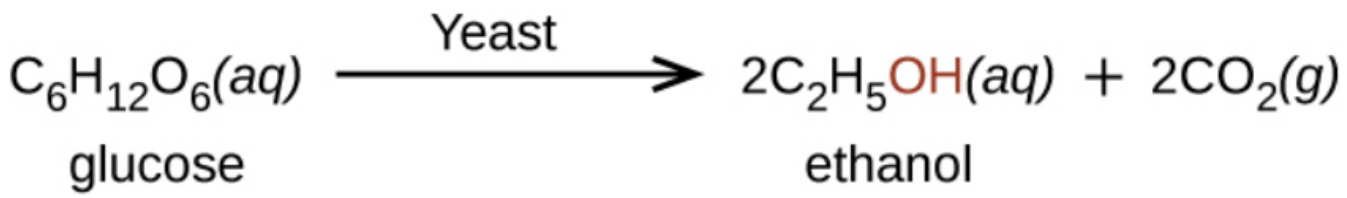


Figure 2. OpenStax, CC BY 4.0, via Wikimedia Commons

To measure fermentation, we will capture the CO_2 produced by yeast. We will place yeast in several environments with different substrates: some with varying levels of glucose, one with artificial sweetener, and one with only water. First, let's develop a hypothesis for this exercise.

Table 3 Fermentation Hypothesis

Yeast Environment	Fermentation? (High/Low/No)
Glucose	
Fructose	
Artificial Sweetener	
Water	

Materials:

- 4 large test tubes or jars, labeled
- 8g baker's yeast
- Scale with weigh boat
- 4 small balloons
- Glucose
- Fructose
- Artificial Sweetener
- Distilled water
- String and ruler

Procedure:

1. Label the tubes according to Table 4.
2. Add 2g yeast to each tube.
3. Add 1 g glucose to Tube 1.
4. Add 1 g fructose to Tube 2.
5. Add 1 g artificial sweetener to Tube 3.
6. Add 100 mL water to tubes 1-4.
7. Shake the tubes.
8. Place a balloon over the top of each tube. Ensure it is secure.
9. Measure the diameter of each balloon and record it in Table 5.4.
10. Leave the tubes alone for 40 minutes, checking every 10 minutes.
11. After 40 minutes, measure the diameter of each balloon.

Table 4 Fermentation Data and Results

Yeast Environment	0 Minutes	10 Minutes	20 Minutes	30 Minutes	40 Minutes
Glucose					
Fructose					
Artificial Sweetener					
Water					

Graph your data from the table above.

Graph from Table 4

Title:_____

What does it mean if the balloon inflated?

What gas do you think the bubbles are made of?

Which substrate had the highest rate of fermentation? How do you know?

The Process of Photosynthesis

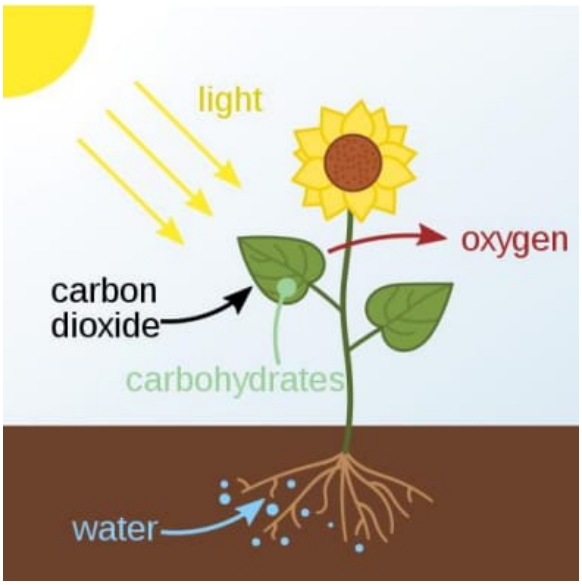


Figure 3. At09kg, Wattcle, Nefronus At09kg: original Wattcle: vector graphics Nefronus: redoing the vector graphics, CC BY-SA 4.0, via Wikimedia Commons

Photosynthesis is composed of two main steps -- the *light dependent* and *light independent reactions*. The light dependent reaction is where energy from light is used to harvest electrons from water. Light is absorbed by chlorophyll in the form of photons. When chlorophyll absorbs a photon, one of its electrons gets knocked away. A water molecule is then split and one of its electrons replaces the one chlorophyll lost.

These electrons are then passed on to the light independent reaction. In the light independent reaction (also called the *Calvin cycle*), these electrons are used to “fix” carbon. Carbon dioxide is taken in through the leaves’ stomata and brought into the chloroplast. Then, an enzyme called RuBisCO strips oxygen away and fixes the carbon to another molecule called RuBP. After cycling through the Calvin cycle, a three-carbon molecule called G3P is formed, with RuBP being recycled to be used again later.

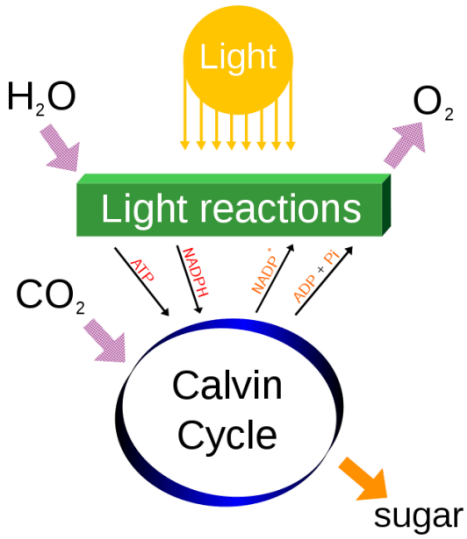


Figure 4. Daniel Mayer (mav) - original imageVector version by Yerpo, CC BY-SA 4.0, via Wikimedia Commons



Figure 5: *Elodea* in phenol red solution (By CB)

For this exercise, we will observe the consumption of CO_2 from photosynthesis. Carbon dioxide (CO_2) is taken in through the leaves of a plant to be used as a carbon source when building carbohydrates. We will use phenol red to detect the use of CO_2. Phenol red appears red when in the presence of a neutral and basic pH, but becomes yellow when the pH becomes acidic. CO_2 becomes carbonic acid when in water, making the liquid slightly acidic. Prior to class, your instructor has added CO_2 to your phenol red, giving it a yellow color. If CO_2 is used up, the solution will become less acidic, turning the solution red.

Materials:

- 2 test tubes with a test tube rack
- 1 sprig of *Elodea*
- Phenol Red (with CO_2 already added)

Procedure:

1. Obtain 1 small branch of *Elodea*, approximately 2-3 cm long.
2. Place 1 branch of *Elodea* in Tube 1.
3. Add enough phenol red (now yellow from the added CO_2) to cover the *Elodea* in Tube 1.
4. Add the same amount of phenol red to Tube 2. (This tube should only have phenol red.)
5. Leave the test tubes in a sunny part of the room (such as a window sill) or outside in the sun for 45 minutes.
6. After 45 minutes, record the color in the space below.

Table 5

Tube	Color Before	Color After
With *Elodea*		
Without *Elodea*		

The Chloroplast

In plants, the organelle responsible for photosynthesis is the chloroplast. Since chloroplasts are responsible for harvesting light for photosynthesis, they are capable of moving within the plant cell to effectively harvest light. When light is weak or low, chloroplasts will move towards the brightest area. When light is too intense or strong, chloroplasts will move away. This may seem counterintuitive, but very intense light can cause damage to the chloroplasts. For this activity we will observe this movement of chloroplasts -- called photorelocation -- in *Elodea*.

Materials:

- Fresh *Elodea* leaves
- Microscope slide and coverslip
- Dropper bottle of distilled water
- Compound light microscope
- Lamp



Figure 6: *Elodea* leaf on microscope slide with coverslip. (By CB)

Procedure:

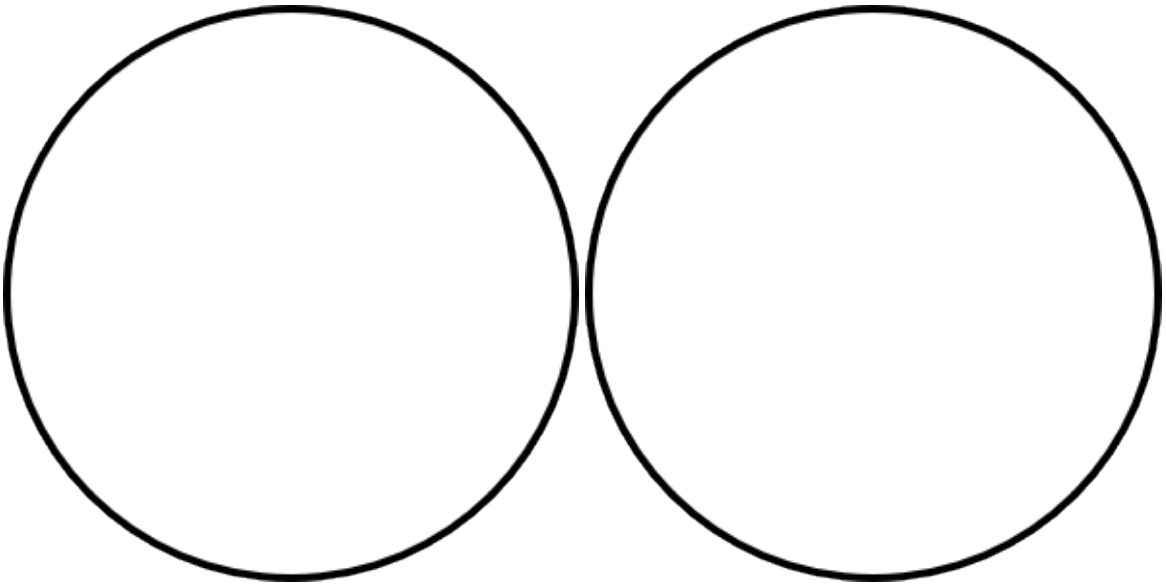
1. Place one fresh *Elodea* leaf on a slide.
2. Add 1 drop of distilled water to the leaf on the slide and cover with the coverslip.

3. View the *Elodea* slide under the microscope using the high objective.
4. After viewing the *Elodea* under normal conditions, shine the lamp on one side of the slide.
5. Leave the lamp shining on one side of the slide for 10 minutes.
6. After 10 minutes, view the *Elodea* slide again.

How were the chloroplasts dispersed in the *Elodea* cells before exposure to bright light?

How were the chloroplasts dispersed in the *Elodea* cells after exposure to bright light?

Draw the field of view before and after the exposure to bright light.



Wet mount slides should NEVER go into the regular trash. Make sure to follow in-class disposal instructions for wet mount slides.

Pigments and Photosynthesis

Within the chloroplasts are photosynthetic pigments. These pigments, such as *chlorophyll a* and *chlorophyll b*, help capture light energy for the plant. However, these pigments cannot just capture any wavelength of light. Chlorophylls are limited in which light wavelengths they are able to absorb. Any wavelength of light they cannot absorb is reflected. This is why plants appear green, as green wavelengths (500-600 nm) are reflected, while blue wavelengths (425-500 nm) and red wavelengths (600-700 nm) are readily absorbed.

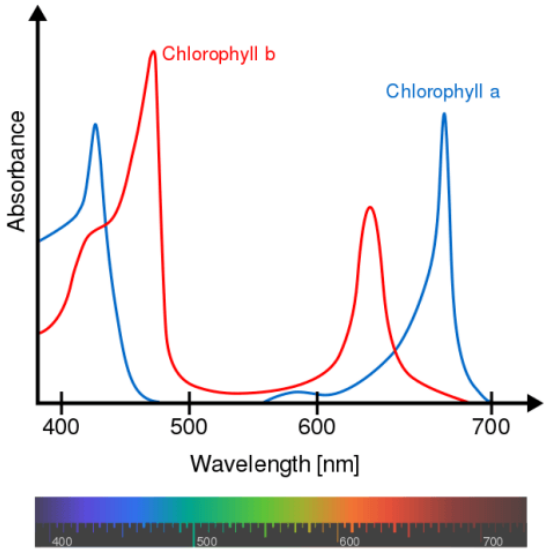


Figure 7. Chlorophyll_ab_spectra2.PNG: Daniele Pugliesi derivative work: M0tty, CC BY-SA 3.0, via Wikimedia Commons

Chlorophylls are not the only pigments found in plants, however. Other pigments such as *carotenoids* (which appear yellow and orange) and *anthocyanins* (which take on shades of red and blue) may also be found alongside the chlorophylls. For this final exercise, we will discover which pigments are found within a spinach leaf.

Hypothesis: Based on the above information, which pigment(s) do you think you will see in a spinach leaf?

Materials:

- 2-3 fresh spinach leaves
- A pencil, paperclip, and coin
- A 10 cm length of chromatography paper
- Chromatography solvent or acetone
- A small beaker
- A metric ruler

Procedure:

1. Fold the chromatography paper in half lengthwise.
2. Measure 2 cm away from one edge and draw a point or line at the 2 cm measurement.
3. Place a spinach leaf on the point or line and use the coin to press the leaf into the paper.
4. Continue pressing, using fresh parts of the leaves until a solid line of spinach extract has been pressed into the paper. (See example image below.)
5. Obtain 10 mL of chromatography solvent (or acetone) from the instructor in the beaker.
6. Place the chromatography paper in the beaker with the spinach end closest to the solvent and the other end facing up. (See example image below.)
7. Use the paperclip to hold the paper to the beaker.
8. Let sit for 45 minutes. Check periodically to ensure that the solvent does not fully evaporate. If the solvent runs low, obtain more from your instructor.
9. After 45 minutes, record and draw your results.

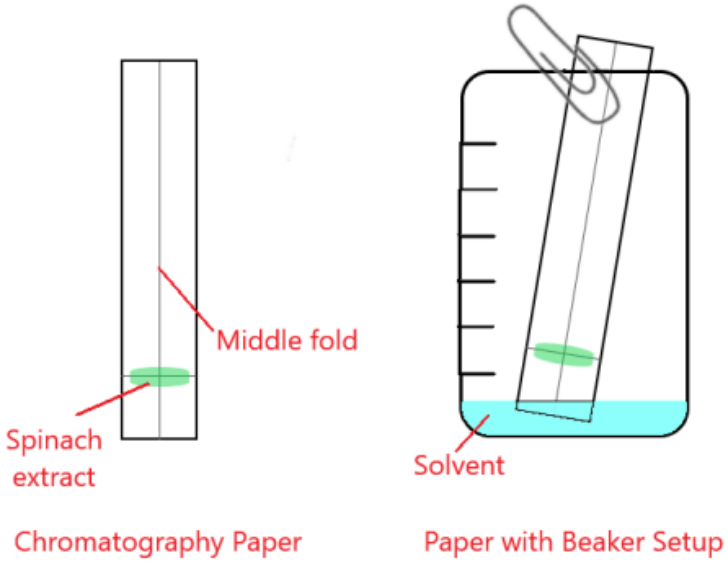


Figure 8. by Karen Marks, Reedley College is licensed under CC BY 4.0



Figure 9: Liquid chromatography set up Oxnard College. (By CB)

Table 6 Paper Chromatography Results

Pigment(s) Present (include color)	
Drawing of Chromatography Paper	

How do your results compare to your hypothesis?

Were there any pigments present that surprised you?

In the fall, leaves lose their green color and instead appear in shades of yellow, orange, and red. Based on your knowledge of plant pigments, which pigments disappeared from the leaves? Which ones are likely still present?

Additional Figures

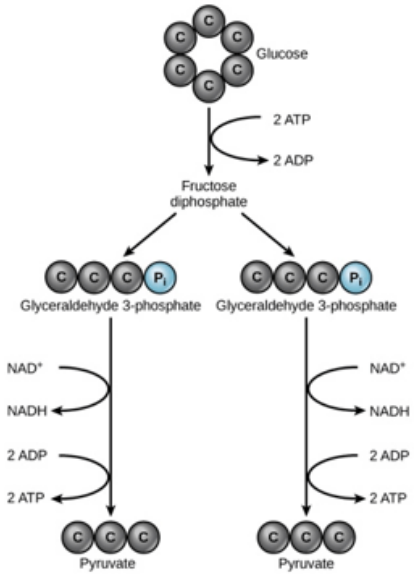


Figure A: In glycolysis, a glucose molecule is converted into two pyruvate molecules. (Glycolysis by Lumen Learning, CC BY 4.0)

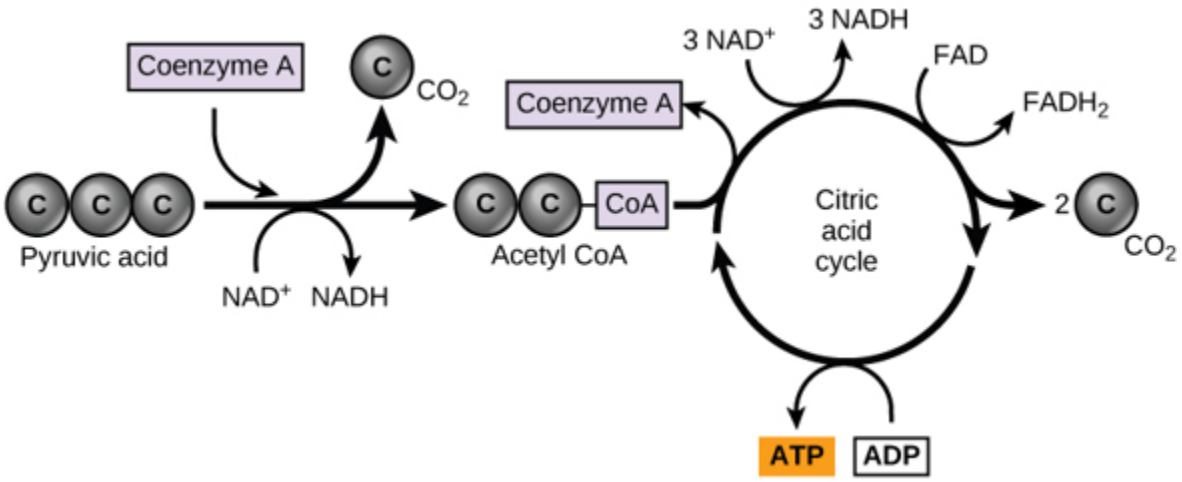


Figure B: Pyruvate is converted into acetyl-CoA before entering the citric acid cycle. (Citric Acid Cycle by Lumen Learning, CC BY 4.0)

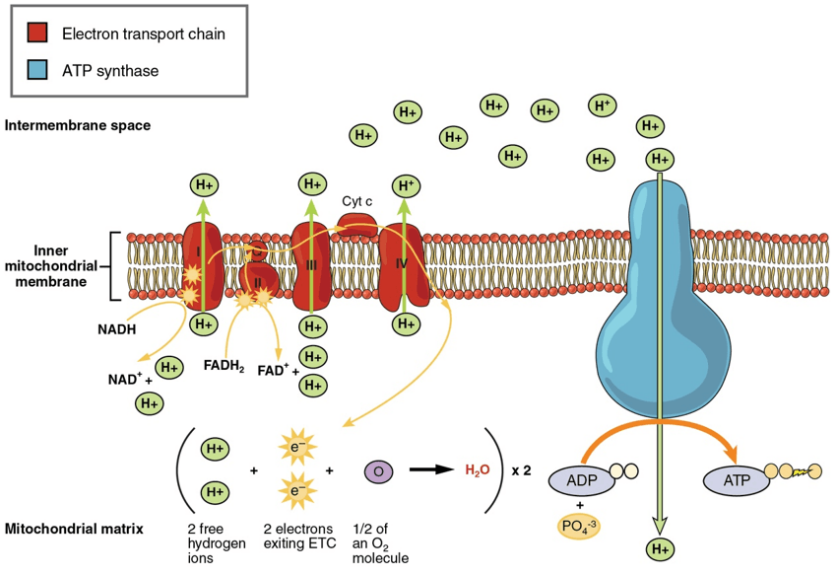


Figure C: Oxidative Phosphorylation - Electron Transport chain and Chemiosmosis (Electron Transport Chain by OpenStax College, licensed CC BY 4.0 via Wikimedia Commons)

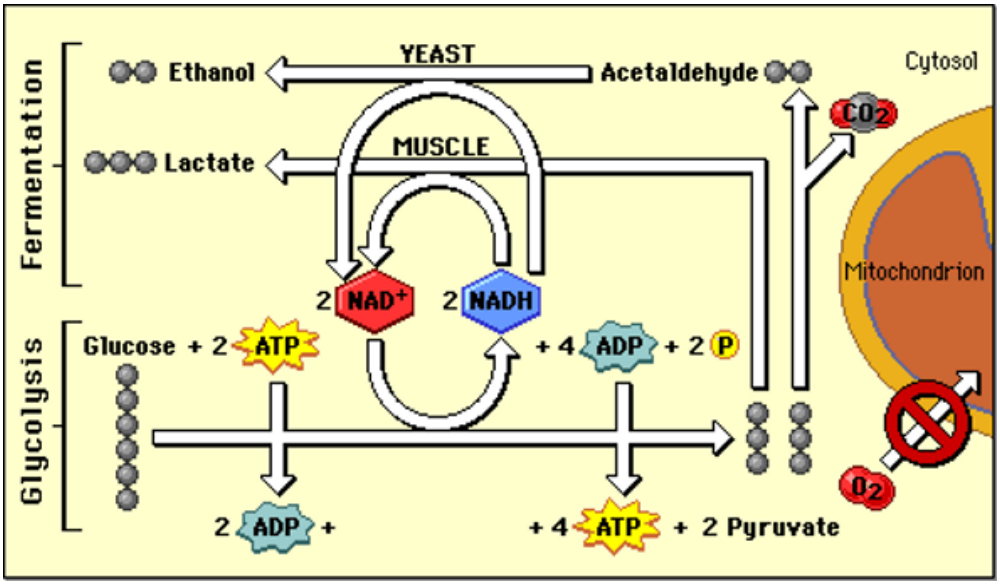


Figure D: The picture shows glycolysis and fermentation. There are two types of fermentation, alcoholic and lactic acid. Fermentation follows glycolysis in the absence of oxygen. Alcoholic fermentation produces ethanol, carbon dioxide, and NAD^+. Lactic acid fermentation produces lactic acid (lactate) and NAD^+. The NAD^+ cycles back to allow glycolysis to continue so more ATP is made. Each black ball in the image represents a carbon atom, and the red balls represent oxygen. (Alcohol fermentation by Vtvu, licensed CC BY-SA 3.0 via Wikimedia Commons)

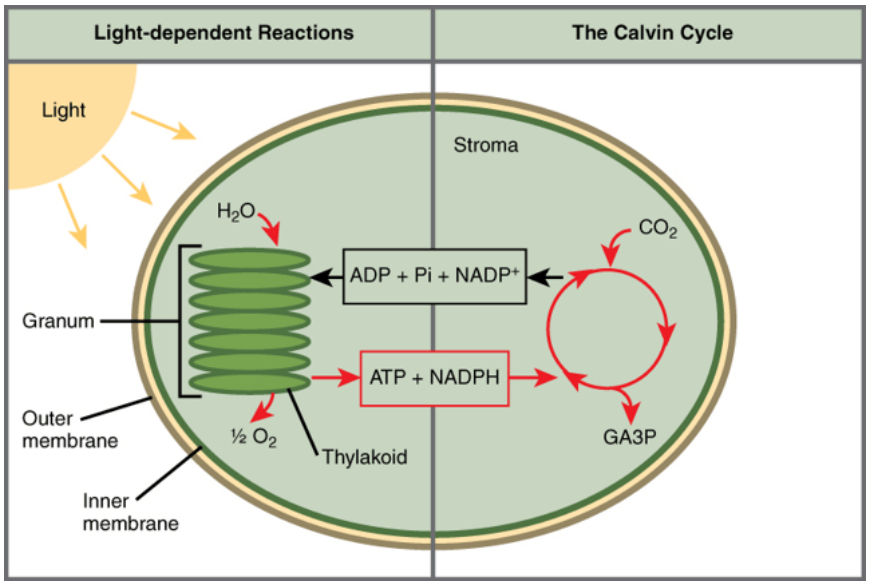


Figure E: Photosynthesis takes place in two stages: light dependent reactions and the Calvin cycle. Light-dependent reactions, which take place in the thylakoid membrane, use light energy to make ATP and NADPH. The Calvin cycle, which takes place in the stroma, uses energy derived from these compounds to make GA3P from CO_2. (**8.1: Overview of Photosynthesis** by OpenStax, is licensed)

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1.9: Mitosis

Introduction

Learning Objectives

- Describe the phases of the cell cycle
- Identify and explain each of the four phases of mitosis

Early in the semester we learned about cell theory. One of the components of cell theory is that cells must come from other cells. How does that happen though? There are actually several ways to replicate cells. Which process a cell uses depends on what type of organism the cell is and the reason why the cell is replicating.

One such method is *binary fission* -- a replication process used by simple organisms such as bacteria and archaea. In binary fission, the bacterium's single circular chromosome is replicated with each copy moving to opposite sides of the cell. Then proteins begin pinching the cell in the middle, a septum forms, and the cell splits into two. This process is both simple and fast, meaning bacteria can replicate very quickly.

Another method is *mitosis*. This process is used by the more complex eukaryotic cells. In multicellular organisms like ourselves, mitosis is used for growth and repair. Consider how big you were when you were born. Are you the same size? Hopefully, no. But rather than your cells getting larger, your body has simply made more cells to create the growth you've experienced over the years. You've also probably experienced a few injuries before. But if you get a cut, it heals. This healing you see is because the cells that were killed and damaged by whatever cut your skin were replaced. Nearby healthy cells used mitosis to replace them.

Cell Cycle

In eukaryotic organisms like plants and animals, cell replication is a tightly regulated cycle. Cells must receive various signals and meet certain criteria before being able to divide. These checks and balances ensure that the cell has enough building material, that the DNA replicates correctly, and that the chromosomes are aligned properly to divide. This helps ensure that the organism is keeping up with normal rates of cell death and not wasting resources. When this system fails, things like cancer can result.

The cell cycle can be broken down into two main parts: *interphase* and the *mitotic phase*. Let's look at interphase first. In interphase, there are three distinct phases: G_1, S, and G_2. In the G_1 phase, the cell is running its daily activities and is gathering needed materials for the next steps. Then the cell enters the S phase. S phase is unique in that this is when a cell replicates its entire genome (the complete set of DNA) of the cell. After all of the DNA has been replicated, the cell then enters the G_2 phase. In the G_2 phase, the cell makes final preparations to divide.

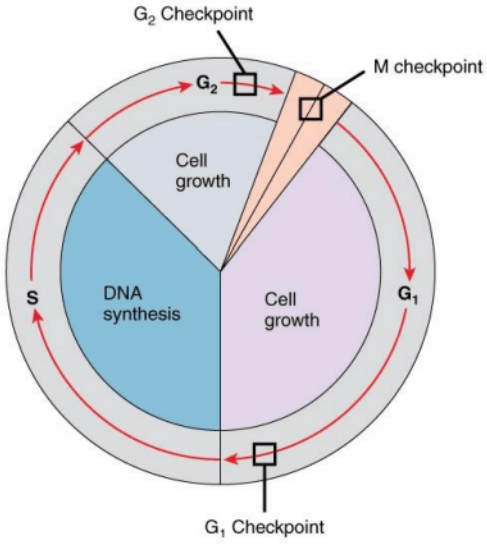


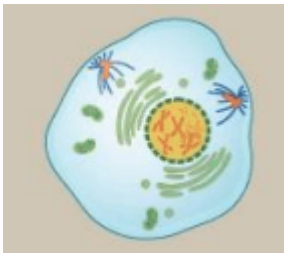
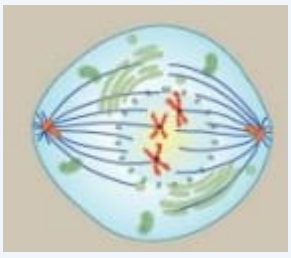
Figure 1: Stages of Cell Cycle. (Modified by Karen Marks, from OpenStax, CC BY 4.0, via Wikimedia Commons)

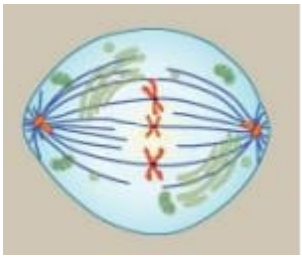
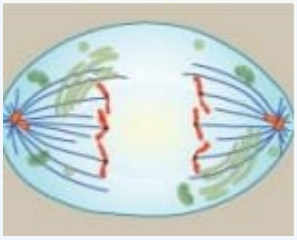
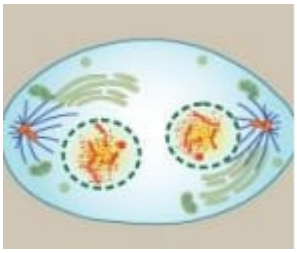
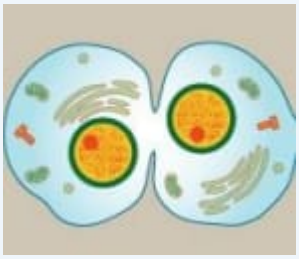
Looking at the figure above, do you think a cell spends more time in interphase or mitotic phase?

Mitosis in Plant and Animal Cells

Mitosis has five phases: prophase, prometaphase, metaphase, anaphase, and telophase. Once the cell has completed these phases, it will split in two via cytokinesis.

Table 1 Phases of Mitosis

PHASE	DESCRIPTION	APPEARANCE
Prophase	• Chromosomes condense and become visible • Nuclear envelope disappears • Centrosomes move to opposite sides (poles)	
Prometaphase	• Kinetochores (attachment points) appear on chromosomes • Spindle fibers form and attach to chromosomes	

PHASE	DESCRIPTION	APPEARANCE
Metaphase	• Chromosomes line up at equator • Spindle fibers attach sister chromatids to opposite poles	
Anaphase	• Sister chromatids are pulled apart and towards opposite poles • The cell begins to elongate	
Telophase	• Chromosomes are on opposite poles • Nuclear envelope reforms around chromosomes • Spindle fibers break down	
Cytokinesis	• The cell splits into two • Forms a cleavage furrow (animals) or cell plate (plants)	

Observe the phases of Mitosis in Plant Cells

Exercise 1: Observing the Phases of Mitosis in the Onion Root Tip

Materials:

- Prepared slide of the onion root tip
- Compound light microscope

Procedure:

1. Examine a slide of a longitudinal section of an onion root tip. Adjust the slide to view the region just above the root cap, where there are likely to be dividing cells.
2. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
3. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase in the boxes provided.

Magnification	Interphase	Prophase	Metaphase	Anaphase	Telophase/Cytokineses
4x Objective					
10x Objective					
40x Objective					

Interphase

DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.

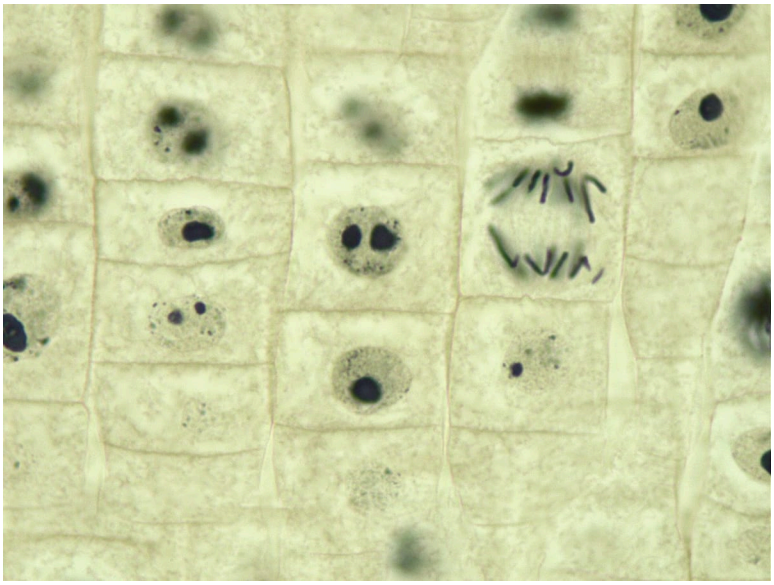


Figure 2: Interphase in plant cells.

Prophase

Chromatin begins to condense into distinguishable chromosomes. These “puffy” structures are seen throughout the nucleus. Nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.

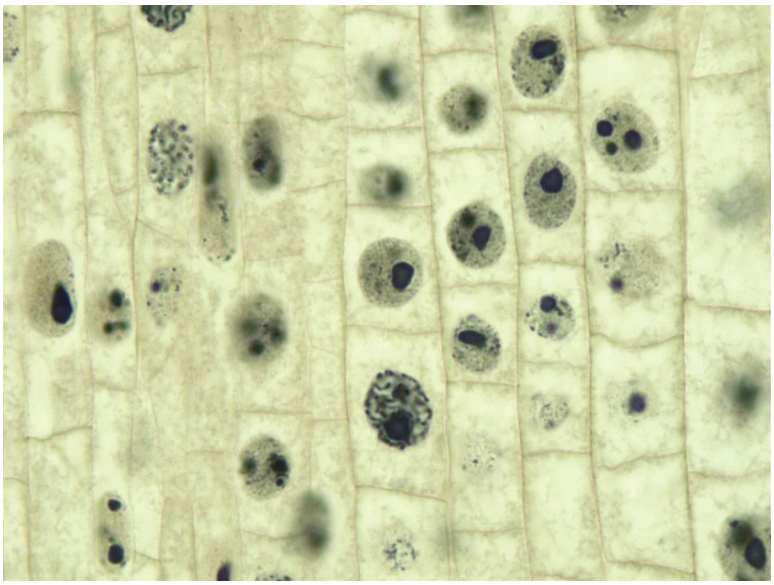


Figure 3: Prophase in plant cells.

Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.

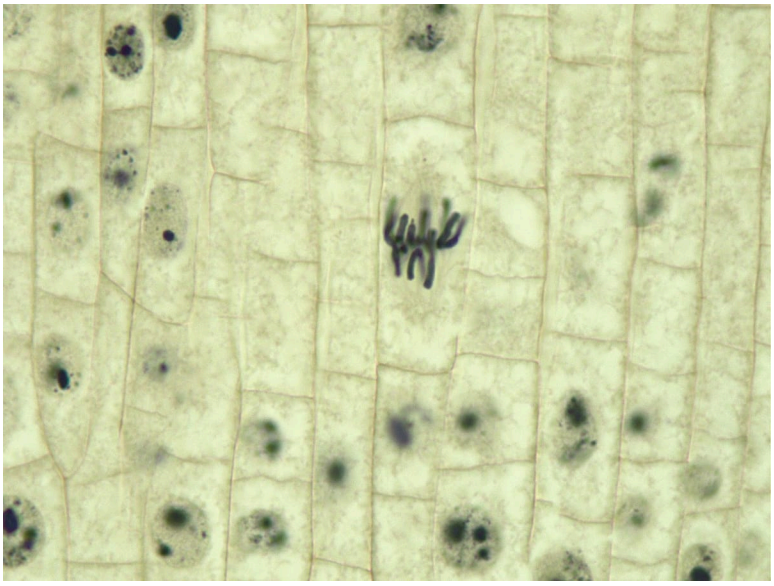


Figure 4: Metaphase in plant cells.

Anaphase

Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.

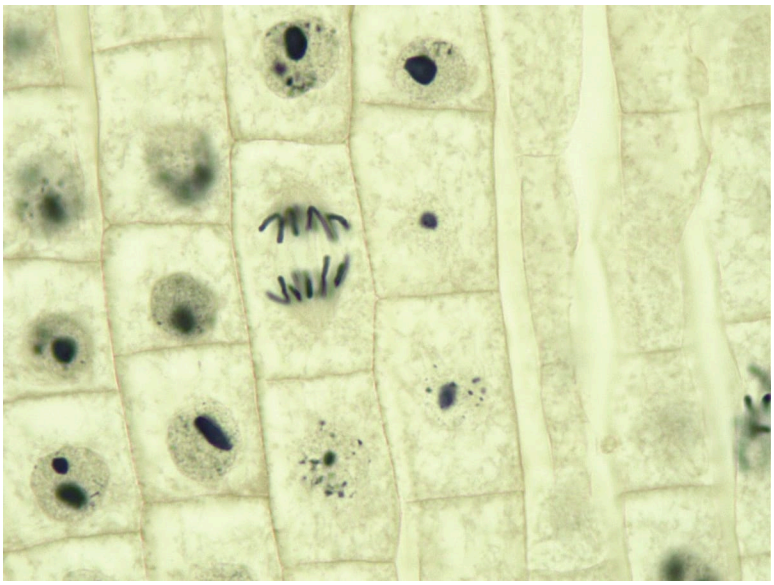


Figure 5: Anaphase in plant cells.

Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. The formation of a cell plate forms between the two cells to carry out cytokinesis.

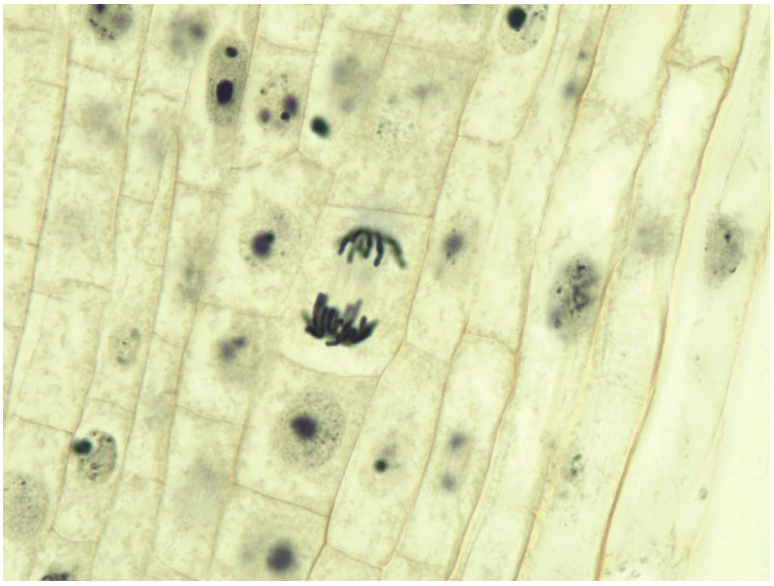


Figure 6: Telophase in plant cells.

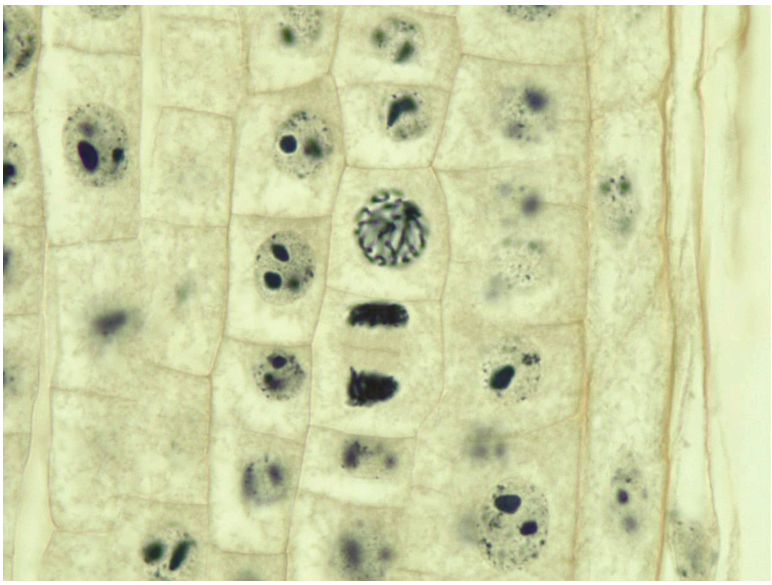


Figure 7: Cytokinesis in plant cells.

Observe the phases of Mitosis in Animal Cells

Exercise 2: Observing the Phases of Mitosis in the Whitefish Blastula

Materials:

- Prepared slide of whitefish blastula
- Compound light microscope

Procedure:

The blastula is an early embryonic stage where many of the cells are dividing at any one time.

1. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
2. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase in the boxes provided.

Magnification	Interphase	Prophase	Metaphase	Anaphase	Telophase/Cytokineses
4x Objective					
10x Objective					

Magnification	Interphase	Prophase	Metaphase	Anaphase	Telophase/Cytokineses
40x Objective					

Interphase

The DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.

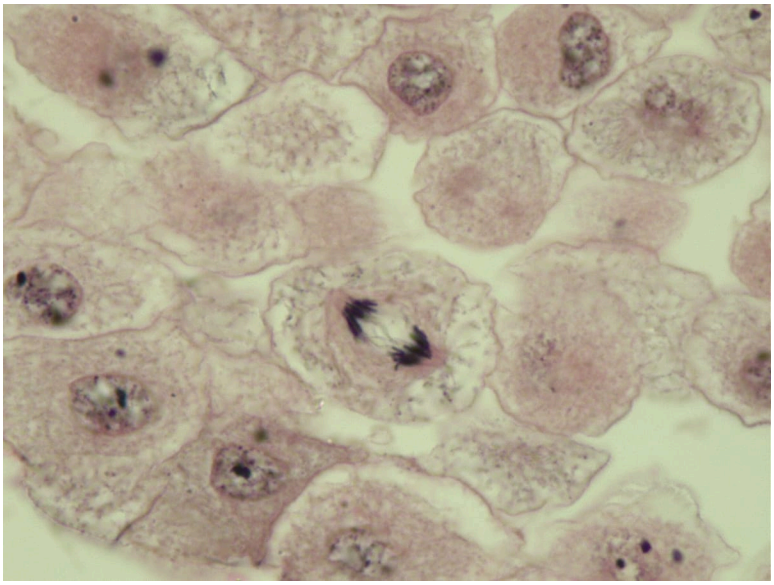


Figure 8: Interphase in animal cells.

Prophase

Chromatin begins to condense and chromosomes are distinguishable. These “puffy” structures are seen throughout the nucleus. The nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.

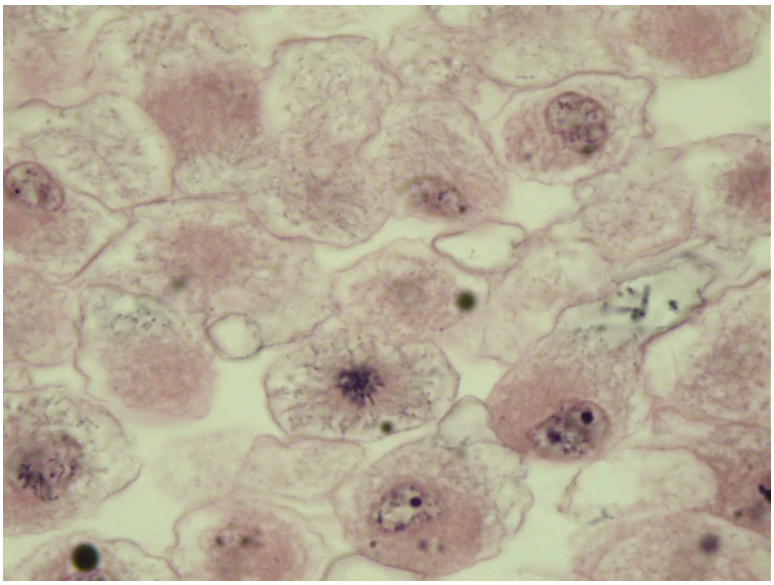


Figure 9: Prophase in animal cells.

Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.

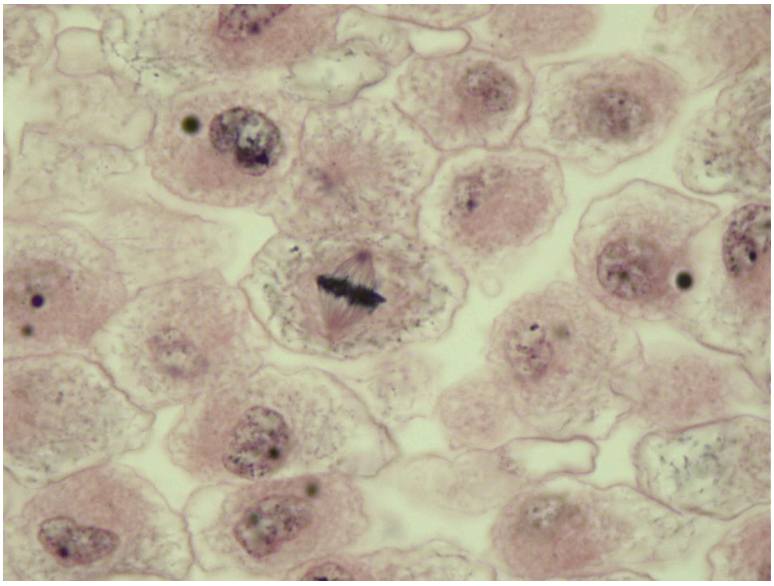


Figure 10: Metaphase in animal cells.

Anaphase

Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.

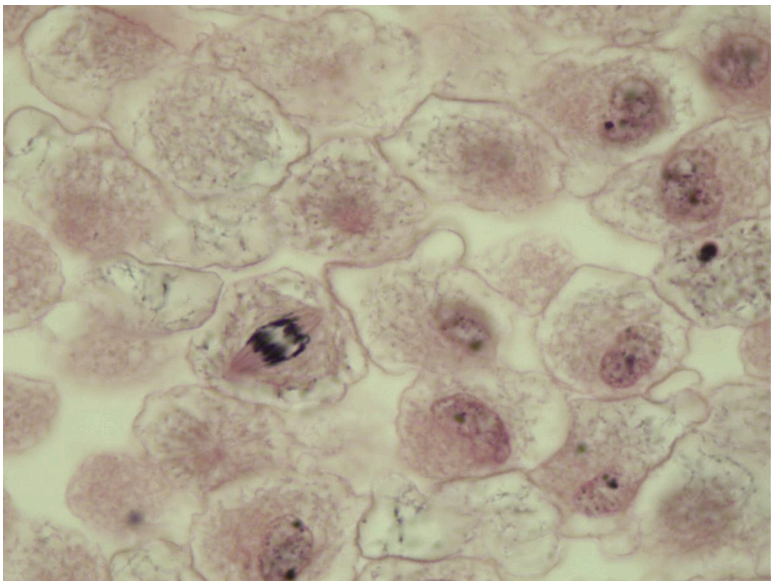


Figure 11: Anaphase in animal cells.

Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. A **cleavage furrow** forms between the two cells to carry out cytokinesis.

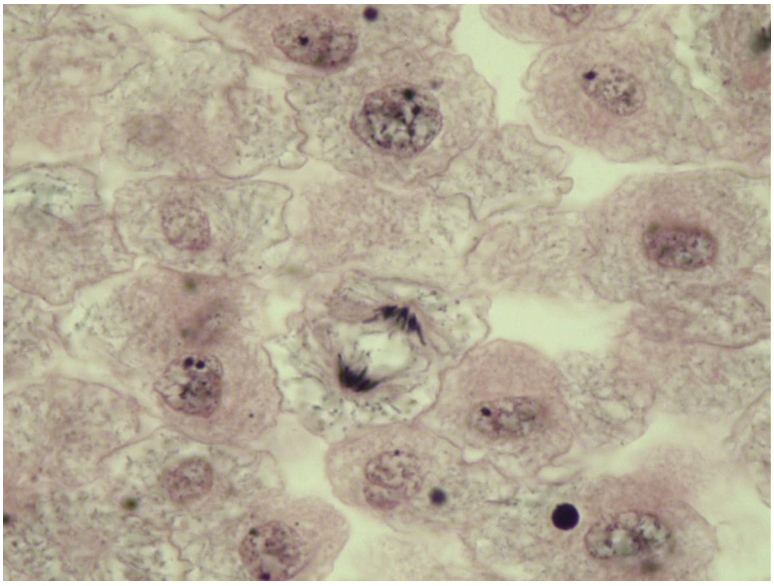


Figure 12: Telophase in animal cells.

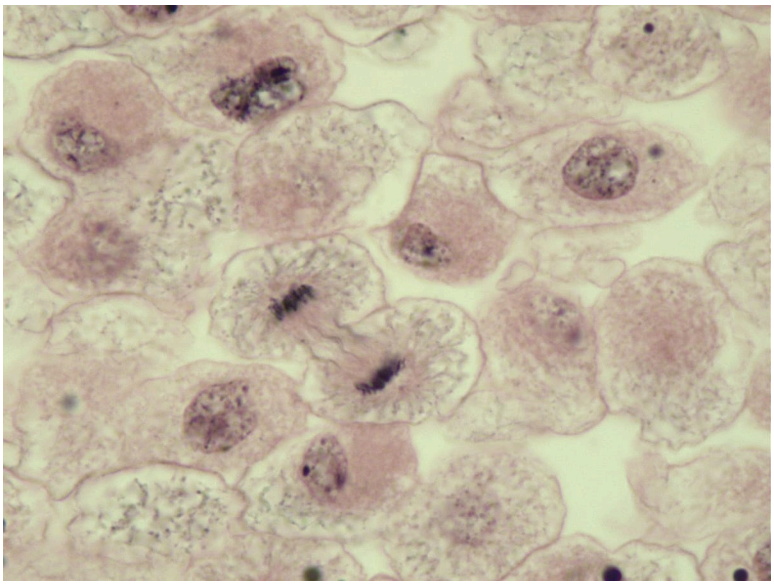


Figure 13: Cytokinesis in animal cells.

- Why would the method of cytokinesis in animal cells not work in plant cells? Explain.

How Long Does a Cell Spend in Each Phase of the Cell Cycle?

Exercise 3: Determining Time Spent in Different Phases of the Cell Cycle (Optional)

Materials:

- Laptop
- Calculator

Procedure:

1. Count ~ 30 cells in total per field of view using the onion root tip slide.
2. Use the formula given below and record your results in the table.
3. The duration of each stage of mitosis can be determined by using the following formula. Compute the length of time for each stage and place your calculations in the table below.

(Number of cells in a stage ÷ Total number of cells) x 1440 (min in a day) = minutes a cell spends in each stage in one day.

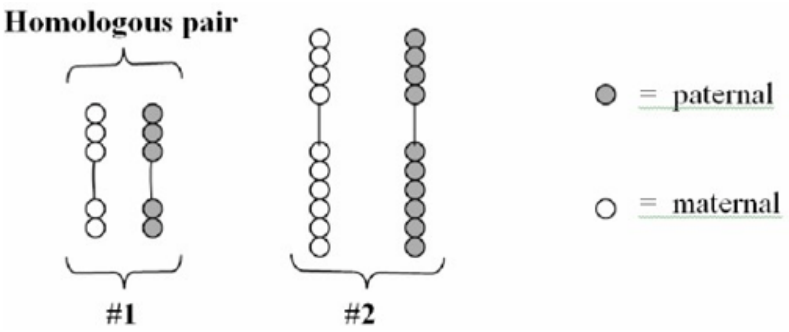
Data Table for Mitosis Stages

Number of cells in each stage	Interphase _____	Prophase _____	Metaphase _____	Anaphase _____	Telophase _____	Total 36
Percent of cells (# of cells / Total)						100%
Time (in minutes) spent in Stage – use calculation above						

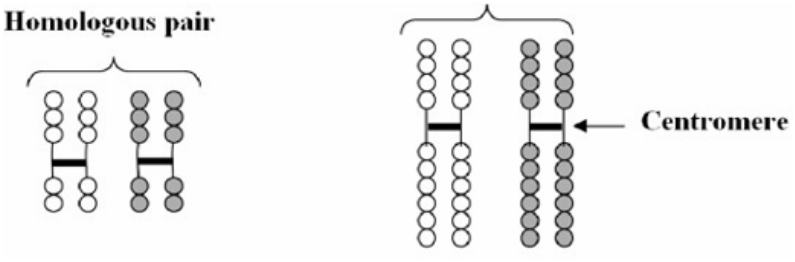
Modeling Mitosis

Now that we've studied cells at the various stages of mitosis, it's your group's turn to model mitosis in the lab. For this exercise, your group will use pop beads to make two pairs of homologous chromosomes. Each pair of homologous chromosomes must be in a different color. Then consider the desk as a cell and arrange the chromosomes to indicate phases of mitosis on the lab bench and explain what happens during each phase to your instructor.

Example of chromosomes made with pop beads:



Replicated chromosomes:



In your explanations, you must follow these "rules":

1. Everyone in the group must participate
2. You must use the proper terminology to explain something
3. You cannot read steps directly from a book or other resource
4. Be prepared before calling the instructor over

Group Members:

Verified complete:_____ (Instructor signature)

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- Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). “Lab 10: Mitosis & Meiosis,” (2019). *Biological Sciences Open Textbooks*. 18. CC-BY (https://oer.galileo.usg.edu/biology-textbooks/18)

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1.10: Meiosis and Gametogenesis

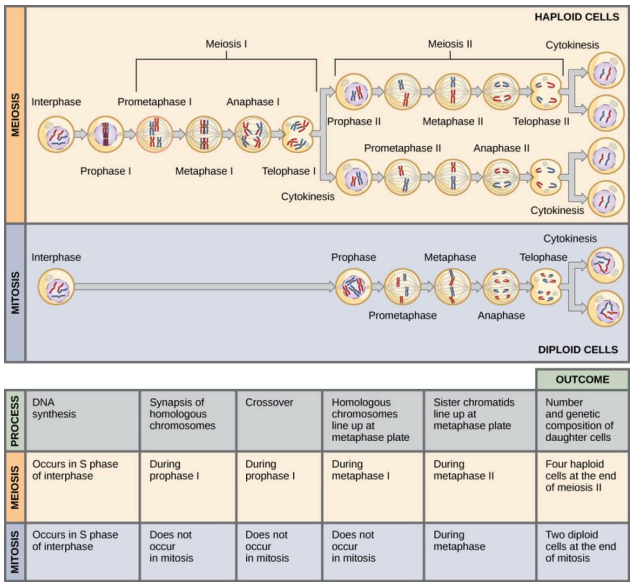
In the previous laboratory exercise, you examined mitosis, the process of cell division used for growth, repair, and replacement of cells. Mitosis produces two genetically identical daughter cells and helps multicellular organisms maintain their tissues throughout life.

In this laboratory, you will investigate meiosis, a different type of cell division found in organisms that reproduce sexually. Meiosis produces **haploid** cells containing half the chromosome number of the original cell. In animals, these cells develop into gametes, such as sperm and eggs. In plants, meiosis produces spores that develop into structures that later produce gametes. During meiosis, chromosomes are shuffled and recombined, creating genetic variation among offspring.

In this exercise, you will compare mitosis and meiosis, model the process of meiosis using chromosome beads, explore how independent assortment and crossing over increase genetic variation, and observe cells undergoing meiosis in the lily anther.

Comparing Mitosis and Meiosis:

In both meiosis and mitosis, the original parent cell is a diploid cell. Remember, this means the cell has two copies of each chromosome. Meiosis and mitosis are both nuclear divisions that result in new daughter cells. However, the two processes have significant differences. Using the diagram of mitosis and meiosis below, fill out the chart below comparing the two forms of nuclear division.



	OUTCOME
PROCESS	DNA synthesis; Synapsis of homologous chromosomes; Crossover; Homologous chromosomes line up at metaphase plate; Sister chromatids line up at metaphase plate; Number and genetic composition of daughter cells
MEIOSIS	Occurs in S phase of interphase; During prophase I; During prophase I; During metaphase I; During metaphase II; Four haploid cells at the end of meiosis II
MITOSIS	Occurs in S phase of interphase; Does not occur in mitosis; Does not occur in mitosis; Does not occur in mitosis; During metaphase; Two diploid cells at the end of mitosis

	Mitosis (begins with a single cell)	Meiosis (begins with a single cell)
# Chromosomes in parent cells		
# DNA replications		
# Nuclear divisions		
# Daughter cells produced		
Purpose		

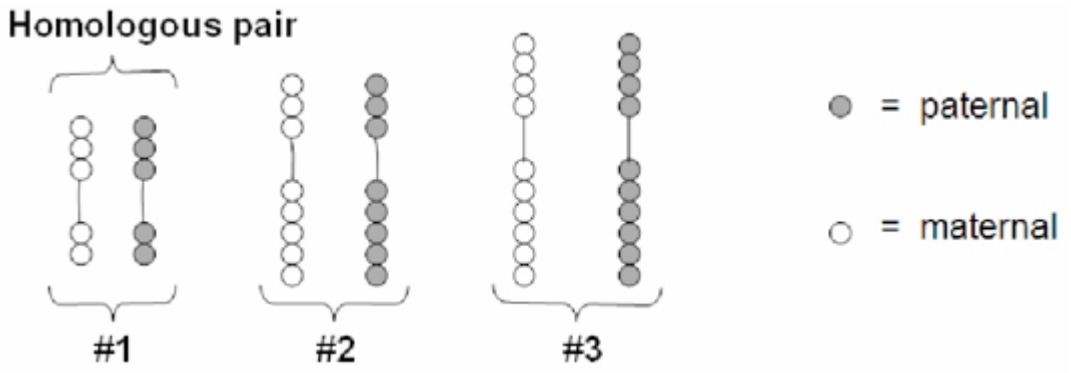
Part 1: Meiosis Bead Simulation

Materials:

- 12 magnets (=centromeres)
- 48 beads of one color
- 48 beads of another color

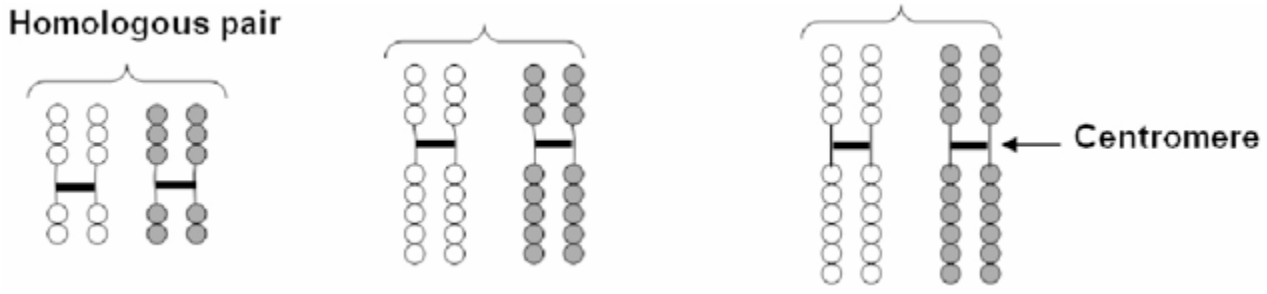
Procedure:

1. Set up half of the beads exactly as follows, representing genes on the chromosome of a hypothetical critter. We will assume that the critter is diploid (2N) and has three different chromosomes. Because the critter has two copies of each of the three chromosomes, the diploid number is 6 (2 × 3 = 6).



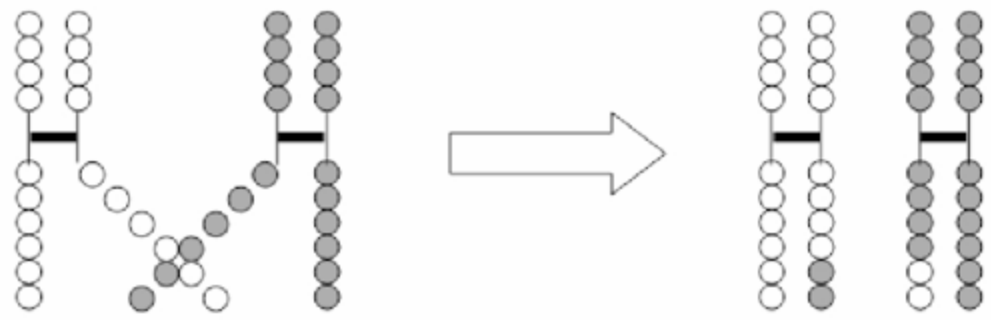
This is what your critter's chromosomes look like in the unreplicated form. Note that there are six chromosomes here consisting of three homologous pairs. Each chromosome pair consists of a maternal and paternal version of the chromosome. The maternal and paternal versions are represented by the respective bead color.

2. Replicate your chromosomes! Make enough copies of each chromosome to represent both paternal and maternal chromosomes in a replicated form, as shown below. Note that the sister chromatids are identical in color. Be sure you can identify the sister chromatids, chromosomes, and the difference between a replicated and non-replicated form.



3. Using your maternal and paternal sets of replicated chromosomes and this lab (or the text) as a reference, practice the process of meiosis until you are very comfortable with it. Each person in the group should practice the entire process.

Part 1: Simulate crossing over by exchanging beads on non-identical sister chromatids as illustrated below. Do this for all the three chromosome pairs.



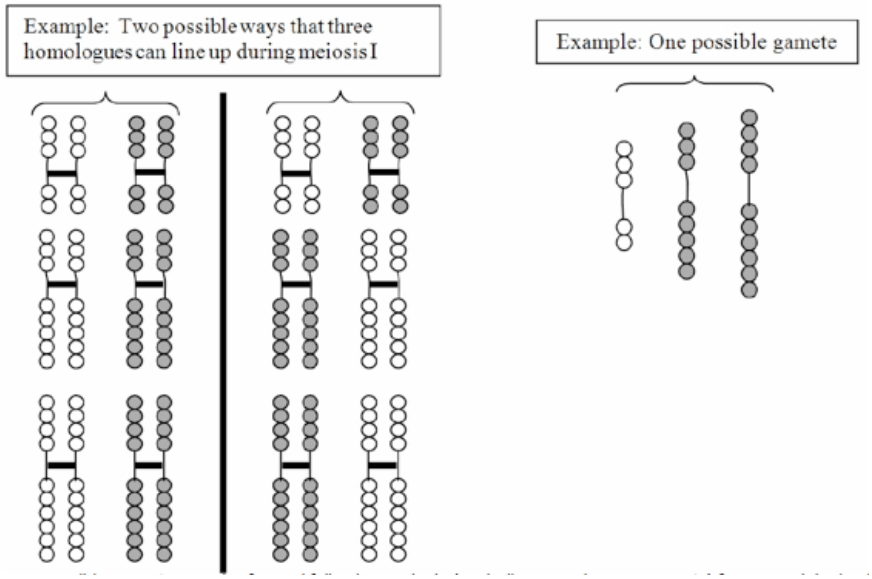
Do NOT proceed until until your instructor directs you to do so.

Part 2: Independent Assortment

There are two possible ways pairs of homologs (also known as tetrads) can line up on the metaphase plate during Metaphase I. This possible number of alignments equals 2_n, where n is the number of chromosomes per set. In humans, n=23, so there are 2_{23} possible ways the homologous pairs can line up on the metaphase plate!

Procedure:

1. Use the beads from the last simulation. This time, demonstrate the principle of independent assortment by determining how many different gametes you can form with three homologous pairs.
2. Use the chromosomes to demonstrate 4 possible ways chromosomes can line up on the metaphase plate.
3. Using simple chromosome drawings like those demonstrated by your instructor, draw a picture of each possible way of lining up. Use different colors to represent maternal and paternal chromosomes.
4. Then draw a picture of **each possible gamete** formed when the chromosomes line up like that.



Lab Questions:

1. How many possible gametes can be formed following meiosis (excluding crossing over events) from an original cell that contains a diploid number of six ($2n$ = 6)? [The number of possible gametes = $2n$ where n is the number of chromosomes per set.]

2. How many possible gametes can be formed following meiosis (excluding crossing over events) from an original cell that

contains a diploid number of 46 ($2n = 46$)?

3. Explain why crossing over can result in a much larger number of possible gamete types than independent assortment alone.

4. Based upon the processes of independent assortment, crossing over, and random fertilization, what is one important difference you would expect to see between a sexually reproducing population of organisms and an asexually reproducing population of organisms?

Part 3: Gametogenesis in the Lily Anther

The formation of gametes, or gametogenesis, is the first stage in sexual reproduction. In single-celled organisms, e.g., many Protista, the vegetative cell acts as a gamete. In more complex organisms specialized regions of the organism are responsible for creating gametes.

In flowering plants, meiosis occurs in specialized reproductive structures. Female reproductive cells develop within the ovary, while male reproductive cells develop within the pollen sacs (microsporangia) of the anther. In this exercise, you will examine meiosis in the lily anther, a tissue that contains many cells undergoing division at the same time. Because the cells do not separate after each division, the stages of meiosis are relatively easy to observe.

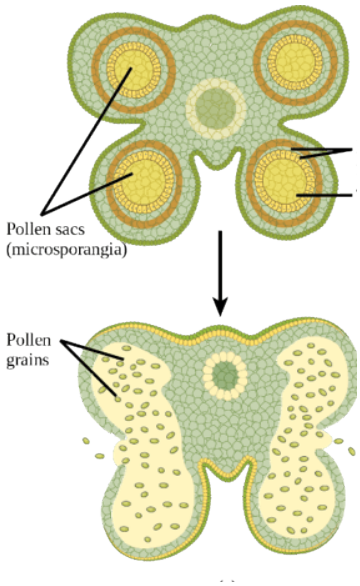


Figure 1: A cross section of an anther at two developmental stages. The immature anther (top) contains pollen sacs. The sacs are filled with immature male gametes undergoing meiosis. When the gametes mature and become pollen grains, the pollen sac walls split open (bottom), and the pollen grains are released.

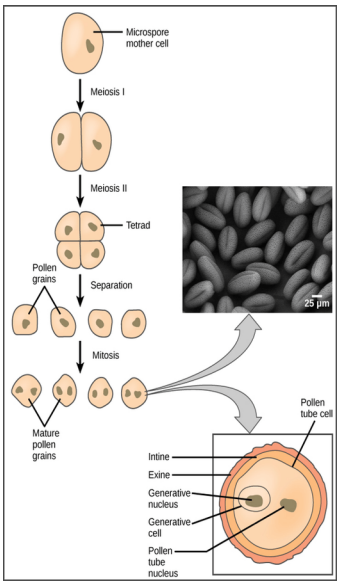
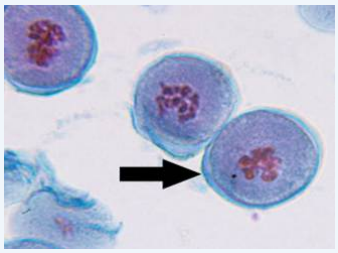
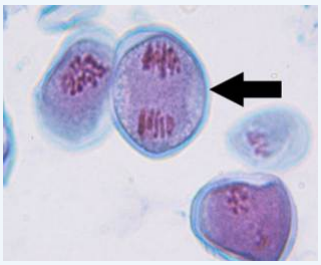
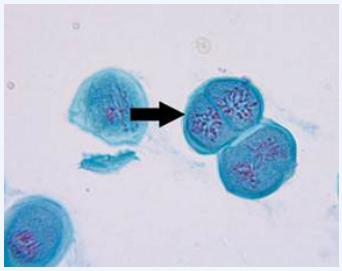
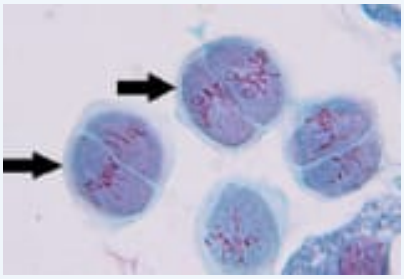
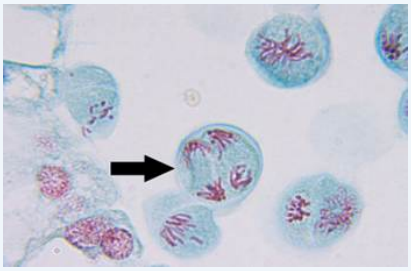


Figure 2: Meiosis in the lily anther. Notice that the cells remain together after each division until they become fully mature

Stages of Meiosis in the Lily Anther

Prophase I	Metaphase I	Anaphase 1
		
Homologues pair	**Homologues line up**	**Homologues separate**

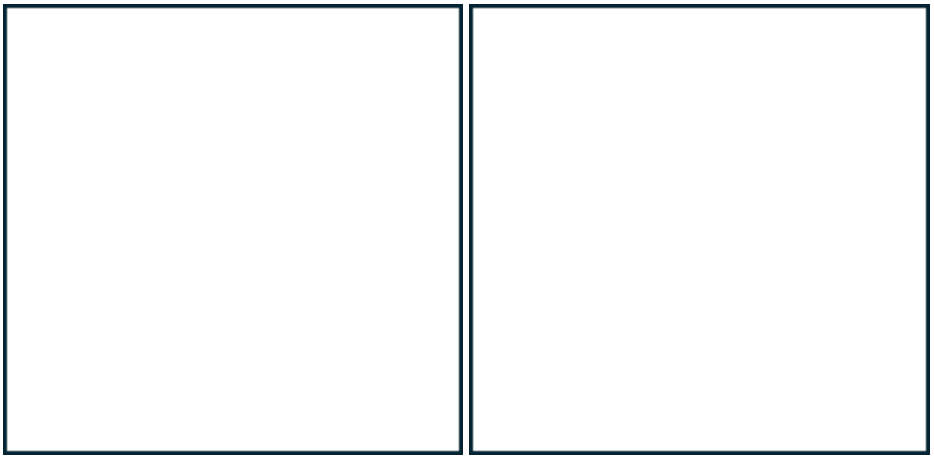
Prophase II	Metaphase II	Anaphase II
		

Chromosomes condense	**Replicated chromosomes line up**	**Sister chromatids separate**

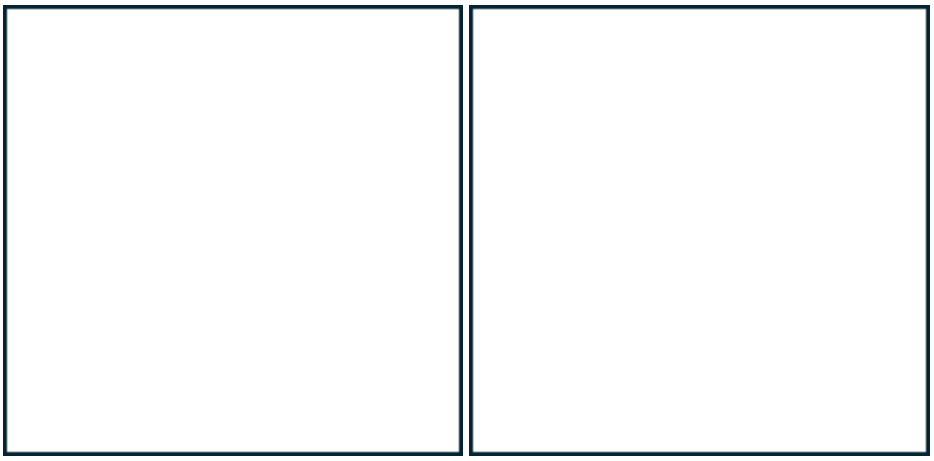
Attribution: Lily anther meiosis photographs courtesy of Dr. Derek Zelmer (University of South Carolina Aiken), used with permission.

Procedure:

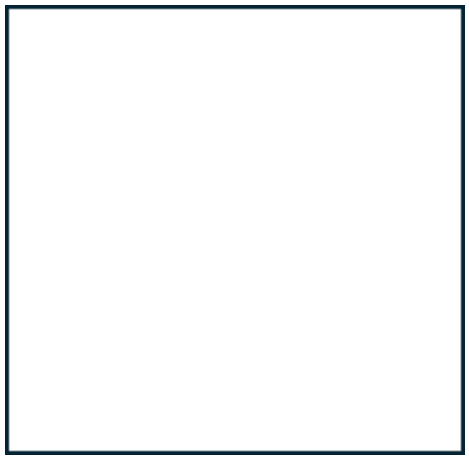
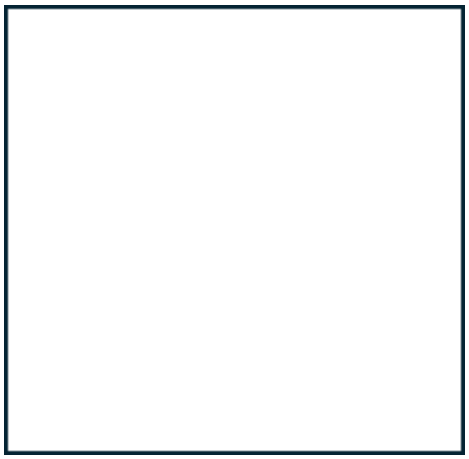
1. Examine prepared slides of lily anther microsporangium under low power (10X). Note the bilobed structure. Immature gametes undergoing meiosis are found in the center of each lobe.
2. With the 40X objective (400X magnification), find one or two cells in each stage of meiosis and draw one in the provided space below. Use the photographs above to identify the stages. Remember that if you see two cells together, the cells are in meiosis II.



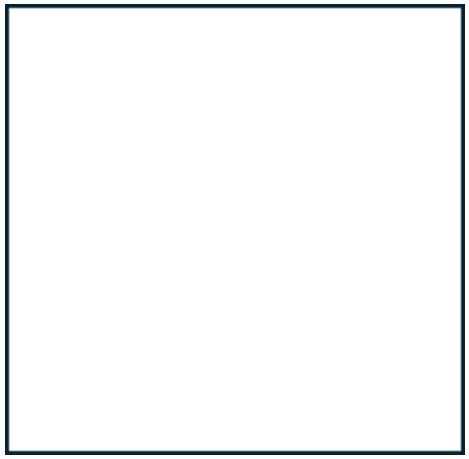
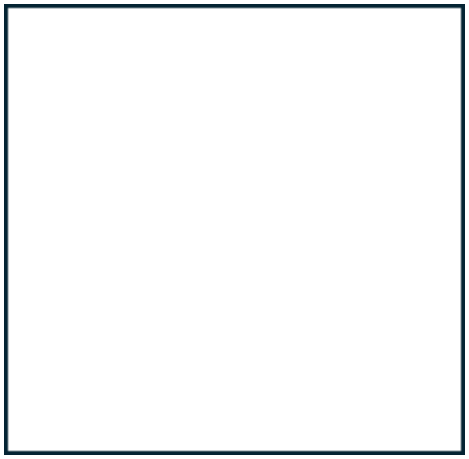
Prophase I Metaphase I



Anaphase I Telophase I

Prophase II Metaphase II

Anaphase II Telophase II

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1.11: Inheritance and Human Genetics

Patterns of Inheritance and Human Genetics

Section Overview

- Introduction: Mendel the father of modern Genetics
- Punnett squares and how to use them
- Laws of Inheritance
- Human Genetics
- Pedigrees

Pre-Lab Introduction

At this point in the semester, we have learned about DNA and how cells replicate. Now, we are going to take a step back and look at another angle -- how we get the traits we see in ourselves and each other. Our traits are determined (at least in part) by our genes. Genes are segments of DNA that encode instructions. As we learned in Lab 7, these instructions tell the cell how to make a protein. These proteins then contribute to how we look, behave, and function. We also learned about our chromosomes in lecture and in Lab 8 and that we have two of each chromosome, meaning we have two copies of every gene.

So, for every gene we have two copies. These copies are called alleles, and they don't have to be identical to each other. Alleles code for the same type of trait but they don't need to code for the same version. So for example, two alleles can both be responsible for eye color, but one allele codes for brown eyes while the other allele codes for blue.

Not all alleles are created equal, in that some alleles are able to dominate over others. These more prominent alleles are called dominant and are written using capital letters while the masked alleles are called recessive and shown using lower case letters. There are also alleles that create a blended appearance (incomplete dominance) as well as others that both show equally (codominance.)

Mendel the Father of modern Genetics

Johann Gregor Mendel (1822–1884) (Figure 1) was a lifelong learner, teacher, scientist, and man of faith. As a young adult, he joined the Augustinian Abbey of St. Thomas in Brno in what is now the Czech Republic.

Supported by the monastery, he taught physics, botany, and natural science courses at the secondary and university levels. In 1856, he began a decade-long research pursuit involving inheritance patterns in honeybees and plants, ultimately settling on pea plants as his primary model system (a system with convenient characteristics that is used to study a specific biological phenomenon to gain understanding to be applied to other systems). In 1865, Mendel presented the results of his experiments with nearly 30,000 pea plants to the local natural history society. He demonstrated that traits are transmitted faithfully from parents to offspring in specific patterns. In 1866, he published his work, Experiments in Plant Hybridization, in the proceedings of the Natural History Society of Brünn.



Figure 1.11.1: Johann Gregor Mendel set the framework for the study of genetics.

Mendel's Experiments

Mendel's seminal work was accomplished using the garden pea, *Pisum sativum*, to study inheritance. This species naturally self-fertilizes, meaning that pollen encounters ova within the same flower. The flower petals remain sealed tightly until pollination is completed to prevent the pollination of other plants. The result is highly inbred, or "true-breeding," pea plants. These are plants that always produce offspring that look like the parent. By experimenting with true-breeding pea plants, Mendel avoided the appearance of unexpected traits in offspring that might occur if the plants were not true breeding. The garden pea also grows to maturity within one season, meaning that several generations could be evaluated over a relatively short time. Finally, large quantities of garden peas could be cultivated simultaneously, allowing Mendel to conclude that his results did not come about simply by chance.

Mendel performed hybridizations, which involve mating two true-breeding individuals that have different traits. In the pea, which is naturally self-pollinating, this is done by manually transferring pollen from the anther of a mature pea plant of one variety to the stigma of a separate mature pea plant of the second variety.

Plants used in first-generation crosses were called P, or parental generation, plants (Figure 2). Mendel collected the seeds produced by the P plants that resulted from each cross and grew them the following season. These offspring were called the F1 generation. Once Mendel examined the characteristics in the F1 generation of plants, he allowed them to self-fertilize naturally. He then collected and grew the seeds from the F1 plants to produce the F2 generation. Mendel's experiments extended beyond the F2 generation to the F3 generation, F4 generation, and so on, but it was the ratio of characteristics in the P, F1 , and F2 generations that were the most intriguing and became the basis of Mendel's postulates.

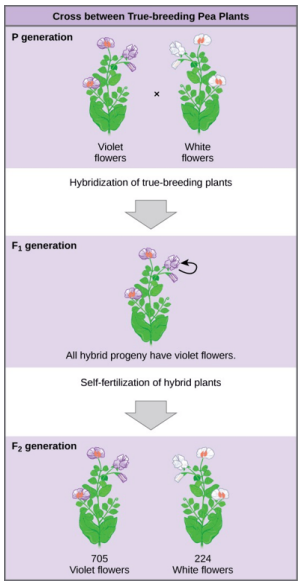


Figure 1.11.2: Mendel's process for performing crosses included examining flower color. (This figure from "BIOL 310: General Biology" by Eric Wada for Folsom Lake College, "Chapter 6: Genetics" is shared under CC BY 4.0 License and was authored, remixed, and/or curated by Openstax.)

The P generation refers to?

_____

_____

What made Mendel's Peas good subjects for studying patterns of inheritance?

_____

_____

Mendel looked at several traits of these pea plants including Stem height, Flower position, Flower color, Seed color, Seed shape, Pod shape, and Pod color (Figure 3). After breeding thousands of pea plants, Mendel noticed consistent ratios of traits would appear in the subsequent generations. These specific and predictable ratios led him to come up with several Law of Inheritance. We still use these laws today in modern genetics.

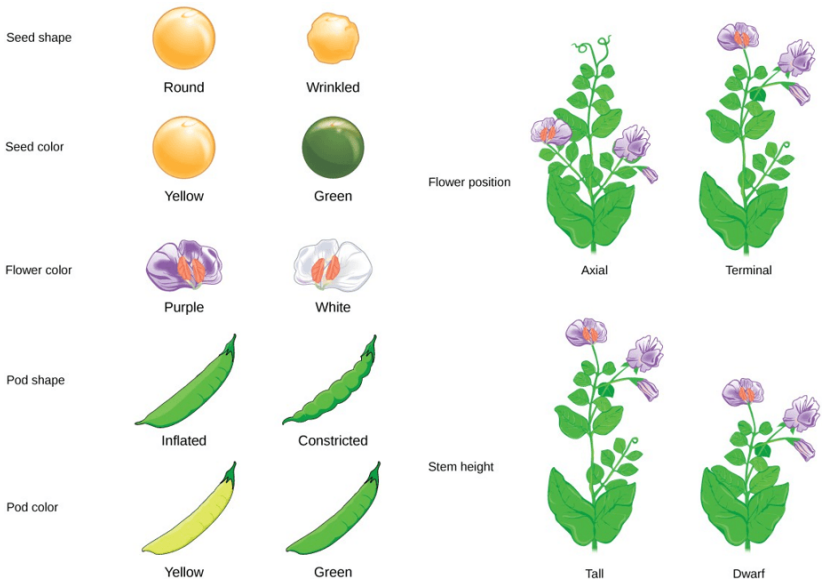


Figure 1.11.3: Mendel identified seven pea plant characteristics. Including seed shape and color, pod shape and color, flower color, flower positon, and stem height. (This figure from "BIOL 310: General Biology" by Eric Wada for Folsom Lake College, "Chapter 6: Genetics" is shared under CC BY 4.0 License and was authored, remixed, and/ or curated by Openstax.)

Genetic Terms used to communicate between Scientists

- **Gene:** The unit of hereditary, represents a portion of DNA that codes for a trait.
- **Allele:** An alternate form of a trait.
- **Dominant allele:** Requires only one copy to be expressed, regardless of what the second allele is.
- **Recessive allele:** Only expressed in the absence of a Dominant allele.
- **Homozygous:** Has two copies of the same allele.
  - Homozygous dominant: Two copies of the dominant allele.
  - Homozygous recessive: Two copies of the recessive allele.
- **Heterozygous:** Has two different alleles.
- **Genotype:** The allelic makeup of an individual for a particular gene.
- **Phenotype:** The expressed trait of a gene, can be observed.

Punnett Squares

Punnett squares can be used to determine the possible genotypic makeup of offspring for a given parental cross. It is important to note the results of a Punnett square are only the what is possible, the actual ratio of offspring traits may differ in some way. Why do you think this is?

_____

_____

_____

_____

_____

Now that we understand the relationship between genotype and phenotype, let's learn how we can use this information to make predictions. We can use the genotypes of the parents to predict their offspring's possible genotypes and phenotypes as well as their probabilities. The best way to chart these possible outcomes is to use the Punnettt square.

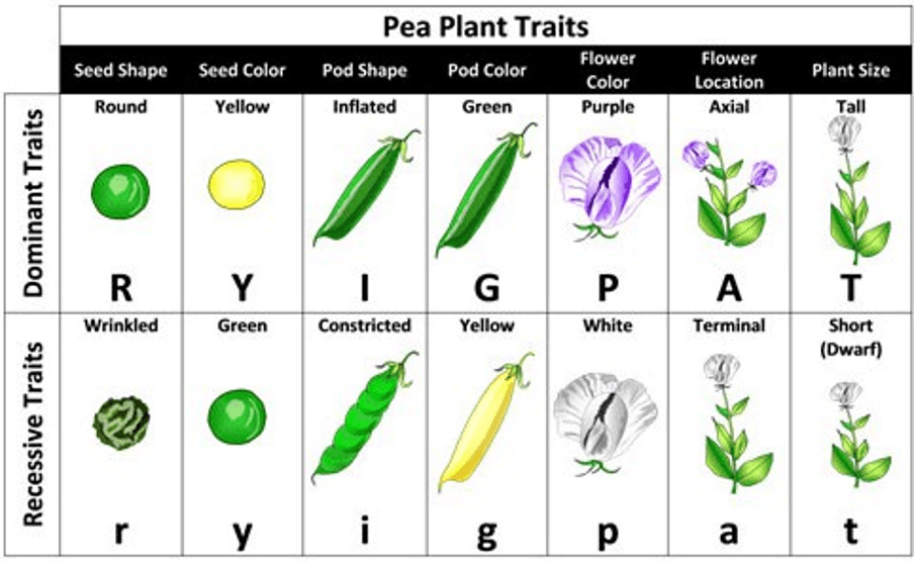


Figure 1.11.4: Giving allele designations to the various plant traits that Mendel was looking at for his experiments. R-for round seed, r- for wrinkled seed, Y- for yellow seed, y- for green seed, I- for inflated pod, i- for constricted pod, G- for green pod, g- for yellow pod, P- purple flower, p- for white flower, A- axial flower location, a- for Terminal flower location, T- for tall plant, t- for short plant. (Image taken from "Environmental Biology: an Open Educational Resource Textbook" Chapter 6 Genetics.)

1. Cross a Tall and short true-breeding pea plants using a Punnett square. Report the Phenotypic ratio.



Phenotype ratio:

_____ tall : _____ short

2. Create a cross using two of the offspring from the previous question. Report the Phenotypic ratio.



Phenotype ratio:

_____ tall : _____ short

3. Cross a Heterozygous Tall plant with a short plant. Report the Phenotypic ratio.



Phenotype ratio:

_____ tall : _____ short

Laws of Inheritance

The repeated ratios that Mendel observed in his pea plants led to his identification of laws pertaining to inheritance of traits.

Law of Dominance- Mendel recognized that one trait was dominant to its counterpart variant, suggesting there are dominant and recessive copies of traits (alleles). A dominant allele will be expressed when there is at least one copy of that allele.

Law of Segregation- The genes segregate into gametes with equal numbers of each allele or copy of a gene. This means that no one allele is more likely to show up the offspring than any other for a given cross.

Law of Independent Assortment- Genes don't affect the inheritance of other genes in that they don't affect the sorting of alleles into gametes. (We will talk about an exception to this law).

Review question: Remembering what we learned previously on Meiosis, how can the Law of Independent Assortment be true when

the particular alleles are each on one of the paired chromosomes?

_____

_____

_____

_____

Simulating a One-Trait Cross:

Procedure:

1. Each group will pick up two paper bags filled with 15 red (R) Poker chips and 15 white (r) Poker chips. This represents two heterozygous parents (Rr x Rr). Red color being dominant to white.
2. At the same time, two students will reach into one bag and pull out one of the Poker chips. What are the only possibilities that can be made from this selection:

_____

_____

_____

3. Record your resulting genotype and phenotype in the data table for the simulated offspring from this one-trait cross of two heterozygous parents.
4. Return the Poker chips back into the bag and conduct the same process 14 more times (15 total trials).

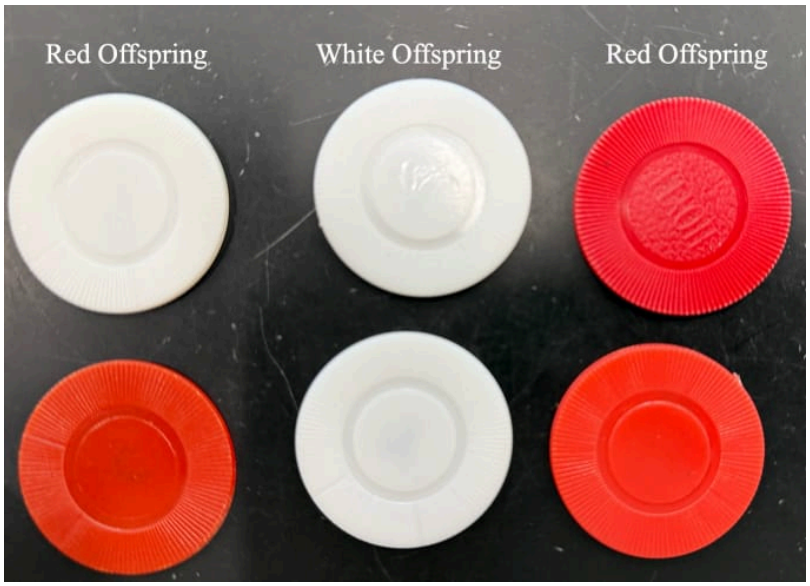


Figure 1.11.5: Example of the crosses made by combing poker chips from the two parental paper bags. The colors representing the different alleles for the trait of color. White and red chip being red offspring, white and white chips white offspring, red and red chips red offspring.

Data Table 1

Trial	Offspring Genotype	Offspring Phenotype

1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		

Follow-up Questions

1. What is the dominant trait? _____
2. What is the phenotype ratio of the above table? _____ Red : _____ White
3. What is the recessive trait? _____
4. What are the genotypes of the parents? _____

5. What are the phenotypes of the parents? _____
6. Fill in the Punnett Square below using the parents given in the procedure:



Phenotype ratio:

_____ Red : _____ White

Genotype ratio:

____ RR ____ Rr ____rr

Color of Tobacco Seedlings

Tobacco plants normally produce chlorophyll, but a mutation of a recessive allele that doesn't code for the production of chlorophyll. The dominant allele C codes for chlorophyll and recessive allele c codes for a lack of chlorophyll. There are two possible phenotypes, producing chlorophyll (green seedlings) and non-pigmented (white seedlings).

Experimental Procedure:

1. Obtain one of the lettered images of freshly sprouted tobacco seedlings. They are all the result of a cross between heterozygous parents Cc X Cc. Using a Punnett square calculate the expected phenotypic ratio.



Phenotype ratio:

_____ tall : _____ short

2. Count the number of green and white seedlings in the image, and record the results.
   Green seedlings: _____ White Seedlings: _____
3. Reduce the counts to their most simplified ratio.
   _____ Green : _____ White
4. Did your predicted ratio match the actual observed ratio of phenotypes? _____

Two trait crosses

It is also common to use Punnett squares to determine the probability of genotype and phenotype of two traits at once. This requires the use of a larger Punnett square 4x4 instead of a 2x2. The possible gametes also need to be determined for each parent of the cross. For this exercise we will be looking at ears of corn, that are a dihybrid cross between two heterozygous parents for kernel color and shape, P= purple p= yellow S= smooth s= rough.

When determining the possible gametes using the FOIL method is helpful, First two alleles of each trait, Outer two alleles of each trait, Inner two alleles of each trait, and Last two alleles of each trait.

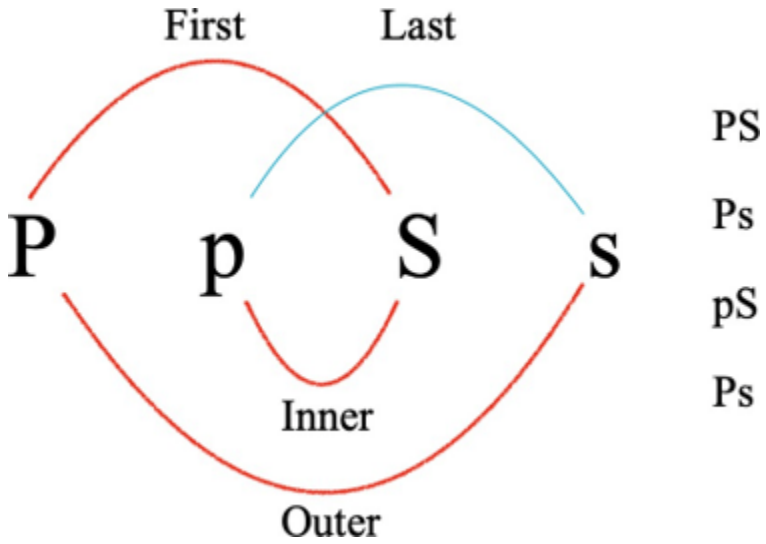
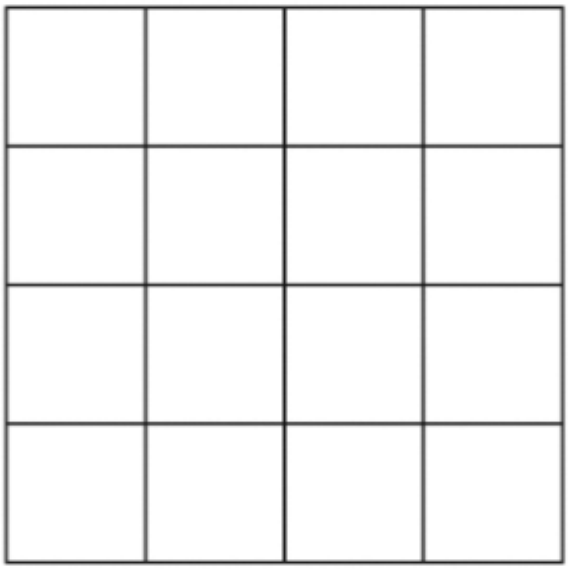


Figure 1.11.7: Demonstrates use the foil method for determining possible gametes for a two trait cross. FOIL method is helpful, First two alleles of each trait, Outer two alleles of each trait, Inner two alleles of each trait, and Last two alleles of each trait. Gives you all the possible gamete combinations for these two traits.

1. Determine the possible genotypes and the phenotypic ratio of a dihybrid cross. Use the foil method to determine the possible gametes.



Phenotype ratio:

_____ Purple Smooth

_____ Purple Rough

_____ Yellow Smooth

_____ Yellow Rough

2. Each lab group should get an ear of Dihybrid cross corn and count the different phenotypes present in the kernels. To avoid double counting any work in an order manner, for instance counting one row at a time. It can be helpful to work with multiple people, one counter and one recorder.
3. After you have your counts of the phenotypes present on your ear of corn fill in the below table with your results. Record your results on the board so that each lab group records one sample.

Data Table 2. Corn Dihybrid Cross Data

Sample #	Purple smooth	Purple rough	Yellow rough	Yellow rough	Phenotypic ratio
1					
2					
3					
4					
Totals					

Follow-up Questions

1. In the corn experiment were the expect phenotypic ratios the same or different from the actual observed ratios?

_____

2. Was this a similar result or different from the previous exercises?

_____

3. What is the reason for the observed and expected ratios to be different?

_____

Human Genetics

Using classroom data, fill in the table below.

Trait Number of Individuals Percentage of Individuals		
Hairline: Widow's Peak (WW or Ww) Straight (w)	Widow's Peak = Straight Hairline =	% with dominant trait = % with recessive trait =
Earlobes: Unattached (E) Attached (e)	Unattached = Attached =	% with dominant trait = % with recessive trait =
Tongue Rolling: Can roll tongue (R) Cannot roll tongue (r)	Can roll = Cannot roll =	% with dominant trait = % with recessive trait =
Freckles: Have freckles (F) No freckles (f)	Freckles = No freckles =	% with dominant trait = % with recessive trait =
Dimples: Present (D) Absent (d)	Present = Absent =	% with dominant trait = % with recessive trait =
Chin: Cleft chin (C) No cleft (c)	Cleft chin = No cleft =	% with dominant trait = % with recessive trait =
Pinky Finger: Bent (P) Straight (p)	Bent pinky = Straight pinky =	% with dominant trait = % with recessive trait =

Hitchhiker Thumb: Straight thumb (T) 90-degree bend in thumb (t)	Straight thumb = Hitchhiker thumb =	% with dominant trait = % with recessive trait =
Interlocking of Fingers: Left over right (L) Right over left (l)	Left over right = Right over left =	% with dominant trait = % with recessive trait =

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Follow-up Question

Based on the classroom data, are the dominant traits always more common?

_____

Blood Type Exercise

Blood type is based on medically relevant surface antigens found on the plasma membrane of red blood cells, and the agglutinins (antibodies) that target those specific antigens. The different surface antigens are A, B, D (also known as Rh factor), and O (a lack of A or B surface antigens). This is a case of a two trait cross represented by the phenotypes A, B, AB, O, and either positive or negative for Rh factor. We use the antibodies that target these antigens to determine a person’s blood type. This is medically relevant for blood transfusions, and can have very negative effects if a person gets the wrong type of blood. The AB blood type is a case of **incomplete dominance** or **codominance** where both the A and B surface antigens are expressed equally, and are both dominant to the type O allele.

1. For this exercise we will be looking at 4 unknown blood type samples and determining possible parentage. Using a provided blood typing kit, you will place a few drops of each sample into the three wells vertically, and then treat each well with a particular antiserum solution containing antibodies for one of three surface antigens (Figure 7).
2. Mary is Angela’s mother, your professor will provide you with their blood types. After testing the possible fathers’ blood samples fill out the below table and use what you know about genetics to determine who might by Angela’s father.

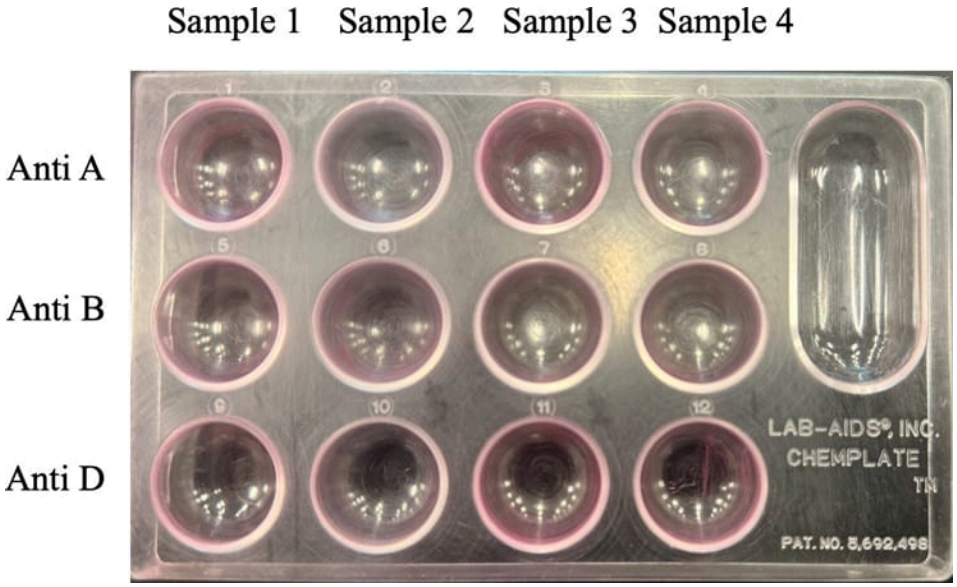


Figure 1.11.7: Example of the testing wells for the blood typing kit. Drops of Anti A serum in wells in row 1, drops of Anti B serum in wells in row two, and drops of Anti D serum in wells in row three. Add drops of sample 1 in wells in column one, drops of sample 2 in wells in column two, drops of sample 3 in wells in column three, and drops of sample 4 in wells in column four.

Blood Typing Results

	Mary	**Angela**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Blood type						

3. Which sample is most likely Angela's father?
_____

_____

Exceptions to Independent Assortment

There are certain traits that are linked to a particular chromosome, the most common effecting humans are sex linked traits. One example being sex linked color blindness, and is a mutated allele that is only found on the X chromosome. In Humans The X and Y chromosomes determine the development of secondary sex characteristics. The X chromosome coding for the development of Female reproductive organs, while the Y chromosome codes for the development of Male reproductive organs. The typical phenotypes being XX - Female and XY - Male. In females it requires two copies of the color blind allele for them to express color blindness, but in males only having one X chromosome only need one copy of the color blind allele to express color blindness. Even though this a two trait cross, we can still use a 2x2 Punnett square to calculate the probabilities of color blindness in the offspring due to the linked nature of the secondary trait.

1. A normal vision female who's father was color blind decides to have kids with a normal vision male, and want to know what are the chances their kids may have color blindness. Use a Punnett square to determine the female's genotype to then do a second Punnett square cross to determine their chances of having a color blind child.
2. Report your findings for the chances of male offspring being color blind and chances of female offspring being color blind.



Genotype of Female:

_____



Chances of Female offspring being color blind:

_____

Chances of Male offspring being color blind:

_____

Pedigrees for tracking autosomal disorders

Pedigrees can be used to trace patterns of inheritance by looking at several generations of a family. They can be used to trace genetic diseases while giving family history and helping provide insight for future generations.

Many conditions are genetic but the pattern of inheritance may be different depending on which chromosome the gene is found on and whether or not the mutated allele is dominant or recessive. When a mutated allele is found on the autosomes, the condition affects males and females equally. But when the mutated allele is found on a sex chromosome (X or Y), then the condition tends to predominantly affect one sex (usually male).

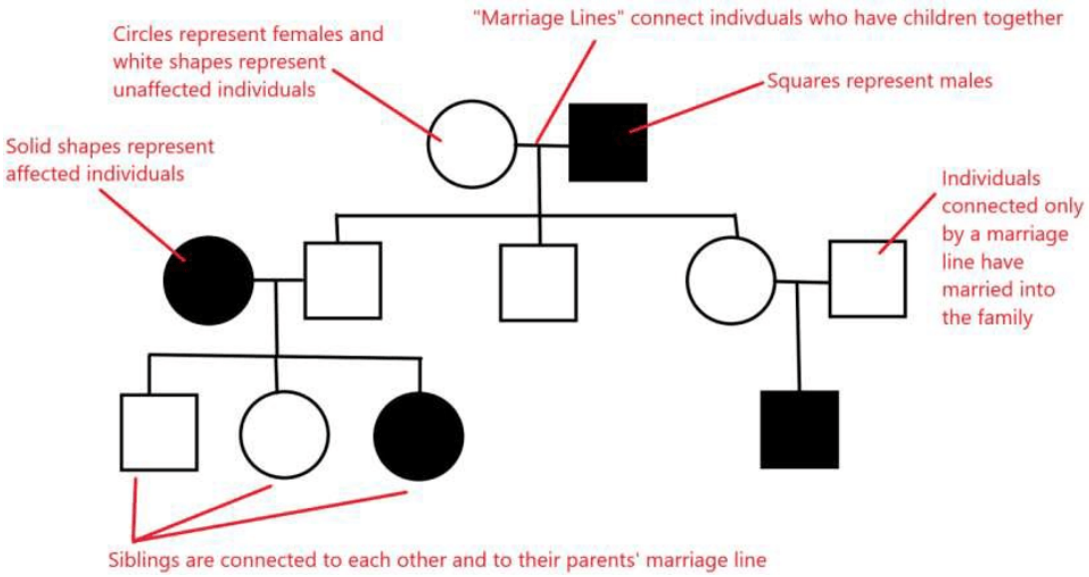


Figure 1.11.8: Example pedigree showing that there are several affected individuals, as noted by the colored-in shapes. By analyzing the relationships and connections of affected individuals, we can determine if the pattern of inheritance is autosomal dominant, autosomal recessive, sex-linked dominant, or sex-linked recessive. In this particular example, the pattern of inheritance is autosomal recessive since males and females are equally affected and the affected male on the left has two healthy parents (the “skipped generation”.) ("Pedigrees Explained" by Karen Marks, Reedley College is licensed under CC BY 4.0)

The following pedigree traces sickle cell disease through three generations of a family. Use the pedigree to answer the following questions:

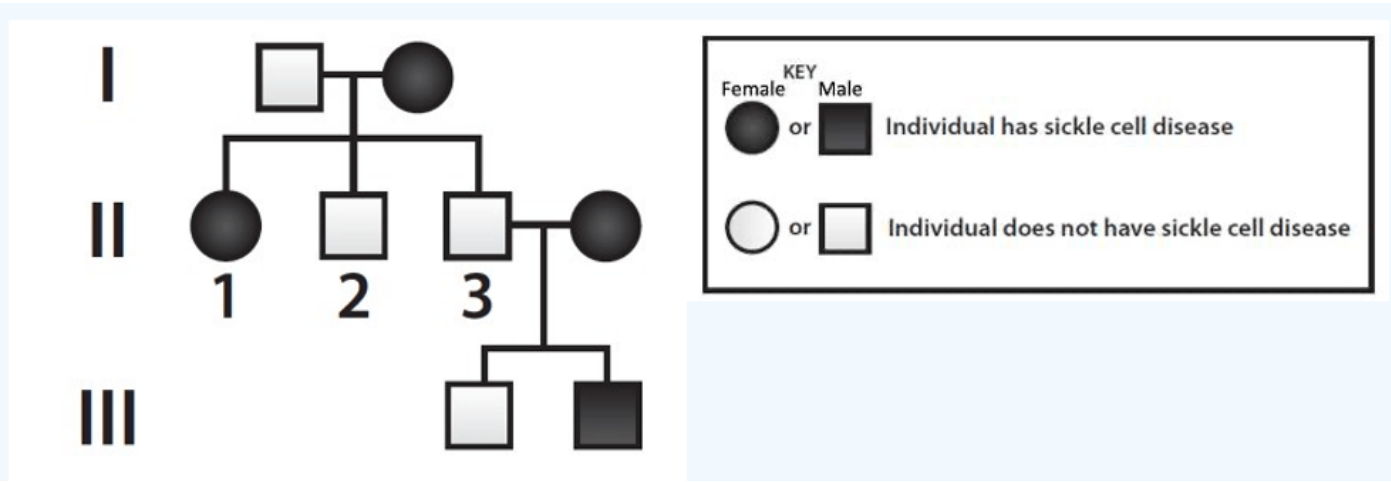


Figure 1.11.9: Example pedigree tracing sickle cell disease through three generations of a family.

1. What is the genotype of the father in the first generation?
2. What is the genotype of the daughter in the second generation?
3. What is the genotype of individual 3 in the second generation? How do you know?
4. If the couple in the second generation has another child, what are the chances the child will have the following:
   A. Sickle cell disease
   B. Sickle cell trait
   C. Normal hemoglobin
5. If the entire family moves to an area where malaria is endemic, four of the five males in the pedigree will have a genetic advantage over the other individuals in the family. Explain why this is.

_____

_____

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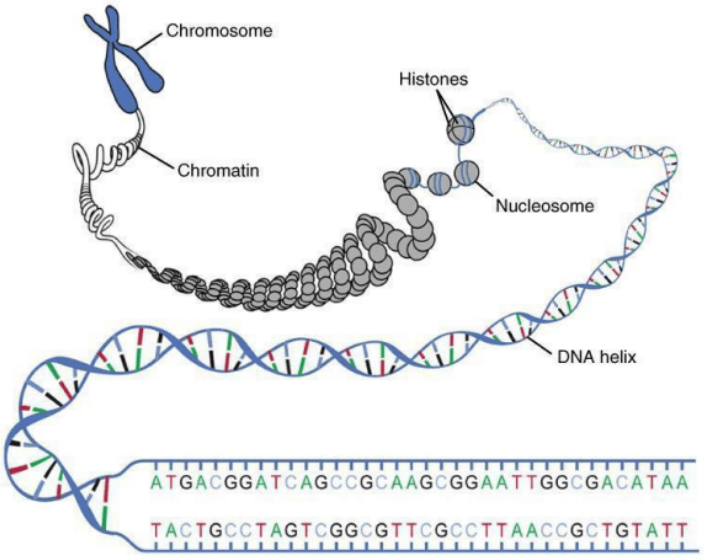
1.12: DNA Biology

Introduction

Learning Objectives

- Identify the structures of DNA and RNA
- Compare and contrast DNA and RNA
- Describe the processes of transcription and translation

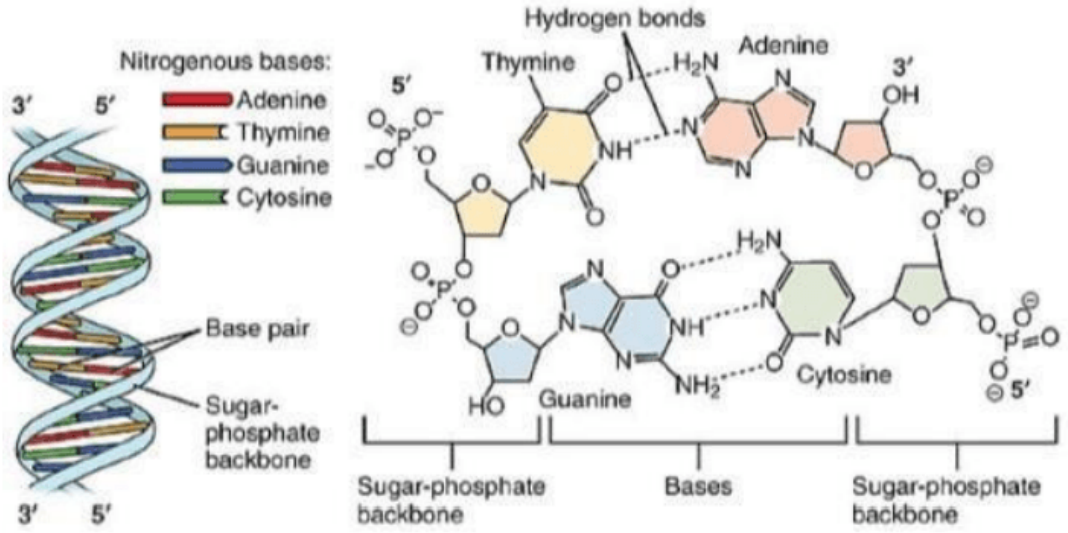
Our cells are constantly doing work, day and night. All of this work is spearheaded by one molecule -- *Deoxyribonucleic Acid* (or *DNA* for short.) DNA is a massive molecule consisting of repeating nucleotides in a double helix shape. These repeating nucleotides can have one of four different bases: adenine, thymine, cytosine, or guanine. It's the unique combination of these four bases that allows DNA to encode all of the information for the cell.



DNA Replication by OpenStax is licensed under CC BY 4.0

The Structures of DNA and RNA

DNA's double helix shape is in part due to it having two nucleotide chains running parallel to each other, forming a ladder-like shape. The phosphate and deoxyribose sugars link together to form what are called the "backbones", while the bases extend outward, forming the "rungs." The two bases forming the rungs are not random though. Each base has only one other base that it can bond with: adenine and thymine always pair together while cytosine and guanine only pair with each other. This is called *complementary base pairing*. This gives us a predictable pattern so that knowing the bases from one side of the DNA molecule means you can easily figure out the other side. A close up of DNA's structure can be seen below.



The Nucleus and DNA Replication by OpenStax is licensed under CC BY 4.0

Let's practice these complementary base pair rules. Complete the table using the appropriate base letter.

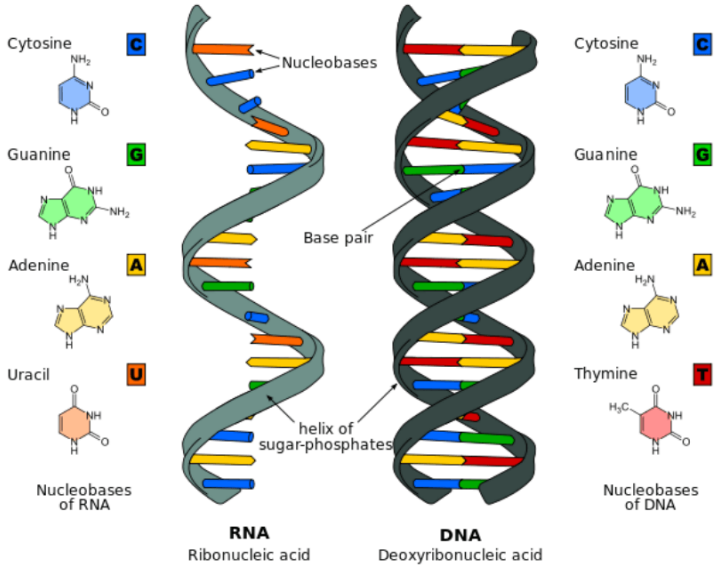
Table 1 Base Pairing Rules

STRAND #1	STRAND #2
	T
G	
A	
	G
T	
	C

DNA is not the only nucleotide important to the cell. *RNA* (ribonucleic acid) is also a necessary molecule, as it helps the cell read and use DNA's instructions. RNA has a similar structure with a few key differences: it is single stranded and DNA's thymine has been replaced with uracil. So, the base pair rules in RNA are A with U and C with G. There are three different types of RNA that we will look at in this class: rRNA, mRNA, and tRNA. Each RNA type can be seen in the table below. RNA compared to DNA is seen in the image below.

Table 2 Types of RNA

Type of RNA	Full RNA Name	Function
rRNA	Ribosomal RNA	Forms part of the ribosome
mRNA	Messenger RNA	Carries instructions from nucleus to ribosome
tRNA	Transfer RNA	Transfers amino acids in translation



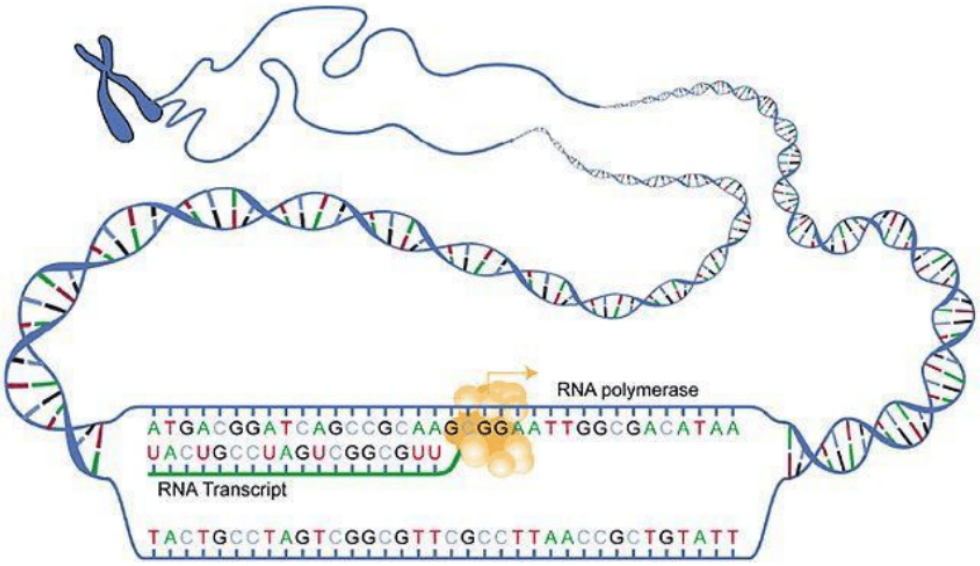
File:Difference DNA RNA-DE.svg: Sponk / *translation: Sponk, CC BY-SA 3.0, via Wikimedia Commons

Transcription and Translation

Transcription and translation put DNA to work. DNA contains many smaller segments called genes -- sections of DNA that code for a protein. These genes cannot directly produce these proteins however, and so the cell must create temporary copies of these segments to use elsewhere in the cell.

To transcribe quite literally means “to put into written or printed form.” This is a very close description as to what is happening in the cell. Since the DNA cannot directly make proteins, mechanisms are in place to read and transcribe DNA’s genes. Instead, mRNA is made by RNA polymerase, which reads the opened DNA strand. When RNA polymerase reads the DNA, it uses the complementary base pair rules to select the correct base for mRNA it’s building. Then, the mRNA molecule is released and leaves the nucleus.

RNA polymerase needs direction on where and when to start the transcription process. Since the cells do not need to use every gene all the time, it’s important that there is a way to regulate when genes are used. The promoter is a special section of DNA that comes before the gene and helps dictate if a gene should be used often, sometimes, or hardly ever.



OpenStax, CC BY 4.0, via Wikimedia Commons

Table 3 Transcription Roles

	Role in Transcription
Promoter	Regulates when a gene will go through transcription
RNA polymerase	"Reads" DNA and builds the mRNA strand
mRNA	Carries the information from the transcribed gene
DNA	Encodes information in the form of genes to be read and used

Translation puts the mRNA produced during transcription to work. The mRNA molecule leaves the nucleus and enters into the cytoplasm. We can describe translation in three steps.

Initiation: Once in the cytoplasm, a ribosome will grab onto the mRNA.

Elongation: The ribosome reads mRNA three bases at a time. These three bases are called a codon and dictate which amino acid is next in the sequence. This codon binds with the anticodon of tRNA in the ribosome, which brings the appropriate amino acid.

Termination: When a STOP codon is reached, it causes the ribosome to let go of the mRNA molecule and protein strand.

Now we will take RNA strands and translate it into a sentence.

For this activity, transcribe the DNA strands below and identify the codons in the message. Then, look up the genetic code to identify the corresponding amino acids in the chain.

1. DNA: T T T A G C C T A G C T A A A T T T C A G T G C
   - mRNA:
   - Protein:
2. DNA: T T T T G A G G C G C T G T G T T T G T C
   - mRNA:
   - Protein:
3. DNA: T T T C A G T T C C A A A A A T T T A G C C T T
   - mRNA:
   - Protein:
4. DNA: T T T G G T A A T G G G G T A C A G T T G
   - mRNA:

    - Protein:
5. DNA: T T T T T A A A G G G A G C T T G G G A G T T T T G C
    - mRNA:
    - Protein:
6. DNA: T A A G A T G T A A G C A A C C C T
    - mRNA:
    - Protein:
7. DNA: T A A A G G C C G A A C G T T
    - mRNA:
    - Protein:
8. DNA: C A G G A A T G T T A G C C A T C T
    - mRNA:
    - Protein:
9. DNA: T A A A G G A C C T C G
    - mRNA:
    - Protein:
10. DNA: T T T T G A G G C G C T C G C T T T C A G C G A
    - mRNA:
    - Protein:
11. DNA: T A A A G G A C C A A G T C C C G T
    - mRNA:
    - Protein:
12. DNA: T A A C G G G T A G G T A A T
    - mRNA:
    - Protein:
13. DNA: T T T A G C C T A T A T T T T T G A C T A
    - mRNA:
    - Protein:
14. DNA: T T T A A C A A T T A C T C A T T T T G C
    - mRNA:
    - Protein:

"Protein Synthesis Activity" by Aimee Mazzoni, Reedley College is licensed under CC BY 4.0

DNA Extraction

DNA may be stored away inside the nucleus of every cell, but that does not mean that it is inaccessible. We can access the DNA if we can get past the plasma membrane. Remember that the membrane is lipid-based, so chemicals that break down lipids are the best bet to releasing the DNA. For this exercise, we will extract DNA from strawberry, although any living thing can be used, such as strawberries or your own saliva. In fact, this experiment can be replicated in your own kitchen.

What do you think DNA will look like when extracted?

Materials:

- 1 set of mortar and pestle
- 2 medium size strawberries or 1 large strawberry (store bought)
- 100 ml saline
- 5 ml of dish soap
- 1 stirring rod
- Ethanol, ice cold
- Test tubes
- Falcon tubes
- Test Tube racks

Procedure:

1. Obtain a clean test tube.
2. Add 1-2 strawberries and add to the test tube.
3. Add enough saline to fully saturate and cover the strawberry.
4. Add 3-5 drops of dish soap.
5. Use the pestle to grind the strawberry in the saline and form a homogeneous mixture
6. Slowly stir the contents of the tube with the stirring rod, carefully avoiding making bubbles, for at least 5 minutes.
7. Set up a falcon tube with a funnel and cheese cloth, filter the strawberry liquid into the falcon tube
8. Discard the strawberry debris collected in the cheese cloth
9. Add 1 mL of ice-cold ethanol to the falcon tube on the side by tilting the tube at a 45° angle and slowly dripping the ethanol down the side of the tube. This will create a layer of ethanol floating over the top of your strawberry slurry.
10. Set the tube aside for 3-5 minutes. The DNA will precipitate up into the ethanol as a white stringy material.
11. Collect the DNA using a glass rod into a clean falcon tube or test tube and add few drops of pH indicator.
12. A phenol red pH indicator will change color to yellow to indicate an acidic pH in the presence of DNA.

Clean-up:

1. Dump the strawberry solids in the trash.
2. Wash the tubes in the sink and return to the test tube rack.

When the DNA precipitated into the ethanol, how did it look?

Why do you think dish soap was added to extract the DNA? Hint: Consider what cell structure serves as a barrier.

Consider the fact that the procedure called for the ethanol to be poured so that it floated over the top of the strawberry slurry. How do you think DNA's visibility would change if the ethanol mixed with the slurry?

Set Up of the DNA Extraction

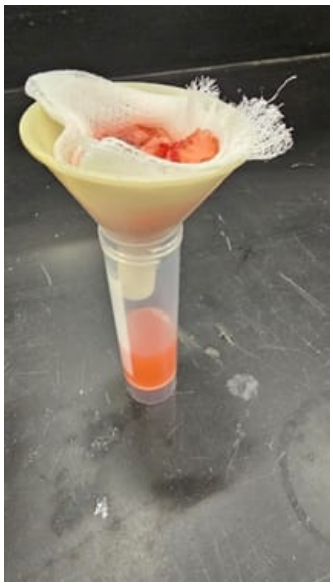


Figure A: DNA Extraction using cheesecloth. (By SB)



Figure B: View of the extracted DNA in the top layer. (By SB)

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1.13: Evolution

Evolution: Mechanisms and Evidence

Overview

- Introduction
- Evolution on the Large and Small Scale
- Modeling Natural Selection
- Evidence of Evolution

Introduction

When looking at the great diversity of life, there are clear differences between many species. Some organisms are photosynthetic, others have scales, some are capable of flying. Although these differences seem great, they are ultimately due to smaller, less noticeable changes that accumulate over time. This change over time is called evolution. There are several mechanisms that can lead to change, such as natural selection, genetic drift, and gene flow. Natural selection is a process where change is driven by selective pressures from the environment, genetic drift is a change in a population's allele frequency, and gene flow is the movement of alleles in or out of a population as seen in the image below. However, the ultimate driver of all change is mutation (random genetic changes), as all other mechanisms of change rely on having a varied genetic pool to work. For this lab we will model two of these mechanisms of evolution -- natural selection and the bottleneck effect.

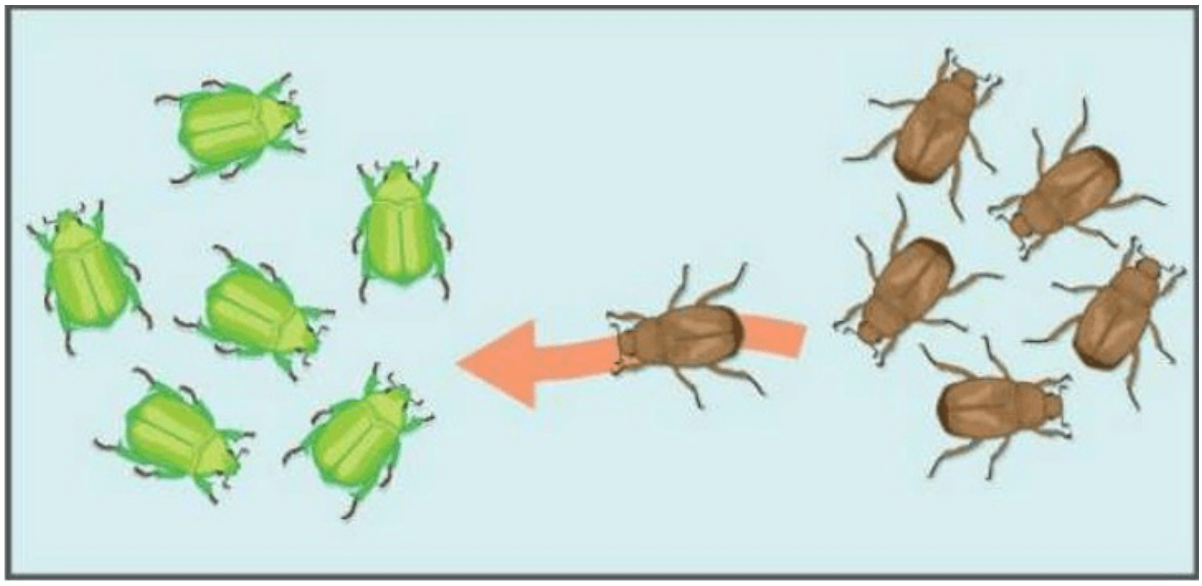


Figure 1.13.1: Example of Gene flow two distinct populations green beetles and brown beetles with their own allele frequencies. When an individual migrates to a different population it brings with it potentially novel genes and alters the allele frequencies. ("Gene Flow" by OpenStax, Rice University, CC BY 4.0, via Wikimedia Commons)

Evolution on the Large and Small Scale

When most people think of evolution, they often think of large, obvious changes such as the differences between a mouse and an elephant. The divergence seen between such drastically different organisms is due to macroevolution -- larger changes that have occurred over millennia. However, evolution does not always lead to such obvious differences. Evolution can also occur on a smaller scale. This smaller scale is called microevolution -- smaller changes that occur within a species over a few generations.

Macroevolution is seen when two species diverge, such as the divergence seen in early hominins and other primates. The image

below demonstrates how macroevolution led to the speciation (development of new species) of the many plants we see today. The flowering plants seen on the right are more closely related to the gymnosperms and more distantly related to the mosses, liverworts, and charophytes.

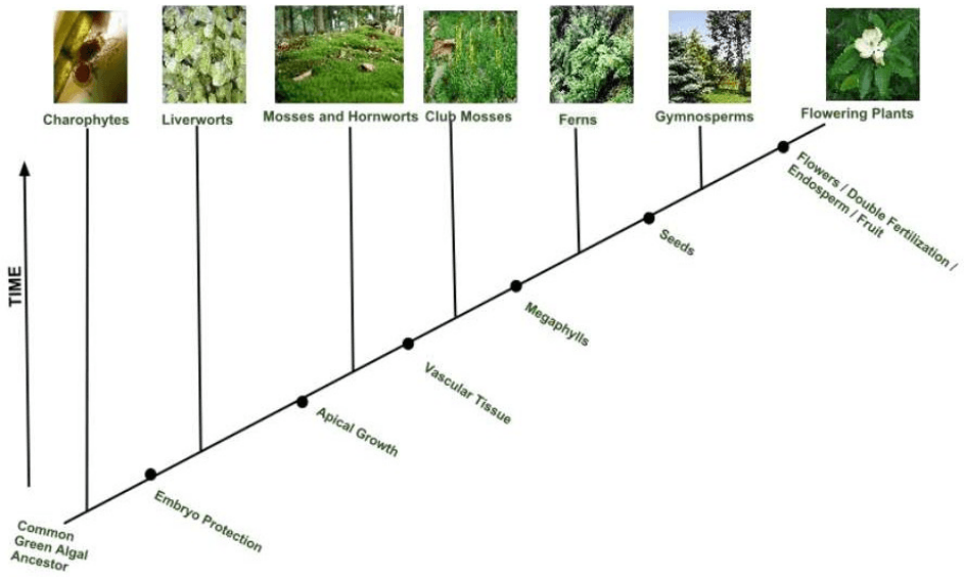


Figure 1.13.2: Cladogram of the Plants including beginning from the common Green Algal Ancestor. Cladogram includes Charophytes, Liverworts, Mosses/Hornworts, Club Mosses, Ferns, Gymnosperms, and Angiosperms (Flowering plants), as well as the derivative trait that defines those different groups. (Laurenprue216, CC BY-SA 3.0, via Wikimedia Commons)

Microevolution is seen when a population changes, such as a change in beak size in finches due to changes in food source. The population is still considered part of the same taxonomic group, but has accumulated some differences when compared to previous generations. The image below demonstrates the development of antibiotic resistance an example of microevolution that is impacting our ability to fight bacterial infections in modern medicine.

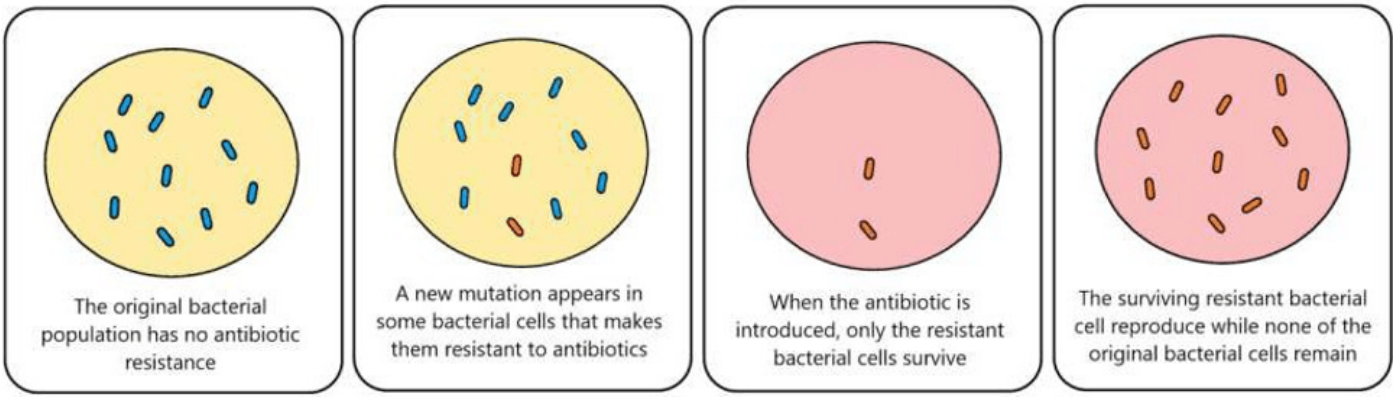


Figure 1.13.3: Bacterial evolution of antibiotic resistance, from left to right non-resistant cultures, cultures including two that have a mutation for resistance, non-resistant cultures die after introduction of antibiotics, resistant cultures spread.("Evolution – Antibiotic Resistance" by Karen Marks, Reedley College is licensed under CC BY 4.0)

Modeling Natural Selection

While in the Galapagos Islands, Darwin noticed that the beaks of various finches were of different lengths, shapes, and sizes. The differences he observed led him to several of his most important ideas about the mechanisms and processes that are responsible for descent with modification, including natural selection.

In order for Natural Selection to occur:

- There must be variation in a population

- The variation must be heritable
- There must be competition (not all individuals will be successful at passing on their traits)

Experiment: Best Beaks

Sialia Mexicana (the Western Bluebird) is a small passerine bird of the family Turdidae. It has a thin, straight beak and feeds primarily on worms, berries, and insects, but it is also known to be a voracious consumer of grapes and raisins. In this exercise, you will simulate the functional consequences of variation in beak shape (short, stout beaks compared to long, thin beaks) of the Western Bluebird and how these might be reflected in subsequent generations. For the purposes of this activity, let's assume:

- There is variation in beak length among individuals in a population.
- Variation in beak length is heritable.
- Individuals with one type of beak length survive or reproduce better than others.

Materials per group:

- 4 wooden craft sticks 2 rubber bands
- 10 pennies Timer Ruler
- 25 large grapes
- 25 raisins
- 1 plate
- 2 bowls

Procedure

1. Make your short, stout beak (Figure 4) by placing three pennies between two of the wooden craft sticks about one inch (2.54 cm) from one end.
2. Secure the wooden craft sticks together with one of the rubber bands.



Figure 1.13.4: The three-penny short, stout beak is made with two popsicle sticks with 3 pennies stacked near one end with a rubber band wrapped around at the pennies. (Image from 10.2: Best Beaks is shared under a CC BY-SA 4.0 license and was authored, remixed, and/or curated by Leah Howell (Dalton State College)

3. Now, make your long, thin beak (Figure 5) by placing seven pennies between the remaining two wooden craft sticks, this time approximately 2.5 inches (6.35 cm) from one end, and secure with a rubber band.

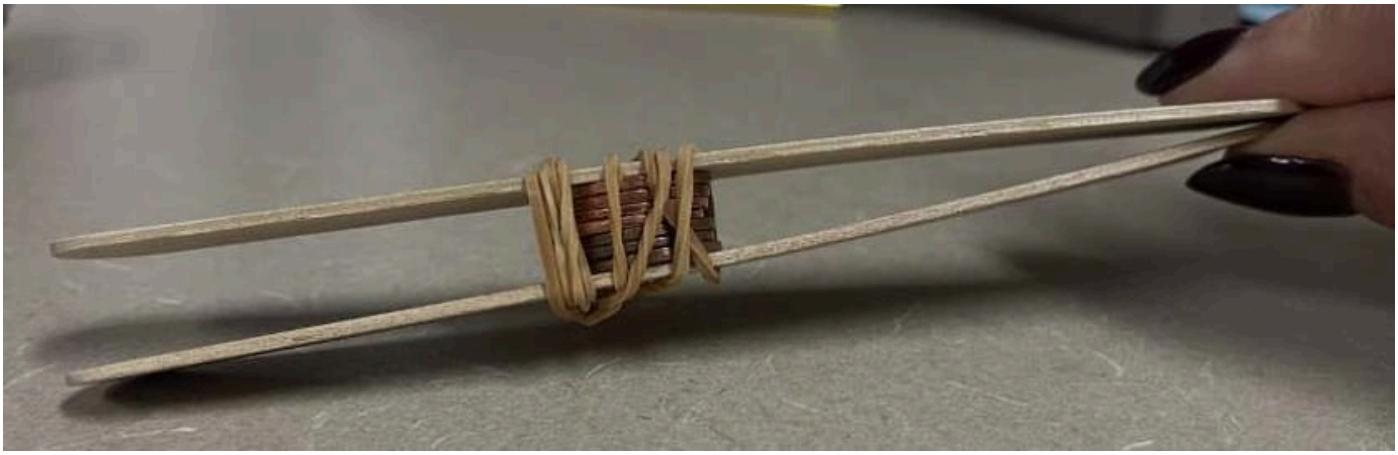


Figure 1.13.5: The seven-penny long, thin beak. Made with 2 popsicle sticks with 7 pennies stacked near the middle, and a rubber band wrapped around at the stack of pennies. (Image from 10.2: Best Beaks is shared under a CC BY-SA 4.0 license and was authored, remixed, and/or curated by Leah Howell (Dalton State College).)

Before continuing make some predictions about the two different beak sizes.

Which beak do you predict will be better suited to eating the typical grapes?

_____

_____

Which beak do you think will be better suited to eating the drought year food raisins?

_____

_____

4. Spread the raisins evenly over the surface of the plate and set your timer for 30 seconds.
5. For 30 seconds, attempt to pick up as many raisins as possible with your small beak and place them in the bowl.
6. Record the number of raisins picked with the small beak in Table Number of Raisin by beak size.
7. Return the raisins to the plate.
8. Complete a second trial, attempting to pick up as many raisins as possible with the small beak in 30 seconds. Record your results in Table Number of Raisin by beak size.
9. Now, attempt to pick up as many raisins as possible with the large beak and place them in the bowl in 30 seconds.
10. Record the number of raisins picked up with the large beak in Table Number of Raisin by beak size.
11. Clear the plate.
12. Spread the grapes out evenly on the plate, and again, set your timer for 30 seconds.
13. Using your small beak, attempt to pick up as many of the grapes as possible in 30 seconds and place them in the bowl.
14. Record the number of grapes picked up with the small beak in Table Number of Grapes by beak size.
15. Now, using the large beak, attempt to place as many of the grapes as possible in the bowl in 30 seconds.
16. Record the number of grapes picked up with the large beak in Table Number of Grapes by beak size.
17. Return the grapes to the plate.

Table 1. Number of Raisin by beak size

Beak Length	Trial #	Trial time	Number of Raisins
Short, stout	1	30 sec.	

Short, stout	2	30 sec.	
Long, thin	1	30 sec.	
Long, thin	2	30 sec.	

Table 2. Number of Grapes by beak size

Beak Length	Trial #	Trial time	Number of Grapes
Short, stout	1	30 sec.	
Short, stout	2	30 sec.	
Long, thin	1	30 sec.	
Long, thin	2	30 sec.	

Follow-up questions

1. Which beak was better for eating the grapes, and was your hypothesis correct? _____

_____

2. Which beak was better for eating the raisins, and was your hypothesis correct? _____

_____

3. If grapes are the typical food source during a normal year, how would you expect the ratio of beak size to change from year to year?_____

_____

_____

4. How would you expect the ratio of beak size to change during a drought year? _____

_____

5. If droughts are relatively infrequent, how is there variation in the population? _____

_____

Evidence of Evolution

The evidence for evolution is compelling and extensive. Looking at every level of organization in living systems, biologists see

the signature of past and present evolution. Darwin dedicated a large portion of his book, On the Origin of Species, identifying patterns in nature that were consistent with evolution and since Darwin our understanding has become clearer and broader.

Fossils

Fossils provide solid evidence that organisms from the past are not the same as those found today; fossils show a progression of evolution. Scientists determine the age of fossils and categorize them all over the world to determine when the organisms lived relative to each other. The resulting fossil record tells the story of the past, and shows the evolution of form over millions of years (Figure 6). For example, highly detailed fossil records have been recovered for sequences of species in the evolution of whales and modern horses. The fossil record of horses in North America is especially rich and many contain transition fossils: those showing intermediate anatomy between earlier and later forms. The fossil record extends back to a dog-like ancestor some 55 million years ago that gave rise to the first horse-like species 55 to 42 million years ago in the genus Eohippus. The series of fossils tracks the change in anatomy resulting from a gradual drying trend that changed the landscape from a forested one to a prairie. Successive fossils show the evolution of teeth shapes and foot and leg anatomy to a grazing habit, with adaptations for escaping predators, for example in species of Mesohippus found from 40 to 30 million years ago. Later species showed gains in size, such as those of Hipparion, which existed from about 23 to 2 million years ago. The fossil record shows several adaptive radiations in the horse lineage, which is now much reduced to only one genus, Equus, with several species.

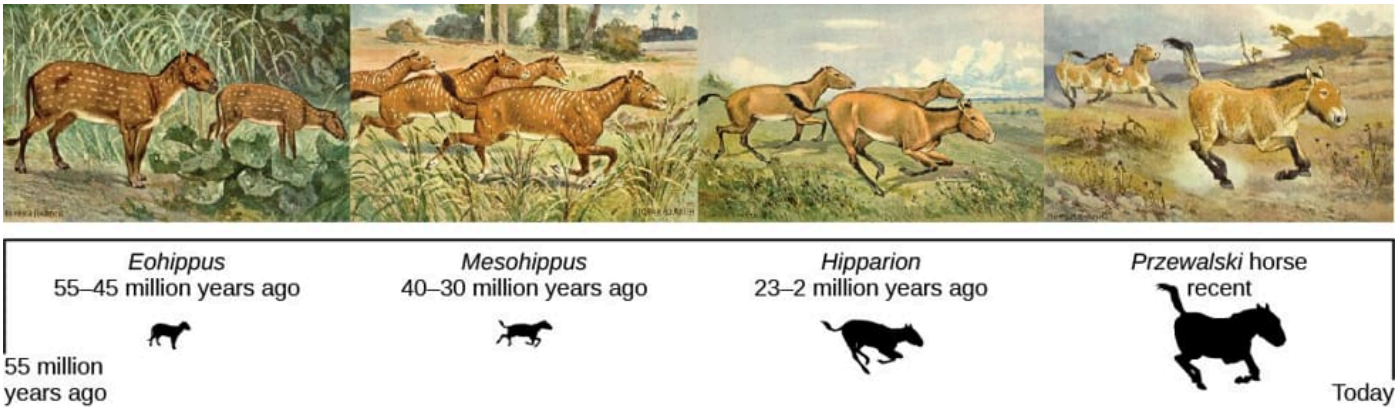


Figure 1.13.6: This illustration shows an artist's renderings of these species derived from fossils of the evolutionary history of the horse and its ancestors. The species depicted are only four from a very diverse lineage that contains many branches, dead ends, and adaptive radiations. One of the trends, depicted here is the evolutionary tracking of a drying climate and increase in prairie versus forest habitat reflected in forms that are more adapted to grazing and predator escape through running. Przewalski's horse is one of a few living species of horse. (Image from "BIOL 310: General Biology" by Eric Wada for Folsom Lake College, "Chapter 7: Evolution, section 7.3 Evidence of Evolution" is shared under CC BY 4.0 License and was authored, remixed, and/or curated by Openstax.)

Fossils Observation Exercise

1. There are several fossils in the lab room, observe the various invertebrate, plant, and vertebrate fossils.
2. In the space below take notes on how old the fossils are and how different or similar they look to modern organisms.

_____

_____

_____

_____

_____

_____

_____

_____

Anatomy and Embryology Evidence

Another type of evidence for evolution is the presence of structures in organisms that share the same basic form. For example, the bones in the appendages of a human, dog, bird, and whale all share the same overall construction (Figure 7). That similarity results from their origin in the appendages of a common ancestor. Over time, evolution led to changes in the shapes and sizes of these bones in different species, but they have maintained the same overall layout, evidence of descent from a common ancestor. Scientists call these synonymous parts homologous structures. Some structures exist in organisms that have no apparent function at all, and appear to be residual parts from a past ancestor. For example, some snakes have pelvic bones despite having no legs because they descended from reptiles that did have legs. These unused structures without function are called vestigial structures. Other examples of vestigial structures are wings on flightless birds (which may have other functions), leaves on some cacti, traces of pelvic bones in whales, and the sightless eyes of cave animals.

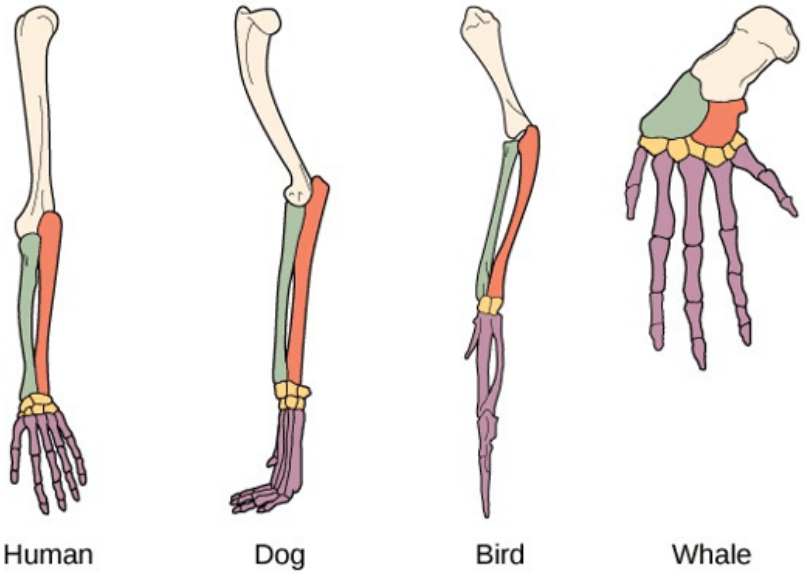


Figure 1.13.7: The similar construction of these forelimbs indicates that these organisms share a common ancestor. The bones are all analogous and allow for wide array of forms and functions with small modifications of the same structures. (Image from "BIOL 310: General Biology" by Eric Wada for Folsom Lake College, "Section 7.3: Evidence of Evolution" is shared under CC BY 4.0 License and was authored, remixed, and/or curated by Openstax.)

Another evidence of evolution is the convergence of form in organisms that share similar environments. For example, species of unrelated animals, such as the arctic fox and ptarmigan (a bird), living in the arctic region have temporary white coverings during winter to blend with the snow and ice (Figure). The similarity occurs not because of common ancestry, indeed one covering is of fur and the other of feathers, but because of similar selection pressures—the benefits of not being seen by predators.



Figure 1.13.8: The white winter coat of (a) the arctic fox and (b) the ptarmigan's plumage are adaptations to their environments. (credit a: modification of work by Keith Morehouse). Image from "BIOL 310: General Biology" by Eric Wada for Folsom Lake College, "Section 7.3: Evidence of Evolution" is shared under CC BY 4.0 License and was authored, remixed, and/or curated by Openstax.

Embryology, the study of the development of the anatomy of an organism to its adult form also provides evidence of relatedness between now widely divergent groups of organisms. Structures that are absent in some groups often appear in their embryonic forms and disappear by the time the adult or juvenile form is reached. For example, all vertebrate embryos, including humans, exhibit gill slits at some point in their early development. These disappear in the adults of terrestrial groups, but are maintained in adult forms of aquatic groups such as fish and some amphibians. Great ape embryos, including humans, have a tail structure during their development that is lost by the time of birth. The reason embryos of unrelated species are often similar is that mutational changes that affect the organism during embryonic development can cause amplified differences in the adult, even

while the embryonic similarities are preserved.

Comparing Vertebrate Skeletons Procedure

1. Look at the skeleton model of Ichthyostega, the ancestral tetrapod or terrestrial vertebrate. In particular look at the forelimb (Figure 7.) made from a humerus (upper arm bones), radius and ulna (forearm bones), carpal bones (wrist bones), metacarpals (hand bones), and phalanges (finger bones)
2. Then compare the same forelimb bones of modern tetrapods: human, lizard, bat, bird, cat, and amphibian.
3. Fill in the table below which of the bones for each organism are still similar to the ancestral form and which ones are different (modified) from the ancestral form.

Table 3. Vertebrate Forelimb comparison table

Animal	Bones resembling ancestral form	Bones differing from ancestral form
Human		
Lizard		
Bat		
Bird		
Cat		
Amphibian		

Follow-up Questions

1. When comparing forelimb skeletons of vertebrates, which organisms seemed more ancestral (more similar to the ancestral tetrapod)?

_____

_____

_____

2. Order the different organisms on a spectrum of ancestral to derived (more modified).

_____

_____

_____

3. What could you say about the organisms based on where along that spectrum they fall?

_____

_____

_____

Embryonic Evidence

The embryonic development of all vertebrates shows remarkable similarities as you can see from these drawings (Figure 9. supplied by Open Court Publishing Company). The drawings in the top row are of the embryonic stage called the pharyngula. At this stage ("I") they all contain a notochord, dorsal hollow nerve cord, a tail extending behind the anus, and a series of paired branchial grooves.

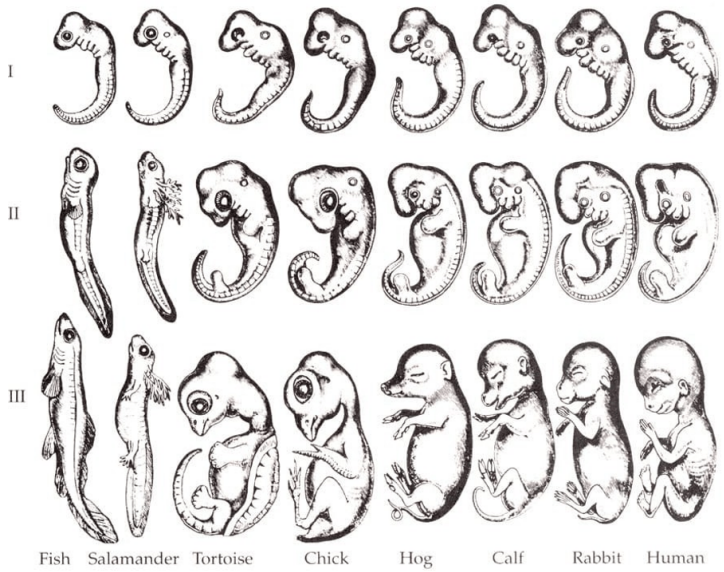


Figure 1.13.9: Embryos of numerous taxa going through similar developmental stages of embryonic development. The branchial grooves are matched on the inside by a series of paired gill pouches. In fishes, the pouches and grooves eventually meet and form the gill slits, which allow water to pass from the pharynx over the gills and out the body. In the other vertebrates shown here, the grooves and pouches disappear. In humans, the chief trace of their existence is the eustachian tube and auditory canal which (interrupted only by the eardrum) connect the pharynx with the outside of the head. George Romanes's 1892 copy of Ernst Haeckel's controversial embryo drawings. (Public Domain)

Comparing Vertebrate Embryos procedure

1. Your professor will have setup three compound microscopes with three different embryos at similar stages as well as have an image of a human embryo up on the screen for you to compare.
2. Look at four embryos and try to identify the common structure found in all of them, and any different structures.
3. Examples include pharyngeal pouches, tail, heart, eyes, yolk stalk, ear. . .
4. Using this information fill in the below table.

Table 4. Embryo Comparison Table

Animal	Features in common	Feature not in common
Human		

Fish		
Bird		
Frog		

Human Evolution procedure

1. In lab there are both a human skeleton and chimpanzee skeleton, list their similarities and their differences.

_____

_____

_____

2. In the lab there a number of skulls including a Gorilla, Human, and other Hominids. While observing them find and list any traits that seem more ancestral and traits that are more derived.
   A. Ancestral traits:

      _____

      _____

      _____

   B. Derived traits:

      _____

      _____

      _____

Molecular Evidence of Evolution

Like anatomical structures, the structures of the molecules of life reflect descent with modification. Evidence of a common ancestor for all of life is reflected in the universality of DNA as the genetic material and of the near universality of the genetic code and the machinery of DNA replication and expression. Fundamental divisions in life between the three domains are reflected in major structural differences in otherwise conservative structures such as the components of ribosomes and the structures of membranes. In general, the relatedness of groups of organisms is reflected in the similarity of their DNA sequences—exactly the pattern that would be expected from descent and diversification from a common ancestor.

DNA sequences have also shed light on some of the mechanisms of evolution. For example, it is clear that the evolution of new functions for proteins commonly occurs after gene duplication events. These duplications are a kind of mutation in which an entire gene is added as an extra copy (or many copies) in the genome. These duplications allow the free modification of one copy by mutation, selection, and drift, while the second copy continues to produce a functional protein. This allows the original function for the protein to be kept, while evolutionary forces tweak the copy until it functions in a new way.

Protein similarity experiment procedure

For this experiment a rabbit is injected with human blood, causing the rabbit's immune system to produce antibodies for that human blood. Those antibodies are recognizing proteins found on the surface of the blood cells, surface antigens. The more

closely related an organism is the more similar these proteins will be to each other, allowing us to now use this rabbit serum to tell in relative terms how closely related organisms are to humans. This is a process called Serology, and we are going to use it to identify 5 mystery serums.

1. For this test there are 5 test serums each one could be Human, Cow, Chimpanzee, Monkey, and Orangutan.
2. Knowing what you've learned so far about evolution construct a list starting with Human ending with the animal least related to Human.
    1. Human
    2.
    3.
    4.
    5.
3. Obtain a chemplate, several stir sticks (enough that you wont contaminate samples), one Rabbit serum, one human serum, one of each of the test serums labeled I, II, III, IV, and V.
4. Add a couple drops of Rabbit serum to wells 1 through 6
5. Add a couple drops of Human serum to wells 1 through 6 as well. The Rabbit serum is now sensitized to Human serum and will react to human proteins.
6. Add 4 drops of serum I to well 1 and stir
7. Repeat step 6 for serums II-V adding them to wells 2-5. Well 6 will be a control showing no reaction, completely unrelated to Human.
8. Wait at least 10 minutes for a reaction to occur.
9. Compare the reactions in the wells and fill in the below table with the amount of precipitate and with which animal you suspect it is.

Table 5. Serology Results

Serum #	Amount of precipitate	Animal
I		
II		
III		
IV		
V		

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1.14: ECOLOGY

PROCEDURE 1: REFLECTION

1. Starting from when you wake up, create a list of all the plastic items you used throughout the day. Then next to the item, put a star if the item is a single use container that would be thrown away after you're done using it (such as a chip bag, yogurt container, etc.). Record your items in Answer Sheet 1.

PROCEDURE 2: SYNTHETIC SAND

In this activity, we will be looking at beach sand and what is commonly found in it. There are many things you might find in beach sand; some things are from animals and plants that live in the ocean or on the shore. Shells are hard material that some animals use to protect themselves. Plant pieces like seeds, sticks, and leaves are from land or water plants. Also, there are commonly protist materials such as seaweed. We can also find anthropogenic items (man-made) such as plastics, metals, glass, papers, and fabrics. Today we will be comparing two sand samples from two local beaches.

Equipment we'll need

- Tweezers (4)- labeled oil, isopropanol, glycerol and water
- 3 small beakers filled with either oil, isopropanol, or glycerol
- Four sample collecting trays
- 600mL beaker
- 1 dropper bottle of acetone
- Sand sifters/sieves
- Sand samples from local beaches

Part 1: Initial Observations

1. Fill in Answer Sheet 2 to indicate where the sand samples were taken and your identification of the sample in this lab.
2. Walk over to the sand samples. Fill the sieve with 3 beakers full of sand then gently filter the sand back into the bucket. Avoid creating large amounts of dust.
3. Transfer all items collected by the sieve into one of the reusable sampling collecting trays.
4. Repeat steps 2 and 3 for the other sand sample.
5. Record the initial identities and number of each item found in Answer Sheet 2.
    a. If the group is unsure of an item's composition, ask for the professor's opinion. We will only further characterize the plastic samples found in sand.

Part 2: Plastic Materials Identification

6. Fill the 600mL beaker with water.
7. Choose a piece of separated plastic material and place it into the beaker of water. Be sure to fully submerge the item in the water and gently let go of it from the tweezers. Make sure not to introduce any bubbles that would cause the item to float. Note if the material sinks or floats.
8. Based on if the item sank or floated, transfer the piece of material to the appropriate beaker based on the next step in Figure 1 flowchart below.
    a. For example, if the material sank the next step is to transfer the piece to glycerol testing if it sinks or floats in this solution.
    b. Use the specific tweezers to transfer into for each specific solution.
9. Follow the flowchart until identification is reached. Record the results in Answer Sheet 2.
10. When finished with a piece of material, remove it from that beaker and place it on a separate reusable collection tray.
11. Repeat steps 7 through 9 with all of the plastic materials separated from the beach sand.

Clean up/Disposal

1. Add the separated plastics to the large beaker on the instructor's desk.
2. Return the used beakers of different solutions to their original locations.. Return the dropper bottle of acetone.
3. Pour water down the drain.
4. Rinse tweezers, beakers, and collection trays. Place on the towel to dry.

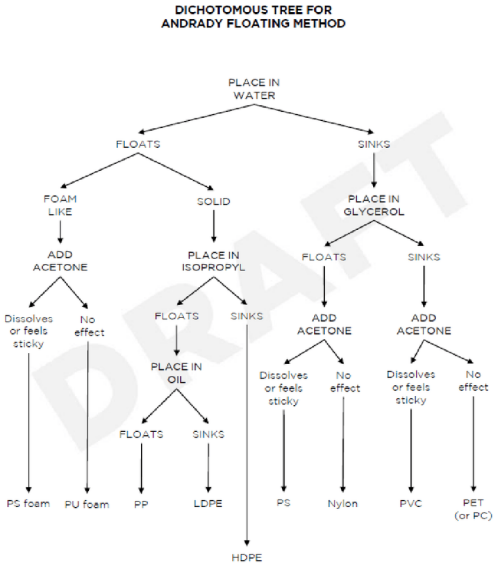


Figure 1. Flowchart of how to identify plastics materials from Algalita.

Minimizing Plastics Usage Resources

- Get active! Look into the events Algalita in Long Beach are hosting.
- Educate others!
- Avoid and reduce usage:
  - Shop used clothes and shoes
  - When buying new items, choose non-plastic options (ex. bamboo toothbrushes, metal water bottles, cloth grocery bags)
  - Go reusable and re-fillable! Stores like Sprouts sell products and foods in bulk- bring small containers or cloth bags to fill them
  - Shop local and at farmers markets

Answer Sheets

Answer Sheet 1: Plastics I used in one day

Item #	Thrown away?	Description

Item #	Thrown away?	Description

Answer Sheet 2: Identification of Plastics Found in Beach Sand

Number of items/ initial identity	Identities – Beach Sample 1	Identities – Beach Sample 2

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1.14.1: Ecology (Alternate)

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1.14.1: Ecology (Alternate)

Learning Objectives

Content Objectives

- Define ecosystem and ecology.
- Understand how energy flows through trophic levels.
- Define population and community.
- Identify the types of symbiosis.

Skill Objectives

- Design a model food web and identify nutrient flow.
- Explore two different types of symbiosis.
- Model and identify issues associated with eutrophication.

Ecology

Ecology is the study of the interactions of living organisms with their environment. One core goal of ecology is to understand the distribution and abundance of living things in the physical environment. Attainment of this goal requires the integration of scientific disciplines inside and outside of biology, such as mathematics, statistics, biochemistry, molecular biology, physiology, evolution, biodiversity, geology, and climatology.

Levels of Ecological Study

When a discipline such as biology is studied, it is often helpful to subdivide it into smaller, related areas. For instance, cell biologists interested in cell signaling need to understand the chemistry of the signal molecules (which are usually proteins) as well as the result of cell signaling. Ecologists interested in the factors that influence the survival of an endangered species might use mathematical models to predict how current conservation efforts affect endangered organisms.

To produce a sound set of management options, a *conservation biologist* needs to collect accurate data, including current population size, factors affecting reproduction (like physiology and behavior), habitat requirements (such as plants and soils), and potential human influences on the endangered population and its habitat (which might be derived through studies in sociology and urban ecology). Within the discipline of ecology, researchers work at four general levels, which sometimes overlap. These levels are organism, population, community, and ecosystem (Figure 44.2).

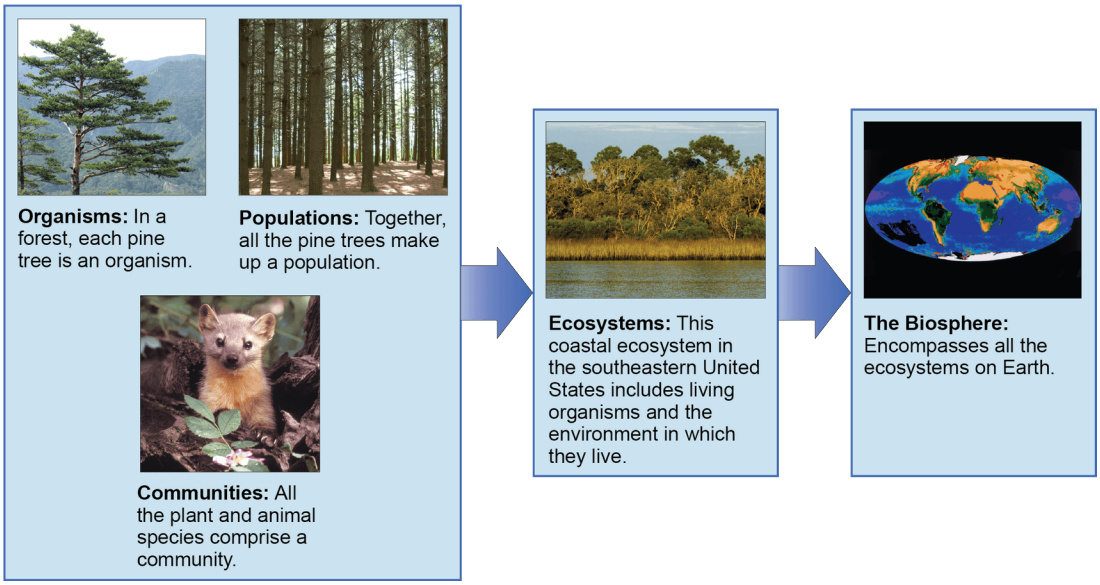
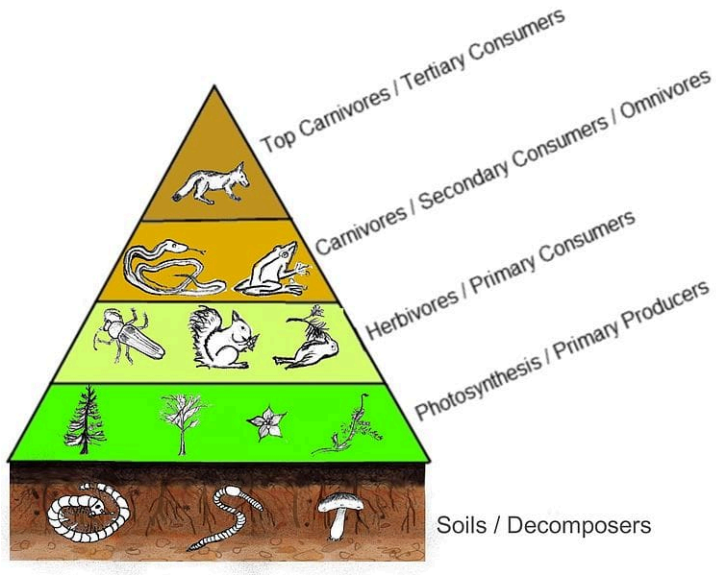


Figure 44.2 Ecologists study within several biological levels of organization. (credit “organisms”: modification of work by yeowatzup”/Flickr; credit “populations”: modification of work by "Crystl"/Flickr; credit “communities”: modification of work by US Fish and Wildlife Service; credit “ecosystems”: modification of work by Tom Carlisle, US Fish and Wildlife Service Headquarters; credit “biosphere”: NASA)

Ecosystem & Eutrophication

An **ecosystem** is defined as an association of life and the physical environment. Ecosystems take into account both living organisms and the nonliving components like water, soil, light, etc. Ecosystems can be either terrestrial (dessert, forest, grassland) or aquatic (coral reef, pond, estuary).

Within ecosystems scientists study **energy transformations**. Energy typically enters the ecosystem from the sun, is transferred to photosynthetic organisms (primary producers) and then to organisms that need to eat others for energy, the consumers or heterotrophs. An ecosystem will typically have several levels of consumers. The transfer of energy from organism to organism can be illustrated through **trophic levels.** A trophic level includes all organisms the same number of transfer steps away from the energy input into an ecosystem. A simple trophic level diagram for a forest ecosystem is illustrated below. Notice the photosynthetic organisms like plants are on the first trophic level, one step away from the energy source. The squirrel is a primary consumer (eating plants directly) but on the second tropic level two steps away from the primary energy source. At each step along the pathway some energy is lost as heat. Therefore at higher tropic levels there are fewer organisms (as noted by the pyramid shape) and most ecosystems support no more than four or five total trophic levels.



Ecosystems are changing due to human behaviors. Humans negatively impact natural ecosystems through activities such as deforestation, hunting, and pollution. Today's lab focuses on the impact of **eutrophication** on aquatic ecosystems. Through eutrophication bodies of water acquire extremely high concentrations of nutrients. The source of these nutrients can be natural or artificial. Humans cause cultural eutrophication through behaviors like run off from agricultural fields, wastewater from sewage treatment plants, and excel detergents running into bodies of water. The excess nutrients fuel photosynthesis causing an increased growth in algae, a photosynthetic primary producer protist, and an algae bloom. During the bloom the algae cover the surface of the water. When the algae die, decomposers in the ecosystem break down the protists using up the oxygen available in the aquatic environment through respiration. As oxygen levels decrease (hypoxia), fish die, and the balance of the ecosystem is destroyed.

In an effort to understand ecosystems and human impact on ecosystems scientists often use models. Modeling of ecosystems serves two main functions. First, the model represents scientist's best understanding of the relationships/functions that define the ecosystem. Second, models allow scientists to investigate questions that would be impossible to pursue in reality.

Ecosystem models fall into two categories, analytical models and simulation models. Analytical models use math to explain simple linear relationships. Simulation models, more widely used, are used to illustrate complex non linear relationships in ecosystems. Since the natural ecosystem has numerous interactions between living and non living components ecosystem models must simplify this real world situation. Models incorporate only the most important components or group similar components in an effort to effectively represent the ecosystem in a straightforward fashion.

Modeling a Food Web

Draw a food web. If you are on a field trip, include organisms that you see in the same area, interacting together. If in the lab, try to include organisms that you have seen or learned about. Make sure to include a **primary producer**, at least **two levels of consumers**, and a **decomposer**. Label which organisms are **autotrophs** and which are **heterotrophs**. Use arrows to show how energy is transferred and lost throughout this web.

What is the ultimate source of energy for your food web?

What happens to this energy as it moves through the food web? Where does it end up?

Nutrient Cycling in Ecosystems

In drawing your food web, you depicted a combination of the flow of energy and the cycling of nutrients through an ecosystem. Where as energy has an input and an output, nutrients are continually recycled. These elements often cycle between the biotic and abiotic components in an ecosystem.

For example, water is composed of hydrogen and oxygen atoms. Water molecules precipitate from the atmosphere and fall as

rain, saturating the soil. A plant absorbs those molecules through its roots, transporting them up to its leaves. Some of these water molecules will be broken apart during the process of photosynthesis, the oxygen exiting the plant as O_2 gas that you will breathe in while the hydrogen might be used to form molecules of glucose that you will eat. The rest of the water exits the plant through its stomata, evaporating back into the atmosphere in a process called **transpiration**, the evaporation of water from plant tissues. The oxygen atoms that you breathed in will be used for cellular respiration and be joined back to hydrogen molecules to once again form water. This water might exit your body as vapor on your breath or as perspiration and return directly to the atmosphere. It might also soak into your clothes as sweat or exit as urine, be processed in a wastewater facility, then sent out to the ocean where it will evaporate back into the atmosphere.

The atoms of hydrogen and oxygen are broken apart and reassembled into other molecules multiple times in this process, but the overall outcome is a cycle, with atoms traveling between the atmosphere, hydrosphere, lithosphere, and biosphere, but rarely ever exiting our Earth system.

In the space below, diagram the flow of water through the ecosystem around you.

What is the role of plants in the global cycling of water?

Mycorrhizal Networks

A **symbiosis** (sym- meaning shared, bio- meaning life) is when two or more organisms of different species live in close proximity to one another, sharing some aspect of their life cycle. A **mutualism** is a type of symbiosis in which both partners get a net benefit from the interaction. In other types of symbiosis, only one partner benefits and in a **parasitic** symbiosis, it is to the detriment of the other partner.

Most plants form a mutualistic symbiosis with fungi called a **mycorrhizal** relationship (myco- meaning fungus, rhiz- meaning root). In this particular partnership, the fungus enters the plant through the roots and takes sugars from the plant. In exchange, the plant takes water and dissolved nutrients (particularly nitrogen and phosphorus) from the fungus. Though both of these organisms lose something in the exchange, it is something that they tend to have a surplus of, while the thing they get in return is one that they would not have access to alone.

The fungus obtains its sugars from the living plant tissue. What terms could you apply to this organism?

The plant synthesizes its own sugars during photosynthesis. What terms could you apply to this organism?

Somewhat recently, a researcher named Suzanne Simard began tracking the exchange of nutrients through mycorrhizae. Not just from tree to fungus, but from tree to tree via connections to the same mycorrhizal partner. By providing certain trees with carbon dioxide containing a heavier isotope of carbon (C14), Dr. Simard could track the sugars formed from those heavy carbon atoms as they moved from tree to tree. What she found was that the connections extended beyond just one tree to another, but that almost all of the trees within her plot were connected through a network of different mycorrhizal fungi. Trees of different species and fungi of different species could exchange nutrients with each other. Further study revealed that the exchange was not limited to nutrients and water, but also included transfer of plant defense compounds that worked like raising an alarm, cuing the other plants to start producing defensive chemicals.

How might this information change the way we study ecosystems?

Sketch a few of the above ground plants that you can see (or imagine) and connect them below ground via a mycorrhizal network. Use arrows to indicate the flow of sugar through this network. Add in some disturbance that would set off a plant's immune system (attack of the caterpillars, fungal infection, etc...) and depict the movement of the signal throughout the plant community.

Some plants have evolved to be parasitic by taking advantage of the mycorrhizal network. These plants no longer photosynthesize. Instead, they form relationships with mycorrhizal fungi that are attached to other plants, siphoning sugars from those plants through the fungal connection. For example, the parasitic plant *Allotropa* parasitizes the Matsutake mushroom, which is mycorrhizal with tanoaks and a few other trees.

Add a parasitic plant into your ecosystem above and use arrows to depict the movement of sugars.

Are these parasitic plants autotrophic or heterotrophic? How would you classify them, using ecology terminology? Explain your reasoning.

Each species in an ecosystem is considered a **population**. This represents a group of actually or potentially interbreeding organisms and is an important area of study for both ecologists and geneticists. When multiple species in an ecosystem are considered together, this is called a **community**. For example, a forest could be composed of a single population of redwood trees, but it is more likely to be a community, including other tree species like Sitka spruce and Douglas-fir, and animals like the Northern spotted owl or Coho salmon. All living organisms are considered to be a part of the community in a given ecosystem.

What is the difference between a community and an ecosystem?

Community Interactions

Pollination

One important community interaction we study in botany (in addition to mycorrhizae) is **pollination**. This is the transfer of pollen from one plant to another and can be mediated by animals, water, wind, or another mechanism. Since we are talking about community interactions, we will focus on animal pollination. Many flowering plants have evolved over time to attract a specific animal pollinator. Because the plant often provides food in the form of nectar or pollen, the pollinator often relies on this relationship as well and coevolves with the plant. One interesting example of this coevolution is the pollination of figs by parasitic wasps.

The Story of the Fig and the Wasp

Figs are made of many small flowers in an inside-out inflorescence called a syconium. How, then, are these flowers pollinated to develop into fruits? Figs are pollinated by tiny chalcid wasps known as fig wasps. In the spring, female fig wasps laden with pollen and fertilized eggs enter into the fig syconium through a small opening at the top. As she enters, her wings are ripped off, making her unable to leave again. She has reached her destination.

As she walks along the tops of the flowers, which are in their female stage, she lays her eggs inside the ovaries by inserting a long tube called an ovipositor down through the style.

Pollen falls off of her body onto the flowers and travels down the styles as well to fertilize the ovaries. Each flower she deposits an egg into will develop into a wasp instead of a fruit. However, some of the styles are longer than others, and in these she will not be able to lay her eggs. These flowers will develop into the fig fruit, while the others will serve as nurseries for the fig's strange pollinators.

The male wasps hatch first. Blind and wingless, they roam around the enclosed inflorescence, impregnating their still-sleeping sisters. As carbon dioxide builds up within the chamber, they begin to suffocate and burrow out through the sides of the figs, allowing oxygen to flow back in.

After their brothers have died, the females hatch, climbing over the flowers that are now in their male stage, collecting pollen and stuffing it into pouches on their bodies. The young female wasps, laden with pollen and fertilized eggs, leave the fig through the tunnels their brothers made and fly off to find a developing fig to lay their eggs and pollinate the enclosed florets. The timeline of a fig wasp's life is intricately interwoven into the phenology of the fig tree.

View the diagram on the following page and identify the stages in the connected life cycles of these organisms. If available in lab or on your field trip, cut open a fig to view the florets developing inside the syconium.

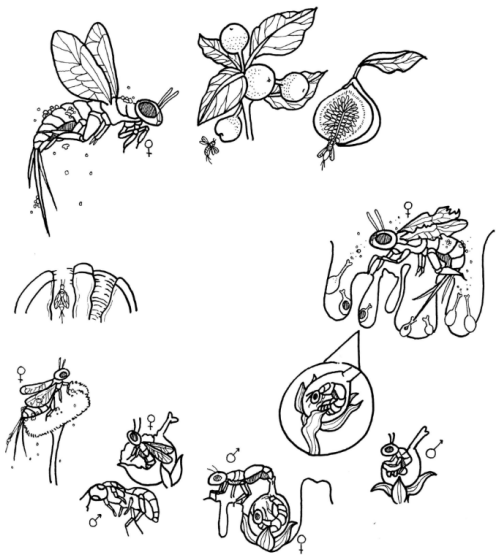


Figure 1.14.1.1: Fig Wasp Pollination Diagram

Is this relationship a parasitism or mutualism? Explain your reasoning.

Contributors and Attributions

- Maria Morrow (College of the Redwoods)

Ecosystem & Eutrophication - Silver Springs

This lab will utilize the Silver Spring model, an analytical model, developed by H. T. Odum in 1957. Silver Springs is a real aquatic ecosystem located in central Florida. Odum developed his model to illustrate energy flow through the different trophic levels. The main organisms in Sliver Sping organized by trophic level are:

- *First trophic level*: Eelgrass and algae are the main photosynthetic organisms.
- *Second trophic level*: Invertebrates, turtles, and fish are the herbivores.
- *Third tropic level*: Both fish and invertebrates are carnivores and prey upon the herbivores.
- *Fourth tropic level*: The top carnivores consist of gar and bass, which eat the other fish species at lower trophic levels.
- *Decomposers*: Bacteria and crayfish are the main decomposers.

The Silver Springs model is on Blackboard as an Excel file. The general model structure is pictured on the next page. Odum's model contains five state variables representing energy inputs into the four tropic levels and the decomposers. Flux rates describe the addition/removal of energy to/from the various parts of the system and the rate of transfer of energy between the components of the system. In the Excel file you can change the initial values **(X)** of any or all of the state variables and coefficients **(P)**, and control the length of time simulated. Following a simulation "run" you will be easily able to view graphs of the state variables or community respiration versus time. To change the initial values, simply type over the current value and press return. Use the model to investigate the scenarios below:

We will use the model in two different scenarios in today's lab, to understand eutrophication and to determine the impact of bread on the ecosystem.

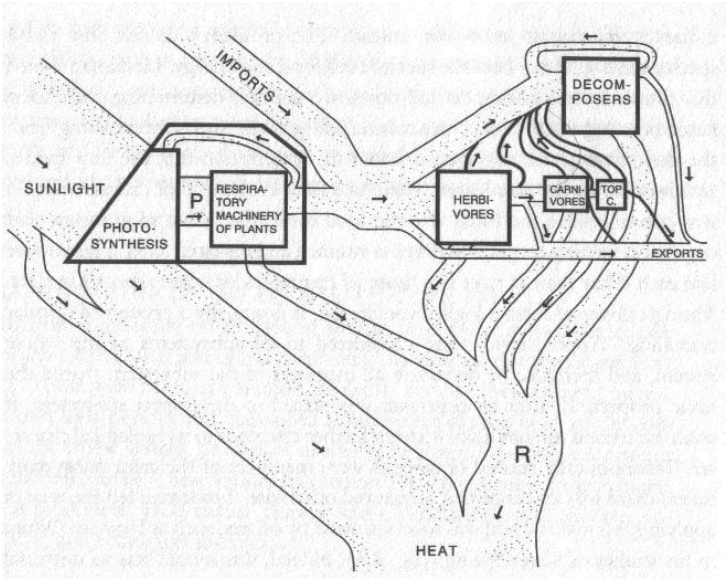


Figure 1.14.1.2: Odum's Silver Spring Model

Activity I

To understand more about eutrophication, you will conduct a laboratory experiment (Part 1) and use the Silver Springs computer model (Part 2)

Part 1

One potential way to decrease cultural eutrophication is by having higher trophic level organisms consume the algae. We will investigate this possibility using *Daphnia*, a zooplankton that feeds on green algae, and an alga species called *Chorella*. Although the information we gain from this activity is useful, it is an over simplification as to what might really occur in the aquatic environment.

We will use a **colorimeter** to measure the algae population. A colorimeter measures absorbance, the amount of light that is absorbed by a solution rather than the amount of light that can pass through. As the algae population increases (number of algae cells), the absorbance will also increase. It is a direct relationship.

Procedure

1. Obtain two cuvettes and a transfer pipette.
2. Fill one cuvette with distilled water. This cuvette is the blank
3. Place the blank into the colorimeter and zero it. This provides a baseline measure for the experiment. Save the blank cuvette for later use.
4. Using the disposable pipette, fill the second cuvette with the *Chorella* algae. Use the pipette to try and disperse the algae evenly throughout the cuvette.
5. Add 10 *daphnia* to the cuvette and immediately measure the absorbance using the colorimeter. Record your reading in the table below.
6. Remove the cuvette from the colorimeter and allow it to sit, undisturbed for 30 minutes.
7. After 30 minutes, place the blank cuvette (with distilled water) back into the colorimeter. Use the blank to re-zero the machine.
8. Remove the blank cuvette and put the algae/*Daphnia* cuvette into the colorimeter. Measure the absorbance and record your reading in the table below.

Table 1: Colorimeter data of *Daphnia* feeding on *Chorella*

	Absorbance value

Table 1: Colorimeter data of *Daphnia* feeding on *Chorella*	
Before feeding (time zero)	
After feeding (time 30 minutes)	

Questions

1. How did the absorbance change from time 0 to time 30 minutes? Did the absorbance increase, decrease, or stay the same?
2. What does the absorbance value change tell you about the concentration of the *Chorella*? Did it increase, decrease, or stay the same?
3. What does the absorbance value chance tell you about the behavior of the *Daphnia*? Did they consume *Chorella?* How do you know?
4. In a real aquatic ecosystem, do you think zooplankton could decrease the impact of cultural eutrophication and algae blooms? Explain why or why not.

Part 2

Now that you have observed trophic interactions and cultural eutrophication in a lab experiment, apply that knowledge to the computer model(opens in new window). Use the model to analyze the sensitivity of the Silver Springs ecosystem to cultural eutrophication. Remember that alga is a photosynthetic producer on the first trophic level. The *Daphnia* would be one of the herbivores in the environment on the second trophic level. Change the levels in the model to simulate a eutrophication situation. View the graphs to see how the different trophic level populations change through the simulation.

Questions

Based on your findings with the model, answer the following questions:

1. What is the maximum number of producers the ecosystem can support before higher trophic levels begin to decline?
2. What happens to community respiration in the simulated algae bloom? Does it increase, decrease, or stay the same?
3. What happens to the decomposer population in the simulated algae bloom? Does it increase, decrease, or stay the same? Why?
4. Which group of carnivores (which trophic level) is more greatly impacted by the algae bloom? Why do you think this is the case?

Activity II

Eutrophication is not the only way that human activities affect aquatic ecosystems. Tourism in the Silver Spring area has grown lately as a result of more of vacationers visiting Central Florida. When visiting Silver Spring, tourists enjoy feeding the ducks. But, the ducks are gaining weight and becoming dependent on the tourists as a food source disrupting the normal food chain in the environment. Some environmental organizations have voiced displeasure regarding the dependence of ducks on bread handouts from visitors to the lake. Is it feasible to stop feeding the ducks or have they become too reliant on the tourists and the bread handouts?

Task

Use the model to analyze the sensitivity of long-term duck populations to an increase or decrease in bread input. There is a separate variable for bread and the ducks would be on the second trophic level as herbivores. View the graphs to see how the different trophic level populations change through the simulation.

QUESTIONS

Based on your findings with the model, answer the following questions:

1. Is an elimination of bread feasible? Why or why not?
2. How would the system respond to increasing levels of tourism, assuming bread levels also increased? What trophic levels are most impacted by increased levels of bread?
3. What is the role of bread in the system, and how does its presence or absence impact the ecology of Silver Springs?
4. What would be your suggestion to the managing board of Silver Springs? Should they completely eliminate the tourists feeding the ducks? Why or why not?

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Ecology Game:

Islands have always provided scientists unique locations to learn about the natural world. Many of Charles Darwin's key observations that led to his understanding of natural selection came from species living on the many islands that make up the Galapagos archipelago. For example, Darwin observed different species of finches were found on different islands and that the shape of the finches' beaks corresponded to the type of food they consumed. Further research on the finches of the Galapagos by Dr. Peter Grant and Dr. Rosemary Grant found that even within an island, changes in environmental conditions (i.e. drought, disease) could influence the adaptations observed in the finches.

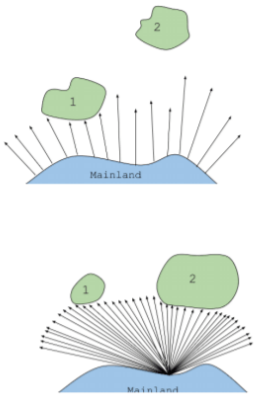


Figure 1 A & B – Both the distance of an island from the mainland (1A) and the size of an island (1B) can influence the number of species it can support.

Because islands are isolated from the mainland and each other by water and often long distances, they can be **colonized** by new species that have intentionally or unintentionally dispersed to an island. In biology, we call this the **founder effect**, which is when a new population is established. A founding population is often small and has a different allele frequency than the original population. It is important to remember that every species that finds itself in a new habitat may not be ideally suited for the environmental conditions. Is the habitat, food, climate appropriate for the new species needs? Is there competition for any of

these resources with species already living on the island? Large islands that are close to the mainland tend to support more species than small islands that are far from the mainland. As we discuss in class, we know that no two species can occupy the same **ecological niche**—basically, two species cannot co-exist if they are competing for the same resource. While niches can overlap significantly between two species, they cannot be exactly alike. Any habitat has a limited number of species that can be supported by the available resources. We know that the most successful species are those with adaptations best suited to the environment in which they find themselves and those that can best utilize the available resources. These individuals are able to survive and reproduce on the island and a new population is established there. Occasionally, if there is a mismatch in the individual's traits and the environment in which they are located or the environmental conditions change unfavorably the individual may no longer succeed in that environment. This can lead to local extinction which is also known as **extirpation**. An extirpated species can recolonize if environmental conditions become favorable.

Today, you will be playing the role of an individual of one of the six different species on an ancient continent to the East in a game we call the Ecology Game. The objective of the game is to cross the ocean, colonizing islands as you go. However, every species in the game has its own ecological needs and each island along the way has specific environmental conditions and the ability to support different numbers of species. If you are a successful colonizer, a population of your species will exist on the island even after you have moved on. Please note that in order to colonize and establish a new population in real life, you would need a sexually mature male and female or a pregnant female. However, in this game, we are simplifying this concept by using a model of a single individual. Every species will not be able to colonize every island successfully and you have to compete with the other species who are also trying to make it to the new continent. The islands are of varying sizes and successful colonization may cover a small area or a large area depending on the size of the island. The winner is determined by a combination of the order that you reach the new continent and the island area (not how many islands, but how much island space) you have successfully colonized. Remember to think strategically! The Ecology Game is not always about speed, but thinking about your species, its ecological niche, and what we know about colonization and adaptation. Just because you are the first species to reach the new continent that does not mean you win the game. Think of today like an ecological game of chess!

Set up (per group—groups should have no more than 6 students):

- 1 board map
- 2 six-sided dice
- 1 cup of 6 species sticks
- 4 bags of colored game pieces (if groups are larger than 4 and additional game pieces are required use coins or see Manal for additional colored pieces)
- 6 species cards
- 24 island cards
- Environmental factor cards

To Begin:

1. Each person in the group should draw a species stick out of the cup. You will play the role of an individual of this species for the entire game.
2. Read the associated species card. This will tell you about your ecological niche and any movement limitations you will have in the game.
3. Each player can place their colored game piece at any location on the mainland to start the game.
4. Roll the dice. The player with the highest roll will go first and play will move counterclockwise around the group.

To Play:

1. Player 1 will roll both die. The sum of the numbers shown on the roll indicates how many squares the player can move.

Note

You cannot move diagonally but you can move vertically and horizontally and you can move forwards or backwards. Some species have limitations on the maximum number of moves they can make. If your roll exceeds the maximum number of moves for your species, you must stop at your maximum number. Every species can stop in the ocean.

2. With each roll, player 1 will also draw an environmental factor card which may influence your movement along the way. This card

may affect all players, a subset of players, or just the player drawing the card.

3. If you land on any part of an island, choose the island card and determine if your species can successfully colonize the island. If so, fill that player's name/species name in the appropriate slot on the card (remember, some islands can only hold a few species) and place a colored game piece on the island to indicate that it has been colonized by you.

> **Note**
>
> On the next turn, a species on an island can leave from any part of the island.

4. Play continues in a counterclockwise fashion.

5. The game is complete for an individual species when they have reached the new continent. At this point, they no longer roll the dice and are no longer affected by environmental factor cards.

6. Play continues until all species have reached the new continent.

7. Calculate the winners using the formulas below.

Who Won?

- Each player's score is determined by adding up the total area your species covered (including bonuses) and dividing by your finish place modifier.
  - 1st place = 1
  - 2nd place = 1.5
  - 3rd place = 2
  - 4th place = 2.5
- For example: If my species covers 144 units of land and I had a bonus of 25 units of land for a total of 169 and I came in second place. My score = 122.7

$$\frac{(144\ units\ covered + 25\ bonus\ units)}{1.5\ (finish\ place\ modifier)} = 122.7\ (final\ score) \tag{1.14.1.1}$$

Questions:

1. What did the game clear up for you that you might not have understood fully about ecology and evolution?

2. Movement from the mainland to an island or from one island to another island represents what force that can cause a population to evolve?

3. If once your species has colonized an island and remains there and also becomes isolated from other populations over time, what possible outcome might occur given the passage of enough time?

4. When an island has multiple habitat types what does this say about the island's ability to support populations of different species?

5. How suitable are small islands to support multiple species? Explain your answer.

6. Once colonizing an island, a species may thrive or fail there. Describe how a species can either thrive or fail using terms and ideas from lecture. To help you answer the question, draw how a population's size will change over time if the species is K-selected.

Figure 1 is adapted from Wikimedia Commons material and this lab is licensed under a Creative Commons Attribution License License (3.0).

Modeling a Food Web

1. Draw a food web.

2. What is the ultimate source of energy for your food web?

3. What happens to this energy as it moves through the food web? Where does it end up?

Nutrient Cycling in Ecosystems

1. In the space below, diagram the flow of water through the ecosystem around you.

What is the role of plants in the global cycling of water?

Mycorrhizal Networks

1. The fungus obtains its sugars from the living plant tissue. What terms could you apply to this organism?
2. The plant synthesizes its own sugars during photosynthesis. What terms could you apply to this organism?

Somewhat recently, a researcher named Suzanne Simard began tracking the exchange of nutrients through mycorrhizae. Not just from tree to fungus, but from tree to tree via connections to the same mycorrhizal partner. By providing certain trees with carbon dioxide containing a heavier isotope of carbon (C14), Dr. Simard could track the sugars formed from those heavy carbon atoms as they moved from tree to tree. What she found was that the connections extended beyond just one tree to another, but that almost all of the trees within her plot were connected through a network of different mycorrhizal fungi. Trees of different species and fungi of different species could exchange nutrients with each other. Further study revealed that the exchange was not limited to nutrients and water, but also included transfer of plant defense compounds that worked like raising an alarm, cuing the other plants to start producing defensive chemicals.

3. How might this information change the way we study ecosystems?
4. Sketch a few of the above ground plants that you can see (or imagine) and connect them below ground via a mycorrhizal network. Use arrows to indicate the flow of sugar through this network. Add in some disturbance that would set off a plant's immune system (attack of the caterpillars, fungal infection, etc...) and depict the movement of the signal throughout the plant community.

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6. Are these parasitic plants autotrophic or heterotrophic? How would you classify them, using ecology terminology? Explain your reasoning.

7. What is the difference between a community and an ecosystem?

Community Interactions

8. Is the relationship between the fig and the wasp a parasitism or mutualism? Explain your reasoning.

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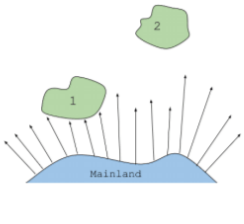


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