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EXPERIMENTS

Leaves as Thermometers

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ABSTRACT

Plant morphology reflects evolutionary solutions to environmental constraints. In this experiment, students use this principle to predict the climate at their site. They collect and measure a set of traits on local tree leaf samples during the first meeting. At the following meeting(s), they pool their data and are introduced to a global database of climate and leaf morphology. In groups, they create regressions across sites and use their leaf data to predict mean annual temperature, which they compare to long-term observations from a local weather station. The experiment emphasizes quantitative analysis, data synthesis, and ecological responses to climate.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** plant ecology, environmental adaptation, forest ecology, species diversity

- **Science Methodological Skills Keywords:** quantitative data analysis, correlation versus causation, field observation skills, graphing data, statistics, use of spreadsheets, model development and testing

- **Pedagogical Methods Keywords:** guided inquiry, cooperative learning, problem based learning (PBL)

CLASS TIME

Four to six hours separated into two or three class meetings.

OUTSIDE OF CLASS TIME

One to two hours for writing a group report.

STUDENT PRODUCTS

Students obtain local leaf data and predict local temperature based on regressions between leaf morphology and climate from a global database. *Turned in as groups:* data sheets from leaf observations and a report with results and discussion of findings.

SETTING

This experiment was initially conducted with trees found on an urban college campus (Beloit College, Beloit, WI). It requires sampling leaves of woody angiosperms from a diverse community to obtain sufficient species observations (recommended at least 15 different species). After collection, samples can be identified in a laboratory room or in the field. Data analysis is done in a computer lab.

COURSE CONTEXT

This experiment was used in an introductory Botany class with two sections of 26 students each. The students were a mix of biology majors and non-majors from multiple grade levels.

INSTITUTION

When teaching this experiment, I was a Visiting Instructor of Biology at Beloit College, a private liberal arts college with approximately 1,300 undergraduate students.

TRANSFERABILITY

This experiment can be adapted to focus on different aspects (taxonomy, computation, physiology, ecology) and meet different educational needs. Since the experiment is based on global cross-site information, the concepts can be tested in a range of settings (urban or natural) across biomes, although the database only includes data for woody angiosperms, so access to tree samples is required. The exercise should be applicable for native or non-native species from local forest patches or open-grown plantings (e.g., around campus). It is also possible to conduct this experiment with local herbarium samples if collecting samples is undesirable.

ACKNOWLEDGEMENTS

developed the experiment while I was a visiting instructor at Beloit College. Substantial feedback on the exercise was provided by Carol Mankiewicz, John Jungck, and Yaffa Grossman, as well as other members of the Ecological Research as Education Network. Comments from Kathy Winnett-Murray and anonymous reviewers at TIEE greatly improved the final submission.

SYNOPSIS OF THE EXPERIMENT

Principal Ecological Question Addressed

In this exercise, students test the hypothesis that tree leaf morphology reflects local average temperature.

What Happens

In the first meeting, students are introduced to leaf morphology and then collect leaf samples in small groups. They identify their samples to the species level and record a set of morphological traits. In the following meeting(s), the student groups analyze the global database from CLAMP, create predictive regressions, and estimate the climate at the site based on the compiled samples from the entire class. Then, they compare the leaf-based climate estimates to long-term climate records from a nearby weather station.

Experiment Objectives

This exercise introduces students to leaf morphology, species identification, data synthesis, and prediction.

Equipment/ Logistics Required

Access to woody angiosperm trees, regional tree species identification guides, measurement tools (rulers or calipers), and computers with a spreadsheet program for data analysis.

Summary of What is Due

Students turn in their raw data at the end of the first meeting and prepare a short report as a group at the end of the experiment to submit for a grade.

DETAILED DESCRIPTION OF THE EXPERIMENT

Introduction

Plant morphology reflects evolutionary solutions to environmental constraints (Niklas 1992, Little et al. 2010). Variation in leaf morphology is a

critical adaptation for plants because leaves play a central role in gas exchange, which is constrained by climate. Plants with certain morphologies are more successful than others in particular climates.

Of all plant organs, leaves are critical for adaptation to environmental conditions. Plants must maintain photosynthesis and avoid dehydration, which are influenced by the size and structure of leaves. For example, trees with large leaves may have a greater surface area for gas exchange but may also experience greater transpirational water loss, which could be costly for plants in a hot and dry environment. We expect these ecological constraints to be reflected in leaf morphology (Holdridge 1947, Niklas 1992). Cross-site comparisons have allowed ecologists to examine how and why leaf morphology responds to changes in climate (Royer and Wilf 2006), and to consider how plant fossil records can be used as indicators of past climate, especially mean annual temperature (Bailey and Sinnot 1915, Wolfe 1990, Spicer 2000). One of these projects is called the Climate Leaf Analysis Multivariate Project (CLAMP), a global data set that has been used to predict climates in Earth's history by examining fossilized leaves and making calculations from relationships between leaf characteristics and climate variables (Wolfe 1990). However, these relationships may not be able to accurately predict climate variables like temperature, due to variation in species composition, evolutionary history, or differences among sites.

To test the predictability of relationships between leaf characteristics and temperature, you will:
1. observe relationships between leaf morphology and temperature from the CLAMP data set,
2. analyze leaf data collected from local trees,
3. use these data to predict the temperature at the site, and
4. compare these predictions to observed temperatures.

Materials and Methods

Study Site(s): Leaf samples will be collected from local trees on campus or in a natural area.

Overview of Data Collection and Analysis Methods:

Data collection (meeting one)
In small groups (two to three people), collect tree leaf samples. This activity has three goals:
- to introduce students to the deciduous trees at the site,

- to help students explore connections between leaf morphology and the environment, and
- to build a large data set from all of our groups for analysis in the next class period.

Each group should try to identify at least **three** tree species and collect at least **one** leaf specimen (up to five) from each. Use a local or regional tree species guide to identify the species based on their characteristics. Many guides include example pictures of the tree and leaves. *Keep track of which specimen belongs to which species* (this is important for the data compilation step). Record the species name in the table at the end of this section. Some common tree species in eastern and central North America include: oaks, maples, elms, shag bark hickories, ginkgos, horse chestnuts, walnut, lilac, dogwood, and honey locust. For example, white oak (*Quercus alba*) may be 'lobed' with 'no teeth' and a greater length than width, while slippery elm (*Ulmus rubra*) may be 'not lobed' with 'regularly-spaced' and 'closely-spaced' teeth and a much smaller leaf to width ratio.

When collecting the leaf specimen, collect the whole leaf. For example, **pinnate** leaves have multiple leaf parts that are all on the same leaf. If you have a leaf but are having trouble identifying the species, bring it back to the class for identification help. If collecting single leaves, please note whether the leaves are **opposite** (directly across from one another) or **alternate** (not opposite) on the stem.

Once you have identified and collected the specimens, return with the samples to the laboratory. Record six morphological variables (outlined in Table 1) from the collected leaves. For each leaf, record four morphological characteristics (with 0s or 1s), plus the leaf length and the leaf width. Figure 1 may be helpful.

Leaf characteristics to record in Table 1:
1. **Lobed** – Leaves can be either pinnately lobed (e.g., *Quercus*) or palmately lobed (e.g., *Acer*)
   0 - not lobed
   1 – lobed
2. **No teeth** – Some leaves have small teeth on the edges (called **serrate margins**).
   0 - teeth present
   1 - no teeth
3. **Regularity of tooth spacing** – Teeth are regularly spaced if the lengths of the bases of two adjacent teeth differ by less than one-third (*or, rather, if the teeth are similar in width*).

0 - no teeth are present, or teeth are not regularly spaced
1 - over 50% of teeth are regularly spaced

4. **Closeness of teeth** – Teeth are closely spaced if the bases of the teeth are no longer than three times the apical (outer) side of the teeth (*or, rather, if the teeth are pretty close together*).
   0 - no teeth are present, or teeth are not close
   1 - over 50% of teeth are closely spaced

5. **Length** – distance from the base of the leaf/edge of the petiole to the tip (cm)

6. **Width** – width at widest part of leaf (cm)

*Leaf character definitions modified from the CLAMP. Based on Wolfe (1993).*

Table 1: Tree data. Species get a "1" score if the condition is true and a "0" score if the condition is false.

Species (sci. name)	Lobed?	No teeth?	Teeth regular?	Teeth close?	Length (cm)	Width (cm)

Try to get at least three (3) species. Find and add more species if there is time. More species/samples will enhance the final data set for the entire class!

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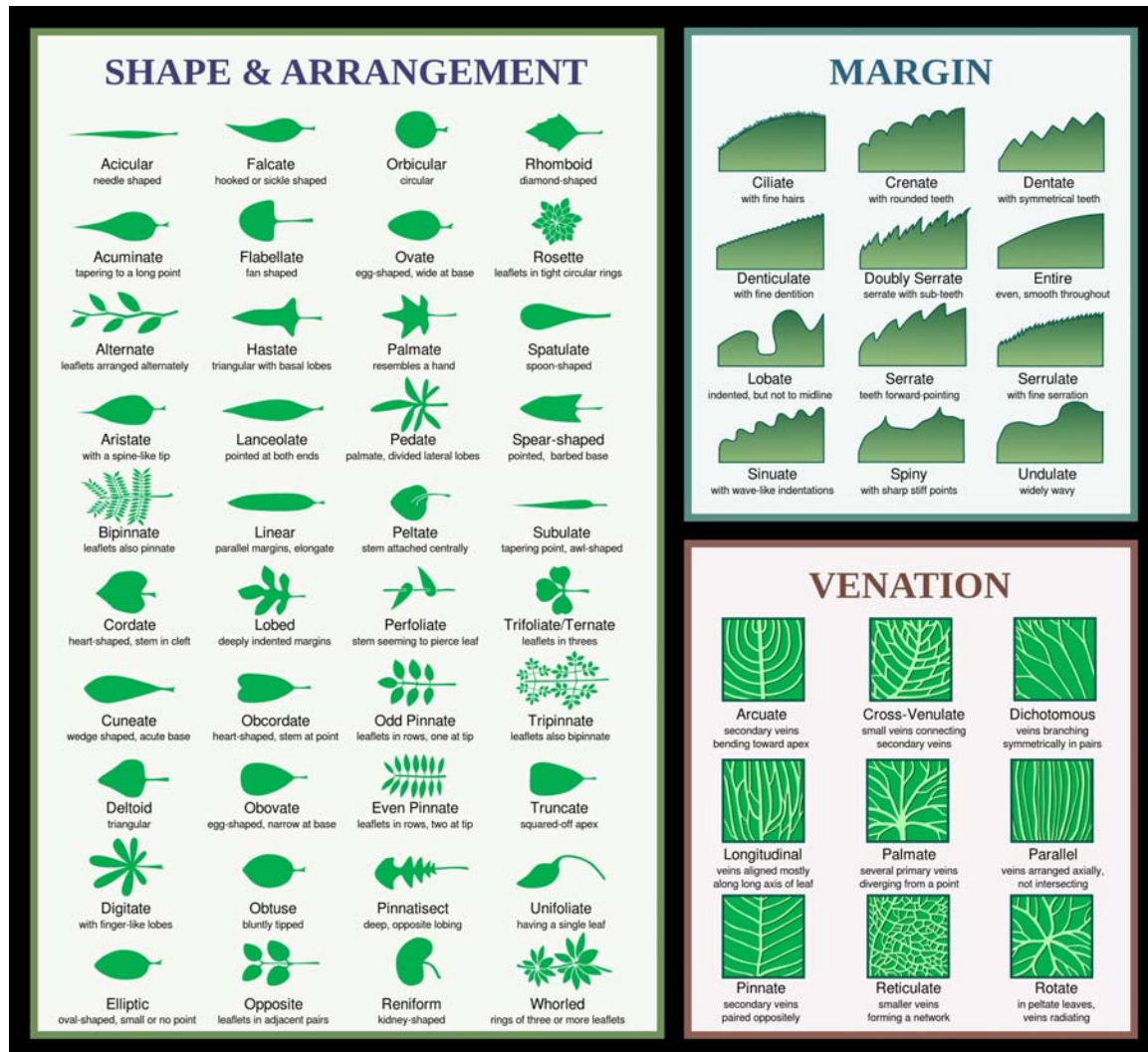
Figure 1. Chart of leaf morphology characteristics.
Obtained from: http://commons.wikimedia.org/wiki/File:Leaf_morphology.svg and licensed under the "GNU Free Documentation License."

Data analysis and comparison (meeting two and/or three)

Follow these steps:

1. Enter the leaf data into the group's spreadsheet. (The instructors may choose to compile the data before class, or have students email them the data, or enter it into an online spreadsheet before this class period.)

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2. After all groups have entered data and a "class data set" has been created, <u>copy the total class data</u> into the spreadsheet. Be sure to average within species if they are repeated.
3. Now, calculate a **proportional average** of each leaf variable. This average will represent the characteristics of the "average tree" at the site (averaged across species), and it will be a percent value (making it proportional). For example, the proportional average of trees with lobed leaves at the site may be around 25%.
    Calculate the averages of each variable with these steps:
    a. In the "Average (x)" cell for the *Lobed* variable, type =AVERAGE(to start a formula.
    b. Then, click once on the first cell of data in the *Lobed* variable and, without releasing the click, drag the mouse to the last cell of data in the Lobed variable. This will select all of the data in a colored rectangle.
    c. Then, type) to close the formula, type *100 to obtain the percent, and hit return.
    d. This should produce a percent value for the *Lobed* variable. Notify the instructor if you need help. Repeat these steps for each variable.
4. Access the CLAMP calibration data (see the "Comments on the Data Collection and Analysis Methods Used in the Experiment" for information about the data set). This is a global data set with 144 modern vegetation sites collected from trees around the world (primarily Northern Hemisphere temperate regions).
    Create **scatterplots** of each leaf variable (on the x, horizontal, axis) and mean annual temperature (MAT on the y, vertical, axis). This shows the relationship between each variable and temperature. Use the spreadsheet program to draw lines (**regressions**) through the scatterplots and show the equation of those lines. These equations take the form of:
    $MAT = slope * leafVariable + intercept$
    $Y = m * x + b$
    and they can be used to **predict** MAT with data from the leaf observations and the **coefficients** – slope (m) and intercept (b) – of the line.
    In the group's spreadsheet, enter the coefficients of each regression line into designated "Slope (m)" and "Intercept (b)" cells.
5. Now, make MAT predictions for our site with the relationships that were developed from the global data set and the leaf variable data that were collected as a class.
    a. In the "Prediction (y)" cell for the *Lobed* variable, type = to start a formula.

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      b. Click once on the "Slope (m)" cell for the *Lobed* variable. Then type * to add a multiplication symbol to the formula.

      c. Click once on the "Average (x)" cell. Then type + to add an addition symbol to the formula.

      d. Click on the "Intercept (b)" cell, and hit return.

      e. This should produce a MAT prediction based on the *Lobed* variable. Notify the instructor if you need help. Repeat these steps for each variable.

6. There are five **independent predictions** of mean annual temperature for the site, based on different measures of leaf morphology from data that were collected. These different predictions will allow you to consider "how confident" or, rather, "how uncertain" the estimates are. Compare the estimates with the calculations made by other groups in the class, either one-on-one or by writing each group's estimates on the board.

7. Collect mean annual temperature statistics from a weather station near the site for comparison. These values can be downloaded from the [National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center](#). Each group can choose what time period to use for comparison to the predictions, considering at what timescale trees may respond to climate (Annual? Decadal?).

Questions for Further Thought and Discussion:

1. How well does the model predict temperature at the site? Do these predictions fit within the other observations in the database?

2. What factors influence the uncertainty in your predictions?

3. How are the different leaf traits related to one another? How does this influence your temperature predictions?

4. What are some possible explanations for the variation observed at different sites? For example, do the site-average traits change across sites because there are changes in species or because there are changes in morphology within species?

5. Choose one or two traits to think about the mechanisms behind the patterns that you observed. What are some possible ecological or physiological explanations for why leaf morphology responds to temperature?

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Teaching Issues and Experiments in Ecology - Volume 9, July 2013

References and Links:

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Links

CLAMP Online. Physg3brcAZ and Met3brAZ calibration datasets. (http://clamp.ibcas.ac.cn/, accessed 25 February 2013)

NOAA National Climate Data Center. 2013. Climate data online. (http://www.ncdc.noaa.gov/cdo-web/, accessed 25 February 2013)

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Tools for Assessment of Student Learning Outcomes:

This experiment will be graded based on two components: collecting leaf data (25%) and a brief report (75%).

Collecting leaf data
Since the exercise depends on compiling a class data set, your participation in the data collection phase is important. Data sheets will be submitted at the end of the first period so that instructors can verify that you have collected the necessary information and to identify any potential issues with the data.

Report
As a group, write a brief report (1-2 page write up) documenting your findings. **Identify** how each morphological trait responds to mean annual temperature. **Discuss** how well this combination of traits can predict the temperature at our site. Are the predictions from certain traits closer to the observations than others? Why is there uncertainty in our predictions? **Include** graphs showing the temperature by trait relationships from CLAMP and add data points for the observed climate and observed site-trait variables. How well does the site fit within the other sites in the data set? Finally, using information previously discussed in class, **develop** some possible hypotheses to explain these relationships. Why might leaf traits respond to mean annual temperature?

NOTES TO FACULTY

Comments on Challenges to Anticipate and Solve:

1. Difficulty identifying species: Students may not have experience using a species guide to identify species. Encourage students to identify species in the field, where they have a view of the entire tree. Identification with just a single leaf sample can be difficult. Using a regional species guide will narrow possible options, and I find that my students found guides with pictures to be the easiest to use.

2. Little prior experience with spreadsheets: I guide my students through a spreadsheet exercise at the beginning of the semester so that they are familiar with some of the graphing and calculation procedures that we will use. If students are unfamiliar with spreadsheets, some more guidance may be necessary to calculate average trait values and predict temperatures based on the fitted regressions.

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Teaching Issues and Experiments in Ecology - Volume 9, July 2013

Comments on Introducing the Experiment to Your Students:

I introduce this experiment by giving a brief (10 minute) presentation about the history of leaf morphological analysis (especially Bailey and Sinnot 1915) and about the CLAMP project. In my presentation, I show a map of the sites that we are comparing and discuss the use of this analysis in paleoclimate reconstruction. The students then have background and motivation for going out to collect samples. The instructor could additionally have students analyze the global data set before their own data collection (rather than after) to further introduce the concepts.

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Comments on the Data Collection and Analysis Methods:

Leaf samples can be collected and identified in the field, or brought back to a laboratory for later identification. The instructor may want to have each group collect multiple leaves from each species to minimize uncertainty, although this might require an introduction to subsampling and averaging. I found that replication naturally occurred across groups, as many groups will collect samples from the same species.

The CLAMP calibration data can be obtained from the CLAMP website in the "Calibration Datasets" link <http://clamp.ibcas.ac.cn/CLAMP_Datasets.html>, either by the instructor or the students. The leaf (Physg3brcAZ) and climate (Met3brAZ) data are downloaded separately. I used Google Spreadsheets for much of this exercise, which allowed each group to upload their data into a common file. To facilitate the compilation step, I merged all of the data files by myself and then shared it with the class. I also downloaded the leaf trait and climate data from the CLAMP website and local climate data from the NOAA website and made my own files to share with them. In the CLAMP data file that I shared with the students, I plotted the leaf trait by climate graphs and made the regression fits (Figure 2), and only included leaf variables that we examined (there are more variables in the database than are included here). Depending on the students' familiarity with spreadsheet programs, instructors may want to allow students to download CLAMP and NOAA data from the internet on their own and/or create their own scatterplots and regressions.
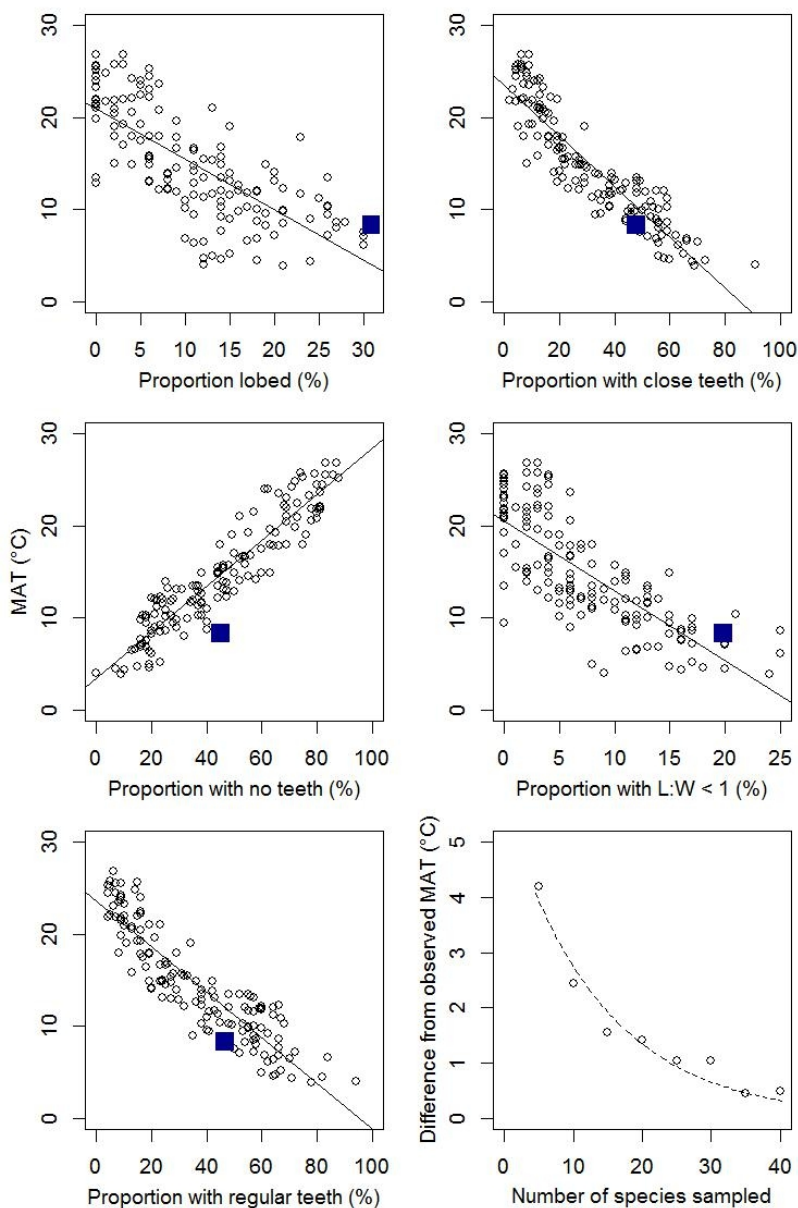
Figure 2: Observations of leaf morphology and mean annual temperature from Beloit College, Beloit, WI compared to the CLAMP data set. The different morphological variables have different predictive abilities. The last panel shows the decrease in predictive error (temperature) with increased sample size (number of species), obtained by randomly re-sampling the observed data.

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There are many different options for the level of detail in the analysis. For my students, we calculated individual predictions for each trait and averaged them, but students with more advanced statistical knowledge could perform multivariate regressions with uncertainty and calculate goodness of fit measures.

Comments on Questions for Further Thought:

1. *How well does the model predict temperature at the site? Do these predictions fit within the other observations in the database?*

   Students can calculate deviance from the observations for each trait. In the comparison with observations from other sites, students should discuss whether their points fit within the distribution of observed values.

2. *What factors influence the uncertainty in your predictions?*

   Students may highlight how the number of species that they sample will influence the predictive ability of the model. They can also discuss whether they obtained a diverse or representative sample. They could also highlight the number of traits that are used to create predictions.

3. *How are the different leaf traits related to one another? How does this influence your temperature predictions?*

   Correlation between traits could create a problem in prediction.

4. *What are some possible explanations for the variation observed at different sites? For example, do the site-average traits change across sites because there are changes in species or because there are changes in morphology within species?*

   Both factors will be important, but it is important to recognize that both changes will lead to changes in mean trait values. Students may consider species similarity across sites. For example, some sites may have very similar species compositions but very different trait values. Additionally, within species trait values change across environments (Royer et al. 2008).

5. *Choose one or two traits to think about the mechanisms behind the patterns that you observed. What are some possible ecological or physiological explanations for why leaf morphology responds to temperature?*

Hypothetical mechanisms behind leaf morphological responses to climate relate to ecological variation in physiology. For example, regulation of gas exchange (Royer and Wilf 2006) and maintenance of leaf temperature (Niklas 1992) are both thought to play a role. Ecologically, these responses may affect the ability of species to compete with species that exhibit different traits.

Comments on the Assessment of Student Learning Outcomes:

The data collection submission is designed to make students accountable for collecting and recording data. Students are given credit for participating in this portion. In this experiment, I focused on presenting and discussing results, which are the main criteria for the written report. The questions for further thought and discussion can be incorporated into the report requirements so that students can apply their knowledge from this experiment to other topics in the course. The report can be graded based on expectations for responses based on prior knowledge. I taught the experiment in a botany class, and we had discussed gas exchange and water use, so the students were familiar with some of the basic mechanisms and this provided an opportunity to apply their knowledge to these issues. Other components (like species interactions or ecological variation) could also be emphasized in assessment.

Comments on Formative Evaluation of this Experiment:

Formative evaluation can occur at multiple points during this experiment. The data collection submission allows the instructor to perform a preliminary check on the data and to identify any potential problems in analysis before they arise. If students are identifying the species themselves, then sampling inaccuracies may be brought up in the reports as a source of error. Because the data for this experiment are pooled by the entire class, the analysis is insensitive to the performance of any particular group. Groups that do not perform well on the data collection portion can learn from their peers when they receive the compiled class data set.

In the second meeting, I walk through the regression models for each leaf trait and ask students to identify the patterns in the relationships (for example, sites in colder climates have more trees with lobed leaves). Once students understand the structure of the models, they could complete a short **transfer and apply** exercise to make predictions from some hypothetical site, helping them prepare for making predictions at their site. Data from my class at Beloit College, Beloit,

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Teaching Issues and Experiments in Ecology - Volume 9, July 2013

WI is included in the "Student Collected Data from this Experiment." This could be used to generate predictions for Beloit, WI and may be used in class as an example for formative evaluation or by the instructors to familiarize themselves with the experiment and analysis. **Note**: when I teach this experiment, almost all of the students have no formal statistical experience, but they are able to understand the patterns in the data and then apply that understanding to predict out of sample.

If this experiment is taught multiple times, the instructor can evaluate both the students' understanding of the concepts and the accuracy of their predictions. The instructor could allow students to compare their predictions to the performance of other class sections. The opportunity to out-perform peers from other classes could be a motivation for obtaining a representative sample and recording careful measurements. When I taught this to two sections of the same class, both classes performed similarly in prediction (statistically indistinguishable), but their pooled prediction was better than each individually.

Comments on Translating the Activity to Other Institutional Scales or Locations:

This experiment is transferable across locations/environments within the range of sites in the CLAMP database. It requires access to local tree samples. With larger classes, students may need to be more independent in the identification portion of the exercise, but more groups should lead to more species samples, which will increase the robustness of the results. With a smaller number of groups, I emphasize that the goal is to obtain a diverse sample and encourage them to find samples that their colleagues might not collect. If groups overlap in their species observations, though, the students can calculate variation within species.

I examined the effect of the number of collected species on the accuracy of prediction by randomly resampling different numbers of species from the pooled class data with replacement. The difference between the observed and predicted temperature values decreased exponentially with increasing number of species. Predictions were within 1.5°C of observations with >15 species observed and within 0.5°C of observations with >30 species. The number of species that each class sampled was close to the average number recorded in a random sample of 60 of the CLAMP sites (29 species).

The exercise could be adjusted for pre-college settings or for students with physical or other disabilities by providing print-offs of pre-identified samples to

measure. It can also be modified for courses where species identification is not a goal with local herbarium samples. The questions and goals can change also. Some examples include:

- climate prediction
- physiological hypotheses for leaf morphology
- multivariate prediction and comparison with a global database
- comparisons between predictions from natural and modified environments at a single site

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

- Example calculation sheet for generating model predictions. Students or instructors can insert CLAMP data that they download and leaf data that they collect. [xls]

- Morphological leaf data collected by students at Beloit College, Beloit, WI, for example calculations. Values for each species are averaged across groups; if multiple groups recorded the same species then the recorded value will be an average of their observations. [csv]

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Lastly, we request that you return your students' and your comments on this activity to the TIEE Managing Editor (tieesubmissions@esa.org) for posting at this site.

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