Exploration 6.3: Annual Growth Increments of Primary-Forest and Pioneer Trees in Forest Understory

Comparing Two Means: Theory-Based Approach

LEARNING GOALS

- Identify when a theory-based approach would be valid to find the p-value or a confidence interval when evaluating the relationship between one binary variable and one quantitative variable.
- Use the Theory-Based Inference applet to find theory-based p-values and confidence intervals for a comparison of two means.

Background

Even though most trees live in forests, not all trees are well-adapted to the same ecological conditions. Some trees are best adapted to living in the deep shade of the forest understory even as seedlings; such trees can survive for many years there, growing slowly and biding their time until the canopy trees that shade them die or get blown over by wind. When that happens, they grow more rapidly, but it sometimes takes 3 or 4 such "releases" from suppression by shade for such trees to reach the canopy where they're not overtopped by taller trees. Because trees that have the ability to survive and grow in the deep shade of the understory usually come to dominate a mature forest, they're often called "primary forest" species, and in much of the eastern United States sugar maple (*Acer saccharum*) and American beech (*Fagus grandifolia*) are good examples. These trees often produce dense, sturdy wood, and can continue to grow and produce offspring for hundreds of years.

Some other species are adapted to survive and grow rapidly only in high-light conditions, in places where the forest canopy has been removed by some physical disturbance – e.g., where a tree has blown down or been killed by lightning or insect attack, or has been removed by human activity. Most of these species cannot grow or even survive for long in the deep shade under an intact canopy, and some species even have seeds that remain dormant in the soil until the canopy overhead is disturbed. These high-light conditions never last for very long, in part because branches of the trees surrounding the canopy opening grow rapidly to fill it, and in part because saplings in the understory of the "canopy gap" grow more rapidly while the light is available. As a result, within a few years the forest floor becomes as shady as it was before the canopy disturbance. For this reason, tree species that specialize on the high light levels in canopy gaps produce wood that is much less dense than that of most primary forest species, so that they can grow in height more rapidly and take full advantage of the light while it's available. The tradeoff of that strategy is that their low-density wood breaks more easily and is more easily attacked by insects than that of primary forest species, so that they come to be rare in a mature forest. Because these kinds of trees often dominate the early stages of ecological succession but become more rare as a forest matures, they're often called "pioneer" species. They usually start reproducing at a relatively young age and produce lots of offspring over a short time period, as they're unlikely to survive as long as primary forest species. Good examples in the eastern U.S. include sassafras (*Sassafras albidum*) and black cherry (*Prunus serotina*).

Step 1: Ask a research question

Because trees grow in discrete yearly increments with a pause over winter, researchers wanted to explore if there are differences in the annual growth increments of pioneer species and primary species trees in a forest? Specifically, are there differences in the annual growth increments of black cherry trees and sugar maple trees?

Step 2: Design a study and Collect data

In this exploration, you will use data gathered by students in nine lab sections of an introductory Biology course at Hope College. These students measured the 2017 growth increment on a large number of sugar maple and black cherry seedlings or saplings (between about 0.5 and 2.0 meters tall, so that the main vertical stem could be reached) at the Hope College Nature Preserve (HCNP) in Allegan County, Michigan during the last week of August 2017. Care was taken to have each lab section work in a different part of the forest, so as not to sample a given plant more than once. Because most of the HCNP is a pretty intact forest, we assume that most of the seedlings and saplings sampled by these classes would be in the shaded understory.

Measuring growth responses of tree seedlings or saplings

The students only made one trip to the forest, so were not able to measure the height one year and then the height the following year. They also did not want to harm any of the trees, so were not able to cut them down to measure the distance between rings. Instead, they used a little trick to determine how much a given tree stem has grown during the current growing season. Every autumn when the tree is about to lose its leaves and go dormant for the winter, it forms several layers of **bud scales** around each of its terminal buds (the buds at the ends of each shoot). These scales protect the meristematic tissues in the buds from drying out over the winter. The next spring, the bud scales fall off as the bud starts to grow again, and the stem lengthens behind the bud.

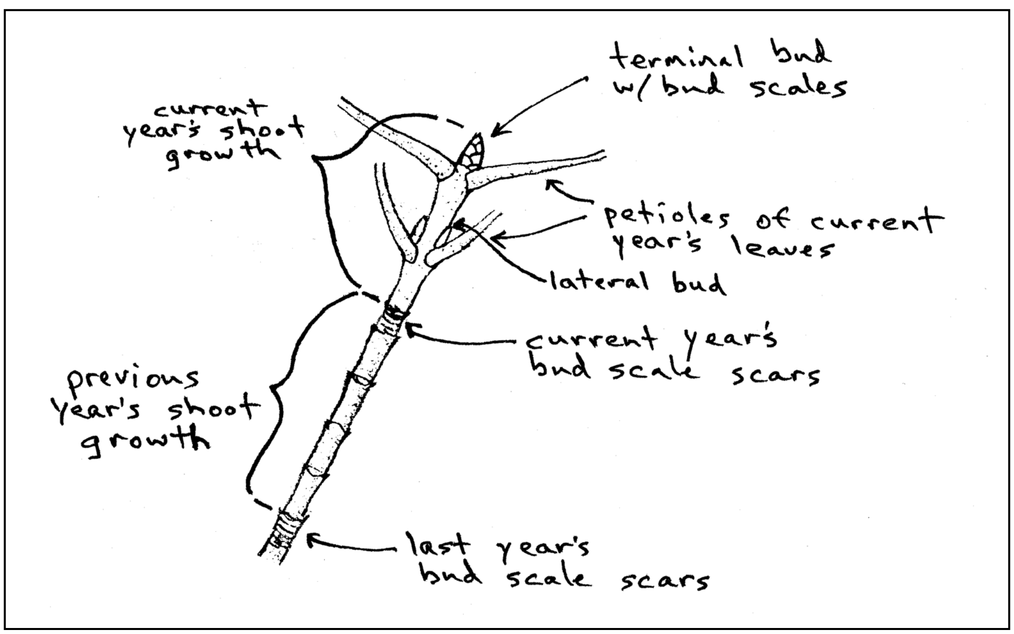

Figure 1. Detail of sugar maple shoot, showing rings of bud scale scars left by successive year's terminal bud scales.

If you look carefully, you can see the bud scale scars from the beginning of a growing season as several close-spaced rings around the stem (fig. 1). The current season's growth will be the distance between the tip of the terminal bud on a shoot and the most distal of last winter's bud scale scars. With many species, you can even see the bud scale scars from several previous years, and measure those growth increments as well.

1. Identify the variables recorded. Also classify each as either categorical or quantitative, and identify each variable's role: explanatory or response.

2. What other variables will you measure that might explain some of the variability seen in the different amounts of annual growth?

3. Did this study make use of random assignment, random sampling, both, or neither?

4. Was this an experiment or an observational study? Explain how you are deciding.

5. In words, state the null and alternative hypotheses to test whether sugar maple saplings and black cherry saplings tend to differ in their annual growth increments.

6. Define the parameters of interest and assign symbols to them.

7. State the null and alternative hypotheses using these symbols.

Step 3: Explore the data

Let's explore the data from the nine lab sections. Data can be found in SaplingData where As denotes annual increment measurements in mm taken on sugar maple saplings and Ps denotes annual increment measurements taken in mm on black cherry saplings.

8. Obtain numerical summaries (statistics, such as mean and SD) of the sapling data using the **Multiple Means** applet.
   - Open the data file SaplingData to access the raw data. Copy the data (e.g., CTRL-A and CTRL-C) to your clipboard. Make sure to include data labels.
   - Open the **Multiple Means** applet and press **Clear**. Click inside the **Sample data** box and paste (e.g., CTRL-V). Make sure to delete the last empty row if there is one. Then press **Use Data.**
     a. Report the sample size, sample mean, and sample SD of growth in mm for each species of saplings. Based on the sample statistics, which species tended to have a larger annual growth: sugar maple or black cherry? How are you deciding?
     b. Based on the sample statistics which species tends to have more variability in annual growth: sugar maples or black cherries? How are you deciding?
     c. Describe the shapes of the distributions of annual growth for each species, are they symmetric or skewed? If skewed, what direction?
     d. Calculate the observed difference in mean annual growth between the two species (As – Ps). Verify your calculation by noting the **Observed diff** output in the applet.

e. Do you think this difference could be due to random chance (sample-to-sample variability)?

Step 4: Draw inferences from the data

Even though ecologists recognize a broad distinction that characterizes what we call "pioneer" vs. "primary forest" species, we realize that these are just endpoints in a continuum of shade tolerance, growth rates, wood density, seed production, and other traits. Nevertheless, we can use this theoretical framework to propose testable hypotheses about the relative growth rates of tree seedlings under different conditions. In the forest understory, for example, we might predict that seedlings of a primary forest species like sugar maple would grow more rapidly than those of a pioneer species like black cherry, and that in treefall gaps the pioneer species would grow more rapidly than the primary forest species.

With the data gathered from the Hope College Nature Preserve, we want to know how different would these sample means have to be before we'd be willing to conclude that they resulted from a real, biological difference between the growth increments of sugar maples and black cherries, rather than from mere random sampling error? A good approach to this question might be to determine how large a difference between sample means could occur ***if there really were no difference between sugar maple and black cherry growth increments, that is, if the null hypothesis (H_0) above were true***.

9. We can use the Multiple Means applet to generate possible values of the difference in sample means under the null hypothesis by shuffling which response values go with which explanatory variable values. Check the **Show Shuffle Options** box and enter 1,000 for the **Number of Shuffles**. Press the **Shuffle Responses** button. The histogram on the right is a simulated distribution for the difference in sample means assuming the null hypothesis is true (i.e., the null distribution).

Enter the observed difference in sample means from #8d in the **Count Samples** box and press **Count**.

a. Record your estimated p-value.
b. Fill in the blanks of the sentence, to complete the interpretation of the p-value.
   The p-value of _______ is the probability of observing
   __________________________________ assuming
   ____.

10. If the difference in means were larger (more different than 0), how would this impact the size of the p-value?
11. How would increasing the sample size (all else remaining the same) change the p-value? Why?
12. How would increasing the standard deviations of the annual growth measurements for both sugar maple and black cherry (all else remaining the same) change the p-value? Why?

Another measure of the strength of evidence against the null hypothesis would be to standardize the statistic by subtracting the hypothesized value and dividing by the standard error of the statistic (e.g., from the null distribution). When comparing these two means, this standardized statistic is referred to as a *t*-statistic.

13. On the left side of the applet (below the **Sample data** window) use the **Statistic** pull-down menu to select the ***t*-statistic**. Record the value of the *t*-statistic that is computed for your data. Write a one-sentence interpretation of this value.

T-values larger than 2 (in absolute terms) are often considered strong evidence against the null hypothesis because they indicate that the observed statistic is more than two standard errors from the hypothesized parameter value. We can use a theoretical distribution to convert the *t*-statistic into a p-value. With *t*-statistics, we use a *t*-distribution. These *t*-distributions are very similar to normal distributions especially when sample sizes are large.

14. In light of the value of the standardized statistic, should you expect the p-value to be large or small? How are you deciding?

15. The histogram on the far right now displays the null distribution of simulated *t*-statistics. Describe the characteristics (shape, center, variability) of this distribution.

16. Below the null distribution check the **Overlay *t* distribution** box. Does the *t*-distribution appear to adequately predict the behavior of the shuffled *t*-statistics? What do you think this suggests about whether the validity conditions will be met for these data?

17. Enter the observed value of the *t*-statistic from #13 in the Count Samples box and press **Count**. (Remember to use the pull-down menu to specify what direction(s) you want to consider more extreme based on the alternative hypothesis.) How does the p-value from the *t*-distribution (in red on the applet) compare to the simulation-based p-value for the difference in means (from #9(a))?

Validity Conditions

The validity conditions required for this theory-based approach (a two-sample *t*-test) to be valid are show in the box.

Validity Conditions
The quantitative variable should have a symmetric distribution in both groups or you should have at least 20 observations in each group and the sample distributions should not be strongly skewed.

18. Do the validity conditions appear to be satisfied for these data? Justify your answer.

Step 5: Formulate conclusions.

19. Based on the analysis you have just carried out, state your conclusion in the context of the study. Be sure to comment on the following:
    a. **Statistical significance:** Do the data provide evidence that the long-run mean annual growth in mm of sugar maple saplings differs from black cherry saplings in the population? How are you deciding?
    b. **Causation:** Do the data provide evidence that the difference found in annual growth in mm is caused by sapling type? How are you deciding?
    c. **Generalization:** How broadly can you apply the results of this study? Any forest? All sugar maple trees and black cherry trees? All primary forest and primary species? How are you deciding?

20. **Estimation:** You can also use this applet for estimating the parameter of interest. (You can also go directly to the **Theory-Based Inference** applet which will also allow you to change the confidence level.) Check the box on the left next to **95% CI(s) for difference in means**.
    a. Identify, in words related to the context of this study, the relevant parameter to be estimated here.
    b. Report the 95% confidence interval for this parameter.
    c. Does the 95% confidence interval calculated from the sample data contain the value 0? What does that imply? (*Hint*: Recall that a confidence interval is an interval of plausible values for the parameter of interest, and the interval is calculated using the sample statistics.)
    d. Does the 95% confidence interval agree with your conclusion in #17? How are you deciding?
    e. Fill in the blanks of the sentence to complete the interpretation of the 95% Confidence interval:

        We are ____% confident that the mean annual growth of sugar maple saplings is ____________ than the mean annual growth of black cherry saplings, by between ___________ and ____________mm.

    f. How would the width of the interval change if you increased the confidence level to 99%? Why?
    g. How would the width of the interval change if you increased the sample size? Why?
    h. How would the width of the interval change if the variability of the annual growth increased for both sugar maple and black cherry saplings? Why?

Step 6: Look back and ahead.

21. *Looking back*: Did anything about the design and conclusions of this study concern you? Issues you may want to critique include:
    - The match between the research question and the study design
    - How the observational units were selected
    - How the measurements were recorded
    - The number of observational units in the study
    - Whether what we observed is of practical value

    Looking ahead: What should the researchers' next steps be to fix the limitations in this study and/or build on this knowledge?

Reference

Murray, K.G., K. Winnett-Murray and L. Hertel. 2005. Photosynthetic Strategies and their Consequences for Plant Community Structure. Pages 233-254, in Tested studies for laboratory teaching, Volume 26. (M.A. O'Donnell, Editor). *Proceedings of the 26th Workshop/Conference of the Association for Biology Laboratory Education (ABLE)*, 452 pages.

Additional References