Principles of Biology

Bi 2152L
Laboratory Manual

Portland State University
Winter 2016
Principles of Biology

Bi 21552L

Laboratory Manual
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Bi215: Principles of Biology Winter Term ‘16  
Laboratory Syllabus

You are required to attend each lab, and to arrive for all of your labs on time. Missed labs cannot be made up.

You can find a list of the lab TAs and their office hours on ‘d2l’.

The lab handouts are available for download from ‘d2l’. You are expected to read these in advance of your scheduled lab meeting.

Please be conscientious of how much paper you are using when printing out items such as PowerPoint slides and the lab manual. Consider printing multiple slides per page and always print double sided.

Grading
The Lab, while a component of the first term of Principles of Biology, is its own 1-credit course as Bi214. Points will be earned from quiz scores, lab write-ups, a formal lab project, and participation.

Grading Policy
Grades will be assigned according to the percentage of possible points earned. As a rough guide, the highest cumulative score can be thought of as 100%. If you earn at least 90% of the highest score you will receive an A- or higher; if you earn at least 80% you will receive a B- or higher; if you earn at least 70% of the possible points you will receive a C- or higher; if you earn at least 60% of the possible points you will receive a D- or higher.

PSU’s policy on the temporary grade of Incomplete (“I”) is strictly adhered to in this course. Please note, you must be passing the course (with a C- or better) in order to be eligible for an “I” grade. See the PSU Bulletin for more information: http://www.pdx.edu/oaa/psu-bulletin.

Quizzes
At the beginning of each lab, you will be given a quiz. The content will be 90% on the previous week’s lab and 10% on the current week’s lab, so read the lab before you arrive. If you are more than 10 minutes late, you will not be permitted to take the quiz.

There will be a quiz every week except the first. There are 9 quizzes, worth 10 points each. The lowest quiz score is automatically dropped. 80 points total.

NO MAKE-UP QUIZZES.

Lab write-ups
You must complete each lab, and answer all questions to receive full credit. There are 10 labs, worth 20 points each. The lowest lab report score is automatically dropped. 180 points total.

NO MAKE-UP LABS.

Lab write-ups are due the week after each lab is performed, no later than 30 minutes into the lab. Labs that are turned in late will automatically have 40% (8 points) deducted before grading. Labs may not be turned in more than one week late.
Drawings need to be large enough for clarity, and labeled with the name of the item, its size, and any of its parts named in the lab handout.

Lab questions and their answers need to be typed on a separate sheet in 12 point font, in full sentences. Show your calculations.

Be sure that your lab reports include your name, TA’s name and lab time, in the upper right corner of the first page. If you miss the lab for any reason and do not discuss with your TA, you will not get any points for that lab write-up.

Lab Project

You will be completing an animal and plant lab project in Bi215 that will be due at the beginning of Lab 10. This project is worth **20 points**. You will be receiving the specific details of this assignment during Lab 3.

Attendance

There is a participation component to each lab report. You must stay for the lab and actively participate. Lack of participation will result in the automatic loss of points for that lab’s write-up. If you arrive more than 30 minutes late to the lab, you may not participate in the day’s lab and will receive a zero on the lab report (extreme circumstances that are approved by both the TA and professor may be considered).

Labs will not meet on the PSU MLK Jr Day holiday. These labs will be made up on a different day—your TAs will have the details.

Laboratory Rules

- Read the lab ahead of time.
- Turn your phone off for the duration of the lab.
- Arrive on time and prepared for 3 hours of work.
- The computers are for Bi 215-related work only, e.g. data collection, lab report write-ups, or investigation of topics related to the lab exercise or lectures.
- **Cheating and any other academic dishonesty will result in a zero for the assignment, and will be reported to the University administration.**
- Leave the lab benches cleaner than you find them. Messes that someone else has to clean up will result in point deductions.

I. Safety

- Report any injuries or broken equipment to the TA immediately.
- No sandals or open shoes of any kind. You will be asked to leave the lab if you are not in compliance.
- Leave backpacks and jackets in the cubby holes next to the door.
- Absolutely no eating or drinking in the lab.
- Waste containers and bins must be used for all designated substances and disposable lab materials. Your TA will tell you what can be disposed of in the sink and what needs to go into the red waste bins.
- Each lab room is equipped with a phone that can be used in case of emergency. Important safety numbers are listed on each phone.
II. Your lab manual, table, and partners

- Read over the current material **before your lab period**. If hypotheses are required, think about how to state them succinctly, but wait until the lab material has been introduced before stating hypotheses in final form. Fill out the required questions, drawings, etc. as much as possible during lab. Your lab records are important for your grade. Use a pencil for drawings.
- Lab work should be completed in cooperation with your table partners (see “expectations for students” below), but all work turned in must be unique and stated in your **OWN words**.
- When you are finished, make sure all equipment and chemicals are stored in the proper places (either at the end of the table or on the center tables). Unless instructed otherwise, all glass- and plastic-ware must be cleaned and returned to your lab table for use by the next class. Leave them to dry upside down on a paper towel (or in a test tube rack) on your lab bench. Do not leave them in or by the sink! Used test tubes should be discarded in the red waste bins, as instructed by your TA.
- Make sure your table is wiped down and push in the chairs.
- Stick around for discussion after the lab. This will help you understand the lab more thoroughly and will help you complete the questions in your lab manual. Participation in this discussion is a component of overall participation and therefore influences your grade.

III. Microscopes and Slides

A. Microscopes: The microscopes will be out and on your table ready for use. Do not move the microscope from your bench unless instructed by your TA and NEVER slide your microscope on the lab bench. Use two hands to lift and move or to carry it.
   1. When you are through with the microscope, make sure oil is wiped off the oil immersion lens, and make sure it is stored in the correct position (stage down, on 4X, lamp off, and without a slide on it). Clean objectives and oculars using only the special lens paper (not paper towels or Kim Wipes).
   2. Report any microscopes that are not stored properly from the previous lab.

B. Slides: Each table will have one box of clean slides and cover slips. Prepared slides will be on each table in the small green boxes.
   1. *Prepared slides* must be returned to the box where they were obtained, with the label right side up. Do not mix them up, and do not place them in the wrong box.
   2. Dispose of slides and coverslips you prepared yourself in the **sharps** container or red waste bins.
## Bi 215 Laboratory Schedule

<table>
<thead>
<tr>
<th>Week</th>
<th>Topics</th>
<th>Quiz?</th>
</tr>
</thead>
</table>
| 1    | Orientation and safety  
Lab 1: Developmental Biology                                             | no      |
| 2    | Lab 2: Natural Selection                                               | Yes     |
| 3    | Lab 3: Cnidarians, Platyhelminthes, Nematodes, and Annelids             | Yes     |
| 4    | Lab 4: Mollusks; species accounts assigned                              | Yes     |
| 5    | Lab 5: Arthropods                                                      | Yes     |
| 6    | Lab 6: Phylogeny and Comparative Anatomy of Deuterostomes              | Yes     |
| 7    | Lab 7: Photosynthesis and Plant Pigments                               | Yes     |
| 8    | Lab 8: Vegetative Structures and Function                              | Yes     |
| 9    | Lab 9: Stomatal Density                                                | Yes     |
| 10   | Lab 10: Reproduction; species accounts due                             | Yes     |
| Finals | No Lab                                                                |         |
IMPORTANT INFORMATION: Please fill out on Day 1

Your TA’s name: ________________________

Office hour and location: ________________________

Your lab mate’s names and email addresses:

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________________________
Principles of Biology -- Bi 215

Expectations for Students

Learning in the University environment should be stimulating, demanding, and also fair. We encourage the open discussion and exchange of ideas, particularly in the laboratory setting. However, it is essential that all graded work in this course be your own work, and in your own words.

Below are the expectations for students in this class. This set of expectations is intended to maximize debate and exchange of ideas in an atmosphere of mutual respect while preserving individual ownership of ideas and written words. If you feel you do not understand or cannot agree to these expectations, you should discuss this with your instructor and classmates.

1. Students are expected to work cooperatively with other members of the laboratory and show respect for the ideas and contributions of other people.

2. When working as part of a group, students should strive to be good contributors to the group, to listen to others in the group and try not to dominate, and to recognize the contributions of others. Students should try to ensure that everyone in the group makes a contribution, and recognize that everyone contributes in different ways to a group process.

3. Students should conduct experiments and discuss problems as part of a group, but must write lab reports and exams alone, without copying anyone else’s work.
I have read and understood the expectations for students in this class. If I am uncertain about appropriate behavior in the class I will ask one of the instructors for clarification. I understand that any instances of academic dishonesty (see the PSU "Code of Student Conduct and Responsibility" for more information: http://www.pdx.edu/dos/conductcode) will result in a zero for that assignment or exam, and will be reported to the University administration, as outlined in the course syllabus.

Signed, ____________________________ ____________________________

Date: __________________

__________________________ ____________________________
Printed name

Bi252: Principles of Biology Winter Term 2014
Laboratory Syllabus

You are required to attend each lab, and to arrive for all of your labs on time. Missed labs cannot be made up.

A badge access ID is required to enter Science Building 1 for labs that take place at 6am and on Saturdays. This is different than your student ID. Please make sure you have obtained a badge access ID from Neuberger by the second week of classes, if you do not already have one.

You can find a list of the lab TAs and their office hours on ‘d2l’.

The lab handouts are available for download from ‘d2l’. You are expected to read these in advance of your scheduled lab meeting.

Grading

Lab work makes up 25% of the total grade in Bi252. The points in lab add up to 280, which will be scaled at the end of the term to be 25% of your points in the class.

Quizzes

At the beginning of each lab, you will be given a quiz on the previous week’s lab. There will also be questions on the current lab, composing of approximately 10% of the total quiz score, so read the lab before you arrive. If you are more than 10 minutes late, you will not be permitted to take the quiz.

There will be a quiz every week except the first. There are 9 quizzes, worth 10 points each. The lowest quiz score is automatically dropped. 80 points total.

NO MAKE-UP QUIZZES.

Lab write-ups

You must complete each lab, and answer all questions to receive full credit.

There are 10 labs, worth 20 points each. The lowest lab report score is automatically dropped. If you are given a ZERO for a lab write-up because of plagiarism, that lab will NOT be dropped. 180 points total.
NO MAKE-UP LABS.
Lab write-ups are due the week after each lab is performed, no later than 30
minutes into the lab. Labs that are turned in late will automatically have 40% (8 points)
deducted before grading. Labs may not be turned in more than one week late.

Drawings need to be large enough for clarity, and labeled with the name of the
item, its size, and any of its parts named in the lab handout.

Lab questions and their answers need to be typed on a separate sheet in 12
point font, in full sentences. Show your calculations.

Sign your lab with your name, TA's name and lab time, IN PEN, in the upper right
corner of the first page. If you miss the lab for any reason and do not discuss with your
TA, you will not get any points for that lab write-up.

Lab Project
You will be completing an animal and plant lab project in BI252 that will be due at
the beginning of Lab 10. This project is worth 20 points. You will be receiving the
specific details of this assignment during Lab 3.

Attendance
There is a participation component to each lab report. You must stay for the lab
and actively participate. Lack of participation will result in the automatic loss of points for
that lab's write-up. If you arrive more than 30 minutes late to the lab, you may not
participate in the day's lab and will receive a zero on the lab report unless of extreme
circumstances, approved by both the TA and professor.

Labs will not meet on the PSU MLK Jr Day holiday. These labs will be made up
on a different day—your TAs will have the details.

Laboratory Rules
- Read the lab ahead of time.
- Turn your phone off for the duration of the lab.
- Arrive on time and prepared for 3 hours of work.
- The computers are for BI 252-related work only. E.g., data collection, lab report
  write-ups, or investigation of topics related to the lab exercise or lectures.
- Cheating and any other academic dishonesty will result in a zero for the
  assignment, and will be reported to the University administration.
- Leave the lab benches cleaner than you find them. Messes that someone else
  has to clean up will result in point deductions.

I. Safety
- Report any injuries or broken equipment to the TA immediately.
- No sandals or open shoes of any kind. You will be asked to leave the lab if you
  are not in compliance.
- Leave backpacks and jackets in the cubby holes next to the door.
- Absolutely no eating or drinking in the lab.
Waste containers must be used for all designated substances. Your TA will tell you what can be disposed of in the sink.

- Dispose of cover slips and broken glass in the red sharps container.
- The phone by the elevator is a direct line to campus safety.

II. Your lab manual, table, and partners
- Read over the current material **before your lab period**. If hypotheses are required, think about how to state them succinctly, but wait until the lab material has been introduced before stating hypotheses in final form. Fill out the required questions, drawings, etc. as much as possible during lab. Your lab records are important for your grade. Use a pencil for drawings.
- Lab work should be completed in cooperation with your table partners (see “expectations for students” below).
- When you are finished, make sure all equipment and chemicals are stored in the proper place (either in the center of the table or on the back bench). Unless instructed otherwise, all glass- and plastic-ware must be cleaned and returned to the center of your lab table for use by the next class. Leave them to dry upside down on a paper towel (or in a test tube rack) in the center of your lab bench. Do not leave them in or by the sink! Used test tubes should be discarded in the sharps waste container, as instructed by your TA.
- Make sure your table is wiped down and push in the chairs.
- Stick around for discussion after the lab. This will help you understand the lab more thoroughly and will help you complete the questions in your lab manual. Participation in this discussion is a component of overall participation and therefore influences your grade.

III. Microscopes and Slides
A. Microscopes: You will be assigned a specific microscope at your bench. DO NOT MOVE THE MICROSCOPE FROM YOUR BENCH.
   - When you are through with the microscope, make sure oil is wiped off the oil immersion lens, and make sure it is stored in the correct position (stage down, on 4X, lamp off, and without a slide on it). Clean objectives and oculars using only the special lens paper (not paper towels or Kleenex).
   - Report any microscopes that are not stored properly from the previous lab.
   - If for some reason you need to move the microscope, do so carefully—always use two hands.

B. Slides: Each table will have one box of clean slides and cover slips. Prepared slides will either be on each table or on the back counter.
   - Prepared slides must be returned to the box where they were obtained, with the label right side up. Do not mix them up, and do not place them in the wrong box.
   - Dispose of slides and coverslips you prepared yourself in the sharps container.
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<th>Topics</th>
<th>Quiz?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Orientation and safety&lt;br&gt;Lab 1: Developmental Biology</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>Lab 2: Natural Selection</td>
<td>Yes</td>
</tr>
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<td>4</td>
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<td>10</td>
<td>Lab 10: Reproduction; species accounts due</td>
<td>Yes</td>
</tr>
<tr>
<td>Finals</td>
<td>No Lab</td>
<td></td>
</tr>
</tbody>
</table>
IMPORTANT INFORMATION: Please fill out on Day 1

Your TA's name: ________________________

Office hour and location: ________________________

Your labmate's names and email addresses:

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Principles of Biology -- Bi 252

Expectations for Students

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Signed, __________________________

_______________________________ Date: __________________

Printed name

_____________________________
Lab 1: Developmental Biology

Introduction

Amphibians of several types, fish and chickens have historically been used as model organisms for development. There is much scientific literature going back over 100 years on their developmental stages. Why have amphibians and some species of fish been so popular for studies of developmental biology? In general amphibians are easy to find, collect and keep in the laboratory. Under laboratory conditions they will often mate and since fertilization is external, this process can be observed and manipulated easily. A female amphibian will shed a very large number of eggs and they are among the largest non-shelled eggs of any animal. The lack of a hard shell means that the developing embryo may be observed directly without affecting further development. Although there are differences in the development of different types of amphibians, for the purpose of this lab we will consider frogs & toads (anura) and newts & salamanders (caudata) to be the same. That is, the differences are small for our purposes. Chickens too are favorite models for the study of development. They are also convenient to obtain and keep, inexpensive, and of course the eggs are very large. Birds are the most complex of the non-mammalian species, but much easier to study than mammals because, with a few exceptions, mammals have in utero development. Furthermore, raising chicks has always been commercially important and historically there was good financial support to investigate, for example, the optimum conditions to get healthy hatchings.

Background Information

Amphibians

Within the subphylum Vertebrata, the class Lissamphibia represents all extant amphibians. Amphibians are members of the clade vertebrata, possessing a vertebral column that surrounds the spinal cord, and tetrapoda exhibiting digits on limbs and loss of internal gills. Unlike lizards, snakes, birds and mammals, amphibians do not produce amniotic eggs. Amphibian eggs must be deposited in water, which has tied their life cycles to water habitats. Amphibian eggs are mesolecithal (with moderate yolk) and telolecithal (with separate animal and vegetal poles). During fertilization, the diploid genome is restored when a haploid egg and sperm unite. Opposite where the sperm enters, the gray crescent forms. A furrow appears longitudinally through the separate poles bisecting the gray crescent. This is the onset of cleavage. The first cleavage results in two equal cells. Then second cleavage begins when another furrow runs through the poles at a ninety degree angle to the original furrow resulting in four cells. As this process continues creating stages of 8, 16, and 32 cells, the animal pole begins dividing more rapidly than the vegetal pole creating smaller cells at the animal pole. Finally, from these divisions, a hollow ball of thousands of cells, a blastula, is created. Within the blastula, a fluid filled cavity (blastocoel) forms. The blastula undergoes gastrulation which is marked by an invagination (pushing inward) of the cells producing a
blastopore. Gastrulation establishes the three germ layers: ectoderm, mesoderm, and endoderm. Also during gastrulation, the primary body axis is constructed which establishes the body plan. After gastrulation, the embryo proceeds into neurulation. During neurulation the neural tube, neural crest and epidermis are created. The mesoderm develops into the notochord and induces the ectoderm above it to begin forming neural tissue. The ectoderm becomes two longitudinal folds, resulting in the "neural folds" stage. The lips of the folds will begin to fuse and form the neural tube which will then develop into the brain and spinal cord. The embryo continues to develop until it becomes a tadpole and hatches. The fully-formed tadpole has no more organic matter than when it started, and can only begin growing once it can feed. In most, but not all species of amphibians, the larval tadpole will eventually undergo metamorphosis and become a fully formed adult amphibian.

Fish
The term "fish" most precisely describes any non-tetrapod craniate (i.e. an animal with a skull and in most cases a backbone) that has gills throughout life and whose limbs, if any, are in the shape of fins[1] Unlike groupings such as birds or mammals, fish are not a single clade but a paraphyletic collection of taxa, including hagfishes, lampreys, sharks and rays, ray-finned fish, coelacanths, and lungfish[2,3] Indeed, lungfish and coelacanths are closer relatives of tetrapods (such as mammals, birds, amphibians, etc.) than of other fish such as ray-finned fish or sharks, so the last common ancestor of all fish is also an ancestor to tetrapods. As paraphyletic groups are no longer recognized in modern systematic biology, the use of the term "fish" as a biological group should be avoided.

Teleost fish ("bony fish") eggs are macrolecithal and teleolecithal (Greek: (telos) = end, (lekithos) = yolk), referring to a large yolk with the uneven distribution of yolk in the cytoplasm respectively. The yolk is concentrated at one pole of the egg, separate from the developing embryo. During fertilization the diploid genome is restored when a haploid egg and sperm unite. In fish eggs, cleavage occurs only in the blastodisc, a thin region of yolk-free cytoplasm at the animal cap of the egg. Most of the egg cell is full of yolk. The cell divisions do not completely divide the egg, so this type of cleavage is called meroblastic (Greek, meros, "part"). Thus, the yolky end of the egg (the vegetal pole) remains homogenous while the other end (the animal pole) undergoes cell division. Since only the cytoplasm of the blastodisc becomes the embryo, this type of meroblastic cleavage is called discoidal. Scanning electron micrographs show the incomplete nature of discoidal meroblastic cleavage in fish eggs (Appendix; Figure 1.6).

The fate of each of these first cells, called blastomerises, is determined by its location. This contrasts with the situation in some other animals, such as mammals, in which each blastomere can develop into any part of the organism. Gastrulation begins at about the tenth cell division, the onset of the midblastula transition can be detected: zygotic gene transcription begins, cell divisions slow, and cell movement becomes evident. At this time, three distinct cell populations can be distinguished. The first of these is the yolk syncytial layer (YSL). The YSL is formed at the ninth or tenth cell cycle, when the cells at the vegetal edge of the blastoderm fuse with the underlying yolk cell. This fusion produces a ring of nuclei within the part of the yolk cell cytoplasm that sits just beneath the blastoderm. Later, as the blastoderm expands vegetally to surround the yolk cell, some of the yolk syncytial nuclei will move
under the blastoderm to form the internal YSL, and some of the nuclei will move vegetally, staying ahead of the blastoderm margin, to form external YSL (Appendix; Figure 1.7). The YSL will be important for directing some of the cell movements of gastrulation. The second cell population distinguished at the midblastula transition is the enveloping layer (EVL; Appendix; Figure 1.7). It is made up of the most superficial cells of the blastoderm, which form an epithelial sheet a single cell layer thick. The EVL eventually becomes the periderm, an extraembryonic protective covering that is sloughed off during later development.

Neurulation, the formation of the central nervous system, is different in fishes than in most other chordates. The neural tube begins as a solid cord formed from the ectoderm. This cord then sinks into the embryo and becomes hollow, forming the neural tube. This process contrasts with the process in other chordates, which occurs by an infolding of the ectoderm to form a hollow tube.[2]

In this lab we will be looking at an ‘Annual fish’; the annual killifish (*Austrofundulus limnaeus*). This species can maintain permanent populations in temporary aquatic habitats since the population survives dry seasons in the form of diapausing eggs. Populations persist even though they are subject to erratic environmental cycles and recurrent ecological catastrophes. It finishes its reproductive cycles are finished within one year (=annual).

In the development of killifish, so-called ‘developmental arrest’ can occur at one or all of the following stages: Diapause I (Dispersed cell phase); Diapause II (Long somite embryo); Diapause III (Prehatching). Embryonic diapause is a state of suspended metabolic, developmental, and cellular activity.

**Birds**

The chicken egg consists of the *ovum*, commonly called the yolk, and the accessory materials, commonly called *albumen*, the shell membranes and shell. The yolk or ovum is formed in the ovary of the female bird and discharged into the abdominal cavity near the opening of the oviduct. The ovum is swept by ciliary action into the oviduct where fertilization may occur. The egg cell or ovum released from the hen’s ovary consists of the yellow yolk mass plus a small area of yolk-free cytoplasm, the germinal disc, which is located on the surface of the yolk. Because of the large amount of yolk, cleavage occurs only in the germinal disc and the embryo grows by absorbing raw materials for its metabolism from the yolk.

During its passage down the oviduct, several accessory features are added to the ovum (note: the term “egg” is best reserved for the shelled entity laid by the hen, while the egg cell, that structure released from the ovary, is called an "ovum"). In the first section of the oviduct, a viscous stringy albumen is secreted and adheres to the ovum. As the ovum moves along, rotation twists the stringy albumen into a pair of strands which surround and project beyond the ovum. In the next oviduct region, a more- watery albumen is added. Finally, two shell membranes and a calcareous shell are applied enclosing the entire structure.

The egg is a remarkable piece of architecture. The hard protective shell is porous, permitting gas exchange with the environment. Albumen serves as a water reservoir for the early embryo and a source of food for the later embryo; it also contains substances that retard the growth of bacteria. The yolk of the ovum contains proteins, carbohydrates, lipids and vitamins that sustain the metabolic needs of the rapidly growing young chick during its normal 21-day incubation. The yolk is surrounded by a
The delicate vitelline membrane. The germinal disc lies just under the membrane and can be easily seen as a small grey spot. In unfertilized eggs, it contains the haploid nucleus. In fertilized eggs, cleavage occurs in the germinal disc either while the egg descends the oviduct or while it is retained in the cloaca. By the time it is laid, it has reached the gastrula stage and appears as a flat patch of cells called the **blastoderm**.

When the egg is laid and cools, division of the cells ceases, but this does not result in the death of the embryo. It may resume its development after several days of rest if it is again heated by the hen or in an incubator.

Here is the detailed sequence of chick development. You may follow along the descriptions by looking at the chick development photos at the end of this document.

- **24 HOUR STAGE:** After cleavage of the blastoderm, it separates from the underlying cavity, the blastocoel. In gastrulation, the blastoderm becomes two-layered, with the endoderm lying ventrally and the ectoderm on the surface. The mesoderm arises by proliferation of cells laterally between the ecto- and endoderm on either side of the primitive streak. Note the primitive streak, neural groove and somites.

- **48 HOUR STAGE:** Some of the prominent features include primitive streak, somites and neural groove (note that the notochord lies immediately beneath the neural groove).

- **72 HOUR STAGE:** The embryo has a distinct C-shape at this stage. It is covered by a thin-walled sac, the **amnion**. Note the development of the somites. The embryo has well-developed circulation at this stage. Observe the heart and its chambers. The smaller, slightly more anterior portion is the atrium and the larger, more ventral portion is the ventricle. Observe the auditory vesicles and optic cups.

- **96 HOUR STAGE:** Extending out from the embryo is a thin delicate membrane with blood vessels at its surface. This membrane, called the **allantois**, expands as the embryo develops until it completely lines the shell. The transparent sac surrounding the embryo is the amnion. The material in the amnion is principally water from the egg white. The embryo is actually developing in the amniotic fluid. Look for limb buds. Examine the eyes and compare their size with the size of the embryo. The large eyes are a characteristic that separates bird embryos from all other vertebrate embryos at the same stage.

- **SEVEN 20 DAYS:** The embryo grows and develops rapidly. By the seventh day, digits appear on the wings and feet, the heart is completely enclosed in the thoracic cavity, and the embryo looks more like a bird. After the tenth day of incubation, feathers and feather tracts are visible, and the beak hardens. On the fourteenth day, the claws are forming and the embryo is moving into position for hatching. The supply of albumen is exhausted by the sixteenth day, so the yolk is the sole source of nutrients. After twenty days, the chick is in the hatching position, and normal pulmonary respiration has begun. That is, the chick no longer depends on the vascularized extra-embryonic membranes for gas transport. The normal position of the chick for hatching is with the head in the large end of the egg, under the right wing, with the legs drawn up toward the head. If the head is positioned in the small end of the egg, the chick’s chances of survival are reduced by at least one-half! Just as a wrong position makes birth more difficult in mammals, a wrong position of the chick makes hatching more difficult, or impossible.

- **21 DAYS:** The chick begins to peck through the shell. A sharp horny (and temporary) structure on the upper beak (egg tooth) helps with this. Once the chick pops its head...
through the shell it kicks free of the bottom portion of the shell. At this point the chick is typically exhausted and rests while its down feathers dry.

Exercises

In this lab there will be five stations set up for different activities. Some use preserved materials, some use models, and some use live animals. You can do the exercises in any order. The stations are:

1. Models of amphibian development.
2. Observation of preserved amphibian embryos.
3. Observation of live fish embryos.
4. Observation of preserved chick embryos.
5. Candling Eggs and Observation of live chick embryos.

Exercise 1: amphibian development models

There are a number of models showing amphibian development. Using one of the models that shows different tissue layers (they are painted different colors), make a sketch in your notebook. Then, using your textbook or other materials, identify each of the layers or structures in the developing amphibian egg. The models are all cross-sections and allow you to see the internal structures of the developing egg and embryo.

* In your lab write up, be sure to include the sketch of the model with all identifying layers and structures labeled for full credit. (1 pt)

Exercise 2: observation of preserved amphibian embryos

Your TA will have set up dissecting microscopes. There are small jars containing amphibian embryos arrested at different stages of development. In this part of the lab you can gain an appreciation of the large size of amphibian eggs, see differences early in the eggs before cleavage has begun, and then track changes in the eggs through cleavage divisions to early embryo formation.

It is important to be very careful when selecting a specimen for observation. Even though they are preserved, they are still delicate. Using a pipette you will carefully aspirate from its jar one embryo. Immediately make a note of which jar or stage embryo you have, so that after observation you can return it to the same jar. It will be more than a little confusing if, by the end of the week, the embryos of different stages are all mixed up!

1. Place a pre-cleavage egg in a depression slide and observe either with a dissecting microscope, or with a compound microscope using the lowest magnification objective (typically 10X). Can you see the gray crescent in the egg before cleavage has begun? When does the crescent disappear?
2. Move to a scope with an egg around the 32-cell stage. Is there a difference in size between this embryo and the pre-cleavage egg you just examined? Can you tell a difference in blastomere size between the animal and vegetal poles?
3. Now, move to a scope with a later stage embryo. Has neurulation begun? Again note the size and compare with the previous stages. At what stage do you find evidence of the developing nervous system?
For you lab write up, please answer ALL questions above that are highlighted in BOLD. (3 pt)

In your lab write up, be sure to include ONE sketch of a frog embryo from either of the two later stages present in lab. (1 pt)

Exercise 3: observation of live fish embryos
Your TA will have set up dissecting microscopes. Our plan is to have four different stations each with embryos at a different developmental stage. Here you can observe real live and/or preserved embryos. Please be careful so as to not harm them. Even though these animals can survive under harsh conditions embryos can be really sensitive to mechanical damage. With luck, many of the embryos will survive. The species you will observe is the annual killifish, *Austrofundulus limnaeus* (Appendix; Figure 1.4). This species is popular for lab exercises (check out Podrabsky website: http://killifish.pdx.edu/home.html). This species has been studied for many years, and is currently developing into a model system in physiological research as embryos can tolerate extremely low levels of oxygen.

Use a pipette to aspirate one embryo and put it in a watch glass and observe under a dissecting microscope. Try to observe several specimens at different stages of development. For each two of the embryos you observe, make a sketch. Note whether there is movement. **If you see movement, what does this tell you about organ and tissue development?**

- In your lab write up, be sure to include the TWO embryo sketches at different developmental stages. Note the stage of development and at least one defining characteristic of that stage. (2 pt)

![Figure 1.1 Annual killifish (*Austrofundulus limnaeus*). You can see a distinct sexual dimorphism (male larger and more colorful than female).](image)

Exercise 4: Chick embryos

1. **Prepared Slides.** There are a number of prepared microscope slides showing the early stages of chick development. Use a compound microscope to view these, and make a sketch of two stages. You should pick stages that are not similar in time, because the point is to compare two different stages and get an idea of how far the embryo has progressed. You should also compare your two chick stages with two amphibian stages. For example, does the gastrula stage look the same between an amphibian and a bird?**
How about the beginning of nervous system development? Are the embryos the same size at the same point in development? Does one type of embryo develop more quickly than the other?

4) For your lab write up, include answers to the FOUR highlighted questions above. (2 pt)

In your lab write up, include ONE sketch of a chick embryo in or near the gastrulation stage from one of the prepared slides you looked at in lab. (1 pt)

Figure 1.2: How chicks fit in eggs.

2. Observation of preserved chick embryos. We have a number of preserved chick embryos at different stages of development. Most of these are mid- to late-stage embryos. You should take one of the containers holding a chick embryo and place it under a dissecting microscope. Try to avoid sloshing the embryo about. They are fragile. Note the cephalization and that the head end of the embryo is much larger than the rest of the body. What does this suggest about the development of different tissue and organ systems? At what point can you identify all the major body features of a bird?

Here is another way to think about this question: Is there a point where you could not identify the taxonomic group of your embryo? If someone said, "Hey look at this developing kangaroo" would you immediately respond with "That's not a kangaroo, it's a bird!"?

- In your lab write up, include the answers to the THREE bolded questions written above. (2 pt)

Figure 1.2: How chicks fit in eggs.

3. Candling. Before technological development, farmers who raised chickens would determine if eggs were fertile or not by holding them up to a candle in
dim ambient light. The candle light shining through the egg would reveal a shadow if the egg contained a developing chick. This method only works between certain limits of development. Too early in development the embryo is microscopic and cannot be detected by candling. Much later in development the embryo is so large as to be completely opaque, so that no light will transmit through the egg. If we are able to secure eggs at the right stage in development, your TA will demonstrate candling, and you may be able to try it for yourself. The TA will place 2 different eggs on the candling apparatus, one fertilized the other not fertilized. In a dark room, hold the egg to the light of the candler to observe the contents of the egg. Cooling that occurs for short periods (less than 10 minutes) during careful examination of eggs does not harm the development of the embryo. However, limit the exposure of the egg to the hot light source. Even a brief period at 104 degrees F kills all embryos.
Figure 1.3: Chick embryos at six stages of development

4. Embryo viewing. If there are eggs at a suitable early stage of development your TA may demonstrate how you can observe the developing chick by making a small window in the egg. A portion of the egg shell is chipped away to make a viewing window. If done just right, a cover can be placed back over the window after viewing and the embryo will continue to develop normally. It is also possible to remove the embryo completely at this stage so that you can observe whether a beating heart has developed. Note that at this early stage in development the nervous system is completely primitive and there is no sensation, cognition or possibility that the embryo experiences any discomfort in this procedure. At this point the chick is a large mass of developing cells. Vascularization is one of the first steps to allow the embryo to grow large enough for other organ systems to expand.

Study Questions

1. Pick one similarity and one difference that you observed in developmental stages of the chicken, fish and amphibian in lab today. Discuss how these two characteristics may benefit the lifestyle of the organism. What is the difference between an egg, zygote, embryo and larva?

Commented [RWS]: The way this question is worded lends itself to students using mere definitions. Maybe we can reword it. Can it be reworked to something like: “Discuss the differences and similarities between eggs, zygotes, embryos and larvae in fish, amphibians, and chickens?” Or just something they actually have to think about.
2. **What is neurulation?** And why is it one of the first gross changes visible during development? (1 pt)

3. Why are there numbered developmental stages from egg to hatchling? (0.5 pt)

4. The lab manual discusses why chicken, fish, and amphibians are ideal organisms to study developmental biology. Using the reverse logic, discuss two developmental characteristics would make an organism not an ideal candidate for study. (2 pt)

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Commented [RW6]: Again, definition questions can lead to problems. Instead, we could ask how they can identify the stage of neurulation in the organisms discussed in lab.

Commented [RW7]: Or three or four?

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The lab manual mentioned the ability of Killifish embryos to undergo developmental arrest. Why might it be beneficial for them to do this?

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The lab manual mentioned the ability of Killifish embryos to undergo developmental arrest. Why might it be beneficial for them to do this?
Exercise 1: Amphibian development models
1. Make one sketch of the amphibian development model, and identify each of the layers or structures. (1 pt)

Exercise 2: Preserved amphibian embryos
1. Make one sketch of amphibian embryo and identify developmental stage. (1 pt)
2. Answer questions.
   a. Can you see the gray crescent in the egg before cleavage has begun? When does the crescent disappear? (1 pt)
   b. Is there a difference in size between this embryo and the pre-cleavage egg you just examined? Can you tell a difference in blastomere size between animal and vegetal poles? (1 pt)
   c. Has neurulation begun? Again note the size and compare with the previous stages. At what stage do you find evidence of the developing nervous system? (1 pt)

Exercise 3: Fish embryos
1. Make two sketches at two different developmental stages. Note the stage of development and at least one defining characteristic of that stage. (2 pts)

Exercise 4: Chick embryos
1. Make one sketch of chick embryo prepared slides. (1 pt)
2. Answer questions.
   a. Does the gastrula stage look the same between an amphibian and a bird? How about the beginning of nervous system development? Are the embryos the same size at the same point in development? Does one type of embryo develop more quickly than the other? (2 pt)
   b. What does this suggest about the development of different tissue and organ systems? At what point can you identify all the major body features of the bird? Is there a point where you could not identify the taxonomic group of your embryo? (2 pt)

Study questions
1. Pick one similarity and one difference that you observed in developmental stages of the chicken, fish and amphibian in lab today. Discuss how these two characteristics may benefit the lifestyle of the organism. (2 pt)
2. What is neurulation? Why is it one of the first gross changes visible during development? (1 pt)
3. The lab manual discusses why chicken, fish, and amphibians are ideal organisms to study developmental biology. Using the reverse logic, discuss two developmental
characteristics which would make an organism NOT an ideal candidate for study. (2 pt)

4. What is one advantage and disadvantage of having a soft-shelled egg? What about a hard shelled egg? Which organisms you observed today have which? (3 pt)

Bibliography

[3] Tree of life web project – Chordates

Some web sites of interest for this lab:

_ http://www.umanitoba.ca/Biology/lab14/biolab14_4.html
_ http://www.msstate.edu/dept/poultry/avianemb.htm
_ http://www2.vaes.vt.edu/resources/4h/virtualfarm/poultry/poultry_development.html
_ http://www.fao.org/docrep/005/AC802E/ac802e08.htm
_ http://www.avianweb.com/candlingeggs.html
_ http://www.luc.edu/faculty/wwasser/dev/axolotl.htm
_ http://www.axolotl.org
_ http://www.ambystoma.de/html/axolotl/axolotl_development.html
_ http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/F/FrogZygote.gif
Appendix

The following pages show images of “developmental series”, that is, views of the development of an organism, in order, at defined stages. Early embryologists carefully described the development and decided, on morphological grounds, when there was enough progression to define a new stage. Although this is a bit arbitrary, so long as everyone agrees on the staging, then it becomes quite useful. For example, if you were publishing a scientific article today and want to say at what point a particular gene switches on, you only need to write something like “…we extracted material from amphibian stage 41 embryos” and other scientists would know what you are talking about.
Figure 1.4: The Gosner (1960) Staging System for Anurans (stages 1-25)
Figure 1.5: The Gosner (1960) Staging System for Anurans (stages 26-46)
Figure 1.6: Discoidal cleavage in a fish egg. (A) 1-cell embryo. The mound atop the cytoplasm is the blastodisc region. (B) 2-cell embryo. (C) 4-cell embryo. (D) 8-cell embryo, wherein two rows of four cells are formed. (E) 32-cell embryo. (F) 64-cell embryo.
Figure 1.7 A schematic representation of the two developmental trajectories observed in embryos of *A. limnaeus*. Early development through about the 10-somite stage is indistinguishable for the two trajectories using the morphological characters. By the 18-somite stage, the embryos are distinguishable by both morphological and physiological characters. Both of these trajectories occur naturally at temperatures of 25°C and above, but increasing the incubation temperature to 30°C can force all the embryos onto the escape trajectory.
Figure 1.8. Fish blastula. (A) Prior to gastrulation, the deep cells are surrounded by the EVL. The animal surface of the yolk cell is flat and contains the nuclei of the YSL. Microtubules extend through the yolky cytoplasm and through the external region of the YSL. (B) Late-blastula stage embryo of the minnow Fundulus, showing the external YSL. The nuclei of these cells were derived from cells at the margin of the blastoderm, which released their nuclei into the yolky cytoplasm. (C) Fate map of the deep cells after cell mixing has stopped. The lateral view is shown, and not all organ fates are labeled (for the sake of clarity). (A and C after Langeland and Kimmel 1997; B from Trinkaus 1993)
Figure 1.89: A complete series of chick development
Lab 2: Simulating Evolution by Natural Selection

SYNOPSIS

In this lab you will simulate the process of natural selection by taking the role of predators hunting for prey. Predators (you) will have varied feeding morphologies, and prey (beans) will have varied colors and shapes. As predators hunt for prey and as both predators and prey “reproduce,” you will observe and interpret how the distribution of traits in each population changes.

LEARNING OBJECTIVES

At the conclusion of this lab you should have a clearer conception of how the process of natural selection works, and you should better understand how this process can drive evolutionary change in populations. You should also be able to graph the change in frequency of a trait over time.

INTRODUCTION

Evolution is the central idea that unifies all of biology. As the geneticist Theodosius Dobzhansky once remarked, “Nothing in biology makes sense except in the light of evolution.” As you have learned in lecture, natural selection is one of the major processes driving evolutionary change. Before Darwin, many biologists had considered the idea that populations of organisms evolve, or change in their characteristics through time, but these early scientists lacked a feasible mechanism or causal explanation. Charles Darwin and Alfred Russel Wallace then put forth a theory, evolution by natural selection, which was both logical and testable. Once combined with Gregor Mendel’s insights about genetics and inheritance, this enabled us to understand how and why organisms look and behave the way they do, how organisms adapt to their environments, and how traits in populations change through time.

In order for a trait to evolve by natural selection, three conditions must hold:

1) There must be variation in the trait among individuals in a population.

2) The trait must be heritable (able to be inherited). This means it must be encoded in the genes, and can be passed from parents to offspring.

3) There must be differential reproductive success among individuals, based upon the variation noted in condition (1), such that those with one form of the trait survive to produce more or more fit offspring than those with another form of the trait.
These three conditions are necessary and sufficient to result in evolution by natural selection; if these conditions hold, then natural selection will take place. Individuals that leave the most descendants also pass the most genes to future generations, so the genes of the more successful individuals will be better represented in the population in the future.

Thus, while natural selection acts on an individual’s phenotype (its morphological, behavioral, or physiological traits), this causes change through the generations by affecting the genotype (the alleles, or gene copies, that underlie phenotypic traits). As particular genotypes become more prevalent in a population through time, their corresponding phenotypes become more prevalent as well. We most often notice the effects of evolution by natural selection by observing changes in phenotypes. However, most phenotypic traits are determined by some combination of genetic, environmental, and developmental influences, so strictly speaking, evolution by natural selection is best documented by measuring change in gene frequencies through time. The extent to which a phenotype reflects a genotype determines how effectively natural selection can change allele frequencies in a population.

A characteristic that promotes reproductive success (directly or through longer survival) is termed an adaptation. Evidence for natural selection is all around us in the adaptations of animals, plants, and microbes to their environments. Some of the evidence most familiar to us comes from artificial selection, natural selection guided by human influence. By selectively breeding organisms, we have selected for specific traits in dogs, cats, cattle, pigeons, and the countless crop plants we depend on for our diet. By directing the dynamics of selection, we have invented agriculture and have created hundreds of varieties of organisms that are useful and appealing for our society.

While the end results of selection are readily observable all around us, actually witnessing natural selection as it takes place in real populations is tricky. Evolutionary changes generally accumulate over many generations, which can be a long period of time (on a human time scale). Moreover, natural selection usually acts in concert with other evolutionary forces, such that it can be difficult to isolate and identify.

Running a simulation lets us observe how natural selection works in a real population. All we need is a system that shows the three conditions for evolution by natural selection outlined above. With a little imagination we can get almost anything to replicate these conditions.

Imagine that beans are organisms that live out their lives in two habitats: (1) in the mountains, on alpine slopes that are often covered by snow, or (2) within the adjacent sun-dappled forest. The only concern our hypothetical bean creatures have is the disquieting presence of ravenous predators (that would be you). Beans vary in size, shape, and color, and any given bean’s phenotype could potentially influence the likelihood that it gets snatched away by a hungry predator. The predators in our simulation vary as well, having different implements with which to capture the beans. Different feeding morphs — different predator phenotypes — might potentially vary in their ability to capture beans. Both predator and prey “reproduce”. Beans and
predators born into their populations “inherit” the traits of their parents. With this simple set-up, we should be able to replicate evolution by natural selection.

LAB PROCEDURE

You and your classmates are all individuals in a population of Beaneaters (Biestudentis beanivorus), a species of predator that survives by hunting for a prey species known as Beans (Beanus polymorphus). In this predator-prey simulation you will attempt to capture and “eat” as many Beans as you can within the time allotted.

Getting Set Up

Your Beaneater population will be split into five subpopulations (one per lab table), with each table containing an area of habitat that is home to your tasty Bean prey. Three tables are of one habitat (HABITAT A = sun-dappled forest), while two tables are of the other habitat (HABITAT B = snowy alpine slope). Four Beaneaters will compete for food at each table, and any additional students will serve as “Roamers” who will help the T.A. patrol the groups watching for infractions of the rules.

The beans come in four different phenotypes that vary in color, size, and shape. Examine these bean morphs in conjunction with the habitat of your table. Formulate a hypothesis as to which bean type will be easiest to prey upon. Now propose a hypothesis as to which predator will be most successful. Write your hypotheses in the spaces allotted for Questions 1 and 2 in this lab handout, and explain why you made the choices you did.

Your T.A. will give each table a supply of beans that has equal numbers (100) of all four phenotypes (for a total of 400 beans). Mix and spread these evenly across your table’s patch of habitat.

Now it’s time to arm yourselves as predators! Beaneater phenotypes are determined by their feeding appendages. In our simulation, we will have four different feeding types: forks, knives, spoons, and forceps. Your T.A. will distribute the feeding appendages so that there are equal numbers of the four phenotypes in the initial predator population (this will mean one of each type per table).

Now, pick up a cup. This will serve as your “stomach.” You will forage by picking up beans ONE AT A TIME using ONLY your feeding appendage and placing each bean in your “stomach”. You must follow the rules below for feeding.

Using the data sheets provided, record the initial number and frequency of each prey and predator phenotype at your table.
Running the Simulation

The rules are as follows:

1. Stomachs must be held in one hand and feeding appendages in the other hand at all times.
2. Stomachs must remain upright. You may not tilt them or use them to scoop up beans.
3. Beans cannot be swept off the table into stomachs. Stomachs must be kept above the level of the table.
4. Beans must be deposited in stomachs ONE AT A TIME. If you accidentally place multiple beans into your cup at once, you must stop and remove all beans that you captured on that particular scoop.
5. Once a bean is legally in a stomach, it cannot be removed by a competing Beaneater.
6. All beans are of equal value (1 unit of food), regardless of their phenotype.

Don’t be shy about ruthlessly competing with your fellow predators . . . like spooning a bean away from a clumsy fork. Remember — you are in a race to survive and reproduce, and only the fast, agile, and clever will succeed!

At your T.A.’s signal, start feeding!

When your T.A. calls **TIME**, stop feeding. (Each feeding bout last 60 secs.)

Count the number of each type of bean in your stomach. Enter these data and those of your table’s fellow Beaneaters into the data sheet provided for “Prey Consumed”.

Now figure out how many beans of each phenotype remain “alive” (uncaptured in the habitat) at your table, and enter these numbers into the data sheet.

It is now time for the beans to reproduce. For each bean of a given phenotype that remains uneaten on the table, add one additional bean of that phenotype. Thus, the number of beans of each type will double. For example, if 7 lima beans remain after your first round of predation, the number of lima beans would double to 14 to begin the second generation. Record the number of each phenotype of bean starting this second generation and enter it in your data table.

Among Beaneaters, the individuals who caught more prey than average in the first generation get to survive and reproduce, passing their genes to the second generation. In contrast, the individuals who caught less than average die of starvation, and do not reproduce. Each surviving predator “reproduces” one offspring by bestowing a replicate of his or her feeding appendage on an empty-handed classmate. For example, if Knife and Forceps do better than Fork and Spoon at your table, then in the next generation the Fork student and the Spoon student are “reincarnated” as a Knife and Forceps.
(Alternatively, “Roamer” students can be given a chance to compete as Beaneaters, and losing Beaneaters can take a turn as Roamers.) Your T.A. will distribute the new feeding appendages accordingly. Calculate and record the new number of each feeding type in this next generation of predators.

Run the second round of feeding as you ran the first round, and repeat all the steps of bean reproduction, Beaneater reproduction, and data entry as described above.

Finally, run a third round of hunting using the new distributions of bean and Beaneater phenotypes as calculated for the third generation, and enter all your data. At the end of the simulation be sure to count out beans and replace into bags as they were at the beginning of lab.

Interpreting the Data

Once all three generations of the simulation are over, your T.A. will reconvene the class so that tables can share their results. Your T.A. will lead you through the compilation of data from each of the two habitats. Follow along and record the compiled data, and determine frequencies of each of the different phenotypes.

Across the three generations you should notice changes in the frequencies of some bean phenotypes and some predator phenotypes. To clearly visualize these changes, you will graph some of these data in line plots on the axes provided:

1. Plot the increase in frequency of the most-successful predator phenotype in the first habitat type over the three generations.
2. Plot the increase in frequency of the most-successful predator phenotype in the second habitat type over the three generations.
3. Plot the changes in frequency of the four phenotypes of beans in the first habitat type over the three generations.
4. Plot the changes in frequency of the four phenotypes of beans in the second habitat type over the three generations.
5. For your habitat, use histograms (bar charts) to graph the changes in distribution of the four phenotypes of beans over the three generations.

Finally, your T.A. will lead a discussion of the questions below. As you discuss these issues, think about your experiences in this lab simulation, and consider how the dynamics of the game may be similar to (or different from) the forces of natural selection acting on organisms in the wild. Write in answers to these questions during or after this discussion. You will hand in your typed answers to these questions (15 pts) and your graphed data (5 pts) at the beginning of your next lab. Your TA will provide you with additional instructions regarding your lab write-up.
QUESTIONS

Answer the first two questions before you begin the simulations:

1. (1 pt) Formulate a hypothesis as to which bean type will be easiest to prey upon, and explain why you think so.

2. (1 pt) Propose a hypothesis as to which predator morphology will be most successful, and explain your choice.

Answer questions 3-8 after graphing the data compiled from the simulations:

3. (2 pts) Was your hypothesis in Question 1 supported or falsified by the data? Which bean type was least successful (preyed upon most easily) in your habitat, and why? What happened to the frequency of this phenotype in the population?

4. (2 pts) Was your hypothesis in Question 2 supported or falsified by the data? Which predator type was most successful in your habitat, and why? What happened to the frequency of this phenotype in the population?

5. (2 pts) Which prey type was most successful in your habitat, and why? Was this true in both habitats? Explain these results.

6. (2 pts) Are there any characteristics of successful Beaneaters that you would call an adaptation? Can you name any adaptations of successful Beans?

7. (2 pts) Does natural selection tend to increase or decrease variation within a population?

8. (3 pts) How did each of the three conditions for natural selection given in the introduction to the lab play out in the simulation?
Your Habitat: Alpine or Forest (circle one)

GENERATION 1:

<table>
<thead>
<tr>
<th>PREDATOR PHENOTYPE</th>
<th>WHITE BEANS</th>
<th>RED BEANS</th>
<th>BLACK BEANS</th>
<th>BROWN LENTIL BEANS</th>
<th>TOTAL CONSUMED, by Predator</th>
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<tbody>
<tr>
<td>Fork</td>
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<td>TOTAL CONSUMED, by Bean Type</td>
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Table 2: Raw Numbers and Frequencies of each prey (bean) phenotype across generations

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<th>BEAN PHENOTYPE</th>
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(HINT: The “frequency” of a given phenotype simply means the percentage of the total that that phenotype represents. Thus, if you start with 1 knife, 1 fork, 1 spoon, and 1 forceps, the frequency of each type is: 1/4 = 0.25 (or 25%). If in a certain generation there are 45 white beans, 20 red beans, 60 black beans, and 6 lentil beans, then the total number of beans is: 45 + 20 + 60 + 6 = 131, and the frequencies of each bean type would be: white = 45/131 = 0.34; red = 20/131 = 0.15; black = 60/131 = 0.46; and lentil = 6/131 = 0.05.)
WHOLE CLASS, FOREST HABITAT: COMPILED DATA
(Combine numbers from all lab table groups in this habitat, and then calculate frequencies)

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Name________________________________

FREQUENCY GRAPHS

1. Most successful predator phenotype in Alpine Habitat

2. Most successful predator phenotype in Forest Habitat

3. Changes in the 4 bean phenotypes in Alpine Habitat

4. Changes in the 4 bean phenotypes in Forest Habitat

5. Histogram of changes in 4 bean phenotypes in your habitat
Lab 3: Cnidarians, Annelids, Platyhelminths, & Nematodes

SYNOPSIS
In today’s lab you will examine representatives of four very different phyla — Cnidaria (jellies, corals, and their kin), Annelida (earthworms, marine worms, and their kin), Platyhelminthes (parasitic tapeworms, free-living planaria, and their kin), and Nematoda (free-living and parasitic roundworms). In the exercises, you will be observing different modes of feeding, movement, and reproduction (asexual and hermaphroditic). To accomplish this, you will observe living specimens as they feed and move, you will examine the external reproductive anatomy of annelids (earthworms) and cnidarians (Hydra), and you will examine the internal reproductive anatomy of nematodes (Ascaris). Work with a partner for all activities. Microscopes will be necessary for most of the exercises.

LEARNING OBJECTIVES
You will become familiar with the basic body plans of the phyla Cnidaria, Annelida, Platyhelminthes, and Nematoda. Given these body plans, you will then learn, via observation, the diverse feeding, reproductive, and locomotion strategies employed by different taxa within these four phyla of animals.

INTRODUCTION
Phyla are characterized by their unique body plans. The four phyla in today’s lab have quite distinct body plans. Cnidarians have polyp (sea anemone-shaped) or medusa (jelly-shaped) bodies, and are radially symmetrical. Annelids, platyhelminthes (flatworms), and nematodes have elongated worm-like bodies, but — as you will learn more about in lecture — a worm-shaped body cannot be used to define a group of organisms. Instead, the internal anatomical features of the annelids, flatworms, and nematodes, in addition to their modes of locomotion and feeding, are better characters for defining these phyla. Highlighted below are some of the characters that are used to distinguish among these phyla.

PHYLUM CNIDARIA
The word “cnidaria” is Greek for stinging thread, an apt description of one of the key features of cnidarians. Cnidarians possess structures called cnidae, which can explosively discharge a long thread-like structure that is tipped with stinging barbs or
venom (see Figure 1). The discharge of the cnidae is triggered either by touch or by chemical stimulation. Cnidae serve to capture prey or defend against predators. The possession of cnidae is a synapomorphy that unites the Cnidaria. There are three types of cnidae: nematocysts, spirocysts, and ptychocysts. All toxic cnidae are nematocysts, whereas spirocysts are sticky, and the everted tubules of ptychocysts are used for constructing felt-like tubes. Most cnidae are nematocysts, and these are present in all cnidarian subgroups. Spirocysts and ptychocysts are found only in Anthozoa (sea anemones and corals). A generalized term for a cell that produces cnidae is a cnidocyte.

![Figure 1. Cnidocyte containing cnidae, before and after discharge.](image)

There are two body plans found within cnidarians: a polyp form that is typically not highly mobile, and a medusa form that is free-swimming. Many cnidarians alternate between polyp stage and medusa stage during different stages of their life cycle (see Figure 2). Each of the 4 major classes of cnidarians is largely defined by which of these stages in which it spends most of its lifecycle.
Cnidarians also possess very different methods of reproducing, often alternating between asexual and sexual reproduction (see Figure 2). Asexual reproduction may occur by budding, when a small piece of the animal buds off, and then detaches from the parent and develops into a separate organism. Sexual reproduction often occurs in the medusa stage. In sexual reproduction both male and female individuals are present in a species, and they each produce gametes that join to develop a zygote, which then grows into a polyp. However, there are many different variations on reproduction and sexuality in the cnidarians. For example, the Hydra polyps that you will look at today possess both male and female reproductive organs. The cnidian body consists of a central blind sac, the coelenteron (gastrovascular cavity), enclosed by a body wall comprising two epithelia, the outer epidermis and the inner gastrodermis (see Figure 3). The mouth opens at one end of the coelenteron and therefore this is termed the oral end. The mouth is usually surrounded by one or more circles of tentacles. The opposite end is called the aboral end. The imaginary line connecting the oral and aboral ends is the axis of symmetry around which the radial symmetry of the body is organized. All cnidarians are carnivores that feed on live prey, which they usually capture using tentacles armed with cnidae. Digestion occurs in the coelenteron, which is typically equipped with ciliated canals for distribution of partly digested food. Gas exchange is across the general body surface.

**Class Hydrozoa**

Hydrozoa is a diverse taxon of about 3000 species of mostly marine cnidarians. Cnidarians are chiefly marine, but *Hydra* is an exception, and can be found in freshwater ponds and lakes. The life cycle usually includes both polyp and medusa generations, but may be entirely polyp or entirely medusa. Polyps typically are...
colonial and medusae usually solitary. Some form colonies of combinations of polyps and medusae. Hydrozoan polyps are usually small, about 1 mm in length, and colonial. Nematocysts are the only cnidae.

Figure 3. *Hydra* drawn with a combination of testes, ovary, and asexual bud. Cnidocyte batteries are shown on only one tentacle.

**Feeding Behavior**
As in most cnidarians, food is captured by the tentacles, stung with the cnidocytes, and then transferred to the mouth and coelenteron. Once inside the coelenteron, there is initial extracellular digestion, followed by intracellular digestion as molecules and food particles are distributed by gastrodermal ciliary currents in the coelenteron and then endocytosed by gastrodermal cells. Notice the row of cnidocytes (also called batteries of cnidocytes) on the tentacles. The mouth in *Hydra* is temporary, being present only when there is food to ingest. At other times the cells surrounding the mouth position adhere to each other and the mouth disappears.

**Activity:** Place 2 or 3 *Daphnia* (tiny crustaceans) in the dish and watch the *Hydra* with the dissecting microscope. Note the effect of contact with tentacles on the prey.
1. (1/2 pt) Based on your observations, do you think the nematocysts are toxic or sticky?

2. (1/2 pt) Does Hydra actively stalk its prey or does it seem to wait for food to blunder into its tentacles?

3. (1 pt) Describe how the Daphnia are transferred to the mouth and coelenteron.

**Prepared Slides**

Use the compound microscope to study stained slides of cross and longitudinal sections of Hydra. Use the scanning lens (40X) to search the slide and locate the longitudinal section. Once found, use a higher magnification (100X) to begin your study.

Allowing for variations due to the location of the plane of section, you should see a cylinder of stained cells. The cylinder is the column (Fig 3). The open space in the interior of the column is the coelenteron. It may contain food items and parts of it may be obscured by the gastrodermis. Below the mouth opening is the stomach region. This region accounts for most of the column. Below it is the shorter, often constricted, stalk. Finally, at the extreme aboral pole, is the pedal disk by which the animal attaches to the substrate.

**Microscopic Anatomy of the Reproductive System**

**Ovary Wholemount.** Use 40X and 100X to study the wholemount of an adult Hydra with an ovary (Fig 3).

4. (1/2 pt) How many ovaries are present?

**Testis Wholemount.** Use 40X and 100X to study the wholemount of an adult Hydra with a testis (Fig 3).

5. (1/2 pt) How many testes are present?

6. (1 pt) Why might an individual have many testes but only one ovary?
Class Scyphozoa (the jellies or jellyfish)
The Scyphozoa are characterized by having primarily medusa stages. You will examine living specimens from the Class Scyphozoa, order Rhizostomeae (the "root-mouth" jellyfishes). The specimens we have are in the genus Cassiopeia (also known as the "upside down jellyfish"). Cassiopeia live in shallow tropical seas, often among mangroves. They are somewhat unusual among the scyphozoan cnidarians in that they have zooxanthellae (symbiotic algae) that live within them and provide them with some nutrients. They lie on the bottom of the ocean, with their oral side facing up. Cassiopeia don't have a central mouth—instead, the edges of their eight oral-arms are fused and folded into elaborate frills containing hundreds of tiny mouth openings. The arrangement of these mouths on the highly branched arms is the basis for the name rhizostome, or "root mouth". These small secondary mouths are connected by channels to the stomach. By pulsing its bell, it forces zooplankton into the nematocysts on its mouth openings. This zooplankton diet is supplemented by food produced by symbiotic algae.

Activity: Observe the living (or view the video of) Cassiopeia in the aquarium.

7. (1 pt) Why do you think Cassiopeia typically lay on the bottom of the ocean with their oral side facing upwards, unlike most other jellies which swim with their oral side facing downwards?

Class Anthozoa (the anemones and corals)
Anthozoans lack a medusa stage, and exist in polyp form only, attached to a substrate. Like other cnidarians they are primarily carnivorous, but differ in that their mouth opens into a tubular pharynx, rather than directly into the gastrovascular cavity. This region has many infoldings, which greatly increases its surface area, allowing greater absorption of nutrients.

Activity: Observe the preserved museum specimens of anemones and corals.

8. (2 pts) Describe or sketch the differences between the anemone and coral. Where on each of these two animals do you think their cnidae are located? Explain.
PHYLUM ANNELIDA

The word annelid comes from the Latin word “annulus” which means ring. The segmented, worm-shaped bodies of these animals give them the appearance of being composed of many rings, and indeed much of an annelid’s internal anatomy is segmentally arranged. However, neither worm shape nor segmentation has proven to be a synapomorphy for the phylum. One trait that is a synapomorphy for annelids is the possession of one or more pairs of chitinous setae. Currently, there are two major classes of annelids that are described, the Clitellata, including earthworms and leeches, and the Polychaeta, a very diverse group of marine worms. The state of annelid systematics is currently in an upheaval, however, as we discover how diverse and divergent the polychaetes are. For today’s lab, you will make detailed observations of the class Clitellata, and then observe some museum specimens of polychaetes.

Class Clitellata: Subclass Oligochaeta, earthworms
Members of this class are hermaphroditic, with both male and female reproductive apparatus present within one individual. They have a specialized region of their epidermis called the clitellum, which secretes a cocoon in which their embryos develop (see Figure 4).

Locomotion and Feeding Behavior
Take a Petri dish and scoop out some dirt and an earthworm from the earthworm bin. Observe it under the dissecting microscope (under the lowest overhead light amount possible).

9. (1 pt) Note the setae on each segment, and describe how the worm moves.

Reproduction
For this exercise, you will examine, draw and label the external anatomy of an earthworm, focusing on its reproductive organs. Wear gloves, and place an earthworm in the ethanol solution provided in a Petri dish. This will anesthetize the animal so that you can make observations of it more easily. You will use Figure 4 to help guide your observations, and then make a drawing of your own to show what you observed.

Use a hand lens and/or the low power of a dissecting microscope as you observe all the external parts of the worm. First, locate the conspicuous clitellum, a swelling on the dorsal surface. The clitellum produces a mucus sheath used to surround the worms during mating and is responsible for making the cocoon within which fertilized
eggs are deposited. Now place the worm so that the ventral side faces up. The ventral surface is usually a lighter color than the dorsal surface. The mouth is located on the ventral side of the first segment while the anus is found at the end of the last segment. Find the anterior end by locating the prostomium (lip), which is a fleshy lobe that extends over the mouth. The other end of the worm’s body is the posterior end, where the anus is located.

Oligochaetes are simultaneous hermaphrodites and each individual contains complete and simultaneously functional male and female systems. The female system produces eggs and receives and stores sperm from the partner. The male system produces sperm and delivers it to a partner during copulation. The reproductive system is restricted to a few preclitellar segments (9-15). Most segments do not contain reproductive structures. Locate the clitellum, which extends from segment 33 to segment 37. Look for the worm’s setae, which are the minute bristle-like spines located on every segment except the first and last one. (Setae are also called “chaetae”). Run your fingers over the ventral surface of the earthworm’s body.

10. (1/2 pt) Which direction do the ends of the setae face on the animal?

Refer to Figure 4 to locate and identify the external parts of its reproductive system. Find the pair of sperm grooves that extend from the clitellum to about segment 15, where one pair of male gonopores is located. During copulation, sperm from the testes exit the body via these pores. Look also for one pair of female genital pores (gonopores) on segment 14. These are the openings of the oviducts. Sometimes they are easy to see (and sometimes not). Eggs from the ovary are released from these pores.

11. (2 pts) Make a sketch of the earthworm’s external reproductive parts, and label the features that are in bold font.
Figure 4. Ventral surface of an oligochaete, illustrating its external reproductive anatomy.

**Class Polychaeta: the marine worms**
Look at the museum specimens of a few different species of polychaetes. Polychaetes possess parapodia, or fleshy protrusions, along both sides of their body. The parapodia possess chaetae on them, and in some species, these can be very numerous, giving the animals a bristly appearance. You will look closely at one of these under a microscope.

**Microscope Slide of Polychaete Parapodia.** Look at the slide of *Nereis* parapodia under the compound microscope.

**12. (1/2 pt)** Describe, or quickly sketch, the parapodia and chaetae.
PHYLUM PLATYHELMINTHES

Platyhelminths, or flatworms, are bilaterally symmetrical metazoans with three tissue layers (triploblastic). They lack the segmented bodies that members of Annelida possess. Unlike most triploblastic animals, they are compact and have no coelom (body cavity) surrounding their organs. As their common name suggests, most flatworms are flattened dorsoventrally. They lack specialized circulatory or respiratory systems. The gut, if present, has a single opening to the exterior. Flatworms display cephalization, with an anterior brain and a concentration of sense organs. Flatworms have elaborate hermaphroditic reproductive systems, with internal fertilization. The majority of platyhelminths are parasitic, although the Class Turbellaria contains free-living taxa.

Class Turbellaria

Turbellarians are carnivores ranging in size from less than 1 mm to as many as 60 cm long; however, the majority are small. Their epidermis is ciliated on the ventral side. Their mouth is located somewhere on the ventral midline and opens into a blind gut, or gastrovascular cavity, which lacks an anus. Most are aquatic, inhabiting the oceans and fresh water, but a few are found in moist terrestrial environments. You will observe movement in aquatic planarians today.

Living Planaria

Place a living planaria in a drop of water in a Petri dish full of water. Living planaria can be picked up with a plastic pipet but they must be ejected quickly or they will attach to the pipet using their adhesive glands. They adhere tenaciously and are difficult to remove when this happens.

Locomotion: Observe the planaria with the dissecting microscope. Watch it as it moves across the dish. The major locomotory force is produced by the cilia of the ventral epidermis, but muscular activity also plays a role in locomotion. Peristaltic type waves are generated by sequential contraction of circular and longitudinal muscle. See if you can see this under the microscope. Manipulate the planarian with forceps or probe to encourage it to change directions. Lift the glass dish up to watch the movement from below.

13. (1/2 pt) Describe how the planarian moves.
Push the planarian gently with the probe and look for evidence of adhesive ability.

14. (1/2 pt) Where do the adhesive cells seem to be located?

**Feeding:** Now place a small piece of liver-hardboiled egg yolk in the water, about an inch away from the planarian.

15. (1 pt) Observe how the planarian responds, and record your observations below.

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**Class Cestoda**

Cestodes, or tapeworms, are highly specialized internal parasites, which may have complex life cycles. Typically, the adults inhabit the intestine of a vertebrate. The life cycle almost always includes an intermediate host inhabited by a larval stage. Either invertebrates or vertebrates may be intermediate hosts. The intermediate host is usually the prey of the definitive host. The definitive host (the host in which reproduction occurs) is a vertebrate. The cestode body is composed of an anterior scolex, with which the tapeworm attaches to its host, and a long posterior strobila which is divided into numerous segment-like units called proglottids. Most organ systems are reduced or absent except for the reproductive system, which is well developed. There is no gut. Cestodes are hermaphroditic and may either self- or cross-fertilize.

**Activity:** Examine the small jar with the preserved specimens of adult tapeworms (taenia).

16. (1/2 pt) How long do you estimate this specimen is?

**Microscope Slide of Scolex.** Look at a wholemount slide of a scolex with the scanning lens (40X) of the compound microscope. The scolex is wider than the strobila to which it joined by a narrow neck. The scolex attaches the tapeworm to the gut wall of the host. For this purpose it has a retractable rostellum armed with two rings of rostellar hooks. Examine the hooks with 100X power. Just posterior...
to the rostellum is a **ring of four suckers**; two dorsolateral and two ventrolateral. Scolex morphology varies widely among taxa.

17. **(1 pt)** Draw a picture of the scolex and label the features in bold.

**Microscope Slide of the Gravid Tapworm Proglottids.**

18. **(1/2 pt)** Roughly how many eggs are in each proglottid?

**PHYLUM NEMATODA**

Nematodes, or roundworms, are one phylum of pseudocoelomate organisms, meaning that their central body cavity lies between the endoderm and mesoderm layers. Although they are triploblastic animals, their central body cavity develops very differently from those animals with a true coelom. As their common name suggests, roundworms have a cylindrical body shape with an external cuticle. This thick cuticle, along with the many longitudinal muscle bands these worms possess (unlike annelids, no circular muscles are present in roundworms), allow this phylum of worms to move in a whip-like fashion. As a result, free-living roundworms are sometimes called “whipworms”. Roundworms exhibit a complete digestive tract, with both a mouth and an anus present. Most roundworms are dioecious (separate sexes), and males and females usually exhibit external morphological differences.

There are several different species of both parasitic and free-living nematodes. Some examples of parasitic roundworms include *Ascaris lumbricoides*, *Trichinella spiralis* (the causative agent of trichinosis), hookworms, including *Ancylostoma duodenale* and *Necator americanus*, and the very common pinworm, *Enterobius vermicularis*. Examples of free-living nematodes include *Caenorhabditis elegans*, a very important model organism for studies in genetics, development, and cell biology, the vineger eel, *Anguilloa aceti*, and the sugar beet nematode, *Heterodera schachtii*.

19. **(1 pt)** List some features of *Ascaris* that are possible adaptations to parasitic life. *(This will be easier to answer after you dissect the worm.)*
CAUTION: Although the *Ascaris* specimens you will receive in the laboratory have been preserved in formalin, the eggs found in the female specimens are enclosed in a very resistant “shell”. They may not all be killed, even after several weeks in formalin. Although acquiring an infection in this way is a remote possibility, you should always follow good laboratory safety procedures and practices. Always wear dissecting gloves and avoid putting your hands near your mouth after handling live or preserved specimens. You should also wear protective goggles or glasses while working with your dissected organism. Always wash your hands thoroughly after you complete your work.

**Procedure:**

1. Use forceps to obtain one specimen of *Ascaris* for your group and place it in a dissecting tray.
2. Identify the anterior and posterior ends. **Determine if your specimen is male or female.** Males tend to be smaller than females and have two copulatory spicules located near the anus (see Figure 5).
3. Pin the anterior and posterior ends of the ventral side of the animal to the dissecting tray and carefully slit the dorsal body wall longitudinally (mouth to anus) using a straight pin. Be careful not to slice too deeply into the worm.
4. Pin the sides of the body wall open to expose the internal contents. **Be very careful not to remove or disturb any organs you will need to identify.**
5. After pinning the specimen, separate the different internal structures as best you can. Be cautious not to break them into small pieces.
6. Identify and locate the parts listed below. Once you have located them, **make a sketch of your dissection on a separate piece of paper, including and labeling these parts (as appropriate in your specimen; 4 pts).**
   a. Mouth, intestine, anus, lateral lines (2)
   b. **Female specimen only:**
      i. Ovaries (2)
      ii. Oviducts (2)
      iii. Uterus
      iv. Vagina
      v. Genital pore
   c. **Male specimen only:**
      i. Testis
      ii. Seminal vesicle
      iii. Copulatory spicules (2)
7. Unpin the specimen and using forceps, place the dissected specimen into the disposal container.
8. Wash all dissecting equipment with soap and water. **WASH YOUR HANDS WITH HAND SANITIZER!**
9. Wipe down lab surface.
Figure 5. *Ascaris* internal anatomy. Male *Ascaris* (left) tend to be smaller than female *Ascaris* (right) and have two copulatory spicules located near the anus.
Lab 4: Phylum Mollusca: Phenotypic Diversity

SYNOPSIS

You will observe the great phenotypic diversity present among the molluscs by observing living and preserved specimens. For the living specimens, you will focus on how the molluscs move. With a lab partner, you will dissect a squid and a clam and locate and draw their major features.

LEARNING OBJECTIVES

Your objectives in this laboratory are to learn the distinguishing features of three classes of molluscs presented: Gastropoda, Cephalopoda, and Bivalvia. You will also learn how some of these features function in the particular representatives of these groups that you will examine in the lab.

INTRODUCTION

Phylum Mollusca

The molluscs (or mollusks) boast a huge diversity of different morphologies and behaviors. Molluscs include octopus, squid, and cuttlefish — marine animals with camera eyes and excellent vision — the terrestrial slugs and snails, the aquatic bivalve clams and mussels, and the chitons that are typically found attached to rocks in the intertidal. Mollusca is also a very species-rich phylum, with over 93,000 described species.

Given the great diversity of molluscan body plans, it may not be obvious what characters they all share. However, all molluscs have the same three body regions — the foot, the visceral mass, and the mantle. These three body parts are often so modified to perform specialized functions that it’s hard to believe that some species belong to the same phylum.

The foot is the locomotory organ of molluscs, while the visceral mass contains all of the organ systems. The mantle is a specialized tissue that covers the internal organs and secretes a shell made of calcium carbonate. Most molluscs have either an internal or an external shell. Shells provide protection from predators and from desiccation, and provide a rigid frame to which muscles may attach. However, molluscs vary tremendously in size, shape, feeding behavior, and manner of movement, and some of the largest molluscs lack any hard surface to serve as a skeleton (such as giant squid, which are up to 21 m long). Instead, they utilize what is known as a muscular hydrostat, or a muscle wrapped around muscle.
Molluscs typically also possess **gills**, although they have been secondarily lost in some groups (such as the terrestrial slugs and snails). The gills are located within the mantle cavity. Molluscs have evolved complex organ systems, including respiratory, excretory, and digestive, as well as a circulatory system with a heart. A rasping structure known as the **radula** is found near the anterior end of the digestive tract in most classes of molluscs, although radula are not present in bivalves. The radula is used for scraping or rasping food. All molluscs possess a central nervous system, and that of cephalopods (cuttlefish, octopus, and squid) is highly complex.

There are seven classes of molluscs. In today’s lab you will observe the three most common classes. Below are brief descriptions of the major features of these three classes, followed by the activities for today's lab.

**LAB ACTIVITIES**

**Class Gastropoda (slugs and snails)**

Gastropods possess a large, muscular foot on their ventral side. They move by producing waves of contractions down the length of the foot. This is often aided by production of slime, which allows gastropods to slide across a surface. Gastropods undergo a phenomenon known as **torsion** during development, in which their visceral mass rotates as much as 180 degrees. Most gastropods possess a shell, although it is greatly reduced or absent in most slugs and nudibranchs. Gastropods have a distinct head with 1 or 2 pairs of tentacles that bear sensory organs.

Reproduction in gastropods is typically via fertilization with another individual. Most are hermaphroditic, with each individual possessing both male and female reproductive organs. While some species are able to self-fertilize, typically two individuals mate with each other. Courtship behavior can be extremely complex and lengthy in gastropods, lasting several hours in some species of slugs.

**Living Snail Observations**

For this exercise, we have **one or two** different species of snails for you to observe: **possibly** the common land snail, *Helix spp.*, which breathes using lungs, and/or the Black Japanese Trapdoor snail (or other species of aquatic snail), *Viviparis malleatus*, an aquatic snail species that breathes using gills. Your observations of these animals will focus on watching how they move.

Put each type of snail in a glass dish (add a little water for the Trapdoor snail), and cover the top with Saranwrap. Let the snails relax for a few minutes, until they extend their tentacles. Observe the available species in the tank. Watch them move across the glass. Use a hand lens to look at the underside of the animals as they glide across the glass.
1. (1 pt) Describe how the snails move.

2. (1 pt) Describe or sketch the eyes at the end base of the snails’ tentacles. (It helps to use a hand lens to observe these, but do not put it under the lights of the microscope.)

**Microscope slides of snail radula**

Look at the slide of radula from the snail, using the compound microscope.

3. (1 pt) Describe or sketch the radula.

**Nudibranchs**

Nudibranchs (sea slugs) are marine gastropods, typically lacking a shell. They are often brightly colored, and are considered some of the most beautiful animals of marine environments. However, this beautiful, bright coloration often serves as a warning signal to other animals telling them “keep away, I’m dangerous”. Indeed, many of these bright animals are poisonous.

**Activity: Watch the DVD of nudibranchs.**

4. (1 pt) Where do the poisons that nudibranchs have come from?

**Class Cephalopoda (octopus, squid, cuttlefish, nautilus)**

Octopuses lack a rigid skeleton, but squid and cuttlefish have an internal skeleton (that is, a shell). They have large and prominent heads and complex camera-type eyes composed of lens, retina, and optic nerve. They are active predators, and capture their prey with the 8-10 tentacles that surround their mouth. Their mouth has been modified to have a beak-like structure used to bite their prey. Their bodies have been highly modified over evolutionary time, and their head and foot are now closely associated (the
word “cephalo” = head and “pod” = foot). They have an excellent capacity for learning and memory, and an amazing ability to change color almost instantaneously in response to environmental stimuli.

**Squid Dissection**
With a lab partner, get a waxed pinning tray, scissors, pins, probes, and a preserved squid. Each person should wear gloves, and follow the directions in the “Illustrated Squid Dissection Guide” that will be provided in the lab. This manual will show you how to dissect the squid, and what parts to look for. Distinguish whether you have a male or a female squid. Females possess two nidamental glands which secrete parts of the eggs; males lack these glands, but instead possess a single spermatophoral gland. After your squid has been dissected and pinned open, **make a drawing** on a separate sheet of paper and label all the internal anatomical features you can find.

5. **(1 pt)** What is the sex of your squid?

6. **(5 pts)** Make a drawing of your dissected squid **on a separate sheet of paper**, with at least 6 body parts labeled.

**Squid Eyes**
Cephalopods are well known for their elaborate camera eyes that resemble those of vertebrates. The eyeball is surrounded by the transparent cornea on the lateral surface. The iris is visible inside the cornea on the lateral surface. It is a tough, thick layer with a silvery, opaque layer. The pupil is an opening in the iris. You will perform a close examination of the squid eye.

First, dissect the eye away from the squid.

Cut through the **iris**, beginning at the edge of the pupil, to reveal the interior chamber of the eye. The spherical **lens** is located just behind the pupil. The lens is supported by **ciliary ligaments** and muscles. The inner wall of the interior of the eye is the **retina**, containing sensory pigments.

7. **(2 pts)** Make a sketch of the dissected eye **on a separate sheet of paper** and label the 4 parts indicated in bold.
Squid Radula
Spread the 10 appendages away from the mouth of your squid. Look at the mouth of the squid, and note the hard, beak-like jaws which allow the animal to bite. Cut away one of the halves of the jaw to reveal the buccal cavity. On the bottom of the buccal cavity is a radular sac, which contains the radula. Dissect the radula away from the squid. Place it on a microscope slide/watch glass and examine it under the dissecting microscope.

8. (1 pt) Make a sketch of either the beak-like jaws or the radula (as seen under a dissecting microscope) on a separate sheet of paper.

Class Bivalvia (clams, oysters, scallops, mussels)
Bivalves have a visceral mass that is contained in a shell. The shell has two halves that are hinged (hence the name “bi-valve”). When they move, their foot extends out of shell; however, locomotion is very limited. Typically, the foot is used for digging into substrate (sand, mud, and even wood). Their head region is greatly reduced in size. Bivalves possess gills, which are used for respiration and prey capture.

Activity: Examine the preserved bivalves we have on display.

9. (2 pts) Suggest a reason why you think bivalves have evolved to have smaller head regions than cephalopods.

Activity: Clam Dissection
(5 pts) Make a sketch of your clam dissection on a separate piece of paper, and label all of the parts indicated in bold.

Procedure (adapted from Marine Science - Mr. Black)

1. Put on your safety glasses and dissection gloves.
2. Place a clam in a dissecting tray and identify the anterior and posterior ends of the clam as well as the dorsal, ventral, & lateral surfaces.

3. Locate the umbo, the bump at the anterior end of the valve (see Fig. 1). This is the oldest part of the clam shell. Find the hinge ligament which hinges the valves together and observe the growth rings.

4. Turn the clam with its dorsal side down and CAREFULLY insert a scalpel between the ventral edges of the valves. Carefully work the scalpel between the valves so you do not jab your hand.

5. Locate the adductor muscles (see Fig. 2). With your blade pointing toward the dorsal edge, slide your scalpel between the upper valve & the top tissue layer. Cut down through the anterior adductor muscle, cutting as close to the shell as possible.

***ALWAYS CUT TOWARDS THE DISSECTION TRAY AND AWAY FROM YOURSELF. DO NOT HOLD THE CLAM IN YOUR PALM AS YOU ARE CUTTING IT.***

6. Repeat step 6 in cutting the posterior adductor muscle.

7. Bend the left valve back so it lies flat in the tray.

8. Run your fingers along the outside and the inside of the left valve and compare the texture of the two surfaces.

9. Examine the inner dorsal edges of both valves near the umbo and locate the tooth-like projections. Close the valves & notice how the tooth-like projections interlock.

10. Identify the mantle, the tissue that lines both valves & covers the soft body of the clam. Find the mantle cavity, the space inside the mantle.

11. Locate two openings on the posterior end of the clam. The more ventral opening is the incumbent siphon that carries water into the clam and the more dorsal opening is the excurrent siphon where wastes & water leave.

12. Using scissors and your index finger, carefully remove the half of the mantle that lined the left valve. After removing this part of the mantle, you can see the gills, which are the clam’s respiratory structures.

13. Observe the muscular foot of the clam, which is ventral to the gills. Note the hatchet shape of the foot used to burrow into mud or sand.

14. Locate the palps, flap like structures that surround & guide food into the clam’s mouth. The palps are anterior to the gills & ventral to the anterior adductor muscle. Beneath the palps, find the mouth.

15. With scissors, cut off the ventral portion of the foot. Use the scalpel to carefully cut the muscle at the top of the foot into right and left halves.

16. Carefully peel away the muscle layer to view the internal organs.

17. Locate the spongy, yellowish reproductive organs (gonad).

18. Locate the digestive gland, a greenish structure that surrounds the stomach.

19. Locate the long, coiled intestine extending from the stomach.
21. Follow the intestine through the clam. Find the area near the dorsal surface that the intestine passes through called the pericardial area. Find the clam's heart (2 auricles + 1 ventricle) in this area.

22. Continue following the intestine toward the posterior end of the clam. Find the anus just behind the posterior adductor muscle.

23. Use your probe to trace the path of food & wastes from the incumbent siphon through the clam to the excurrent siphon.

24. When you are finished with your dissection and drawings, dispose of the clam properly and clean, dry, and return all dissecting equipment to its proper location. Wipe down the lab bench with cleaner.

25. Wash your hands thoroughly with soap and water before leaving lab.

Figure 1. Some external anatomy of the clam.
Figure 2. Internal anatomy of the clam.

Water enters the mantle cavity from the rear and is pulled forward by the beating of cilia to the gills and mouth. Water flowing over the gill is filtered, tiny food particles are caught in the mucus coating and carried by cilia, in a mucus string, to the mouth. Sand and other inedible debris are moved to the mantle cavity and removed.

A. Feeding mechanism of clam.

B. Clam anatomy.
Lab 5: Sexual Selection and Adaptations: Arthropods

SYNOPSIS
In this lab you will observe a variety of organisms from the planet’s most species-rich phylum. You will work with a partner and move through the stations, examining arthropods that are alive and preserved. You will study phenotypic traits of these animals, and form hypotheses as to whether and how these traits may be adaptations for particular lifestyles. You will also make educated guesses as to whether sexual selection likely influenced the evolution of some of these phenotypes. You will make measurements and use your data to test the hypothesis that sexual selection has influenced the evolution of cheliped size in crayfish, and finally, you will dissect a crayfish to view both internal and external anatomy.

LEARNING OBJECTIVES
This lab provides you a tiny sampling of the amazing diversity of arthropods. You will familiarize yourself with what arthropods look like, how they capture prey, and how to recognize some of the adaptations these organisms possess. In addition, you should learn how sexual selection has influenced the evolution of some organisms, and you will form and test hypotheses to determine whether sexual selection may have caused sexual dimorphism to evolve.

INTRODUCTION
Arthropods (Phylum Arthropoda) are the most species-rich phylum of organisms on Earth. Over half of all described species on our planet are arthropods, and more than four of every five animal species is an arthropod. Arthropods have adapted to nearly every habitat on Earth. On land, the insects and arachnids are abundant, while crustaceans predominate in water and are key components of marine ecosystems. Arthropods help build soil and cycle nutrients, pollinate wild plants and crops, and provide food for organisms higher up the food chain. If arthropods were to suddenly vanish from the Earth, our planet’s ecosystems would collapse.

Whereas vertebrates such as ourselves have hard skeletons underneath layers of soft tissue, arthropods possess hard skeletons outside of their soft tissues. These hard sclerotized exoskeletons (exo = outer) protect them from predators, desiccation, and damage to their internal organs, while providing them with a rigid structure that can withstand great forces. Arthropods also are characterized by segmented body plans and by jointed appendages (the word “arthropod” comes from the Greek arthros (joint) and poda (foot)). The appendages have often been highly modified by selection, and have allowed arthropods to adapt to a diversity of habitats and diets. You will learn more about arthropods in lecture.
**Sexual selection** is a type of natural selection in which the selective pressures happen to be from members of an organism's own species and affect reproductive success. Sexual selection favors phenotypes that increase an individual's probability of attracting mates. It may take the form of intrasexual competition (typically male vs. male) or mate choice (most often female choice of males). Sexual selection is defined as variation in reproductive success among members of the same sex of a species. Sexual selection often results in **sexual dimorphism**, the condition whereby males and females differ in some phenotypic traits, and is generally responsible for producing elaborate and gaudy features that seemingly serve no survival purpose. Identifying sexually dimorphic traits often suggests that sexual selection is acting or has acted in the past. Sexual selection occurs widely throughout animals and plants, so as you examine arthropods in this lab, think about how the patterns you see may also apply to other organisms. As you observe the various arthropods in this lab, you are encouraged to share with your classmates any interesting observations that you make.

**LAB PROCEDURE**

**Class Arachnida** (Greek for "spider")

After the insects, arachnids comprise the most species-rich group of animals. Within arachnids, there are 11 extant orders. The two orders that contain the most species are the Acari (mites and ticks) and the Araneae (spiders), each of which live in diverse habitats on every continent. For instance, different mite species can be found living in the hair follicles of mammals, on the feathers of birds, in ephemeral ponds, and even at deep-sea hydrothermal vents! Uropygids can be found in deserts, while amblypygids thrive in tropical rainforests. As you might guess, these varied arachnids have different adaptations that allow them to live in these diverse habitats. However, all arachnids share certain general characteristics: 2 body segments (tagmata), 4 pairs of legs, 1 pair of palps, and 1 pair of chelicerae. (*cheli* is Greek for claw or hook — an apt description of these feeding appendages). All 6 pairs of appendages attach to the anterior segment, the cephalothorax (see diagram of a spider below). The chelicerae are the most anterior appendage, followed by the pair of palps, followed by the 4 pairs of legs. In spiders, the male's palps are modified for sperm transfer, and typically are much larger than the female's palps.

Most arachnids are carnivores, and feed only on living prey. However, unlike carnivorous insects, arachnids lack chewing mandibles. Instead, they use their mouthparts to suck up their food in a liquid or semi-liquid form. This means that once they catch and subdue their prey, they need to liquify it. Arachnids may immobilize their prey with venom (as in spiders or scorpions), wrap them in silk (spiders), or grab them with spiny appendages (amblypygids and uropygids). Then they vomit digestive juices onto their victim, or else tear and macerate their prey to a mushy pulp. There are a number of unique adaptations for doing this among the various arachnid orders. In some spiders, for instance, the chelicerae often have tiny teeth that aid in macerating
prey. By observing the anatomy of the spiders, scorpions, uropygids, and amblypygids in this lab, you should be able to hypothesize what adaptations they have for feeding.

We have animals from four orders of arachnids for you to observe. Your T.A. will feed one or two of these animals during the lab, so try to observe at least one of these feeding events.

**Scorpions**
Examine the scorpions. Compare the appendages of scorpions to the appendages of a spider in the diagram labeled below. Note that the relative location of each of the appendages is the same in all arachnids (chelicerae are in the most anterior position, followed by palps, followed by the four pairs of legs), but that the appendages may be adapted for different uses.

1. (1 pt) Which pair of scorpion appendages appears to be adapted for grasping prey? Explain.

![Diagram of a spider, illustrating the location of the chelicerae, the palps, and leg pairs 1-4, as found in all arachnids.](image-url)
Amblypygid
First, observe one of the living animals. Some of you may be fortunate enough to watch these animals feed on crickets. However, these animals do not feed very often, and they are nocturnal, so you will most likely only see them hiding in the dark during the day. Like all arthropods, amblypygids must undergo a molt, shedding their old cuticle (exoskeleton), so that they can grow to a larger size. Examine the molt of the amblypygid we have placed under the microscope. In particular, note the first pair of legs and the palps. You will need to look closely at the molt and compare it to the spider diagram above to make sure you correctly identify these appendages that are anatomically homologous.

2. (1/2 pt) Describe or quickly sketch the first pair of legs. What function do you think they may serve?

3. (1/2 pt) Describe or quickly sketch the palps. What function do you think they may serve?

Uropygid
Like many arachnids, uropygids (commonly known as “vinegaroons”) are nocturnal. Therefore, the two in the lab will likely be in their burrows during the day. Although they look superficially like scorpions, they lack a stinger with venom. Their defense against predators is to emit a vinegar-like spray from an organ at the base of the whip-like telson. Either examine one of the living uropygids, or examine a preserved specimen under the microscope.

4. (1/2 pt) Describe or quickly sketch the first pair of legs. What function do you think they may serve?
5.3. (1/2 pt) Describe or quickly sketch the palps. What function do you think they may serve?
Spiders
We have living spiders (in addition to a few preserved specimens). Have your T.A. put food in with the tarantula. If the spider feeds, you can watch it using either a hand lens, or under low magnification of a dissecting microscope.

- Hint #1 = Use the overhead light only, and NOT the stage light of the microscope!
- Hint #2 = Avoid making rapid movements that may startle the spider and prevent it from feeding.
- Hint #3 = Be prepared to watch the spider catch its prey immediately after you put it in the vial; sometimes this happens fast!

5.4. (1/2 pt) Describe either the feeding behavior of the spider you observed (if it fed), or the chelicerae of one of the preserved tarantulas.

Subphylum Myriapoda (Greek myria = many; poda = foot)
Myriapods consist of the centipedes and the millipedes. Myriapods are primarily soil-dwellers. Their bodies are elongated because they have evolved a large number of body segments. As with an earthworm or a snake, a long and flexible body allows them to wriggle through soil and leaf litter.

Class Chilopoda (centipedes)
Observe the preserved specimens of Scolopendra centipedes. These are carnivorous animals, feeding mostly on invertebrates in the soil.

6.5. (1 pt) How does the body segmentation in the centipede differ from the arachnids and insects at other stations in this lab?

Class Diplopoda (millipedes)
Examine the large, preserved specimens of Spirobolus millipedes. Look at one in a petri dish in 80% ethanol and turn it so that you can examine the ventral side of its head and segments.
Subphylum Hexapoda (Greek hexa = six; poda = foot) Insects and their kin

The hexapods are the most species-rich group of organisms on Earth. Most hexapods are insects (Class Insecta), of which there are about 1 million species described, as well as many more not yet discovered or described by scientists. One reason proposed to explain the great diversity of insects is that they evolved wings and the ability to fly. This allowed them to invade more ecosystems and fill more niches than their flightless terrestrial or aquatic kin.

Insects have three main body parts (head, thorax, and abdomen), and three pairs of legs (which grow from their thorax). Insects also have antennae for chemically sensing their environment. Insects often communicate with each other via the pheromones they release, and the antennae serve as detectors of these chemical signals. The more complex and finely divided the antennae are, the better they are for sensing pheromones. Within insects, the beetle family (Coleoptera) is the most species-rich group. The basic body plan of a beetle is illustrated below.

You will see two trays of pinned beetles, containing two different species. Examine these specimens and note that they all possess a hard exoskeleton, including modified forewings called elytra, which are sclerotized and protect their softer delicate hindwings when the animals are not flying. By covering their wings, the elytra adapt beetles for many different habitats and behaviors, including burrowing through soil or wood.

7.6. (2 pts) Sketch the first few segments of the millipede and label the

What is different about the legs and segments of the millipede as compared to the centipede?
Figure 2. Diagram of a beetle, illustrating the 3 body regions, 3 pairs of legs, and pair of antennae.

Carefully examine the beetles (Coleoptera) that we have marked in the trays. The box of Tenlined June Beetles contains both males and females.

8.7. (1/2 pt) Does the Ten-lined June Beetle show sexual dimorphism? In what

Examine the butterflies and moths (Lepidoptera) that we have marked in the trays. Note that they have the same general body plan as the beetles, but that their forewings are large and delicate, and better adapted for flight than those of beetles. Note how their antennae differ from those of beetles.

9.8. (1/2 pt) Do the antennae of moths and butterflies differ? In what

Note the different colors and patterns on the moths and butterflies. Many species of Lepidoptera have evolved circles or colored spots, called eyespots. In some species, eyespots may serve to deter predators, while in other species these rings of color may be sexual signals. Now look at one of the butterfly wings we have placed under a microscope. Look closely at the spots of color and how they are formed. Lepidoptera wings are covered with tiny scales.

10.9. (1 pt) Describe how the color varies from the inside to the outside of colored spot. Does color vary within a single scale?
Subphylum Crustacea

Crustaceans are a diverse group that includes such animals as crabs, shrimp, lobsters, crayfish, and barnacles. Most species are aquatic, and crustaceans are among the few arthropods to be widespread in the oceans. Because of their aquatic lifestyles, many crustaceans breathe using gills. Like insects, they have three main body segments, and as in insects the abdomen is often subdivided into multiple segments. The head and thorax may sometimes be fused into a single body part called the cephalothorax. In most crustaceans, the appendages are biramous, or branched into two parts. Many of the most familiar crustaceans, such as lobsters, shrimp, and crabs, are decapods (deca = ten), having five pairs of legs.

Crustaceans show extremely diverse morphologies. Examine the preserved museum specimens on hand in the lab.

Next, examine a species of crayfish, a type of decapod crustacean that lives in streams and rivers. With your lab partner, get one crayfish and lay it carefully in a dissecting tray and examine its external surface. Note the cephalothorax and abdomen. Examine the dorsal surface of the head and note the portion that extends anteriorly beyond the antennae and mouthparts. The compound eyes are located to either side of the carapace, as is the long pair of antennae. The short pair of antennae tend to point forward of the carapace.

Now flip the animal over on its back. Note the many appendages. Three pairs make up the inner jaw (mandibles and maxillae), 3 pairs make up the outer jaw (maxillipeds), 5 pairs are used for walking and digging (all located stemming from the “thorax”), and 6 pairs are used mainly for swimming (the swimmerets, all stemming from the abdomen). The most anterior of the 5 pairs of walking legs are modified into specialized structures called chelipeds. These are used for combat, defense, and obtaining food.

In crayfish and lobsters, the most anterior of the swimmerets of males is modified to function in sperm transfer: males fertilize females by using this first swimmeret to transfer sperm to her genital opening. You can identify whether you have a male or female by noting the appearance of the first swimmeret. If it is longer and stiffer than the others, it is male. If it is about the same size or smaller than the others, the animal is female. Females also possess an oval opening called the seminal receptacle, located just anterior to the first pair of swimmerets.
Now that you can identify males and females, let's do some hypothesis testing! As noted above, the chelipeds serve several purposes. Both males and females fight over resources such as shelter and food, so it is likely that large chelipeds would be advantageous for both sexes. However, it is conceivable that chelipeds are also used in a sexual context. Perhaps their phenotype has been influenced by sexual selection.

As a class, we will test the hypothesis that chelipeds are a sexually selected character whose size results from either male-male combat for access to females or female preference for males with large chelipeds. To assess this we might ask whether males have significantly larger chelipeds than females. Following the scientific method, we would phrase this as a testable prediction ("Males have significantly larger chelipeds than females"), and then collect data (by measuring lengths of chelipeds) to test whether or not this prediction is supported by the data.

However, in testing this prediction we could encounter a serious problem if cheliped size varies with overall body size (i.e., length), and if males and females differ in overall body size. Talk this over with your lab partner and discuss how to go about testing the prediction while controlling for the potentially confounding effect of body size. Do you expect males or females to be larger? Why? Think not only about competition for mates, but also the costs of reproduction (egg production and incubation).

In this lab, we will pursue one simple way to control for the effects of potential sex differences in body size. For each crayfish we measure, we will divide cheliped length by total body length. This will give us the size of the cheliped relative to body size, so that we can make meaningful comparisons between males and females. This is crucial to understand, so before we move on, let's consider a quick example....

Let's say we gathered 25 males and 25 females at random from the crayfish population and measured their total body length and right cheliped length. Computing the averages, we obtained the following results:
We can see that males clearly have larger chelipeds than females. Chelipeds are larger in males in their absolute size, BUT we can also see that males are larger in total body size. The difference in cheliped size might therefore only be due to this difference in overall body size. If we truly want to assess whether chelipeds are a sexually selected trait, we need to look at the proportional size of chelipeds. So let’s calculate proportional sizes (right cheliped/total length) for our data:

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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</thead>
<tbody>
<tr>
<td>Total Length</td>
<td>162 mm</td>
<td>122 mm</td>
</tr>
<tr>
<td>R. Cheliped</td>
<td>34 mm</td>
<td>26 mm</td>
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</tbody>
</table>

As you can see, the proportional cheliped length is the same for males and females. This tells us that the difference we observed in absolute size of chelipeds was merely due to the difference in body size. This result would not support our hypothesis that chelipeds are under sexual selection.

Now that you understand the importance of controlling for absolute size, you are ready to get started!

1. With your lab partner and along with another pair of classmates, obtain 10 crayfish (5 from each bucket). Each pair of students will measure 5 crayfish.

2. Gently flatten each individual out, dorsal side up.

3. Accurately sex your crayfish and sort them into male and female piles.

4. For each crayfish, take the following two measurements (in millimeters) using calipers:
   a. total length (tip of the rostrum to tip of the tail)
   b. length of right cheliped

5. As you work, write your data into the table on the accompanying sheet. Each group of 4 students will fill out one table, with each pair of students entering 5 crayfish each onto the table.

To compare how a character (such as proportional cheliped length) differs between two groups (such as male and female crayfish), we could simply compare the means (averages) for the character in each group. However, this would be valid only had we...
sampled the entire crayfish population. Instead, we are sampling only a small number of individuals that we hope may be representative of the entire crayfish population. As a result, we must use statistics to tell us the likelihood that any difference we observe in our samples really exists in the whole population.

To do this, we can use a statistical test called the **student's t-test**. A t-test compares the data in our two categories (male and female) and tells us the likelihood that proportional cheliped length truly differs between the categories. That is, the t-test tells us whether to reject our prediction and thus our hypothesis that cheliped length is sexually selected, or whether our data lends support to our prediction and our hypothesis. It does this by providing us with a **P-value**. Think of the P-value as the probability of being wrong in making the claim that the two groups being compared are different. So if P=0.60, it means that 60% of the time your conclusion that there is a difference would be incorrect. This is clearly an unacceptable level of error. If however, P=0.04, it means that only 4% of the time you would be incorrect in your conclusion. This seems like a much more acceptable level of error. In biology the standard acceptable error rate is 5% (P≤0.05). Thus, in our case, to lend support to our hypothesis that male cheliped length is greater than female cheliped length, our P-value must be ≤0.05.

An easy way to perform a t-test is to go online and access the website:

http://faculty.vassar.edu/lowry/t_ind_stats.html

Once you are done making your measurements, go to the first of the two computers your T.A. has designated for the class’s data analysis. Upon entering this website, it will ask you to “Please enter the size of the larger of your two samples”. Enter the total number of males OR females that you measured, whichever is larger (not the size of the measurements — the number of samples).

Now you will be presented with two columns: X_a and X_b. Enter your data for proportional cheliped length into these columns, placing the **male data into X_a** and the **female data into X_b**. Hit the “Calculate” button. The program will automatically calculate a number of statistical values. Scroll toward the bottom of the screen and note the “one-tailed” P value. Record this number — this is the P value you will use to test your prediction and answer Question 11 below.

Now your data will be compiled with the data from all your classmates. Take your data sheet to the second of the two computers your T.A. has designated for the class’s data analysis. (Make sure your 4 names are on the data sheet). Leave your data sheet here for the designated data-entry person to enter into the program. This computer will be open to the same website, but on this computer the entire class’s data will be entered. When all data has been entered by the designated data-entry person, your T.A. will hit “Calculate,” and will supply the class with the overall one-tailed P-value for the class’s data as a whole. Record this number — this is the P value you will use to test your prediction and answer Question 12 below.
44-10. (3 pts) Consider our prediction that proportional cheliped length in males would be greater than proportional cheliped length in females. Was this prediction supported by the data measured by your table? What was your P value? Was your result statistically significant? Does the data support or reject the hypothesis that cheliped length is a sexually selected trait?
12.11. (3 pts) Now consider the overall data from your entire class. Was our prediction supported by the class data? What was the class’s P value? Was the result statistically significant? Does the data support or reject the hypothesis that cheliped length is a sexually selected trait?

13.12. (1 pt) Let’s say that sexual selection does favor large chelipeds. There be other reasons that large chelipeds could be disadvantageous? Speculate about a few potential fitness costs of having large chelipeds.
Crayfish Dissection: (5 pts) Make a sketch of your crayfish dissection on a separate piece of paper, and label all of the parts indicated in bold.

**Procedure**: (modified from Crayfish Dissection © John R. Sowash | May 2009 Permission to redistribute granted)

1. Working with your lab partner, place a crayfish on its dorsal side in a dissection tray. Locate the cephalothorax and the abdomen. The carapace, a shield of chitin, covers the dorsal surface of the cephalothorax. On the carapace, observe an indentation, the cervical groove that extends across the midregion and separates the head and thoracic regions. On the thoracic region, locate the prominent suture or indentation on the cephalothorax that defines a central area separate from the sides. Note the individual segments of the abdomen.
2. Turn the crayfish on its side, and locate the rostrum, which is the pointed extension of the carapace at the head of the animal. Beneath the rostrum locate the two eyes. Notice that each eye is at the end of a stalk.
3. Locate the five pairs of appendages on the head region. First locate the antennules in the most anterior segment. Behind them observe the much longer pair of antennae.
4. Locate the mouth. Then observe the mandibles, or true jaws, behind the antennae.
5. On the thoracic portion of the cephalothorax, observe the three pointed maxillipeds.
6. Next observe the largest prominent pair of appendages, the chelipeds, or claws. Behind the chelipeds locate the four pairs of walking legs, one pair on each segment.
7. On the abdomen, observe the six distinct segments. On each of the first five segments, observe a pair of swimmerets.
8. On the last abdominal segment, observe a pair of pointed appendages modified into a pair of uropods. In the middle of the uropods, locate the triangular-shaped telson.
9. Now turn the crayfish ventral side up. Observe the location of each pair of appendages from the ventral side.
10. Using one hand to hold the crayfish dorsal side up in the dissecting tray, use your scissors to make an incision through the back of the carapace. Cut along the indentations that separate the thoracic portion of the carapace into three regions. Start the cut at the posterior edges of the carapace, and extend it along both sides in the cephalic region.
11. Use forceps to carefully lift away the carapace. Be careful not to pull the carapace away too quickly. Such action would disturb or tear the underlying structures.
12. Place the specimen on its side, with the head facing left. Using your scissors, start cutting at the base of the carapace along the side of the crayfish. Extend the cut line forward toward the rostrum (at the top of the head). Repeat this cut on the opposite side of the crayfish.
13. Use forceps to carefully lift away the remaining parts of the carapace, exposing the underlying gills and other organs.
14. Locate and identify the organs of the respiratory system. Locate the gills, which are featherlike structures found underneath the carapace and attached to the chelipeds and walking legs. A constant flow of blood to the gills releases carbon dioxide and picks up oxygen.

15. Locate and identify the organs of the digestive system. Locate the maxillae that pass the pieces of food into the mouth. The food travels down the short esophagus into the stomach. Undigested material passes into the intestine. Observe that the intestine is attached to the lobed stomach. The undigested material is eliminated from the anus.

16. Locate and identify the organs of the circulatory system. Locate the dorsal tubular heart and several arteries. The crayfish has an open circulatory system in which the blood flows from arteries into sinuses, or spaces, in tissues. The blood flows over the gills before returning to the heart.

17. Locate and identify the organs of the excretory system. The blood carries cellular wastes to the disk-like green glands. Locate these organs just in front of the stomach. The green glands excrete waste through pores at the base of each antenna.

18. Locate and identify the organs of the reproductive system. If your specimen is a male, locate the testis. The testis is the long, white organ under the heart and a bit forward. The sperm ducts that carry sperm from the testis open at the fifth walking leg. If your specimen is a female, locate the bi-lobed ovary. It is in the same relative position as the testis, but the ovary appears as a large, reddish mass under the heart. Then locate the short oviducts that extend from near the center of each side of the ovary and open at the third walking leg. Exchange your specimen with a nearby group who has a crayfish of the opposite sex. Then study its reproductive system.

19. Clean up your work area and wash your hands before leaving the lab.
Figure 5. Some external anatomy of the crayfish.

Figure 6. Some external anatomy of the crayfish.
Figure 7. Some internal anatomy of the crayfish.

Figure 8. Some internal anatomy of the crayfish.
Name:_______________________________

DATA SHEET FOR CRAYFISH MEASUREMENTS

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total Length</th>
<th>R. Cheliped</th>
<th>Proportional Cheliped Length (R. Cheliped/Total Length)</th>
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Lab 6: Comparative Anatomy and Phylogenetics of Deuterostomes

SYNOPSIS

In this lab you will dissect one specimen after choosing among 5 deuterostomes. You will sketch your animal and label its key anatomical features. You will then examine your classmates’ dissections of the other 4 deuterostomes and record comparative anatomical data. Next, you will observe other museum and living specimens stationed around the lab and record their phenotypic features. The recorded phenotypic characters will be traced onto the phylogenetic tree of deuterostomes provided in your lab handout.

LEARNING OBJECTIVES

This lab will allow you to become familiar with the basic body plans of vertebrates and echinoderms, while learning to recognize structural homologies. Structural homologies are typically determined by comparisons of internal anatomy; therefore, you will learn how to compare internal features from dissected animals. You will learn how to compile comparative data into a table, or matrix, and then use this to map the evolution of each trait or character onto a phylogenetic tree. Finally, from examining how the characters evolved on a phylogenetic tree, you will be able to determine whether they most likely evolved due to shared common ancestry, or due to convergent evolution.

INTRODUCTION

Taxonomists have traditionally classified organisms into categories based upon shared similarities. The classification system we currently use is based upon the work of Carolus Linnaeus, who developed a hierarchical system of classifying organisms in his book *Systema Naturae* almost 300 years ago. In the traditional Linnaean system, each kingdom of organisms is subdivided into phyla (for instance, Kingdom Animalia, which includes all animals, consists of about 35 phyla). In turn, phyla (singular = phylum) are subdivided into smaller groups called classes, which are in turn divided into orders, families, genera (singular = genus), and species.

In the three centuries since Linnaeus developed his scheme of classification and nomenclature (naming species), we have learned much more about the diversity of life on Earth. Therefore, scientists have modified the Linnaean system to better categorize the many new species that have been described since Linnaeus’s time. For example, as discussed in lecture, we now recognize three major domains of life: Bacteria, Archaea, and Eucarya. Therefore, we now use the term “domain” as the largest category to encompass organisms with shared similarities. We have also further
subdivided the seven basic levels that Linnaeus recognized (kingdom, phylum, class, order, family, genus, and species). For example, a subclass is a smaller taxonomic group than a class, but larger than an order. A superorder is larger than an order, but smaller than a subclass.

Each phylum has a particular anatomical organization, or body plan, that distinguishes it from other phyla. Not all features need to be present in every member (some features may have been secondarily lost or modified during evolutionary history), and some features may be found in other phyla. However, each phylum possesses a unique combination of features that distinguish its members from those of other phyla. These statements also hold for the other hierarchical levels (class, order, etc).

Organisms that are genetically related because they have evolved from a common ancestor may share certain characteristics that the ancestor possessed and that have been retained since that time. This type of similarity due to common descent is called homology. When comparative anatomists compare the anatomies of different types of animals and try to determine whether a given organ, structure, or trait is homologous, they focus on its position relative to other entities in the body. Thus, the positional context of the structure is important. For instance, recall from lecture that the bones in the forelimbs of vertebrates provide examples of structural homology.

A trait that is shared by multiple types of organisms because of homology is termed a synapomorphy. A synapomorphy is a shared derived character — one that is shared by members of a clade, or monophyletic group, and that evolved in that clade but not others. When evolutionary biologists construct phylogenetic trees, they use synapomorphies to determine clades. As you’ve seen in lecture, once we have a phylogenetic tree, we can use it to trace the evolution of traits. When we map a homologous trait on a tree, it originates only once, and is retained by the taxa that descend from the lineage with the trait. Some traits are not homologous, but instead show homoplasy or convergent evolution. Such traits may originate independently multiple times on a tree.

In today's lab, we will focus on organisms from the Superphylum Deuterostomia, a major group within the Kingdom Animalia and the Domain Eucarya. The deuterostomes share an important developmental feature: the blastopore (through which tissues migrate inward during gastrulation) will become the anus (whereas in other animals with a gastrula, the blastopore will become the mouth). The mouth will then form afterwards. There are two major deuterostome phyla: the Echinodermata (sea urchins, sea stars, sea cucumbers, etc.), and the Chordata (vertebrates and their kin, including us). Each of these phyla is subdivided into multiple classes.

By dissecting organisms and comparing their anatomy, you will become acquainted with deuterostome body plans and with some of the homologous features shared among the various groups. You will then map your findings onto a phylogenetic tree of deuterostome taxa, determine where each trait most likely originated, and interpret which anatomical features represent synapomorphies for which groups.
LAB PROCEDURE

With a lab partner, you will select ONE of the animals listed below for dissection. Be sure you are dissecting the organism you were assigned in last week’s lab. After your dissection is complete, you will sketch the internal anatomy of the animal, and label the diagram on the instruction manual posted on Blackboard that accompanies each animal. Leave that diagram next to the dissected animal to help guide your classmates, but keep your sketch to turn in as part of your lab write-up.

Now, go examine the dissections that your classmates have made of the other 4 taxa. Feel free to ask students who have dissected the other animals to share with you their findings and observations. Record the animals’ internal and external anatomical features in the table provided.

Next, observe the museum specimens and the living animals on display, and record their phenotypic features in the table provided. Put a question mark in the table if you cannot confidently determine whether the animals possess that feature.

Finally, use this table of data and record where on the provided phylogenetic tree of deuterostomes each of the traits most likely originated. For instance, if you see that a particular trait is shared by the sea star and the sea urchin but by no other animals, you would draw a hash mark across the branch of the lineage leading to sea stars and sea urchins and label the hash mark with the name of the trait. One example is already entered on the tree for you: the deuterostome developmental pathway (anus developing before mouth) is shown to have originated at the base of the tree, on the lineage leading to all deuterostomes. Likewise, if you infer a loss of a character, indicate this with a hash mark.

Once you are done, answer the questions at the end of the lab handout.

Animals for dissection and comparative anatomy:
- Sea stars
- Lamprey
- Fish (Perch)
- Frogs
- Rats

Other animals for phenotypic comparisons:

Museum specimens
- Bird skin and skeleton
- Bat skin and skeleton
- Sea Urchins

Living animals
- Turtle
- Lizards
Goldfish
Various tropical fish

General Dissection Instructions

You will be given a set of dissecting tools and supplies (dissecting pans, large and small dissecting scissors, forceps, pins, scalpels, gloves, towels). Wear gloves at all times when handling the preserved animals. For specific dissection instructions, please refer to the instructions provided for the animal of your choice on BlackboardD2L. After the animals are dissected, please clean up your area thoroughly, and put all your dissection tools away! After your classmates have observed your dissection, place the dissected animals in the disposal bags that are provided.

Required Labeling for Dissections:

Sea Star
Label: Ampullae, Gonad, Madreporite, Pyloric ceca & Stomach

Lamprey
Label: Eyes, Gill slits, Heart, Intestine & Liver

Perch
Label: Gills, Heart, Intestine, Liver, Pyloric ceca & Stomach

Frog
Label: Heart, Intestine, Liver, Lungs & Stomach

Rat
Label: Heart, Intestines, Liver, Lungs(2), Pancreas & Stomach
A phylogenetic tree is a hypothesis, based upon the data at hand. We have provided you with the most-accepted tree for deuterostomes that is available, given current scientific knowledge. Assume that the tree provided is correct, and imagine that the organisms you have just examined comprise all you know about deuterostomes. Answer the following questions based upon the specimens you have just observed.

Your lab write-up should include the following:

1) a drawing of your dissection (4 pts)
2) your completed data matrix (4 pts)
3) your phylogenetic tree with characters mapped on it (4 pts)
4) your 5 typed questions and answers (8 pts)

Questions:

1. (1 pt) Which characters (if any) are synapomorphies for chordates?

2. (1 pt) Which characters (if any) are synapomorphies for mammals?


4. (2 pts) What characters exhibit homoplasy, and therefore may have evolved due to convergent evolution?

5. (2 pts) List two additional characters that you observed (that are not listed in your table) that are synapomorphies for bony fish.
Data matrix in which to record observations

Record your data as "present" or "absent". You can simplify data entry by using an "X" if present and a "0" if absent.

<table>
<thead>
<tr>
<th>Taxa (species)</th>
<th>Internal Skeleton</th>
<th>Cartilaginous Skeleton (as opposed to bony)</th>
<th>Jaws</th>
<th>Four Limbs</th>
<th>Heart</th>
<th>Lungs</th>
<th>Liver</th>
<th>Bilateral Symmetry</th>
<th>External Spines</th>
<th>Eyes</th>
<th>Eyelids</th>
<th>Wings</th>
<th>Ribs</th>
<th>Ears</th>
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<tbody>
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</table>
Phylogenetic tree of some deuterostome relationships

- sea star
  - sea urchin
- lamprey
  - perch
  - goldfish
- frog
  - rat
  - bat
  - lizard
  - bird
  - turtle

embryo develops anus first, mouth second
Lab 7: Photosynthesis and Plant Pigments

Background on Photosynthetic Activity

It has been said that photosynthesis is the most important biochemical process that occurs on earth, and that Rubisco, the key enzyme that catalyzes the carbon fixation stage of photosynthesis (the Calvin Cycle), is the most abundant enzyme on earth. Why is this process considered so important? All forms of life require the capture of energy from the environment and the storage of this energy in chemical bonds from which it can be retrieved when required. Photosynthesis is the process that initiates this transfer for the vast majority of organisms, whether they are plants conducting photosynthesis, animals that consume these plants (or that consume animals that once consumed plants), or other organisms that consume living or decaying plants and animals. In this lab, you will examine in detail part of the process by which energy is harvested from light for incorporation into the bonds of carbohydrate molecules.
From Fig. 10.2 in P. H. Raven et al. (2005), *Biology*. McGraw-Hill, Boston.

The net result of photosynthesis is often described by this equation:

\[ 6 \text{CO}_2 + 12 \text{H}_2\text{O} + \text{light} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{H}_2\text{O} + 6 \text{O}_2 \]

However, photosynthesis actually occurs in two linked but physiologically distinct steps: the light-harvesting electron transport stage, and the carbon-fixing Calvin cycle (see figure on previous page). Today’s lab will focus on the first stage in which energy from visible light is captured by a series of electron transfers among molecules in a precise series (the electron transport chain). This electron transport stage transfers light energy to chemical bonds in **ATP** and **NADPH**, both of which are used to fuel the carbon-fixation reactions.

The electron transport stage is composed of two linked complexes, Photosystem II and Photosystem I. These **photosystem** complexes are located in the thylakoids of chloroplasts. Molecules embedded in the **thylakoid** membranes include pigments (such as chlorophyll) that absorb energy from light that strikes the chloroplast, enzymes that catalyze electron (and, thereby, energy) transfer, and molecules that readily accept or donate electrons. The molecule at the end of the photosystem chains that ultimately receives the energized electron is **NADP**\(^+\), which when bound to two such electrons picks up a hydrogen ion and becomes **NADPH**.
Electron transfer is not a perfect mechanism for energy conversion—some energy is lost at every point of transfer. As an electron is passed from one transfer molecule to another, it gradually loses a fraction of its energy in each step. This "lost" energy is actually used to drive the attachment of a free phosphate ion to ADP, forming ATP. The transfer of energy from light to a phosphate bond in ATP is called photophosphorylation. Both the electron chains that follow Photosystem II and I involve photophosphorylation.

The electron lost in Photosystem I to NADPH is replaced by one from Photosystem II. An electron lost in Photosystem II is replaced by one stripped from a water molecule, which is split into protons and oxygen in the process. Thus, both water and a new input of light energy are needed for Photosystem I to operate again. The energy stored in the bonds of ATP and NADPH produced by the light reactions is used in the Calvin cycle. The net result of the two photosystem reactions is represented by the following chemical equation:

$$H_2O + ADP + P + NADP^+ \rightarrow O_2 + ATP + NADPH + H^+$$

Given the light reaction equation above and the overall net photosynthesis equation given at the top of page 286, you should be able to discern the simplified chemistry that constitutes the net outcome of the Calvin cycle.

Introduction to today’s lab exercise

In this lab, you will recreate the Hill reaction. In 1937, Robert Hill demonstrated that energy acquisition from light and electron transport could operate independently from carbon fixation in photosynthesis. This discovery contributed greatly to the understanding that photosynthesis took place in the stages that were described above. Hill observed that O2 is produced by chloroplasts in the presence of light, even when no carbon is fixed. In the Hill reaction, a dye is added to a test tube containing chloroplast membranes that have been isolated from plant cells in order to demonstrate that electron transport is responsible for the conversion of light into chemical energy. This blue dye is called 2, 6 dichlorophenolindolphenol for long (or DCPIP for short). DCPIP takes the place of electron receptors, like NADP+, in the electron transport chain. When DCPIP accepts electrons, it becomes reduced to DCPIPH2, which is colorless. Thus, the electron transport activity of the photosystems can be measured by the rate at which blue DCPIP is converted to colorless DCPIPH2. The net result of the photosystem reactions in the presence of DCPIP is represented by the following chemical reaction:

$$H_2O + DCPIP \text{ (blue)} \rightarrow DCPIPH_2 \text{ (colorless)} + \frac{1}{2} O_2$$

For additional background information, refer to your textbook (Chapter 10).
Terminology:
To successfully complete this lab, you should be able to define the following terms:

- absorbance
- ATP
- carbon fixation
- colorimeter
- cuvette
- DCPIP
- enzyme
- Hill reaction
- linear regression
- NADPH
- photophosphorylation
- photosynthesis
- photosystem
- thylakoid

Objectives:
1. Learn the use of controls in a scientific experiment.
2. Learn to interpret scientific data using graphical analysis.
3. Estimate the photosynthetic rate of isolated thylakoid membranes using a dye indicator.
4. Estimate the effects on photosynthetic rate of applying darkness and denaturing the thylakoid membranes.

Materials:
- flood lamp
- 2-liter beaker
- fish bowl
- beaker of tap water
- 1L beaker of tap water
- isolated thylakoid membranes (must be kept on ice, in the dark)
- DCPIP solution (250 M) (Use with care!)
- latex gloves
- 1 beaker of ice bucket
- 150 ml test tubebeaker
- beaker for waste
- transfer pipettes
- marking pen
- aluminum foil
- colorimeter
- colorimeter cuvettes
- beaker for waste

Procedure:
1. Each table will work as a team.
2. Obtain the following materials for each team:
   3. Mark the 6 cuvettes (ribbed side only) and corresponding lids as follows:
      - U (unboiled)
      - UC (unboiled control)
      - D (dye only)
      - F (foil-covered cuvette)
      - B (boiled)
      - C (clear: blank for spec)

4. Start the computer program LoggerPro. Open the file “Biology with Vernier.” Select “07 Photosynthesis.” LoggerPro should automatically recognize the colorimeter if it is plugged and then open the corresponding experiment. A meter window will display the absorbance readings from the colorimeter. Set the wavelength to 635 nm on the colorimeter.

5. Calibrate the colorimeter by following these steps:
a. Prepare a “blank” by filling the cuvette with the lid marked C with DI H₂O. This cuvette is a reference to which all experimental samples are compared.

b. Clean and dry the outside of the cuvette with tissue/kiwi wipe.

c. Handle cuvettes only by the top edges of the ribbed sides.

d. Tap the side of cuvettes to remove any bubbles that may be present.

e. Always position the cuvette in the colorimeter with its reference mark facing toward the white mark to the right of the cuvette slot.

f. Press and hold the CAL button on the colorimeter until the red LED begins to flash. Release the button. When the LED stops flashing, calibration is complete.

g. Unless directed otherwise, never invert cuvettes.

6. Place the water-filled beaker/fish bowl/beaker in front of the flood light. This will be your light source for the Hill reaction; the beaker will act as a heat shield so that your experimental tubes receive light, but not heat. Be sure to position the cuvettes so that they receive equal amounts of light directly behind the fish bowl/beaker. Do not turn on the light until your experimental tubes are ready.

7. Obtain boiled and unboiled thylakoid suspensions. Gently swirl before transferring to cuvettes.

8. Prepare five experimental cuvettes according to Table 1, below. Add DCPIP and DI H₂O to all cuvettes first, then quickly add thylakoid suspension to each. Chloroplasts should always be added to each cuvette LAST, and only when the group is completely prepared for the following procedures.

Note 1: Use the appropriate transfer pipette for each suspension (boiled and unboiled and for the DCPIP do not cross-contaminate).

Note 2: Thylakoid suspensions are measured in drops, whereas the DCPIP is measured in mL (see gradations on the side of the pipette).

Note 3: ALWAYS WEAR GLOVES when using DCPIP.

Table 1: Summary of experimental cuvette contents.

<table>
<thead>
<tr>
<th>Tube</th>
<th>DCPIP (mL)</th>
<th>DI H₂O</th>
<th>Thylakoid Suspension(drops)*</th>
<th>Foil?</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>2.5</td>
<td>0</td>
<td>1 unboiled</td>
<td>No</td>
</tr>
<tr>
<td>UC</td>
<td>0</td>
<td>2.5 mL</td>
<td>1 unboiled</td>
<td>No</td>
</tr>
<tr>
<td>D</td>
<td>2.5</td>
<td>1 drop</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>F</td>
<td>2.5</td>
<td>0</td>
<td>1 unboiled</td>
<td>Yes</td>
</tr>
<tr>
<td>B</td>
<td>2.5</td>
<td>0</td>
<td>1 boiled</td>
<td>No</td>
</tr>
</tbody>
</table>

*1 drop is generally enough; if the reaction is weak, use more in following trials.

9. As quickly as possible, but only as the solutions are prepared (i.e. as soon as suspensions are added), mix each solution without introducing bubbles by gently inverting the cuvettes several times (do not mix later!). Read the absorbance of each sample with the colorimeter. Be sure to handle the cuvettes carefully. Remember to take the foil off the F tube for its reading, and then replace it.
10. Place each tube on the side of the beaker away from the lamp and turn on the lamp now (not before). Record the time or start a timer.

11. Read the absorbance (abs) for all 5 tubes every 2 minutes, for a total of 12 minutes. Record the measured absorbance below:

<table>
<thead>
<tr>
<th>Tube</th>
<th>0 min. (abs)</th>
<th>2 min. (abs)</th>
<th>4 min. (abs)</th>
<th>6 min. (abs)</th>
<th>8 min. (abs)</th>
<th>10 min. (abs)</th>
<th>12 min. (abs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>UC</td>
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<td></td>
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<tr>
<td>D</td>
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<tr>
<td>F</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
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</tr>
</tbody>
</table>

12. Repeat all procedures for a complete replicate of the experiment. Record data below:

<table>
<thead>
<tr>
<th>Tube</th>
<th>0 min. (abs)</th>
<th>2 min. (abs)</th>
<th>4 min. (abs)</th>
<th>6 min. (abs)</th>
<th>8 min. (abs)</th>
<th>10 min. (abs)</th>
<th>12 min. (abs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
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<tr>
<td>B</td>
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</tr>
</tbody>
</table>

13. Repeat all procedures for a third replicate of the experiment. Record data below:

<table>
<thead>
<tr>
<th>Tube</th>
<th>0 min. (abs)</th>
<th>2 min. (abs)</th>
<th>4 min. (abs)</th>
<th>6 min. (abs)</th>
<th>8 min. (abs)</th>
<th>10 min. (abs)</th>
<th>12 min. (abs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Clean-up:
Dispose of all liquids in the **silver-red waste container**, not down the sink.

Analysis:
1. Enter your data in an MS Excel spreadsheet, following the instructions of your TA. For each of the three trials, create a single graph that illustrates the absorbance measured at each time period, for each of the 5 treatments.

2. Calculate the rate of photosynthesis for each of the five treatments by following these steps:
   a. Follow the instructions of your TA to calculate a **linear regression** (best-fitting line) for each of the 5 treatments. This line will estimate the rate at which absorbance is changing over the course of the experiment.
b. In the following table, record the slope of the regression, \( m \), which is the rate of change in color of the solution.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slope (abs/min) Trial 1</th>
<th>Slope (abs/min) Trial 2</th>
<th>Slope (abs/min) Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>D</td>
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<td>B</td>
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</tbody>
</table>

c. Your calculated slope is measured in absorbance per minute. This rate is proportional to the rate of photosynthesis. (Why is this?) You can easily convert the rate of color loss to the actual photosynthetic rate (measured as milimoles of \( \text{O}_2 \) produced per minute) by dividing each value by the following factor: -0.3125 abs/mmol. Record the converted data below.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slope (mmol ( \text{O}_2/\text{min} )) Trial 1</th>
<th>Slope (mmol ( \text{O}_2/\text{min} )) Trial 2</th>
<th>Slope (mmol ( \text{O}_2/\text{min} )) Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
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<td></td>
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<tr>
<td>B</td>
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</tbody>
</table>

Photosynthesis Questions:

1. (2 points) Recreate your completed data tables in Excel (5 total) and identify the treatment that demonstrated the highest photosynthetic rate. Explain why you think this is.

2. (4 points) Provide a graph that demonstrates the rate of photosynthesis for the treatment you named in #1, using data from any one of your three trials. Label and title your graph, and report the slope of the best-fit line. (This graph must be recreated in Excel on a full page, printed, and attached as a separate page).

3. (2 points) What was the purpose of running this experiment on thylakoids with no dye (UC) and dye without thylakoids (D)?

4. (2 points) What effect did boiling have on photosynthetic rate? Explain why you think this occurred.

5. (2 points) What effect did darkness (foil) have on photosynthetic rate? Explain why you think this occurred.

Background on Plant Pigments Activity
Paper chromatography is a technique that is commonly used to separate and identify pigments from cell extracts that contain a complex mixture of molecules. In this activity, you will use paper chromatography to determine if a spinach leaf contains only one pigment or a mixture of pigments. This is done by first grinding up the spinach leaves with a mortar and pestle. A 1:1 acetone-hexane solvent mixture is used, along with about 1 gram of sand. The sand is used to help break up the cell walls so that the solvent can get to the pigments inside. After the spinach solution is decanted, a line of spinach pigment is placed near one end of a sheet of chromatography paper. After the pigment is transferred onto the chromatography paper, the paper will be placed into a jar containing a (highly volatile) solvent mixture of petroleum ether and acetone. The solvent will move up the paper by capillary action, which occurs as a result of the attraction of the solvent molecules to the paper and with the attraction of the solvent molecules with one another.

Four pigments are widely found in leaves: carotene, xanthophyll, chlorophyll a, and chlorophyll b. Carotene is an orange pigment, but may appear as a yellow band on the chromatography paper. Beta carotene is the most abundant carotene found in plants, and is carried near the solvent front because it is highly soluble in the solvent and because it forms no hydrogen bonds with cellulose. Xanthophyll differs from carotene in that it contains two hydroxide (\(-\text{OH}\)) groups. Xanthophyll will also appear as a yellowish band on the chromatography paper, but this pigment is found farther from the solvent front because its movement is slowed down by hydrogen-bonding with cellulose, and it is less soluble in the solvent. The chlorophylls contain both nitrogen and oxygen, binding them more tightly to the paper than the other pigments. Chlorophyll a is the primary photosynthetic pigment in plants.

**Materials:**
- chromatography jar w/lid
- chromatography paper
- mortar and pestle
- ruler
- pencil
- spinach leaves
- transfer pipette
- 1:1 acetone-hexane solution
- 9:1 petroleum ether-acetone solution
- 10mL graduated cylinder
- sand
- 50ml beaker

![Diagram showing the process of paper chromatography](image)
Procedure:

1. Obtain a 4 x 8 cm piece of thin layer chromatography paper and draw two pencil marks 1.5 cm from the bottom.

2. PUT ON GLOVES.

3. Pour 10 mL of the pre-mixed 1:1 acetone-hexane solution, along with a pinch of sand into your mortar. Add a couple healthy looking spinach leaves. Use your pestle to grind up the leaves. **Try not to directly inhale over the mortar.

4. Once the leaves are sufficiently macerated, use a transfer pipette to decant the top organic layer of the solution. Place this into a 50 mL beaker and then put the beaker under the fume hood so that some of the solvent will evaporate.

5. When your beaker appears to have about 2 mL of solution in it, use a transfer pipette to move this liquid from the beaker to the chromatography paper. Be sure to follow your 1.5 cm marks and make a straight line with the spinach solution all the way across the paper.

6. Place the chromatography paper in the jar so that the pigment-streaked end of the paper is barely immersed in the solvent. **There should be about 5 mL of the 9:1 petroleum ether-acetone solution in the bottom of the jar. If there’s not, please add 5 mL. The pigment stripe itself should not be in the solvent!* **Avoid inhaling the fumes from the solvent.

7. Tightly cap the jar. Place jar on a flat surface and do not disturb for several minutes. Carefully observe the chromatography paper within.

8. When the solvent appears to be about 1 cm from the top margin of the paper, remove the paper from the jar and mark with a line the location of the solvent before it evaporates.

9. Mark the bottom of each pigment band.

10. Use a ruler to measure the distance traveled by the solvent front and each pigment band, starting from the origin line. Record these results in Table 1. Number the bands so that Band 1 is the pigment band nearest the origin line.
Table 1:
Chromatography of Plant Pigments

<table>
<thead>
<tr>
<th>Solvent Front</th>
<th>Band #</th>
<th>Distance from Origin (mm)</th>
<th>Band Color/Identification</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Analysis:

For a given solvent and substrate system (in this case, cellulose is the substrate and petroleum ether-acetone is the solvent), each pigment will move a distance that is proportional to the distance moved by the solvent. This is expressed as the Rf (Retention factor) value, and is a constant for the solvent/substrate/pigment. For example, suppose that a pigment moves 60 mm while the solvent moves 100 mm. Then,

\[
Rf = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}
\]

\[
Rf = \frac{60 \text{ mm}}{100 \text{ mm}} = 0.60
\]

1. Calculate Rf values for each pigment band you have identified and record these data in Table 1 (recreate table 1 in Excel for question 1 on the following page).

2. Using the data you have collected, make a tentative identification of the chlorophyll band(s) and other major bands on your chromatography paper. Record these in the “Band Color/Identification” column of Table 1.

3. Sketch the chromatography paper on a blank piece of paper to hand in with your lab report (Question 6 on the following page).
Chromatography Questions:

6. (4 points) Sketch your chromatography data on a blank piece of paper (or in the space above) and hand the drawing in with the rest of your lab report. Recreate your completed data table in Excel and include it in your report. Label the color/identification of the various bands and their respective Rf factors. The solvent front, or leading edge, should be at the top. The pencil line where the pigment was added originally should be at the bottom. Give your diagram an appropriate, descriptive title.

7. (2 points) Recall that carotene and xanthophyll have very similar molecular structures except for the presence of two hydroxide groups in xanthophyll. Use this information to explain the difference in Rf values for these two pigments given the substrate/solvent combination employed in this exercise.

8. (2 points) Given your understanding of how deciduous trees behave in the autumn, which of these pigments tend to be retained the longest before leaves fall? How do you expect photosynthesis rates change during this time? Explain your answer by employing your observations from both of the exercises completed in lab this week.
Laboratory 8: Plant Vegetative Structure, Function, & Development

Background

In this lab, you will be investigating the basic structure and function of the most species-rich group of vascular plants—namely, the **angiosperms**, or flowering plants. However, all the cell types and tissue types discussed and examined here are typical of all vascular plants. Although there is less organismal complexity in the non-vascular plants, similar functions are achieved (as needed) through similar but not necessarily homologous modifications of form. In vascular plants, there are three tissue systems: ground, dermal, and vascular. Bryophytes (the non-vascular plants) have only ground and dermal tissue systems.

Simple cell types of the ground tissue typical of flowering plants:

**Parenchyma**

*Parenchyma* cells are living, undifferentiated, generally thin-walled cells that may vary in size and shape. These are the most abundant cell type; found throughout the body of a plant. Primary functions may involve photosynthesis or storage, depending largely on the location within the plant.

**Collenchyma**

*Collenchyma* cells are elongated, thick-walled cells. Walls are often fortified with extra *pectin*, a structural polysaccharide that provides support and that helps bind adjacent plant cells to each other. *Collenchyma* helps support plant stature, particularly in young or herbaceous plants and in *petioles*, the short stalk-like portion of the leaf. Clusters of these cells are often found just beneath the epidermis, in "ribs" or along the entire periphery.

**Sclerenchyma**

*Sclerenchyma* cells have a thick, lignified *secondary* wall. Sclerenchyma may or may not be living at functional maturity. Clusters or rings of elongate sclerenchyma cells form supporting *fibers*. Fibers are generally found in a thin concentric ring around the vascular bundle or as clustered fibers exterior to the vascular bundle. Sclerenchyma may also be found sporadically amid parenchyma as *sclereids*; these cells tend not to elongate and instead form hardened “stones” scattered throughout some plant organs in some species.
Vascular tissue systems and component cells typical of flowering plants:

In all vascular plant species examined to date, xylem (the water-conducting tissue) and phloem (the sugar-conducting tissue) occur together in bundles that connect leaf, stem, and root organs. Vascular bundles may be few or plentiful depending on the organ in which they are found and on the age of the plant, and their arrangement within the stem is a distinguishing characteristic often used to classify fossil specimens to their appropriate phyla (especially since xylem in particular is well-preserved in the fossil record).

**Xylem: The water-conducting tissue:**

Xylem walls are lignified which provides a waterproof channel for water to move through and increases the support of above-ground structures. Furthermore, xylem are empty (dead) at functional maturity. Side walls of xylem elements often contain pits (small pore-like areas lacking secondary walls). The conducting cells are often interspersed with fibers and/or parenchyma. In longitudinal sections, cells of the xylem appear to have rings or spirals of lignin (xylem formed prior to elongation of the stem) or solid lignin walls with scattered pits (late-formed xylem).

**Tracheids** are cells with vertical ends that are generally strongly tapered. Individual tracheids are connected longitudinally at their top and bottom end walls by **pits**.

**Vessel elements** have only mildly tapered end walls (at top and bottom of the element). Cells connect at to each other longitudinally via ruptures in the cell wall called **perforations**. Vessel elements are considerably thicker in diameter than tracheids.

**Phloem: The sugar-conducting tissue:**

**Sieve tube elements** are elongated cells connected vertically by porous sieve plates. These cells are living at functional maturity but lack nuclei, ribosomes, and cytoskeletal elements.
**Companion cells** are small specialized parenchyma cells, each connected to a single sieve tube element by multiple plasmodesmata. These function in “loading” phloem from nearby parenchyma into sieve tubes and in supporting sieve cell metabolism. Companion cells are narrower in diameter and drastically shorter in length than sieve tube elements. Interestingly, a sieve tube element and its companion cell arise from a single unequal mitotic division of the same parent meristem cell.

---

**Monocots vs. Dicots**

Most angiosperms are **dicots** (loosely classified in the Dicotyledonae, although this group is not monophyletic). Dicot embryos contain two “seed leaves,” or **cotyledons**. Cotyledons are storage structures developed during embryonic growth before the seed has germinated (sprouted). Cotyledons nourish the young plant during its first week or so of life after germination, giving it the energy it needs to grow large enough to effectively photosynthesize and to meet its own metabolic needs. The remaining angiosperms are **monocots**, belonging to the monophyletic Monocotyledonae. Monocot embryos have only a single seed leaf. Dicots and monocots differ in a number of other important features, many of which should become apparent as you work through today’s lab exercises. Some (but not all) other distinguishing characteristics of monocots and dicots are listed below.
In monocots, there are usually a number of major leaf veins that run parallel to each other along the length of the leaf. In dicots, there are usually numerous axillary veins that reticulate between the major veins, appearing netlike.

In most dicots (and in most seed plants) the root develops from the lower end of the embryo, growing from the root apical meristem, and forming a distinct dominant taproot. By contrast, the taproot aborts in monocots, and new roots arise adventitiously from nodes in the stem. This is what is called a fibrous root system.

If you count the number of petals, stamens, or other floral parts, you will find that monocot flowers tend to have a number of parts that is divisible by three. Dicot flowers on the other hand, tend to have parts in multiples of four or five (four, five, ten, etc.). This character is not always reliable, however, and is not easy to use in some flowers with reduced or numerous parts.

*An epicotyl is the portion of the embryonic shoot above where the cotyledons attach.*
Terminology

To successfully complete this lab, you should be able to define the following terms:

- blade
- chloroplast
- collenchyma
- companion cell
- cortex
- cystoplasmic
- streaming
- dermal tissue
- dicot
- epidermis
- fiber
- fibrous root
- ground tissue
- lateral meristem
- lignin
- monocot
- parenchyma
- pectin
- perforation
- petiole
- phloem
- plasmodesmata
- plasmolysis
- pit
- pith
- root
- root hair
- sclereid
- sclerenchyma
- shoot
- sieve tube element
- stele
- taproot
- tracheid
- trichome
- vacuole
- vascular bundle
- vascular cambium
- vascular tissue
- venation
- vessel element
- xylem

Materials

- Compound microscope
- Slides and coverslips
- Razor blades
- Dissecting microscope
- Dissection kit
- Diagrams
- Plant materials
- Stains (phloroglucinol, aniline blue)
- Prepared slides

Procedure

Work in PAIRS. Prepare your own cross sections of living material provided. Directions on how to properly (and safely) prepare sections will be provided by your TA. Mount sections on a glass slide, and choose either of the staining techniques below. Except where otherwise noted, be sure to produce enough sections from each specimen to stain some with the first stain and others with the second stain. Place a coverslip over the specimen, and examine your sections on the compound microscope.
Staining Methods:

a. **Phloroglucinol**: Place the section on a slide, and add 1-2 drops of phloroglucinol solution on top. Wait 2-4 minutes. Add additional water to the mount if necessary. Phloroglucinol stains **lignin** red. Sclerenchyma cells and the walls of the xylem contain lignin. *Note: this stain contains a small amount of hydrochloric acid to render it effective. Wash your hands thoroughly if you come in direct contact with it.*

b. **Aniline Blue**: Soak sections in a pool of stain for 5 minutes. Remove sections from the stain using fine forceps, then rinse sections by dipping them in clean water. Mount sections in water before adding the coverslip. Aniline blue stains **callose**, a minor component of most cell walls. Depth/darkness of stain is indicative of callose concentration. Callose accumulates at the sieve plate in the phloem.

Make detailed drawings and label the features you see. Note the level of magnification you employ, the approximate size of major anatomical features, and the color of structures depending on the stain you use. You may use the prepared slides to orient you to your hand sections and to elucidate anatomical detail, but your drawings must correspond to the specimens dissected in lab today. Features you should note are listed for each of the specimens separately.

Sunflower Stem: Note the location of the three cell types, the arrangement of the vascular bundles, and the arrangement of vascular tissues within those bundles. Also note the position and relative frequency and size of the **trichomes** (modified elongate cells of the dermal tissue).
Iris Stem: Note the location of the three cell types, the arrangement of the vascular bundles, and the arrangement of vascular tissues within those bundles.

Celery stalk: Note the location of the three cell types, the arrangement of the vascular bundles, and the arrangement of vascular tissues within those bundles. Make particular note of the location of collenchyma.
The sunflower is a representative dicot. Describe the arrangement of vascular tissues that is typical of dicot stems. What other typical dicot features are apparent on the sunflower? (1 point)

1. The iris is a representative monocot. Describe the arrangement of vascular tissues that is typical of monocot stems. What other typical monocot features are apparent on the iris? (1 point)

2. The iris is a representative monocot. Reference your drawing and describe the arrangement of vascular tissues that is typical of monocot stems. What other typical monocot features are apparent on the iris? (1 point)

3. Based on the vascular tissue arrangement from your drawing, would you hypothesize that the celery as a monocot or a dicot? (1 point)

4. Celery has very small flowers clustered into an inflorescence called an umbel. Based on what you noted about flower morphology in the sunflower and the iris, what do you predict would be the morphology of an individual celery flower? (1 point)

5. If you have ever eaten celery, you have likely “experienced” its collenchyma. What do you notice about the texture of collenchymas when you eat it? (1 point)

6. A celery stalk is actually a very long petiole. Reference your drawing and answer why do you think collenchyma is so prevalent here? In other words, what function does it serve to the plant? (2 points)

Parsnip root: Note the arrangement of vascular bundles and the arrangement of vascular tissues within those bundles. This vascular arrangement is the ancestral condition—all roots of all vascular plants share this arrangement, and this pattern is also apparent in the stems of both fossil and extant lycophytes.
| Aniline Blue | Phloroglucinol |
Many fossil lyocphytes were tree-sized plants. It is hypothesized that seed plants now outnumber lycophytes because of the adaptive advantage of the seed, but there may also be an adaptive advantage to novel vascular tissue arrangement. What might be advantageous about the arrangement of vascular tissues in the dicot stems compared to the ancestral condition? Defend your answer. (2 points)

8. Roots *might* all share this “ancestral” arrangement of vascular tissue because the ancestral condition is selectively favored in the root. What might be a reason that root organs with this phenotype provide a fitness benefit to the plant relative to the other arrangements you have observed today? (1 point)

9. Roots might instead have retained the ancestral conditions for some other reason. Postulate one such reason. Explain your answer. (2 points)

Apple and Pear fruits: Prepare thin hand sections of the fruits and stain them with lignin (not aniline blue), then view each with the compound microscope. *Compare* the cellular anatomy of the fleshy tissues of the apple and pear fruits.

<table>
<thead>
<tr>
<th>Apple</th>
<th>Pear</th>
</tr>
</thead>
</table>

10. *Reference your drawing.* What cell type is most prevalent in both fruits? Why, functionally, is this important to fruit function in these species? (1 point)
11. If you have ever eaten these fruits, you have likely “experienced” the cellular difference between them. What do you notice about the texture of a pear as compared to the texture of an apple? What anatomical difference explains this textural difference? Explain. (2 points)

Bean and Corn Seeds and Seedlings: Carefully dissect the seeds to find the embryo within. Identify the cotyledon(s) in each, plus the stem/root axis and the first “true” leaves of the embryos themselves. Dissect, section and stain, uproot, and otherwise “sample” both seeds and seedlings to obtain the most thorough data for answering the questions below.

12. Which seed—bean or corn—is a dicot and which is a monocot? (1 point)

13. Draw and label all the evidence apparent from the seeds and from the seedlings that substantiates your conclusion. Use the next page of the handout for these drawings and hand it in with your answers to questions 1-12, or copy your drawings neatly onto a separate sheet of paper. (4 points)

*Turn in all of your drawings with your lab write up next week. They will contribute to your overall score.*
Laboratory 9: Environmental Correlates of Leaf Stomatal Density

Background

Phenotypic Plasticity

Plants almost without question are literally stuck in the place where they are rooted—they cannot move away or hide if environmental conditions are not to their liking. This means that plants must deal with their circumstances in the moment and that they should also have the ability to deal with the changing circumstances from day to day, or across their lifetimes. When whole populations adapt, as a unit, to the selective forces of their environment, this is evolution. When an individual alone adapts its environment, it is exhibiting phenotypic plasticity. Likewise, natural selection chooses among existing phenotypes but results in alterations in population-level frequencies of genotypes. Plastic adaptations, on the other hand, are not heritable. However, it has been widely demonstrated that the ability to plastically adapt to environmental conditions can be genetically determined (some plants have narrow environmental tolerances, others have broad environmental tolerances). Therefore, somewhat paradoxically, differences in plastic ability can be driven by natural selection—ie., plastic ability can evolve.

Stomatal Density

Leaf stomata are the principal means of gas exchange in vascular plants. Stomata are small pores, typically on the undersides of leaves, that are opened or closed under the control of a pair of banana-shaped cells called guard cells. When open, stomata allow CO\(_2\) to enter the leaf for synthesis of glucose, and also allow free oxygen, O\(_2\), to escape. When stomata are open, molecules of water will also escape. In addition to opening and closing the stomata (stomata behavior), plants may exert control over their gas exchange rates by varying stomata density in new leaves when they are produced (usually in the spring or summer). The more stomata per unit area (stomatal density) the more CO\(_2\) can be taken up, and the more water will be released. Thus, higher stomatal density can greatly amplify the potential for behavioral control over water loss rate and CO\(_2\) uptake. Stomatal density is determined during leaf formation, but will not vary within that leaf as it ages. Leaf size is also predetermined—the young leaf emerges from the bud almost fully grown, and it will not grow further after it has unfurled.

Why might it be adaptive for a plant to control its rates of water loss and CO\(_2\) uptake? Generally, plant photosynthetic apparati are only designed to function well over a rather narrow range of temperatures. When heated, cytochromes, pigments, and membranes critical to phosphorylation (ATP regeneration) and to carbon fixation rapidly denature (i.e., they cook). To avoid this, an individual plant may open its stomata and allow the evaporation of water from the leaf surface. Evaporation of water will lower the
leaf temperature. Thus, one may hypothesize that leaves in the sun should have higher stomata density than do leaves in the shade—all else being equal.

But, on the other hand, if water is not available, such as under drought conditions, excessive evaporation might lead to desiccation and an equally severe disruption of photosynthetic function. Thus, one might expect plant leaves exposed to drought conditions to have fewer stomata in sunlit environments.

The above discussion illustrates a very important concept in experimental biology—there are often competing alternative hypotheses to explain the variation observed in nature. In this case, stomatal density may increase or decrease in response to environmental variation in sunlight and/or water availability.

**Before lab meets:**

Before lab meets, begin thinking about a specific environmental difference that might affect stomatal density and formulate a hypothesis about which way you would expect stomatal density to vary and why. To help you think about possible hypotheses and better understand the factors affecting stomatal density, begin thinking about the three questions below.

As you will see in this lab activity, plants confronted with different environmental conditions vary the number of stomata per unit area by quite a lot. Yet, in theory, the same result due to having more stomata could be attained by simply having bigger stomata with no difference in stomata number—however, plants vary stomata number and not stomata size. Why?

How might stomatal density serve as a bioindicator for estimating CO$_2$ concentrations in the past (paleoclimates) and in the future?

Given that plant leaves face a tradeoff between carbon dioxide uptake and evaporative water loss, speculate upon the “behavioral” features in stomata you would expect to evolve in plants adapted to dry environments with variable and unpredictable water supply. Might microgeographic differences correlate with stomatal density? If so, what environmental conditions might predict stomatal density (and in what pattern)?

**Go outside and collect ten leaves, total from two locations (five per location) appropriate to your choice of environmental variable. Locations can be different positions on one individual plant, or different plants of the same species in different locations. You should not, however, sample different species to assess environmental effects on plastic traits. (You should be able to logically explain why interspecific sampling would not be helpful in addressing a question about environmental effects and phenotypic plasticity. Discuss this with your group.)**
REVIEW: The Scientific Method

For the purposes of understanding plant biology in general, we are really interested in understanding what sorts of variables might cause differences in phenotypically plastic characters. This is an extremely broad question, however, so we have to narrow our approach to consider only one, particular, measurable phenotypic feature at a time. In this lab, the feature we are investigating is known to be plastic, is known to vary as a function of at least some aspects of the environment, and (conveniently) is really easy to measure. To proceed, though, we have to hypothesize what specific environmental parameter we think might affect this plastic character, and in what direction change in the plastic character will manifest. Your task this week is therefore to propose a suitable hypothesis about environment and phenotype, then devise an experiment that samples from two microhabitats that you think vary in the environmental parameter about which you hypothesize, carry out that experiment, analyze your data, accept or reject your hypothesis, then think critically about how what your experiment tells you about more general features of plant biology. The questions provided as a part of this lab handout (and which are your assignment for this week) are designed to guide you step-by-step through the application of the scientific method—the lead-up to the experimental design, the collection and analysis of data, and the interpretation of those data. As with all research, graphical representation of the data is key to aiding your own (and others’) understanding of it. Likewise, statistical rigor is required so that you may make more authoritative conclusions about the patterns you observe (or about the absence of patterns).

Statistics

Whenever we sample a population, we do so in order to estimate some average feature of a population without measuring every individual in it. Samples are not perfect representations of the whole, but the science of statistics helps us to estimate the confidence we have that our sample is a fair representation. When we sample two different populations, and we really want to know if they are different from each other, we must test to see if the average feature we estimate for the first population (and our confidence in that estimate) is indeed—statistically, significantly—different from our estimate of the average in the second. Note that statistical populations need not necessarily contain whole organisms: for example, the leaves at the top of the tree are distinct from and separated from the leaves at the bottom and can legitimately be treated as separate populations of leaves!

One way to analyze differences between two statistical averages (means) and our confidence in those means is by a t-test. T-test calculation and interpretation should be familiar to you, but if you need a review see the website below and click on "t-test for independent samples."

http://www.statsoft.com/textbook/basic-statistics
To run a t-test, you may either use the data analysis tools in Excel, or plug your data into this website:

http://faculty.vassar.edu/lowry/t_ind_stats.html

Terminology

To successfully complete this lab, you should be able to define the following terms:

- correlation
- p-value
- stoma (pl. stomata)
- cuticle
- phenotypic plasticity
- t-test
- guard cell
- pore
- statistical significance
- epidermis
- stoma (pl. stomata)

Materials

- Compound microscope
- Glass slides
- Plant materials
- Clear nail polish
- Clear packing tape

Helpful Calculation

Field of View:

**Mag: 10x objective = 100x total.**

Equation: \( A = \pi (r^2) \)

\( r = 1.8 \text{mm}/2 \)
Procedure

Work in groups. Together, decide upon a single hypothesis to address and design a sampling scheme to test your group’s hypothesis. Note that since you will not be measuring sunlight or water availability, you should use caution in how you word your hypothesis (and your acceptance or rejection of that hypothesis). Clear your approach with your TA, and answer these questions (#1-3) before proceeding.

1. What is your hypothesis about stomatal density as it relates to some environmental variable? (2 points)

2. What logical justification can you provide that your hypothesis is reasonable? (2 points)

3. How will you put your hypothesis to the test? In other words, what will you sample and why will comparing samples address the hypothesis? (2 points)

Go outside and collect ten leaves, total from two locations (five per location) appropriate to your choice of environmental variable. Locations can be different positions on one individual plant, or different plants of the same species in different locations. You should not, however, sample different species to access environmental effects on plastic traits. (You should be able to logically explain why interspecific sampling would not be helpful in addressing a question about environmental effects and phenotypic plasticity. Discuss this with your group.)

Bring out your leaves that you collected for back to the lab, and follow the instructions below to make leaf impressions in order to detect stomata on the leaf surface.

Method for obtaining and visualizing leaf impressions:

- Wash the leaf.

- Paint a rather thick swath of clear nail polish on the underside of the leaf. You will most likely see more features on this side, since the upper side (sun-facing side) often has a thicker cuticle than the underside.

- After the nail polish has dried (several minutes), obtain a square of very clear packing tape. Stick your tape piece to the area that contains the swath of dried nail polish.
- GENTLY, peel the nail polish swath from the leaf completely. You will see a cloudy
impression of the leaf surface now attached to the tape. This is the leaf impression.

- Tape the leaf impression onto a very clean slide and use scissors to cut off the excess
tape.

- Examine the leaf impression at up to 400x total magnification. No cover slip is
necessary. Focused, somewhat condensed light is helpful.

Next, quantitatively compare stomatal densities of leaves collected in different
environments. Statistically assess the validity of your hypothesis by comparing
differences in mean stomatal density between environments, using a t-test.
Answer the remaining questions.

4. Produce a suitable (hand drawn) graph to demonstrate the differences you have
observed. Hand the graph in today, attached to your answer sheet with the rest
of the questions from this lab. (4 points)

5. Is there a significant difference in stomatal density between the two environments
that you sampled? Statistically defend your answer. (4 points)

6. What can you qualitatively conclude about environmental variation and stomatal
density, and about developmental plasticity in general, as a result of your
experiment? (2 points)
7. Is the justification for your hypothesis warranted, now that you have analyzed your data? Why or why not? (2 points)

8. How would you modify your project design (sampling, analysis, etc.) in the future to get a more in-depth understanding of environmental effects on stomatal density or on developmental plasticity? (2 points)
Laboratory 10: Reproductive Structure and Function

Background:

In this lab, you will be investigating the structure and function of the distinguishing features of angiosperms—namely, the flower and the fruit.

Reproduction and the flower:

Heterospory, the production by the sporophyte generation of two morphologically distinct types of spores, is a necessary feature of all seed plants. The gametophyte generation in seed plants consists of unisexual individuals—male gametophytes that produce only sperm and female gametophytes that produce only eggs. Different gametophyte sexes arise from the different spore types, but heterospory is not a necessary condition for separation of the sexes in the next generation. (Recall that many moss species have unisexual gametophytes but are homosporous). Rather, heterospory in seed plants also provides for a system in which male function and female function are each maximized by the formation of distinct and separate microsporangia and megasporangia—highly specialized organs for the production of unisexual spores and the subsequent retention of male gametophytes and female gametophytes, respectively.

The sperm-producing microgametophytes (the male gametophytes) grow from microspores within chambers called microsporangia. Microsporangia in the flowering plants are located in the anthers, structures at the tips of the male floral organs called stamens (see floral diagram, below). The grown but sexually immature male gametophytes are called pollen grains. Pollen grains—whole male gametophytes—
are released from the anthers and transported by wind or by animal vector away from the parent sporophyte.

The egg-producing megagametophytes (the female gametophytes) grow from the megaspores that never leave the megasporangia that produce them. Megasporangia in the flowering plants are located in chambers within the ovary, itself located at the base of the female floral organ, the carpel (see the diagram below). The megagametophyte is called an embryo sac. The wall of the ovarian structure that encases the megasporangium and within which (eventually) the embryo sac grows, together with the megasporangium and the embryo sac itself, is called an ovule.

When a released pollen grain lands on the stigma of a receptive flower, it produces two sperm and begins to grow a hollow extension down toward the ovule. This extension is the pollen tube, and it will be used to deliver the sperm to the female gametophyte held in an ovule below. Two sperm are released, one of which will fuse with the egg to form the zygote and the other of which will fuse with two additional nuclei to form the nutritive endosperm. The zygote grows into a diploid, sporophyte embryo and the endosperm enlarges. The wall of the ovule, and the embryo sac now bearing the embryo and endosperm are collectively called the seed. Successful formation of the seed triggers cellular changes in the surrounding ovary, which ripens and matures to become the fruit.

In today's lab, you will review the overall structure of flowers and fruits. You will also closely examine the structure of stamens and carpels and how they function in the life cycle.
Life Cycles

Recall that all plants have the sporic life cycle, in which multicellular diploid sporophyte generations alternate with multicellular haploid gametophyte generations. In flowering plants, the gametophyte generation is so tiny as to be visible only with a microscope. Also, the gametophyte generation is retained by the parent sporophyte for all or part of its lifespan, so in many cases it is invisible. The importance of the gametophyte generation must not be understated, however—without the production of gametophytes, there would be no production of gametes, and hence no production of new sporophytes!

As part of your exercises today you will examine prepared slides containing male gametophytes and female gametophytes. Make note of where each is produced within the flower, and of how many can be produced by each flower. Typically, in discussing the reproductive fitness (or reproductive potential) of a flower, we talk about the production of male gametophytes as “male function” and the production of female gametophytes as “female function.” Some plants invest more than others in male function; other plants invest more in female function. Investment in both male and female function is intrinsically correlated to the number of flowers produced by an individual plant. Consider how natural selection might act on any of these floral characters independently or in concert. In other words, what might selection “favor” about the number of flowers produced or about relative investment in male versus female function?

Fig. 29.3 from P. H. Raven et al. (2005), Biology, McGraw-Hill, Boston
**Terminology:**

To successfully complete this lab, you should be able to define the following terms:

<table>
<thead>
<tr>
<th>Term</th>
<th>Term</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>achene</td>
<td>embryo</td>
<td>ovary</td>
</tr>
<tr>
<td>androecium</td>
<td>embryo sac</td>
<td>ovule</td>
</tr>
<tr>
<td>anther</td>
<td>fleshy fruit</td>
<td>perianth</td>
</tr>
<tr>
<td>berry</td>
<td>fruit</td>
<td>polar nuclei</td>
</tr>
<tr>
<td>calyx</td>
<td>gynoecium</td>
<td>pollen tube</td>
</tr>
<tr>
<td>corolla</td>
<td>haploid</td>
<td>seed</td>
</tr>
<tr>
<td>dehisce</td>
<td>legume</td>
<td>sperm</td>
</tr>
<tr>
<td>diploid</td>
<td>megagametophyte</td>
<td>stigma</td>
</tr>
<tr>
<td>double fertilization</td>
<td>megasporangium</td>
<td>style</td>
</tr>
<tr>
<td>drupe</td>
<td>megaspore</td>
<td>triploid</td>
</tr>
<tr>
<td>dry fruit</td>
<td>microgametophyte</td>
<td>zygote</td>
</tr>
<tr>
<td>egg</td>
<td>microsporangium</td>
<td></td>
</tr>
<tr>
<td>endosperm</td>
<td>microspore</td>
<td></td>
</tr>
</tbody>
</table>

**Materials:**

- Compound microscope
- Slides and coverslips
- Dissecting microscope
- Dissection kit
- Diagrams
- Plant materials
- Prepared slides
Procedures:

Observe each example of a reproductive structure of angiosperms, make drawings as requested, and answer the questions related to what you’ve observed.

Specimen: *Lilium*

I. Perianth

All the sepals of the flower are referred to collectively as the **calyx**; all the petals are referred to as the **corolla**. The collection of sepals and petals **together** is referred to as the **perianth**. Important aspects to note about the perianth of a flower are: 1) whether both sepals and petals are present, 2) the numbers of these structures, and 3) whether they are separate from one another or united into a single, lobed structure. Each of these important features may have important consequences for reproductive fitness. Because reproductive structures tend to be constrained by natural selection relative to vegetative characters, features of the perianth that seem similar in two species are most likely similar by virtue of shared ancestry—for this reason, characteristic features of a particular flower are the most reliable traits to use to determine the phylogenetic clade to which that particular species belongs.

Examine the perianth of the lily.

**DRAW (2 points):** Draw the features of the lily (*Lilium*) perianth. Label the parts of the **perianth**.

Questions:

1. Flowers are key to successful pollination in angiosperms, but modes of pollination vary greatly across species and are intrinsically correlated with floral morphology. Describe any notable features of the **form** of the lily perianth, including any markings that you observe on the petals. Hypothesize what you think might be the specific pollination-related **function** of the particular floral form of the lily you observe.

2. If the form you describe has the function you hypothesize, what would be the effect on an individual’s fitness of a mutation that marred or obliterated this aspect of form? How would a population in which this mutation arose evolve as a result? Explain.
Androecium

The stamens of the flower can be referred to collectively as the androecium. Single stamens are most often composed of a stalk with four sporangia at the apex. The stalk is called the filament. The sporangia and surrounding tissue are collectively referred to as the anther. Meiosis (spore production) and growth of the spores into pollen grains occur within the anther.

Examine stamens of fresh lily flowers as well as prepared slides of cross sections of lily anthers at two different stages of development. Some prepared slides show anthers containing spores; other slides show anthers containing pollen grains.

**DRAW (3 points):** Draw fresh stamens as seen with the dissecting scope. Label the filament and anther. Also draw two anther cross-sections depicting stages of development (and the production of pollen) as seen with the compound scope. Label the spores, if present, or pollen grains, if present, and indicate which of your two drawings is developmentally younger and which is older. Be sure to include the magnification at which the structures were observed.

**Questions:**
3. Which tissues that you’ve observed in the androecium are haploid (gametophytic)? Which tissues are diploid (sporophytic)?

III. Gynoecium

The carpels of the flower are referred to collectively as the **gynoecium**. The ovules, which contain the megasporangia, are contained within a chamber of the carpel called the **ovary**. Most carpels have a specialized region for receiving pollen called a **stigma**. The stigma usually has elongate epidermal cells that secrete a sticky substance. These traits improve the ability of the stigma to capture pollen from the wind or the body of an animal. Often, there is a narrow, elongate passage between the stigma and ovary called the **style**.

The ovule is a remarkably compact package of multiple generations of the angiosperm life cycle. The outer part of the ovule is a sterile (non-reproductive) sporophytic tissue surrounding the sporangium. Meiosis of only one cell occurs in the sporangium to produce four megaspores, only one of which survives. The megaspore undergoes only three mitotic divisions to produce a few-celled gametophyte, the **embryo sac**. All of the mitotic divisions of the embryo sac as it grows occur before cell membranes form to separate the nuclei. The membranes then to form seven distinct uninucleate cells plus an unseparated pair of nuclei (**polar nuclei**) that remain at the center of the embryo sac while the other cells migrate to the ends. The uninucleate cell closest to the opening of the ovule is the **egg**.
Examine carpels of fresh lily flowers whole and in cross and longitudinal sections. Also observe prepared slides of cross-sections of carpels, in which one (but only one) ovule contains a mature gametophyte.

**DRAW (3 points):** Draw whole and sectioned fresh carpels as seen with the dissecting scope. Label the **ovary, style, stigma, and ovules.** Also draw prepared carpel sections depicting **ovules** and **megagametophytes** as seen with the compound scope. Label the **polar nuclei** and the **egg** within the megagametophyte. Be sure to **include the magnification** at which the plant was observed.

**Questions:**

4. Which tissues that you’ve observed in the gynoecium are haploid (gametophytic)? Which tissues are diploid (sporophytic)?
Specimen: Unknown

Examine the perianth, androecium, and gynoecium of whole and dissected flowers of the other angiosperm specimens provided at your table. Make note of the features you think are most relevant to this other plant’s reproductive fitness.

Questions:

5. Identify one of the unknown flowers provided in lab, and describe how you think it and the lily differ in reproductive function. Defend your answer by describing the appropriate differences in form.

IV. Pollination

If a pollen grain is successfully transported to the stigma of a carpel, the microgametophyte will germinate (begin growing) and extend a pollen tube towards the ovule. Pollen tubes emerging from multiple pollen grains on the stigma are effectively in a race to deliver their sperm to the eggs inside ovules. Since there is only one egg inside each ovule of a flower, the degree of competition among pollen tubes depends on how many ovules there are in the ovary. Some species have many ovules per carpel, but others have only a single ovule. Competition for access to a single ovule could be quite intense if many pollen grains have landed on the stigma.
The winning pollen tube delivers its two sperm into the ovule and one of these unites with the egg to form a zygote. The second sperm fuses with the two polar nuclei. The fusion of these three nuclei is referred to as a second “fertilization” and angiosperms are thus said to have double fertilization. The fusion product is triploid (having three haploid genomes). It undergoes mitotic divisions (grows) and forms a tissue called endosperm, which is used as a food source by the growing embryo, or, later, by the young seedling.

From Fig. 21-19 in P. H. Raven et al. (1999), Biology of Plants. W.H. Freeman, NY
In addition to mediating the fertilization process and protecting ovules, the carpel has another important function in angiosperms. As the ovule matures into a seed following syngamy and the initial growth of the resulting sporophyte embryo, the carpel also matures into a modified structure. This structure, in all its various forms across the plant kingdom, is the fruit. The function of a fruit is mainly dispersal of the seed, although some fruits also serve to protect maturing seeds. At maturity, however, the seed often has a tough protective seed coat of its own (derived from the wall of the ovule itself).

Fruits may develop from carpels in various ways. Added sclerenchyma may make fruits rigid for seed protection or for the formation of structures that attach to animals. Sclerenchyma in the fruit wall may also cause fruits to dry and crack open, or dehisce, thereby ejecting seeds. Added sugar-rich pulpy parenchyma may make fruits palatable to animals, which eat them and defecate or regurgitate the intact seeds. Fruits are extraordinarily diverse in structure and function. You will observe fleshy fruits and dry fruits in lab today.

The two fruits provided at your table in lab are representative of the two most common fruit types among agriculturally important species. One fruit type is a
**drupe** (a generally fleshy fruit containing one large seed); the other type is a **berry** (a fleshy fruit that does not dehisce, containing many seeds).

Also examine two other fruits that have been placed on the back bench. These are both examples of dry (i.e., not fleshy) fruit types—a **legume** (a dry elongate fruit with multiple seeds that splits open along two seams when the fruit dehisces) and an **achene** (a dry single-seeded fruit, which generally will not dehisce).

**DRAW (2 points):** Draw examples of two dissected fleshy fruits: a **drupe** (one large seed) and a **berry** (many tiny seeds). Title your diagrams to indicate both the common name of the fruit and the type of fruit. Label the structures that have arisen from the **ovary** and those that have arisen from the **ovule**.

**Questions:**

6. Examine all the drupes and berries available in class today. (There is one of each type per table, and no two tables have the same things). Qualitatively contrast how you think drupes versus berries facilitate the dispersal of seeds.

7. What do you think is the type of fruit that is produced by the lily? Defend or explain your answer by employing relevant observations that you made in lab today.
TYPE HAND WRITE THE ANSWERS TO THESE QUESTIONS (worth 14-10 points), ALONG WITH YOUR FOUR LABELED DRAWINGS (worth 6-10 points).

Questions:

1. (2 points) Flowers are key to successful pollination in angiosperms, but modes of pollination vary greatly across species and are intrinsically correlated with floral morphology. Describe any notable features of the form of the lily perianth, including any markings that you observe on the petals. Hypothesize what you think might be the specific pollination-related function of the particular floral form of the lily you observe.

2. (2 points) If the form you describe has the function you hypothesize, what would be the effect on an individual’s fitness of a mutation that marred or obliterated this aspect of form? How would a population in which this mutation arose evolve as a result? Explain.

3. (2-1 points) Which tissues that you’ve observed in the androecium are haploid (gametophytic)? Which tissues are diploid (sporophytic)?
4. (2 points) Which tissues that you've observed in the gynoecium are haploid (gametophytic)? Which tissues are diploid (sporophytic)?

5. (2 points) Identify the unknown flower, and describe how you think it and the lily differ in reproductive function. Defend your answer by describing the appropriate differences in form.

6. (2 points) Examine all the drupes and berries available in class today (there is one of each type per table, and no two tables have the same things). Qualitatively contrast how you think drupes versus berries facilitate the dispersal of seeds.

7. (2 points) What do you think is the type of fruit that is produced by the lily? Defend or explain your answer by employing relevant observations that you made in lab today.