Laboratory One

Hypothesis Testing and Experimental Design

Prelab

Study this Hypothesis Testing and Experimental Design lab description and textbook section 1.5 "Doing Biology," then do the Lab 1 Prelab linked to the course website. See "Submitting Catalyst Exercises" in this course manual; Prelabs are due by Tuesday at 8:00 AM. (2 points)

Learning Objectives

By the end of this lab, students should understand:

- How to design an experiment that controls for all variables except one
- How to distinguish among hypothesis, null hypothesis, and prediction
- How to design an experiment with statistical analysis of results in mind
- How to create a well-designed graph

Introduction

Though they have no eyes, termites follow trails laid down by colony-mates that lead to distant food sources. In today's lab, you'll generate a hypothesis related to this trail-following behavior and test it by conducting an experiment. Before you begin, let's review the elements of experimental design.

Designing Experiments

State the question. Scientific investigations start by posing a question that can be answered by observing or measuring something. For example, pill bugs are often found under stones. (Pill bugs are small arthropods; they're sometimes called sow bugs or potato bugs.) If you made this observation, you might ask a simple question, "Why do pill bugs live under stones?

The question leads to one or more hypotheses. Initial observations lead to a testable **hypothesis**. A hypothesis is a proposed explanation. For example, you might hypothesize: Pill bugs are found under rocks because they prefer dark environments versus light environments. Every hypothesis has a corresponding **null hypothesis**—a claim that the explanation is not correct. In this case, the null can be stated as: Pill bugs have no preference for dark vs. lighted environments.

It's also important to consider other explanations. For example, pill bugs might live under rocks because the environment is moister there. So another alternative hypothesis is: Pill bugs are found under rocks because they prefer moist environments. In this case the corresponding null hypothesis is: Pill bugs have no preference for environments with higher humidity.

A hypothesis allows you to make a prediction. The next step is to formulate a testable prediction—something that must be true if the hypothesis is correct. A prediction states something that you can go out and measure or observe (this is the "testable" part). Predictions often take the form of an "If..., then..." statement. For example: *If* pill bugs do not prefer being in the dark or the light, *then* they should distribute themselves equally in each type of place when given a choice. This prediction follows from the null hypothesis in our example.

Different hypotheses make different predictions. As a result, an experiment that tests those predictions tests the underlying hypotheses. You **test** a prediction by collecting data.

Predictions can be tested with observations. Some predictions can be tested by making observations directly. In the pill bug example, you might survey a habitat and compare pill bug density (# of pill bugs per unit area) in dark versus sunlit areas. But for this comparison to be valid, you would have to be sure that the areas surveyed were similar in all ways except exposure to light. This can be difficult, as other factors vary along with light levels. For example, dark places tend to be moister than sunlit places. A critic could argue that termites are choosing their habitats on the basis of humidity, not light. Because termites are prone to dehydration, this is a reasonable competing hypothesis. In cases like this, it's difficult to test the alternative hypotheses on the basis of observation alone.

Predictions can be tested experimentally. To document the effect of varying only one factor at a time, you need to perform an **experiment**. To do this, test subjects are typically divided into two groups, the **experimental** and the **control**. The groups are treated exactly the same in all ways except for the one factor, or **variable**, that you are investigating. That is the only thing that differs between the groups. This is what makes experiments so powerful. A well-designed experiment eliminates the possibility that other reasonable hypotheses could explain the results.

In the pill bug example, you might add a pill bug to the middle of an experimental chamber that has a lighted half and a dark half, with equal humidity on both sides. After a set time, you could record which side the pill bug is on. You would then repeat this with more pill bugs—to make sure that the individual you tested wasn't unusual. You'd also want to do the same test with the light and dark sides switched, to make sure that the pill bugs aren't orienting to a certain side instead of to the presence or absence of light.

There are several types of variables. Factors that are kept the same in experimental and control treatments—and which might affect the result—are called **controlled variables**. The variable that you manipulate—the one that differs between experimental and control groups—is the **independent** (or explanatory) **variable**. The independent variable is the factor that you think is driving change in a **dependent** (or response) **variable** (see Appendix A).

Note that you can create several different experimental groups or treatments, each of which has a different level or state of the independent variable. For example, in an experiment in which light is the independent variable, you might examine the effect of high, medium, low, and no light.

Now, suppose you did the pill bug experiment that was just suggested. List some controlled variables:

___________________ ___________________ __________________ ______________________

What is the independent variable? ______________________

What is the dependent variable? ______________________

Experimental results support the hypothesis being tested or the null. If the dependent variable differs between the control and experimental groups (also called treatments) in the way that you predicted, then the results support your initial hypothesis. But if there is no difference between groups, then you accept the null hypothesis. Based on the results, you might also modify your working hypothesis and start over again.

It's critical to realize, though, that you are almost certain to observe some amount of difference between the treatments just by chance. To decide whether the difference you observed is really due to the factor you are testing and not just due to chance variation—meaning that the null is actually correct—you have to do a **statistical test**. The type of statistical test you choose depends on the nature of the data you've collected. In later labs, you will learn statistical methods of evaluating data.

Also, it's important to note that an experiment that supports your hypothesis doesn't "prove" it. All scientific knowledge is contingent—it reflects the best understanding we have, given the data collected to date. There is no guarantee that further study won't reveal limitations or flaws in your conclusions.

Termite Experiments

Your lab group will have some termites, pens of various colors and brands, a ruler, a petri dish, and paper towels, to use in investigating trail-following behavior. To begin, spend about 10 minutes observing how termites move on paper towels in relation to each other and to lines drawn on the paper towel. (Lines should be freshly drawn, with several coats of ink; use the ruler or petri dish cover as a guide to keep the lines thin.)

Between trials, keep your termites on a **slightly damp** paper towel under a petri dish cover, and don't let any wander off. Be gentle and respectful with your organisms. Keeping your termites in good condition is good ethics, and will also improve the quality of your data.

What questions are suggested by your observations?

Think about how termites' sensory capabilities and their normal physical environment might influence trail-following behavior. Create a hypothesis that is relevant to one of the questions you've asked, and make a prediction based on that hypothesis. Then design an experiment to test the hypothesis.

As you work on the experimental design, consider the following:

- Because you will analyze today's results later in the quarter using a t-test, your data must fall into *two categories* and the *dependent variable must be one that varies continuously*. In particular, avoid experiments where you *count* the number of times something happens. Your TA will explain why this is important.
- Are you controlling other factors that might affect termite behavior, such as: the amount of ink or pencil mark; the age, width and depth of lines; the number of termites that have already been on the lines? Does your design control for the possibility that termites might have a tendency to follow the first material they encounter, or to turn consistently to the right (or left)?
- Are you testing one individual at a time? If so, is each termite exposed to alternative treatments in random order? Are you controlling for fatigue, habituation, and prior experience?
- If you are testing more than one individual at a time, how will you identify individuals, and eliminate interactions between individuals that could affect your results?
- Are you measuring your response variable in an explicit and objective way? Could someone else repeat the experiment and measure exactly the same thing?
- Are you using a large sample of individuals? If not, how can you be sure that your results are not due to one or two unusual individuals?

Be sure that the data you record is organized and well labeled; you will use it again in a later lab.

Graph your data, following guidelines found in the Appendix A of this manual ("Presenting Your Data"), and complete the Experimental Report pages. In the "Results" box, indicate what pattern or lack of pattern is supported by your graphed data. Also, while interpreting your results and filling out the "Conclusions" box, it's critical to avoid making statements that are not directly and explicitly supported by your data.

Note: Your lab group will turn in one graph and one copy of the Experimental Report. Discuss each of the boxes in the table as a group. Then have each person pick 3 different boxes to answer in their own words, so the group hands in a complete report. Put your initials in the left-hand part of each box you answer.

Experimental Report Names________________________ ____________________________

________________________ ____________________________

What question are you trying to answer?	
Hypothesis to be tested:	
How does your hypothesis relate to termite biology?	
Null hypothesis:	
Experimental set-up: (include a sketch)	
Prediction 1: outcome under your hypothesis	
Prediction 2: outcome under null hypothesis	

Independent variable:	
Dependent variable:	
Controlled variables:	
Raw data:	Record your raw data in an organized table on the back of your graph.
Results (a 1- to 2-sentence summary):	
Discussion and Conclusions *	

Discussion and Conclusions should include:

- Interpretation of your results: What you can conclude about your original hypothesis, based on this experiment? Do you think your findings are statistically significant? (You will find out for sure during the Introduction to Statistics lab.)
- Limitations of the experiment—factors that might affect the quality of the data or the interpretation.
- Additional questions suggested by the results, or ideas on how to extend the experiment.