

Diversity— The Tracheophytes (Vascular Land Plants)

LABORATORY

24

OVERVIEW

Despite the variety of their forms and the diversity of the environments to which they have become adapted, all vascular land plants, or tracheophytes, share some characteristics that reveal a common evolutionary organ.

Alternation of generations is a characteristic common to all land plants. As you examine the evolutionary progression of land plants, from nonvascular to vascular forms, you will notice a continuous reduction in the size and complexity of the gametophyte until it ultimately becomes greatly reduced and nutritionally dependent upon a much larger, more complex sporophyte.

Also associated with the evolutionary trend toward sporophyte dominance is a change from the **homosporous** condition (the simplest vascular plants produce only one type of spore) to the **heterosporous** condition (more advanced vascular plants produce two different types of spores—megaspores, which develop into female gametophytes, and microspores, which develop into male gametophytes). Another factor essential to the success of tracheophytes on dry land is the change from unprotected zygotes to the protection of the embryo sporophyte within a seed. In addition, modern tracheophytes contain networks of vascular strands (xylem and phloem). Leaves in the most primitive vascular plants have only single vascular strands.

During this laboratory period, you will study representatives of the many types of vascular plants, both with and without seeds.

STUDENT PREPARATION

To prepare for this laboratory, read the text pages indicated by your instructor.

Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency.

PART I TRACHEOPHYTES (VASCULAR PLANTS) WITHOUT SEEDS


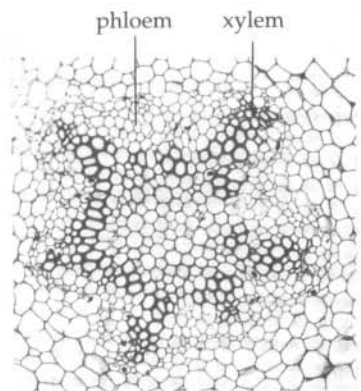
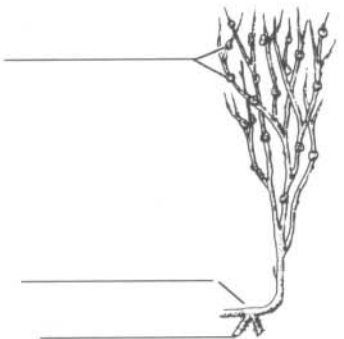
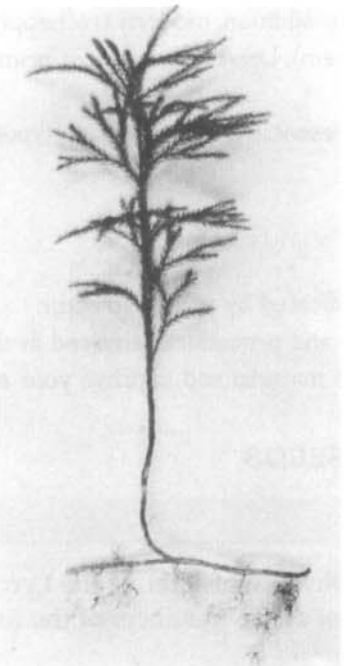
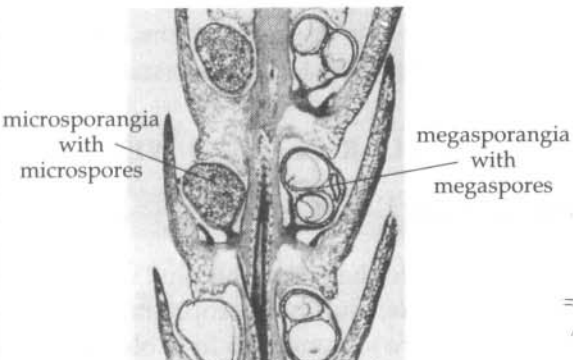
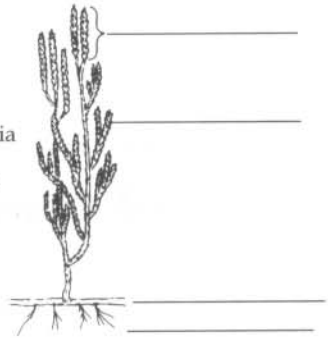


EXERCISE A Examining Seedless Tracheophytes

The tracheophytes without seeds include the following phyla: the Psilophyta, whisk ferns; the Lycophyta, club mosses; the Sphenophyta, horsetails; and the Pterophyta, ferns. Most of the members of the first three phyla are extinct; their characteristics are summed up in Table 24A-1.

Extinct Lycophyta once loomed as giants in the forests of the Carboniferous period. All living lycopods, however, are small. *Lycopodium* species are commonly called ground pine or club moss. An entire patch of *Lycopodium* may be connected by a single **rhizome**, a characteristic that once made this plant a favorite for wreath-making (collection is now prohibited in most states).

Table 24A-1 Vascular Plants, Divisions Psilotophyta, Lycophyta, and Sphenophyta

Phylum	Characteristics	Method of Observation
Psilotophyta Rep. <i>Psilotum</i> (whisk fern)	<p>Sporophyte lacks both leaves and roots. Dichotomously branched triangular stem with small scalelike appendages in place of leaves.</p> <p>Rhizomes and rhizoids in place of roots. Vascular tissue present (see below).</p> <p>Spores produced in trilobed sporangia (spore-producing organs). Homosporous.</p>	<p>Study preserved and living material on demonstration. Label sporangia, rhizomes, and rhizoids on the diagram below. Observe vascular tissue (phloem and xylem) in a cross section of a <i>Psilotum</i> stem, if available.</p>
		
Lycophyta Rep. <i>Lycopodium</i> (club moss)	<p>Leaves considered to be true leaves since they contain vascular tissue. Sporangia produced in the axils of leaves called sporophylls; these form conelike structures called strobili. Roots and rhizomes are present.</p> <p><i>Lycopodium</i> is homosporous. <i>Selaginella</i> is heterosporous.</p> <p>In the <i>Selaginella</i> strobilus below, note the microsporangia and megasporangia.</p>	<p>Identify specimens of <i>Lycopodium</i> and <i>Selaginella</i> on demonstration. Identify microphyllous leaves (sporophylls), strobili, roots, and rhizomes. Label these structures on the diagram below.</p>
		

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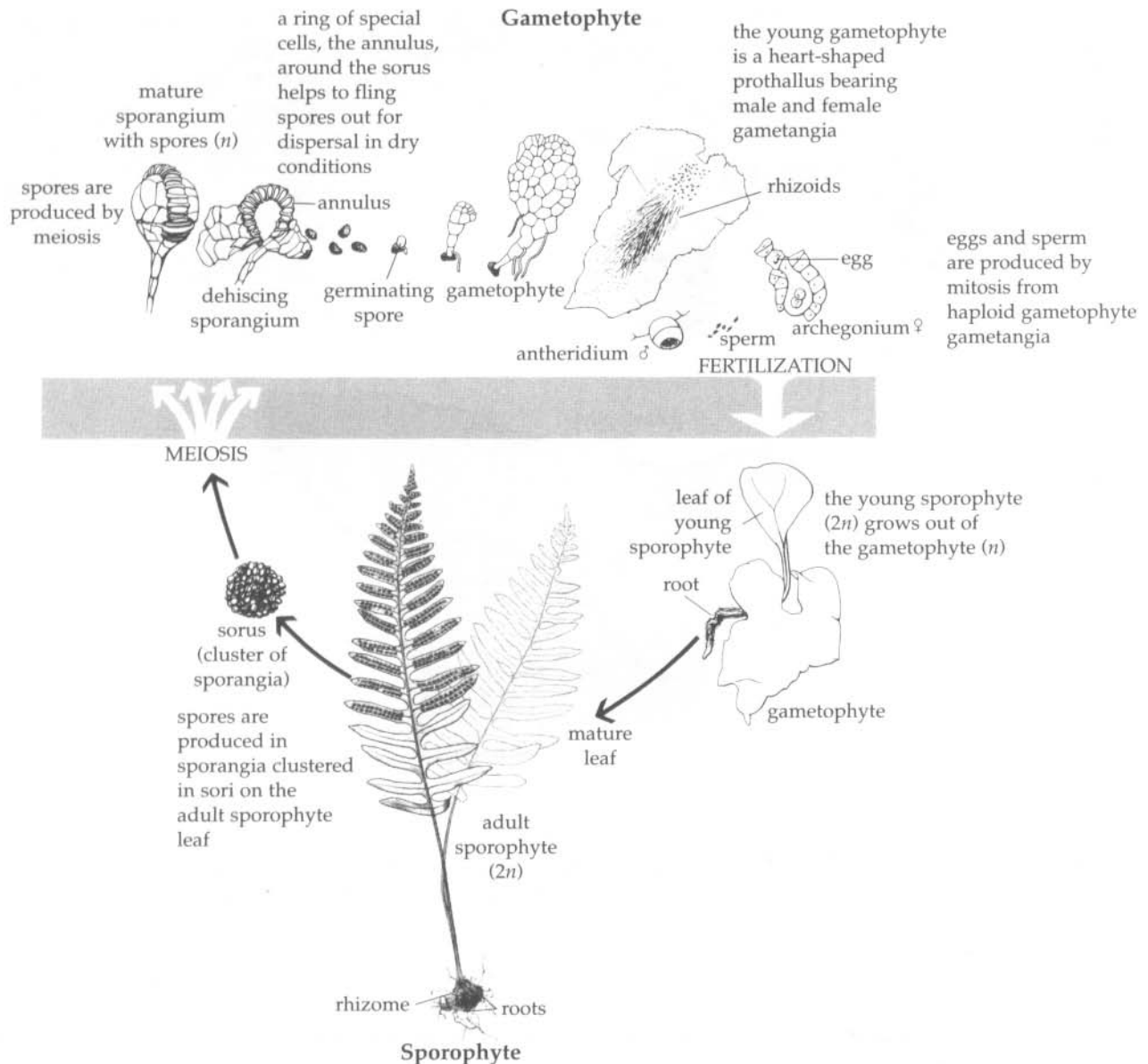


Figure 24A-2 The life cycle of the fern.

3. Prepare a wet mount of a living heart-shaped fern gametophyte (prothallus) or obtain a prepared slide of a young fern gametophyte (whole mount). Observe the specimen using the $10\times$ and $40\times$ objectives of your microscope. Identify the following parts and study their function as part of the fern life cycle (Figure 24A-2).

Rhizoids Specialized cellular filaments extending from the lower surface of the gametophyte into the substrate.

Antheridium Sperm are produced within the antheridia located on the lower surface of the gametophyte among rhizoids. When an adequate supply of water is present, mature antheridia burst and release sperm (Figure 24A-3a).

Sperm Flagellated male gametes produced in antheridia.

Archegonium This flask-shaped organ contains the female gamete (egg) and is located on the lower surface of the gametophyte (Figure 24A-3b). Sperm swim down the neck of the

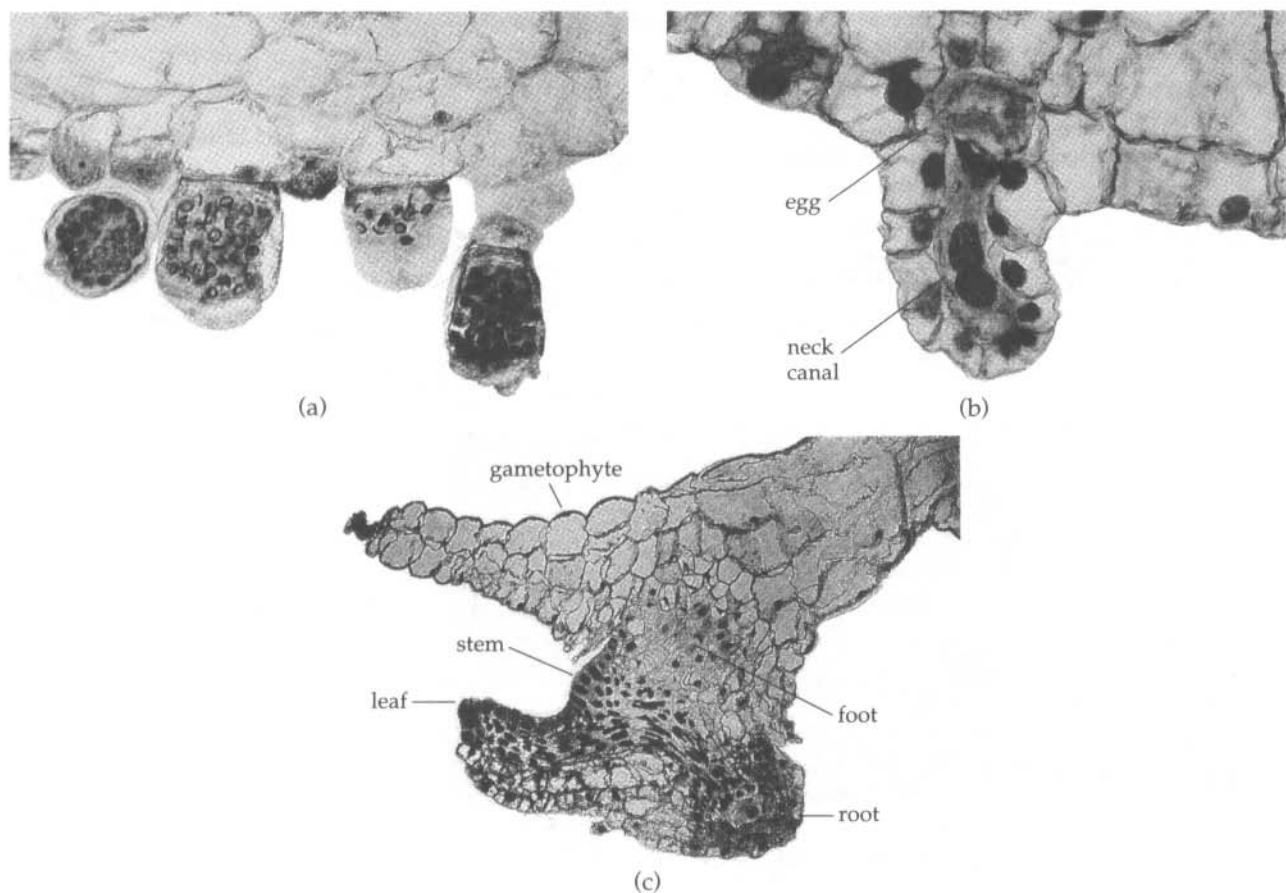


Figure 24A-3 (a) Antheridia with nearly mature archegonia on the prothallus of *Osmunda*. (b) Archegonia. (c) Young sporophyte growing out of the gametophyte.

archegonium to fertilize the egg, forming the zygote, which develops directly into the young embryo sporophyte.

Eggs Female gametes produced in archegonia.

c. How many cell layers are there in the young prothallus? _____

4. Obtain a prepared slide of a gametophyte with an attached sporophyte, or use living material (Figure 24A-3c). Fertilization has occurred several days earlier. The resulting sporophyte consists of one or more leaves and a root.

d. Is the new plant haploid or diploid? _____

e. What will be the fate of the gametophyte? _____

PART II TRACHEOPHYTES (VASCULAR PLANTS) WITH SEEDS—GYMNOSPERMS AND ANGIOSPERMS

What is the ecological advantage of a seed? Seeds provide a mechanism for a plant to survive during adverse environmental conditions, such as lack of water and extreme cold. Seed plants achieved prominence during a time when the earth's climate became colder and drier following the warm and humid Carboniferous period.

Gymnosperms are plants with “naked” or unprotected seeds, and **angiosperms** are plants with enclosed, protected seeds. In gymnosperms, seeds develop directly on the surface of the scales of the female cone; in angiosperms, seeds are enclosed in a fruit.

The evolutionary trend toward **sporophyte dominance** is most strikingly apparent among gymnosperms and angiosperms. In angiosperms, the mature female gametophyte is often as small as seven cells (one of which is the egg) and the mature male gametophyte may consist of only three cells (two of which are sperm). The inconspicuous gametophyte generation is completely dependent upon the sporophyte generation for nutrition.

Understanding the life cycles of heterosporous seed plants is made easier by first studying a generalized life cycle (Figure 24II-1). Keep in mind that the particulars may differ for individual gymnosperms and angiosperms.

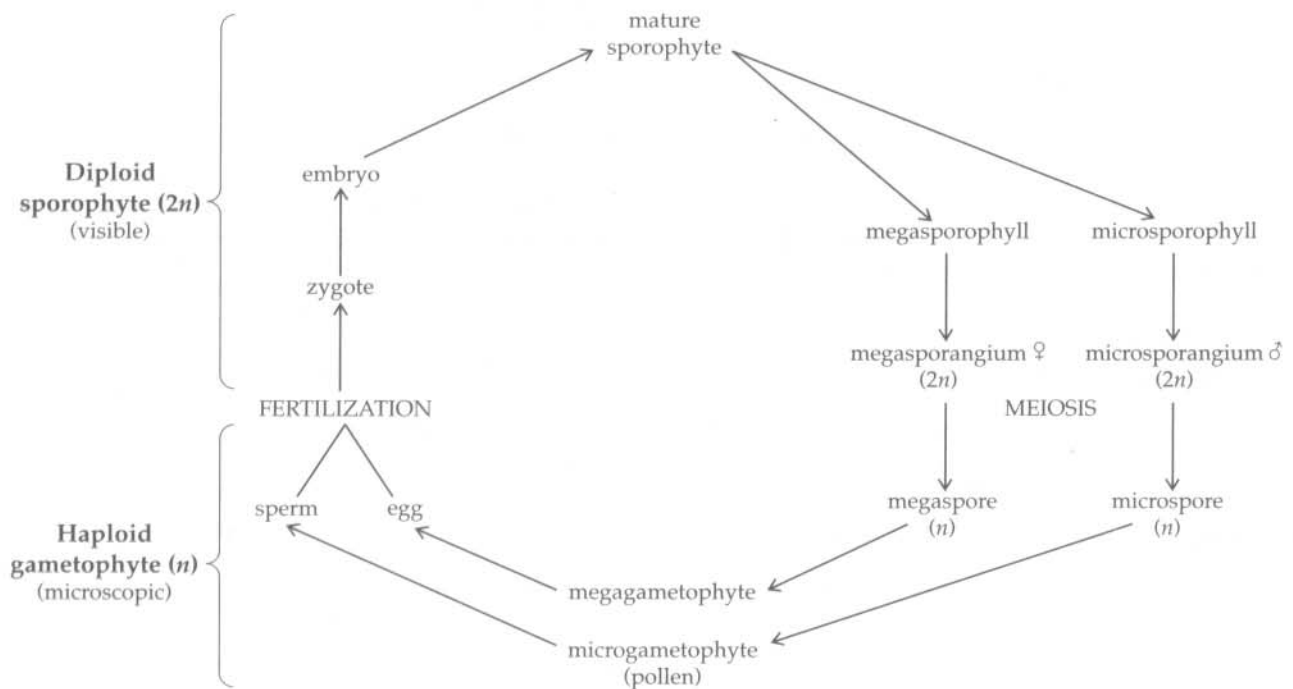


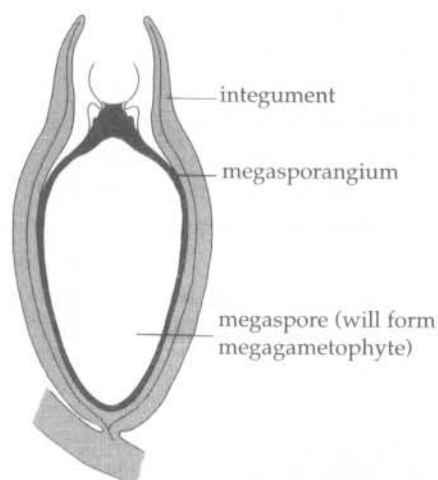
Figure 24II-1 Generalized life cycle of the vascular plants. This life cycle is characterized by an alternation of sporophyte and gametophyte generations.

The types of spores produced by heterosporous seed plants, called **megaspores** (n) and **microspores** (n), are produced by meiosis from **megasporangia** ($2n$) and **microsporangia** ($2n$). Within the megasporangium (*nucellus*), one megaspore divides many times to develop into the multicellular female gametophyte (*megagametophyte*) containing, as a rule, two archegonia. Inside each archegonium, an egg develops.

Microspores develop into the male gametophyte (**microgametophyte**). The mature microgametophyte is the **pollen grain**, which can be dispersed by the wind. After landing on an ovule, the pollen grain produces a special structure called a **pollen tube** that transports the sperm produced within the pollen grain into the vicinity of the egg. (In contrast to the nontracheophytes and seedless vascular plants, the heterosporous land plants do not require water for fertilization; thus, these plants thrive in a greater diversity of environmental conditions.)

The egg, developed from the megaspore, remains inside the female megasporangium (Figure 24II-2), often borne on a leaflike **megasporophyll** of the sporophyte plant. The fertilized egg develops into the young embryo within the megasporangium, which, in seed plants, is covered by one or two layers of

Figure 24II-2 Sectional view of the ovule of the ancient seed plant *Eurystoma angulare*, showing the spatial relationship of the integument, the megasporangium, and the megaspore.



protective tissue called **integuments**. The entire structure (megaspore within the megasporangium covered by integuments) is the **ovule**. The ovule develops into the **seed** with the integuments serving as the **seed coat**.

The seed, with its specialized structures for the nutrition, protection, and dispersal of the young sporophyte, is one of the most important innovations to arise among the land plants and is probably the major reason for their dominance.



EXERCISE B Gymnosperms

There are four phyla of **gymnosperms** with living representatives: the phylum Coniferophyta, conifers; phylum Cycadophyta, cycads; phylum Ginkgophyta, ginkgos; phylum Gnetophyta, gnetophytes.

Objectives

- ☐ Describe the relationship between microsporophyll, microsporangium, microspore, microgametophyte, and pollen.
- ☐ Describe the relationship between ovule, megasporangium, megaspore, and seed.

Procedure

1. Examine the branches and cones from different types of conifers on demonstration in your laboratory. Most conifers are large evergreen trees or shrubs with needlelike or scalelike leaves. Conifers can be identified by the length, appearance, and number of modified leaves held together in a single group attached to the stem. For each of the conifers you study, write a brief description that distinguishes it from the others (Table 24B-1). (*Note:* Your instructor may provide you with a dichotomous key to help you identify the conifers.

Table 24B-1 Types of Conifers on Demonstration

Conifer	Characteristics

2. Locate a male cone (staminate or pollen cone) from one of the conifers on demonstration.

a. How does it differ from the typical (female) pine cone? _____

Carefully remove one of the scales (**microsporophylls**) that aggregate to form the cone. Observe with a dissecting microscope. Note the two attached microsporangia (Figure 24B-1).

3. Place the microsporophyll in a drop of water on a slide and carefully crush it to release the **microspores** from the microsporangium. A mature microgametophyte is known as a pollen grain (Figure 24B-2).

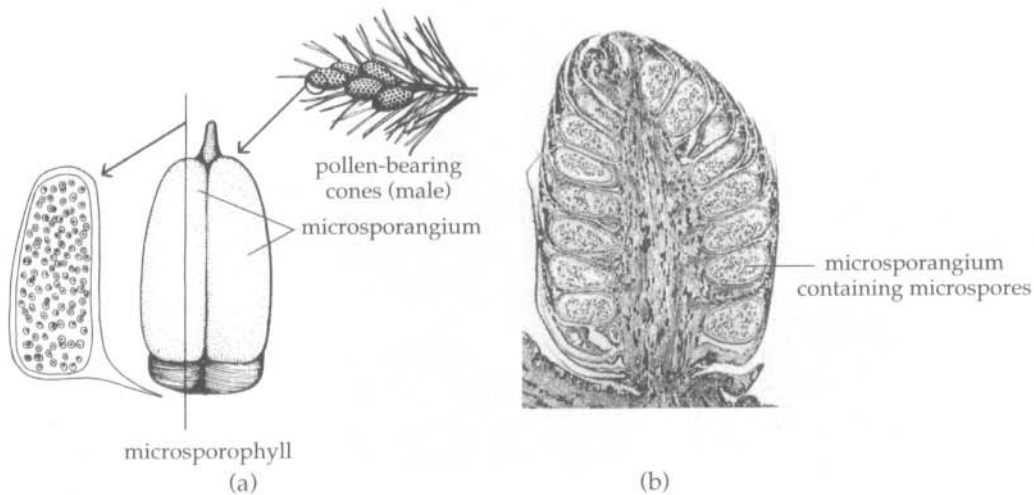


Figure 24B-1 (a) Microsporophyll with attached microsporangia containing developing microspores. (b) Longitudinal view of a pollen-producing cone, showing microsporophylls and microsporangia containing mature pollen grains.

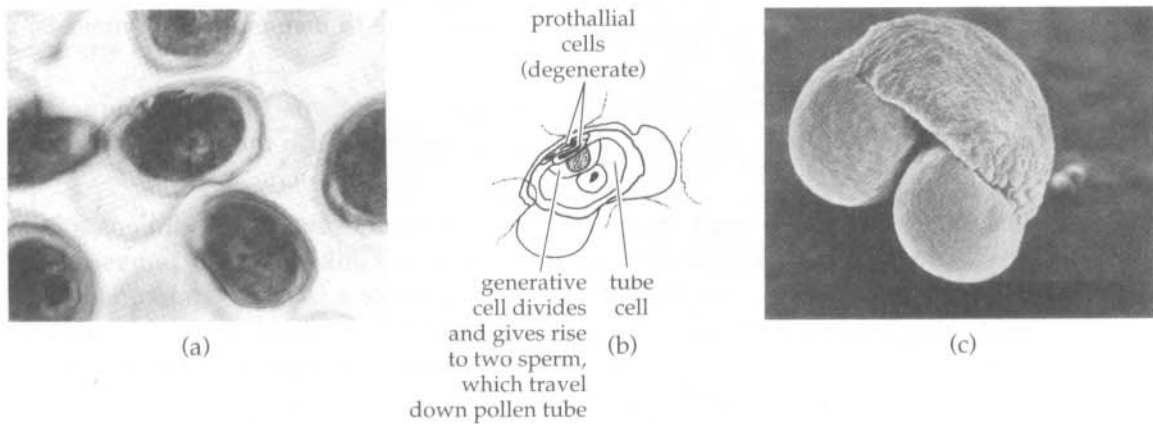


Figure 24B-2 (a) Immature pollen grain. (b) The pollen grain consists of several cells; the generative cell will eventually divide to give rise to sperm cells. (c) Scanning electron micrograph of a pine pollen grain. When the pollen grain germinates, the pollen tube emerges from the lower end of the grain, between the wings.

4. View a prepared slide of a longitudinal section of a staminate cone and look for microsporangia, microspores.
5. Examine a female cone (ovulate or seed cone) and locate the ovules (Figure 24B-3). The seeds of gymnosperms are said to be naked because they are produced on the surface of scales constituting the ovulate cone. At maturity, each scale bears two seeds or ovules containing embryos. Locate and examine a seed.

b. How are conifer seeds dispersed? _____

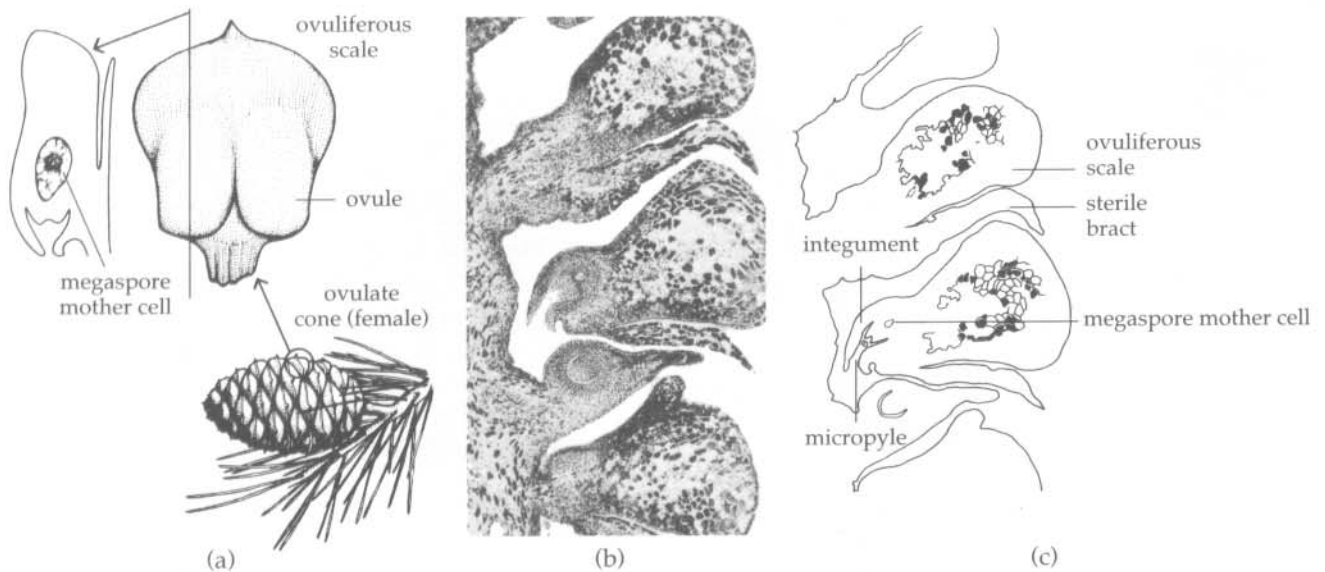


Figure 24B-3 (a) Ovules on the scale of an ovulate cone. (b) Cross section of ovuliferous scales. (c) The megaspore mother cell divides to form the megaspores. The micropyle opening allows the pollen tube to enter so that fertilization can occur later.

6. View a prepared slide of a longitudinal section of an ovulate cone. The **ovule** consists of **integuments** containing the **megasporangium** (Figure 24B-3). The **megaspore mother cell** develops within the megasporangium and gives rise to **megaspores** by meiosis. Locate these structures on your slide.
7. *Optional.* If assigned by your instructor, review the life cycle of pine in Figure 24B-4. Write the letter that follows each structure described in the caption in the appropriate box in the life-cycle diagram.
8. Examine living and preserved cycads (phylum Cycadophyta) and ginkgos (phylum Ginkgoophyta) on demonstration in the laboratory. Phylum Ginkgoophyta is represented by only a single living species, *Ginkgo biloba*. This tree was once a favored landscape planting for city sidewalks. However, the seeds have a fleshy outer layer that contains a foul-smelling compound, butyric acid. The putrid, slimy messes (not to mention the smell) created by the prolific production and dropping of seeds onto city sidewalks soon discouraged the planting of female *Ginkgo* trees. You will find the most recently planted *Ginkgo* trees are male.

Fewer than a dozen living genera of Cycadophyta exist today; they occur naturally only in the tropics or subtropics. Note the fernlike leaves and short, thick stem. Also study the cones if present. Cycads and ginkgos are *dioecious*—pollen and seed cones are found on different plants. Look for ovules in female cones and microsporangia in male cones.

c. How do representatives of these other gymnosperm phyla resemble the conifers? _____

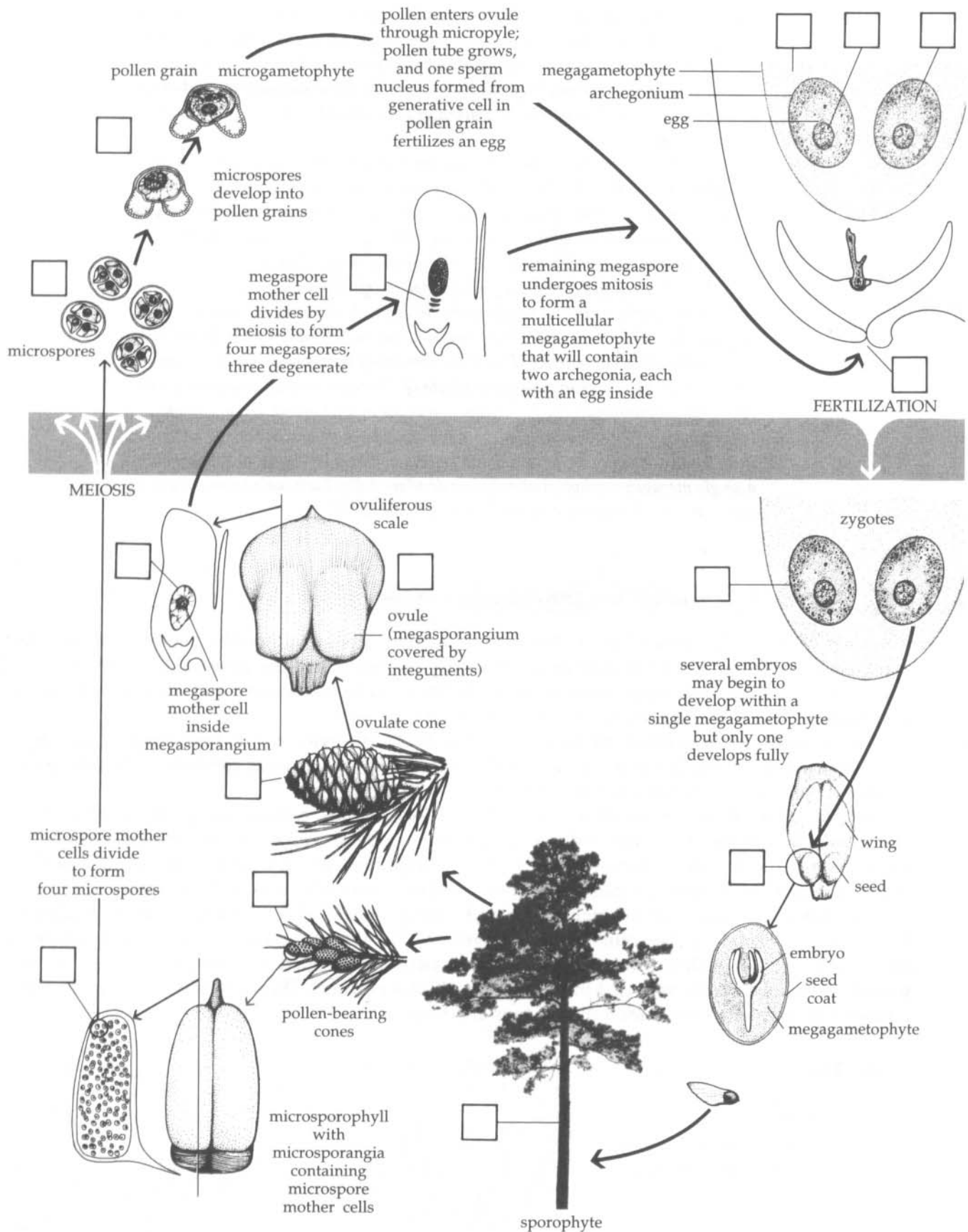


Figure 24B-4 Beginning at the bottom of the diagram: The *immature sporophyte* (gymnosperm seedling) develops into the *mature sporophyte*

(a). **Male cones (b)** are usually produced in clusters of up to 50 or more at the tips of low branches. The male cones produce pollen within the **microsporangia (c)** that occur in pairs on the microsporophylls (leaves) of the cone. Microspore mother cells within the microsporangia produce **haploid microspores (d)** that develop into four-celled immature **pollen grains (e)**. At this stage, the pollen grains are shed.

After landing in the vicinity of a micropyle of a female cone, one of the cells of the pollen grain, the tube cell, will develop into a pollen tube during the germination of the pollen grain. Another of the cells will divide to form two sperm cells as the pollen grain matures. A mature pollen grain is the mature male microgametophyte. **Female cones (f)** are generally larger than male cones and consist of woody scales containing paired **ovules (g)**. The ovule is a megasporangium covered by integuments. Inside each megasporangium is a **megaspore mother cell (h)**, which produces four haploid megaspores by meiosis. Three of these cells degenerate (**i**) and the remaining one divides mitotically to produce a multicellular **megagametophyte (j)** that will contain two **archegonia (k)**, each containing an egg (**l**). Pollen enters the ovule through the micropyle (**m**) opening in the integument. One sperm nucleus unites with the egg to fertilize it. Generally, all eggs are fertilized and several embryos (**n**) begin to develop within a single megagametophyte, but only one develops fully. Each scale bears only two seeds (**o**), which separate and are dispersed by the wind.



EXERCISE C Angiosperms (Phylum Angiospermae)

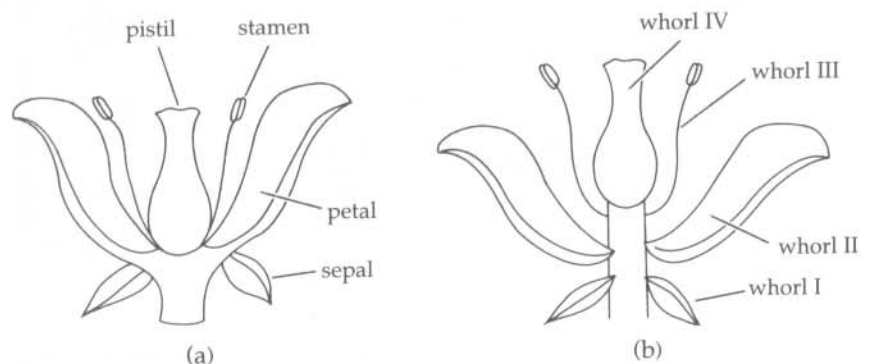
Angiosperm means “covered seed.” Following fertilization, seeds produced by angiosperms develop from ovules and are covered by a structure called the **ovary** located within the **flower**. The ovary eventually develops into a **fruit** containing one or more seeds. Enclosed seeds, flowers, and fruits are all unique to angiosperms, which are, at present, the dominant plants on earth.

Angiosperms can be divided into two classes: the **dicots** (dicotyledons) and **monocots** (monocotyledons). These names refer to the fact that the dicot embryo has two seed leaves or **cotyledons** (leaflike parts of the embryo), while monocot embryos have only one.

Among monocots are the familiar grasses, lilies, irises, orchids, cattails, and palms. Dicots include many herbs and almost all shrubs and trees (other than conifers). The many distinctions between monocots and dicots will be discussed during later laboratories, which examine the structure of the leaves, stems, and roots of angiosperm plants. Here, you will turn your attention to the flower.

A **flower** can be considered a highly modified and specialized shoot: a stem tip where modified leaves, the petals and sepals, occur “bunched together.” Floral parts and leaves have many similarities. Working from the outside toward the inside, similar parts are grouped as whorls (Figure 24C-1). Monocots usually have flower parts arranged in threes (or multiples of three). Dicots usually have flower parts arranged in fours or fives (or multiples of four and five).

Figure 24C-1 (a) Generalized flower parts. (b) Similar parts are grouped in whorls. In monocots, whorled structures occur in groups of three. In dicots, they occur in groups of four or five.



■■■■ Objectives ■■■■

- ☐ Distinguish between monocot and dicot flower types.
- ☐ Describe the structures of the male and female parts of a flower.
- ☐ Differentiate between pollination and fertilization.

■■■■ Procedure ■■■■

1. Obtain a flower from those provided. *a. Is your flower a monocot or dicot?* _____
2. Identify the parts of the flower in the following list. Locate as many parts as you can by simply looking at the flower. Then, using forceps and a dissecting microscope, start at the first whorl of the flower and work inward, locating each of the parts. Label these structures on the flower diagram (Figure 24C-2). Be sure that you understand the function of each part.

Peduncle Narrow stalk that bears a flower or group of flowers (**inflorescence**). The stalk of an individual flower in an inflorescence is called a **pedicel** (Figure 24C-3).

Receptacle Part of the flower stalk to which floral parts are attached.

Figure 24C-2 Parts of a flower.

Note: The receptacle is located at the base of the sepals and is not visible in this figure.

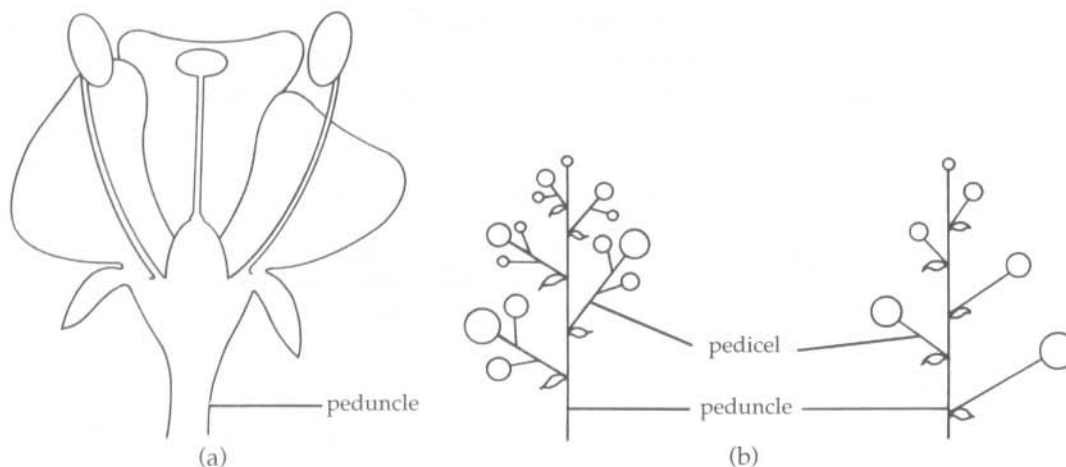
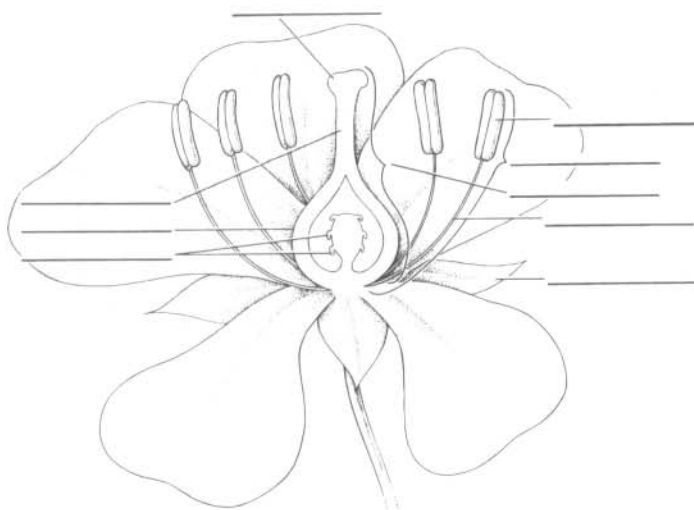


Figure 24C-3 (a) A single flower attached at the peduncle. (b) Inflorescences supported by peduncles; each flower is attached to the peduncle by a pedicel.

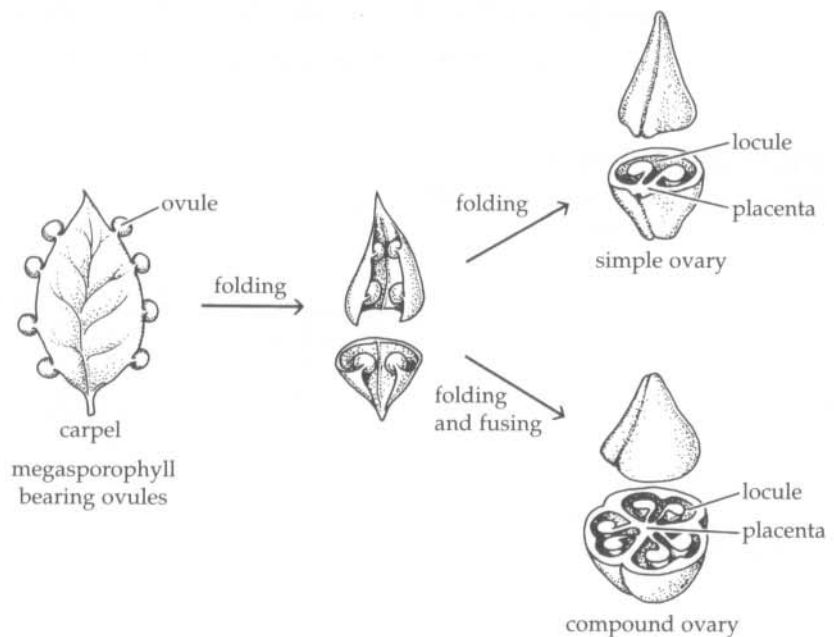
Sepals Outer leafy parts of the flower collectively called the **calyx**. Sepals usually enclose the outer flower parts, protect the bud, and later surround the ovary.

Petals Often brightly colored, forming the conspicuous inner whorl of flower parts. Collectively, petals are called the **corolla**.

Stamens Produce pollen grains, the male gametophytes. The gametes, sperm, are not produced until after pollination, as in gymnosperms. Stamens are actually microsporophylls consisting of a **filament** and a two-lobed **anther** containing the microsporangia.

Carpels Carpels may be individual (free) or fused. The pistil (composed of one or more carpels) is differentiated into a lower part, the **ovary**, and an upper part, or **stigma**, which receives pollen. The **style** connects the ovary to the stigma. The portion of the ovary to which the **ovules** are attached is called the **placenta** (Figure 24C-4).

Figure 24C-4 Presumed evolutionary development of simple and compound ovaries. A leaf-shaped carpel with ovules along its edge (left) folded in on itself, and the edges fused to form a simple ovary. Compound ovaries were formed by the fusing of separate infolded carpels.



In angiosperms, pollination results in **double fertilization**: one sperm nucleus fuses with the egg cell to form a $2n$ **zygote** and the other sperm nucleus fuses with the two polar nuclei to form a polyploid (usually $3n$ or $5n$) **endosperm**. Endosperm provides a source of stored energy for the developing embryo. The fertilized ovule then develops into a seed. In monocots, endosperm persists, forming a food reserve to be used during germination. Most dicots absorb all of the endosperm during embryo development, storing nutrients in fleshy cotyledons for use during germination.

- Examine pollen grains by tapping the anther of a flower over a drop of sucrose solution on a microscope slide. Add a coverslip and examine your preparation, first using low power ($10\times$ objective) and then high power ($40\times$ objective). Keep this slide until the end of the laboratory period (do not let it dry out). Before you leave, check to see if any of the pollen grains have produced pollen tubes.

b. Why did you mount the pollen in sucrose solution rather than in water alone?

- Optional. If assigned by your instructor, study the life-cycle diagram for angiosperms in Figure 24C-5. Write the letter that follows each structure or process described in the caption in the appropriate box in the diagram.

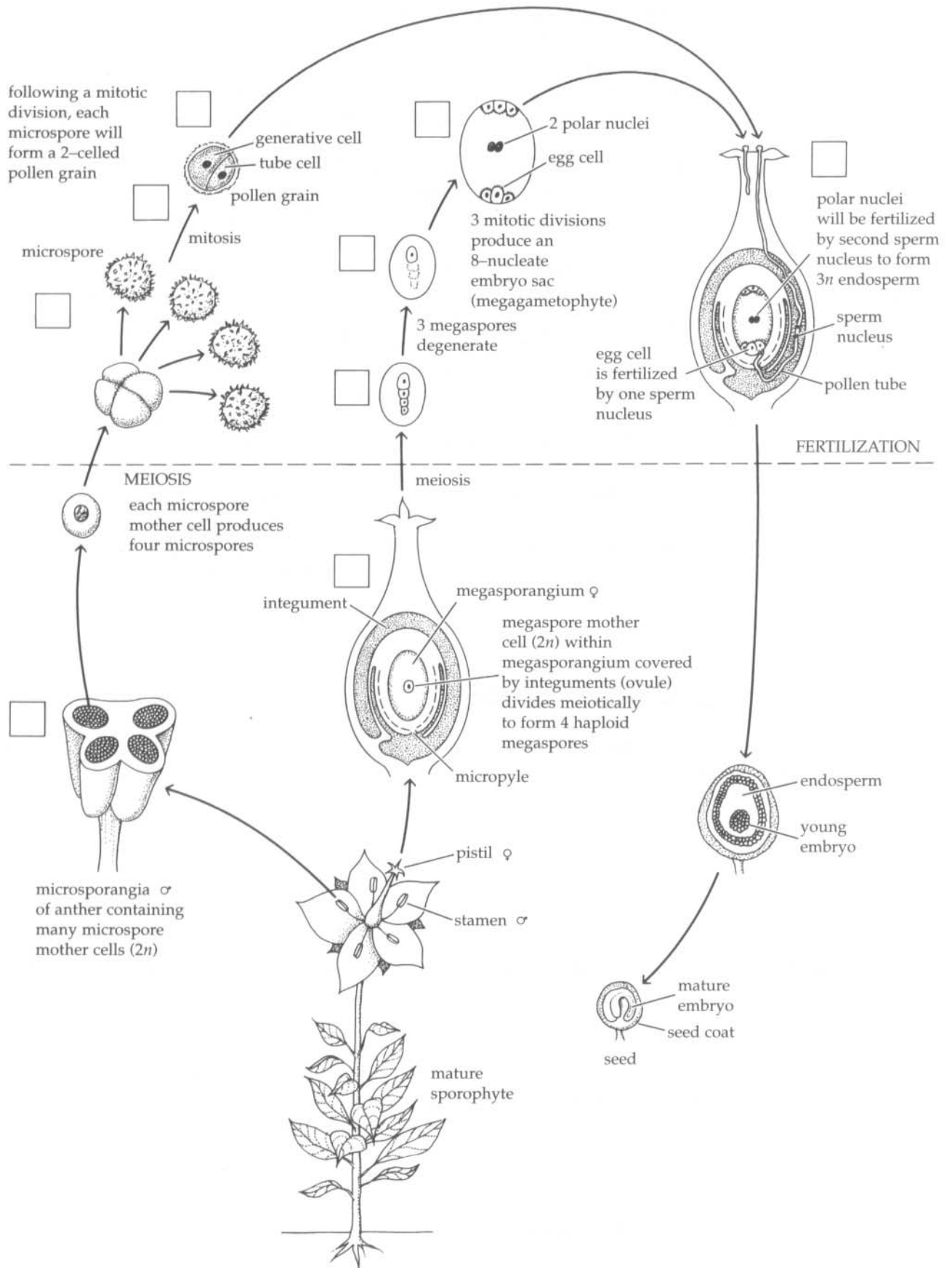


Figure 24C-5 (page 24-15) Beginning in the center of the diagram: Within the **ovary** of the flower pistil, one or several **ovules** are attached to the ovary wall. Each of these ovules contains a megasporangium covered by **integuments** (**a**). Inside the megasporangium is the **megaspore mother cell** which produces four **megaspores** by meiosis (**b**), three of which degenerate (**c**). The remaining megaspore enlarges and undergoes three mitotic divisions to form eight nuclei. Of these, one nucleus develops a cell wall and becomes the egg; upon fertilization, two other nuclei (polar nuclei) will develop into a food source for the embryo (**d**). This structure represents the mature female gametophyte—the microscopic gametophyte generation in the sporophyte-dominated life cycle of seed plants.

Microsporogenesis takes place within the **anther** of the flower. Microspores are produced by meiosis within the pollen sacs (microsporangia) of the anther (**e**). Each microspore develops a resistant outer wall and the specialized structures common to **pollen grains** of that species (**f**). The microspore also divides mitotically (**g**) to form two cells, the **tube cell** and the **generative cell** (**h**). Most pollen grains are at this stage when released. A mature pollen grain containing these two cells represents the male **microgametophyte**—one of the only visible parts of the gametophyte generation in the sporophyte-dominated life cycle of angiosperms.

Pollination occurs when wind or insects carry the pollen to the stigma of the flower. After pollination, the tube nucleus forms a **pollen tube**. The pollen tube grows down through the style of the pistil and enters the ovary and then the ovule through an opening in the integuments (**micropyle**) (**i**). Fertilization can then occur.

Laboratory Review Questions and Problems

1. Compare the life cycle of a moss (Laboratory 23) with that of a fern. Which is the visible generation of each?
2. How do the tracheophytes (vascular land plants) differ from the nontracheophytes?
3. Do whisk ferns, club mosses, horsetails, and ferns have vascular tissue? Seeds? Based on your answer, how do we group these phyla in terms of a general category?
4. In what group of land plants do we first observe true roots, stems, and leaves?

5. What three major advances are obvious among the vascular plants with seeds?
6. Are ferns heterosporous or homosporous? _____ Gymnosperms?
 _____ Angiosperms? _____
7. In the table below, indicate whether each structure is haploid (n) or diploid ($2n$), gametophyte or sporophyte.

Structure	n or $2n$	Gametophyte or Sporophyte
Fern archegonium		
Moss antheridium		
Fern leaf		
Moss "leaflet"		
Pollen grain of pine		
Megagametophyte of angiosperm		
Microsporangium of angiosperm		
Pine tree		
Flower		

8. Flowers play an essential role in sexual reproduction of angiosperms. What types of insects or animals pollinate flowers? _____ What do these flowers look like?
 _____ What types of plants are pollinated by wind? _____