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A Scaffolded Approach to Introducing Biology Students to Primary Literature

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This workshop suggests a scaffolded introduction of students to biological literature. Students are presented with deep-lobed and shallow-lobed leaves (*Quercus alba*) and led to design methods to quantify depth of lobing and determine stomatal density. After students learn that the leaves were obtained from the top and bottom of the same tree, they are asked where each type (deep-lobed/more stomata; shallow-lobed/ fewer stomata) would be located. Finally, students read a journal article in which stomatal density of fossil oak leaves is used to predict paleoatmospheric CO_2 concentrations. Students' familiarity with laboratory procedures facilitates their understanding of the article.

Keywords: primary literature, scaffolded, stomatal density

Introduction

Interpreting primary literature is one of the more challenging skills for students of biology. They are stymied by complex vocabulary, obscure protocols and information-dense sentences. This major workshop suggests a scaffolded approach to introducing first-year students or non-majors to biological literature by framing it in a familiar conceptual and experimental context. Students are presented with two sets of oak leaves—one with deep lobes and another with shallow lobes. The two sets of leaves were obtained from different locations on the same oak tree (*Quercus alba*); one set was taken from the top (sun leaves) and the other was taken from the bottom (shade leaves). Students are led by the instructor to design a method by which they can quantify the depth of lobing and determine if the difference is significant. Next, they develop a method by which they can determine stomatal density of the leaves and determine if that difference is significant. In the last part of the laboratory study, students are told that the leaves actually were obtained from the top and bottom of the same tree and are asked where each type of leaf (deep lobed/ more stomata; shallow lobed/ fewer stomata) would best be positioned, given what they know about the requirements for photosynthesis. Finally, students read a journal article in which researchers use stomatal density of fossil oak leaves to predict the paleoatmospheric concentration of CO_2. While the discussion of the journal article is still a challenge, students' familiarity with the biological concepts (phenotypic plasticity in development of stomata and depth of lobing due to light exposure of sun and shade leaves) and the laboratory protocol (determining stomatal density from casts of leaves) enables them to more easily understand what the researchers are doing and interpret their results.

This exercise requires about 4 hours for the actual laboratory component and at least two hours for the discussion of the journal article. It is intended for first-year students or non-majors. It requires little preparatory and clean-up time. Perhaps the greatest challenge is finding an oak tree from which upper leaves can be removed easily.

Student Outline

As preparation for class/lab, students should locate vocabulary words that will help them to describe the parts of a leaf (lobe, sinus) as well as the structure of the stomata/guard cells in the epidermis. A website such as http://uptreeid.com/glossary.htm is sufficient. It is also presumed that students have a basic understanding of the events of photosynthesis

Materials

The following materials are needed for a class of 20 students. This exercise works well if students work in groups of no more than three.

- Two sets of oak leaves (we use *Quercus alba* because of the availability of the tree on campus); if students work in pairs, each pair needs two leaves from the top of the tree (sun leaves with deep lobes) and two leaves from the bottom (shade leaves with shallow lobes). Therefore, about 24 leaves of each type are needed.
- Engineer-grid graph paper that has been photocopied onto cardstock (alternatively, engineer-grid graph paper could be glued onto card stock); for ease of reference use two colors—one for each type of leaf. About a dozen sheets of each color are needed, unless the leaves are exceptionally large and won't fit two to a sheet.
- Metric balance (sensitive to 0.01 g); three or four positioned in the room/lab works well

Materials per group:

- 2 shade leaves (from the bottom of a tree) and 2 sun leaves (from the top); don't identify them as such at the start of the exercise, though
- Ruler
- Pencil
- Scissors
- 2-4 sheets of each of two colors of engineer-grid graph paper that has been photocopied onto cardstock
- Clear nail polish
- Clear wide packing tape
- Microscope slides
- 80% ethanol (to clean slides)
- Kimwipes
- Sharpie
- Microscope with camera
- Computer (for statistical tests and Google search)

Notes for the Instructor

As preparation for class/lab, students should locate vocabulary words that will help them to describe the parts of a leaf (lobe, sinus) as well as the structure of the stomata/guard cells in the epidermis. This information constitutes their "ticket" to lab. A website such as http://uptreeid.com/glossary.htm is sufficient. It is also presumed that students would have a basic understanding of the events of photosynthesis.

This laboratory exercise is conducted as a guided inquiry—students are led by the instructor to develop procedures for determining the depth of lobing of leaves and for determining stomatal density. These notes are presented in Table 1 as a series of questions that the instructor would pose to the students (in regular font) and the anticipated answers to those questions (*in italics*). The actual procedures are shown here **in bold.**

Table 1. Steps in guided inquiry.

Step 1.	**Distribute the leaves to the students, making sure each pair/group receives one deep-lobed and one shallow-lobed leaf. Don't mention that the leaves come from the same tree.**	
	Instructor's Questions	*Elicited Student Response*
	How are these leaves similar to each other?	*They have the same general shape.*
	Can we agree on terminology to use when we describe these leaves?	*Students introduce the terms lobes and sinus.*
	How are the two leaves different from each other?	*In one set the sinuses are much deeper than in the other.*
	Can we agree on a designation that we can use when we're referring to these leaves?	*Shallow-lobed and deep-lobed leaves*
	Can you think of something we could do to quantify the difference in depth of lobing of these two sets of leaves?	[The goal is to steer them toward tracing the outline of the leaves onto graph paper.] *Students may suggest various strategies here.*

	If we're interested in the space of the sinuses, what would we have to do with the outline to indicate where the sinuses were?	*We'd have to connect the tips of the lobes.*
Step 2.	**Students connect the tips of the lobes on both outlines. See Figure 1.**	
	Now that we've completed that step, can we determine how many areas we are working with?	*We have the area of the sinuses and the actual area of the leaf*
	What about the sum of those two areas? What could we call that?	*Total leaf area*
	What would be the equation that represents their relationship?	*Sinus area, Actual leaf area, Total leaf area.*
	What would be the equation that represents their relationship?	*sinus area + actual leaf area = total leaf area*
	How could we represent that equation with symbols?	*SA + ALA = TLA*
	So how can we actually determine the values for those areas?	*We'd have to count all the "boxes."*
	That would certainly work, but wouldn't it take a lot of time? Can you think of a way we could arrive at a value more quickly?	[The goal here is to steer them toward cutting out the leaf along the lines they drew to connect the tips, determining the mass of the paper leaf and converting that mass to area.]
	Which of the three areas would we start with?	*Total leaf area*
	What would you have to do to get that by itself?	*Cut it out*
Step 3.	**Students cut out the two leaves along the lines that they drew to connect the tips of the lobes. They can cut off the petiole. They measure and record the mass of each of the two leaves.** [I find it helpful for each group to record their values in a table on a large dry-erase board—it's easy to walk around and monitor what they're doing.]	
	What would you have to do to determine a value for ALA? [The mass of the sinuses is pretty small and students may lose one or two of these pieces when they cut them out. That's why I go this route rather than measure the SA.]	*We'd have to cut out the sinuses and determine the mass of what was left.*
Step 4.	**Students cut out the sinuses and measure and record the mass of the ALA of each leaf.**	
	Now, what about the SA? How can we determine a value for that?	*We could subtract the ALA from the TLA.*
Step 5.	**Students carry out the subtraction and record the values for SA for each leaf**	
	What do we have so far?	*Values of mass for each of the three areas of each type of leaf.*
	Right. But what do we want to know about each of those three areas?	*We want to know the actual area values.*
	Let's think about other situations in which we've measured along one line but want our values to be expressed along another line. Can you offer some examples?	*Measure temperature in ^{o}F when you want ^{o}C. Measure length in inches when you want cm. Measure water in mL when you want grams.*
	Right. What do you have to use in each of those situations to change one measurement to the other?	*Guide students to the concept that they need a conversion factor.*

	So what's the role of a conversion factor?	*It changes a measure from one form to another, usually using a formula.*
	What would we want our conversion factor to do?	*Convert mass to area.*
	How could we do that?	*[Guide students to the idea that they could cut out 10cm^2 of the card stock (1 cm^2 is probably too small) and determine its mass. Then they could set up a conversion factor.]*
Step 6.	**Students cut out a 10 cm^2 piece of each color of graph paper/card stock and determine its mass.** [I usually have students record their mass values on the board and then we calculate a class average that we work with from that point on. It simplifies the process.]	
	What do we do to get a conversion factor out of these values?	*We set up a ratio:* *mass of 10cm^2 piece of cardstock (g)/area of 10cm^{2}card stock = mass of 1cm^2(g)* */ area of 1 cm^{2}cardstock*
	Explain how you would use this conversion factor.	*We would multiply each mass value that we obtained for our leaves by the conversion factor.*
Step 7.	**Students use the conversion factor to convert the mass values for each of the three leaf areas (SA, ALA, and TLA) to area values.**	
	Can we now make a comparison between the lobing in the two sets of leaves?	*[Lead students to understand that we can't because the size of each of the various leaves that they measured is not the same.]*
	What can we do mathematically to put both sets of leaves on "equal ground" in our comparison, then?	*We can convert the values to percentages.*
	How would you do that?	*We'd form the fraction: SA/TLA = % depth of lobing.*
Step 8.	**Students convert their area values to depth of lobing percentage values.**	
	Does there appear to be a difference between the two sets of values?	*There does.*
	What can we do mathematically to simplify the comparison?	*We can calculate the average depth of lobing percentage for the two sets of leaves.*
	How can we determine if the difference is significant?	*[If the students have conducted t tests already in the class, they should recognize this as an appropriate use of an unpaired t test. If they have not carried out t tests, they will need further assistance.]*
Step 9.	**Students conduct unpaired t test to determine if the difference between the depth of lobing in the two sets of leaves is significant. www.graphpad.com is an easy website to use.**	
	We have now determined that there is a macroscopic difference between these two sets of leaves. How might they be different microscopically?	*[Guide them to think about the stomata/guard cell relationship.]*
	How might the stomata be different in the two sets of leaves?	*They might differ in number or in size.*
	On what surface of the leaf would we find stomata?	*On the under surface only.*
	Can we find a procedure that would enable us to	*www.biologyjunction.com/leaf_stomata_lab.htm;*

	see the stomata? Let's look online.	
Step 10.	**a. Obtain a leaf from each of the two sets of leaves. Apply clear nail polish to about a 1 cm^2 area of the under surface of each leaf, trying to avoid large veins. Allow the nail polish to dry completely. (This takes ~ 30 minutes.)** **b. Pull off a length of clear packing tape about 3 cm long. Fold over one end of it to form a "handle" that you can hold easily.** **c. Place the tape over the dried patch of nail polish and press firmly on the entire piece of tape with your thumb.** **d. Using the handle, peel the packing tape away from the leaf. As you do this, the nail polish will be removed, also. The impression of the leaf made by the nail polish and tape is called a cast.** **e. Wipe a microscope slide with alcohol and dry it carefully. Tape your peeled impression to the slide. Use scissors to trim away any excess tape. Label the slide with the sharpie.** **f. Examine the leaf impression at 400X. Find an area in which there are few veins and no fingerprints. Use the camera to take a picture of the field of view. Count the stomata in the entire field of view.**	
	What we're interested in here is not just the number of stomata we can see and count but a concept called stomatal density. What does that sound like to you?	*[Guide them to think about this, perhaps using "population density" as a starting point.]*
	To determine stomatal density, what would we have to know?	*The number of stomata in a given area of leaf epidermis.*
	So how could we determine the area of the field of view we were observing?	*[Guide them to explain that they would measure the length and width of the field of view with a clear plastic ruler and multiply to determine the area.]*
Step 11.	**Students use a clear plastic ruler to measure the length and width of the field of view; they multiply to obtain the area. Then they convert the number of stomata in each field of view to the number of stomata/mm^2.**	
	Does there appear to be a difference between the two sets of values?	*There does.*
	What can we do mathematically to simplify the comparison?	*We can calculate the average stomatal density for the two sets of leaves.*
	How can we determine if the difference is significant?	*We can conduct an unpaired t test.*
Step 12.	**Students conduct an unpaired t test to determine if the difference between the stomatal density in the two sets of leaves is significant using www.graphpad.com.**	
	Suppose I told you that the two sets of leaves came from the same tree. What do you know about the sun exposure of the two sets (Figure 2)?	*The ones at the top receive the full sun but the ones at the bottom are mostly in the shade and receive much less sunlight.*
	Knowing what you know about photosynthesis, which leaves do you think came from the top of the tree and which leaves came from the bottom?	*The deep-lobed / high stomatal density leaves came from the top of the tree where they would receive a greater amount of sunlight. Because they receive more sunlight, they'd also need more CO_2 to carry out a higher rate of photosynthesis. Therefore, they'd need more stomata to allow more CO_2 to enter the leaves. The deep lobes would enable them to allow sunlight to pass down to the leaves that are located at the bottom. The shallow-lobed leaves came from the bottom part*

		of the tree, where they would receive less sunlight in the shade. Because of this, they'd need less CO_2. Therefore, they'd need fewer stomata.
	Do the leaves in the two parts of the tree, top and bottom, have the same genetic information or do they have different genetic information?	*They have the same information.*
	What happened, then, to bring about the difference in structure for the leaves at the top and the leaves at the bottom?	*[Guide the students to realize that the intensity of light determined how they responded in their development of lobes and stomata.]*
	What is the term that biologists use to describe the appearance of an organism?	*Phenotype*
	Based on what you've observed here, would you say the phenotype of these leaves is flexible and adaptive or inflexible and nonadaptive?	*It appears to be flexible and adaptive.*
	Can we find a term that we can use to name this characteristic?	
Step 13.	**Students find "phenotypic plasticity" in a Google search.**	

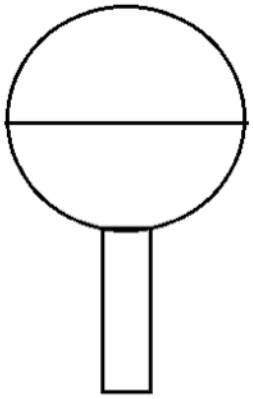

Figure 1. How the tips of the lobes should be connected.

Figure 2. Diagrammatic representation of two areas of oak tree.

At this point, the instructor can take a number of different directions. Students can write an essay or lab report that summarizes what they did with the leaves and what they learned. They can use Excel to design a graph that illustrate their results. Students can also be led through a guided reading of a journal article, Table 2. The one that is suggested is: Van Der Burgh, *et al*. (1993).

Students are told to prepare for the class discussion of the article by skimming it and making a list of all vocabulary words—both biological and non-biological—with which they aren't familiar. This list constitutes their "ticket" to class on the day of the discussion.

We read the journal article as a group. I ask a student to begin reading (skipping the abstract) and instruct the others to interrupt as the need arises (usually every few sentences). Then we work together to "de-code" the sentences.

- Paragraph 1—The concentration of CO_2 in the atmosphere can be directly measured as far back as 160,000 years. For concentrations earlier than that, indirect measurements can't be made. A few of these indirect procedures are described. Fossils, in particular plant fossils, provide a potential source of this information.
- Paragraph 2—It's important that students understand that stomata can be involved in the regulation of photosynthesis because they determine how much CO_2 enters the leaf This paragraph requires substantial time to make sure students understand this relationship. The authors use the term phenotypic plasticity here.
- Paragraph 3—The authors explain how previous research supported the connection between stomatal density and CO_2 concentration. They present the concept that since CO_2 concentrations can be used to predict stomatal density, the opposite could also hold: stomatal density could be used to predict CO_2 concentrations.
- Paragraph 4—The authors explain why they chose *Quercus petraea* as the focal tree in their research.
- Paragraph 5—The authors explain how they obtained casts of fossil leaves.
- Paragraph 6—I generally skip this paragraph.
- Figure 2—The authors present data for geological time periods, climate fluctuations, and stomatal density values in *Q. petraea.*
- Figure 3—The authors integrate stomatal density/CO_2 concentration values from recent time with values from Neogene time.

Table 2. Guided reading of article.

	Instructor's Questions	***Elicited Student Response***
	Which time periods did the authors have information about?	*They knew information about recent leaves (about 200 years old) and Neogene leaves.*
	Which of these did they know more about?	*They knew more about the more recent leaves.*
	How would they have graphed the information they had about the leaves?	*They would graph the stomatal density on the y axis and CO_2 concentration on the x axis.*
	Where did the authors obtain these data?	*They got the stomatal density values from herbarium materials and the CO_2 concentration from one of the proxy methods described at the beginning of the article, most likely polar ice analysis*.
Step 14.	**Show the students a version of the graph that presents only the recent data as points.**	
	What do you observe about these values?	*They fall in a straight line.*
	What can we do mathematically to emphasize their relationship?	*Connect them with a line of best fit.*
Step 15.	**Add the line of best fit (the regression line described in the figure legend).**	
	How can we indicate the variability shown by these data?	*We can add error bars.*
Step 16.	**Similar to the bars that show 95% confidence limits (as included in the original figure).**	
	Now, what do we know about Neogene leaves?	*We know their stomatal density values.*
	How can we fit these into our expanding figure if we only know the y axis values.	*Guide the students to realize that the data points (from Figure 2) can be located on the line of best fit.*
Step 17.	**Plot the values from Figure 2 onto the line of best fit and complete Figure 3.**	
	So what can we say about the paleoatmospheric CO_2 concentration shown by this graph?	*If you follow each Neogene data point down to the x axis you can identify the CO_2 concentration that existed at the time each leaf actually lived.*

We conclude the discussion by reading the abstract which, at this point, the students find they understand.

> "An increase in the atmospheric carbon dioxide ($C0_2$) concentration results in a decrease in the number of leaf stomata. This relation is known both from historical observations of vegetation over the past 200 years

> and from experimental manipulations of microenvironments. Evidence from stomatal frequencies of fossil *Quercus petraea* leaves indicates that this relation can be applied as a bioindicator for changes in paleoatmospheric CO_2 concentrations during the last 10 million years. The data suggest that late Neogene CO_2 concentrations fluctuated between about 280 and 370 parts per million by volume."

Finally, to determine whether students understand the connection between CO2 concentration and stomatal density, they are given this question which is based on another journal article (Maherali *et al*., 2002). http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1452&context=usdaarsfacpub

A group of researchers studied stomatal conductance (g_s), a measure of the rate at which water evaporates from a leaf through the stomata. Their research focused on three types of grass: *Solanum dimidiatum*, *Bromus japonicus,* and *Bothriochloa ischaemum*. In this experiment, the researchers constructed two elongated chambers over parallel and adjacent plots of grassland, each 60 m long, 1 m wide, and 1 m tall. The chambers were covered with a transparent plastic film. Air was introduced into one end of each chamber and was moved down the length of the chamber by a blower. As the air moved down the chamber, CO_2 was taken in by the plants. CO_2 concentrations were maintained at a range of 200-550 μmol. Both chambers were watered regularly; the temperature and humidity were kept constant throughout the length of the chambers. At the end of the growing season, the researchers prepared casts of the surfaces of the leaves of the three major types of plants growing in the two chambers. They determined the density and size of the stomata.

Figure 3 is taken from the journal article that reports this experiment. Study the figure and answer these questions:

a. Hand sketch a detailed diagram of the main parts of the experimental set up (don't try to do it with the computer).
b. Write a one-sentence explanation of what it means that the "slope is significantly different from zero) in the figures below.
c. Write a one-sentence description of the difference in stomatal density in the three species of plant grown at various concentrations of CO_2.
d. Write a one-sentence description of the difference in stomatal size in the three species of plant grown at various concentrations of CO_2.
e. Stomatal conductance is a combination of stomatal density and stomatal size. Write a one to two-sentence comparison of how each of the three species of plant adjusted the stomatal conductance in response to changes in CO_2 concentration.

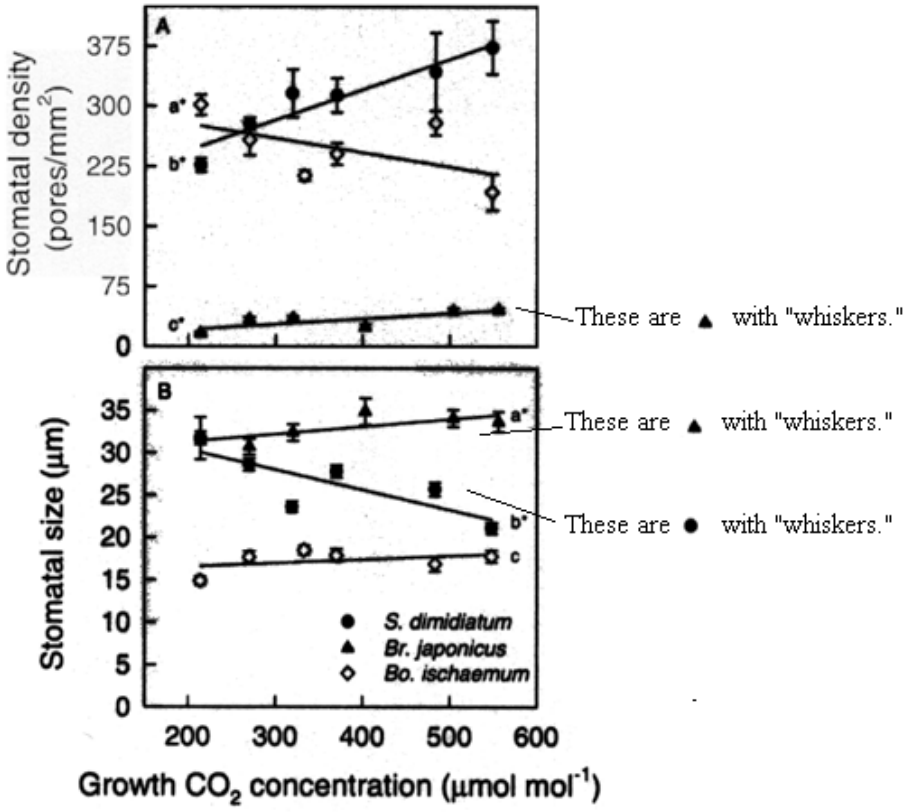

Figure 3. The response of three species of grass to changes in carbon dioxide concentration: mean stomatal density (A) and stomatal size (B. In A, all the slopes are significantly different from zero; in B, only slopes b and c are significantly different from zero. The "whiskers" in the graph show standard error (similar to standard deviation) (Maherali *et al.,* 2002).

Literature Cited

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About the Author

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