Leaves are the main appendages of the stem, and in most vascular plants, the principal structure for photosynthesis. Although leaves vary tremendously in form and internal structure, most consist of a petiole and a blade. Some of the variation in leaf structure is related to habitat. Aquatic leaves and leaves of dry habitats have special modifications to permit survival in those different habitats. Leaf shapes, margins, tips, and venation patterns are characteristics used to identify different species of flowering plants.

A. Dicot Leaf Structure

Examine a prepared slide of a lilac, *Syringa*, or similar leaf. Note the large midvein. As you scan your section locate the many branching veins, some of which will be in longitudinal section while others are in cross section.

Observe a portion of the blade to one side of the mid vein. Identify the
- **Upper epidermis**, which has a relatively thin but discernible **cuticle**
- **Palisade mesophyll**
- The **veins**, with their bundle sheaths of sclerenchyma
- **The spongy mesophyll**
- **Lower epidermis**, which also has a cuticle.

The palisade and spongy mesophyll are composed of parenchyma cells, which contain many chloroplasts for photosynthesis. Note the presence of intercellular **air spaces** among the spongy mesophyll cells and the relative distribution of **stomata** and guard cells in the lower epidermis. Most stomata open into an air space within the spongy mesophyll. The mushroom-shaped structures of the epidermis are **trichomes**, or **epidermal hairs**.

Dicot Leaf Cross Section
B. A Monocot Leaf

Observe a corn (Zea mays) leaf section. Note the distribution of veins. Monocot leaves generally have parallel veins rather than the branching network of veins common to dicot leaves. Note too that the corn leaf has a uniform mesophyll region rather than distinctive palisade and mesophyll areas.

In the corn leaf the veins are surrounded by a sheath composed of large parenchyma cells. These cells are involved with C-4 photosynthesis. The larger vascular bundles contain extensions of sclerenchyma which connect to the epidermis for support. Identify the xylem and the phloem regions of the veins.

Where are the stomata and guard cells located in the corn leaf?

Monocot Leaf Cross Section

C. Environmental Adaptations of Leaves

Xeromorphic Leaves

Plants which live in arid environments are subject to drought, and often, intense sunlight. Such plants are called xerophytes. These plants are subjected to intense evaporation of water, a resource which is often in short supply. Many such plants have a number of modifications which minimize water loss through transpiration, the evaporation of water from the plant surfaces. Some plants drop their leaves during periods of drought; cactus plants photosynthesize with modified stem tissue, and lack leaves entirely. Those plants which do produce and retain leaves often have special features which we associate with the xeromorphic leaf. Nerium oleander is a good example of a plant with xeromorphic leaves.

Examine the prepared slide of Nerium oleander leaf, xs. Note the very thick cuticle as you focus on the upper epidermis. The epidermis is several layers thick, too. The palisade parenchyma, beneath the epidermis layers, is in two layers. The spongy mesophyll is loosely packed and quite wide. The unusual structures seen in the spongy mesophyll are a type of crystal, called druses.

Veins may have bundle sheath extensions in additional to the bundle sheath layer. Look for the mid vein. It has phloem on both sides of the xylem, which is unusual.

As you turn to the lower epidermis, note that it, like the upper epidermis, has several layers and a thickened cuticle. As you move your slide along the lower epidermis, note the deep invaginations of the epidermis layer into the lower leaf. These invaginations are called stomatal crypts. There are a number of epidermal hairs in the crypts, along with the stomata. All of the stomata are located in the crypts. Why do you think this is?
Nerium oleander leaf, xs.

Hydromorphic Leaves

The leaves of the water lily float on the surface of ponds and lakes, although the water lily is rooted in the lake bottom. Examine a prepared slide of Nymphaea leaf, xs, to observe modifications water lilies have for flotation.

Look first at both epidermis layers. Where do you find stomata? Why? Look for small hairs in the lower epidermis layer. Now refocus on the upper epidermis layer. Can you find the cuticle? It is very thin. Below the epidermis cells the palisade mesophyll consists of three or four overlapping layers of cells, which are fairly loosely packed, allowing for gases to enter from the upper epidermis. Note the huge intracellular spaces in the spongy mesophyll layer. The buoyancy of the water lily comes from these large air spaces. The spongy mesophyll also contains large, branching, thick-walled sclerids for support. There are crystals within the sclerids, too.

Note the reduced size of the veins in Nymphaea, compared to most leaves. The vascular tissue, especially the xylem, is minimal in most hydromorphic leaves. You should find more phloem than xylem in the vascular tissue as you observe the scattered veins.

Nymphaea leaf, xs
Compare the adaptations of the hydromorphic and xeromorphic leaves with the typical mesomorphic dicot leaf, such as *Syringa*.

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<th>Ecological Leaf Type</th>
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D. **Stomata Structure in *Zebrina* leaves**

The epidermal surfaces of plants are covered with a protective cuticle. However, CO$_2$ must enter the leaf for photosynthesis and the O$_2$ produced during photosynthesis must be released from the plant. To solve this dilemma plants have specialized cells in the epidermis, called guard cells, which form **stomata** (pores) in the epidermis. Stomata can be open or closed, depending on the turgor of the guard cells. When stomata are open, gas exchange can occur. Unfortunately, large amounts of water are lost from the plant through the open stomata as well. (For example, as much as 90% of the water absorbed by the roots of a corn plant growing in Kansas may be lost through the stomata of its leaves.) To avoid excessive water loss, the guard cells have a mechanism to open the stomata during photosynthetic periods (i.e., daylight hours) and close the stomata when photosynthesis is not occurring.

You will observe guard cells and stomata in the lower epidermis of leaves of *Zebrina*. Since the regular epidermal cells of *Zebrina* contain anthocyanin (purple) pigments, the guard cells, which contain chloroplasts, are particularly conspicuous.

Leaf epidermis showing stomata and guard cells
Zebrina Epidermal Peel
- Cut a portion of a leaf from a Zebrina plant.
- With your fingernail or a sharp razor blade, peel a portion of the lower epidermis from the leaf, starting at the cut edge. Note: The lower epidermis is purple-pigmented. The upper epidermis is silver and green striped.
- Make a wet mount of the epidermal peel. Try to have the peel flat on the microscope slide; wrinkled portions have too many layers of cells and trap air bubbles.
- Observe your slide with your microscope. After locating guard cells with the lower power magnification, use the 45x objective to observe one of the stomata closely. Can you see the chloroplasts in the guard cells?
- What is the shape of the guard cells? Note the thickness of the inner walls of the guard cells. Are any of the stomata open?

Recall from your observation of the prepared slide of a leaf that a stoma opens into an air space of the spongy mesophyll. Of what advantage is this arrangement to the plant for photosynthesis?

E. C₄ Photosynthesis and Leaf Structure
Most higher plants use a photosynthetic pathway known as the C₃ photosynthetic pathway, where the Calvin cycle of the "dark reactions" begins with CO₂ (carbon dioxide) combining with ribulose biphosphate (RuBP) to form the 3-carbon compounds, PGA (phosphoglyceric acid) and PGAL, (phosphoglyceraldehyde). Both the light reactions of photosynthesis and the Calvin cycle occur within the same chloroplasts in all of the mesophyll cells. The Ligustrum or Syringa dicot leaf cross section you observed shows the typical leaf structure of a C₃ plant.

Some plants, known as C₄ plants, use a different pathway for carbon fixation, in which CO₂ first combines with PEP (phosphoenolpyruvate) to produce 4-carbon acids, such as oxaloacetic acid or malic acid. The reaction serves as a CO₂ trap, since the CO₂ taken into the leaf can now be stored in the form of the 4—carbon acids. This is especially beneficial for plants in hot dry areas, which lose lots of water through their open stomata when CO₂ is absorbed. Many C₄ plants can "stockpile" CO₂ this way, freeing CO₂ from the acids for the Calvin cycle as needed.

Some monocot C₄ plants also separate the reactions of photosynthesis into different chloroplasts within different types of cells, another energy conserving measure. When plants do a lot of photosynthesis, the oxygen produced during the light reactions competes with CO₂ for the ribulose biphosphate (RuBP) enzyme. The light reactions of C₄ plants occur in mesophyll cells which surround the veins' enlarged and modified bundle sheath cells. The Calvin cycle occurs in chloroplasts of the enlarged bundle sheath cells. This separation of reactions keeps oxygen away from the cells performing Calvin cycle steps. C₄ photosynthesis has several benefits for the plant, resulting in a more efficient rate of photosynthesis. It also results in an interesting modification of the typical leaf anatomy.
Observing a C4 leaf

Corn (Zea mays) is a C4 plant. Observe again the prepared slide of a corn leaf to see the differences in C3 and C4 leaf structure.

- Note especially the layer of round cells which surround the veins in the corn leaf. This layer is formed by the bundle sheath cells, which contain the chloroplasts in which the Calvin cycle occurs.

- Note, too, that the mesophyll cells are not separated into well-defined palisade and spongy mesophyll layers, such as you observed in the Ligustrum leaf. In the corn leaf, the mesophyll cells surround the bundle sheath cells. Only the light reactions of photosynthesis occur in the chloroplasts of the mesophyll cells. This C4 leaf structure is known as Kranz anatomy.

- Observe the electron micrographs of the C4 mesophyll and bundle sheath cell chloroplasts shown above. Note the different chloroplast structures in the two cells. Why does the mesophyll cell have chloroplasts containing lots of grana composed of many thylakoid layers? Why are well-developed grana absent in the chloroplasts of the bundle sheath cell?

- Note the many plasmodesmata which connect the two cells. Why would you expect to see so many plasmodesmata between the mesophyll cells and the bundle sheath cells in the C4 plant?