Impressions of a Stoma

Overview
Students use two different methods to view stomata on the underside of leaves.

Introduction
Plants exchange the gasses involved in photosynthesis and respiration through stomata, pores in a leaf’s epidermis. Water also evaporates from the surface of the leaf through the stomata—a process called transpiration—which is one of the driving forces of water uptake and transport throughout a plant.

The plant can regulate gas exchange or water loss through its stomata by opening and closing them. This function is regulated by the guard cells, oval-shaped, photosynthetic cells surrounding the pores. Stomata are generally open under optimal conditions, but close when the plant is under heat, light, or physical stress. When the cells are turgid and full of water, they unevenly swell, and the stomata are opened up. To close the stomata, the plant sends water out of the guard cells and as they go flaccid, the pores close up.

Plants also can reduce transpiration by having stomata in recessed pits and/or surrounded by hairs. This will create local areas of humidity even in dry conditions. Many arid-environment plants will have stomata with these adaptations.

Motivation
To get your students to consider the importance of the tiny cells they will see, suggest that they consider how plants obtain the carbon they need for photosynthesis and the oxygen they need for cellular respiration. For example: “We as humans take in and exhale air through our mouths; how do plants take in and release air?” Tell your students that they will prepare a slide for viewing the structures that the plant uses for gas exchange, named from the Greek word for “mouth”. These “mouths” are also the sites where water vapor evaporates from the plant, completing the system of water transport from root through to air.

Objectives
Upon completion of this lab, students should be able to
1. Correctly locate and identify stomata on a leaf.
2. Explain the role of stomata in the daily functioning of a plant.
3. Evaluate the effect of daily light cycles on stomata opening/closing.

Materials
- Razor blades
- Zebrina (Tradescantia zebrina, a common houseplant which should be available at local nurseries or florists).
- A variety of other plants adapted to different moisture/light levels, such as succulents and tropical houseplants. You may be able to use weeds or landscape plants in your schoolyard.
- Glass slides
- Slide coverslips
- Clear nail polish
- Clear Scotch tape
- Compound microscopes
- Permanent markers

**Associated California State Biology Standards**

1a. Students know cells are enclosed within semipermeable membranes that regulate their interaction with their surroundings.

1f. Students know usable energy is captured from sunlight by chloroplasts and is stored through the synthesis of sugar from carbon dioxide.

**Procedure**

**Part I: Viewing zebrina stomata**

1. Give each student group a leaf from a zebrina plant.
2. Have students carefully use a razor blade to slice a small square of the leaf, tiny enough to be placed on a microscope slide. **You should perform this step for the students if there are safety concerns in your classroom with students’ use of sharp objects.**
3. Students should place the leaf section on a slide. It may help to use a dropper to apply some water to the section to prevent it from drying. Next, they should place a coverslip over the sample.
4. Have the students view the sample under a compound scope, first at the lowest magnification. Have students look for and focus on the green guard cells. In zebrina, the guard cells are surrounded by other epidermal cells that are purple, so the stomatal areas stand out quite clearly. The space between the guard cells is the stoma.
5. Your students may notice that the “mouths” appear to be closed. Ask the students why stomata from the zebrina leaf sections tend to be closed when viewed under the scope. They should begin to make connections between the plant stress and the action of guard cells to close stomata.
6. Students should draw and label the cells and features in their viewing field.
7. Repeat steps 5 and 6 with higher magnification. At some magnification levels, students may be able to see chloroplasts as small green dots in the guard cells.

**Part II: Making an imprint slide of stomata**

8. For this part of the assignment, students will use Table #1 to make comparisons of stomata among plants of different species. You may wish to break the students into groups; each student being responsible for the information for one plant, with the group then compiling and sharing results to complete the table and make conclusions together.
9. Have student groups obtain leaves from a variety of available plants. It may help to leave the leaf on the plant for the experiment (so the stomata stay open—they often close when stressed). They should record information about the source plant on Table #1.
10. Each student should carefully apply clear nail polish to a section on the underside of the leaf and then let it dry.
11. While the polish is drying, ask the students to consider the reasons why they are going to be making an imprint of only the *underside* of the leaf when looking for stomata. Based on class discussion, they should understand that one role of the stomata is to regulate transpiration, and stomata on the top of the leaf would have very high exposure to the evaporating influence of the sun. As an extension, you may wish to have students compare and explain impressions taken from the upper surface of the leaves to impressions from the lower.
12. When the polish is dry, have students place clear tape to the area and peel it off. Make sure that each group of students has a sample for each of the specimens they’ve described in Table #1.

13. Each student in the group should now apply the tape from their peel to a microscope slide, label the slide with the name of the plant the sample was taken from, and then view the tape and impression under increasing magnifications.

14. Again have students sketch and label what they see under each magnification. Each student should try to view many of the species their team has listed in Table #1.

15. Each student should count all the stomata visible in the field of view under a particular magnification for the slide they have created. Students should record this information as stomatal density in Table #1. Again, groups should share and compile the results with team members.

16. If you wish to have your class convert their numbers into stomata per square millimeter, you will need a micrometer to measure the diameter of the field of view for the microscope your class is using. When you have that number, students should use it to find the radius and then the area of the field of view, which can be calculated with \(\pi r^2\), the area of a circle. Finally, have students divide the number of stomata by the field of view area to get the stomata per square millimeter.

17. Have groups make conclusions about stomatal density in different plants under different conditions. Based on the observations from this lab, students should form hypotheses about the stomatal densities on plants adapted to a given environment. Some leading questions you may wish to use to guide student investigation:
   - Compare the stomatal density among different species of plants. Do they differ? Why or why not?
   - On which plant(s) are stomatal densities highest? Why?

**Evaluation**

The following questions are listed under the Analysis section of the student handout and maybe used as part of a report, class discussion or assessment.

1. Compare the impressions from the under and upper surface of the leaves. Explain your findings.

2. Compare the stomatal density among different species of plants. Do they differ? Why or why not?

3. Compare the stomatal density between two plants of the same species grown in different conditions. Do they differ? Why or why not?

4. On which plant(s) and where on each plant are stomatal densities highest?

5. Why do stomata from the Zebrina leaf sections tend to be closed when viewed under the scope?

6. When might stomata be found more on the upper surface of a leaf?

**Extension Activities**

1. Students can repeat Part II as a research project by using plants of the same species grown under different conditions. You can use this as an opportunity to conduct an individual or class research project. The students should be able to ask a question and form a hypothesis about density of stomata on the same species in different conditions; choose control and experimental groups while minimizing variables; conduct Part III again as their experimental
procedure; then make conclusions and ask new questions about stomatal density. The experiment can be written up as a scientific paper and even presented in a poster or slide show.

Test Preparation:
1. Which of the following, circle all that apply, occur during the light-dependent reactions?
   (A) Oxygen is released
   (B) Carbon gets reduced
   (C) Oxidative phosphorylation
   (D) ATP is produced
   (E) Electrons flow through an electron transport chain
   (F) Oxidation of NADPH
   (G) Reduction of NADP

2. Which of the following, circle all that apply, occur during the light-independent reactions?
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   (B) Carbon gets reduced
   (C) Oxidative phosphorylation
   (D) ATP is produced
   (E) Electrons flow through an electron transport chain
   (F) Oxidation of NADPH
   (G) Reduction of NADP
Student Sheet: Impressions of a Stoma

Name: __________________________

**Procedure**

**Part I: Viewing Zebrina stomata**

1. Obtain a leaf from a Zebrina plant.
2. Carefully use a razor blade to slice a small square of the leaf, tiny enough to be placed on a microscope slide.
3. Place the leaf section on a slide or Petri dish and view under a dissection scope. It may help to use a dropper to apply some water to the section to prevent it from drying.
4. Focus on the green guard cells surrounding the stomata. The surrounding epidermal cells are purple, so the stomatal areas stand out quite clearly.
5. On a separate sheet of paper, draw and label the cells and features in the viewing field. Label your sketch with the level of magnification.
6. Repeat steps 4 and 5 with higher magnification. At some magnification levels, you may be able to see chloroplasts as small green dots in the guard cells.

**Part II: Making an imprint slide of the stomata**

7. Obtain leaves from a variety of available plants. It may help to leave the leaf on the plant for the experiment. On the table below, record the appropriate information about your source plants.
8. Carefully apply clear nail polish to a section on the underside of the leaf, let dry, then place clear tape to the area and peel off. Repeat this procedure for a few different leaves, from different species and/or from the same species in different conditions. Pick one leaf do a peel on the upper surface of the leaf instead.
9. Apply the tape from each peel to a microscope slide then view the tape and impression under magnification.
10. Make approximations of the stomatal density (in stomata per millimeter) for each peel. To do this:
   a. Count all stomata in a clear field of view on a given magnification. Record your count on a separate sheet of paper.
   b. Your teacher will use a micrometer to measure the diameter of the field of view for the microscope your class is using.
   c. When you have that number, use it to find the radius of your viewing field and then the area of the field of view, which can be calculated with \( \pi r^2 \) (the area of a circle).
   d. Divide the number of stomata you counted by the field of view area you just calculated to get the stomata per square millimeter. Record your findings in the table provided below.
<table>
<thead>
<tr>
<th>Species</th>
<th>Part of leaf from which peel was taken</th>
<th>Leaf description (color, succulence, texture, etc)</th>
<th>Description of plant’s environment (wind, temp, shade, etc.)</th>
<th>Stomata density (stomata/square mm)</th>
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<tbody>
<tr>
<td>Sample A</td>
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**Analysis**

On a separate sheet of paper please complete the following:

1. Compare the impressions from the under and upper surface of the leaves. Explain your findings.
2. Compare the stomatal density among different species of plants. Do they differ? Why or why not?
3. Compare the stomatal density between two plants of the same species grown in different conditions. Do they differ? Why or why not?
4. On which plant(s) and where on each plant are stomatal densities highest?
5. Why do stomata from the Zebrina leaf sections tend to be closed when viewed under the scope?
6. When might stomata be found more on the upper surface of a leaf?