# What’s *Stomata* With You?

***Using Leaf Epidermal Peels to Determine Stomatal Density***

**Authors:**

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**Field tested with:** (e.g., 11th grade Marine Biology students, Watsonville High School, Watsonville, CA (Fall, 2012)

**Module Type:** Lecture, lab activity, and discussion

**Duration:** One 2-h class session

**Key materials:**

* Zebrina plant (*Tradescantia zebrina)* Temperature gun
* Forceps Beaker of water with dropper
* Microscope, microscope slides and cover slips

**Concepts:** guard cells, leaf anatomy, photosynthesis, stoma, stomata, transpiration

**Skills:** Data collection, using the microscope, leaf epidermal peels, testing hypotheses, calculating averages

**Science Education Standards:**

**•Next Generation Science Standards (NGSS)**

Crosscutting Concepts

Structure and Function (**HS-LS1-1**); Stability and Change (**HS-LS1-3**)

**•National:** Science As Inquiry; Science and Technology; Science in Personal and Social Perspectives

**•California:** Biology-Life Sciences: Cell Biology (1f), Ecology (6b,d); Investigation and Experimentation

# Overview:

This project is an opportunity for students to learn:

* The basic structure and function of stomata
* That plants must make trade-offs between photosynthesis and water loss
* About interactions between plants and environmental conditions
* How to observe stomata under a microscope
* How to form hypotheses and argue whether hypotheses are supported by evidence

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# Background for Teachers

**Why this matters:** This module provides an opportunity for students to observe leaf stomata and make predictions about interactions between plants and environmental conditions. After introducing students to the basic form and function of stomata and discussing photosynthesis and transpiration, students will hypothesize about the distribution of stomata on the upper and lower leaf surfaces. Then, they will make wet mount slides of leaf epidermal peels to view stomata under a microscope and calculate average stomatal densities for the top and bottom of the leaf.

**Assumed background:** This activity assumes that students:

* Have some knowledge about the basics of photosynthesis
* Are familiar with using a microscope and preparing a wet mount slide
* Know how to calculate mathematical averages

Before the lab activity, the instructor should clearly explain or demonstrate to students how to make a leaf epidermal peel.

**Special context:** A plant’s chief task is to balance photosynthesis and water loss. Stomata are microscopic **pores** in the **epidermis** of leaves where **carbon dioxide (CO2)** enters and **oxygen (O2)** exits during **photosynthesis**. **Water (H2O)** also exits the leaf through stomata due to evaporation in a process called **transpiration**. For each molecule of CO2 that enters, many more molecules of H2O are lost. Thus, the two major functions of stomata are:

 1) To facilitate **gas exchange** (*i.e.*, CO2 in and O2 out) with the atmosphere

 2) To **control loss of water** molecules from the plant

Specialized cells, called **guard cells,** border each stoma and control the opening and closing of stomata by **gaining and losing water**, which **changes their shape**. When guard cells fill with water and become **turgid**, they bend and open the pore between them. When guard cells lose water and become **flaccid**, they shrink and the pore closes.

Many factors can cause stomata to open or close:

* Light: Light causes stomata to open.
* CO2 concentration: Low CO2 concentrations will cause stomata to open.
* Internal biological clock (Circadian rhythms): Most plants operate on a 24-hour cycle.
* Temperature: Stomata will close if temperatures are very high to avoid excessive water loss.
* Water balance: A wilting or water-stressed plant will close its stomata.

Upon completion of this activity, students should be able to:

* Correctly identify stomata under a microscope
* Explain the role of stomata in plant functioning
* Evaluate the effects of environmental stimuli on stomatal opening/closing

**Scaffolding supplements:**

1. “Stomata Student Worksheet.docx”: This worksheet is designed to accompany the lecture and lab activity. Students fill in the blanks and answer the pre-lab questions during and after the lecture. Then, the worksheet guides students through the lab activity and sets up a discussion at the end.

2. “What’s *Stomata* With You?” Video (<https://www.youtube.com/watch?v=Qh9evk8TNXE>): This video includes a brief review of photosynthesis and transpiration, a general review of stomatal structure and function, and provides some of the answers to the questions on the student worksheet. The video also demonstrates the lab protocol.

# Module Description

## Materials:

* Zebrina plant (*Tradescantia zebrina)*
* Forceps
* Microscope
* Microscope slides
* Slide cover slips
* Beaker of water with dropper
* Temperature gun

## Preparation:

The teacher will need to obtain a Zebrina plant (*Tradescantia zebrina*), a common purple and green houseplant that should be available at local nurseries, or online.

## Timeline:

1. Review/introduce photosynthesis, transpiration, and stomatal structure and function – 20 min.
2. Describe/demonstrate how to make a wet mount slide of a leaf epidermal peel – 5 min.
3. Break into groups of 2-4 students and set up (students retrieve microscopes and lab materials) – 5 min.
4. Students create wet mount microscope slides of leaf epidermal peels and collect data – 60 min.
5. Clean up (students return microscopes and lab materials) 5 min.
6. Students analyze results – 25 min.
7. Review/discussion of findings – 10 min.

## Starting Point For Inquiry:

To get students motivated, begin by asking the class what functions/services plants provide for our planet and listing their answers on the board. Prompt students to ponder *how* plants take in and release air. It may be useful to use humans as an analogy, *e.g.*, “We breathe through our noses and mouths, but how do plants breathe? Do plants have mouths?” The singular form of stomata is stoma, which means “mouth” in Greek.

Stomata are microscopic **pores** in the **epidermis** of leaves where **carbon dioxide (CO2)** enters and **oxygen (O2)** exits during **photosynthesis**. **Water (H2O)** also exits the leaf through stomata due to evaporation in a process called **transpiration**. A plant’s chief task is to balance photosynthesis with water loss. For each molecule of CO2 that enters the leaf, many more molecules of H2O are lost through transpiration. For example, a large redwood tree transpires up to 200-500 gallons of water per day! Thus, the two major functions of stomata are:

 1) To facilitate **gas exchange** (*i.e.*, CO2 in and O2 out) with the atmosphere

 2) To **control loss of water** molecules from the plant

Specialized cells, called **guard cells**, border each stoma and control the opening and closing of stomata by **gaining and losing water**, which **changes their shape**. When guard cells fill with water and become **turgid**, they bend and open the pore between them. When guard cells lose water and become **flaccid**, they shrink and the pore closes.

Many factors act as stimuli that cause stomata to open or close:

* Light: Light causes stomata to open.
* CO2 concentration: Low CO2 concentrations will cause stomata to open.
* Internal biological clock (Circadian rhythms): Most plants operate on a 24-hour cycle.
* Temperature: Stomata will tend to close if temperatures are very high to avoid excessive water loss.
* Water balance: A wilting or water-stressed plant will close its stomata to avoid further water loss.

Stomata are typically found in greater densities on the **lower epidermis** than **upper epidermis** because the underside of leaves is less exposed to sun and wind, both of which increase transpiration.

In this activity, students will learn how to make wet mount slides of leaf epidermal peels and then identify and count stomata on the leaf peels under a microscope. They will develop hypotheses based on what they learn about a plant’s trade-offs between photosynthesis and water loss. The Zebrina plant is ideal for viewing stomata under a microscope, as the underside of its leaves have purple epidermal cells and green guard cells that are easily visible under the microscope.

## Detailed Procedure:

Review Photosynthesis and Transpiration, Introduce Stomata

Distribute the student worksheet at the beginning of class. Review the processes of photosynthesis and transpiration with students and introduce stomata through the associated video (<https://www.youtube.com/watch?v=Qh9evk8TNXE>). The video will demonstrate the epidermal peel/wet mount slide technique for the lab. Following the introductory activities, provide a few additional minutes as necessary for students to complete the pre-lab questions on their worksheets.

Introduce Lab Activity

Introduce the lab activity to the students. You may wish to discuss the process of formulating a hypothesis about the activity if the class needs help brainstorming.

Students Make Wet Mount Slides of Leaf Epidermal Peels and Collect Data

Divide the class into groups of 2-4 individuals. Send one student from each group to retrieve the microscope (remind students of the proper way to carry a microscope) and one to retrieve the other materials. Students will begin by measuring the temperature of the upper and lower leaf surfaces with a temperature gun and recording their findings on the worksheet. Students will then create their wet mount slides. Once students have placed their leaf peels under the microscope, they will count the number of stomata in three fields of view per each side of the leaf and record their data on their worksheets.

Students Analyze Results

Once students have collected their data, they will calculate average stomatal density for each side of the leaf and compare these numbers to conclude whether their hypothesis is supported or rejected. Have them speculate about why they found the results that they found (the expected pattern is that stomatal density will be greater on the lower epidermis than the upper epidermis of the leaf).

## Assessment Methods:

Upon completion of this activity, students should be able to:

* Correctly identify stomata under a microscope
* Explain the role of stomata in plant functioning
* Evaluate the effects of environmental stimuli on stomatal opening/closing

Students who master the content will be able to articulate their hypothesis and whether their data support or refute their hypothesis. They will also be able to speculate about why they found the patterns they found (most likely that there are more stomata on the bottom of the leaf).

## Possible pitfalls:

Stomata will likely close under harsh microscope lighting, so it may be difficult to view an open stoma unless students are very rapid with creating their slides and finding the stomata.

## Glossary:

**Guard cell** – each of a pair of cells that surround the stoma, becoming larger or smaller depending on their internal pressure.

**Photosynthesis** – the process by which plants use energy from sunlight to synthesize sugars from carbon dioxide and water, releasing oxygen as a by-product.

**Stoma** – microscopic pore on the leaf epidermis (plural: stomata).

**Transpiration** – process by which water that is absorbed by plant roots is lost to evaporation through stomata

# Science Education Standards Addressed

***Next Generation Science Standards (NGSS)***

Disciplinary Core Ideas

**LS1.A**: Structure and Function

 Systems of specialized cells within organisms help them perform the essential functions of life. (**HS-LS1-1**)

 Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level. (**HS-LS1-2**)

 Feedback mechanisms maintain a living system’s internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system. (**HS-LS1-3**)

**LS1.C**: Organization for Matter and Energy Flow in Organisms

 The process of photosynthesis converts light energy to stored chemical energy by converting carbon dioxide plus water into sugars plus released oxygen. (**HS-LS1-5**)

Crosscutting Concepts

*Structure and Function*

 Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem. (**HS-LS1-1**)

*Stability and Change*

 Feedback (negative or positive) can stabilize or destabilize a system. (**HS-LS1-3**)

Practices

1. Asking questions (for science) and defining problems (for engineering)

3. Planning and carrying out investigations

4. Analyzing and interpreting data

5. Using mathematics and computational thinking

6. Constructing explanations (for science) and designing solutions (for engineering)

7. Engaging in argument from evidence

8. Obtaining, evaluating, and communicating information

**National Science Standards (NSES)**

A. Science As Inquiry (p.175-176)

E. Science and Technology (p.192-193)

F. Science in Personal and Social Perspectives (p.198-199)

**California Public Schools Standards (SCSCPS)**

Biology/Life Sciences, Cell Biology: 1. The fundamental life processes of plants and animals depend on a variety of chemical reactions that occur in specialized areas of the organism’s cells.

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

Biology/Life Sciences, Ecology: 6. Stability in an ecosystem is a balance between competing effects.

 **b.** Students know how to analyze changes in an ecosystem resulting from changes in climate, human activity, introduction of nonnative species, or changes in population size (p. 54).

 **d.** Students know how water, carbon, and nitrogen cycle between abiotic resources and organic matter in the ecosystem and how oxygen cycles through photosynthesis and respiration (p. 54).

Investigation and Experimentation, 1. Scientific progress is made by asking meaningful questions and conducting careful investigations. As a basis for understanding this concept and addressing content in the other four strands, students should develop their own questions and perform investigations. Students will:

**a**. Select and use appropriate tools and technology (such as computer-linked probes, spreadsheets, and graphing calculators) to perform tests, collect data, analyze relationships, and display data.

**c**. Identify possible reasons for inconsistent results, such as sources of error or uncontrolled conditions.

NSES (<http://www.nap.edu/catalog/4962.html>)

SCSCPS (<http://www.cde.ca.gov/be/st/ss/documents/sciencestnd.pdf>);

# Guide to supplemental materials

**Worksheets**

“Stomata Student Worksheet.docx”: This worksheet is designed to accompany the lecture and lab activity. Students fill in the blanks and answer the pre-lab questions during and after the lecture. Then, the worksheet guides students through the lab activity step by step, provides space to record data, and sets up a discussion at the end.

**Videos**

“What’s *Stomata* With You?” Video (<https://www.youtube.com/watch?v=Qh9evk8TNXE>): This video includes a brief review of photosynthesis and transpiration, a general review of stomatal structure and function, and provides some of the answers to the questions on the student worksheet. The video also demonstrates the lab protocol.