REMINDER: Wear long sleeves, long pants, and closed shoes or boots—we will be working with poison oak!

Introduction

Vines and lianas (woody vines) are an important component of many forest communities. They are defined by their growth habit, which involves climbing other plants that they use for structural support (Putz 2006). Because much of their biomass can occur in the canopy, it has been harder to quantify them than the tree, shrub, and herb components of their communities. As a result, vines and lianas are often overlooked in studies of forest dynamics, and even basic questions about them (such as whether they prefer different tree hosts) remain unanswered (Talley et al. 1996).

This hole in our knowledge exists despite recent studies demonstrating the importance of vines and lianas in a range of ecological processes. In swampy forests of the southeastern United States, for example, large infrequent disturbances (such as hurricanes) had greater effects on individual trees with lianas attached than those without lianas (Allen et al. 1997). Similarly, trees with heavy liana loads in the Amazon rainforest were more likely to die than those with lighter loads (Phillips et al. 2005). Lianas in the Amazon, which may contribute up to 25% of the woody stems and up to 40% of the leaf area in a forest, also turn over more quickly than their tree hosts and thus play an important role in forest dynamics (Phillips et al. 2005).

There is some evidence that vines may prefer, or at least perform better on, particular tree hosts. In tropical forests in Malaysia, Australia, and Costa Rica, lianas occurred more often on some tree families than others (Clark and Clark 1990, Campbell and Newbery 1993, Carsten et al. 2002). Similarly, some tree species in Alabama were more likely to host *Rhus radicans* (poison ivy) than others (Talley et al. 1996). Tree size (height and girth), bark characteristics, and tree growth rates have all been suggested as possible reasons for differences in vine prevalence among tree hosts (Putz 1984, Talley et al. 1996).

In the western United States, *Rhus diversilobum* (poison oak) can be a common vine in forests—sometimes more common than we'd like. Poison oak can also grow as a shrub, and differences in its ecology as a shrub vs. vine have been reported (e.g., Gartner 1991). Little is known, however, about the relationship between host tree characteristics and poison oak vine prevalence. In this lab, we will survey poison oak vines in a forested area to answer the following questions:

1. Do poison oak vines occur more often on some tree species than others?
2. Is tree size correlated with the presence of poison oak vines?

Goals & objectives

By the end of this two-week lab, students will be able to:

- explain how to use transects to obtain a random, representative sample, and why that is important;
- describe a belt transect, and lay out one in a forest habitat;
- identify several common tree species as well as poison oak;
- measure tree diameter using a dbh tape;
- use common statistical tests to analyze data related to host tree choice in poison oak.
- determine whether poison oak occurs more often on some trees, and describe the characteristics of those host trees;
- suggest possible reason(s) for the observed pattern of poison oak host tree "choice" and the ecological ramifications for either trees or vines;

Literature cited

Allen, B. P., E. F. Pauley, and R. R. Sharitz. 1997. Hurricane impacts on liana populations in an old-growth southeastern bottomland forest. Journal of the Torrey Botanical Society 124:34-42.

Campbell, E. J. F., and D. M. Newbery. 1993. Ecological relationships between lianas and trees in lowland rain forest in Sbah, East Malaysia. Journal of Tropical Ecology 9:469-490.

Carsten, L. D., F. A. Juola, T. D. Male, and S. Cherry. 2002. Host associations of lianas in a south-east Queensland rain forest. Journal of Tropical Ecology 18:107-120.

Clark, D. B., and D. A. Clark. 1990. Distribution and effects on tree growth of lianas and woody hemiepiphytes in a Costa Rican tropical wet forest. Journal of Tropical Ecology 6:321-331.

Gartner, B. L. 1991. Relative growth rates of vines and shrubs of western poison oak, *Toxicodendron diversilobum* (Anacardiaceae). American Journal of Botany 78:1345-1353.

Phillips, O. L., R. V. Martínez, A. M. Mendoza, T. R. Baker, and P. N. Vargas. 2005. Large lianas as hyperdynamic elements of the forest canopy. Ecology 86:1250-1258.

Putz, F. E. 1984. How trees avoid and shed lianas. Biotropica 16:19-23.

Putz, F. E. 2006. Vine Ecology. *Ecology.Info #24.*

Talley, S. M., R. O. Lawton, and W. N. Setzer. 1996. Host preferences of *Rhus radicans* (Anacardiaceae) in a southern deciduous hardwood forest. Ecology 77:1271-1276.

WEEK 1

Reading/preparation

- Read through this week's portion of the handout carefully before lab (p. 1-4). Be sure you understand the terms in boldface.
- Review p. 14-16 in the CBH textbook.

Background: principles of experimental design in ecological field studies

In many ecological studies, the researchers are collecting data on organisms in the field. For example, an ecologist interested in the effects of different pollinators on plant reproduction might ask how many seeds result from pollination by bees vs. hummingbirds. Answering this question involves censusing the population—collecting data on individuals living in nature. In some cases, a **true census** is possible. In a true census, the investigator counts every single individual in the population; this may be possible with highly endangered species, for example. As an example in another context, each decade the United States attempts to conduct a true census of every person living in the country.

In most cases, however, it is impossible to conduct a true census, usually because it would require too much time, labor, and money (none of which is unlimited!). Imagine trying to measure every single Douglas fir tree in Oregon, or every single *Daphnia* in a pond. In some cases attempting a true census may actually lead to biased results; for example, some animals may always hide when disturbed by people, and thus will never be counted. Therefore, most ecological studies involve **sampling**. The goal of sampling is to collect data on a smaller area and use that information to draw conclusions about the population in a larger area.

There are several key factors to consider when designing the sampling for a study. To avoid bias, your sampling should be **representative**—in other words, the areas you sample should be similar to the entire area. For example, imagine you were interested in estimating the mosquito population in a forest. If you only sampled the wet areas, your estimate would be too high, and if you only sampled the dry areas, your estimate would be too low. There is plenty of evidence that when an investigator chooses a "representative" sample, unconscious judgments lead to biased sampling. Therefore, most studies use **random sampling**, which helps ensure more representative sampling. We will practice different ways to randomly sample populations throughout the course.

If the study area varies systematically, it is usually preferable to do **stratified sampling**. For example, imagine you wanted to estimate the density of amphibians in several wetlands. The density of different species is likely to vary with moisture. A completely random sampling approach might miss some parts of the moisture gradient by chance. A better approach would be to divide the entire area into categories (e.g., standing water, lowland moist sites, and upland dry sites) and then distribute the sampling units across these different categories. If you account for the proportions of each habitat type, this approach can provide a more accurate overall estimate of the population.

A good sampling design also has **replication**. Replication involves collecting data from multiple (different) sampling units. Multiple samples give more confidence in the accuracy of estimates; for example, it would be absurd to count the number of parasites in one fish and conclude that all fish had that number of parasites. It would be better to count the number of parasites in many fish and then calculate the average number of parasites per fish. As in most of biology, deciding how many replicates are “enough” is a challenging question in ecological studies.

Finally, keep in mind the difference between precision and accuracy. **Precision** is the similarity of repeated measurements. For example, if you measure the length of a leaf 10 times, how similar are the 10 measurements? If they are exactly the same, the measurement is very precise. **Accuracy** is how well the measurement captures the true character. It is possible to be very precise but also inaccurate; consider, for example, a balance that consistently underweighs. Although ecologists strive to be both accurate and precise, accuracy is usually more important.

Methods: sampling trees and vines in the forest
Techniques to count or identify individuals to include in an ecological study depend on the biology of the organism. For many sessile organisms (such as trees or mussels), it’s relatively easy to make counts because individuals stay put. A common way to pick sessile organisms to include in a study involves setting out a plot with a known area (also called a **quadrat**) and measuring all the individuals in that plot. Because you know the area, you can also calculate the density of the organisms. Mobile organisms are more difficult to count accurately because they move! The most common technique to estimate densities of mobile organisms is called mark-release-recapture. We won’t practice this technique in lab, but it’s relatively straightforward and explained in your textbook.

There are many issues involved in setting up quadrat. For example, you want to be able to locate and measure individuals without damaging them—if you are measuring small herbaceous plants and you have to walk around a large quadrat, you may crush some individuals. Many factors (such as quadrat shape or size) are especially important when trying to measure the true density of a species; rough guidelines exist for quadrat sizes for various organisms, and this information is available in books that describe census technique (e.g., Sutherland 1996). Another issue is how to locate your quadrats so they appropriately sample the environment.

In this week’s lab, we will sample trees along belt transects. A **transect** is simply a line placed through a habitat, and can be used to randomly sample in an ecological study. For example, you can randomly locate a transect, or you can pick random points along a transect (e.g., at 3, 8.5, 9.4, and 16 m) to sample. A **belt transect** includes area to either side of the line; it’s essentially a long skinny quadrat that extends a set distance (e.g., 1 or 5 m) to either side of the transect line itself. It is often used to test for ecological effects of an abiotic gradient (e.g., high to low elevation). It can also be used as an efficient method to lay a “plot” that you don’t plan to revisit, or to stratify sampling when you want to see whether the density of organisms responds clearly to a gradient. In this lab, we will use belt transects to sample trees in the forest. In each belt transect, we will identify each tree and record the presence or absence of poison oak vines on it.

We will compare the size of trees with and without poison oak by measuring their diameters with a special tape, called a “dbh” (diameter at breast height) tape. A dbh tape translates circumference into diameter. We will measure every tree with poison oak (use gloves!). Because only a small proportion of trees host poison oak at this site, we will measure the diameter of a random subsample of trees without poison oak to compare to the trees with poison oak.

WEEK 2

Reading/preparation, Week 2

- Make sure your data is accurately entered in the Excel file before lab starts.
- Answer the questions below. I will check them before lab starts.
- Review basic statistical principles by reading Pechenik p. 52-67 (6^{th} edition) or p. 51-56 (7^{th} edition).
- Review the information in the WebStats handout (especially p. 11-14).

Prelab questions

1. In your own words, explain what each of the following is, and why it is important in statistics. Use the WebStats review, the reading in Pechenik and your text, and prior knowledge.

Measure	What is it?	Why is it important in statistics?
variance		
mean		
null hypothesis		
degrees of freedom		
P value		

2. Write down a <u>scientific</u> (ecological) hypothesis for question 1. Be sure to phrase it as an if…then statement.

3. Write down the <u>null</u> hypothesis for the Chi-Square analysis for question 1.

4. Suggest one way you might be able to calculate the expected distribution of poison oak vines on different host tree species. It's Ok if you're wrong, but I want you to have one idea written down before lab.

Statistical Analyses: general principles

After you've collected your data, the next step is to figure out what it tells you—in other words, how much support is there for your hypothesis? Ecologists generally use statistical tests to make this evaluation. Statistics provide tools to evaluate the probability that any patterns in the data are real—that they are different from random variation. Distinguishing random variation from the effects of a particular variable is especially important in ecology, where many environmental factors may influence study organisms.

Thus, statistics are a critically important tool. They're also a challenging tool, especially for beginning students. This course is not a math class, so we will not consider the theory behind most of the tests we discuss and I won't expect you to memorize formulas or other details of statistical tests. Instead, we'll focus on some central concepts that you need to understand when using or interpreting statistical tests. You will also practice doing and interpreting some common tests both by hand and using the statistical software package SPSS.

Remember that each statistical test evaluates null hypotheses. The null hypothesis typically states that there is "no relationship" or "no difference." Technically, statistical tests determine the probability that the observed data would arise if the null hypothesis is true; if this probability is very low, we reject the null hypothesis and conclude that the data supports a relationship or difference. Please carefully review the information on Drawing Inferences in the WebStats handout, and be sure you understand the difference between statistical and scientific hypotheses.

Question 1: Do poison oak vines occur more often on some tree species than others?

We will use a Chi-Square (χ^2) test to see whether poison oak vines occur more often on some tree species. The chi-square test compares an observed distribution (e.g., the number of poison oak vines in three different species of trees) to the expected distribution. Any time you use the Chi-Square test, you must think carefully about how to determine the expected distribution.

To carry out Chi-Square test, arrange your data in a table that shows the observed and expected values. For example:

	Poison oak present		Poison oak absent	
Tree species	Observed	Expected	Observed	Expected
Douglas fir				
White oak				

For the observed values, the cells of the table are filled in with the count of the number of trees that fall into each category. We'll calculate the expected values together. The test determines whether the expected values differ from the observed values by calculating a **test statistic**:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

where O is the observed value in each row and E is the expected value in each row. Notice that this equation *explicitly* compares the observed to the expected values. This comparison is at the heart of all statistical tests, although it's not always as obvious as it is in this test.

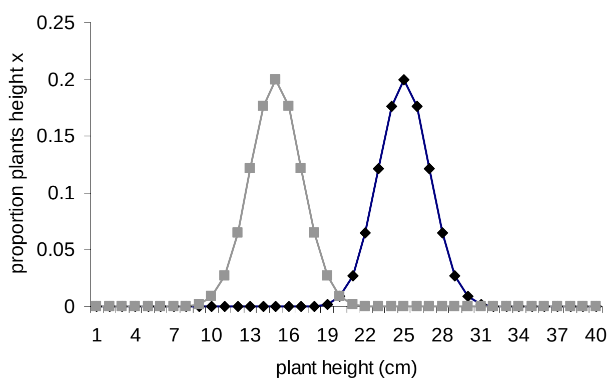
It's very important to look at your expected frequencies before calculating the test statistics. For the chi square test to be accurate, the expected frequency for all categories must be greater than 1. If the expected frequency for any category is less than 1, we will combine categories (e.g., group together two or more tree species).

You can use formulae in Excel to calculate $(O - E)^2/E$ for each cell, and then sum them to get the test statistic. You also can use the following Excel formula to get a precise ***P* value** associated with your test statistic and degrees of freedom:

=CHIDIST(χ^2, df)

where χ^2 is the test statistic you calculated and df is the degrees of freedom. The degrees of freedom for this test is (# rows – 1) × (# columns – 1). A *P* value gives the probability that you'd see a test statistic this extreme or more so if the null hypothesis were true. In this case, our null hypothesis is that the observed values do not differ from the expected values. If our *P* value is small, there is a small probability that our null hypothesis is true. Specifically, for this example, a small *P* value indicates that the data we observed are very unlikely if the poison oak vines are equally common on all tree species.

Question 2: Is tree size correlated with the presence of poison oak vines?
We will use a t-test to see whether trees with and without poison oak vines differ in size. A t-test determines whether two groups are statistically significantly different than each other by comparing the difference in the means of the two groups to the variation around those means. When the difference in means is large relative to the variation around each mean, we conclude that the groups differ. The figure below illustrates this principle.



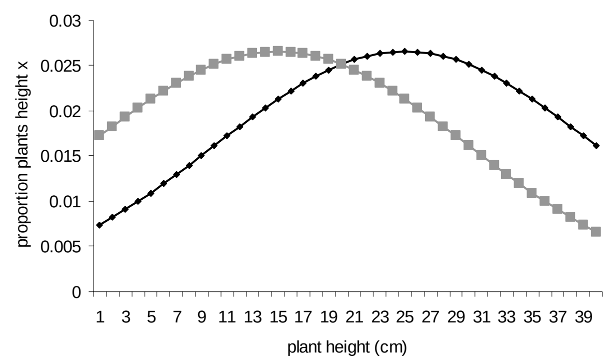


Figure 1. Examples where the difference in means is large (left) or small (right) relative to the variation around the mean. In both cases, the gray line has a mean of 15 cm and the black line has a mean of 25 cm. On the left, the standard deviation = 2 cm while on the right the standard deviation = 15 cm. In which case do <u>you</u> think the means are likely to be different? In which case do you think a t-test would find that the means are statistically significantly different? Why?

Follow the instructions in the WebStats Review for calculating the test statistic, t. You can use Excel to calculate the descriptive statistics that you'll need, such as means and variances (these are also reviewed in WebStats). For a t-test, the degrees of freedom are calculated as:

$$d.f. = n_1 + n_2 - 2$$

.
You can use Excel to calculate the exact *P* value for your t test statistic using this formula:

=TDIST(calculated t value, df, 2)

The 2 at the end refers to the fact that you're doing a two-tailed test. This simply means that you're testing two possibilities simultaneously—whether trees with poison oak are significantly bigger than trees without poison oak, or whether they're significantly smaller than trees without poison oak. In rare cases you have an *a priori* reason to test in only one direction (e.g., are trees with poison oak bigger than trees without poison oak, a one-tailed test), but in most cases the two-tailed test is more appropriate.

Note that the t-test makes some assumptions about the distribution of data (e.g., that the data are normally distributed, that the variances are equal between the two groups). It is similar to the Rank Sum or Mann Whitney test, which does not make those assumptions. We will start with the t-test in this lab, and will discuss how to determine whether it is the best test to use in the future.

Data Sheet—Distribution of Poison Oak Vines on Host Trees at Mt. Richmond

Date: ____________________________ Team members: ______________________________________

Transect length: ___________________ Transect width: ______________________________________

DF = Douglas fir WH = Western hemlock WRC = Western red cedar RA = red alder

BLM = Big leaf maple VM = Vine maple Oak = Oregon white oak GF = grand fir

For this study, we will consider all stems greater than 2 cm in diameter as trees.

Tree species	**Poison oak?**	**dbh (cm)**	**notes**
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		

Data Sheet—Distribution of Poison Oak Vines on Host Trees at Mt. Richmond

Date: ____________________________ Team members: ______________________________________

Transect length: ___________________ Transect width: ______________________________________

DF = Douglas fir WH = Western hemlock WRC = Western red cedar RA = red alder

BLM = Big leaf maple VM = Vine maple Oak = Oregon white oak GF = grand fir

For this study, we will consider all stems greater than 2 cm in diameter as trees.

Tree species	Poison oak?	dbh (cm)	notes
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		
	Y N		

Summary of tree data for your transect

For each tree species, record (e.g., make a tick mark) every individual you observed in your transect that did and did not have poison oak vines.

Tree species	# with poison oak vines	# without poison oak vines