

## Diversity and Life History

# The Angiosperms II

## microscopic aspects of sexual reproduction and the fruit

While the preceding laboratory exercise dealt with floral morphology, this exercise is devoted to additional aspects of sexual reproduction in angiosperms (i.e. flowering plants). To fully understand and appreciate the elaborate reproductive process of this group of plants requires that you review the basic differences between **sporophyte** and **gametophyte** stages of plant life cycles. In angiosperms, the sporophyte (2n) stage predominates and includes the vegetative parts of the plant and even the various parts of the flower. The process involving meiosis, which converts diploid sporocytes (or spore mother cells) into haploid (n) spores, is termed **sporogenesis**. Sporogenesis occurs in the anthers of stamens and the ovules of pistils. Angiosperms, and seed plants in general, are **heterosporous**. This means that two different kinds of spores, **microspore** and **megaspore**, are produced by the sporophyte. Consequently, heterosporous organisms exhibit separate male and female gametophytes. The microspore gives rise to the **microgametophyte** (or **male gametophyte**), and the megaspore gives rise to the **megagametophyte** (or **female gametophyte**).

REFERENCE. Chapter 21, pp. 495–515 in Raven, P.H., R.F. Evert, and S.E. Eichhorn. 1999. Biology of plants. 6<sup>th</sup> Ed. W.H. Freeman and Company. New York.

IMPORTANT TERMS AND CONCEPTS			
seed plant angiosperm heterospory heterosporous spore microspore megaspore gametophyte sporophyte microgametophyte (male gametophyte)	megagametophyte (female gametophyte) meiosis spore tetrad two-celled pollen grain generative cell tube cell germination pollen tube mature microgametophyte	microsporogenesis tube nucleus two sperm cells megasporogenesis megasporocyte (megaspore mother cell) microsporocyte (microspore mother cell) nucellus ovum synergid cells	polar nuclei antipodal cells embryo sac double fertilization triple fusion primary endosperm nucleus endosperm zygote embryo pollination vs. fertilization

### MICROSPOROGENESIS

Microsporogenesis is the process by which haploid **microspores** are produced from diploid **microsporocytes** in the anther of the flower. Of course, meiosis is a critical part of microsporogenesis. Initially, the microspores adhere into a four-celled group called a **tetrad**. However, shortly after its production the tetrad breaks apart into individual microspores.

PREPARED SLIDES. Examine the *series* of prepared slides of transversely sectioned anthers in flower buds of *Lilium* sp. (lily), which show microsporogenesis. Minimally, the following stages should be observed: meiosis I, meiosis II, microspore tetrad, and separate microspores. Identify the following: **microsporocyte**, **microspore tetrad**, **microspore**, and the various **stages of meiosis I and II**.

DRAWING. Prepare the following drawings (see Appendix A): microsporocyte in meiosis I, microsporocyte in meiosis II, microspore tetrad, and separate microspores.

## THE POLLEN GRAIN

While still within the anther, each microspore undergoes mitotic cell division to produce a *two-celled pollen grain*. You will recall that pollen grains are actually immature microgametophytes. It is these two-celled pollen grains that are released from the anther upon **dehiscence** (i.e., opening, or literally *yawning*) and transferred to the stigma during pollination.

PREPARED SLIDE. Examine the prepared slide showing *Lilium* sp. (lily) pollen grains. The larger of the two cells of the pollen grain is the **tube cell**, and the smaller lens shaped one with darker staining cytoplasm is the **generative cell**. The tube cell develops into the pollen tube upon germination of the pollen grain, and the generative cell undergoes mitotic cell division to produce a pair of sperm cells.

DRAWING. Draw (see Appendix A) the sectioned *Lilium* sp. (lily) pollen grain, labeling pollen grain wall, generative cell, and tube cell.

## DEVELOPMENT OF THE MICROGAMETOPHYTE

**Pollination** is the transfer of the pollen grain from the anther to the stigma. Most angiosperm species are pollinated by insects. However, many species are pollinated by one of a number of other agents of pollination, such as birds, mammals, wind, or water. If the pollen grain is deposited on a receptive (i.e. chemically compatible) stigma, it germinates. **Germination** involves the growth of the **pollen tube** from the pollen grain. The **tube cell** of the pollen grain gives rise to the pollen tube. As it grows the pollen tube penetrates the surface of the stigma, grows through the style into the ovary. It then continues to grow along the surface of an ovule around to and into the micropyle. Growth of the pollen tube ceases after it reaches and penetrates the embryo sac contained within the ovule. The two **sperm cells** produced by division of the **generative cell** migrate through the pollen tube into the embryo sac where double fertilization occurs.

PREPARED SLIDE. Examine the prepared slide showing *Lilium* sp. (lily) pollen grains with pollen tubes (i.e., mature microgametophytes). Identify **pollen grain wall, pollen tube, sperm cells, and tube nucleus**. –In nature, where does germination of the pollen grain normally occur? –What is the significance of pollination in the evolution of the seed plants?

DRAWING. Draw (see Appendix A) and label the mature *Lilium* sp. (lily) microgametophyte (i.e., the germinated pollen grain).

## GERMINATION OF POLLEN ON AN ARTIFICIAL MEDIUM

GROUP EXERCISE. The objective of this exercise is to allow students to observe pollen germination and microgametophyte development in the laboratory. Flowers of various species will be provided as pollen sources, and each group will observe and compare pollen germination in *four* different species.

### Materials and Methods

The following materials are needed:

fresh pollen

4 petri plates (100 X 15 mm)

4 filter paper circles (paper towel circles may be substituted)

8 supports (2--3 cm long): wooden splints or small diameter glass rods sufficiently thick to elevate inoculated slide above moistened filter paper in bottom of petri plate

4 microscope slides coated on one surface only with simple nutrient agar nutrient agar: 10% sucrose in 1.5% agar

If slides are coated and inoculated at the beginning of the laboratory period, a three-hour period allows enough time for students to coat slides and still observe germination. Certain species germinate readily and microgametophytes can be observed during the laboratory period; others develop more slowly. Thus, observations made at regular intervals for a 12--24 hour period may be required to detect germination.

**Coating of slides.** Standard glass microscope slides are dipped into the hot nutrient agar. Immediately upon removing the slide from the hot agar, one surface is wiped clean using a moistened Kimwipe. The coated slides are then allowed to cool to room temperature. Water content and degree of hydration of the agar appear to be important variables, and best results have been obtained using freshly coated slides. Do not let the agar coating dry out excessively.

**Inoculation of slides.** A variety of techniques have been used. In all cases it is necessary to select mature anthers that are actively shedding pollen. With some species it is necessary to "smudge" pollen onto the nutrient agar (i.e., to make direct contact between mature anther and surface of nutrient agar). However, the preferred technique is to sprinkle pollen over the surface of the agar by holding the anther with forceps and tapping it sharply with a dissecting needle.

**Culturing microgametophytes.** A moist culture chamber must be provided to promote germination and microgametophyte development. Culture chambers are kept in light and at room temperature. Petri plates are prepared as follows for use as culture chambers. Place a filter paper circle into the petri plate. (Note: Paper towel circles several sheets thick may be substituted for filter paper.) Moisten the filter paper thoroughly with distilled water. Place two supports (wooden splints or small diameter glass rods) parallel with one another on opposite sides of the moistened filter paper. The purpose of the supports is to elevate the inverted slide slightly so that its inoculated, agar-coated surface does not touch the moistened filter paper. Place a lid on the chamber and set it aside for at least one hour. Some species do not show much germination for several hours, and one, *Helianthus angustifolius*, showed no evidence of germination until after 12 hours.

**Observation of developing microgametophytes.** Carefully remove the inverted microscope slide from the culture chamber. Make certain it is re-positioned with the agar-coated surface up before placing it onto the microscope stage. Examine without a cover slip at low power magnification and determine the percentage of germinated pollen grains. If time and interest allow, re-invert the slide and place it back into the culture chamber. Observations may be made and germination percentages may be determined at regular intervals for up to 24 hours, if precautions are taken to maintain a moist culture chamber and to prevent dehydration of the agar. Just before completing the exercise, preparations should be examined with the compound microscope at high power magnification. Although not absolutely necessary, a cover slip can be placed on the agar to gain better resolution. However, because of the greater thickness of these preparations working distance is limited even more than usual and special care should be taken to protect the high power objective.

**Staining.** Toluidine blue and methylene blue stains may be used to enhance sperm cells and tube nuclei in pollen tubes. These should be applied just prior to the end of the observation period. Stains are flooded over the agar surface and allowed to penetrate for 5-10 seconds, then the excess stain is washed off by irrigating with distilled water.

LABORATORY REPORT. A formal laboratory report will be written comparing pollen germination among the species observed. Instructions for writing a laboratory report may be found in Appendix B.

## MEGASPOROGENESIS

Megasporogenesis is the process by which **megaspores** are produced from the **megasporocyte** in the ovule. Although four megaspores are produced meiotically in megasporogenesis, only one of them survives to develop into the **embryo sac**.

PREPARED SLIDES. Examine the series of prepared slides showing megasporogenesis and embryo sac development in ovules of transversely sectioned *Lilium* sp. (lily) flower buds.

DRAWING. Draw (see Appendix A) the immature *Lilium* sp. (lily) ovule with megasporocyte.

## THE EMBRYO SAC

In *Lilium* sp. (lily) the surviving haploid megaspore undergoes three mitotic divisions to produce within the ovule an *eight-nucleate, seven-celled structure* termed the **embryo sac**. The embryo sac is the **megagametophyte** of the angiosperm.

DEMONSTRATION SLIDE. Examine the slide showing the mature lily embryo sac. Identify the following cells and structures: **micropyle, integuments, nucellus, ovum, synergid cells, polar nuclei, and antipodal cells**. –Where does the embryo sac develop? –What is the micropyle? –What is the function of the micropyle? –Into what part of the seed does the integument develop? –How many synergid cells are present in the embryo sac? –What is the function of the synergids? –How many polar nuclei are present in the embryo sac? –What is the function of the polar nuclei? –How many antipodal cells are present in the embryo sac? –What is the function of the antipodal cells? –How many nuclei are present in the mature embryo sac of lily? –How many cells are present in the mature embryo sac of lily?

DIAGRAM. Diagram (see Appendix A) the longitudinal section of the *Lilium* sp. (lily) ovule with mature embryo sac. Label each structure shown in bold in the preceding section.

## DOUBLE FERTILIZATION

Double fertilization is virtually restricted to and characteristic of angiosperms. Two non-motile sperm cells are produced by the male gametophyte and are transmitted through the pollen tube of the male gametophyte to the embryo sac inside the ovule. One of the sperms fuses with the **egg cell** to produce the zygote, and the other sperm fuses with the **two polar nuclei** to produce the triploid primary endosperm nucleus. The **primary endosperm nucleus** develops by mitosis into the nutritive **endosperm tissue**, and the zygote develops into the **embryo**. As the embryonic sporophyte develops within the embryo sac, it is nourished by the surrounding endosperm tissue.

DEMONSTRATION SLIDE. Examine the demonstration slide showing double fertilization in *Lilium* sp (lily). –What is the significance of double fertilization?

## THE FRUIT

The angiosperms (Phylum Anthophyta) are the only plants that produce fruits. Technically, the **fruit** is a ripened ovary. The **ovary** is the enlarged basal part of the typical pistil. After fertilization, the **ovules** within the ovary begin to develop into **seeds**. These developing ovules secrete auxin, a growth regulator, which stimulates the ovary to develop into a fruit. The wall of the ovary develops into the **pericarp** of the fruit, and the seeds are contained within the pericarp. In certain fruits distinct layers are evident in the pericarp. Going from the outer surface of the pericarp inward to the center of the fruit, these layers are **exocarp**, **mesocarp**, and **endocarp**. There are many different types of fruits known, and, in some cases, structures other than the ovary, such as receptacle or other floral parts, may be incorporated into the fruit. Such fruits that include structures other than ovary are termed accessory fruits. Many edible fruits are called vegetables by uninformed persons. However, “fruit” is a botanical term with a precise botanical meaning. --Does “vegetable” have a precise botanical meaning? Information about the major types of fruit as well as fruit classification is summarized in the dichotomous key presented in this exercise. This laboratory exercise deals specifically with basic fruit structure, types of fruit, and fruit modification for dispersal.

REFERENCE. Chapter 22, pp. 543–553 in Raven, P.H., R.F. Evert, and S.E. Eichhorn. 1999. Biology of plants. 6<sup>th</sup> Ed. W.H. Freeman and Company. New York.

IMPORTANT TERMS AND CONCEPTS		
angiosperm	septum (septa, <i>pl.</i> )	legume
ovary	parthenocarpy	follicle
fruit	dispersal	achene
ovule	dehiscence	samara
seed	simple fruit	grain (or caryopsis)
pericarp	drupe	nut
exocarp	berry	aggregate fruit
mesocarp	pome	multiple fruit
endocarp	capsule	accessory fruit

**FRUIT VS. VEGETABLE**

–What is the difference between a *fruit* and a *vegetable*? Cite examples of fruits that are commonly called vegetables. Below is a list of edible plants and plant parts. For each item listed indicate the name of the specific plant part that is eaten (e.g. root, stem, leaf, petiole, seed, fruit, cotyledon, endosperm, etc.).

Edible Plant	Specific Part Eaten	Edible Plant	Specific Part Eaten
squash		lima bean	
beet		kidney bean	
radish		lettuce	
celery		peanut	
white potato		apple	
orange		sweet potato	
garlic		pecan	
sugarcane		peach	
onion		turnip	
watermelon		cauliflower	
asparagus		tomato	
artichoke		pole bean	
spinach		almond	
carrot		okra	
broccoli		kiwi	
rutabaga		pomegranate	
banana		leek	
brussel sprout		green pea	
mustard greens		cucumber	
coconut		snow pea	

**PARTHENOCARPY**

Parthenocarpy is the phenomenon in which seedless fruits develop in the absence of fertilization. --How do parthenocarpic plants reproduce? Below cite examples of **parthenocarpic** fruits.



## DICHOTOMOUS KEY TO FRUIT TYPES

1. Fruit derived from only one pistil (SIMPLE FRUIT).
  2. Fruit fleshy at maturity.
    3. Fruit with one seed enclosed by a hard, stony endocarp..... **DRUPE**
    3. Fruit with more than one seed, derived from a compound ovary.
      4. Entire pericarp fleshy (BERRY).
        5. Fruit with a thin "skin"..... **TRUE BERRY**
        5. Fruit with a leathery "skin" or thick rind.
          6. Fruit with a thick rind..... **PEPO<sup>1</sup>**
          6. Fruit with a leathery "skin"..... **HESPERIDIUM**
      4. Outer layers of pericarp fleshy but endocarp papery or cartilaginous..... **POME<sup>1</sup>**
  2. Fruit dry at maturity.
    7. Fruit dehiscent (i.e. opening at maturity) and generally containing more than one seed.
      8. Fruit derived from only one carpel.
        9. Fruit constricted between seed, and the one-seeded segments separating at maturity... **LOMENT**
        9. Fruit splitting longitudinally.
          10. Fruit dehiscent along only one side or seam..... **FOLLICLE**
          10. Fruit dehiscent along two sides or seams.
            11. Fruit without a partition..... **LEGUME**
            11. Fruit with central partition on which the seed are borne.
              12. Fruit more than three times as long as wide..... **SILIQUE**
              12. Fruit less than three times as long as wide..... **SILICLE**
        8. Fruit derived from two or more carpels.
          13. Fruit with two carpels each with one seed, which split apart lengthwise into one-seeded segments at maturity..... **SCHIZOCARP**
          13. Fruit dehiscent by longitudinal lines or by a transverse ring or by a series of pores near top (CAPSULE).
            14. Fruit dehiscent by one or more pores..... **PORICIDAL CAPSULE**
            14. Fruit dehiscent by longitudinal lines or a transverse ring.
              15. Fruit dehiscent by a transverse ring.....  
..... **PYXIS (CIRCUMSCISSILE CAPSULE)**
              15. Fruit dehiscent by longitudinal lines.
                16. Lines of dehiscence along septa.... **SEPTICIDAL CAPSULE**
                16. Lines of dehiscence between septa.....  
..... **LOCULICIDAL CAPSULE**
        7. Fruit indehiscent (i.e. not opening) and one-seeded.
          17. Pericarp modified into a wing..... **SAMARA**
          17. Pericarp not modified into a wing.
            18. Series of bracts (involucre) completely enclosing the fruit or forming a cup around base of fruit, fruit with one to several seed..... **NUT**
            18. Involucre absent or, if present, then not as above; fruit one-seeded.
              19. Seed enclosed by a thin, loose-fitting, sac-like pericarp..... **UTRICLE**
              19. Fruit not as above.
                20. Pericarp fused tightly to entire surface of seed..... **CARYOPSIS (or GRAIN)**
                20. Pericarp attached only at base of seed.
                  21. Ovary superior..... **ACHENE**
                  21. Ovary inferior..... **CYPSELA**
      1. Fruit derived from more than one pistil in an individual flower *or* from more than one flower in an inflorescence (COMPOUND FRUIT).
        22. Fruit derived from several to many pistils of a single flower..... **AGGREGATE FRUIT<sup>1</sup>**
        22. Fruit derived from multiple flowers of a single inflorescence..... **MULTIPLE FRUIT**

<sup>1</sup>This fruit type is often further classified as an accessory fruit. An **accessory fruit** is derived from tissue of the receptacle in addition to ovary.

**FRUIT MODIFICATIONS AND DISPERSAL**

Plants have evolved a variety of mechanisms to facilitate the dispersal of their fruits and seeds. Many of these mechanisms involve modifications of the fruit. Dispersal is a natural process, which insures survival of the species and its colonization of new habitats. Natural agents of dispersal include animals, wind, and water.

DEMONSTRATION. Examine preserved materials, photographs, and diagrams provided by your instructor. Cite at least one example of a fruit modified for each of the dispersal modes listed below and specifically describe the nature of the modification indicating how it facilitates dispersal.

**dispersal by animal hooves**

Example: \_\_\_\_\_

Nature of Modification: \_\_\_\_\_

\_\_\_\_\_

**dispersal by animal fur**

Example: \_\_\_\_\_

Nature of Modification: \_\_\_\_\_

\_\_\_\_\_

**dispersal by ingestion of fruit and excretion of seeds**

Example: \_\_\_\_\_

Nature of Modification: \_\_\_\_\_

\_\_\_\_\_

**wind dispersal**

Example: \_\_\_\_\_

Nature of Modification: \_\_\_\_\_

\_\_\_\_\_

**water dispersal**

Example: \_\_\_\_\_

Nature of Modification: \_\_\_\_\_

\_\_\_\_\_

## **REVIEW**

1. Relate the angiosperm reproductive cycle to alternation of generations, the plant life cycle pattern.
2. In angiosperms is the gametophyte or the sporophyte dominant? Is the flower primarily a gametophytic or a sporophytic structure?
3. What is heterospory? Review the plant phyla, and reconsider which groups are homosporous and which are heterosporous.
4. How is heterospory related to the production of two types of gametophytes?
5. In angiosperms which structures are thought to be homologous to the gametophytes of the more primitive plants?
6. Specifically, where in the angiosperm plant body are the micro- and megagametophytes produced?
7. Which type of gametophyte, male or female, is retained within the sporophyte plant body? Which type is released?
8. Distinguish between pollination and fertilization and explain why pollination must precede fertilization.
9. Why is pollination considered to be a significant advancement in adaptation to life on land?
10. What is co-evolution? Can you cite examples of co-evolution between plants and pollinators? Are these examples of co-evolution also examples of mutualism?
11. What is a fruit?
12. Distinguish between fruit and vegetable.
13. Cite examples of structures other than the ovary and its contents, which are may be present in the fruit?
14. What is the technical term for a fruit that involves structures in addition to the ovary? Cite specific examples of such a fruit.
15. Why is the pecan fruit sometimes classified as a "pseudodrupe"?
16. What is parthenocarpy? Cite examples of parthenocarpic fruits.
17. How do partheoncarpic plants reproduce.
18. What is dispersal and how is it significant?
19. Review dispersal mechanisms involving modification of the fruit.