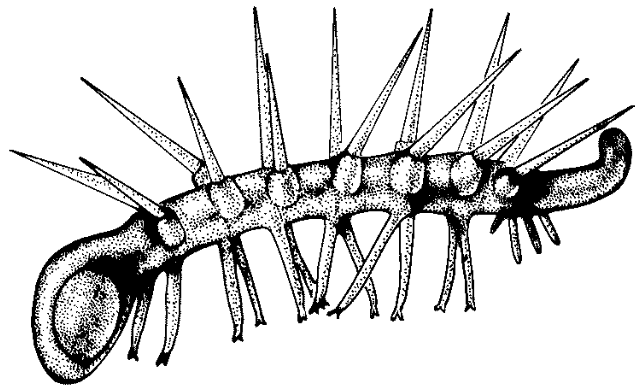


BIOL 111 —Laboratory Manual

The Faculty of MSU Department of Biology[1]

Spring 2017
Swain Hall, Room 304

[1] Links in this PDF are *active*, you may click on them.

Contents

Introduction

Sections & Attendance

If you have a legitimate reason (see the Syllabus), it is possible to come for the lab with different section. Instructor e-mail(s):

Ihli, Lori	lori.ihli@minotstateu.edu

Sections

BIOL	Section	Instructor	Class	Start	End	Day	Room
111	111-1	Lori Ihli	3281	3:00 pm	4:50 pm	Th	Swain 304
111	111-2	Lori Ihli	3282	10:00 am	11:50 am	Th	Swain 304
111	111-3	Lori Ihli	3283	1:00 pm	2:50 pm	Th	Swain 304
111	111-5	Lori Ihli	3284	10:00 am	11:50 am	Tu	Swain 304
111	111-6	Lori Ihli	3285	1:00 pm	2:50 pm	Tu	Swain 304
111	111-9	Lori Ihli	3286	3:00 pm	4:50 pm	Tu	Swain 304
111	111-10	Lori Ihli	3287	5:00 pm	6:50 pm	Tu	Swain 304

About labs

If you learn anything from the lab portion of this course, we hope you gain some appreciation of the following:

- First, *Biology is a huge topic.* Those who study it must be familiar with the very small (e.g., how individual molecules interact) and the very large (e.g., how forests affect global CO_2). It can be easily argued that no other field of study encompasses such a broad range. In addition, no other area of science is so directly connected to how we live our lives. Recent advances in technology are constantly causing humans to think about what sciences offers and whether or not one ought to use these technologies (e.g., DNA fingerprinting, cloning, stem cell research, environmental degradation, threat of bio-terrorism).
- Second,*Biology is studied by scientists.* This method of knowing (i.e., science) provides the most efficient means of understanding the breadth and complexity of the hugeness and importance described above. It is our central goal that we communicate how scientists think about and approach biological problems. There are other "ways-of-knowing" but none is as effective in pursuing cause-effect relationships.

The lab portion of this course will show you how to approach these two areas, i.e., *biology* and *science*. Because of the influence and underlying significance of biology and science, this course is intended make you a better citizen in this world where breakthroughs usually out-pace the capacity of the general public to make informed decisions.

In contrast to other laboratory courses, this lab portion is not always meant to work hand-in-hand with the lecture. We view this part of the course as our opportunity to expose you to a greater variety of biological principles than will be covered in lecture. This may seem awkward at first. As with all things, just play along and try hard!

Electronic Devices

Attentiveness matters! It is a component of attendance and participation. Unless otherwise specified, **electronic devices should NOT be utilized, or even visible during class**. If it is absolutely necessary that you make or take a call during class, please step outside so as not to disturb your instructor or peers. If this becomes a habit, points will be deducted from your grade based upon my discretion.

Folders

The report ("requirements") section (and only the that section) of each lab must remain in your folder. The folder will be turned in at the end of each lab period. Keeping all your work in a single folder ensures that we can examine your work each week and will allow to ascertain your progress and detect problems in understanding. Additionally, it is your responsibility to make sure that instructors can find each lab. Do not "hide" your work behind older labs. If instructors cannot find it, it will be assumed that you were not here and you will get a zero for the day!

Grading

At Minot State University, **the lab and lecture grades are combined to equal one overall score for the course.** This means that attending, and doing well in lab can considerably improve your overall grade.

There are a total of 12 labs. Each lab is worth 15 points. The lab portion of the course is worth a total of 180 points (12 labs $\times$ 15 points each = 180).

Please keep it in mind that it is not always easy to get full credit on each lab. In order to earn full credit, students must follow all rules described in the lab requirements section.

If, for some reason, lab non-attendance is "excused", you still have zero points.

Lab Requirements

The ability to understand and assimilate this material requires you to be a willing participant, i.e., read and think about the lab exercises before lab, and then continue to be thoughtful during lab and as you complete the requirements due at the end of each lab. **Lack of preparation will be reflected in your grade.**

You must have the lab print out when you arrive in class. **This means that you cannot print it in class while we all wait for you!**

All labs must be submitted on the lab report page (last page of the lab). Students submitting report sections in any other form will automatically lose 5 points for that lab. Illegible labs will not be accepted.

Since this is a science class, so you are expected to write in a scientific way! This means that simple answers are generally unacceptable (e.g. "Yes", "No", "5", "It didn't work", etc.) and will be graded as such. All answers/hypotheses must be thoroughly explained. All equations and mathematical work must be shown. All units must be defined. All units should be metric.

Below is list of rules for writing good labs. It would be a good idea to review this list prior to turning in each lab (hint, hint). If you have further questions, please ask!

1. **Use complete sentences.**
2. **Proofread.**

3. **DO NOT PLAGIARIZE!**

   Copying someone else's work (including your neighbor's) is illegal in academia. You can be expelled from the ND University System for plagiarizing. Don't do it; use your own words instead!

4. **Hypotheses must be worded properly.**

   Ex. "*I hypothesize that ... because ...*"

5. **Answers must be specific. Explain, explain, and explain some more.**
6. Answers should be simple. Someone who has never taken biology should understand your answer.

Disabilities

In coordination with Disability Support Services, reasonable accommodations will be provided for qualified students with disabilities (LD, Orthopedic, Hearing, Visual, Speech, Psychological, ADD/ADHD, Health-related, and other). Please meet with or call Evelyn Klimpel during the first week of class to make arrangements. Accommodations and alternative format print materials (large print, audio, disk or Braille) are available through Disability Support Services, located in the lower level of Lura Manor, phone number 858-3371 or email `evelyn.klimpel@minotstateu.edu`.

MSU nondiscrimination statement

Minot State University does not discriminate on the basis of sex, religion, creed, national origin, race, age, disability, or any other basis prohibited by law. If you believe you have been discriminated against unlawfully, please bring this matter to the attention of your instructor or the MSU Human Resource Office at 701-858-3352.

Minot State University interpersonal abuse statement (Title IX)

Title IX makes it clear that violence and harassment based on sex and gender are Civil Rights offenses subject to accountability and support. If you or someone you know has been harassed or assaulted, you can find the appropriate resources off/on Minot State University campus. These resources include:

Lisa Dooley
Title IX Coordinator
Memorial Hall, 4th floor, Room 412
701-858-3447
`lisa.dooley@minotstateu.edu`

MSU Counseling (Confidential)
701-858-3371

Domestic Violence Crisis Center (Confidential)
24/7 Crisis Line: 701-857-2200
24/7 Rape Crisis Line: 701-857-2500

MSU Campus Safety & Security
701-858-HELP (4357)

Minot Police Department
911/701-852-0111

Laboratory 1

Science and the Scientific Method

The **scientific method** is the "program" that scientists use to understand the patterns (e.g., how far away is Mars?) and processes (e.g., how did the rocks on Mars form?) that we observe in the natural universe. Because the method is used to understand the natural universe, it cannot be used to ask more metaphysical questions (e.g., did God create Mars?). Anyone who uses the scientific method considers themselves a **scientist**. You will also be a scientist this semester when you use this method to understand the biological patterns and processes explored in lab.

The scientific method is recognized as a step process. In order, these steps are:

1. observe;
2. hypothesize and explain;
3. experiment;
4. conclude/interpret

Initial observations suggest a hypothesis. A hypothesis is "a proposition (or set of propositions) that *explain* some aspect of the physical universe" (i.e. the observation). Experiments are attempts to *test predictions that follow from a hypothesis*. The results of an experiment allows a conclusion/interpretation of whether to reject or fail-to-reject the hypothesis.

This might seem complicated, but in fact people tend to do this automatically.

Take a simple example: You switch on a flashlight and **observe** that the bulb does not light-up. Immediately, your observations suggest a **hypothesis and explanation**: the flashlight does not work because the batteries are dead. You then **experiment** by testing the prediction that *replacing the batteries will fix the flashlight.* If new batteries do not make the bulb light up, your **conclusion/interpretation** would be to *reject* your hypothesis. Something else must be the problem.

Notice that experiments are always *designed to **attempt to reject** the hypothesis*. If replacing the batteries had resulted in the flashlight lighting up, you would have *failed-to-reject* your hypothesis. You would not, however, have "proven" your hypothesis. While you can have some confidence in your hypothesis, it could still be incorrect. For example, it is possible that corrosion on the old battery terminals was the problem; perhaps the batteries themselves were fine.

1.1 Procedure

1. Each team should obtain one box. Each box has some unknown item inside. You will be attempting to characterize (i.e., describe) the item without ever seeing it or touching it.
2. Make some **preliminary observations** (e.g. lift, rotate, shake the box). Form a hypotheses and justify or explain them, (e.g., "I hypothesize that box #4 contains two coins, because those items would be light, as is box #4, and make the kind of 'clanking' sounds we hear from the box."). **Your entire group must agree on the contents!**

3. Make predictions that follow from your hypotheses (**hypothesize and explain**) (If there are two coins in the box, slowly tipping the box should produce sounds like one coin sliding, followed by another.) Write it down, being as explicit as possible. What were you looking for and why? Test your predictions.
4. Draw **conclusions/interpret** the results, i.e., would you reject or fail-to-reject your hypothesis? Make sure you explain your conclusion/interpretation.
5. Interpret the results, i.e., would you *reject* or *fail-to-reject* your hypothesis? Make sure you explain your interpretation.

Keeping track of each hypothesis on scratch paper, repeat steps 1 & 2 with each of the other boxes. **Remember, your team must agree upon the contents of each box.** Further instruction will be given by your instructor.

Importantly, you may never look inside the box. This is an important lesson of this exercise. Scientists often do not get to look "inside the box." Your sense of this idea will increase during this semester. Thus, good scientists always entertain the notion that what they think is completely wrong. The scientific method is what defines the scientist. Science is a process by which things can be known.

Lab 1 report

Your name ____

1. (*Observe*) Record your initial observations of the box your team has been given (sounds, weight, size, etc.). What characteristics do the contents possess? (3 pts)

2. (*Hypothesize and Explain, Experiment, and Conclude/Interpret*) Using the **back of this page**, describe **three rejected hypotheses**, prediction(s) that follow from each hypothesis, and the experiments used to reject them. For example:

   "I hypothesize box #1 contains a medium, white, Hanes, tag-less t-shirt, without a decal because I initially observed the contents of the box are not heavy. I therefore predict the item inside should sound soft and slide around easily. Instead, the item made a thumping sound. I rejected this hypothesis."

   Be sure to follow this format! (9 pts)

3. Give a hypothesis for the contents of the box that you have been unable to reject (i.e. *failed-to-reject*). Describe your prediction(s), experiment(s), and the results that led you to your conclusion. Be sure to follow the format above! (3 pts)

Laboratory 2

Sensitive Plant

2.1 Background

Now that you are familiar with the scientific method, it is time to practice using it in a laboratory setting. Let us start with the sensitive plant, *Mimosa pudica*.

Mimosa pudica is a short-lived sub-shrub that is native to Brazil, but has become pan-tropical. It has prickly stems that can grow to a height and spread of about one meter. In some areas, it is considered a noxious weed. *Mimosa* can grow in most well-drained soils regardless of nutrient content, but it is not shade tolerant. As a member of the bean family, Leguminosae, *Mimosa* roots contain nitrogen-fixing nodules. In cultivation, *Mimosa* will produce pink, fluffy from multiple stamens flowers from which viable seeds may be collected. All parts of the plant are toxic, and should not be ingested. As you see, plant is well-defended, even roots (after the touch) have strong garlic odor which repels soil animals.

Mimosa pudica is commonly called the sensitive plant, as it responds to stimulus. Yes, it moves. Of course, most plants move to some extent (like young sunflowers twisting to face the sun all day), but *Mimosa* actually retracts from stimuli! The response of *Mimosa pudica* to seismic stimulus is immediate and captures the attention of anyone observing it. Even Charles Darwin was intrigued enough to devote time to describing the leaf-closing response of this plant to external stimuli.

Note that plants recover best under the *bright light*.

In addition to *Mimosa pudica*, you might also have today couple of plants of *Biophytum sensitivum*, another sensitive plant of Asian origin, from wood sorrel family, Oxalidaceae. This one also reacts to stimuli, but not exactly like *Mimosa*. You can use it to answer the last question.

2.2 Procedure

1. Before obtaining your plant, your team will develop specific hypotheses and associated experiments for all questions.

   Hypotheses must be testable within our lab setting. As with every hypothesis, it should be written properly and contain a sensible explanation. Remember, you are stating what you think will happen and why the plant would do such a thing. Note that last question is one that you will come up with on your own.

   Plan your experiment. Explain *exactly* what you are going to do to test this hypothesis. This should be clear enough that another student who has never spoken with you can do the experiment again *exactly* the same way. Indicate any supplies you will use to get measurable results. **Be sure to indicate units of measure!**

2. When ready, carefully obtain your plant and systematically go through your experiments.

   Interpret your data. Clearly and thoroughly indicate your results. Again, be sure to include units! Indicate whether you reject, fail-to-reject, or find your hypothesis inconclusive. Use your data to explain why.

Lab 2 report

Your name ______________________________

Below, describe your experimental plans and the results of each experiment.

1. Which **part** (root, stem, petiole, leaves or leaflets) of the plant is most sensitive to stimuli (touch, movement, light, etc.)?
   - Hypothesize and explain (how the hypothesis is suggested by observation(s)) (1 pt)
   - Describe your prediction and plan your experiment (1 pt)
   - Conclude/interpret your results (1 pt)
2. How **fast** is the response? How **long** is recovery time?
   - Hypothesize and explain (how the hypothesis is suggested by observation(s)) (1 pt)
   - Describe your prediction and plan your experiment (1 pt)
   - Conclude/interpret your results (1 pt)

3. Are responses **all-or-none** or can one get a partial response (the whole plant or just a leaflet)? (Do all leaves respond or only the ones stimulated?)
    - Hypothesize and explain (how the hypothesis is suggested by observation(s)) (1 pt)
    - Describe your prediction and plan your experiment (1 pt)
    - Conclude/interpret your results (1 pt)

4. ____? (3 pt)
    - Hypothesize and explain (how the hypothesis is suggested by observation(s)) (1 pt)
    - Describe your prediction and plan your experiment (1 pt)
    - Conclude/interpret your results (1 pt)

Laboratory 3

Conway's Game of Life

3.1 Background

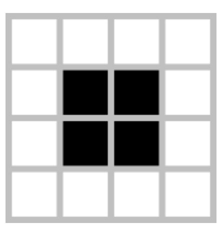
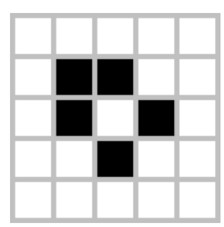
In computer science, there is always a tendency to explore things that are somewhat similar to biological life. Computer scientists try to create programs which simulate real life features: growth, feeding, moving, reproduction and even evolution. These simulations help create robots, self-replicating systems and serve as steps toward artificial intelligence. From a biological point of view, such simulations demonstrate self-organization, or how organized life may emerge from a chaotic system of inorganic molecules. Modeling can support the hypothesis about the way that first cell likely formed billions years ago. And we can visualize how variation in design could give a creature an advantage over others in its population.

The most known simulation of this kind is a **Conway's Game of Life**. This is not a game in a strict sense, it is more like programming. You will have a field of indefinite size, and a time (generations). The field is separated into dead cells (white) and live cells (black). Actually, the field is initially dead (white), and you will introduce black cells yourself. Every cell has 8 neighbors. Rules are simple:

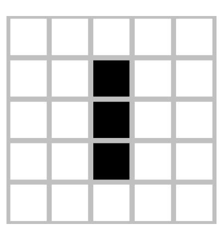
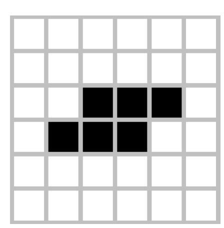
- **A live cell with 2 or 3 (live) neighbors survives, but dies otherwise** (easier to survive in groups).
- **A dead cell with exactly 3 (live) neighbors comes to life, and remains dead otherwise** (reproduction).

As you understand, there may be infinite number of cell combinations. However, some of them are different. They behave, sometimes just like real cells. There is a classification:

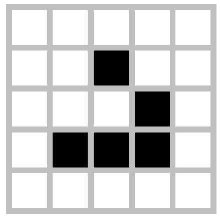
- **Still lives**. Creatures which do not change. Examples: block (left), boat (right) and others.

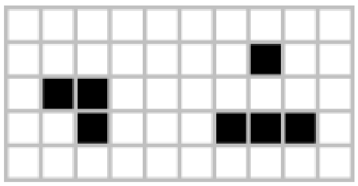
- **Periodic / Quasi-periodic lives**
  - **Oscillators**. They change its shape cyclically. Examples: blinker (left), toad (right), 10 cell row, tumbler and others

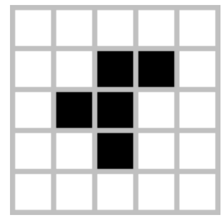



  - **Spaceships (Translators)**. They are moving! Examples: glider (below), lightweight spaceship and others.

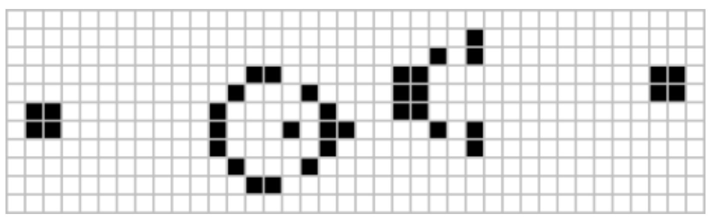


- **Aperiodic lives**
  - **Transient lives**. Sometimes, they just vanish. Sometimes, they live for a long time and only then stabilize. Examples: die hard (left), r pentomino (right), small exploder, exploder and others.

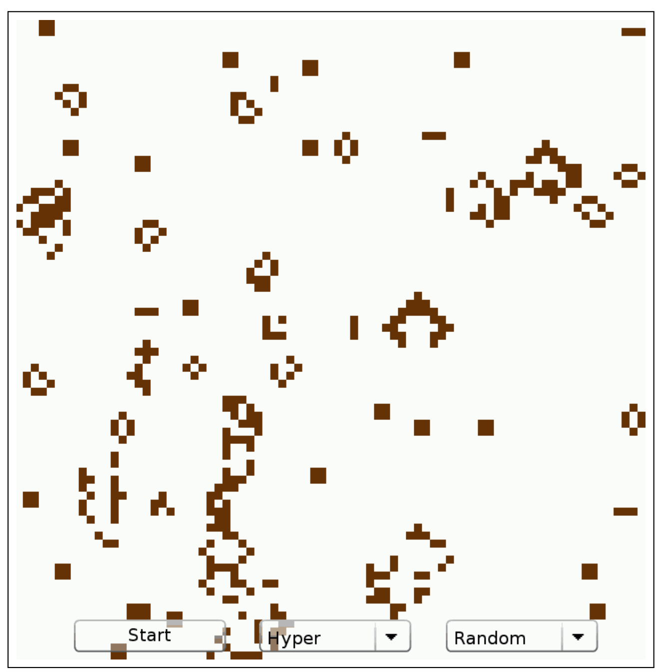




  - **Infinitely growing lives**. They live forever and even produce offspring! These creatures are big and complicated. One of the smallest is Gosper glider gun (below).



3.2 Procedure



1. Open the game from Web site: http://ashipunov.info/shipunov/school/biol_111/game_of_life.htm, switch the sound off, skip the ad (if presented).

2. Start the game, explore start/stop button, speed and creation menus.

3. Try all 8 examples from background part. Creation menu will help you to make a Gosper glider gun.

4. Fill the field with random cells three times, observe the result on different speeds.

5. Invent two your own creatures of different types (not bigger than 10×15 cells), explore their behavior.

Lab 3 report

Your name ____

1. Make a sketch of your two creatures below. Describe how each of them behaves. Hint: look at the varieties listed above. (6 pts)

2. In short words, we may define the self-organization as the *emergence of order from a chaos.* How is Conways Game of Life a model for self-organization? (4 pts)

3. How are Conways creatures similar to real cells? Find as many similarities as possible. (3 pts)

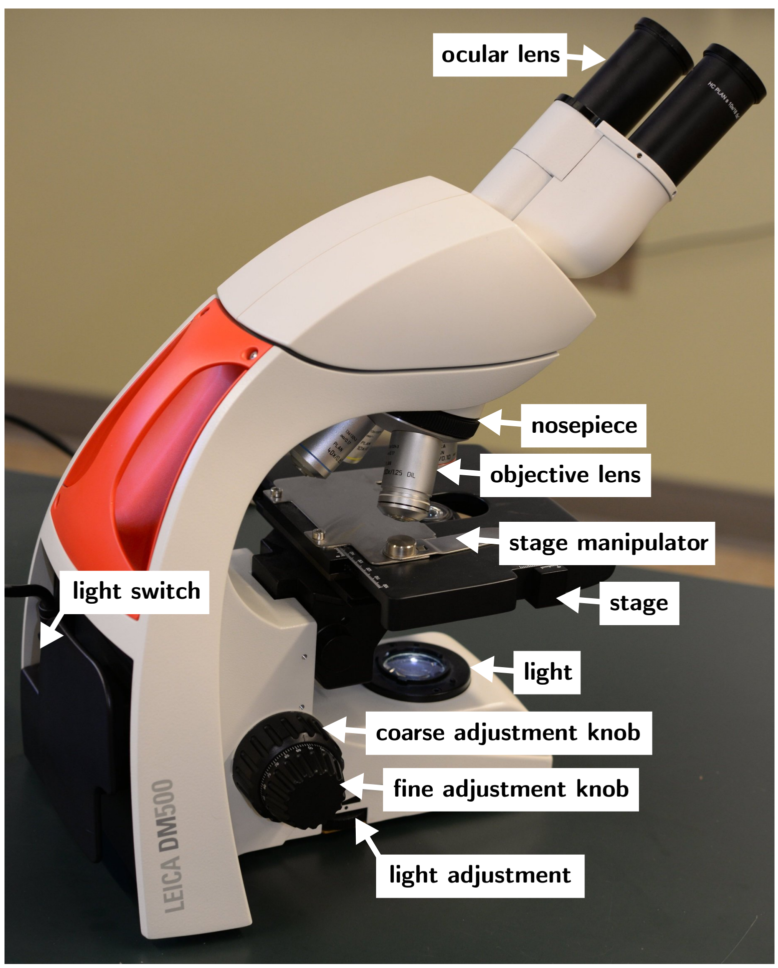
4. How are Conways creatures different from real cells? (2 pts)

Laboratory 4

Microscope and Cells

4.1 Microscope

To be able to distinguish two objects (i.e. to resolve them), they must be separated by some minimum distance, i.e., the *limit of resolution*. If they are any closer they will appear as one object. The best human eyes have a limit of resolution of about 0.1 mm.



To observe objects that are too small to be seen (or resolved) with the naked eye, we must use a **microscope**. The simple purpose of the microscope is to observe things that are too small to be seen clearly. All microscopes are designed to both magnify and to increase resolution. The limit of resolution of the light microscope is usually 0.2 μm. This means that many objects of biological importance, from cells and some sub-cellular structures to small tissues and organs are visible .

The compound light microscope is used for magnifying objects, typically 100× (or "100 times larger than actual") to 1000×. There are several additional features of this microscope. First, there are two adjustment knobs: *coarse* and *fine*. The coarse adjustment knob is only used when using the *lowest* power objective lens. When the higher power objective lenses used, focus using only the fine adjustment knob.

Actually, there are several important safety rules which everybody must remember:

- Good microscope is a really expensive equipment, and it is not easy to mend or replace it.
- **Never use the coarse adjustment knob with high magnification lenses** (more than 30×)!
- **Never ever leave microscope on high lenses!** If you finish your work or go out of microscope for a while, switch to smaller lenses (e.g., 5×).
- In our labs, **we do not use the highest lenses available** (100×). Do not switch to it. Our highest is 40×. Also, remember that with 40× you must always use the slides with **cover glasses**.
- **Do not move microscope, move yourself!** It is made heavy for the reason.
- **Always use manipulator**, do not move slide with your fingers.

A magnifying glass is a simple microscope because it consists of only one lens; our microscope is called *compound* because it consists of two or more lenses. The two lenses on the microscope are called the *ocular lens* (the eyepiece, or lens closest to your eye) and the *objective lens* (the lens that is variable and can be changed). The ocular lens typically magnifies objects 10×, whereas the objective lenses (used one at a time and mounted on a nosepiece) vary from 4× to 100×.

As two or more lenses are used together, their effects are *multiplicative*; this means that a lens that magnifies objects 10 times used with a lens that magnifies objects 4 times will together magnify an object 40 times (i.e., $4 \times 10 = 40$).

Total magnification refers to this magnification produced together by the ocular lens and the objective lens in use. When discussing magnification, the microscope user should be clear about whether "objective lens magnification" or "total magnification" is being used!

4.2 Measuring everything in anything

It is easy to calculate the size of microscopic object. All what you need is a diameter of field (the whole visible circle) and number of objects which fit in this diameter. If, for example, the field diameter is 1 mm and 10 objects will fill this diameter, then the size of object is 1 mm/10 = 0.1 mm.

Today, we will use mostly 400× (or less) total magnification ($40 \times 10 = 400$):

The field diameter for this magnification is **0.43 mm**.

If you use higher magnification, you should decrease the field size *proportionally*. If you use lower magnification, you should proportionally increase it. For example, if you use 10× objective lens instead of 40×, the magnification is four times smaller, and the field diameter is four times **bigger**.

Now, when field diameter is known, you need to count how many objects of question (e.g., cells) will fit in the line crossing the center of the field. If 10 objects fit the line, and magnification is 400×, then size of each object is 0.43/10 = 0.043 mm.

Very helpful animation movie "38 parrots" (your instructor could provide the link or simply show it) explains how to measure everything in anything. If you saw this movie, then imagine that your "boa" is a field diameter, and your "parrot" is an object of question.

4.3 Characteristics of cells

The basic unit of life is the cell. Some organisms are composed of a single cell (e.g., a bacterium like *Esherichia coli*) whereas others are multicellular (e.g., us). Regardless of what type of organism one considers, all are composed of cells and these cells are remarkably similar, from algae to anteaters and from *Hirudo* leech to *Homo*. This means that examining a single cell can give you a picture of the cell anatomy of almost any living thing.

There are two classes of cells: prokaryotic ("before nucleus") and eukaryotic ("true nucleus"). Organisms that have prokaryotic cells are called "prokaryotes"; if organisms have eukaryotic cells, they are called "eukaryotes." No organism has both kinds. Prokaryotes are the bacteria; eukaryotes are everything else (protists, plants, and animals). Prokaryotes are *very* small and difficult to see easily.

We will be examining eukaryotic cells (from plants and animals). Eukaryotic cells differ from prokaryotic cells in that there is *compartmentalization*. All the functions that were accomplished by prokaryotes in the mishmash of *cytoplasm* are now separated into their own chambers. The result is a much higher level or organization. This organization appears to be necessary in order to achieve higher levels of complexity seen in eukaryotes (all of you would agree that a cottonwood appears to be a more organized and specialized form of life than a bacterium). The compartments are called *organelles*.

When you examine the cells, you will see two types of organelles: implicit and explicit. Explicit organelles are those which are plainly visible (e.g., the *nucleus* will be clearly seen); implicit organelles are those that we cannot truly observe but must exist (i.e., if there is a nucleus to see, then there must be a *nuclear membrane* around it). If we include implicit organelles, then you will observe many different aspects of cells today. Cell membrane is also almost implicit but plant cell wall is not.

We will examine plant (from *Allium cepa* onions and an aquatic plant called *Elodea*) and animal cells (*Homo sapiens* inner cheek cells of your own mouth). We examine both plants and animals because plant cells have three structures not seen in animal cells, and these three structures are responsible for the obvious differences between these two kinds of life that you have always known about: plants make their own food (via *chloroplasts*), don't move much and may grow very tall (via *cell walls*), and plants can store stuff in their cells much longer than animals (via the *vacuole*).

4.4 Procedure

1. Using the directions provided to you by your instructor, prepare three slides—one with onion skin (epidermis), one with an *Elodea* leaf, and one with human cheek cells:

   Slide 1. Using a drop of IKI (iodine in potassium iodide) prepare a wet mount slide of onion skin from the bulb leaf. The sample must be extremely thin! Observe the cells at different magnifications.

   Slide 2. Using a drop of water, prepare a wet mount of an *Elodea* leaf.

   Slide 3. Prepare a slide of your own cheek cells stained with methylene blue.

2. Cover your slide with the cover glass (instructor will show how).
3. Place your slide inside manipulator, locate the smallest lens (5×) over the object on a distance about 3 cm, and looking into the microscope, simultaneously adjust the coarse knob.
4. When your object is in focus, switch to bigger lenses. When using bigger lenses, it is recommended to look on the object, simultaneously rotating the fine knob to and fro.
5. Using the approach discussed above, estimate the size (maximal length) of each kind of cells.

Lab 4 report

Your name ______________________________

1. On the back of this page:

   (a) Prepare a *diagram* of one (or more) onion skin cells;

   (b) *Label* the cell wall and the nucleus, *list* their definitions and functions;

   (c) Include a *scale bar* indicating the approximate length and width of an average cell. Scale bar is a short line labeled with particular size. With scale bar, everybody should guess what is the real size of the object on the diagram.

   Please show your math! (6 pts)

2. What is the length and width of a cell from an *Elodea* leaf? How big is a chloroplast from an *Elodea* leaf?(Hint: the same way as you "fitted" *Elodea* cells into field diameter and then calculated cell size, you need to "fit" chloroplasts *into the cell* which size you already know.) Please show your math! (3 pts)

3. Which cells are bigger: cheek cells or onion skin cells? Please show your math! (3 pts)

Laboratory 5

Osmosis

One of the most obvious and prominent processes in the Universe is *diffusion*, which is defined as "the movement of a substance from an area of high concentration to an area of low concentration." For example, when you exhale air, it is composed principally of carbon dioxide (a toxic gas). As soon as it leaves your mouth, the carbon dioxide is at a high concentration (relative to the surrounding air) and so diffuses away from your mouth to all the surrounding air. It is important to understand the distinction between "concentration" and "abundance." In the example, air is more abundant in the atmosphere than in a balloon. But the critical factor is "concentration." There is more air per volume in a filled balloon than in the atmosphere. So the air in a filled balloon is at a higher concentration.

Everything, including water, is subject to diffusion. *Osmosis* is a specific type of diffusion.

*Osmosis is the diffusion of water across a **semi-permeable** membrane.*

A "semi-permeable membrane" is any object that allows free passage of only some substances. The prime example of a semi-permeable membrane is the covering around every cell in your body, i.e., the plasma membrane. The plasma membrane allows free passage of water, oxygen, carbon dioxide and other small compounds, but resists the crossing of larger molecules like sugars or salts (namely, chlorine, sodium and potassium ions).

Because many solutions contain different amount of compounds (e.g., pure freshwater has nothing in it; saltwater is water *and* salt), osmosis is a very important biological process. For example, a walleye has saline (i.e., salty) solution coursing through its body, yet it swims in freshwater that has very little salt. This means that the salt is more concentrated in the walleye's body than in the environment that it swims in. Conversely, the water in the walleye is less concentrated than it is in the lake (i.e., salt is taking up some of the space in the walleye where there could be water). Because the walleye is composed of cells (which have cell membranes that are semi-permeable; they allow free passage of water but prevent the passage of salts), the water will move from the area of high concentration (the lake) to an area of low concentration (within the walleye's body). The result would be a walleye that gains water and swells. Eventually the walleye would burst from all the excess water flowing in by osmosis. Fortunately, the walleye has kidneys that prevent this; walleyes urinate often and their urine in very dilute (i.e., rich in water and low in salts). The opposite process occurs in fishes that live in the ocean.

(Because freshwater fishes constantly have water pouring into their body by osmosis, there is little need to drink. However, marine fishes are constantly losing water and so must drink constantly. This has the interesting everyday result that the statement "drinks like a fish" needs to be modified to "drinks like a saltwater fish.")

This example also applies to cells. In today's procedure, you will use cells of carrot root to observe the cumulative effect of osmosis. Cells (in our case, pieces of carrot which contain thousands of small cells) will be placed in two "environments" that differ in amount of salt: no salt (distilled water) and 1 M solution of salt (1 M NaCl). You can observe osmosis in action by monitoring the change in length.

5.1 Procedure

1. Each pair of students will work as a team. Each team should obtain 2 vials, 1 carrot, 1 metric ruler, a digital scale and a small knife. Sorry, no stabbing allowed!

2. Fill each of the 2 vials 3/4 full of the appropriate solution (either H_2O/distilled water or 1 M sodium chloride/NaCl).

   Note: the "M" refers to how much stuff there is in the water. The larger the number, the more stuff there is in the water. "M" stands for "molarity." By the way, typical molarity of NaCl *inside* a plant cell is 0.15. This means that 1 Molar salt water is really salty!

3. Prepare 2 carrot sticks from the *same* carrot. Slice narrow rectangular strips as close to 100 mm long × 10mm wide × 5mm thick as possible. (The instructor will give a demonstration of this).

4. Measure each strip's length in *millimeters* and weigh each strip in *grams*. Place them in the cups according to the table (question 2). Be careful to keep track of which strip is in which cup! Leave the strips in the environments for 20 minutes.

5. Meanwhile, you might want to answer some questions from the report page.

6. After 20 minutes, remove the strips, blot dry, re-measure and re-weigh them. Record the final length and weight for each strip. Compute the change (final minus initial length). Be sure to record changes with "+" or "−".

7. There are lots of random factors which all influence our results. This is why we need statistics. Your instructor will lead you through a statistical analysis to determine if the group data represent significant changes.

   For each set of before and after length data, we will calculate the probability of the *null hypothesis* ("Carrots strips *did not* change in length") using so-called t-test.

   We will use an **on-line t-test calculator** (`http://studentsttest.com/`). Enter initial data on the left and final data on the right. Make sure that "groups are matched" and "two tails" are selected. Then press "calculate". You will be given the p-value which reflects the *probability of the null hypothesis*. This is the probability that there was *not* a change. Record the p-value.

8. Address question 3. If p-value is less than a threshold (0.05), this is an indication that the null hypothesis ("Carrots strips *did not* change in length") is *unlikely*. Consequently, if p-value < 0.05 it is likely there was a significant change so we reject the null hypothesis and fail-to-reject the hypothesis indicating change!

9. Clean up as instructed.

Lab 5 report

Your name ____________________

1. Provide two hypotheses that *explain* water movement by osmosis in regard to the carrot cells. (2 pts)
   (a) freshwater experiment:

   (b) saltwater environment:

2. Give predictions that follow from your hypothesis for the following. As in, will the carrot gain length/weight, lose length/weight, or remain the same (4 pts):
   (a) freshwater experiment (length)
   (b) freshwater experiment (weight)
   (c) saltwater experiment (length)
   (d) saltwater experiment (weight)

3. Which change should be a better indicator of osmosis, length or weight? Explain why or why not. (2 pts)

4. Fill out the table below. Don't forget units! (3 pts):

	freshwater experiment		saltwater experiment	
Initial length				
Final length				
Length change				
p-value				
Significant?				
Initial weight				
Final weight				
Weight change				
p-value				
Significant?				

5. Does your data reject or fail-to-reject each hypothesis? Explain why or why not using p-values and common sense. Be thorough! (4 pts)
   (a) freshwater experiment (length)

   (b) freshwater experiment (weight)

   (c) saltwater experiment (length)

   (d) saltwater experiment (weight)

Laboratory 6

Plasticizoa: Volume and Surface

6.1 Background

Why the sparrow, relative to size, eats more than a lion? Why are there no insects bigger then rat? Why then in Carboniferous were insects bigger than rat? Why are dinosaurs suspected to be warm-blooded? Why are leaves of plants flat and often dissected?

To answer these, and many more, questions, you need to understand one of the deepest law of biology, **law of volume and surface**.

We require you to start with your own simple experiment. Take two cans, one big and one small, boil water in both, simultaneously take them from fire to the cooler place. Wait 30 minutes. Which can is now warmer? Why?

Explanation lays in geometry. When the linear size grows, the volume of object grows as the *cube* of it whereas the surface grows only as *square*. As a result, there will be more and more volume on the unit of surface. The relative surface will shrink when size grows!

This is the true limit to the life of Earth. In general, evolution always tries to make organisms bigger. It is beneficial for everybody: bigger predators catch more prey, bigger prey escape from predators better, bigger plants take more sun and shade more competitors, and so on. However, bigger organism will have more volume per the unit of surface (i.e., *smaller relative surface*). Therefore, the most important feature of life, *exchange* between living body and environment will be hindered because it goes through the surface. The pressure of weight (which corresponds tightly with volume) will also increase. How to overcome these restrictions?

Today we will practice volume and surface law with two "animals" made from the modeling clay. We call them *plasticizoans* since they are made from plasticine.

6.2 Procedure

1. Instructor will provide everybody with two *differently colored* plasticizoans of cylindric shape, one about 20 mm height (animal A_1), and another about 70 mm height (animal B_1).
2. Measure them with ruler (height h and diameter $2r$ of cylinder base, all in millimeters) and calculate their *volume* and *surface*.

   Hint: to calculate volume, use

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

   and to calculate surface, use

   $$A = 2\pi r h + 2\pi r^2$$

3. Calculate the *relative surface* which is the *surface divided by volume*:

$$R = A/V$$

4. Now, *transform* the A_1 into A_2. You need to make the relative surface of A_2 **as small as possible** to decrease contacts with the environment. How does A_2 looks now?
5. Measure the length and calculate volume, surface and relative surface for A_2.

   Hint: to calculate volume of sphere, use

   $$V = \frac{4}{3}\pi r^3$$

   and to calculate surface of sphere, use

   $$A = 4\pi r^2$$

6. *Transform* B_1 into B_2 to make its relative surface **as big as possible**. How does B_2 looks now?
7. Answer questions and provide sketches on the report page.

   Note. It is recommended to show your A_2 and B_2 to instructor first.

Lab 6 report

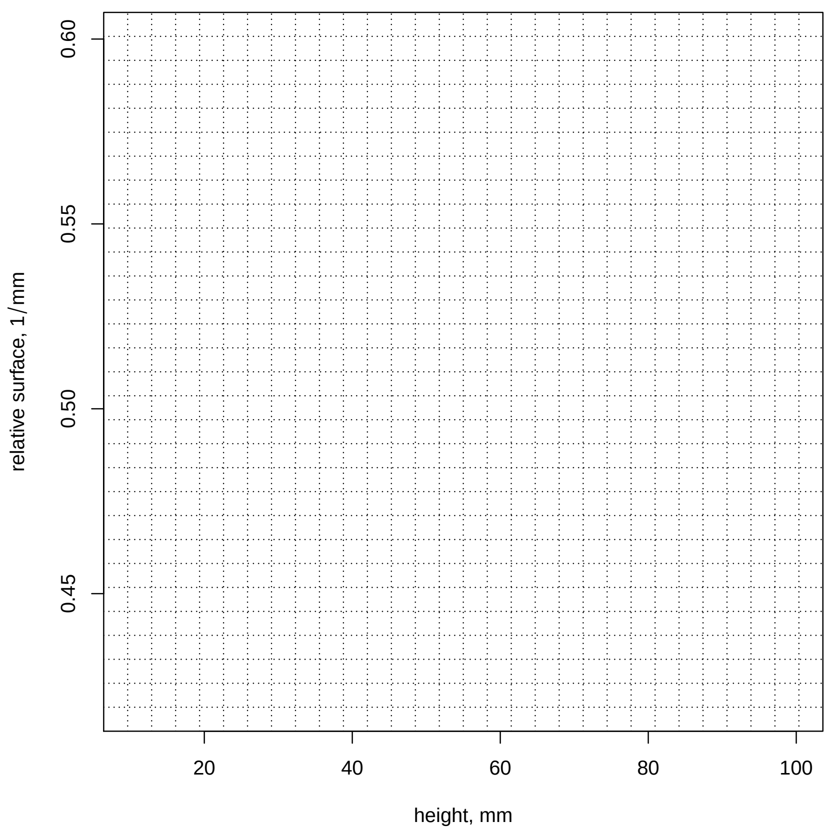
Your name ______________________________

1. In many languages, there is a saying "To make an elephant out of a fly" (i.e., "To make a mountain out of a molehill", "exaggerate"). How will this Highly Magnified Fly feel? Will it have any problems? Describe. (3 pts)

2. List below the volume, surface, and relative surface of A_1, A_2 and B_1. Which of these plasticizoans is more isolated from the environment? Why? (2 pts)

	plasticizoan A_1	plasticizoan A_2	plasticizoan B_1
volume V, mm^3			
surface A, mm^2			
relative surface R, mm^{-1}			

3. Using the formulas above, *calculate* relative surfaces of plasticizoans similar to B_1 (narrow cylinders) but with heights exactly 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mm and base diameter exactly 10 mm each. Make the plot below. If the relative surface is *less* than 0.45, cylindric plasticizoan *will not survive*. Show it on the plot (5 pts).



4. **On the other side of this paper**, *sketch* your A_2 and B_2. What A_2 and B_2 are good at? Which role could they play in ecosystem? Explain (5 pts).

Laboratory 7

Photosynthesis and Respiration

7.1 Background

There are two great processes that make the world of life go around: **photosynthesis** and **respiration**. One is about *converting solar energy into chemical energy* (to make the sugar, glucose); the other is about *converting this chemical energy into a useful form* (to convert glucose into ATP) to drive everything us organisms do. One seems to be the opposite of the other, and when you consider where the first process begins and where the other ends, then these two processes indeed form a circle. If there is actually a circle of life this is it: photosynthesis makes energy and respiration uses it. All cells (and thus all organisms) perform respiration (often in an organelle called the mitochondria), whereas only certain kinds of organisms that have a specific organelle called a chloroplast carry out photosynthesis. Those organisms that are capable of photosynthesis are known as producers, and all the remaining organisms can be termed consumers.

Photosynthesis can be defined as the transfer and storage of solar energy to a chemical form called glucose (a type of sugar). Glucose (and other kinds of sugar) is an arrangement of atoms of carbon (C), hydrogen (H) and oxygen (O). Much like a battery stores energy used to power your portable CD player, glucose is designed to be a temporary holding pen for some of the energy that arrives from the sun. From the standpoint of chemistry, photosynthesis is written like this:

$$CO_2 + H_2O \xrightarrow{\text{solar energy}} \text{carbohydrates} + O_2$$

Carbon dioxide comes from the air, the water comes from the soil or the surrounding environment, the glucose is either used by the plant or gets stored (we eat the stored stuff), and the oxygen gets released into the air. This reaction requires energy input from the sun.

Respiration can be defined as the release of the stored energy from glucose; this stored energy is transferred to a molecule called ATP that is used to drive any process in your cells that needs energy input.

$$\text{carbohydrates} + O_2 \rightarrow CO_2 + H_2O + \text{ATP}$$

This reaction releases energy in the form of ATP.

An example of this reaction occurs during intense physical exertion. While contracting muscles, you need lots of ATP. This is because your muscles need ATP to do what they are supposed to do (i.e., contract and release, contract and release). Where does this ATP come from? You obtain it by retrieving sugars that are stored in your liver (they got there by digesting more complex foods in your digestive tract) and carrying them via your bloodstream to your muscles where respiration occurs to move the energy from glucose (that began as energy in the sun) to ATP which make your muscle cells work.

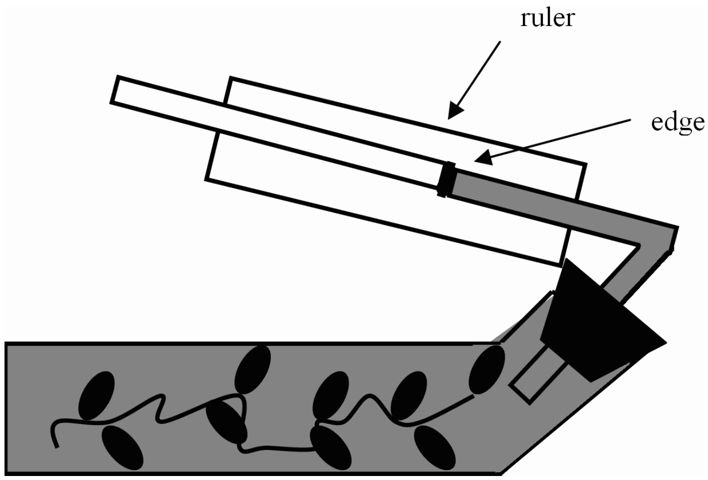
Note that the balance sheet is even. The only discrepancy is the energy budget. A lot more solar energy is available than gets stored as glucose, and more energy is available in the glucose than gets transferred to ATP. All the energy that is not stored is "lost" as heat. Have you ever noticed that you warm up during intense physical exertion? Duh.

In an earlier lab, you learned about osmosis and diffusion. These processes are free; they require no energy because stuff is moving from a high concentration to a low concentration. It just happens. However, life often requires that we move stuff the opposite way: from a low concentration to a high concentration. It also requires that this movement happens in an organized fashion. This takes energy. In short, life needs energy. ATP is this energy.

In this lab you will perform a simple procedure that will illustrate the extent to each of these processes in an aquatic plant.

7.2 Procedure

1. Place an *Elodea* sprig in a test tube that is equipped with a rubber stopper and a bent glass tube. Before capping the tube, fill the tube with water. Add enough so that when the stopper is inserted into the tube, the solution comes to rest at about one-fourth the length of the bent glass tubing.



   Formation of O_2 bubbles displaces the water and moves the edge to the *left* in this diagram. Consumption of O_2 makes the edge move to the *right* in this picture. The distance moved should be proportional to the amount of O_2 produced or consumed.

2. Place the tube near a light source on the lab bench.
3. Record the position of the solution "edge." As soon as the edge of the solution in the thin glass tube begins to move away from the *Elodea*, time the reaction for 10 minutes.
4. At the end of the 10 minutes, record the new position of the edge of the solution in the tube. *Record in millimeters (mm) how far the edge moved.*

   Note: always pay attention to the direction that the fluid is moving. Moving in opposite directions must mean that something is either being consumed or produced. Be sure to record direction of movement with "+" or "−".

5. Move tube away from under the lamp, and wait 10 min to introduce break between "day" and "night". Meanwhile, you might answer some questions from the report page.
6. Wrap the test tube in aluminum foil. Wait 10 minutes and measure the distance the edge recedes. This is usually the *negative* number.
7. Return the *Elodea* and clean and dry the equipment and lab area.

Lab 7 report

Your name ____

1. Give hypotheses for oxygen production and usage during each of photosynthesis and respiration. (4 pts)
   (a) photosynthesis

   (b) respiration

2. Based on these hypotheses, predict when (e.g. during "day" and/or "night") the bubble will move *toward or away* from the test tube in each experiment. (2 pts)

3. How far (in mm) did the bubble move during the "daytime" (photosynthesis and respiration)? How far (in mm) did the bubble move during the "night" (respiration only)?

	photosynthesis + respiration ("day")	respiration only ("night")
initial		
final		
distance moved (use + or −)		

   If the *Elodea had not been respiring* during photosynthesis, how far would the edge have moved? (Tell exactly how far, i.e., a number with + or −.) Please show your math! (5 pts)

4. Why exactly do plants carry out photosynthesis? When do plants photosynthesize (day, night)? (2 pts)

5. Why exactly do plants carry out respiration? When do plans respire (day, night)? (2 pts)

Laboratory 8

Genetics and Inheritance

8.1 Background

This lab is an introduction to how one analyzes genes without ever seeing or touching them. The first person to do this was **Gregor Mendel**, also known as the "Father of Genetics". In 1866, Mendel published a paper that documented a breeding experiment in bean plants. Using several different traits (including seed coat color and texture), Mendel was the first to understand that the traits of an organism are determined by bits of genetic information, or **genes**.

Mendel's work was unappreciated and/or undiscovered by the biological community until the beginning of the 20th century. Because of this, **Charles Darwin**'s principle of natural selection (the other revolutionary theory of the time) fell into disrepute. Darwin's critics thought that all variation would be lost (or "blended away") by interbreeding. Without variation, natural selection does not occur. Thus, the rediscovery of Mendel's principles at the turn of the century would spark new interest in natural selection. Interestingly, both revolutionary thinkers were dead by the time that their ideas gained full support.

Mendel understood that the genes were hidden away from view (in the DNA of the nucleus). Therefore he had to infer that the genes of an organism determined what the organism would look like. **The appearance or traits of an organism are called its phenotype. The genetic information (genes) can be referred to as the genotype**. A phenotype is what we can see, therefore it is observable. Until recently, a genotype was unobservable and had to be inferred. Based on his principles, Mendel was able to make predictions (*hypotheses*) about how many different phenotypes should result from crossing one type of parent to another.

Mendel's principles of inheritance follow from his idea of genes as particulate and that each individual contained a pair of **alleles** (the variant forms of the genes):

Law of Segregation: the alleles for each gene are not blended;

Law of Dominance: the alleles of each gene are dominant or recessive to each other. The dominant allele is expressed in individuals with one or two dominant alleles. The recessive allele is only expressed in individuals with two recessive alleles;

Law of Independent Assortment: genes for different traits are unaffected by one another, therefore the presence/absence of one trait in offspring is not affected by the presence/absence of another trait.

What is amazing is that Mendel had no knowledge of DNA, the nucleus or the principle of meiosis. However, his laws predicted the existence of the properties for all eukaryotic organisms. His work remains a classic example of how the scientific method can allow one to explain unobservable phenomena.

A gene is most simply defined as a position on a chromosome that codes for a trait. Because humans spend most of their life cycle as diploid organisms (i.e., possessing two sets of identical chromosomes), each person has two copies of each gene, i.e., **two alleles per gene**. It is the alleles that determine how individuals differ from one another. For example, there is a gene for eye color, and alleles for blue eyes and brown eyes.

HERE'S WHAT ALL THIS MEANS

Your parents each have 46 chromosomes. Nonetheless, **it's better to say that they have 2 sets of 23 chromosomes**, because the chromosomes come in pairs. (Having 2 sets of chromosomes makes humans, and many other organisms, diploid.) When your mother and father made their gametes (egg and sperm), they split up the pairs of chromosomes so that each gamete received only 23 chromosomes. Because they each contributed 23 chromosomes, you now have 2 sets of 23 chromosomes (= 46 chromosomes). This is good because more/less than 46 chromosomes is problematic (e.g., Klinefelter's syndrome, Down's syndrome).

Why say "2 sets of 23 chromosomes" instead of 46? Each chromosome carries only one allele of a gene; its matching chromosome also carries only one allele of the same gene, but the alleles may be different! This is why we say you have two alleles per gene. Since you have two alleles for every gene, this explains why some alleles are expressed more than others (dominant and recessive). It also explains why a trait that did not appear in your parents may appear in you.

(The process that determines which of your parents' chromosomes ended up in the gametes is called **meiosis**.)

We use symbols (usually using letters) to keep track of genetics and inheritance. **Capitalized or uppercase letters refer to dominant traits while lowercase letters refer to recessive traits**. Every individual has two alleles and we list them both. For example, "*E*" will be used to indicate the allele that codes for "unattached earlobes" and "*e*" indicates the allele for attached earlobes. "*e*" is recessive, so anytime it appears with a "*E*", it's expression is masked by this dominant allele (i.e., "*Ee*" is a genotype that means a person has both alleles, but whose phenotype is unattached earlobes).

Keep in mind that we, like Mendel, will never see the genotype. It is hidden away in the nucleus of your cells. But if we know the phenotype of the parents and their children, then we can infer the genotypes of everyone involved. For example, person with *unattached earlobes* phenotype could be described as *E*– which means that second gene in the pair is either *E* or *e*. However, if one of her/his parents has *attached earlobes* (*ee*), then second gene in the pair must be *e* because one of these genes must come from the parent with *ee* genotype. (By the way, the other parent must have *unattached earlobes*. Please think why.)

Moreover, if the person of question has sibling(s) with *attached earlobes*, then the second parent should also have one recessive gene!

You can calculate all these combinations in mind, but if you like, *Punnett square* will help you:

	e	*e*
E	***Ee***	***Ee***
e	*ee*	*ee*

(Unattached earlobes are boldfaced.)

Punnett squares are especially handy when you need to analyze simultaneously two (or more) traits.

Keep in mind also that most traits are not controlled by only one gene (e.g., height is controlled by many genes and by environmental pressure as well). In this lab we keep things simple by examining traits that are controlled by one gene only (e.g., skin freckles).

8.2 Procedure

8.2.1 Analysis of several single gene characteristic in humans

If individuals are **homozygous dominant** (e.g., AA) or **heterozygous** (e.g., Aa), their phenotype will show the dominant characteristic. Dominant phenotype could be encoded as "A–". If individuals are **homozygous recessive** (aa), their phenotype will show the recessive characteristic.

1. Work with a partner to determine your phenotype for the traits listed in the table below.
2. Record your phenotype and possible genotypes (circle the letters on the appropriate line).
3. After the totals for class are tallied, calculate the percentage of the class with each characteristic.

8.2.2 Human genetics and pedigree analysis

An important and useful tool provided by Mendel is that one's genotype can often be inferred by knowing the phenotype of the individual's parents, grandparents, children, etc. Furthermore, one can also infer whether the alleles are dominant or recessive.

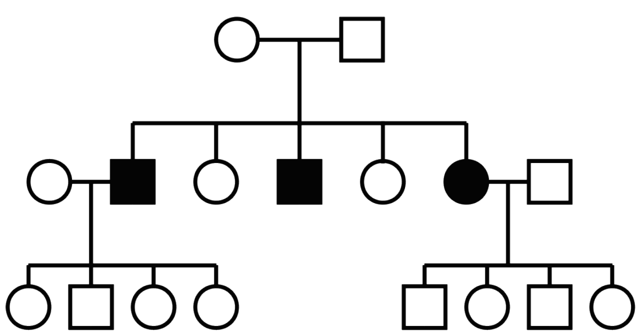
Genetic counselors are sometimes able to identify parents who are likely to produce children with genetic disorders. Fetal cells can then be tested to determine if the newborn does indeed have the disorder. This is called prenatal analysis.

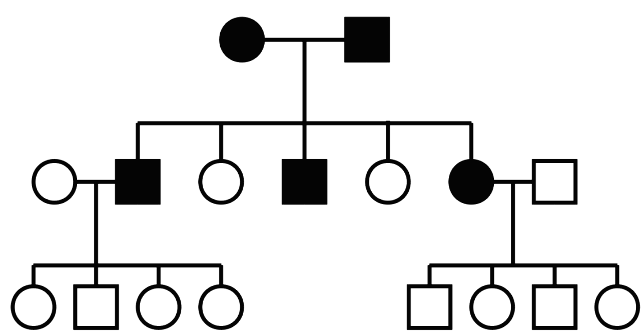
Another type of genetic counseling uses **pedigree analysis**. Pedigree charts show the inheritance of a genetic disorder within a family and make it possible to determine whether any particular individual has an allele for that disorder.

In pedigree charts, symbols are used to indicate:

- normal (clear) and affected (filled-in),
- males (squares) and females (circles),
- reproductive partners (linked at midline), and
- siblings (linked from above)

1. For the below practice pedigrees, determine how the characteristic is passed. Is it dominant or recessive (attempt to use both)? Determine as many genotypes as possible:





Lab 8 report

Your name ______________________________

1. Based on the table, do you think that recessive traits are rare? Why or why not? Explain your answers with references to your data! *(3 pts)*

Your phenotype	Your genotype	Number in class with each phenotype	% of class with trait
		Hairline:	
widow's peak	*W*–		
continuous	*ww*		
		Earlobes	
unattached	*E*–		
attached	*ee*		
		Skin pigmentation:	
freckles	*F*–		
no freckles	*ff*		
		Toe length:	
2nd toe longer	*T*–		
1st toe longer	*tt*		
		Thumb hyperextension:	
cannot be bent back	*H*–		
can be bent back 60°	*hh*		
		Bent pinky:	
pinky bends	*L*–		
pinky straight	*ll*		
		Interlacing of fingers:	
left thumb over right	*I*–		
right thumb over left	*ii*		

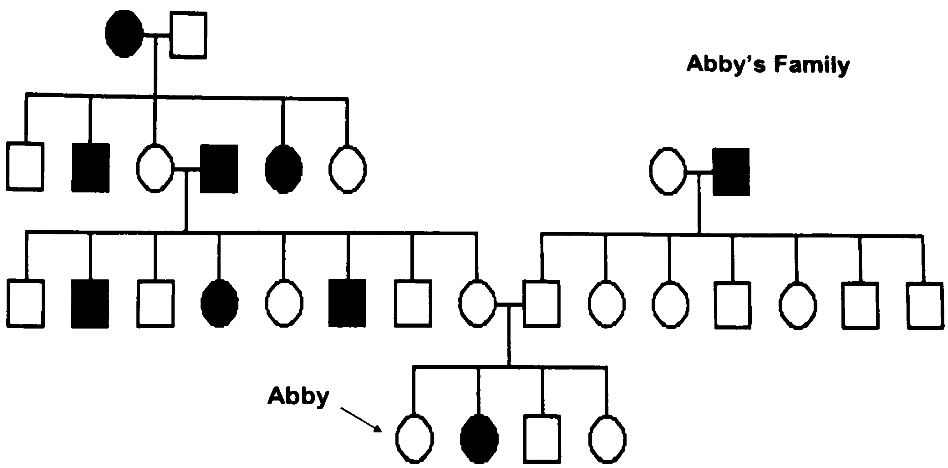
2. Below, you will be given with different cases of pedigrees. Typically, instructor controls that every table receive at least four different cases. Please note that by default it is assumed that the **spouse does not have the "diseased" gene of the partner**.

   Do not forget to calculate likelihoods. It is also recommended to show Punnett squares.

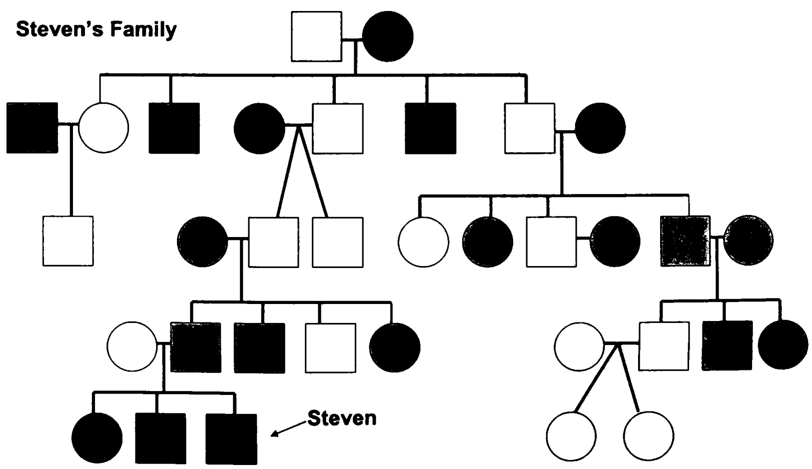
   Note: to avoid confusion, always use different letters for different traits!

   Write down the number of case and answer all questions from case(s) chosen (*12 pts*).

Case 1



Abby's family has a history of Phenlketonuria (PKU). Individuals with PKU lack the enzyme that converts a non-essential amino acid (phenyalanine) into a useful one (tyrosine). High levels of phenyalanine accumulate in the blood affecting neuronal development—especially in children. Mental retardation can be a result. Since phenylalanine is found in many proteins, patients afflicted with PKU can escape the disease by strictly limiting themselves to low protein diets.



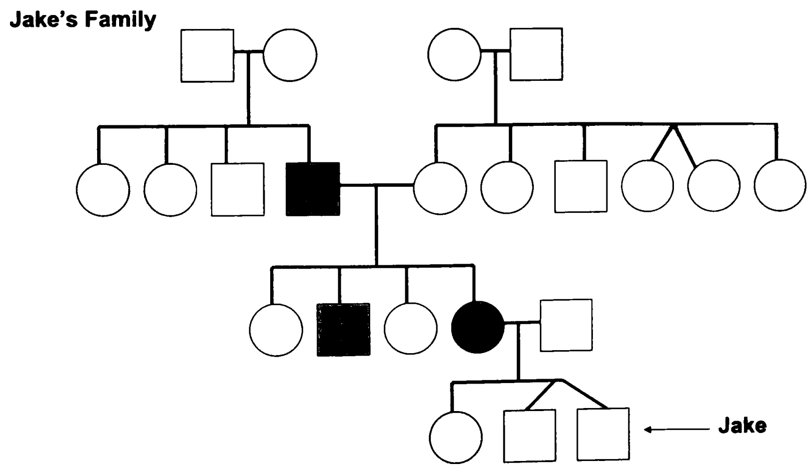
Steven has Huntington disease. Huntington's disease is characterized by degeneration of the nervous system causing uncontrollable movements, dementia, and psychiatric disturbances. Loss of motor skills eventually prevent swallowing and speech. Huntington's generally develops in a person's thirties or forties, leaving the afflicted children and spouse to care for them.

(a) Is it possible for Steven and Abby's children to have PKU? Explain.

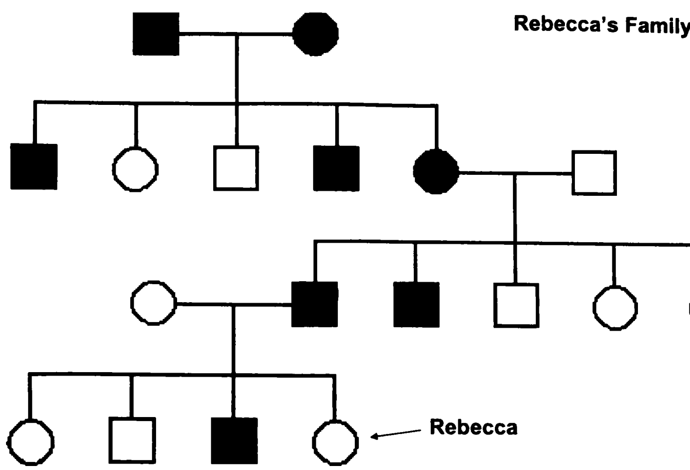
(b) Is it possible for Steven and Abby's children to have Huntington's disease? Explain.

(c) Are these traits dominant or recessive?

Case 2



Jake's family has the most common lethal genetic abnormality in the United States—Cystic Fibrosis. Cystic Fibrosis is characterized by excessive secretion of mucus from the lungs, pancreas, and other organs. The mucus is very thick, causing problems with breathing, digestion and liver function. It also makes the person especially vulnerable to infections like pneumonia. Without a special diet, and frequent pounding on the chest and back (to clear the lungs of mucus), most children with CF die by the age of 5.



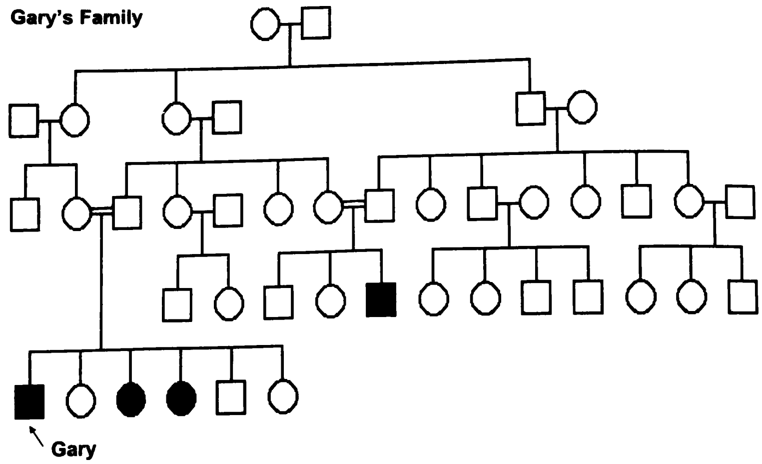
A rare trait in Rebecca's family causes Marfan's syndrome, in which the connective tissue is not as rigid as it should be. The long bones of the body continue to grow causing an abnormally tall, thin individual, with a narrowed face. The lens of the eyes may be dislocated. The heart valves and arteries stretch and leak, Lung, skin and neurological problem are also common. Rebecca does not have Marfan's, but is concerned she might be a carrier of the disease.

(a) Is it possible for Rebecca and Jake's children to express Marfan's syndrome? Explain.

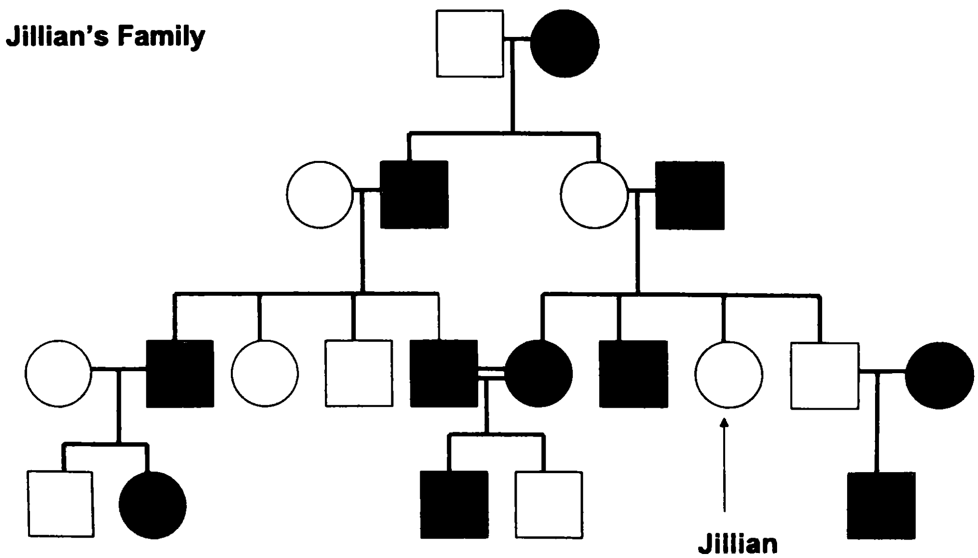
(b) Is it possible for Rebecca and Jake's children to express Cystic Fibrosis? Explain.

(c) Are these traits dominant or recessive?

Case 3



Gary is an albino. Albinos lack pigment, causing very pale skin, hair and eyes. They tend to suffer more UV damage than normal individuals, and are therefore forced to wear long sleeves, pants, sunglasses, hats and gloves whenever exposed to sunlight.



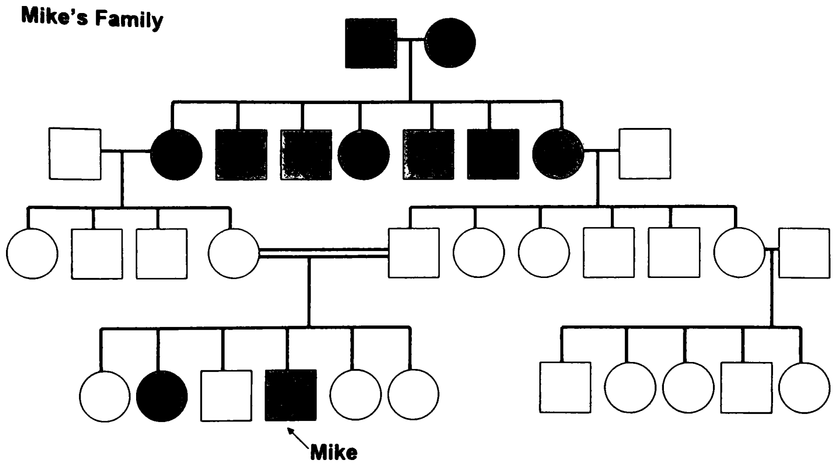
Many of Jillian's family have a type of dwarfism called achondroplasia. In this condition, the head and torso develop normally, but the limbs are short. Only heterozygous individuals have this disorder. The homosygous genotype causes death of the embryo. Jillian does not have this achondroplasia, but wants to be sure she will not pass on the disorder.

(a) Is it possible for Gary and Jillian's children to be dwarfs? Explain.

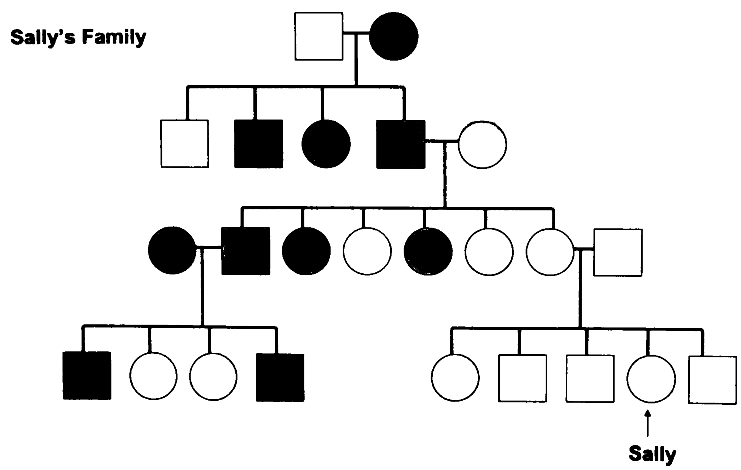
(b) Is it possible for Gary and Jillian's children to express albinism? Explain.

(c) Will the cousin marriage in Jillian's family produce albinos? Explain.

Case 4



Leptodigitalia occasionally shows up in Mike's family. Leptodigitalia is a condition where the fingers and toes are excessively long and slender. Such digits are extremely fragile and often suffer frostbite in conditions considered normal to unaffected individuals. Mike has this trait, but does not wish to pass it on to his children.



Sally's family has genetic predisposition to syndactyly, a condition in which two or more digits are fused together. While she does not exhibit this condition, she is concerned that she might be a carrier of this trait.

(a) Is leptodigitalia conferred by dominant or recessive allele(s)? Does Mike possess the leptodigitalia allele?

(b) Is syndactyly conferred by dominant or recessive allele(s)? Does Sally possess the syndactyly allele?

(c) What are the odds that Sally and Mike's children will have both syndactyly and leptodigitalia?

Laboratory 9

Animal Diversity: Creating a Phylogeny

9.1 Background

Phylogeny is the genealogy (i.e., "family tree") of organism. In other words, the phylogeny represents the ancestor-descendant relationships. The inference of phylogeny is one of the foci of evolutionary biologists. It is also one of the most difficult tasks that these scientists undertake. Because one can never replay the "tape of history", one can never "know" the true phylogeny. Species are subject to extinction and parallel evolution, and these phenomena obscure phylogeny. At best, a "phylogeny" is really a "phylogenetic hypothesis." In no other field is appreciation of the strengths and weaknesses of the scientific method more appropriate.

The basis for inferring phylogeny is the **synapomorphy** = *a shared derived characteristic*. For example, the presence of a cranium (= skull) is a synapomorphy for the *Vertebrata*. No other animals have a skull. Thus, this synapomorphy supports a phylogenetic hypothesis that states that all vertebrates (fishes, mammals, amphibians, etc.) are more closely related to each other than to any other groups of animals. For example, fish and frogs are more closely related to each other than they are to crayfish.

To infer a synapomorphy, one must have some idea what the ancestral (**plesiomorphic**) state of that character is. For example, the ancestral amphibian had lungs. Because there is a large group of salamanders that do not have lungs, we call "lunglessness" a synapomorphy that unites all lungless salamanders into a single family. Lunglessness is a *derived condition* that all these species of salamanders share.

Using synapomorphies, one can place species on a "family tree" or phylogeny. Because traits are subject to natural selection, it is uncommon to find concrete synapomorphies that define 100% of the group. Terrestrial vertebrates are called *Tetrapoda* in reference to a significant synapomorphy: presence of four limbs. However, snakes are a member of the *Tetrapoda* even though they lack external evidence of legs. Yet there are numerous other characteristics that clearly make snakes reptiles, thus members of the *Tetrapoda*. Use of only one characteristic (presence or absence of four limbs) would lead one to an incorrect phylogeny that did not include the snakes with the reptiles. Use of as many characters as possible is critical to developing a rigorous phylogenetic hypothesis.

Loss of legs (synapomorphy of tetrapods) in the snakes is an example of what is referred to as *evolutionary reversal*. The best phylogenetic hypothesis is one in which the number of evolutionary events (achievements, reversals *etc.*) is minimal. This is known as the **principle of parsimony**. There is a consensus among evolutionary biologists that the most parsimonious phylogenetic hypothesis is the one that is most likely to represent the true genealogy of a group of organisms.

* * *

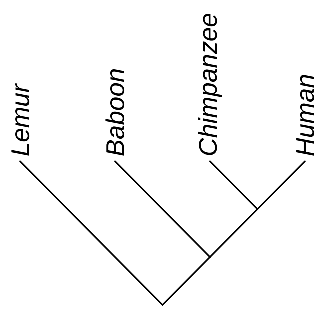
Most organisms have common names, such as the "red maple" (*Acer rubrum*) or the "brown-headed cowbird" (*Molothrus ater*). However, these common names are often misleading. Many different species are called the same thing in different parts of world, and many identical species are called different names. Formal Latin names are used by scientists to establish a unique name for each species on the earth. Each Latin name is made, approved and used by scientists worldwide. Every species name consists of two parts: the first part is the **generic name** (or genus, e.g., *Homo*); the second part is the

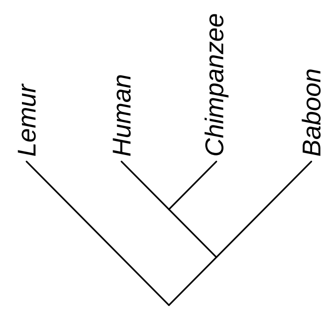
specific epithet (e.g., *sapiens.*) This Linnaean binomial system of nomenclature was introduced by Carolus Linnaeus in the 18th century and has been in use ever since. The study and practice of naming organisms is known as **taxonomy**. Taxonomic groups have ranks: they may be species, genera, families, orders, classes, phyla, kingdoms or stay between them like subclass or superfamily.

Biologists would like taxonomy to reflect phylogeny. For example, all the frogs in *Ranidae* are hypothesized to be more closely related to one another than to any frogs from other groups. However, because phylogeny is difficult to infer, taxonomy is always changing. As scientists' opinions of phylogeny change, so does taxonomy. As more information is gathered, phylogenetic hypotheses may change; this often results in a change in taxonomy. This is the reason that textbooks often present different taxonomies. This is also the reason that the taxonomy presented in any textbook will not be the same one that is found in textbooks in 20 years.

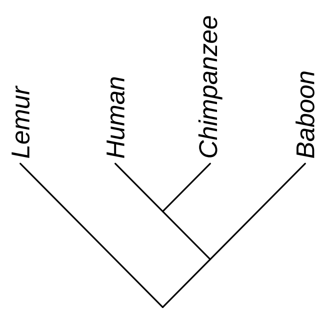
9.2 Understanding phylogenetic trees

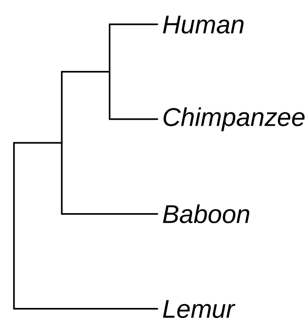
1. Phylogenetic tree is a *dichotomously branching diagram* where all terminals (tips representing objects) are connected. Phylogenetic tree is *related with time*; start of the diagram is always older then end. All terminals must be labeled. Nodes which have more than two branches are typically not allowed.
2. Tree edges may be freely rotated in any direction. For example, these trees are same:



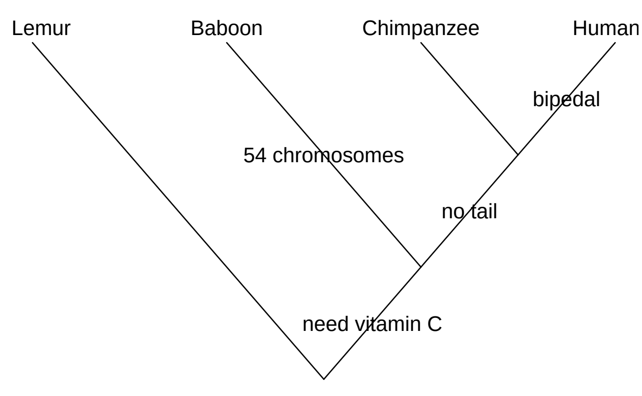


3. Direction of branches also does not matter. These trees are same:





4. Apomorphies (and reversals) could be shown as edge labels:

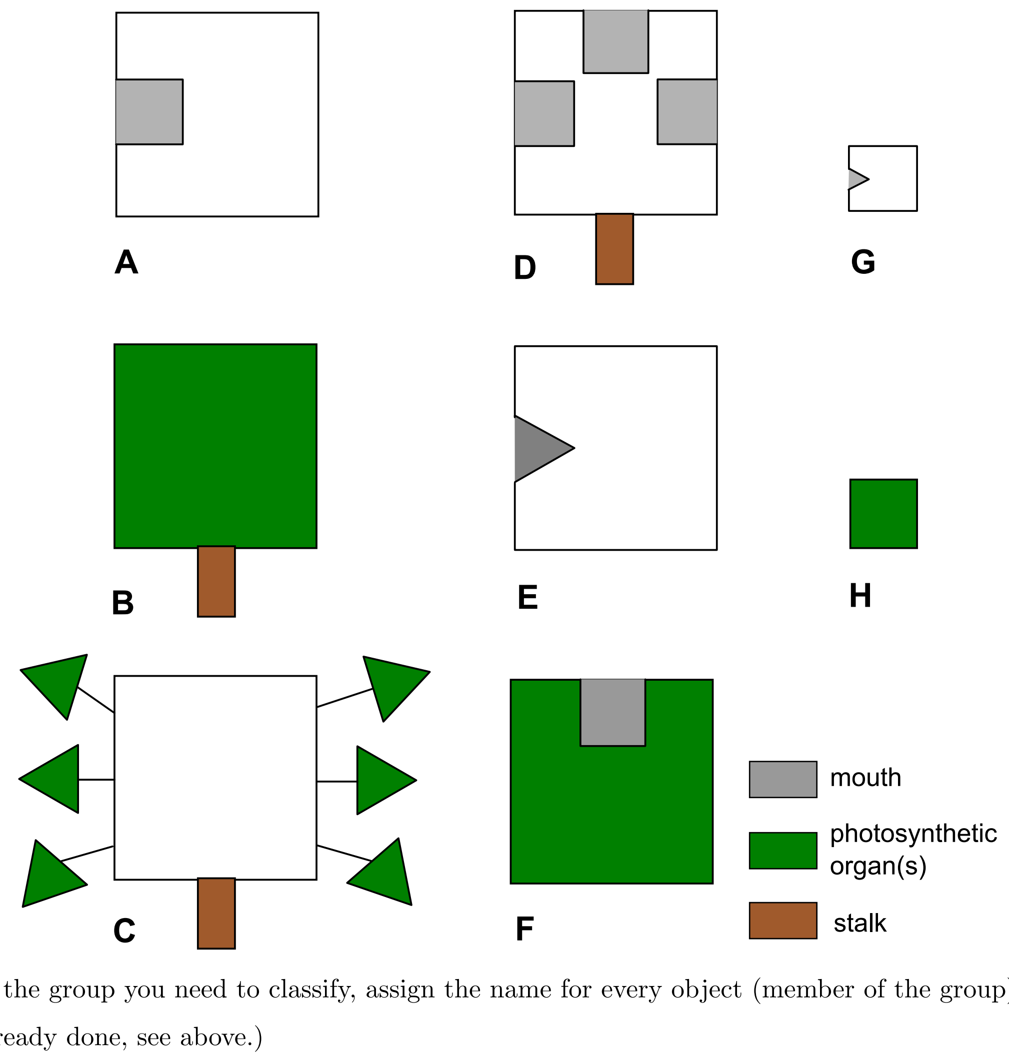


5. Phylogenetic tree is directly related with taxonomy (classification):
   - Suborder Strepsirrhini: lemur
   - Suborder Haplorrhini:
     - Superfamily Cercopithecoidea: baboon
     - Superfamily Hominoidea:
       * Genus *Pan*: chimpanzee
       * Genus *Homo*: human

9.3 How to make a phylogeny tree

This instruction will explain it on the example of imaginary creatures:

Planet Aqua is entirely covered with shallow water. The ocean is inhabited with various flat organisms (see the figure). These creatures (let us call them "kubricks") can photosynthesize and/or eat other organisms or their parts (which match with their mouths), and move (only if they have no stalks). We need to make a phylogeny of kubrick species A–G because kubrick H is an **outgroup** and has only plesiomorphic (ancestral) characters.



1. Start with the group you need to classify, assign the name for every object (member of the group).

   (This is already done, see above.)

2. Find as many characters as possible. Make number of characters $N \geq n + 1$ where n is the number of objects. Therefore in this example we need $N \geq 8$. Review all characters and make the *character list*. Note that plesiomor-

phies and *autapomorphies* (unique derived characters) do not help to make a tree so **concentrate on finding synapomorphies**, characters which unite two or more (but not all) objests.

For every character, guess its plesiomorphic (ancestral) and apomorphic (derived) states, either with the help of outgroup, or using the other methods like comparative anatomy, paleontology or embryology. Encode plesiomorphic character state as "0", and apomorphic as "1":

Character list:

1. Mouth(s) presents 1, no mouth 0

2. One mouth 1, not 0

3. Triangle mouth 1, not 0

4. Square mouth 1, not 0

5. Stalk 1, no stalk 0

6. No photosynthesis 1, photosynthesis 0

7. Body big 1, body small 0

8. Mouth on top 1, no mouth on top 0

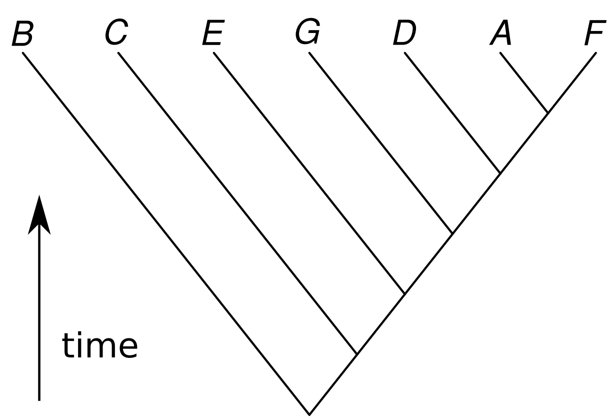
Note character #6: since outgroup *is* photosynthetic, we must make the absence of photosynthesis apomorphic state.

Make the *table of characters* where rows are names of objects, columns are characters, and each cell contains 0 or 1:

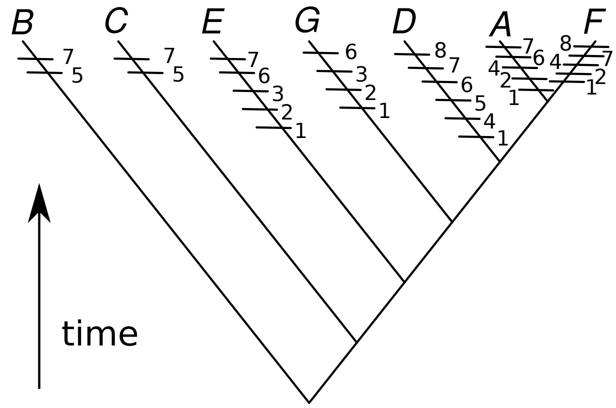
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	1	1	0	1	0	1	1	0
B	0	0	0	0	1	0	1	0
C	0	0	0	0	1	0	1	0
D	1	0	0	1	1	1	1	1
E	1	1	1	0	0	1	1	0
F	1	1	0	1	0	0	1	1
G	1	1	1	0	0	1	0	0

Note that outgroup is not here; this is because we already polarized our characters and therefore do not need outgroup anymore.

3. Start to draw the tree, preferably from the most ancestral ("primitive") member (which has most zeroes). This is the first branch. Continue to grow the tree attaching branches, preferably (but not necessarily) placing most similar objects closer. Actually, you may even attach members at random because this tree will be optimized anyway. All terminal branches should end with objects.

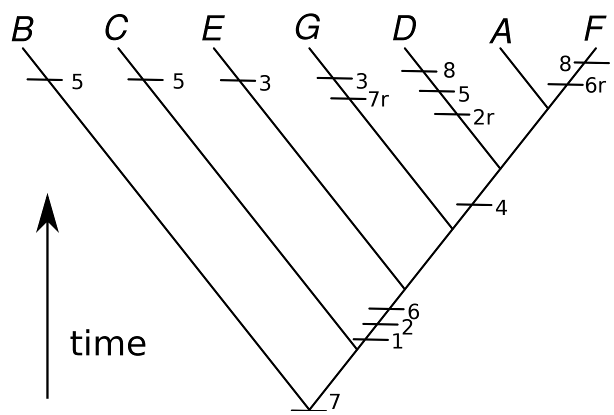


4. Look at the complete tree, label all apomorphies (from the character table) as edge labels. Now calculate the length of tree which is a **number of evolutionary events**.



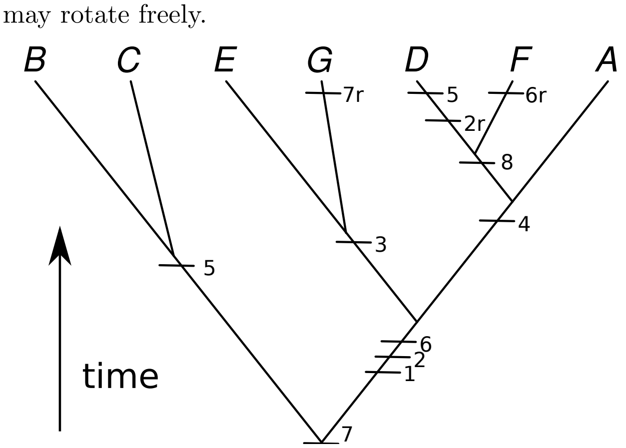
Length of tree is simply the number of tickmarks, so $L = 29$ (this is exactly how many "ones" are in the table). This is a bit too much since good tree should have length $L < 2N$ where N is the number of characters. In our case, $L < 16$ is strongly recommended.

5. Now start to think how to make your tree shorter. First, decrease length without altering tree. You can shift characters down, deeper in time. Then all descendants from this point should have this character. If some do not have it, you may introduce *reversal*, the loss of apomorphic character. Label reversals with "r" letter, like "2r":



Length of this tree is the number of apomorphies plus reversals so $L = 15$ now.

6. Then start to alter the actual tree. The basic method is to prune (take off) branch and attach it to another place, re-label tree and calculate the length again. If the length is shorter, this is *more parsimonous* tree. Try to find the *most parsimonous* (shortest) tree, i.e. the tree with as low number of apomorphies and reversals as possible. Do not forget that tree branches may rotate freely.



Now $L = 12$! This is likely the *most parsimonous tree*. If you find the shorter one, you will receive extra points!

9.4 Procedure

1. We will work in small teams today.
2. Every team should choose 7 *different* plastic dinosaur plastic toys, name them, make character table, make initial tree, calculate its length, modify the tree to make it as most parsimonous as possible, label apomorphies and reversals (if any).
   (a) To name dinosaurs, use shortcut of their scientific name (e.g., “Drom” for Dromeosauridae). Check the image below. We have:

Pteranodontidae (dinosaur “bats”)

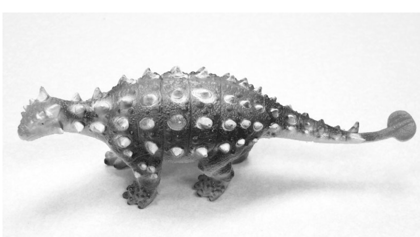
Dromaeosauridae (“raptors”)

Tyrannosauridae and relatives

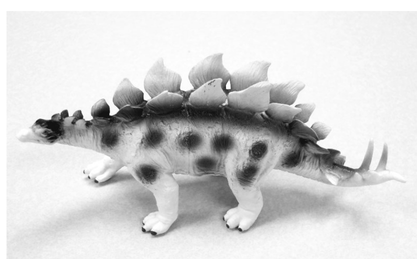
Diplodocidae (“titans”)

Hadrosauridae (“duck-billed”)

Pachycephalosauria (“skull-domed”)



Ankylosauria (“club-tailed”)



Stegosauria (with osteoderms)

Ceratopsidae (“frilled”)

Disclaimer. Not all toys are imaged here. These toys are not supposed to serve as proper reconstructions of dinosaur groups mentioned above.

   (b) To polarize character states (i.e. decide which of them are plesiomorphic and which are apomorphic), you may use either the outgroup like imaginary “turlte” or “salamander”, they both should work for dinosaurs. Alternatively, you can employ comparative and paleontological arguments (developmental arguments are not applicable here.)

   You must state clearly which approach you took and why you call some characters apomorphic (derived, “1”) and some plesiomorphic (ancestral, “0”). Avoid terms “advanced” and “primitive”, phylogeny does not work with these!

Lab 9 report

Your name ______________________________

1. Write your dinosaur *character list* and draw the *character table* below. How did you determine which character state is plesiomorphic and which is apomorphic? Explain. (5 pts).

2. **On the back of this page**, draw **two** of your dinosaur phylogeny trees below. Do not forget to label all apomorphies and reversals (if any). Note the length of trees. Which tree is most parsimonous? Why? (10 pts).

Laboratory 10

Plant Diversity: Dichotomous Keys

10.1 Background

Anyone with even a mild interest in studying organisms must be able to differentiate similar species from one another. This sounds easy, but can be very difficult since many species appear almost identical. The most common way of determining plant species is to use a descriptive or dichotomous key. The idea came from an 18th century French naturalist, Jean-Baptiste Lamarck. Legend has it that when Lamarck first demonstrated the key, he gave it to a random stranger. The stranger (some baker), who did not know much about plants, was able to determine plant species without trouble!

A key consists of a series of steps. Each step has 2 or more choices that systematically lead you to species identification. Flow charts are an example of a simplified dichotomous key.

How to make a key? The example is below:

Phase 1. Start with "players". In this example, it will be three plants:

Alpha
Beta
Gamma

Phase 2. Assess descriptions of these three plants:

Alpha: Flowers red, petioles short, leaves whole, spines absent
Beta: Flowers red, petioles long, leaves whole, spines absent
Gamma: Flowers green, petioles short, leaves dissected, spines present

Phase 3. Start with a character which splits the list into two nearly equal groups. Then add other character(s). It is always good to use more characters!

1. Petioles long . . . . **Beta**.
– Petioles short . . . . 2.

2. Flowers red, leaves whole, spines absent . . . . **Alpha**.
– Flowers green, leaves dissected, spines present . . . . **Gamma**.

As you see here, key consists of steps. Every step has a number and two choices. Number is attached to the first choice whereas the second choice is marked with minus "–". The choice will lead either to the name, or to another step.

To make a key, you will need plants, but you will also need to describe them. Today we will use leaf-related characteristics such as (1) arrangement, (2) position, (3) dissection, (4) shape, (5) bases, (6) tips, (7) margins and (8) venation.

Be careful! Even on same plant, leaves may be diverse. Always use the typical, average leaves. If all leaves are different, use the middle leaf from a main stem.

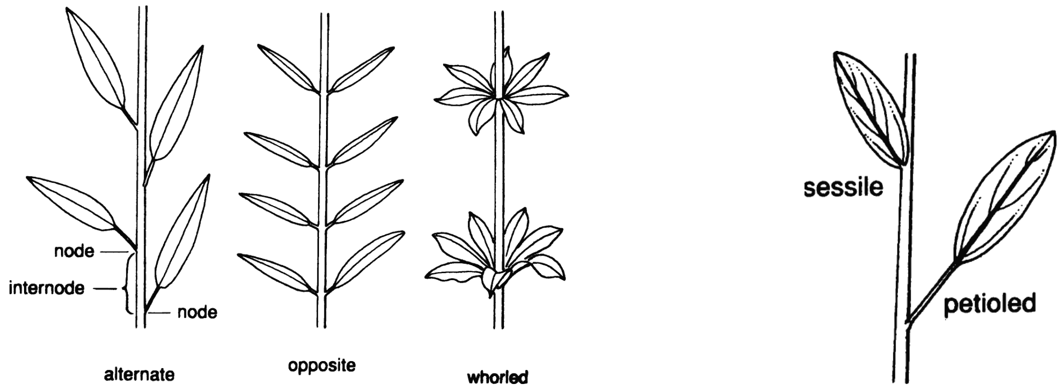


Table 1. Leaf arrangement (left) and position (right)

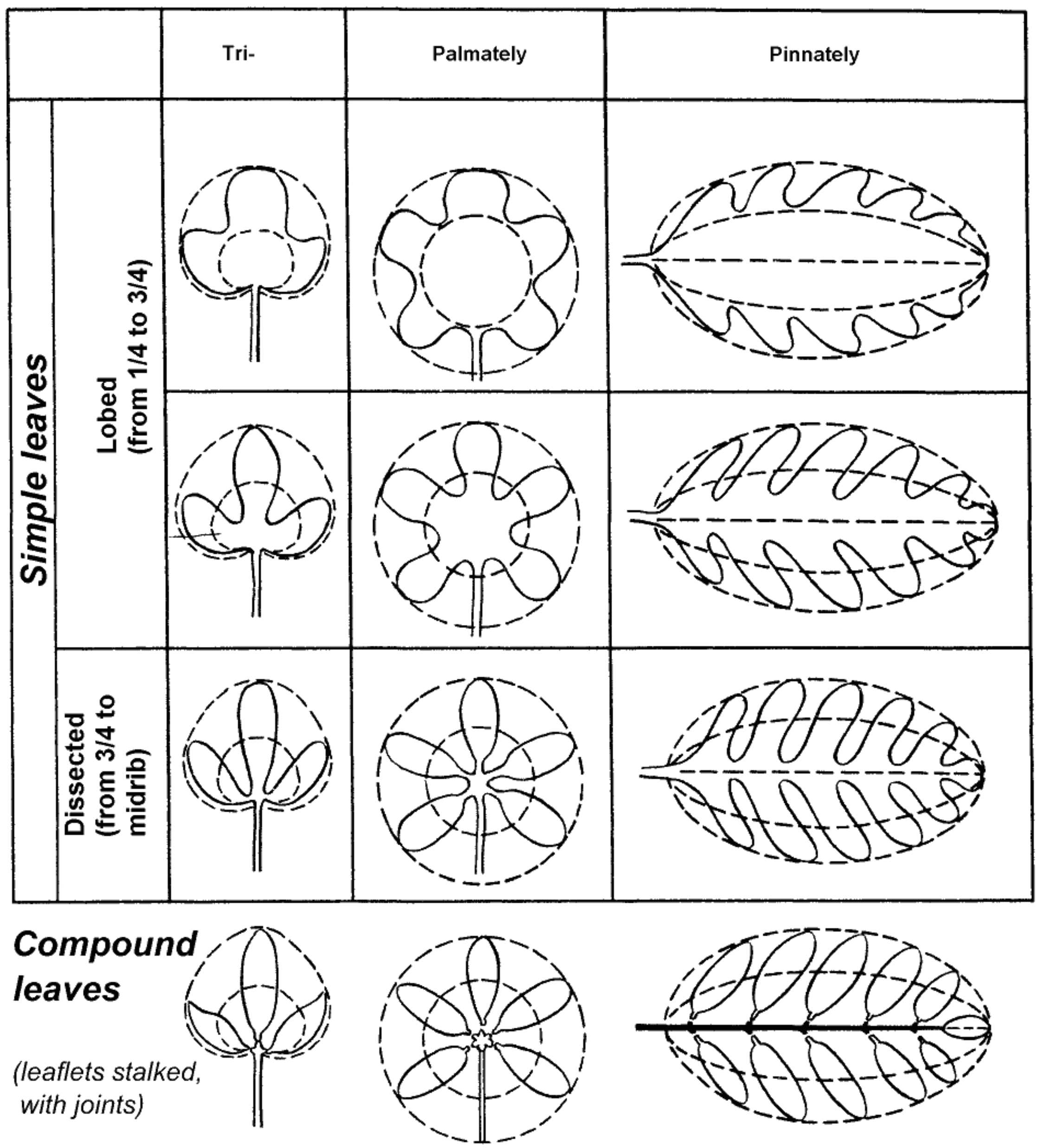


Table 2. Variants of leaf dissection

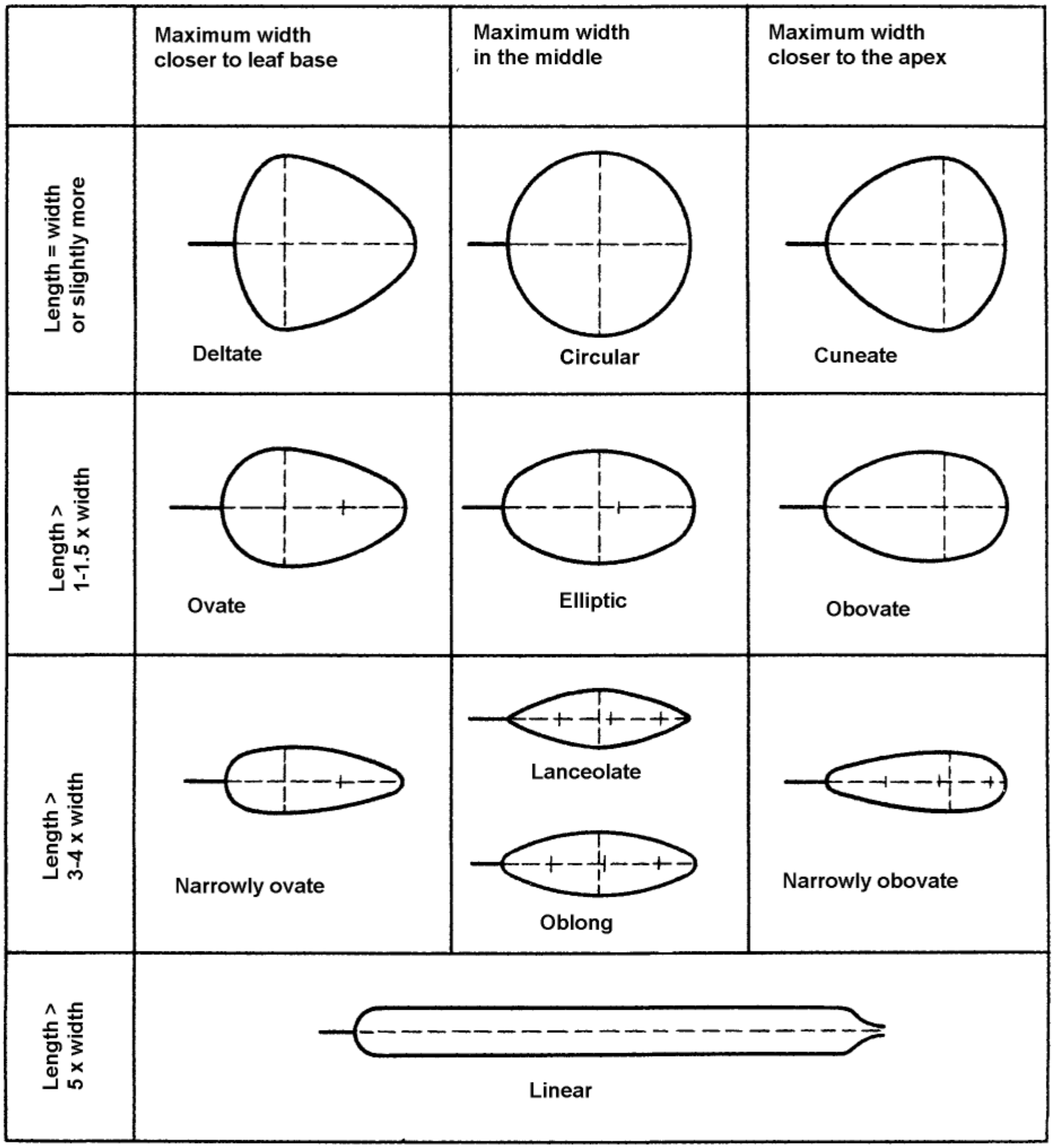


Table 3. Variants of leaf shapes

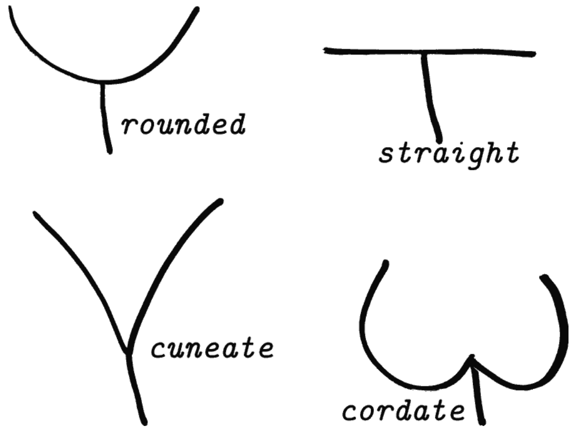


Table 4. Bases of leaf blade

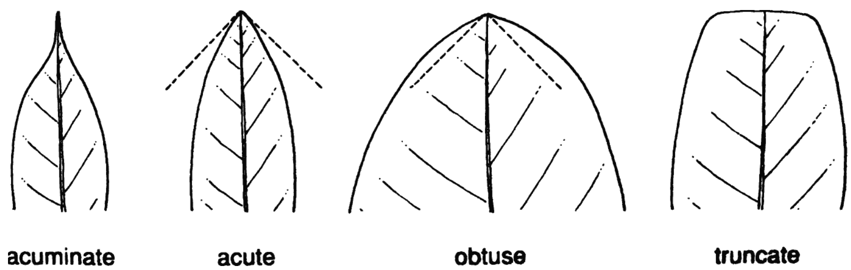


Table 5. Tips of leaf blade

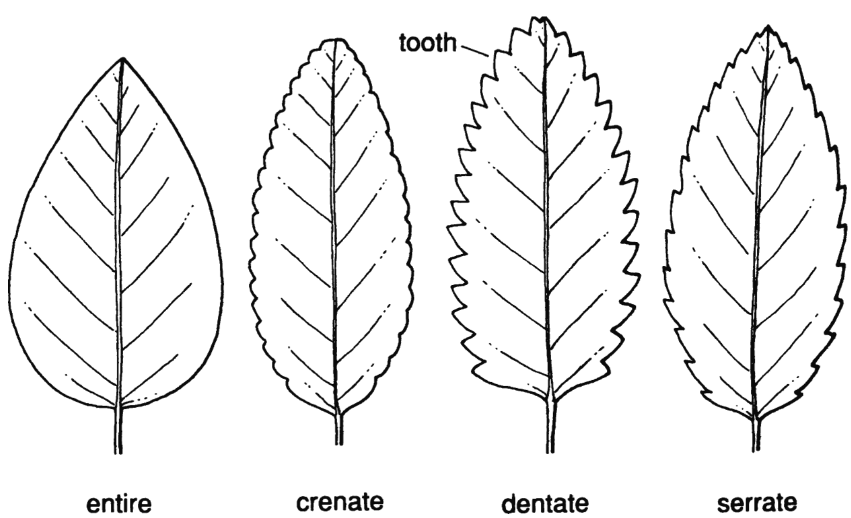
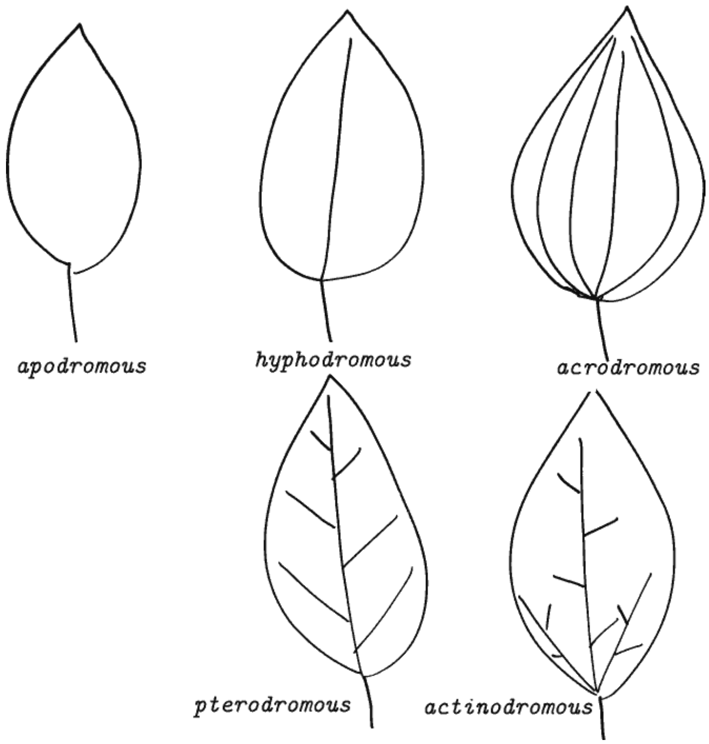


Table 6. Margins of leaf blade



Lateral veins \ Main vein	No	One	Several
No	Apodromous	Hypho-	Acro-
Several	Acrodromous	Ptero-	Actino-

Table 7. Types of leaf venation

10.2 Procedure

1. Each table is a team. Each team should obtain 6 different plants. Start by determining which characteristics given fit each plant. Gather as many characteristics for each plant as possible.
2. Now you will determine which characteristic you will use to begin splitting the plants up. Begin with a 3:3 or a 4:2 split. 5:1 splits really do not work out well and you end up starting over.
3. In this step your team will build a complete key using the characteristics you have gathered. Name each plant using the number written in the lower right hand corner of the paper the plant is mounted on.
4. Now you should make a key without the final solutions (the plant number). **Test your key**: have a neighboring team try to determine the number of couple of your plants. Revise the key if necessary.
5. Continue with the additional questions.

Lab 10 report

Your name ______________________________

1. Copy your team's key here. Be sure it is neat, orderly and most of all that it works! (10 pts)

2. Does your key reflect evolution of these plants? Why and why not? (3 pts)

3. Imagine you that have eight (not six) plants. How many steps minimum will be in your key? (2 pts)

Laboratory 11

Natural Selection

11.1 Background

We have been studying genetics and the biological explanations for variation of traits in living things. Mendel described "particles" that were passed from parent to offspring. We now know those particles were **genes**, and that variation in the traits he studied was the result of inheriting different variants, **alleles** of each gene. It is important to have a good foundation in genetics to understand evolution.

Evolution is formally defined as a **change in allele frequency over time**. In other words, if you analyze a population of organisms today, and then sometime in the future, you may note a shift in the percentages of specific alleles. Since evolution is merely a change in allele frequency over time, then all life is constantly evolving. What fuels these changes?

The principal process involved in evolutionary change is **natural selection**, which is defined as "differential survival and/or reproduction."

There are three requirements that must be met for natural selection to actually take place:

1. There must be variation among the members in the population for the trait in question
2. The variants of the trait must result in differences in survival and/or reproduction.
3. The trait in question must be controlled to some degree by genes (e.g., when you dye your hair, this new variant is not controlled by genes).

Note that if no variation exists in a population, then natural selection cannot work. Other modes of evolution exist as well (mutation, genetic drift, migration). These can work alone or in conjunction with one another.

There is one well-known classic case of natural selection: melanism mutation in peppered moths. Prior to the Industrial Revolution in England, nearly every peppered moth had light-colored speckled wings, only some months had the mutated black peppered phenotype. This coloration enabled the moths to blend in on the lichen-covered tree trunks. The much rarer black peppered moths were so obvious that birds (the primary killer [i.e., the important "selector"] of peppered moths) easily caught and killed them. The result is that these forms differed in survival probability and thus the light-colored moths survived and reproduced. Black moths were only maintained because of the rare mutation.

The Industrial Revolution was responsible for the production of huge amounts of soot (from factory smokestacks). The soot was produced so rapidly that it covered the once-light-colored tree trunks. The now-dark tree trunks exposed the light-colored moths and the dark moths now were blending in. The birds now caught mostly light-colored moths, thus dark moths had higher survival and reproduction. Because they were producing more offspring (which had better survival probability), the dark moths now became abundant. The population now contained a larger fraction of dark moths, and this follows the definition of evolution (i.e., change in allele frequencies).

11.2 Procedure

Today, we will have two species: a predator (you) and prey (bean species). Every table will become a battleground between a population of prey and a population of predators.

In all, there will be 4 predators (you and your neighbors) and 200 prey (different beans). In each round, you will "eat" beans by collecting them into your "stomach". To catch beans, every predator will be assigned one (and only one) **tool**:

- fork
- knife
- teaspoon
- soup spoon

Before the game starts, all varieties of prey should be equal. Therefore, there should be:

- 50 white **small** beans, Navy or Great Northern beans (*Phaseolus vulgaris*, multiple cultivars)
- 50 white **large** beans, Lima beans (*Phaseolus lunatus*)
- 50 brown **medium** beans, kidney beans (*Phaseolus vulgaris* cv. 'Red Kidney')
- 50 speckled **medium** beans, Pinto beans (*Phaseolus vulgaris* cv. 'Pinto')

Rules:

1. Every table becomes a team of players. Carefully read instructions, formulate hypotheses to answer question 1.
2. Put all prey (200 beans, 50 of each variety) to the center of the table, **mix** them, place your dishes in each corner.
3. Ready tools, remove all items from the table other than beans and dishes.
4. Start the hunt: **you have a given number of seconds to catch and "eat" as many beans as possible. The prey may be caught only with the designated tool in your non-dominant hand, everything else is forbidden!**

   You are allowed to catch more than one bean at a time, but you cannot move your "stomach"! Try not to hinder your teammates' progress. After the "stop" command, stop catching immediately. If you just caught the prey but did not put it into "stomach", release it.
5. Calculate the results for predators: the predator who caught most of prey will *reproduce* to replace the most unsuccessful predator. In other words, if the predator with fork caught the maximal number of prey, and the predator with teaspoon caught the minimal number of prey, the teaspoon will starve and be replaced by the fork.
6. Prey reproduce: all surviving beans should be doubled. For example, if 15 Limas, 18 Navys/Great Northens, 28 Kidneys and 12 Pintos survived, add 15, 18, 28 and 12 beans of each variety, respectively.

Lab 11 report

Your name ____

1. Hypothesize which predator will be the most successful. Hypothesize which prey species will be the most successful. As with all hypotheses, be sure to include a reason for your thoughts (3 pts)

2. Did you reject or fail-to-reject your hypotheses? Describe below the final ecosystem on your table. Who survived and why? (3 pts)

3. Why did we start from 16 predators but 200 prey? What happen if numbers of predators and prey are equal? Explain (3 pts).

4. Did the other tables end up with an ecosystem similar to yours? Why or why not? (3 pts)

5. Apart from natural selection, the real evolution process will also be influenced by (a) mutations, (b) migrations from other populations and (c) random processes ("genetic drift"). How you would change the rules of game in order to accommodate one or more of these processes? (3 pts)
   - mutations
   - migration
   - genetic drift

Laboratory 12

"*Evolution. The Origin of Species*" Board Game

12.1 Background

"Evolution" is the game based on the theory of **Charles Darwin**. It offers players to create their own species of animals with their own abilities all the while fighting to control the one important resource—**food**. By regulating the population of your creatures, obtaining new useful abilities and fighting off opponents, you must survive till the end of the game and stand at the head of the food chain.

Note that this board game is not about evolution in populations of single species. It mostly concerns with *ecological evolution*, formation of ecosystems with prey and predators and so on.

At the beginning all players receive 6 cards; with them you can either create a creature or place an ability on an already created one: for example make it Huge or Poisonous.

The game is turn based and each turn is divided into separate phases:

1. Players create creatures and apply abilities to them;
2. With the help of a dice the amount of food is decided;
3. Players turn-by-turn take food tokens from the pile to feed their creatures: some need only one, while others, depending on their abilities may require two, three or even more to satisfy their hunger;
4. Animals that are not completely fed will starve and become extinct. The completely fed animals survive and grant their player more cards to create new creatures and new abilities.

Once the deck is empty everyone counts their points. Points are awarded for each creature that survived and for each ability on them. The winner is the one who creates the most balanced ecosystem.

12.2 Procedure

Every table becomes a team. Instructor will show you a YouTube video (http://youtu.be/OzDigM1vcNU) explaining how to play. The game's instructions are included below for reference. The team will then play one full game. When game is finished, prepare your report.

Note: there is also an extended, newer version of this game (*orange boxes*). If you team chose this one, it could take a bit longer to play (about 10–15 min). Also, extended game has slightly different rules so study them carefully! Instructor has a right to add up to 3 extra points for playing the extended game.

EVOLUTION

The Origin of Species

Rules of the Game

An astonishing diversity of life forms inhabits our planet. The evolutionary theory explains what differences have arisen from the struggle for existence, when each species used a different approach for survival. Some species adapted to forage on previously unfit resources; others gained advantage by learning to defend themselves from predators effectively; still others moved into habitats where they alone could survive. New adaptations arose in different groups of animals. For example dinosaurs, birds and mammals evolved flight independently. All the adaptations "invented" by evolution were recombined and tested by natural selection. During this process some species went extinct, while others came to dominate the planet.

In Evolution, you are the one who combines different traits (adaptations) of animals, and grows your own population while dealing with an ever changing amount of food resources. By regulating the number of animals, gaining new useful traits and struggling with other players, your population can fight for survival, and by the end of the game, dominate the planet!

Winner

The player with the largest number of victory points at the end of the game is the winner. Points are awarded to a player for all his surviving animals and their traits.

Preparation

Mix the deck thoroughly. Each player gets 6 cards from the top of the deck; these cards are now the player's hand. The deck is put at the center of the table with facing up. The food tokens are placed near the deck: red ("food bank"), blue ("extra food") and yellow ("fat"). Then each player rolls a die. The player whose throw is the highest starts the game

The game turn

Each turn of the game consists of four phases:

- development phase
- food bank determination phase
- feeding phase
- extinction and draw phase

During each phase players act in order moving clockwise from the first player. The phase of development and the phase of feeding may include several rounds; after the first round is over, the first player acts again, etc. The player who can't or doesn't want to act passes.

Development Phase

During this phase you may play your cards by putting them from your hand onto the table in front of you. You may play each card either as an animal (with facing up) or as a trait of an existing animal (with facing down). If you play a card as a trait, put the card underneath the corresponding animal (or pair of animals).

Some of the cards carry two traits, with one trait at the top and the other (e.g. Carnivorous) on the bottom of the card. When playing such a card, you should decide which of the two traits you will use, and place the card with the desired trait on the top. This decision is final, and you can't change your mind and use the second trait later in the game.

Some cards, such as "Communication", can only be played onto a pair of animals. Such cards are placed between the two cards onto which they are played.

The development phase includes several rounds. Each player can play just one card at a time, starting from the first player and going clockwise in order. During this phase you may play any number of cards from your hand. If you don't want to play a new animal or add a new trait to existing animals, you say: "I pass". You must pass if you have no cards left. After passing, you can't choose to play more cards during this phase. The phase ends after all players pass.

Food Bank Determination Phase

The amount of food available during this turn is determined at this time. The amount is indicated by the dice, depending on the number of players:

2 players - number indicated by **one dice + 2**

3 players - sum of **two dice**

4 players - sum of **two dice + 2**

The first player rolls the dice. When the amount of food is determined, the corresponding number of red tokens is put in the center of the table; this is **the food bank** for the current turn.

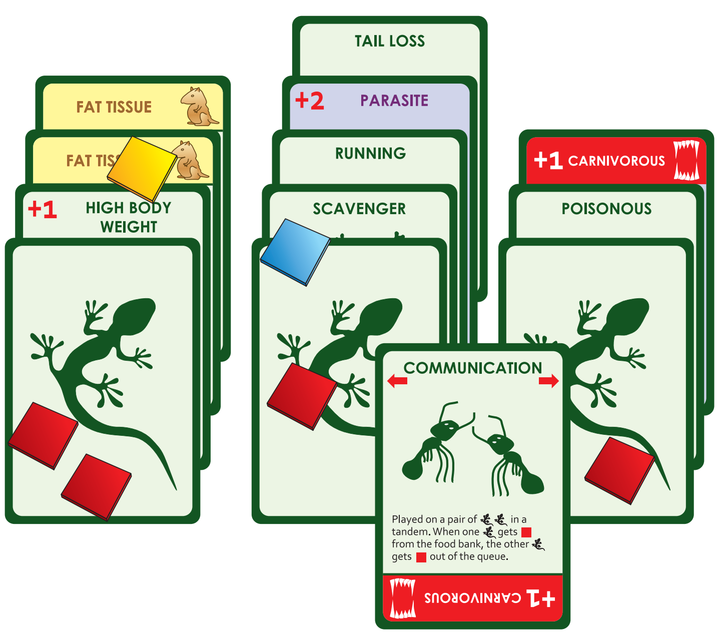
Feeding Phase

During this phase players in order take one red food token from the food bank and put it on the top of one of their animals. The first player takes the first token, and other players continue clockwise, going in several rounds if necessary and if there are enough tokens.

You may only take one red token from the food bank at a time. However, some traits, such as "Communication", allow to take several tokens.

As a result of their additional traits, your animals can also get blue "extra food" tokens during this phase.

An animal with **one food token** on the top of it is considered **FED,** unless it carries traits which increase its food requirements. Such cards show a number at the top left corner, indicating how many additional food tokens are required to feed the animal. For example, an animal (which, by itself, requires one food token) with the HIGH BODY WEIGHT trait (+1) carrying a PARASITE (+2) is only considered fed if it has four food tokens on the top of it.



Any animal can be fed by red or blue food tokens, or any combination thereof.

Important! A fed animal can't get more food tokens, except to fill its FAT TISSUE (see Fat Tissue section below). If all your animals are fed and their FAT TISSUE is filled you can't obtain more tokens from the food base or in other ways.

After all animals are fed and their FAT TISSUE is filled, or the food bank is empty and all players have used any traits of their animals they wanted to use, the feeding phase is over. Any remaining red tokens in the food bank are set aside.

Extinction and Draw phase

At the beginning of this phase all animals which are not fully FED are put into a discard pile, along with their traits, and all the pairwise traits associated with them. Each player has his own discard pile. The cards are put into a discard pile with facing up. You may look at cards in your own discard pile, but not in other players' piles.

The first player now deals new cards to players from the top of the deck. The cards are dealt one at a time in order, beginning with the first player. Each player gets in total the following number of cards: **1 + the number of surviving animals** belonging to the player. If the deck is empty it's possible that one or more players get fewer cards than they are due.
If a player has no surviving animals and his hand is empty then he takes **6 cards** from the deck during this phase.

After the cards are dealt the turn is over. All food tokens except the fat tokens are removed from the cards and set aside. The new turn starts with the development phase; the role of first player passes clockwise from the first player of the previous turn.

End of the Game

After the deck is empty the last turn begins. After the extinction phase of the last turn the victory points are counted. Each player is awarded victory points as follows:

- **+2 points** *for each surviving animal;*
- **+1 point** *for each trait of a surviving animal;*
- **additional points** *for the traits which increase food requirements:* **+1** *point for Carnivorous or High Body Weight;* **+2** *points for Parasite.*

If several players have an equal number of victory points, the one who has the most cards in the discard pile is the winner.

Traits

An animal may combine any number of traits. However, **no animal can have two identical traits,** with the exception of the FAT TISSUE trait and pairwise traits.

You may only add traits to your own animals. The only exception is the Parasite trait, which you may only add to another player's animal.

The sign in the description of a trait on a card denotes an "animal". The sign denotes a red token from the food bank. The sign denotes a blue extra food token which you can get as a result of using some traits.

Some traits such as High Body Weight or Swimming have permanent effects; others can only be used at specific moments during the game. For example the Running, Tail Loss, and Mimicry traits can only be used when an animal is to be eaten by a Carnivore.

If your animal has several traits which can be used at the same time, you decide in which order to use them. For example if your animal is attacked by a Carnivore, you can first use the Running trait (i.e. try to run away), and, if your attempt failed, use the Tail Loss trait (i.e. survive by loosing one of your traits).

Some of the traits may be only used during your feeding round. This means that when you are to take a red token from the food base, **you may use this trait together with, or instead of, taking a token**. This is possible even if there are no tokens left in the food bank.

Some traits, such as Carnivorous, Piracy, or Hibernation Ability, may only be used once per turn or every other turn. After using such a trait, rotate the card of the trait horizontally to identify that it has already been used in this turn. At the end of the turn, you may rotate the cards back into the vertical position.

Carnivorous Trait

This trait may be used during your feeding round. At this round **don't take food tokens from the food bank**. Instead, use your Carnivorous animal to attack one of the animals on the table, and if it succeeded in eating this animal get **two blue extra food tokens**. Each Carnivorous animal may only use its Carnivorous trait once a turn. In each feeding round only one of your Carnivorous animals may use its Carnivorous trait. Your Carnivorous animal may attack any animal on the table that is not protected by additional traits, including your own animals or another Carnivorous animal. For the animal eaten, all its traits and all pairwise traits associated with it are put into a discard pile. A Carnivorous animal can't attack and eat other animals if it is fully fed and has no empty Fat Tissue. You can use the Carnivorous trait even if there are no tokens left in the food bank.

Fat Tissue

A single animal may have several Fat Tissue traits. At any time during the game, when your animal with this trait which is already FED and can receive another food token, you can deposit it into its Fat Tissue. In this case, exchange the food token for a yellow fat token and put it on the top of the Fat Tissue card. You can put no more than one fat token on the top of each Fat Tissue card. If there are fat tokens on the top of each Fat Tissue, the Fat Tissue is considered filled and the animal can't get additional food tokens during this turn.

You may use the yellow fat token only during your feeding round. **Instead** of getting one red token from the food base take **any number** of yellow fat tokens from **one of your animals** and convert them into blue food tokens. This conversion isn't considered the same as **getting food tokens** and isn't bound up with using other traits of the animal.

Pairwise Traits

Pairwise traits are played on a pair of animals simultaneously. You can't play two identical pairwise traits onto the same pair of animals. If one of the animals is put into a discard pile, all pairwise traits associated with it are also put into the discard pile. During any player's action during a feeding round you can use each pairwise trait only once; however, you can use each trait during each feeding round. You decide in which order to use your pairwise traits to get food tokens for them. For example, if both Communication and Symbiosis traits are played onto the same pair of animals, you can first take a red token from the food bank to feed the symbiont, and if the symbiont is then fed, immediately take a second red token for the other animal.

If there are not enough tokens of a certain color at some point during the game, you can use tokens of another color or self-made tokens to substitute for them.

Playing with Two Game Sets

You can mix cards from two game sets to accommodate up to 8 players. The size of the food bank is then determined as follows:

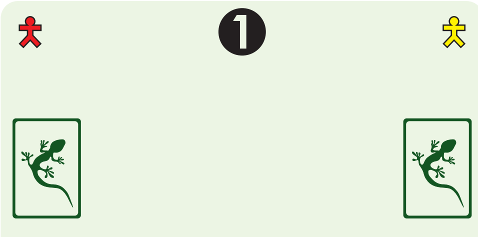
5 players - sum of **three dice + 2**
6 players - sum of **three dice + 4**
7 players - sum of **four dice + 2**
8 players - sum of **four dice + 4**

Special thanks: D. Bazikin, E. Bulyshkin, I. Gambashidze, A. Glagoleva, Richard Ham, L. Machina, A. Mironov, A. Pahomov, I. Tulovsky, D. Shahmatov.

FIRST TURN

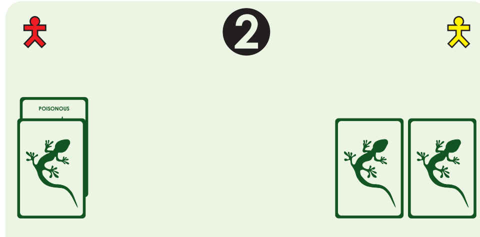
The game starts. Two players are playing: Alex ( ) and Dan ( ). Each has 6 cards in his hand. Alex makes the first move.

Development phase



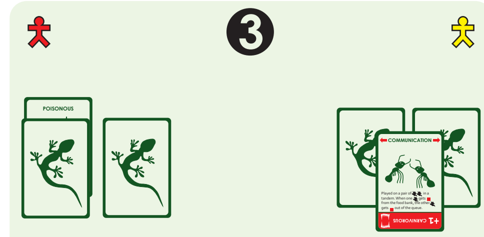
Alex plays the first card as an animal ( )

Dan also plays the first card as an animal ( )



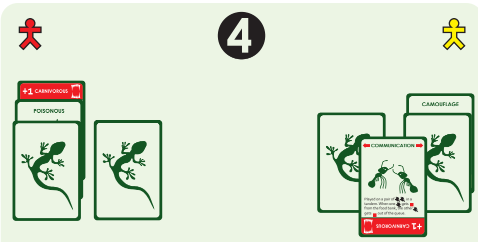
Alex adds a new trait Poisonous to his

Dan plays the second card as



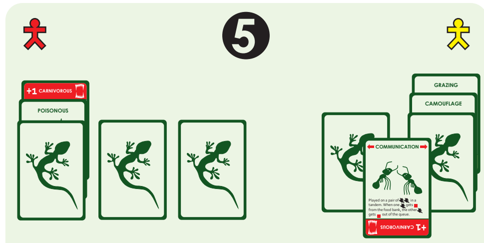
Alex also plays the second

Dan plays the pairwise Communication trait onto both his



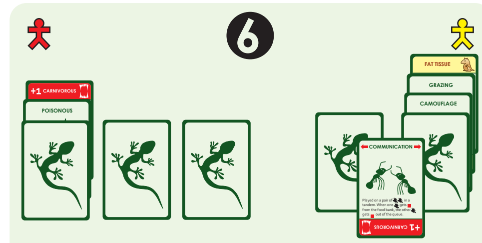
Alex adds a new trait Carnivorous to his poisonous

Dan adds the Camouflage trait to one of his to defend it from the carnivore



Alex puts the third into game

Dan adds Grazing trait to his camouflaged to deprive the opponent of food.

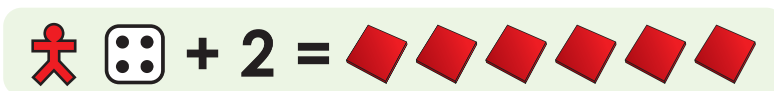


Alex says "Pass", deciding to save one card for the next turn.

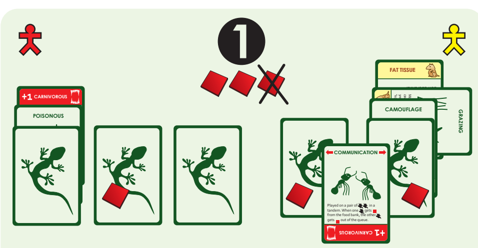
Dan adds the Fat Tissue trait to his , hoping to save food for the next turn.

Food Bank Determination Phase

Alex rolls a die. The roll is . This is a two-player game, so **2** should be added to the die result. Therefore, the food bank during this turn is **6 food tokens**

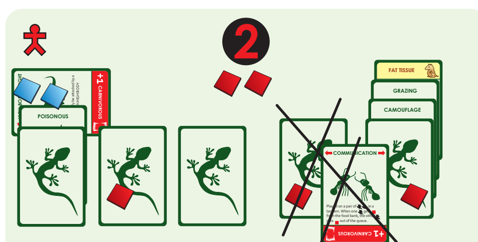


Feeding Phase



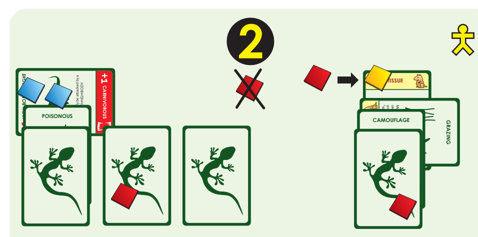
Alex is the first to take . He puts it on one of his

Dan takes for his (because they are Communicating) He uses the Grazing trait and destroys another .



Alex uses his Carnivorous animal to attack and eats one of Dan's . The Carnivorous animal gets .

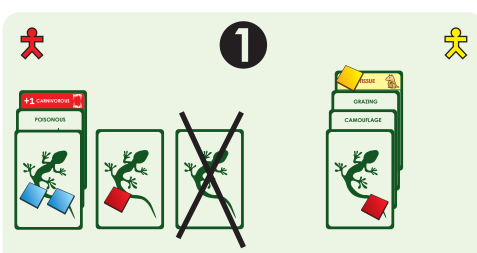
Dan looses the eaten , all its associated traits, and the obtained in the previous round.



Dan turns one of the into , filling the Fat Tissue of the surviving , and destroys the second remaining with the Grazing trait.

Extinction and Acquisition of New Cards

There are no tokens left in the food bank. All animals that are not fed die.



Alex loses one of the which is not fed.

Dan's survives.

All food tokens except the fat tokens are removed from . The players get new cards from the top of the deck, for a total of **1 + the number of surviving animals.** Alex gets **3** cards; Dan gets **2** cards.

The turn is now over. In the next turn Dan will make the first move in all phases.

Comment: in order to win, it is very important to play the right cards in the development phase and to distribute food properly in the feeding phase. Note that in this example, in the second round of the feeding phase, Alex could have taken the red food token for his second , and could have used the Carnivorous trait in the third round. In that case all his animals would have been fed, and he would have had a tactical advantage by the end of the turn.

Lab 12 report

Your name ____________________

1. Describe the most successful ecosystem (from the winner). Explain how the traits utilized made it the most successful (11 pts)

2. Why do carnivores require more food than "ordinary" ones? (2 pts)

3. Give *two reasons* why parasites and carnivores are necessary to maintain a healthy ecosystem. (2 pts)