Lab 13
Seed Plants - Gymnosperms

DOMAIN **Eukarya**

**KINGDOM Plante**

- **Phylum: Cycadophyta**
- **Phylum: Ginkgophyta**
- **Phylum: Coniferophyta**
- **Phylum: Gnetophyta**

During the peak of dominance of dinosaurs, the forests and swamps were not scented with the sweet aroma of wildflower blossoms. Instead, the air was likely filled with the copious pollen from the (now extinct) seed ferns and cycads, and perhaps the odor of resins from other cone-bearing species. This landscape was dominated by the gymnosperms which represent a series of evolutionary lines of seed-bearing plants. There are four phyla of gymnosperms with living representatives. Of these, by far the most numerous in terms of species diversity and abundance are the conifers. The gymnosperms today include the tallest and oldest of all trees.

**EXERCISE 1:**

* **Cycadophyta: The Cycads**

Cycads are so abundant in the fossil record of the Mesozoic (65 to 230 million years ago) that this era is sometimes referred to as the "Age of Cycads and Dinosaurs." Cycads are widely distributed in tropical and subtropical regions of the world. When cycads thrived on earth, the continents were united into an enormous super continent named Pangaea (approximately 200 million years ago). As the large plates of earth slowly moved, cycads were widely distributed across the globe. Despite their former dominance of the global flora, today many relict cycad populations are seriously threatened with extinction due to extensive collecting and degraded habitats.

Cycad cones can reach 3 feet in length, the largest of all living cone-bearing plants. The most massive seed cones may weigh up to 95 pounds. The cones of cycads are
composed of numerous overlapping scales called sporophylls. Sporophylls are actually modified leaves, and in male cones they bear numerous sporangia on their lower surface.

Two large seeds are produced at the base of each sporophyll in female cones of most species. In some species of cycads the cluster of seed-bearing "leaves" superficially resembles a large blossom. In fact, some botanists have suggested that ancient cycad like plants may have given rise to flowering plants. Other botanists believe the connecting link may be extinct cone-bearing species in the Division Gnetophyta, while others say these are merely examples of parallel evolution.

*Examine the prepared slide of the cycad Zamia archegonia with a dissecting microscope. Draw what you see. Label the nucellus, the archegonium, and the integuments. Can you see the micropyle? Should you expect to see it on every slide? Why or why not?*

Cycads have a slow-growing trunk, 3 to 50 feet tall, and a crown of leaves that superficially resembles a palm. Unlike palms, cycad trunks produce rings of xylem surrounding a wide, pithy central core.

*Look at the prepared slides of a Zamia stem cross section and draw what you see. Label the xylem cells emanating from the large central pith. What do you call this kind of stele?*

Cycads are unique among gymnosperms because for associations with nitrogen-fixing cyanobacteria living exterior of root outgrowths at the base of the plant. Like bacteria in the root nodules of legumes, the cyanobacteria supply the cycad with inorganic nitrogen by converting inert atmospheric nitrogen into ammonia. Since the photosynthetic cyanobacteria are not exposed to vital sunlight, the cycad supplies its microscopic symbiotic partner with organic nutrients.

*Examine the roots on a live specimen and look for a pale greenish layer of filamentous cyanobacteria (usually Nostoc or Anabaena).*
Cycads of the genus Cycads have some striking taxonomic affinities with ferns, including large leaves that uncoil like a fern. Cycads also produce remarkable multiciliate swimming sperm. The later characteristic is especially interesting when you consider that each sperm may have 20,000 to 40,000 spirally arranged cilia and may be up to 400 micrometers (1/60 of an inch) long, visible to the naked eye. This is larger than a grain of table salt and roughly equivalent to an entire plant of Wolffia globosa, the world's smallest flowering plant. Because of multiciliate sperm, cycads show a nice connecting link with the more primitive spore-producing ferns, which must depend on free environmental water for the transport of their sperm, and the advanced seed plants in which the sperm are non-flagellated and non-motile. In a sense, the primitive ferns are like amphibians with external fertilization because their motile sperm require environmental water in order to swim to the egg.

EXERCISE 2:

**Ginkgophyta: Maidenhair tree**

Leaf imprints resembling the present-day maidenhair tree have been found abundantly in sedimentary rocks of the Jurassic and Triassic Periods (135-210 million years ago). The branching pattern of the veins of Ginkgo leaves is primitive. Many extinct members of Gingophyta only known from the fossil record show the same pattern.

*What is this pattern called? If you are unsure, look at the leaf veins under the dissecting microscope on the leaves in the acrylic mount.*

The leaves of Ginkgo biloba have long been recognized for their benefit as a geriatric drug in China. A tea made from the leaves has been used for a variety of ailments, including asthma, vascular (circulatory) diseases and urinary problems. The leaf extract contains several valuable compounds, including 24 percent flavonol glycosides and 6 percent terpene lactones. Recent medical research indicates that Ginkgo biloba leaf extract may also be an effective treatment for memory loss and the early stages of Alzheimer's disease in elderly patients. During the past few years Ginkgo has become a popular herbal remedy sold in natural food stores across the United States.

**MALE STRUCTURES**

*Look at the prepared slide of the Ginkgo male strobilus. Draw what you see.*
Does it differ in any dramatic way from the strobili we saw in some seedless vascular plants?

Once the spores are released, the life cycle is quite different. How?

Look at the prepared slide of Ginkgo pollen. Draw what you see. Can you find the four cells of the male gametophyte generation? Label them.

FEMALE STRUCTURES

Female Ginkgo trees are typically not used for landscaping because the seeds are foul-smelling when they fall to the ground. The stench of the seed coats comes from butyric acid, named from its presence in rancid butter. Butyric acid (also known as butanoic acid) is a common by-product of many plants and animals, and is responsible for the distinctive odor of Limburger cheese. It is also produced by anaerobic bacteria in the digestive tract of animals, and may be present in vomit. Nevertheless, the large, nutritious seeds of Ginkgo biloba are a popular food in China and Japan.

Look at the longitudinal section of a Ginkgo archegonium. Draw what you see. Label the integument, microspore, and micropyle.

Now look at the prepared slide of the ovule with embryo. Draw what you see. Label the nutritive tissues that feed the embryo. Which generation do these tissues belong to?
EXERCISE 3.

Coniferophyta: The Conifers

LIFE CYCLE
What the series of life cycle videos on your lap top. Follow along on the gymnosperm life cycle handout from lecture.

MALE REPRODUCTIVE STRUCTURES

Get an acrylic mount of male and female conifer cones to examine under the dissecting microscope.

Examine one of the male cones under a dissecting microscope. Note that the microsporophylls are arranged helically along the axis of the cone. Each microsporophyll bears two microsporangia or pollen sacs in which microspore cells are formed. Each microspore mother cell eventually undergoes meiosis to give rise to four microspores, which develop into the pollen grains, or immature male gametophytes (microgametophytes).

Look at a prepared slide of a longitudinal section of a Pinus male strobilus. Draw what you see. Label a pollen grain.

Look at the prepared slide of Pinus pollen grain. Identify the cells in the pollen grain diagramed below.

FEMALE REPRODUCTIVE STRUCTURES
Now examine an ovulate cone in acrylic. There are two kinds of female cones in the mount.

*Think about the lifecycle of Coniferophyta and provide an explanation for why there are one-year old and two-year old cones on a single tree. Can you find one-year old and two-year old cones in the fresh samples on the center bench? What are female cones doing outside this time of year?*

Take a scale off of a female cone. Each scale is subtended by a sterile bract which is morphologically equivalent to a leaf. The combination of ovuliferous scale and subtending sterile bract is referred to as the seed-scale complex. Dig into the cones and find a seed. At maturity, two seeds are associated with each scale.

*Study prepared slides containing longitudinal sections of ovulate cones (longitudinal section Pinus female cone). Find a scale that shows a median or near median section of one of its ovules. Draw what you see and label the ovuliferous scale, sterile bract, integument, the micropyle, the “pollen chamber,” and the nucellus (megasporangium).*

If you examine the ovules about 15 months after pollination, you will find that they have developed a great deal. After 15 months, the ovules contain a female gametophyte (megagametophyte), which develops from the functional megaspore and which contains two or more archegonia near the “pollen chamber.” Soon after pollination, the pollen grain germinates and the pollen tube begins to form. However, the sperm do not appear until the female gametophyte is mature. Each male gametophyte gives rise to two sperm, one of which may eventually fertilize an egg. The other sperm has no function and disintegrates.

*What is happening in the megasporangium in the series of illustrations below?*
THE EMBRYO
The fertilized egg, or zygote, develops into an embryo, the young sporophyte. The embryo is surrounded by tissue of the female gametophyte, which serves during germination as source of food for the seed. During embryo development, the integuments develop into the seed coat. A seed, consisting of embryo, seed coat, and stored food, has now been formed.

Get a pine nut from the center bench and gently pry it apart. You should be able to see the mature embryo with cotyledons inside. Draw what you see. Observe the prepared slide of the Pinus embryo. Draw what you see. Label the cotyledon, root cap, and apical meristem of root and shoot. What is the function of the cotyledons?

THE SEEDLINGS
Look at the potted pine seedling on the center bench. The browned leaves are the cotyledons and the new leaves are spiraled around the stem. After some growth, some species present their leaves in bundles called “fascicles.” Find a fascicle on the mature branch on the center bench.

THE LEAVES
Observe the prepared slide of a cross-section of a pine needle. What structures can you identify that confer drought tolerance to these leaves?
**EXERCISE 4:**

*Gnetophyta* – The Gnetophytes

Observe the two living examples from Gnetophyta on display:

1. *Gentum*: tree in the front of the lab
2. *Ephedra*: two smaller plants on the center bench.

*Ephedra* is a genus of drought-resistant shrubs with jointed stems and whorls of minute scale-like leaves in 2's or 3's at each node. One very unusual characteristic of the *Gnetophyta* is that many members possess vessels in their xylem tissue, an angiosperm trait that is not found in most gymnosperms.

*Look at the prepared slides of an Ephedra root cells. Why do xylem and phloem stain different colors?*

*Look at the demo slide that shows a close-up xylem cells in Ephedra. There are two kinds of cells, trachieds and vessel elements. Aside from this phylum, vessel elements are only found in angiosperms. Sketch out the difference between a trachieds and vessel element.*

*Why do you think vessel elements are considered more “advanced?”*

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**EXERCISE 5. – from the previous lab!**

Chemotaxis in ferns

Chemotaxis is a widespread biological phenomenon that occurs in essentially every form of life, from single cells to multicellular organisms. Chemotaxis involves the ability by specific cell types to recognize the presence of a chemical gradient and to respond either positively or negatively to that gradient. Familiar examples of chemotaxis are the movement of bacteria toward or away from a particular chemical (e.g., a food source).
and the nearly universal ability for single celled gametes to locate one another in order to accomplish fertilization.

Chemotaxis is important for fertilization of lower plants that depend upon swimming sperm to find the egg. In this exercise we will examine 12-day old C Fern™ gametophytes. The gametophytes of this species has two distinct sexual types, small thumb shaped males and larger heart shaped hermaphrodites. While the males contain only antheridia (male sex organs), hermaphrodites contain both antheridia and archegonia (female sex organs). Antheridia are composed of a few outer cells that enclose 16 sperm at maturity. Archegonia consist of a short neck that protrudes from the surface of the gametophyte directly behind the actively growing meristem region located in the notch of the heart. An egg is located at the base of each archegonal neck.

By adding water to a mature culture, it is possible to observe the release of thousands of swimming sperm (mostly from males). The sperm are positively attracted to receptive archegonia. How do sperm know where the archegonia (and eggs) are? Sperm are attracted to chemical substances that are contained in a small drop of liquid that is discharged from the necks of receptive archegonia. This attraction is an example of positive chemotaxis. One sperm eventually succeeds in fertilizing each egg.

Today we will use a simple technique to obtain a suspension of sperm from 12-day-old C-fern gametophytes. The C-fern is a special rapid cycling fern that is used in biotechnology. We will test five different test substances to determine whether their chemical structure resembles the natural chemoattractant produced by archegonia.

**Procedures:**

1. Using the razor blade, carefully sharpen 6 of the wooden toothpicks provided so that one of the ends has a very fine point. Do not touch the sharpened end with your fingers. Carefully lay the toothpicks down on a clean surface.

2. Use the pen to make from 1 to 5 small dots along the side of the toothpicks near the unsharpened ends, so that each toothpick has a unique marking (i.e., 1, 2, 3, 4, 5) and one is kept unmarked. Take the toothpick with one dot and dip the sharpened end of it into the vial containing test solution # 1. Place it, sharp end up, in the toothpick holder (foam block). Repeat with the remaining 4 test solutions, leaving the final unmarked toothpick dry.

Which of the toothpicks will serve as a control? Why is the one you chose appropriate to be used as the control?

3. Obtain a depression slide and place one drop of the Sperm Release Buffer (SRB) in the central depression.
4. Unscrew the light on your dissecting microscope so that you can shine the light on the gametophytes from the side. You may want to have two dissecting microscopes set up side by side – one for picking out male gametophytes and one for observing sperm.

5. Now obtain a petri dish containing mature C Fern™ gametophytes. Open the dish and observe it under a stereomicroscope using transmitted (bottom) illumination. Note the two types of gametophytes that are present smaller, thumb shaped males that have many bumps on them, and larger, heart shaped hermaphrodites (See diagram on the chalk board). Take the dissecting needle and carefully pick up males only and transfer them to the drop in the concavity slide. Be careful not to damage or wound the gametophytes during transfer. If one is wounded, discard it. Transfer a total of 7-10 male gametophytes to the drop. It is not necessary to submerge them completely, only to place them within the drop of buffer.

6. Place the slide on either the lid or bottom of an empty petri dish, edges up, and observe it under the dissecting under low magnification (12x or higher). The use of the petri dish will keep the slide and sperm suspension cooler and provide a clearer view. In a few minutes, usually less than five, sperm should begin to be released from antheridia. Adjust the illumination on the stereomicroscope to provide the best contrast for viewing the sperm.

7. After a large number of sperm are released (about 3-5 min), begin testing the response to the test solutions as follows:

   a. Using 12-20x magnification, carefully focus on the TOP surface of the drop of sperm suspension, in an area free of male gametophytes.

   b. Take a test toothpick and, while looking through the microscope, gently and briefly touch the sharpened end of the toothpick to the surface of the drop. Do not stick the toothpick fully into the drop only touch the surface briefly.
c. Observe what happens, if anything, during the next minute, and record your observations in Table 1.

d. After you have made your observations, repeat the procedure with the remaining toothpicks. If necessary, use the dissecting needle to stir the sperm suspension and redistribute sperm after each of the tests. This is typically needed only after a strong chemotactic response is observed.

e. For comparison of the relative strength of the chemo attractants, you can test more than one substance side by side. Briefly touch the pair of tips to the drop of suspension as before. Observe the two points of contact and compare the responses.

Results:
You may have observed a very weak response by the sperm to the control toothpick. How could this be explained, and does it invalidate the experiment?

Which of the test chemicals elicited the strongest response?

The chemical structure of each substance is shown on the on center bench in the lab. Can you relate the biological reactivity of the sperm to the chemical structure of the test substances?
Table 1. Chemotactic response of sperm to test substances.

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<th>Test Substance</th>
<th>Swarming response</th>
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