Developmental and Phenotypic Plasticity in Leaves

Objectives:
1. Learn some ways to quantify morphology
2. Collect and analyze data
3. Learn about phenotypic plasticity

Introduction:
The traits of an organism are determined jointly by its genes and the environmental conditions it experiences. The primacy of “nature” (genetics) or “nurture” (environment) has long been argued in biology, and has had philosophical implications for how humans think about their place in the world. Today we will explore these ideas by sampling organisms in (semi-)natural habitats.

We could study “nature” vs. “nurture” by comparing different individual organisms, but we may have difficulties because both the genes of the organisms and the environments they experience will likely differ…. it will be difficult to assign any differences between the individuals to either cause.

One way to isolate the environmental effect would be to compare things that have the same exact DNA, for instance by studying many leaves from the same tree. Genetically the leaves are the same - all are part of a plant that arose from a single fertilized egg. Although all somatic cells in an organism have the same DNA, cells may develop different final phenotypes in response to variation in environmental conditions. Thus, any differences we see between leaves would be due to environmental causes.

We will look at leaves in two different micro-environments: sun and shade.

Broad Questions:
Does the environment affect the phenotype of a leaf?
Specifically, does a difference in light availability affect the phenotype of a leaf?
What features of a leaf might respond to light availability (think about the function of a leaf)?

Methods:

Although many traits of a leaf might respond to environmental conditions, today we will focus on morphology, or shape.

Step 1: Collect the leaves, label, and press for quantification next week.
Choose a tree with a full crown that can be reached from the ground. Collect several sun leaves from the outside edge of the canopy, and several shade leaves from darkest part of the interior.

Avoid leaves that have been partially eaten by insects. Label the leaves "Sun" or "Shade". Then, put the leaves into a plant press to be dried overnight.

Step 2: Quantify leaf morphology. As you calculate the measurements below, record the data in Table 1.

We can quantify leaf morphology in several ways:

1. Area -- trace leaf onto graph paper. Count squares, and multiply number of squares by their area, to find total area of leaf.

2. Sinus area -- One way to quantify shape would be to measure how much leaf area is lost to sinuses, or the inward-curving leaf edges.

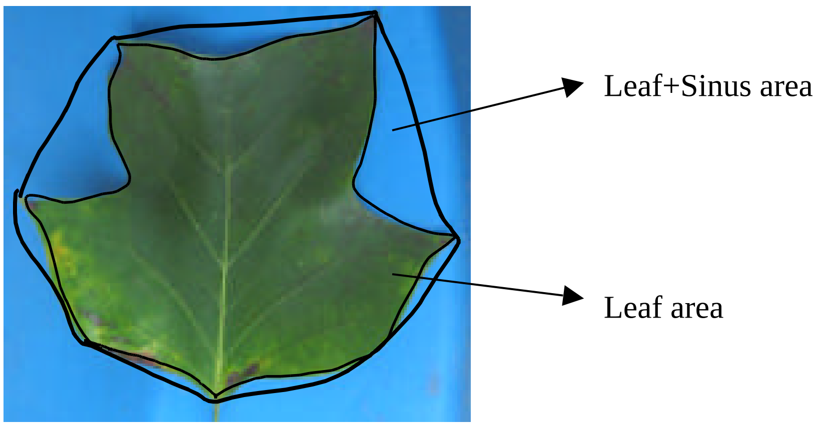

Figure 1. A yellow poplar (*Liriodendron tulipifera***) leaf. The thicker hand-drawn line connects the outermost leaf edges and is used to quantify the relative depth of the leaf's sinuses.**

Find the "leaf+sinus" area by connecting the leaf lobes with a straight line (see Figure 1) and estimating the area of that entire enclosed region. Divide the "leaf+sinus" area by the leaf area to get the "shape ratio." A leaf with deep sinuses and narrow lobes will have a large "shape ratio".

3. Leaf thickness -- The leaf weight divided by the leaf area will provide an index of leaf thickness. The thicker the leaf, the higher the ratio will be.
Weigh each leaf to the nearest 0.1 g and divide by leaf area.

Hypotheses:
From the above broad questions, and knowing what data we can collect, formulate three hypotheses about the effect of micro-environment on leaves. Think again about the functions of leaves, and what conditions are like where “sun” and “shade” leaves will be collected.

1.)

2.)

3.)

Figure 2. Divergent leaf morphology among common Virginia tree species. Why do leaf shapes differ so much between species? Why haven’t the tree species evolved towards one, “ideal”, shape?

Sugar maple (*Acer saccharum*) **Flowering Dogwood (*Cornus florida*)** **Pignut Hickory (*Carya glabra***

Results:

Table 1. One datasheet is completed by each group, then entered into a computer program.

Leaf Number	Leaf type (sun/shade)	Leaf area (cm^2)	Leaf+sinus area (cm^2)	Shape ratio	Leaf weight (g)	Thickness (g/cm^2)

Tree species:__________________________________

Data Analysis:
Enter the data into a computer program, creating separate columns for the type of leaf, the shape ratio, and specific leaf weight. Calculate the mean and standard error for each dependent variable. Compare the means to see if there are any differences and whether those differences are significant.

Conclusions:
Did the data you collected support or refute your hypotheses?

What differences were observable between the leaves from different micro-environments? What might explain these differences?

What are the relationships between acquiring sunlight, reducing water loss, and the size/morphology of a leaf?

Leaves from the same tree can differ in shape, but leaves from different tree species surely differ more (see Figure 2). Considering what you learned about leaf shape and light availability, can you speculate about which tree species (or general leaf shapes) do better in shady environments?

In this lab we studied the effect of environmental variation on the traits of organisms, but genes influence the traits of organisms as well. How could we examine just the effect of genes on the phenotypes of organisms, separate from the effects of environmental conditions?

Compare results with classmates who sampled a different tree species. What similarities or differences exist between the species in their response to light availability?

Adapted from: Westmoreland, D. 1995. Developmental plasticity in oak leaves. Pages 115-119, *in* Tested studies for laboratory teaching. Volume 16. (C. A. Goldman, Editor). Proceedings of the 16th Workshop/Conference of the Association for Biology Laboratory Education (<u>ABLE</u>), 273 pages.

Part II. Leaf Anatomy

With a microscope, examine a prepared slide of a cross-section of a leaf. The prepared slide may be labeled: "Typical dicot leaf" or "privet leaf" or "*Ligustrum* leaf, c.s."

OBSERVE the cross-section on MEDIUM (100x) or HIGH (400x) power.

The cross-section of the leaf is several cell layers thick. Each layer of cells has a different function and a different morphology ('form fits function'), though each cell of the leaf has the same DNA and genes as every other cell in the leaf. Just as the micro-environments of sun and shade have an effect on leaf development, so too does the location of cells in the embryonic leaf bud have an effect on the development of individual cells.

In the cross-section, the uppermost and bottom most layers of cells are the **upper epidermis** and **lower epidermis,** respectively. Each of these epidermal layers is just one cell thick. These cells have relatively thick walls and they secrete a layer of wax called the **cuticle** on the surface of the leaf. What do you think would be a function of the cuticle?

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Observe the cells which make up the lower epidermis; you may (or may not) see gaps in this array of cells. These gaps are called **stomata**; each one (a stoma) is surrounded by two **guard cells**. The guard cells are able to open and close the stoma, thus regulating the entrance and exit of air, and thus of oxygen , carbon dioxide, and water. For example, the stomata may close if the leaf becomes too hot or dry, in order to stop water loss from the leaf. Air which enters the leaf through the stomata first encounters a layer of cells called the **spongy parenchyma**. The spongy parenchyma, like a sponge, has relatively large air spaces amongst the cells. These spaces allow for the circulation of air throughout the leaf.

Above the spongy parenchyma lies a layer of cells that are rectangular and tightly packed. This layer is called the **palisade parenchyma**. The cells of the palisade parenchyma contain chloroplasts where photosynthesis takes place. Is it obvious why the chloroplasts are found in the upper side of the leaf ?

This leaf is from a shrub (a privet or a lilac) that is a common ornamental plant grown in yards in the eastern United States. These plants are adapted to a moderately moist, or 'mesic,' environment. Aquatic environments are those that have much more water. Arid or 'xeric' environments have much less water. (xero = dry; Xerox brand copiers were so named because they produced dry copies; earlier copy methods produced copies that were wet when they first came out of the machine.)

Knowing what you do about the functions of the various layers of a leaf, how do you think layers of the leaf anatomy of plants from xeric or aquatic environments might compare to this leaf adapted to a mesic environment ?