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.

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).

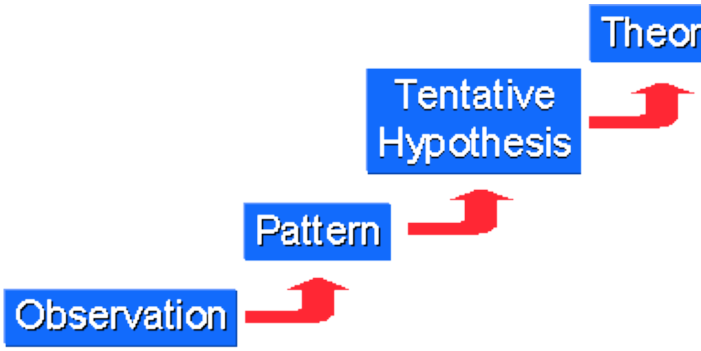

Figure 1. Inductive reasoning: "from the bottom up"

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).

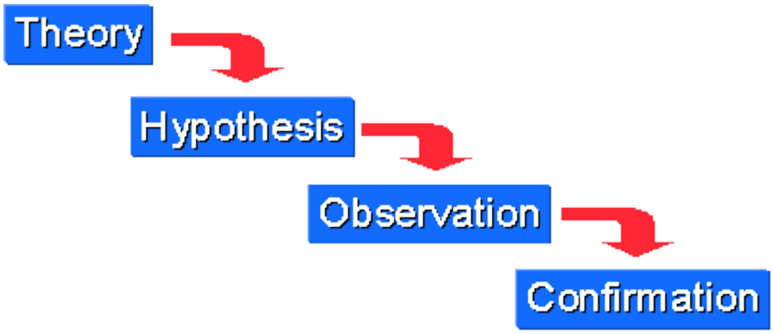

Figure 2. Deductive reasoning: "from the top down"

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 of subjects, 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, excludes 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.)

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 now a body of knowledge available that gives background information and previously gained knowledge about the system that interests you. When the 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 often 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 really 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 collect 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. This is covered in more detail in Appendix II.

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**. Discoveries about biological systems made using a particular species of model organism can often 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, at least in early stages of study.

We can learn about general biological principles from model organisms because, at some time in the distant past, we all 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 scientific discoveries are made in simple model organisms, and then later found to be true of more complex organisms, as well.

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 cereviseae*), Wall Cress (*Arabidopsis thaliana*), a nematode (roundworm) named *Caeorhabditis elegans* (or just *C. elegans* to most researchers), the House Mouse (*Mus musculus*), to name just a few.

The model organism most appropriate for a particular study 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.

In our first laboratory exercise this semester, we will use the handy and adorable Baker's Yeast (*S. cereviseae*) as a model organism. They are easy to handle and grow, they look very cute under the microscope, and they give us wonderful gifts, such as bread and beer. Who among us could not love yeast?

VI. Understanding a Biological System

Today's lab discussion of the scientific method is only the beginning. Next week, you will learn about statistical testing and hypothesis formulation.

Almost all multicellular organisms produce enzymes that break down a harmful by-product of cellular metabolism, hydrogen peroxide. In many different species, this enzyme is known as **catalase**, which evolved from a catalase-like enzyme found their common ancestor. Over the years, the study of catalase in various model organisms by many different researchers has shed light on the basic nature of enzymes themselves. You will now join their ranks. Our goal is to equip you and your colleagues (team members) with enough biological know-how to be able to pose an interesting and relevant question about catalase.

Near the end of today's lab, your instructor will give you a brief overview of catalase and the reaction is catalyzes. Your team's job, over the next two weeks, is to start doing a literature search and reading as much as possible about your model organism, the enzyme catalase, what it does, and what is known and not known about it. Once you have this background information, your team should be able to confer and pose a problem based on observations and knowledge you have gained about catalase in Baker's Yeast (and perhaps other organisms).

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 next two weeks. 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 live yeast and various lab supplies, you should be ready to perform your experiments and well-equipped to analyze your results.

Refer to the Lab Chapter posted as Laboratory #3 (linked to the course syllabus at www.bio.miami.edu/dana/151) to see what materials will be available for you. If your team will require something different or in addition to what's listed, then you must let us know at least a week in advance so we can provide it.

Most of all…enjoy the scientific experience!

Literature Cited

1. **Platt, J.R.** Strong inference. *Science* 146: 347–353, 1964.

2. **Hiebert, S.M.** The strong-inference protocol: not just for grant proposals. Adv Physiol Educ 31: 93-96, 2007

3. **Popper, K.** Science as Falsification. *Conjectures and Refutations*, London: Routledge and Keagan Paul, 1963, pp. 33-39; from Theodore Schick, ed., *Readings in the Philosophy of Science*, Mountain View, CA: Mayfield Publishing Company, 2000, pp. 9-13.