Pinus Life Cycle

You have observed some gymnosperm diversity last week; today you will observe the details of the life cycle of Pinus, one of the most common conifers (Coniferophyta). This life history is similar to that of all the gymnosperms, although the details might vary. For example, while the life cycle of some pines is a two-year process, in other conifers and most other gymnosperms it is a one-season process. All the gymnosperms are heterosporous with the megasporophylls and the microsporophylls grouped together in separate cones, but most gymnosperms are dioecious with only one type of cone (male or female but never both) on a particular individual. Pine, however, is monoecious; the microsporangiate cones occur near the ends of branches and the megasporangiate cones are found at the apex. Other conifers include: spruce, fir, hemlock, redwood, etc., as well as the southern hemisphere podocarp and Araucarias. It includes the largest and the oldest living individual organisms known.

Observe branches of a few representative genera available in the laboratory. On your walks around campus, you will see others and might make some observations as you go along. Note that some genera, such as Juniperis, have two types of foliage leaves: longer needle-like leaves and much smaller scale leaves. Since the needle leaves are characteristic of young plants, they are referred to as JUVENILE leaves. Heterophylly such as this occurs in a number of angiosperms as well.

The prefix Hetero- means ________________; the suffix -phylly means ____________.

Observe a Pinus resinosa leaf cross section. This species of pine has lots of resin (no, duh!).

The mesophyll [has!!!!!lacks!] resin ducts in the mesophyll.

The epidermis [has!!!!!lacks!] cutin.

The guard cells [protrude!from!!!are!even!with!!!!!are!sunken!below!] the epidermis.

How would you describe the shape of mesophyll cells? ____________________________

Inside the mesophyll there is an endodermis surrounding the vascular tissue that has awesome Casparian strips; if you have not seen a strip well, this is your best chance this semester!

What color are the Casparian strips? ____________________________

Between the endodermis and the vascular bundles there is a layer called transfusion tissue.

Is transfusion tissue [parenchyma!!!!collenchyma!!!!!sclerenchyma!].

Observe the xylem and phloem. The arrangement is [radial!!!colateral!!!bicolateral].

Considering the position of xylem and phloem, the flat epidermis is the [upper!!!!lower] epidermis.

Observe a cross section of a young pine stem. This stem is surrounded by pairs of leaves (also caught in the cross-section).

The leaves face each other with [upper!!!!lower] epidermi in contact.

The leaf pair closest to the stem (at 1 o’clock) shows more leaves than just two.

This makes the whole collection of leaves a _________ shoot called a ____________________

The stem has a [protostele!!!!!siphonostele!!!!!eustele!].

The xylem/phloem arrangement is [radial!!!!!colateral!!!!!bicolateral].

The xylem maturation is [endarch!!!!mesarch!!!!exarch].

A pith [is!!!!!is!not!] present.

The cortex shows resin ducts and cells with lots of chemistry (take up the red dye).

The outermost layer of phloem is made of what kind of cells? ____________________________
Observe a cross section of an older pine stem.
The epidermal functions are taken over by a ___________________.
The cortex [has!!!lacks] resin ducts. A secondary plant body is [present!!!absent].
The color of the secondary phloem is _____________________________.
The color of the tracheids in secondary xylem is _____________________.
The secondary xylem shows (#)________ annual growth rings.
The blue strips radiating out through the secondary xylem are called _________________.
Go to high power to look at the blue strips. They are [parenchyma!!!!sclerenchyma!].
What is your evidence for this? ____________________________________
A pith is [present!!!!!absent!].

Observe a cross section of a pine root.
Please do not move the slide on the stage! Just focus!
Is this an absorptive region of the root? [yes!!!!!no!]
What tissue has taken over the epidermal functions? ____________________________
What are the parenchyma cells just outside the xylem/phloem?____________________
Is there an endodermis? [yes!!!!!!no!]
The vascular cylinder is: [diarch!!!!triarch!!!!tetrarch!!!!polyarch!].

Observe a slide showing a longsection of a microsporangiate cone and relate what you see on the slide to what may be available as a whole cone.
What processes occur in the microsporangia? _____________________________.
The loose cells found within the mature microsporangia on this slide are [microspores!!!!pollen].
What feature tells you which one it is? _____________________________.
What is the ploidy level of the microsporophyll? [1N!!!!2N!!!!3N!!!!∞N]!

Observe a slide showing a cross-section of a microsporangiate cone and relate what you see on the slide to the gross structure you have looked at. Three of the microsporophylls are shown attached to the stem, and two of those are shown nicely.
How many microsporangia are there in each microsporophyll? [one!!!!two!!!!more!]
What is the ploidy level of the sterile jacket? [1N!!!!2N!!!!3N!!!!∞N]
While here, you might want to look closely at the loose cells inside the microsporangium. Some of these are maturing and have become pollen grains. Technically what is a pollen grain?
Also interesting here, is that the three attached microsporophylls show their vascular connections. They each have a vascular bundle, but notice how the bundle seems to head toward a space between stem vascular bundles (use the 10x lens). This space caused by the departure of a leaf is called a ___________ ___________.

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Observe a slide which shows mature pollen grains. The cellular composition of pollen grains at the time of shedding from the microsporangium is: two disintegrated prothallial cells, a generative cell, and a tube cell. The two wings are expansions of the outer layer of the pollen-grain wall.

The microspore produced the "wings".

The generative cell has the larger cytoplasm inside the pollen wall.

What is the ploidy level of all cells in the pollen wall? 1N

What is the function of the tube cell? ___________________________

What is the function of the generative cell? _______________________

Observe the fresh specimens of megasporangiate cones of different ages. Dissect further, if necessary, to determine the structure. Note that each "megasporophyll" is not really a leaf but is a double structure: a bract with a flattened structure (called a CONE SCALE) in its axil. It is on these cone scales that the megasporangia are borne.

How many megasporangia are there on each cone scale? one two few many

On which surface do they occur? upper lower

Observe a slide showing a longisection of a megasporangiate cone which is at the pollination stage. Please do not use the stage knobs.

Set the microscope at the 4x (red) lens. The view depicts a megasporangiate cone at the time of pollination (Spring 1). You can see a megasporophyll with a few resin ducts, and a curved bract beneath. This is a compound cone scale. The ovule shown on the sporophyll is in median longitudinal section. You can clearly distinguish the integument and its micropyle. In this specimen the megasporophyll is not connected to the cone axis in the plane of section.

Switch to the 10x (yellow band) lens. What is in the micropyle? ___________________________

When pollen is being shed and carried by the wind in the spring, the young megasporangiate (ovulate) cones open; pollen which sifts between the cone scales is caught in a drop of fluid formed by the disintegration of cells at the tip of the nucellus. As the drop dries, the pollen grains are sucked into the ovule. The transfer of pollen from microsporangium to ovule is called pollination.

Most of what is contained inside the few layers of integument is nucellus. What word on the generalized life cycle is equivalent to the nucellus? ___________________________

What is the ploidy level of the nucellus? 1N

Switch to the 40x (blue band) lens. This is a near-median section of the nucellus inside the integument. Note that there is a dome-shaped central portion, which is the nucellus. If you look carefully in the middle of the nucellus, and focus clearly, you should find one cell that is different from all the others. This cell has a larger and more obvious nucleus with at least three nucleoli (red spots). It is the megasporocyte. Why is the nucleus larger in this cell than those in the cells of the nucellus? ___________________________

What is the ploidy level of the megasporocyte? 1N

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Observe the slide of *Pinus young ovulate cones*.
This slide shows a later stage (Summer 1). The micropyle has closed by growth of the integument. There is a purple area in the nucellus that looks like it is disintegrating.

What is eating it?
Deeper in the nucellus, the megasporocyte has divided meiotically and three of the meiotic products degenerate, the fourth one remains as the functional megaspore. As you focus up and down through this specimen you should be able to determine which cell is the functional megaspore and where the deteriorating meiotic products are.

What is the ploidy level of the megaspore? \[ 1N!!!2N!!!!3N!!!!\times N! \]
What is the ploidy level of the disintegrating cells? \[ 1N!!!2N!!!!3N!!!!\times N! \]

Observe the Pine two-year female strobilus l.s.
The view depicts a megasporangiate cone at the beginning of its second year (Spring 2). The nucellus is further digested. The megaspore has been dividing slowly by mitosis without cytokinesis. This is called a free-nuclear stage. You may ignore the partial collapse of this cell...

it is caused by the very large central ____________________________.
The ovule below this one shows that organelle better...it is light blue!

What is the ploidy level of the meiotic products? \[ 1N!!!2N!!!!3N!!!\times N! \]
What is the ploidy level of each nucleus in this cell? \[ 1N!!!2N!!!!3N!!!\times N! \]

This free-nuclear cell is technically the early stage of the ____________________________.

Observe the longitudinal section of a *Pinus archegonium with an egg*.
This slide shows an ovule in Summer 2. The micropyle is at the top of the view. The digested nucellus is nearly purple here. Contained in the nucellus is now a megagametophyte. The free-nuclear stage has finally divided up to make perhaps 2000 cells. There are two archegonia shown in this slide. The one on the right is cut at near-median. You should be able to make out the sterile jacket and the neck cells above the egg. Compare the size of the nucleus of a nucellus cell and a megagametophyte thallus cell.

Which one is smaller? [nucellus!!!!megagametophyte!]
What is the ploidy level of the megagametophyte nucleus? \[ 1N!!!2N!!!!3N!!\times N! \]
The ventral canal cell has a large nucleus with a prominent red nucleolus, but the egg. Oh the egg! How big is it? [tiny!!!!same!as!the!canal!cell!!!!huge!]
The egg nucleus has how many nucleoli (red)? [none!!!!one!!!!two!!!!three!]
The cytoplasm of the egg has some protein bodies in it. They take up some reddish tinge in the stain.
In the pollen tube, one of the derivatives of the generative cell divides to form two SPERM nuclei unequal in size and not flagellated. The tube then actively elongates until it reaches and forces its way between the neck cells of the archegonium. There its tip ruptures and the sperm are discharged toward the egg. The larger sperm fuses with the egg.
Observe a slide of *Pinus* proembryos with suspensors.

The view in this slide is of an ovule (fourth from left) in mid-summer. The micropyle is at the top of the view. The egg on the left has not been successful and is degenerating. The archegonium on the right was "found" by the pollen tube and its egg has already united with sperm. The zygote has divided into eight cells. The four most near the micropyle have become suspensors and have elongated to push the other four cells deep into the megagametophyte tissue. The cells being pushed are already half-way down the megagametophyte. One of these cells (called a proembryo) will divide by mitosis and become the embryo. At the chalazal end of the ovule, the integument is growing a wing.

What is the ploidy level of the....suspenisor?  \[1N+2N+3N+\infty N\]
...embryo?  \[1N+2N+3N+\infty N\]
...wing?  \[1N+2N+3N+\infty N\]
...storage tissue around the embryo?  \[1N+2N+3N+\infty N\]

Observe a longitudinal section of a *Pinus* mature embryo.

Starting at the micropylar end (the bottom of this view), there are some purple remains of nucellus (most was removed in preparation along with integument). Just above this is a layer of dark-green stuff: the compressed and crushed suspensor. The mature embryo above the suspensor consists of a root cap and apex, hypocotyl, and a small shoot apex surrounded by approximately eight cotyledons.

How many cotyledons are shown here at least in fragments? ___________________________________________

Center an area where the embryo and surrounding storage tissue touch. Switch to high power (40x blue-band) and observe.

What are most of the organelles in the storage tissue?
Which of the two cell types has the larger nucleus? [embryo!!storage tissue!]

What is the ploidy level of the storage tissue?  \[1N+2N+3N+\infty N\]

In the cotyledon on the left you should be able to observe the three primary tissues:

The embryo, the megagametophyte, remnants of the nucellus, and the integument, now differentiated into fleshy and stony layers, constitute the SEED. The seeds are shed from the parent plant and may remain dormant for various periods of time.

Observe a large megasporangiate cone. Start taking it apart with the pliers. How many seeds form on each megasporophyll? [one!!two!!three!!many!]

Obtain one or more of the contained seeds. Notice that these are winged. Open cones are ones from which seed were shed last year or earlier.

Take a seed apart carefully to see if you can find all the parts: integument (seed coat), nucellus, megagametophyte, and embryo. If you remove the latter carefully, you should be able to count the cotyledons at one end and uncoil the suspensor at the other. The seeds in the cones available are rather small so this is difficult.

Observe a large "shelled" pine seeds, ones from which the seed coat (integument and nucellar remains) has been removed. These were purchased and are available for dissection (and eating!). These consist of megagametophyte plus embryo and may be dissected instead of the smaller whole seeds. Try to remove the embryo whole by splitting the seed longitudinally. Also try to obtain the coiled and compressed suspensor.

How many cotyledons are present on the embryo within? ___________________________________________
An important difference among gymnosperms.

It should be pointed out that the loss of swimming sperm was not so abrupt as our jump from the *Selaginella* cycle to that of the pine might suggest. In the cycads and *Gingko*, as in pine, the megaspore and resultant megagametophyte are retained in the megasporangium (ovule), as is the embryo, so that typical seeds are formed. Pollen grains also are deposited on the nucellus. In these species, however, although pollen tubes form, they are haustorial organs only. Most of the center of the nucellus is eaten away so that when the basal end of the microgametophyte bursts to release two very large multiflagellated sperm, they can swim in the discharged cytoplasmic fluid through the space to and into the archegonia. Although the sperm are motile, they do not depend on an external source of water to achieve syngamy.