EXPERIMENTAL DESIGN & HYPOTHESIS TESTING
Lab 3

Key Concepts
- The Process of Science
- Hypothesis Testing
- Experimental Design
- Confounding Factors
- Bias, Accuracy and Precision
- Replication
- Type I and Type II Errors

Student Learning Outcomes
After Lab 3 students will be able to:
1. ask scientific questions, develop hypotheses, and describe key elements of designing a scientific study.
2. design a scientific study utilizing each step of the scientific method to collect and analyze data and draw conclusions in a biological context.

I. Introduction
“All interpretations made by a scientist are hypotheses, and all hypotheses are tentative. They must forever be tested and they must be revised if found to be unsatisfactory. Hence, a change of mind in a scientist, and particularly in a great scientist, is not only not a sign of weakness but rather evidence for continuing attention to the respective problem and an ability to test the hypothesis again and again.”

Dr. Ernst Mayr
Evolutionary Biologist

Today’s lab introduces you to the process of science. Throughout this course and others, you will use the scientific method to investigate specific patterns and questions. At the heart of this method is the fact that all scientific questions can be translated into hypotheses, which are testable, and that an idea in science is not accepted until the hypothesis is supported by evidence.

Experimental Design
Experimental design refers to the careful planning of an experiment, from beginning to end, before it is conducted. Because there is inherent variability in nature, we design experiments to encompass this range of variability. This is perhaps the most important aspect of a scientific study, as it prevents against bias. The results of ecological experiments should advance our understanding of nature; therefore, experiments must be set up in such a way that we can have confidence in the results and answers we obtain. If studies are carried out with no regard for the experimental design, the results are likely to be invalid or inconclusive (See Type I and II Errors).

The Question
There are a few things you must consider when designing an experiment. First, you must identify the question that you are trying to answer. Researchers spend a good deal of time developing interesting and important questions that can be addressed through scientific methods. Most ecological questions arise from observations of natural phenomena or the human imagination. To develop an ecological question, you must first make an observation of some pattern or event.

Second, you must transform your observations into a question. For example, a fundamental question in ecology is – why do species seem to be found in some places but not others?

Once you have identified your question, you need to think about how you can answer it. This will involve identifying the various factors that you believe may be responsible for the pattern, and ideally, manipulating the various factors (variables) in an experiment. The process of developing and testing hypotheses is called the scientific method.

The Scientific Method
In this lab, we will focus on hypothesis testing, a scientific method developed extensively by Popper (1968). This is the most widely used scientific method in which one develops explicit hypotheses for observations of a natural pattern or phenomenon (Figure 1). In the context of the scientific method, a scientific hypothesis is ideally a description of a mechanism, a proposed explanation that can account for some phenomenon in nature (e.g., male chickadees sing to attract mates). Hypotheses are derived by induction: taking one or many specific observations and developing a general explanation for those
observations. These hypotheses developed from inductive reasoning are often called inferences. Following Popper’s methodology, the best hypotheses lead to logical predictions that can be tested with an experiment. Unlike hypotheses, predictions are derived via deduction, a form of reasoning in which a logically certain conclusion or consequence is drawn from one or more general statements (hypotheses). A prediction (typically stated in the form of an “if…then” statement) forms the basis for an experimental test of a hypothesis (e.g., if recorded male chickadee songs are played in a forest, then females will move toward the sound). If the outcome of an experiment fails to meet the prediction of the hypothesis, then the hypothesis may be rejected.

![Flowchart of the steps involved in the scientific method](image)

**Figure 1.** Flowchart of the steps involved in the scientific method. (1) Before a hypothesis is formulated, some phenomenon must be observed. (2) The hypothesis is formulated to explain the phenomenon. (3) An experiment is designed to test the hypothesis and predictions are made about what the outcome would be if the hypothesis was true. (4) The experiment is conducted, and the results are compared to our prediction. If the outcome of the experiment does not meet our prediction, then our hypothesis is considered falsified (arrow goes back to observation).

If the outcome of an experiment is consistent with the predictions of the hypothesis, then the hypothesis is considered supported. One can never prove a hypothesis, one can only find support for a hypothesis or reject a hypothesis. The reason we do not prove a hypothesis is that there may be another explanation for what we observe that we have not yet considered. Therefore, we say that our results are consistent with or support our hypothesis. It is not yet proven, because we may get results that match a different hypothesis. Support for a hypothesis may be strengthened by developing additional (new) predictions that may be investigated with the scientific method.

Not all scientific studies use the scientific method to identify mechanisms responsible for natural phenomena. Descriptive studies attempt to identify patterns or trends, and do not require the use of the scientific method. They are question-based (e.g., At what time of day do chickadees sing?) but are not designed to test scientific hypotheses about why things are the way they are. Descriptive studies can have predictions, but they are not required as scientists often have no idea what the outcomes of a descriptive study might be. Descriptive studies often provide the observations that lead to the development of good scientific hypotheses than can explain the patterns uncovered in a descriptive study.

**Statistical Hypotheses**

For better or for worse, the word “hypothesis” is used in a different way within the statistical framework that is used to assess the outcomes of an experiment. Statistical hypotheses are statements about whether or not a pattern or trend is present in data collected during an experiment. In hypothesis-driven studies, statistical hypotheses are actually predictions derived from scientific hypotheses, and in the framework of experimental design, an experiment can have two general outcomes referred to as a null hypothesis ($H_0$) and one or more alternative hypotheses ($H_a$). $H_a$ is our prediction about what will happen in the experiment if our scientific hypothesis is true, whereas the null hypothesis ($H_0$) usually refers to an expectation if our scientific hypothesis is wrong. It is important to remember that statistical hypotheses are not the same as scientific hypotheses. Statistical hypotheses are simply statements about expectations that are used to determine if patterns or trends are present in descriptive studies or to test hypotheses in hypothesis driven studies.
The reason we have a null hypothesis is so that we have a clear expectation that we can disprove. \( H_0 \) provides a statistical reference point that you use to gauge the degree to which your data support \( H_a \). After data are collected from a survey or experiment, the data are analyzed and compared with what would be expected according to \( H_0 \). If the data are inconsistent with \( H_0 \) but instead match our prediction (\( H_a \)) then \( H_0 \) is rejected and it can be concluded that the data support \( H_a \) (which in turn provides support for the scientific hypothesis). Always keep in mind that we cannot absolutely prove that \( H_a \) is true using the Scientific Method. We can only show that there is support for it, and we can measure the degree of support using statistics.

Descriptive studies also often use this same statistical framework to look for trends or patterns in data, but the predictions (null and alternative hypotheses are not necessarily derived from a scientific hypothesis). For example, in a previous lab, we measured the heights of all students, and recorded whether each person was male or female. \( H_0 \): There is no difference in mean height between males and females. \( H_A \): Mean height for males will be greater than mean height for females.

**Confounding Factors, Bias and Precision**

There are many pitfalls in designing and interpreting experimental and descriptive ecological studies. A confounding factor is anything that has not been controlled or accounted for but may affect the outcome of an experiment. A great deal of care must be taken into account for possible confounding factors. In a hypothetical study of the relationship between drinking coffee and increased incidence of lung cancer: If coffee drinkers are more likely to be smokers than non-coffee drinkers, a study may incorrectly conclude that coffee increases the risk of lung cancer. Smoking is a confounding factor, which was not accounted for in the study’s design.

Another important concept in experimental design is avoiding bias (a systematic difference from the population parameter of interest). When designing an experiment or set of observations with the goal of estimating some quantity, it is important to minimize bias and maximize precision (the degree to which repeated measurements under unchanged conditions show the same results (Figure 2)). A biased sampling technique is one that does not give you a representative sample of whatever you are trying to estimate in your experiment but gives you a systematic error (i.e. you will repeatedly make a measurement that will alter the data a certain way).

**Randomization and Replication**

One of the best methods of minimizing bias is to employ randomization when assigning treatments, sites or individuals to measure.
Type I versus Type II Error

When testing hypotheses, there are two possible sources of errors that would make your conclusions incorrect, termed Type I and Type II errors. A Type I error refers to the rejection of $H_0$ when $H_0$ is actually true. Most experiments are designed to minimize this type of error. The probability of a Type I error is often referred to as the p-value. When the p-value for your results is equal to 0.05, there is a 5 in 100 chance that the variation we are seeing is due to chance. It is generally accepted that when a p-value is less than or equal to 0.05, it is safe to reject $H_0$ in favor of $H_a$. Increasing the number of replicates generally lowers the risk of Type I error.

A Type II error refers to not rejecting $H_0$ when it is actually false. As with the Type I error, the Type II error rate can be decreased by increasing your sample size (increased replication). However, keep in mind that adding extra replicates can be time consuming and expensive.

Interaction between Type I and Type II error

There is an important relationship between Type I and Type II error that leads to a dilemma. You can choose to make Type I error as low as you want, but as you decrease Type I error, you INCREASE Type II error and vice versa. There is no way around this problem. Most ecological experiments have the Type I error rate (alpha) is set to 0.05, and the resulting Type II error rate (beta) in the range of 0.1 to 0.3. This means that it is more likely (10-30% chance) to commit a Type II error as opposed to a 5% chance of committing a Type I error. One argument sometimes used to justify this situation is that scientists should be conservative – they should be most concerned about not committing Type I error (falsely rejecting the null hypothesis) because in such a case, the scientist would be erroneously claiming support for his/her hypothesis. Any scientist who makes such claims repeatedly will quickly get a bad reputation and will not be taken seriously by others. On the other hand, scientists are often less concerned about making a type II error (falsely accepting the null hypothesis) because no specific claims are made. Rather, the scientist concludes (erroneously) that his or her hypothesis was not supported, and more experiments are needed before a conclusion can be reached. Eventually, the experiment will be repeated and the correct outcome (reject $H_0$) will be obtained.
II. LABORATORY EXERCISES


A crucial step in the scientific method is to design an experiment that clearly supports or rejects the hypothesis. This is one of the biologist's most challenging and creative tasks. Biologists spend considerable time reading the scientific literature and critiquing other experiments before undertaking their own work. In designing a good experiment, scientists must define the variables, outline a procedure, and determine controls. In Exercise 1 you will explore how biologists use the scientific method to answer a question by considering Lyme disease as a case study, while in Exercise 2 you will have the opportunity to develop hypotheses based on your observations.

**Exercise 1. Lyme Disease: A Case Study**

Lyme disease is a prevalent pest-carried disease in the United States (not present in Hawaii). If it is left untreated, it can become debilitating causing heart and nervous system problems and severe arthritis. A spiral shaped bacterium called *Borrelia burgdorferi* causes Lyme disease. The bacterium is transmitted to people by a small, black-legged tick that also feeds on deer and mice. A summary of how we think the life cycle of the tick that carries Lyme disease works (a hypothesis) is shown in Figure 3.

![Figure 3. Life cycle of the tick that carries Lyme disease (From: Lyme Disease, 2000; University of Rhode Island Tick Research Laboratory 2000; and American Lyme Disease Foundation, Inc, 2003.)](image)

The ticks’ hosts are deer and mice. Deer and mice feed on acorns in oak forests primarily in the northeastern and western parts of the United States. A team of researchers observed that the acorn density of the oak forests in New York fluctuates from year to year and that mice populations in the forest were increased when the acorn density was high (Jones et al. 1998). Based on this observation and what was
already known about the life cycle of ticks, these researchers proposed that fluctuations in the abundance of acorns (and mice) regulate the abundance of ticks (a hypothesis), predicting that "if there are more acorns and mice in the forest, then will there be a higher density of ticks that carry Lyme disease."

To test the hypothesis about factors that control tick populations, the researchers studied six unfenced oak forest plots in New York (Figure 4). Mice were initially removed from each plot by trapping. Four tons of acorns were added to three of the plots (with the help of some local girl scouts!). The remaining three plots did not receive acorns. Ticks were collected in 225 sections (15m x 30m) in each plot and were identified and counted. The number of mice in each plot was counted also.

1. Review the elements of experimental design presented in your class discussion by answering the following questions on your work sheet:

What is the key OBSERVATION related to this experiment?

What is the HYPOTHESIS being tested?

What PREDICTION can be made from the hypothesis?
Phrase it in the form of an if/then statement.

2. Determining the variables
When designing an experiment to test a hypothesis, it is essential to identify and carefully consider the variables. Variables are the factors that may change during an experiment. The variables must be clearly defined and measurable. The table below describes the types of variables to consider when designing an experiment.

<table>
<thead>
<tr>
<th>TYPE OF VARIABLE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEPENDENT</td>
<td>This is the variable that the researcher actually measures, counts, or observes. The DEPENDENT variable is what the researcher thinks will change in response to the experimental treatment.</td>
</tr>
<tr>
<td>INDEPENDENT</td>
<td>This is the variable that is intentionally changed by the researcher. An INDEPENDENT variable is selected that the scientist thinks will affect the dependent variable.</td>
</tr>
<tr>
<td>STANDARDIZED or CONTROLLED</td>
<td>These variables are held constant between each group. By keeping STANDARDIZED (or CONTROLLED) variables equal among the treatment groups, the scientist helps prevent these factors from influencing the dependent variable outcomes.</td>
</tr>
</tbody>
</table>

In this case study, WHAT ARE THE DEPENDENT, INDEPENDENT AND STANDARDIZED VARIABLES? Record your answers on your worksheet. DISTINGUISH between dependent and independent variables in the examples on your work sheet.
3. Designing the Procedure

The procedure refers to the actual method or particular series of steps used to conduct the experiment. When designing the procedure, it is important to consider the control treatment, the level of treatment, the number of replications, and the sample size. Components of the procedure are outlined in the table below.

<table>
<thead>
<tr>
<th>COMPONENT OF PROCEDURE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEVEL OF TREATMENT</td>
<td>The value(s) of the independent variable.</td>
</tr>
<tr>
<td>REPLICATION</td>
<td>The number of times the experiment is repeated.</td>
</tr>
<tr>
<td>SAMPLE SIZE</td>
<td>The size of the group or portion of the whole that is being assessed.</td>
</tr>
<tr>
<td>CONTROL TREATMENT</td>
<td>A group in which the independent variable is held at zero or at some standard or established level.</td>
</tr>
</tbody>
</table>

In this case study, WHAT IS THE LEVEL OF TREATMENT, THE NUMBER OF REPLICATES, THE SAMPLE SIZE, and THE CONTROL TREATMENT? Record this on your work sheet.

4. Predictions

Remember that a good hypothesis is testable and can be either supported or proven false. It also can be used to PREDICT the effect of the independent variable on the dependent variable. Predictions can be expressed in the form of if/then statements. (General form: If the independent variable is X and an experiment is conducted in which X is varied, then the dependent variables will be affected in this way.) Predictions are useful when evaluating the experimental results. If the results do not match your prediction, then the hypothesis is rejected. If the results match, the hypothesis is supported.

PREDICT the results of the case study experiment based on the hypothesis. Phrase your prediction in the “if…then” format on your worksheet.

5. Check Your Understanding

To check your understanding of hypotheses and variables, complete Section B of your work sheet.
Exercise 2. Developing scientific hypotheses from observations
For this exercise you will form scientific hypotheses based on our observations of images. An observation is made with our senses. If you look at a picture, you can use your senses to describe the picture. If you smell an aroma, you can describe what the aroma smells like. If you touch an object, you can describe what the object feels like and so on. Your descriptions of a picture, an aroma, or an object are observations. With our observations, we can make inferences using inductive reasoning. An inference is a hypothesis that we formulate based on our observations. For example, if you were to walk outside and see that the ground was wet, you may infer that it rained. However, when you check the weather report in your area (with predictions about what the report will say that were derived from your hypothesis), you could find that it hasn’t rained at all (which would lead you to reject your hypothesis that it rained). We may then make more observations that could lead to a new hypothesis, such as finding nearby that lawn sprinklers or workers repairing a water line. We could then use our new observations to make new predictions, which in turn help us formulate a new hypothesis to explain why the ground was wet.
Recall that a scientific hypothesis is a statement that include variables that are testable and is based on observation and existing knowledge. In order to develop a hypothesis, you must have a question that you want to answer. For example, why do parrotfish make cocoons? Researchers have observed that parrotfish secrete mucus from their gills and wondered what the function of the cocoon was. The researchers honed in on the explanation for the cocoon: protection against parasites. From this hypothesis, the researchers predicted that parrotfish that did not have a cocoon would be attacked more by parasites than parrotfish that did (Grutter et al. 2010).

1. Make Observations
Your TA will give you an image to study. With your group write down all of your observations. Because it is a picture, you won’t be able to use all of your senses. With your sight, what do you see? Remember that you are writing down observations, not what you think are explanations for those observations (scientific hypotheses). Is it an object? What color is it? Does it have patterns?

2. Develop Hypotheses
Discuss your observations with your lab mates. Based on your observations, come up with a list of scientific hypotheses about what is being shown in the photo you were given. What questions does the photo bring to your mind? How might you begin to answer your questions? How might you justify your reasoning? Construct your hypothesis based on the reasoning you think would answer your question. Remember that a good scientific hypothesis is testable and can be proven false. From one or more hypotheses, draft three predictions using an “If…then” statement, which should form the basis for a potential experiment. What are your variables? If [independent variable changes], then [dependent variable changes]. An example: You have a photo of a plant being exposed to the sun. A question you could ask is does the amount of light affect the growth of the plant? For the “If…then” statement could be: If I grow plants under high light, then the height of the plant will increase.

3. Testing your Hypotheses
Design an experiment to test your hypothesis. Consider what kind of data can be collected. Be sure to consider the following elements of experimental design and to include these in your lab report: dependent variable, independent variable, standardized variables, number of replicates, level of treatment. Prepare a table or chart to organize your data. Remember that a good experiment can be repeated.

4. Present Your Research
Biologists share their findings with the scientific community. There are many ways to communicate--giving talks or presenting posters at professional meetings, participating in conferences, and writing papers for journals. Over a half-million new research articles are added to the scientific literature each year. Before an article is published, scientists familiar with the field carefully evaluate it, a process called peer
review. Share the results of your group's photo with the class. What new hypotheses should be tested?
III. ASSIGNMENTS (35 pts.)

Please complete the following assignments individually. You are expected to submit complete answers to each question using complete sentences. Your TA will not read beyond the sentence limit listed for each question. The take home message: although one-word answers are not usually acceptable keep answers brief and to the point!

1. Summarize your observations, hypotheses, predictions, and conclusions for exercise 2 in a concise paragraph. (6 pts.)
   Further discuss any issues with your experimental design or hypotheses and how you could improve them. (10 sentences total max.) (4 pts.)

2. MAKE UP an experimental design, procedure or situation that illustrates how a confounding factor could affect interpretation of the results. You can use the Lyme disease example or anything else you see fit. Make sure to state the question, $H_0$, the treatment(s) and the variables measured, as well as how you would test your hypothesis. (6 sentences max.) (6 pts.)

3. Coral reefs are fragile ecosystems in tropical environments and they attract many tourists. Since sunburn is a danger in the tropics, many of these tourists wear sunscreen. Suppose you are interested in preserving coral reefs and you are concerned with the effect sunscreen might have on the reefs. As part of an initial exploratory or descriptive study, your $H_0$ is that sunscreen has no impact on the health and reproductive success of corals. (see Danovaro et al. 2008; posted on Laulima)
   a) Which type of statistical error would you be most concerned about (i.e., less willing to commit) in your study on effects of sunscreen – Type I or Type II? Explain your answers and refer to the paper by Danovaro et al 2008. (4 sentences max). (6 pts.)
   b) What are the biological implications of making a Type I and a Type II error in this study? Biological implications are the biological consequences that may arise as a consequence of making an error. If you make a Type II error (incorrectly rejecting $H_0$), what might happen if the use of sunscreen continues unabated? What if you make a Type I error (incorrectly finding support for $H_a$) and the use of sunscreen is erroneously discouraged or restricted? Explain your answers (4 sentences max). (2 pts. each, 4 pts. total)

Note: there is no single correct answer, but you must explain your reasoning. Unfortunately, when you decrease the chance of committing a Type I error, you increase the chance of committing a Type II error, and vice versa.

4. Attach the worksheet that you completed in lab to your written assignment. (3 pts. each, 9 pts. total)
IV. REFERENCES


Student Work Sheet

Exercise 1. Elements of Experimental Design: A Case Study.

A. Identify the following elements in the Lyme disease case study by completing the table:

<table>
<thead>
<tr>
<th>Key Observation</th>
<th>Hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediction (Phrase as an if/then statement)</td>
<td></td>
</tr>
<tr>
<td>Variables: Independent</td>
<td></td>
</tr>
<tr>
<td>Dependent</td>
<td></td>
</tr>
<tr>
<td>Standardized</td>
<td></td>
</tr>
<tr>
<td>Components of Procedure</td>
<td></td>
</tr>
<tr>
<td>Level of Treatment</td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td></td>
</tr>
<tr>
<td>Sample Size</td>
<td></td>
</tr>
<tr>
<td>Control Treatment</td>
<td></td>
</tr>
</tbody>
</table>
B. Check your understanding

1. PREDICTIONS
Rewrite the following statements into predictions “if…then” format. Using the two variables given, designate an independent variable and a dependent variable.

   a) High-carbohydrate dry foods, weight of cats

   b) Thickness of ozone layer, levels of chlorofluorocarbons (CFCs)

   c) High-fat and low-carbohydrate diet, levels of low-density lipoprotein (LDL) cholesterol

   d) Serotonin levels of individuals, seasons (Spring, Summer, Fall, Winter).

   e) Students’ exam scores, time spent studying
2. VARIABLES
Identify the independent and dependent variables in the examples below:

<table>
<thead>
<tr>
<th>EXAMPLE</th>
<th>DEPENDENT VARIABLE(S)</th>
<th>INDEPENDENT VARIABLE(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Your lab instructor’s blood sugar is measured daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. The diversity of soil microbes is measured before and after fertilizer application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Light absorption by spinach pigments is measured for red and blue light</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Three different brands of mouthwash are tested for their ability to inhibit bacterial growth</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. CONTROL
Ima Bionerd is considering the following research topics for an Independent Study project. Suggest an appropriate control treatment for each.

<table>
<thead>
<tr>
<th>EXAMPLE</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. The effect of caffeine on reaction time in college students</td>
<td></td>
</tr>
<tr>
<td>b. The effect of water pollution on reproductive structures in alligators</td>
<td></td>
</tr>
<tr>
<td>c. The effect of acid rain on rates of photosynthesis in zucchin plants</td>
<td></td>
</tr>
</tbody>
</table>
Exercise 2. Developing hypotheses based on observations

The Scientific Method

Observation:

Question:

Develop 3 Hypotheses (Theories that Explain Your Observation)

Hypothesis 1:

Hypothesis 2:

Hypothesis 3:

What Are Some Predictions of Your Hypotheses (“If…then” statement)

Prediction from Hs 1:

Prediction from Hs 2:

Prediction from Hs 3:
Design 3 Tests of Your Hypothesis (3 Experiments)

Test #1:

Test #2:

Test #3: