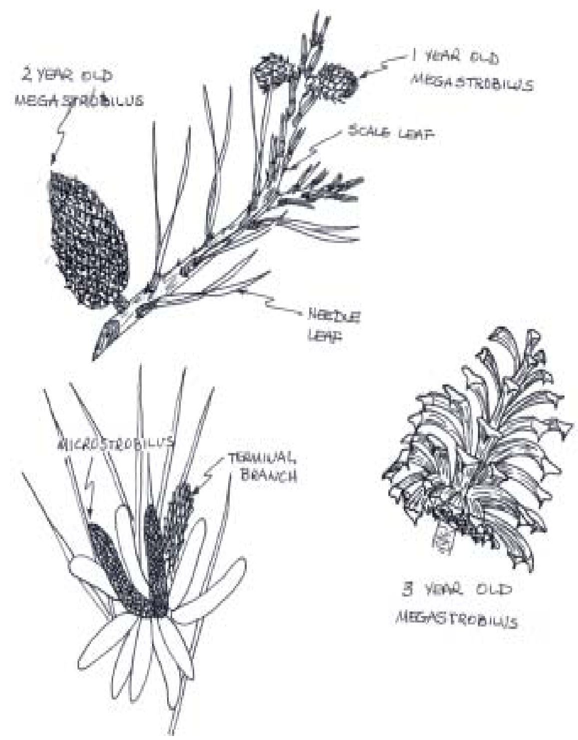
Plant Reproduction

The topic of plant reproduction is a little overwhelming. Not only is there asexual reproduction in plants: fragmentation, budding, and spores, but there's reproduction which may involve sexual spores as well as seed production. In this lab exercise, we'll concentrate on sexual reproduction. The formation of sexual spores was covered with the fern allies and ferns so we'll further restrict our topic by looking a sexual reproduction involving the formation of seeds in gymnosperms and angiosperms.

Microgametogenesis and Megagametogenesis in the Gymnosperm *Pinus*

Because of the commercial uses of pine trees, we know a great deal about sexual reproduction in this genus. In the southeastern United States, the reproductive cycle begins in April and May with the pollination of the first year female pine cone. Pollen in pines is produced on male staminate cones. The cones are actually aggregations of spore containing leaves called **microsporophylls** and the cluster is called a **microstrobilus**. Each microsporophyll has attached to its adaxial surface two **microsporangia**.

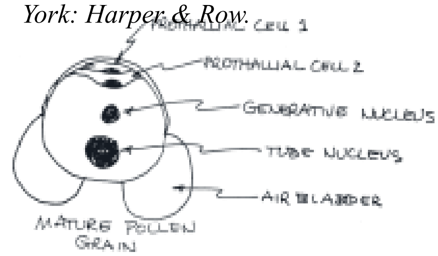
Figure 1. Pinus *male and female cones. From Bold HC. 1973. Pages 517, 519* in *Morphology of plants, 3rd ed. New York: Harper & Row.*



☐ Observe the microsporophylls of the genus *Pinus*. Pull off a couple of microsporophylls and look for the microsporangia attached. Remove one or more microsporangia and place on a clean, dry slide. Rupture the microsporangia with a sharp probe and spread the contents over the slide. Add a drop of distilled water and a coverslip. In this case, you will probably see **mature pollen grains** of pine. Note the Mickey Mouse face type appearance of the pollen grains. The "ears" are called **air bladders** and they help carry pollen on the air currents in the spring when shed by the tree. Pine pollen production can be so prolific it can actually cause a haze in the atmosphere. Remove the slide and set aside. We'll come back to it later.

The microsporangium is composed of a sterile jacket of cells surrounding another group of cells called the **microsporocytes**. These microsporocytes undergo mitotic divisions to form two separate layers within the microsporangium: the **tapetum** and **microspore mother cells**. The tapetal layer will break down and serve as nutritive tissue for the microspore mother cells.

Figure 2. Pinus *mature pollen grain. From Bold HC. 1973. Pages 523* in *Morphology of plants, 3rd ed. New York: Harper & Row.*



The microspore mother cells undergo meiosis to produce **tetrads of spores**. These tetrads break apart to release individual **microspores**. The microspores often are somewhat pyramidal in shape at this time.

The microspores undergo a series of mitotic divisions. The first division produces a **prothallial cell** and an **initial cell**. The prothallial cell has no function and actually degenerates over time. However, the initial divides again by mitosis to produce a **second prothallial cell** and a **antheridial cell**. Like the first prothallial cell, the second is also nonfunctional and degenerative.

The antheridial cell undergoes mitosis to produce a **generative nucleus** and a **tube cell**. The tube cell will form a pollen tube when the pollen grain "germinates." It's at this stage the pollen grain is considered mature and is shed from the microsporangia, usually between April and May. The mature pollen grain is thus composed of two prothallial cells, a generative nucleus, a tube nucleus and two air bladders.

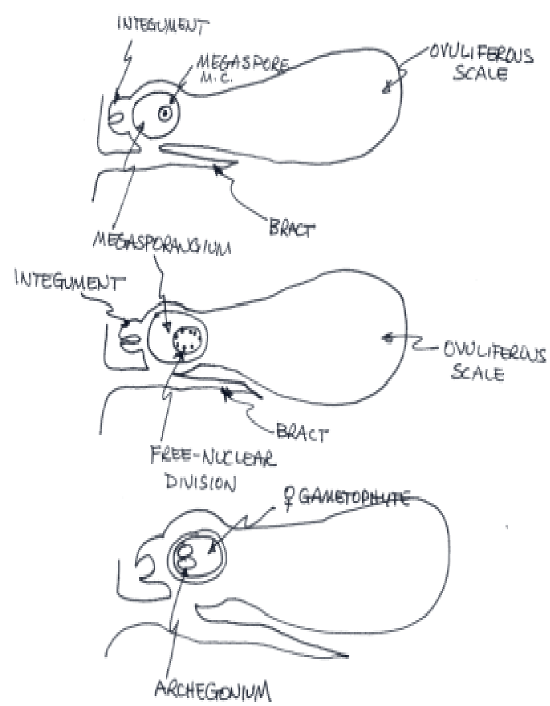
☐ Now look again at the wet mount you just made of the microsporangia. See if you can find the structures of a mature pollen grain. You'll probably need to go to high power. Compare this with a prepared slide of mature pollen grains. Once the pollen in shed, wind currents carry it to the first year female cone.

☐ Observe the first year female pine cone provided. This cone is upright on the pine branch with the bracts pointing upward to receive the pollen. Wind pollination is extremely inefficient. What if the wind is blowing in the wrong direction? To compensate for the inefficiency, the plant makes up for it by producing copious amounts of pollen. Once pollen is trapped by the first year female cone, it takes until September or October of that year for the pollen tube to grow down into the first year female cone and find the female reproductive structures. As you might guess, there is a chemical attraction for the pollen tube to the female reproductive structures held by the cone.

Once on the female cone, the generative cell of the mature pollen grain undergoes mitosis to produce a **stalk cell** and a **body cell**. The body cell divides by mitosis to produce two **ciliated sperm cells** which will swim down the pollen tube and discharge out the tip to fertilize the egg.

☐ Observe the series of prepared slides showing microgametogenesis in the genus *Pinus*. You may wish to make some sketches for review.

Figure 3. Megagametogenesis in Pinus. *From Bold HC. 1973. Pages 521-526* in *Morphology of plants, 3rd ed. New York: Harper & Row.*



Megagametogenesis in Pines

Again, refer to the first year female cone. Every **bract** you see on the cone represents a **megasporophyll** with two **megasporangia**. The aggregation of megasporophylls is called a **megastrobilus** (or female cone). This is what you generally recognize as a pine cone.

Each megasporangium is surrounded by sterile tissue which forms an **ovuliferous scale** and two **integuments**. The integuments will serve as the entry point for the pollen tube and the ciliated sperm. At first, the ovuliferous scale is actually larger than the bract but soon the bract will overtake the ovuliferous scale in size. By the time pollen grains arrive, the bract is much larger and help trap the pollen grains.

The megasporangium is cellular and composed of sterile, nutritive cells and a single **megaspore mother cell.** The megaspore mother cell undergoes meiosis to produce a linear tetrad of megaspores.

Three of these degenerate and we are left with one single functional **megaspore.** Think how this compares with human egg production where one oögonial cell results in the production of one egg cell and three polar bodies. The functional megaspore then undergoes **free nuclear division** where the nuclei divide but no cell walls are formed (at first). Eventually, cell wall formation does take place and series of cells forms around a central egg cell. This mass of cells is called an **archegonium**. The archegonium is similar to those you saw in ferns and the fern allies. Two archegonia form in the megasporangium. There's not much of a neck or neck canal cells nor ventral canal cell. However, additional cells from the free nuclear division form the **female gametophyte** which nourishes the archegonia.

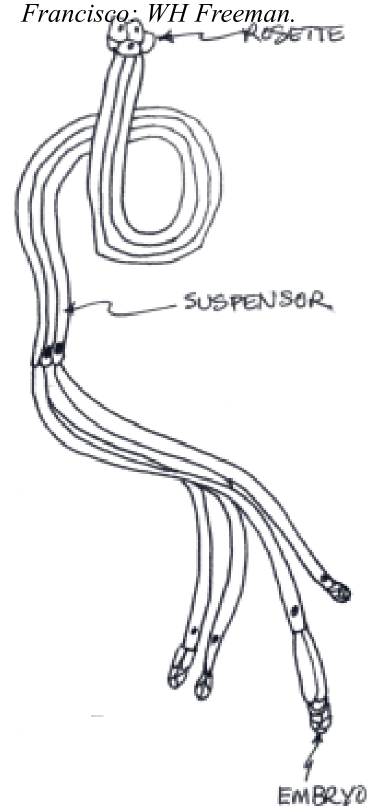
Each bract contains two megasporangia with two ovuliferous scales. Each megasporangium has the ability to produce two archegonia and therefore each scale has associated with it 4 eggs available for fertilization. However, for each ovuliferous scale, only one egg is fertilized. The result is two seeds per bract. The fertilized egg is surrounded by the archegonium, the ovuliferous scale and the integuments. These eventually form the seed coat and wing of a pine seed.

During this process, as the female cone grows, it moves from its upright position on the branch to a downward position where the bracts now point towards the ground. When the cone matures, the bracts open outward to facilitate the release of mature seeds. When the seeds are released, germination does not occur until June of the following year.

☐ Observe the series of slides showing the various stages of megagametogenesis in pines. You may wish to make some sketches for review.

Once fertilization is effected, the zygote in the archegonium undergoes mitosis to produce an **apical cell** and a **basal cell**. The apical cell becomes the embryo. The basal cell eventually continues to divide mitotically and produces four cells which produce a **rosette** of cells. In addition, a series of mitotic divisions produces a series of cells which form a **suspensor**. The suspensor grows and pushes the developing embryo into the female gametophyte for protection and nourishment.

Figure 4. Polyembryony in Pinus. *From Foster AS, Gifford EM. 1974. Page 505* in *Comparative morphology of vascular plants, 2nd ed. San Francisco: WH Freeman.*



☐ Observe the prepared slide of a pine embryo showing polyembryony.

In essence, each pine seed has the potential for four different embryos. This condition is called **polyembryony**. Generally, only one of the embryos survives and the other three degenerate, but on occasions, you may see two pine trees emerging from the ground from a common point. In this case, two of the embryos survived and both germinated.

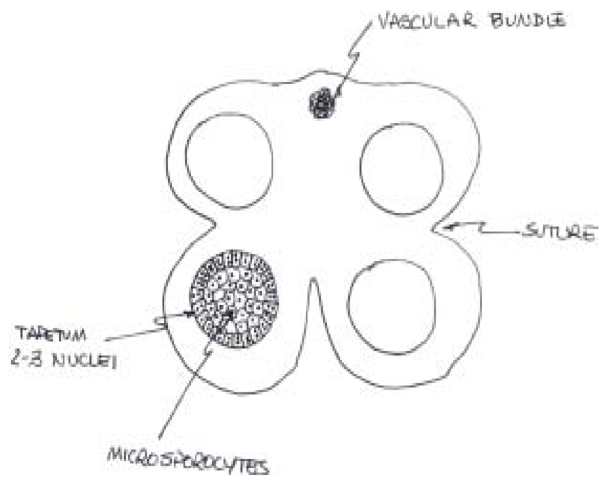
Not all pines shed their seed yearly. Often, mother nature has to lend a hand. If pines shed their seeds upon maturity, often the seeds would germinate and the young pines would be in competition with all the plants around the pine tree. Grasses, shrubs, herbs, all compete for the same nutrients. Pines have evolved an ingenious method to insure their young seedlings get a head start. Many pines will not release seed unless there is great heat in the form of a fire. When fire scorches the pines the intense heat opens the pine cones and after the fire has passed, the seeds are shed from the cones. Each seed has an ovuliferous scale that serves to catch the wind and produce a helicopter motion to the seed as it falls, further spreading it away from the parent tree. Most pines produce a thick corky bark from which only the outer layer burns away in a fire and the tree is save. Of course, in very intense forest fires, the trees are destroyed.

☐ Look at the pine cones provided. One has not opened yet is mature. The other was exposed to a few minutes in a microwave to open the bracts so the seeds could be released.

Microgametogenesis in the Angiosperm *Lilium*

The genus *Lilium* is known commonly as a lily. There are numerous species of lilies, but think of an Easter lily as an example of this type of flower. Microgametogenesis occurs in the male parts of the angiosperm flower.

Figure 5. Lilium *anther, cross section.*



Pollen in the angiosperms is produced not in strobili as in pines but instead in the anthers of flowers. Usually, anthers, when cut in cross section, have four chambers. Inside the chambers are a sterile set of cells, the tapetum and the microsporocytes. An unusual feature of the tapetum of angiosperms is that their may be 2 or 3 nuclei per tapetal cell - they are multinucleate. The microsporocytes, found in the center, undergo meiosis to produce the standard tetrad of microspores.

The tetrad breaks apart to release individual microspores. Each individual microspore undergoes mitosis to produce a generative cell and a tube cell. This is considered to be a mature pollen grain at this point. The generative cell will undergo mitosis to produce two nonciliated sperm cells. As in the pines, the tube cell will grow and form a pollen tube. The sperm move down the pollen tube by ameboid motion and eventually are ejected to effect fertilization.

☐ Look at a prepared slide of a cross section of *Lilium* anther. Look for the four chambers and find the tapetum. See if you can see more than one nucleus in the tapetal cells. Also note whether or not you are seeing microsporocytes, spore tetrads or mature pollen grains in the chambers.

Pollination in angiosperms may occur in many different ways. However, angiosperm flowers have evolved mechanisms to try to insure pollination by a wide variety of insects and other animals. Some cacti are pollinated by bats. Some moths and butterflies effect pollination. Honey bees, however, are the workhorses of pollination in angiosperms. Perhaps one of the more unusual methods of insuring pollination is a particular orchid whose flower resembles a female wasp. A male wasp tries to breed with the "female" and pollinia attach to the male. Since he's unsuccessful in breeding, he flies to another orchid flower to try again, taking the pollen sacs from the first flower to pollinate the second! What are the differences in the mature pollen grain of pine and *Lilium*? ____________________

____

____

Megagametogenesis in the Angiosperm *Lilium*

☐ If a lily flower is available for dissection, remove the petals, sepals, and stamens. Although the production of eggs (and eventually seeds) in lilies is somewhat atypical of angiosperms, the events that take place are close enough for us to use it as a specimen of study since it is often readily available in the biology lab. Look for the pistil composed of a stigma, style and ovary. Notice the stigma is three lobed. The surface of the stigma often has a sticky secretion to adhere the pollen grains which may arrive by wind or insects. The style is the neck-like portion of the pistil. Once a pollen grain lands on the stigma, a chemical change takes place that stimulates the growth of the pollen tube. The pollen tube must grow down the length of the style to reach the ovary.

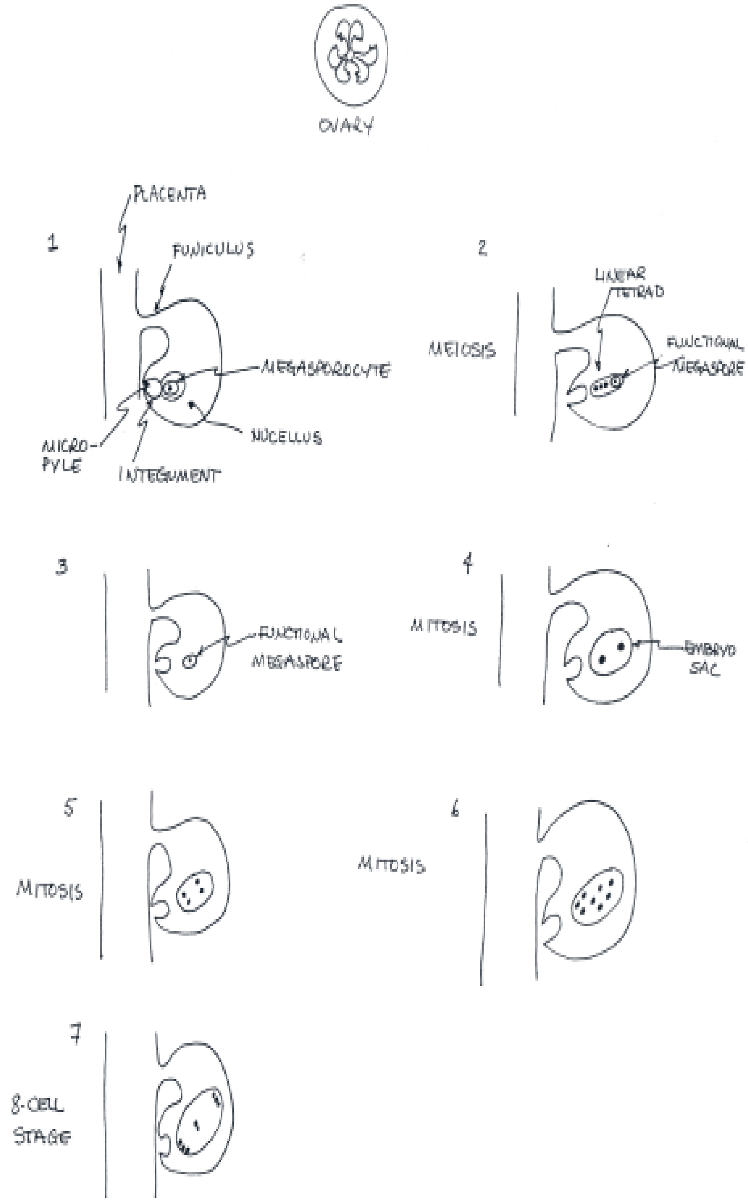


Figure 6. Megagametogenesis in Lilium.

What do you think would happen if a pollen grain from the wrong species of plant stuck to the stigma? It depends. Sometimes the pollen tube simply would not germinate - the chemical signals are not correct. In other cases, the pollen tube may germinate but the pollen tube from the wrong species may not grow long enough to place the sperm in the appropriate place in the ovary. In some cases, the pollen tube of wrong species may actually be too long and grow too far. Sometimes they actually grow down into the ovary and back up the style!

□ Now cut through the ovary in cross section. Look down into the ovary and you should notice at least three chambers (carpels from the ovary). There are typically two ovules (seeds) produced per chamber. Notice the seeds are attached to the ovary wall (placenta) by thin filaments called the funiculus. What you are seeing is the ovary in cross section probably after fertilization has taken place. Let's back up the process and see what takes place prior to fertilization.

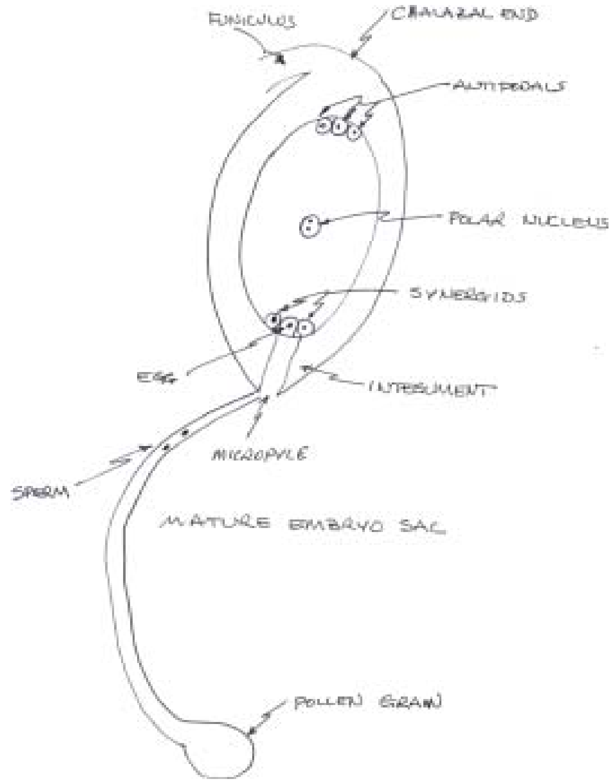
A megasporangium begins to from as an outgrowth of the ovary wall. The megasporangium is surrounded by sterile tissue which is attached to the ovary wall (placenta) by the funiculus. Inside the megasporangium, a **megaspore mother cell** differentiates. At the opposite end from the funiculus of the megasporangium are two leaf-like structures called the integuments. They, along with the sterile tissue of the megasporangium will eventually form the seed coat. The integuments also serve as a point of entry for the pollen tube and sperm.

□ Obtain a prepared slide of a cross section of a *Lily* ovary showing the megasporangium and megaspore mother cell. The megaspore mother cell undergoes meiosis to produce a linear tetrad, just as in pine. Three of these, again like the pine, degenerate and we are left with one **functional megaspore**.

□ Obtain a prepared slide of a cross section of a *Lily* ovary showing the linear tetrad. Try to identify the linear tetrad, the megasporangium, and the nucellus (nutritive tissue).

☐ Obtain a prepared slide of a cross section of *Lily* ovary showing a functional megaspore. The functional megaspore undergoes two successive mitotic divisions to produce first 4 nuclei and then 8 nuclei (no cell walls form at first and thus the division is said to be free nuclear). Look for the functional megaspore, the megasporangium and nucelus.

Figure 7. Mature embryo sac of Lilium *- 8 nucleate*



☐ Obtain a prepared slide of a cross section of *Lily* ovary showing the 4-nucleate stage and the eight nucleate stage. Three of these nuclei migrate to the end where the megasporangium attaches by the funiculus to the ovary wall. This end of what is called the developing **embryo sac** is called the **chalazal end.** The opposite end is called the micropylar end. The three nuclei at the chalazal end are called **antipodals.**

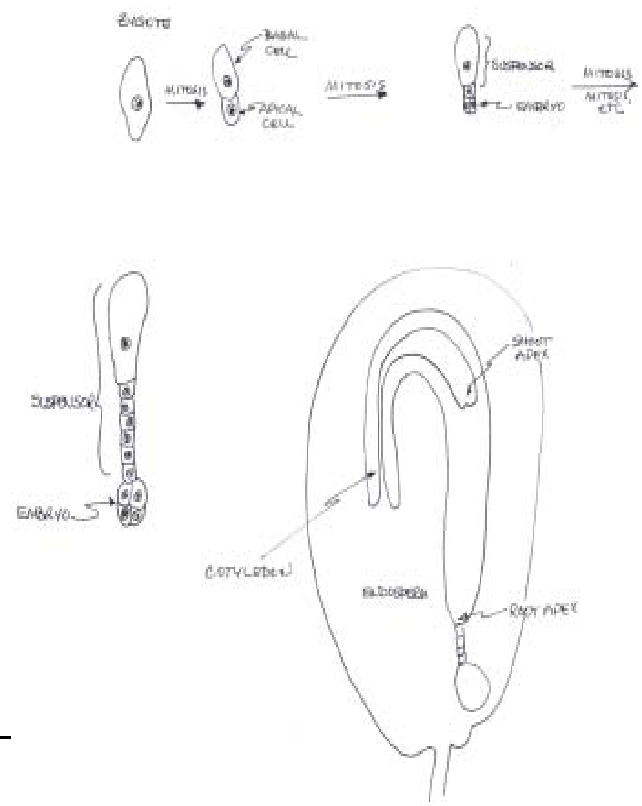
Three other nuclei migrate to the micropylar end and form two **synergids** with an **egg** in the between. Two other nuclei fuse and migrate to the center of the embryo sac. These fused nuclei are called the **polar nucleus.** Note that the 3 antipodals, 2 synergids, and 1 egg are all haploid but the polar nucleus is now diploid.

☐ Obtain a prepared slide of *Lilium* ovary cross section showing a mature embryo sac. Locate the funiculus, chalazal end, 3 antipodals, 2 synergids, 1 egg, 1 polar nucleus and the micropyle. Eventually cell walls form around all these nuclei and the embryo sac thus becomes cellular. It's at this point the egg is ready to be fertilized.

The pollen tube is chemically attracted to the micropyle. When the pollen tube reaches the micropyle, the two sperm cells are discharged into the opening. One sperm fertilizes the egg and the second sperm migrates to the polar nucleus and fertilizes the polar nucleus. This is referred to as **double fertilization**, a characteristic of angiosperms. The fertilized egg or zygote is now diploid and the polar nucleus is now triploid or 3n.

The zygote now undergoes mitosis to produce two cells, an apical cell and a basal cell. The basal cell eventually forms a suspensor and the apical cell eventually forms the embryo. As

Figure 8. Capsella *embryo. From Foster AS. Gifford EM. 1974. Pages 698, 700* in *Comparative morphology of vascular plants, 2nd ed. San Francisco: WH Freeman.*



the embryo continues to divide and enlarge, the suspensor continues to divide and pushes the embryo deeper into the embryo sac.

Figure 9. Corn embryo and germination. From Raven PH, Evert RF, Eichhorn SE. 1986. Page 375 in *Biology of plants, 4th ed. New York: Worth.*

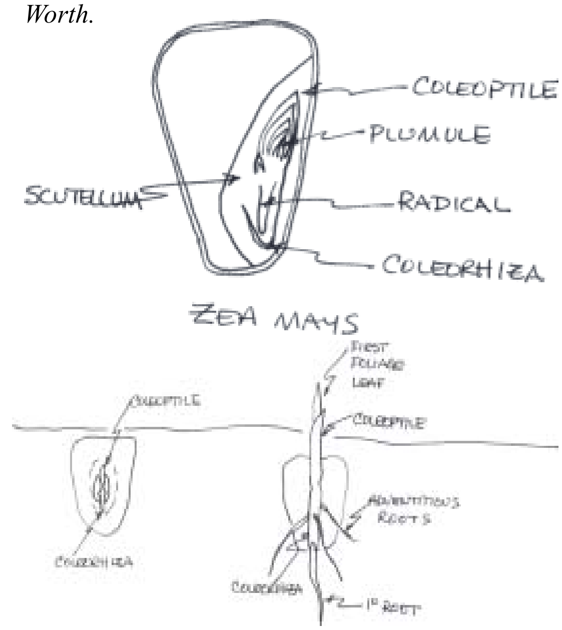
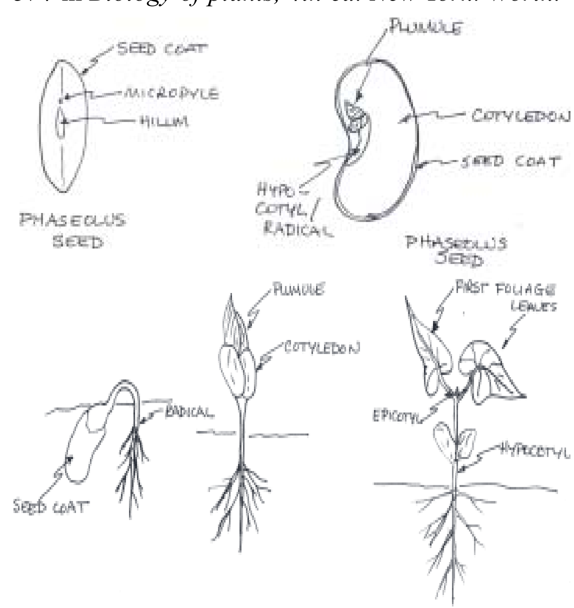


Figure 10. Bean embryo and germination. From Raven PH, Evert RF, Eichhorn SE. 1986. Page 374 in *Biology of plants, 4th ed. New York: Worth.*



The triploid polar nucleus now undergoes a series of mitotic free-nuclear divisions. The nuclei eventually produce cell walls and the tissue which develops is called endosperm (remember, it's triploid). Endosperm serves as nourishment material for the embryo and can keep the embryo for long periods of time before the seed germinates.

The embryo continues to grow and differentiate. Eventually, a shoot apex appears, a root apex, and one or two cotyledons (depending upon whether it's a monocot or dicot).

☐ Obtain a prepared slide of *Capsella* fruit which contains a dicot embryo and look at the developing embryo within the embryo sac. Try to identify the cotyledons, shoot and root apex, and endosperm.

The shoot apex differentiates into the **coleoptile** and has a protective covering over it called the **plumule**. The root apex differentiates into the **radical** and is covered by the **coleorhiza**. We now have a mature ovule or seed.

Each species of angiosperm has variations on the above theme. We'll examine two embryos a little more closely: the lima bean and corn.

☐ Obtain a lima bean and corn grain that have been soaking in water overnight. Begin with the corn grain. With a razor blade, cut the corn grain longitudinally through the center. Inside you should be able to see the developing embryo. Look for the plume, radical, coleorhiza and coleoptile. Part of the coleoptile is extensive and forms a structure called the **scutellum**. The majority of the corn grain is endosperm. The seed coat comes from the original sterile tissue of the megasporangium.

For those of you who like corn meal, corn meal is simply dried corn grains which have been ground into a flour-like consistency. If you have ever heard of the southern dish hominy, hominy is the corn grain which has been boiled and covered in lye (sodium hydroxide) to remove the seed coat. What's left after this destructive process is the white endosperm layer and the embryo. Hominy grits, or more popularly just called grits, is dried hominy ground into the grit-like material served in restaurants for breakfast.

☐ Next, remove the loosened seed coat of the lima bean. The two halves of the bean should now pop apart. Look at each half and on one of the halves should be the embryo with plumule and radical. Where are the cotyledons? The cotyledons are the part you eat of the lima bean, the kidney shaped halves. In the lima bean, endosperm is very small and undetectable to the unaided eye.

Observe the seed flats with germinating corn and lima beans. Note how each comes out of the ground. Corn germinates in a totally different way than lima beans. In corn, the grain remains below the ground and only the coleoptile pokes above ground and from it comes the first seed leaf. Notice there is only one seed leaf emerging, so corn is a monocot.

Next observe the lima bean sprouts. In the case of the lima bean, the main axis of the embryo elongates quickly and pushes the entire embryo with cotyledons above ground. The cotyledons then open and the coleoptile and seed leaves begin to emerge. There are two seed leaves, therefore, lima beans are dicots.

References Cited

Bold HC. 1973. Pages 517, 519 *in* Morphology of plants, 3rd ed. New York: Harper & Row.

_____. 523.

_____. 521-526.

Foster AS, Gifford EM. 1974. Page 505 *in* Comparative morphology of vascular plants, 2nd ed. San Francisco: WH Freeman.

_____. 698, 700.

Raven, PH, Evert RF, Eichhorn SE. 1986. Page 375 *in* Biology of plants, 4th ed. New York: Worth.

_____ 374.