Laboratory Exercise 1  
The Scientific Method

The study of science is different from other disciplines in many ways. Perhaps the most important aspect of science is its adherence to the principle of what is usually called the scientific method: formulating mutually exclusive hypotheses to explain an observed phenomenon, and then deliberately attempting—with rigorous, carefully designed experiments—to demonstrate which proposed hypotheses are false. Most scientists will agree that there is no one, set scientific method. But good science does have a process that should be followed in order to obtain good, repeatable results.

As a science major, you are no stranger to curiosity. It is the beginning of all scientific inquiry and discovery. As you walk through the campus arboretum, you might wonder, “Why are trees green?” As you observe your peers in social groups at the cafeteria, you might ask yourself, “What subtle kinds of body language are those people using to communicate?” As you read an article about a new drug promising to be an effective treatment for male pattern baldness, you think, “But how do they know it will work?” Asking such questions is the first step towards hypothesis formation. But to answer them requires that the investigator understand

1. the biological system in which the phenomenon of interest is framed
2. how to ask the right questions, and
3. how to devise meaningful, testable hypotheses.

A scientific investigator does not begin the study of a biological phenomenon in a vacuum. If an investigator observes something interesting, s/he first asks a question about it, and then uses inductive reasoning (from the specific to the general) to generate hypotheses based upon a logical set of expectations. To test the hypothesis, the investigator systematically collects data, perhaps with field observations or a series of carefully designed laboratory experiments. After analyzing the data, the investigator uses deductive reasoning (from the general to the specific) to state a second hypothesis (it may be the same as or different from the original) about the observations. Further experiments and observations either refute or support this second hypothesis, and if enough data exist, the hypothesis may eventually become a theory, or generally accepted scientific principle.

I. Science as Falsification

The scientific endeavor is largely one in which the investigator attempts to exclude hypotheses that are clearly wrong (as indicated by the results of carefully designed experiments), leaving only those hypotheses “standing” that still could be correct. This process of exclusion is known as falsification.

To give a ridiculously simple example, let’s say you are an ancient explorer who has just arrived at the shore of the Pacific Ocean. Being an inquiring person, you hypothesize that there are fish in this body of water. The alternative to this hypothesis is that there are no fish in the water. To test your hypotheses, you dip a net into the ocean and pull it out. What if you were to put your net in the water many times, and never catch a fish? Does this mean that there are no fish in the ocean? Not necessarily. While none of your trials has falsified the hypothesis that there are no fish in the ocean, you have not performed the infinite number of trials that might be required to know (with this method, at least) that there are no fish in the ocean. In other words, you have not proven your “no fish” hypothesis to be correct. You have only failed to prove that it is incorrect.
On the other hand, if you happen to capture a fish in the net on the first try, then you have falsified the hypothesis that there are no fish in the ocean. All it takes is one instance of catching a fish to do this. While this method of falsification has been in practice for a long time, it was perhaps most eloquently described by German philosopher Karl Popper (3):

1. It is easy to obtain confirmations, or verifications, for nearly every theory — if we look for confirmations.
2. Confirmations should count only if they are the result of risky predictions; that is to say, if, unenlightened by the theory in question, we should have expected an event which was incompatible with the theory — an event which would have refuted the theory.
3. Every "good" scientific theory is a prohibition: it forbids certain things to happen. The more a theory forbids, the better it is.
4. A theory which is not refutable by any conceivable event is non-scientific. Irrefutability is not a virtue of a theory (as people often think) but a vice.
5. Every genuine test of a theory is an attempt to falsify it, or to refute it. Testability is falsifiability; but there are degrees of testability: some theories are more testable, more exposed to refutation, than others; they take, as it were, greater risks.
6. Confirming evidence should not count except when it is the result of a genuine test of the theory; and this means that it can be presented as a serious but unsuccessful attempt to falsify the theory. (I now speak in such cases of "corroborating evidence.")
7. Some genuinely testable theories, when found to be false, are still upheld by their admirers — for example by introducing ad hoc some auxiliary assumption, or by reinterpreting the theory ad hoc in such a way that it escapes refutation. Such a procedure is always possible, but it rescues the theory from refutation only at the price of destroying, or at least lowering, its scientific status. (I later described such a rescuing operation as a "conventionalist twist" or a "conventionalist stratagem.")

One can sum up all this by saying that the criterion of the scientific status of a theory is its falsifiability, or refutability, or testability. (The complete text of Popper’s essay can be found at http://www.stephenjaygould.org/ctrl/popper_falsification.html#see, and like Platt’s work, is a must-read for every student of the scientific method.)

II. Reasoning and Hypothesis Formulation

There are several different ways one can apply the scientific method, and the appropriate hypotheses to apply in a particular situation may depend on the nature of the system, the observation and the problem. In studies of complex, multi-factor systems (e.g., ecology and evolution), or in the initial, exploratory phases of an investigation, a hypothetico-deductive approach is often taken. In other areas, such as cellular and molecular biology, developmental biology, and other areas in which questions are being asked based on a large base of previous knowledge, hypotheses may be reached inductively, and an array of competing hypotheses potentially able to explain a given observed phenomenon may be posed. Each of these is systematically eliminated with carefully designed experiments until only the most likely explanation remains. To better understand each method, we should first review the differences between inductive and deductive reasoning.

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A. Inductive and Deductive Reasoning

Scientists use both inductive and deductive reasoning to address biological problems. **Inductive Reasoning** is sometimes considered a "from the bottom up" approach. When we use inductive reasoning, our specific observations and measurements may begin to show us a general pattern. This might allow us to formulate a tentative hypothesis that can be further explored, and we might finally end up making a general conclusion about a set of observations (Figure 1).

![Figure 1. Inductive reasoning: “from the bottom up”](image)

In this case, one might construct an argument such as:

Items X, Y, and Z all have shown to have characteristic W. Therefore, all items in the same class as X, Y and Z probably also have W.

For example:

This bee stung me. It is a hymenopteran.
This wasp stung me. It is a hymenopteran.
This fire ant stung me. It is a hymenopteran.
I'm starting to see a pattern here. All hymenopterans have stingers.

One potential pitfall is the "inductive leap": When you make the jump from many specific observations to a general observation, your generalization might not be correct every time.

For example, many hymenopterans (stingless bees and ants, male honeybees, etc.) do not have stingers. (You might not discover this unless you test every single hymenopteran species for stinging capability.) Although generalizations are certainly useful, the wise investigator is aware that there may be exceptions to a general rule, and—in some cases—a "general rule" might eventually turn out to be wrong more often than not.

**Deductive Reasoning** is sometimes considered a "from the top down" approach. In this case, we start with a general premise and work down to the more specific (Figure 2).

![Figure 2. Deductive reasoning: “from the top down”](image)
Deductive reasoning is used to test existing theories and hypotheses (general ideas) by collecting experimental observations (specific examples). A syllogism is a specific argument with three simple steps:

- Every X has the characteristic Y.
- This thing in my hand is X.
- Therefore, this thing has the characteristic Y.

For example:
- All hymenopterans have stingers. (General idea previously reached via induction.)
- This thing in my hand is a hymenopteran.
- Therefore, this thing can probably sting me! (specific conclusion)

The experiment necessary to test this hypothesis might be painful.

The results of your experiment may suggest further questions that lead to new hypotheses and predictions. (What types of hymenopterans don't have stingers? Which is the primitive condition: stinger or no stinger? Why has stinglessness persisted?)

**B. Types of Hypotheses**

As mentioned previously, there is no single scientific method used in every field of science. Similarly, different types of hypotheses may be appropriate to apply under different circumstances.

1. **Inductive Hypothesis**
   - An inductive hypothesis is a generalization based upon many specific observations.

2. **Deductive Hypothesis**
   - A deductive hypothesis is derived from a general principle, and testing it is meant to support or refute (contradict) that general principle.

3. **Statistical Hypotheses**
   - When observed phenomena are suspected to be related to each other in a populational sense (For example, a new drug, SmokeBeGone, may help smokers quit smoking), statistical tests are used to determine whether the two phenomena are, indeed, related. Two mutually exclusive hypotheses are compared. The null hypothesis states that there is no relationship between the two phenomena (i.e., SmokeBeGone does not help smokers quit smoking). Its opposite, the alternative hypothesis states that there is a relationship between the two phenomena (SmokeBeGone does help smokers quit smoking). Well-designed and executed experiments will indicate which of these two competing hypotheses should be rejected, and which should be (provisionally) accepted.
   - Statistical alternative hypotheses may be **directional (one-tailed)** or **nondirectional (two-tailed)**. A directional hypothesis specifies how a particular phenomenon is related to another (“Smokers who take SmokeBeGone are MORE likely to quit smoking than those who take a placebo.”). A nondirectional hypothesis states only that there is a relationship between the two phenomena, but does not specify its direction. (“The rate of quitting smoking will be DIFFERENT between two groups, one taking SmokeBeGone, and the other taking a placebo.”)

**III. Methods of Scientific Investigation**

As mentioned previously, there is no single, set scientific method, and different methods and philosophies in science are still the subject of controversy. But it is probably safe to say that certain methods are better employed under certain circumstances than others. For
example, it would be difficult to pose detailed, complex hypotheses about an observed phenomenon without any degree of background knowledge of that phenomenon. Instead, in an exploratory phase of a scientific endeavor, it is sometimes necessary to first identify trends to determine whether a perceived phenomenon is real. Statistical methods are often employed in the exploratory phases of study, and only later, when more is known about the phenomenon of interest, can more specific hypotheses and strong inference be used.

A. Statistical Hypotheses

In the very early phases of investigating a biological phenomenon, the investigator may not have enough background information (and there may even be very little published literature in the area) to allow him or her to formulate detailed possible explanations for an observation. In fact, it is sometimes necessary to simply establish whether a suspected relationship between two factors is real or not. For this, statistical hypotheses are often employed. You will learn more about statistical methods in Appendix 2, and the second laboratory of this semester will be devoted to teaching you a bit of introductory statistics.

In areas of research where complex, multifactorial systems are being studied, it may also not be possible to pose simple, mutually exclusive hypotheses to explain what’s going on. Ecology, systematics, and evolutionary biology are all areas where this is often (but not always) the case, and statistical hypotheses are commonly employed to test whether competing models or hypotheses are correct or incorrect.

Before you and your teammates perform your first original lab experiment, you will be expected to educate yourselves about your model organism, the system you intend to study and what knowledge already exists in the area. The more knowledge you have in an area, the more likely it is that you will be able to ask interesting, meaningful questions of value that go beyond the “science fair mentality” of questions such as “do enzymes work faster at warmer temperatures?” Face it. You already know the answer to that one. A more interesting and relevant question would be “WHY do enzymes work faster at warmer temperatures?” You might also already have some inkling about the answer to this, but at some point in the past, researchers did not. They had to pose hypotheses about the structure and nature of enzymes and devise experiments that would allow them to eliminate all but the correct hypothesis.

At this moment, you and your teammates may or may not have enough background knowledge to propose appropriate competing hypotheses to address the question/problem you have stated and wish to examine. Once you complete your background information, this may change. But in some cases, you may still need to use statistical methods and a populational approach to address your initial hypotheses.

B. Strong Inference

In 1964, John R. Platt (1) wrote an influential paper regarding the scientific method then commonly employed in areas such as molecular biology and physics. He termed this the strong inference method of hypothesis testing, and wrote:

In its separate elements, strong inference is just the simple and old-fashioned method of inductive inference that goes back to Francis Bacon. The steps are familiar to every college student and are practiced, off and on, by every scientist. The difference comes in their systematic application. Strong inference consists of applying the following steps to every problem in science, formally and explicitly and regularly:

1. Devising alternative hypotheses that potentially explain an observation
2. Devising a crucial experiment (or several of them), with alternative possible
outcomes, each of which will, as nearly as possible, exclude one or more of the hypotheses;
3. Carrying out the experiment so as to get a clean result.
4. Recycling the procedure, making subhypotheses or sequential hypotheses to refine
the possibilities that remain, and so on.

It is like climbing a tree. At the first fork, we choose—or, in this case, "nature" or the
experimental outcome chooses—to go to the right branch or the left; at the next fork, to
go left or right; and so on. There are similar branch points in a "conditional computer
program," where the next move depends on the result of the last calculation. And there
is a "conditional inductive tree" or "logical tree" of this kind written out in detail in many
first-year chemistry books, in the table of steps for qualitative analysis of an unknown
sample, where the student is led through a real problem of consecutive inference: Add
reagent A; if you get a red precipitate, it is subgroup alpha and you filter and add
reagent B; if not, you add the other reagent, B; and so on.

(The entire text can be found at www.bio.miami.edu/dana/151/Platt_1964.pdf. You should
read it before you begin this laboratory course.)

Let’s Face It...

When you ask a scientific question these days, you are not like the very first humans who
looked with a curious eye at the natural world around them, but had to “start from scratch.” In
most cases, there is a body of knowledge available that gives background information and
previously gained knowledge about the system that interests you. When an investigator has
sufficient background knowledge to pose relatively detailed hypotheses about the workings of
a particular phenomenon of interest, the strong inference method can, and should, be used.

With sufficient background knowledge, an investigator can have a pretty good idea of the
possible reasons for an observation. S/he could, of course, just rely on a personal hunch to
pose a hypothesis. But this isn’t good practice in our modern age. Rather, a careful and often
exhaustive review of previous research and literature will provide the key.

In the strong inference method, the goal is to create a list of all reasonable possible
explanations for a particular observation. Such a list is useful because:

1. It helps eliminate bias.
   Let’s face it. Almost anyone who has tried to explain something observed has a favorite
   “pet” explanation, even though it might be wrong. By listing all possible explanations, the
investigator effectively forces himself/herself to consider alternatives to his/her favorite idea.

2. It provides a scientific “safety net”
   If your experimental results do not support your favorite hypothesis, and you have not
considered alternatives, then what? Back to the drawing board? In real research laboratories,
that could be prohibitively wasteful of time and resources. Listing all possibilities in advance
not only forces the investigator to see his/her own possible biases, but also provides
alternative explanations, should the “favorite” hypothesis prove to be incorrect.

Like statistical hypothesis testing, strong inference is science by process of elimination. And
once again, one should not say that a particular hypothesis—even if it is the only one left
standing at the end of a strong inference protocol—is true. One can say only that it is the best
explanation, given current understanding of the problem. It may be a very good explanation,
and one that will ultimately prove to be very powerful. But while it remains a hypothesis, it is still subject to testing and falsification with any new methods or in the light of any new information that may become available later.

**IV. Scientific Protocol**
A critical first step in any scientific endeavor is to create a protocol that summarizes:

1. why you are performing your experiments (background information and reasoning)
2. precise experimental design
3. methods for data collection and analysis
4. all possible outcomes of the experiment
5. potential pitfalls in experimental design, data analysis, or assumptions

This document will serve as a template for the final presentation of experimental results and conclusions, which should take the form of a scientific paper.

**A. Introduction**
In this section, your team should explain why the particular study being undertaken is relevant and interesting. General background of the phenomenon should be summarized, including as much pre-existing information with respect to the phenomenon as possible, without being exhaustive. Your introduction should be enough to justify the experiment and its relevance.

As you might already have surmised from the above, one of the MOST important things you must do before embarking on a research project is *background research*. This means using the library resources, scholarly internet resources, and refereed journal articles to educate yourself about the system and model organism you will be studying. Before you start working on a problem in this lab, you and your teammates must become well educated about the problem itself. We leave that up to you, the budding scientist.

**B. Methods**
Here, your team should describe the experimental protocols that will be employed in the experiment. Since your team will design its own experiments, the precise difference between treatment and control groups, the number of the subjects and replications, and all unique aspects of your experiment should be included. You need not write detailed explanations of how to use equipment, since this information is already written in the lab manual. Simply refer to it, and do not re-state it here.

**C. Data Analysis**
The type of data collected and—if a statistical test is being used to analyze data—the type of statistical test must be stated before any data are collected.

**D. Predictions and Interpretations**
The team should discuss all possible results/outcomes of the experiments to be performed. Each outcome should be accompanied by an explanation of the most likely cause of this outcome. Note that the outcome is NOT the same as the interpretation. The outcome is the summary of the results themselves, whereas the interpretation is the logical, reasoned *explanation* for the observed results.

**E. Potential Problems**
As much as we like to think that our experiments are flawless, this often is not the case, especially in a lab with limited time and resources available. In this section, the team should
list all possible sources of error, and especially note any assumptions being made that are not already supported by previous knowledge. Listing these things in advance may help you improve your experimental design even before you begin.

By listing these things before even starting an experiment, the research team has laid the foundation for the scientific report of their conclusions, which will be written after all data have been collected and analyzed.

V. Model Organisms

Laboratory studies addressing biological phenomena often employ model organisms. Biological discoveries from a particular model organism can be further explored in different species to see if the same principles hold true. Model organisms are widely used in medical research where it would not be possible (or ethical) to perform experiments on humans.

We can learn about general biological principles from model organisms because, at some time in the distant past, we shared a common ancestor. The genetic code of all living things consists of DNA sequences, and those sequences often encode similar molecules and more complex structures. While it is important not to extrapolate from one species to another without strong scientific evidence that this is appropriate, many discoveries are made in simple model organisms, and then later found to be true in more complex organisms.

Viruses, while not considered to be truly "living" by most definitions, can be used as models. Various species of bacteria, protists, fungi, plants and animals have been used as model organisms. Our own intestinal resident, Escherichia coli, is one of the most ubiquitous model organisms in biology. Other common model species include pink bread mold (Neurospora crassa), Baker’s Yeast (Saccharomyces cerevisiae), Wall Cress (Arabidopsis thaliana), a nematode (roundworm) named Caenorhabditis elegans (affectionately known as C. elegans to most researchers), and the House Mouse (Mus musculus), to name just a few.

The model organism most appropriate depends on the study. But such organisms are usually chosen because they are amenable to easy manipulation, they reproduce quickly, and they are easy to handle without having your hand bitten off.

VI. Understanding a Biological System

Today’s lab discussion of the scientific method is only the beginning. You will also take some time this period to learn about statistical testing and hypothesis formulation. All this is preparation for your BIL 161 Lab Research Team (to be formed today) term projects. You will choose three of the following four topics:

- Biodiversity in Local Aquatic Ecosystems
- Seed Germination
- Behavior of Betta splendens (Siamese Fighting Fish)
- Water Movement in Vascular Plants

A lab manual chapter is available for each of these, and is linked to the course syllabus at:

http://www.bio.miami.edu/dana/161/summer
Your team should spend some time (between the next two lab periods) reading and discussing these chapters to decide which projects you’d like to tackle over the term. For each project, you will spend:

1. One lab period doing background research and experimental design
2. One lab period actually performing the experiment
3. One lab period presenting your results in the form of either PowerPoint or a Poster presentation (your instructor will tell you which format for each project).

Your instructor will tell you more about how your lab section will maintain an interactive discussion about each team’s proposed experiments (probably via a blog or Discussion Board on Blackboard) over the few lab sessions. Your instructor will give your team a deadline for an experimental proposal, which you will present to the entire class for critique and revision. By the time we’re ready to provide you with various lab equipment and supplies, you should be ready to perform your experiments and well equipped to analyze your results.

Most of all…enjoy the scientific experience!

VI. Plan of Attack: Guidelines for Planning Your Project

Scientific findings are presented in many different ways, including slide show talks, (e.g., PowerPoint), scientific poster symposia, and, of course, publication in refereed scientific journals. You may already be familiar with the traditional format of a scientific paper, which can be modified depending on the medium or venue of your presentation. This consists of the

• **Title**, a brief (one phrase/sentence) but detailed description of your study.
• **Introduction**, in which you explain the background and reason for your study, and pose your hypotheses and predictions
• **Methods**, in which you explain methods and materials used in the experiment in such a way that another research team could replicate your experiment.
• **Results**, in which you present your analyzed data and, if appropriate, your statistical findings.
• **Discussion**, in which you explain your results and pose additional hypotheses about your observations. This is also the place to suggest future research ideas.
• The **Abstract**, which appears as a single paragraph under the title of the paper, gives a summary/overview of the entire study from introduction to discussion.

The Scientific Paper Template below are meant to help you organize your thoughts and prepare your work so that it can be put into the proper format. Use this template each time you begin a research project. Your lab instructor will go over this form with you, and possibly hold a class discussion of each team’s template so that everyone can trouble-shoot before the actual experiment is performed.

Title: 

Group (Name and members): 

**INTRODUCTION**

In a few sentences, give some background on this project. For example, tell us the following.

1. What biological system are you examining?
2. Are you using a model organism? If so, why is it appropriate for your study?

3. What critical question are you asking about this biological system?

4. Is a pilot study necessary to verify that there is a basis for this question? If so, briefly describe such a pilot study and what it is intended to show.

5. What parameter are you going to measure in this study? Why is it appropriate?

6. What is your overall hypothesis regarding the system you are examining?

7. If you are using statistics to examine your system, what is your null hypothesis?

8. What is your alternative hypothesis?

9. What do you predict your results will show? Why?

METHODS

1. What are you measuring? Are there treatment and control groups? Are you comparing two different sub-systems within your biological system? Describe them and explain what you are measuring.

2. Describe your experimental procedure. (Give all details, such as temperature, equipment used to take measurements, etc. Another person should be able to replicate your work by reading this and following the general directions.)
3. What type of statistical test will you use to analyze your data?

RESULTS
1. What are the statistics of your treatment & control groups (as appropriate; e.g. means of weight, length how many days it took to go from stage X to stage Y, or whatever you measured.)

2. What were the results of your statistical test?
   Value of your statistic:
   P value:

3. Did your statistical test indicate that you should accept or reject your null hypothesis?

4. Arrange your analyzed data in neat, tabular or figure form. In the space below, write a legend for the table or figure, telling the reader exactly what is shown. Don't forget the units you used to measure your data (weight in grams; number of days, etc.)

DISCUSSION
1. Which hypothesis did you (provisionally) accept?

2. How does the above correspond (or not correspond) your predictions?

3. What, exactly, do your observations/data tell you?
4. To the best of your ability, using logic and creative reasoning—Pose as many competing hypotheses that could explain your results as possible. These should be stated so as to provide testable hypotheses for additional experiments.

5. What future experiments could be done to further shed light on what this experiment told you about your biological system?

6. Give a summary statement that draws the entire study together.

**Literature Cited**

