Published 1980

2 Reproduction and Seed Development

Nels R. Lersten
Iowa State University
Ames

Cultivated species comprise less than 1% of about 300,000 species of flowering plants; nevertheless, they are quite diverse in their reproductive structures and behavior. This chapter deals mostly with the general life cycle of angiosperms and discusses some differences between dicots and monocots and individual species. It provides the background needed to understand the structures, reproductive processes, and problems of hybridization in the individual crop species discussed in later chapters. The account is based on some broad works (Davis, 1966; Frankel and Galun, 1977; Hayward, 1938; Heslop-Harrison, 1972; Maheshwari, 1950; Palser, 1975), as well as more specialized references that will be cited in appropriate sections.

I. THE INFLORESCENCE

Most plants have many flowers clustered in a predictable pattern called an inflorescence. Most crop plants have one of two basic types, the cyme or the raceme.

The cyme is a determinate inflorescence consisting of primary, secondary, tertiary, and often additional floral branches (Fig. 1). The terminal flower on the primary stem develops first, then secondary floral branches develop below it. Each secondary branch has a terminal flower that develops first, then tertiary branches develop below. This branching process continues, thereby expanding the inflorescence and extending the flowering duration of the plant. The cyme is present in potato, for example (Fig. 2).

A more diverse group of inflorescences is based on the raceme, in which flowering begins at the base and proceeds toward the top. The arrows in Fig. 1 do not imply an evolutionary pathway for inflorescence types, but

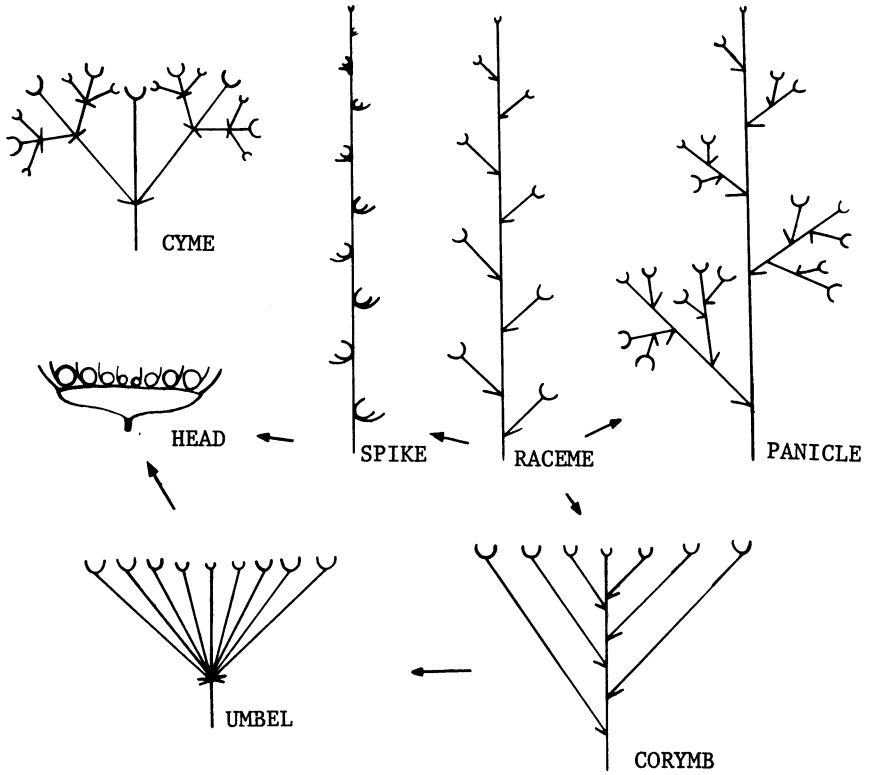

Fig. 1—Common types of inflorescences found in cultivated plants: cyme and various forms of the raceme. Types of racemes include the branched raceme (panicle), raceme with sessile flowers (spike), raceme with pedicels unevenly elongated to produce a flat-topped cluster (corymb), pedicels emerging from a common level (umbel), and umbel with crowded, sessile flowers (head). The arrows are not meant to imply the evolutionary pathways for inflorescence types, but are primarily to show how these types can be derived from a raceme.

show how the various types can be derived from a raceme. Figure 3 shows that a typical raceme consists of a primary stalk (peduncle) with flowers along its length borne on short stalks (pedicels). The soybean has a typical raceme, but various lengths and number of flowers can be found among cultivars and in different environments.

The panicle of oat is a branched raceme (Fig. 4). The spike of barley, wheat, triticale, and many other grasses is a raceme whose flowers are sessile; they are borne on such short pedicels that they seem to be attached directly to the primary stalk (Fig. 5). The spikelet, not the multiple florets that may occur within a spikelet, is the basic unit of the grass inflorescence. The corymb (Fig. 1) is a raceme with pedicels of unequal length that produce a flat-topped cluster of flowers. This type does not occur in cultivated plants, although a corymbose raceme is found in crambe and certain other mustards. The simple umbel has pedicels that elongate from a common point, such as in onion (Fig. 6). An inflorescence of multiple umbels occurs in carrot and other cultivated Umbelliferae. The head (capitulum) may be thought of as an umbel with crowded, sessile flowers like those in sunflower (Fig. 7).

Fig. 2—Inflorescence of potato, an example of a cyme.

Fig. 3—Raceme of sweetpea.

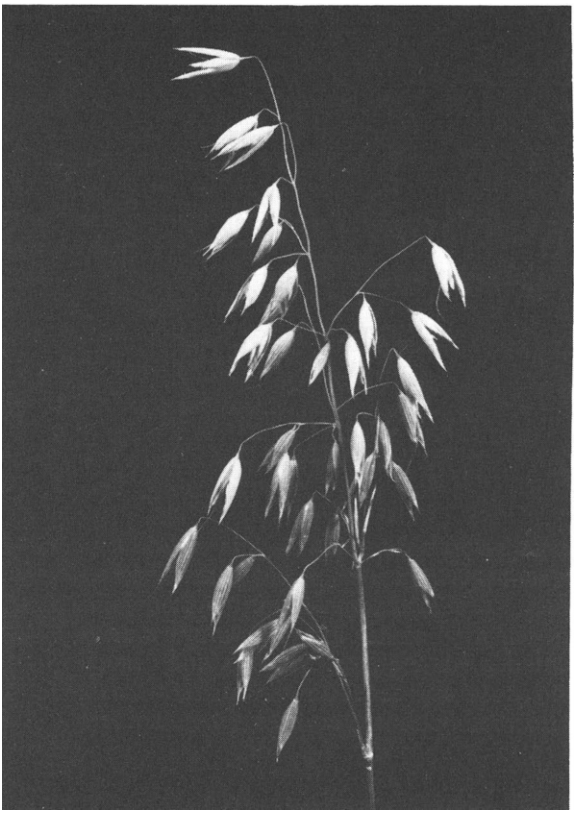

Fig. 4—Panicle of oat.

II. THE FLOWER

A. General Morphology

Flowers of crop plant species differ from each other in size, numbers of appendages, and in other ways. Many species have flowers that consist of a number of leaf-like sepals that compose the calyx, colorful petals that form the corolla, the female pistil (gynoecium), and the male stamens (androecium). The pistil consists of one or more carpels, each carpel having an ovary, style, and stigma. The stamen is composed of a filament and anther. The sepals, petals, carpels, and stamens vary in number and may be separate or fused. In grass species, the calyx and corolla are replaced by glumes, of varying prominence, which embrace the spikelet, and a lemma and palea that enclose the pistil and stamen of each floret within the spikelet.

Flowers of cultivated legumes such as soybean, cowpea, and alfalfa have flowers similar to each other (Fig. 8). The calyx consists of five sepals fused to varying degrees. The corolla includes a large standard petal, two

Fig. 5—Spikes of triticale (left) and hexaploid wheat (right).

small wing petals, and two fused petals collectively called the keel. The pistil has a single carpel whose ovary contains ovules that vary in number among species. The stigma-tipped style varies in shape and length among species. For example, the style of the common bean is spiral and has short hairs near the stigma, whereas the style of soybean is curved and lacks hair. Legume flowers have 10 stamens, 9 in one group with fused filaments, and 1 that is free (Fig. 9). Nectar glands at the base of the ovary make the flowers of many legume species attractive to bees.

A spikelet, the floral unit of a grass inflorescence, consists of a pair of glumes that embrace one or more florets (Fig. 10). Glumes of oat are prominent and enclose several florets, but the glumes of rye are small and at the base of a single floret (Fig. 10). Each floret consists of a lemma that commonly bears an own and a palea (Fig. 10). The grass pistil has an ovary containing one ovule, and a short style with a branched, feathery stigma. Most grasses have three stamens; rice, however, is an example of a grass species with six stamens.

Cross-pollination, also called allogamy, is assured in crops with dioecious plants, such as hemp. A high frequency of cross-pollination also occurs

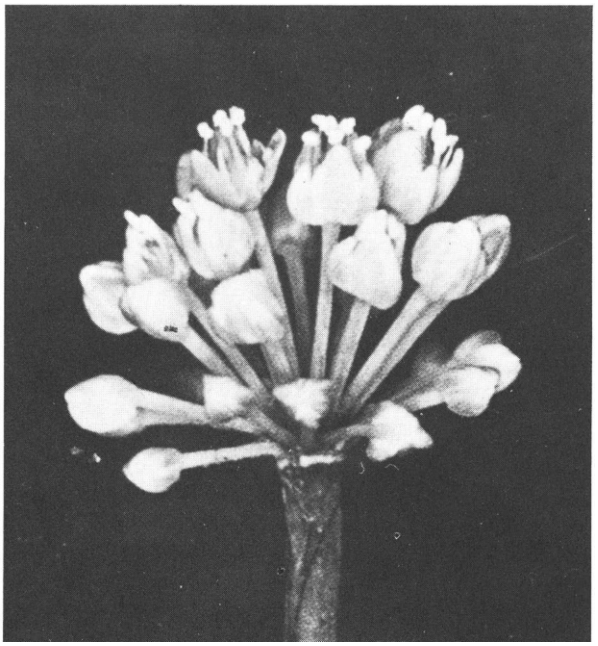

Fig. 6—Simple umbel of wild onion.

Fig. 7—Head (capitulum) of sunflower.

Fig. 8—Intact flower of soybean, a representative legume.

among monoecious plants (Fig. 11), those with pin and thrum flowers (Fig. 12), and those with protandry or protogyny. Self-pollination, referred to as autogamy, is promoted in perfect flowers that are cleistogamous, such as in oat and soybean.

The stamens (♂) and the pistil (♀) are the structures directly involved in sexual reproduction. They occur in various arrangements and may mature at different times (Lloyd and Webb, 1977). Several sets of terms exist to describe the possibilities (Frankel and Galun, 1977). The more common ones are:

A. ♂ and ♀ expression in individual flowers
   1. ♂ and ♀ in one flower: bisexual, hermaphroditic, monoclinous, perfect
      a. pollen shed before stigma is receptive: protandry
      b. stigma matures and ceases to be receptive before pollen is shed: protogyny
      c. stigma receptive, and pollen shed, after flower opens: chasmogamy
      d. stigma receptive, and pollen shed, in closed flower: cleistogamy
   2. Perfect flowers of two types on same plant.
      a. long styles and short stamens: pin flower
      b. short styles and long stamens: thrum flower
   3. ♂ and ♀ in separate flowers: diclinous, unisexual, imperfect
      a. ♂ flower: male, staminate
      b. ♀ flower: carpellate, female, pistillate

B. Flower distribution on plants
   1. ♂ and ♀ flowers on one plant: monoecious
   2. ♂ and ♀ flowers on separate plants: dioecious
   3. ♂, ♀, and perfect flowers: mixed, polygamous
      a. on same plant: polygamomonoecious
      b. on separate plants: polygamodioecious

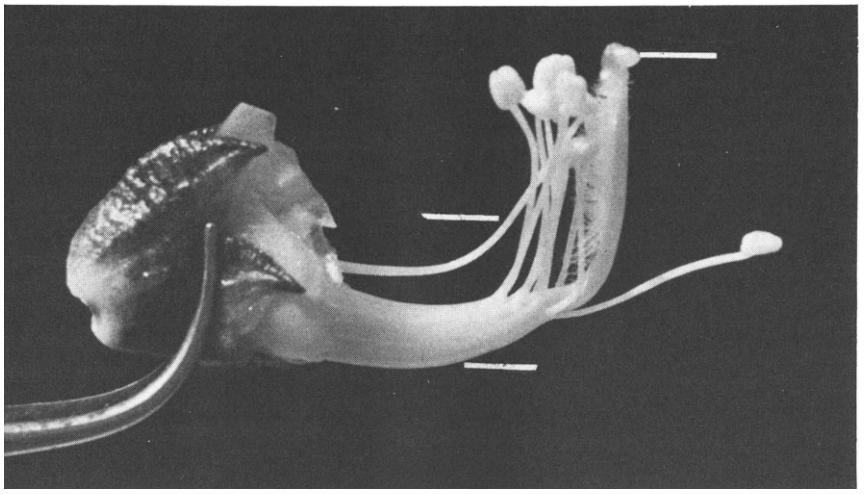

Fig. 9—Cowpea flower with calyx and corolla removed. Upper line indicates stigma at tip of style, middle line touches the solitary stamen, and lower line points to the fused staminal sheath of the other nine stamens.

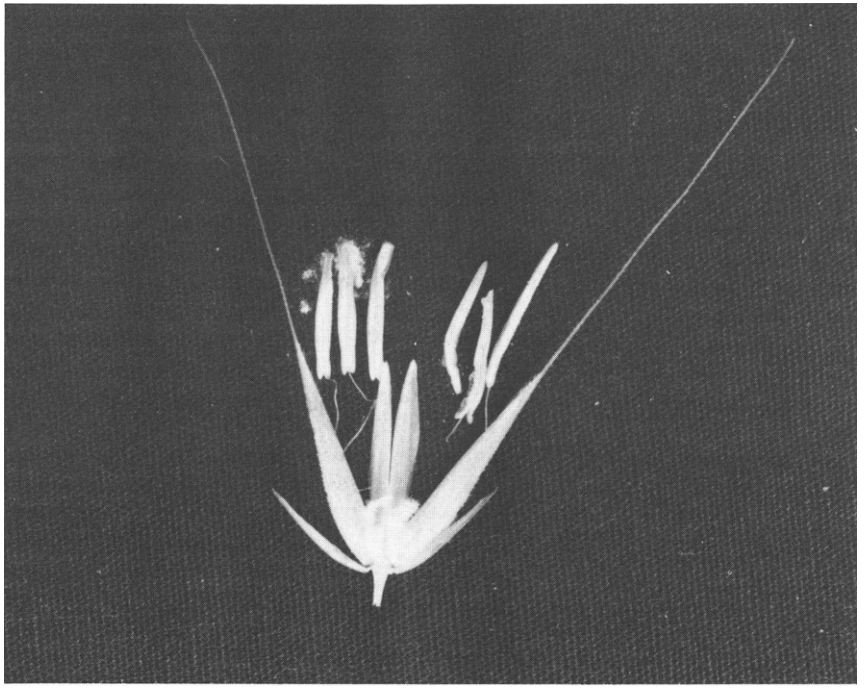

Fig. 10—Spikelet of rye.

Fig. 11—Monoecious inflorescences in corn. Left: terminal male inflorescence. Right: Axillary female inflorescence.

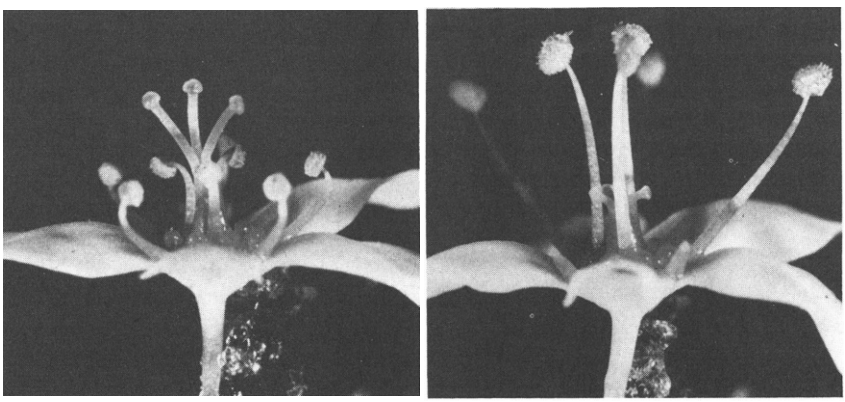

Fig. 12—Pin and thrum flowers of buckwheat. Left: Pin flower with long style and short stamens. Right: Thrum flower with short style and long stamens.

B. Stamen and Pollen Development

A stamen consists of a sterile filament bearing an anther. Four cylindrical pollen sacs (locules) can be first distinguished within the anther as four separate rows of densely cytoplasmic cells (Fig. 13). Cell division continues in each locule until a certain number of microspore mother cells have formed. The microspore mother cells undergo meiosis to form microspores, which develop into pollen. The locules are surrounded by a nurse layer called the tapetum (Fig. 13). There are three to five additional cell layers between the tapetum and epidermis, and a few to many parenchyma cells

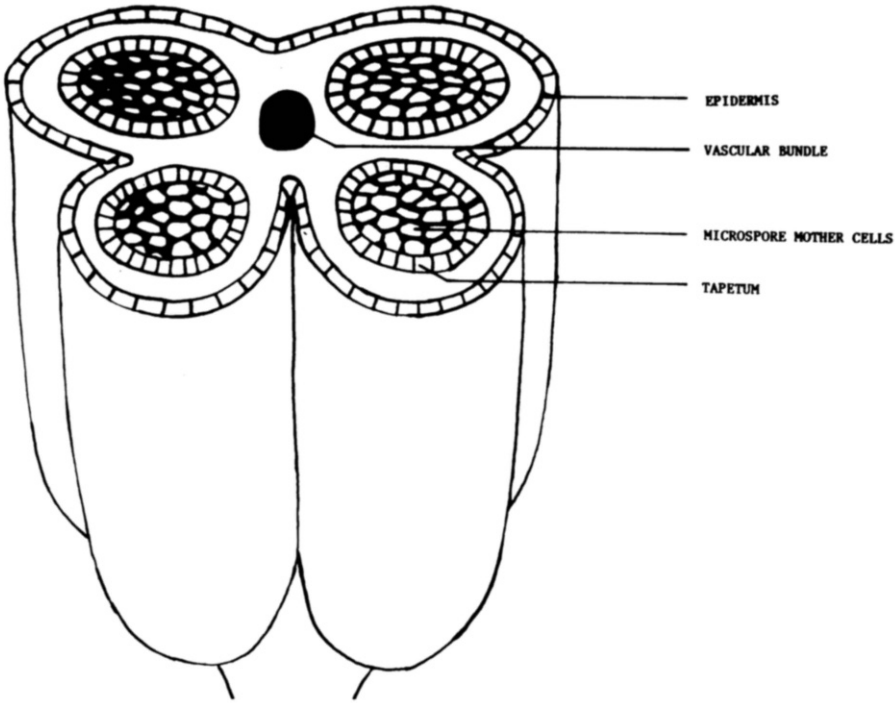

Fig. 13—Three-dimensional view of an anther with upper half removed to show four pollen sacs, each at the microspore mother cell stage.

which serve as connective tissue between the tapetum and the single vascular bundle of the stamen.

Cultivated species differ in stamen length, size of the anther, number of microspore mother cells, and amount of connective tissue. Grasses are unusual because each microspore mother cell remains in contact with the tapetum throughout development, until the mature pollen is shed.

Each microspore mother cell secretes around itself a gelatinous sheath made up of a carbohydrate called callose. This substance isolates each microspore mother cell during meiosis and for some time thereafter. Opinions differ as to why the callose coating is needed.

The tapetal cells of most plants become binucleate before, or during meiosis. In some species, however, tapetal cells become multinucleate through repeated nuclear mitosis. Dandelion may have up to 16 nuclei per tapetal cell. In all legumes, in contrast, the tapetum remains uninucleate.

The tapetum remains as a recognizable layer, even after it loses its cell walls. In most dicots, the tapetum remains in place at the periphery of the locule (parietal tapetum), while in most monocots it grows inward shortly after meiosis and appears to engulf the immature pollen grains (invasive tapetum). There are, however, many exceptions; e.g., sunflower (a dicot) has an invasive tapetum, whereas all grasses (monocots) that have been studied have a parietal tapetum. The tapetum is thought to contribute to pollen growth in one or more ways, depending on the species.

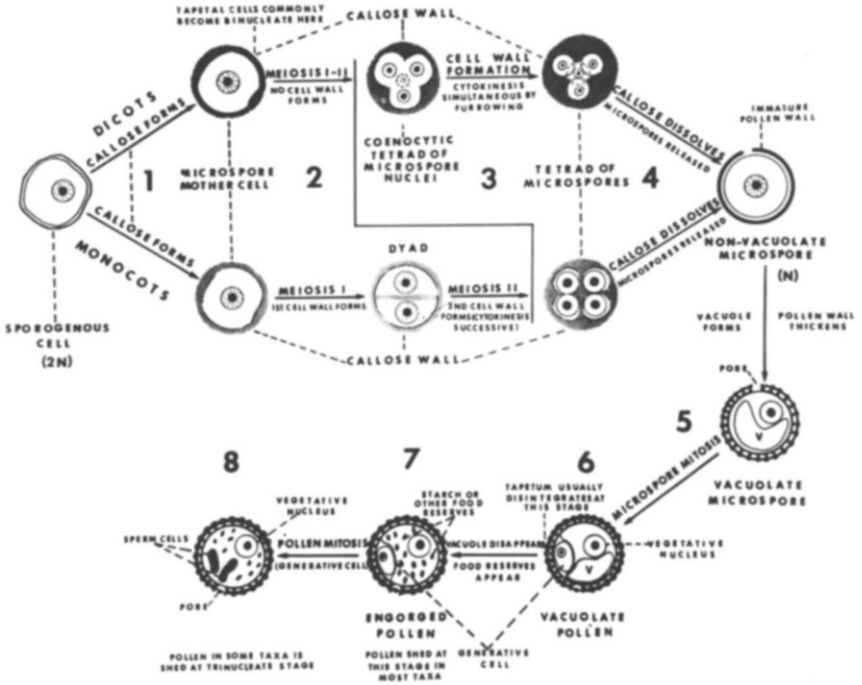

Fig. 14—A diagrammatic scheme showing general features of pollen development of representative monocot and dicot pollen. The numbers delimit successive stages from microspore mother cell (stage 1) to mature pollen (stages 7, 8). From Laser and Lersten (1972).

Each microspore mother cell undergoes the two divisions that constitute meiosis, resulting in four haploid nuclei and, eventually, four pollen grains (Fig. 14). Stages 1 to 4 in Fig. 14 show differences that often occur in dicot and monocot pollen development. Cell walls in dicots do not ordinarily form until after meiosis II. Thus for a short period the four haploid nuclei are in a common cytoplasm. The two walls form simultaneously to delimit the microspores. In monocots, a new wall forms after each meiotic division, so that a cellular dyad stage is followed by a cellular tetrad.

The young microspores, still embedded in callose, begin using food reserves to build the future pollen wall. While this wall is still immature, the callose dissolves and the microspores are released into the fluid of the pollen sac. As the pollen wall thickens, each microspore enlarges and becomes more vacuolate. After the large central vacuole develops, the microspore nucleus divides mitotically (Fig. 14). After one nucleus migrates to the wall, a thin cell wall forms around it and a bit of cytoplasm to delimit the generative cell. The other nucleus remains in the larger cell, now called the vegetative cell. Most pollen is shed in the two-celled condition but, in some plants, most notably grasses, the generative cell divides again and pollen is shed with two sperm cells already formed. Each of these is a true cell with a nucleus, cytoplasm, and cell wall.

The already wall-less tapetum commonly disintegrates completely after the vegetative and generative cells are formed. At the same time, the pollen grains begin to fill with food reserves. Oily, proteinaceous substances from the tapetum may be deposited on the pollen wall and these may be important later when pollen interacts with the stigma.

A mature pollen grain wall has an inner layer (intine), and an outer layer (exine) of considerable complexity in which subdivisions can be seen. The outer part of the exine commonly has a sculptured pattern of ridges, mounds, large or small spines, or other features. These occur singly or in various combinations (Figs. 15 and 16). In addition, the exine fails to form in one or more places, creating pores or furrows which expose the underlying intine (Fig. 16). Grasses and most other monocots have only one pore or furrow (Fig. 15) whereas most dicots have three (Fig. 16). Sugarbeet and spinach, in contrast, are examples of dicot pollen with many pores. These thin places allow the intine to bulge out at the time of germination and become the cell wall of the pollen tube.

Further information on pollen development and mature structure can be found in Heslop-Harrison (1971), Linskens (1974), Mascarenhas (1975), and Vasil (1973).

C. Ovule and Embryo Sac Development

The ovule-bearing or seed-bearing part of a flower is the pistil. The term pistil has lost some of its precision because it is used to describe both a carpel and a gynoecium. A carpel consists of an ovary, style, and stigma. The term gynoecium represents all the carpels in a flower. A gynoecium may have one carpel as in legumes, several separate carpels as in raspberry and strawberry, or several carpels which have varying degrees of fusion, as in potato, cotton, and the majority of cultivated plants. Where there is extreme fusion of carpels, as in buckwheat, sugarbeet, and cultivated grasses, the compound ovary will have a single locule, and will appear superficially to be simple. The terms pistil and gynocoeium will be considered equivalent in this chapter.

Ovules develop as outgrowths from a local area (placenta) on the inner ovary wall. The chamber in which an ovule develops is called the locule. There may be one ovule per locule, as in grasses or buckwheat; two ovules, of which one aborts, as in cherry and related members of the Rosaceae; several ovules per single locule, as in legumes; or many ovules in several fused locules, such as in tomato or cotton.

Whatever the number and arrangement, each ovule at maturity consists of a main body (nucellus) containing the embryo sac, one or two cup-like sheaths (inner and outer integuments), and a basal stalk (funiculus) which connects the ovule to the placenta (Fig. 18J). The integuments have a pore at the apex (micropyle) through which the pollen tube enters. The ovule in the vast majority of species is bent back on itself at least 90°, and in many species, such as legumes, mustard, and potato, it is bent back 180° so that the micropyle is adjacent to the placenta. In a small number of species, e.g., walnut and some grasses, the ovule is straight. In grasses such as corn, the ovule is almost straight (Fig. 17).

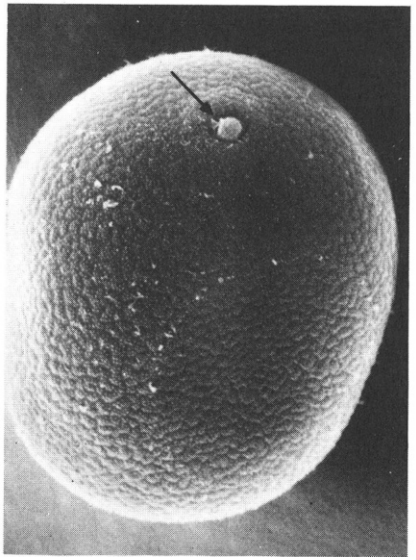

Fig. 15—Scanning electron microscope view of sorghum pollen grain. Grass pollen has inconspicuous exine patterns. The arrow indicates the single pore, characteristic of grasses and monocots in general. The exine plug over the pore becomes a tight cork when the pollen is dehydrated and shrunken. (Photo, courtesy of Dr. H. T. Horner, Jr.)

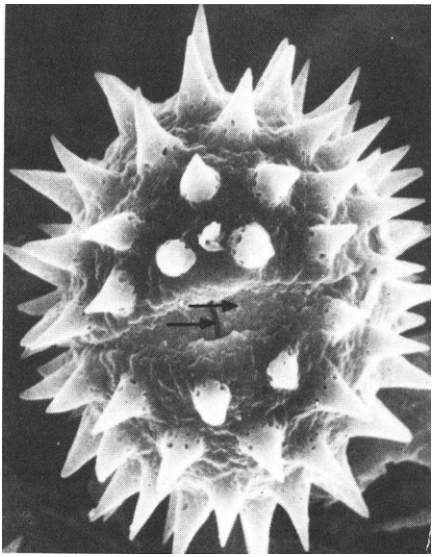

Fig. 16—Sunflower pollen grain, a scanning electron microscope view. Outer exine bears spines with tiny pores. Upper arrow indicates inner portion of exine exposed in a furrow. Lower arrow indicates the pore in the furrow, below which occurs the intine. There are three sets of furrows with pores, two of them obscured on this grain. This is commonest pattern in dicots. Most dicot pollen is not as conspicuously spiny. (Photo, courtesy of Dr. H. T. Horner, Jr.)

The embryo sac is a sac-like structure, commonly composed of seven cells, which develops from one cell of the nucellus. One of the cells in the embryo sac is the egg cell, which becomes the embryo after fertilization. Embryo sacs develop variously and the number and arrangement of cells within the sac vary at maturity, but more than 70% of flowering plants, and nearly all cultivated species (lily and onion are exceptions) are of the so-called normal type.

In the normal type of embryo sac development, one cell in the nucellus enlarges to become the megaspore mother cell (Figs. 18A to E). It undergoes meiosis, resulting in a row of four haploid megaspores (Fig. 18G). Like the microspore mother cell and microspore tetrad, the megaspore mother cell and the megaspore tetrad are coated with callose. The outer three cells almost invariably degenerate (Fig. 18H), while the innermost megaspore enlarges to form the embryo sac. The callose dissolves at about this time.

Three mitotic divisions occur within this cell, resulting in eight nuclei (Fig. 18I). Three nuclei remain at each end and two migrate to the middle to become the polar nuclei. Each of the three nuclei near the micropyle becomes enclosed, along with a small amount of cytoplasm, by a thin wall. These become the egg cell and two accompanying synergid cells. The three nuclei at the other end are also each encased in walls and become antipodal cells. The function of antipodals is unknown; they may remain unchanged, degenerate, or divide and form a mass of cells, as in grasses and crucifers (Fig. 20E). The mature embryo sac consists of the enlarged megaspore, called the central cell, within which are six cells and the two polar nuclei (Fig. 18J).

III. MEIOSIS

Meiosis in angiosperms occurs only in microspore mother cells and megaspore mother cells. The process is virtually identical in both (Bennett, 1977). Figure 19 illustrates the configurations taken by, and the movements of, the chromosomes as they proceed through meiosis I and II.

Premeiosis is recognized as a period during which no visible events occur, but genetic material (DNA) is replicated, so that each chromosome can enter meiosis with double the amount of DNA. During meiosis I, also called reduction division, the two sets of chromosomes in a nucleus pair, exchange portions of their chromosomes by a process called crossing-over, then separate and move into different nuclei. It is divided into four stages, of which the first, prophase I, is the longest and most complex. For this reason prophase I is subdivided into five substages:

a) leptotene. The diffuse chromosomes of premeiosis have coiled and shortened to the extent that they are visible as a jumble of threads.

b) zygotene. As they continue to shorten, chromosomes begin to pair with each other. This pairing is remarkably exact and specific.

c) pachytene. The chromosomes continue to shorten and their subunits, the chromatids, exchange segments by crossing-over.

d) diplotene. Chromosome pairs can be seen separating from each other, except where crossovers have occurred. They continue to shorten.

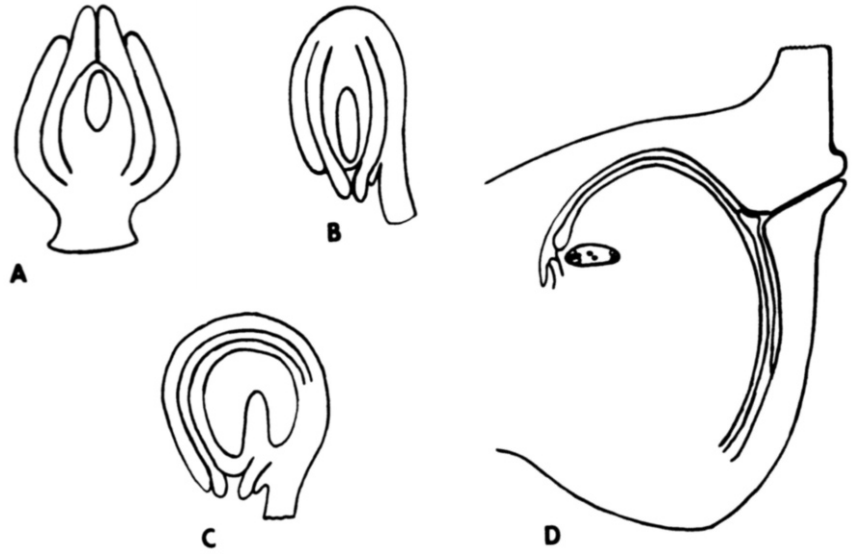

Fig. 17—Ovule shapes shown diagrammatically. A. Straight. B. Bent back 180° with embryo sac also reversed. C. Bent back 180° but embryo sac not completely reversed. D. Ovule of corn showing condition between A and B.

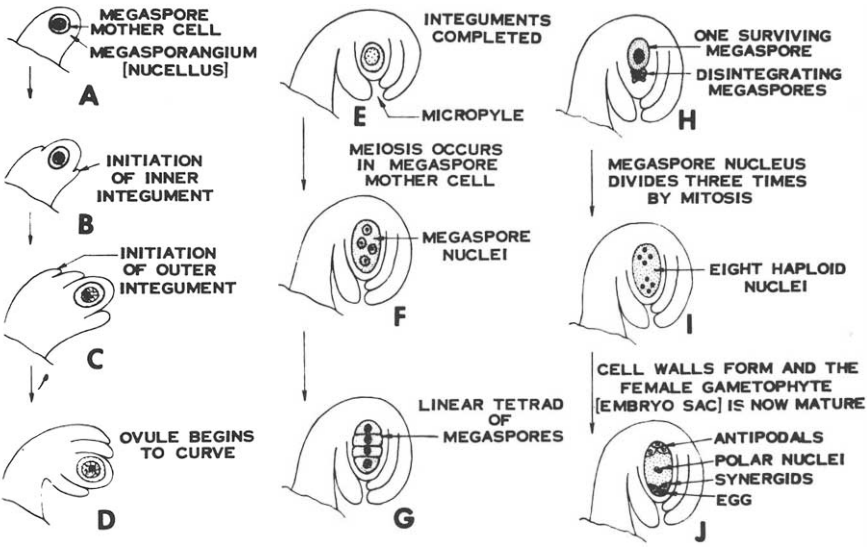

Fig. 18—General features of ovule and embryo sac development. The ovule is bent back on its stalk (funiculus) and embryo sac development is of the normal type. (Reprinted by permission from *Course Book in General Botany* by John D. Dodd © 1977 by Iowa State Univ. Press, Ames.)

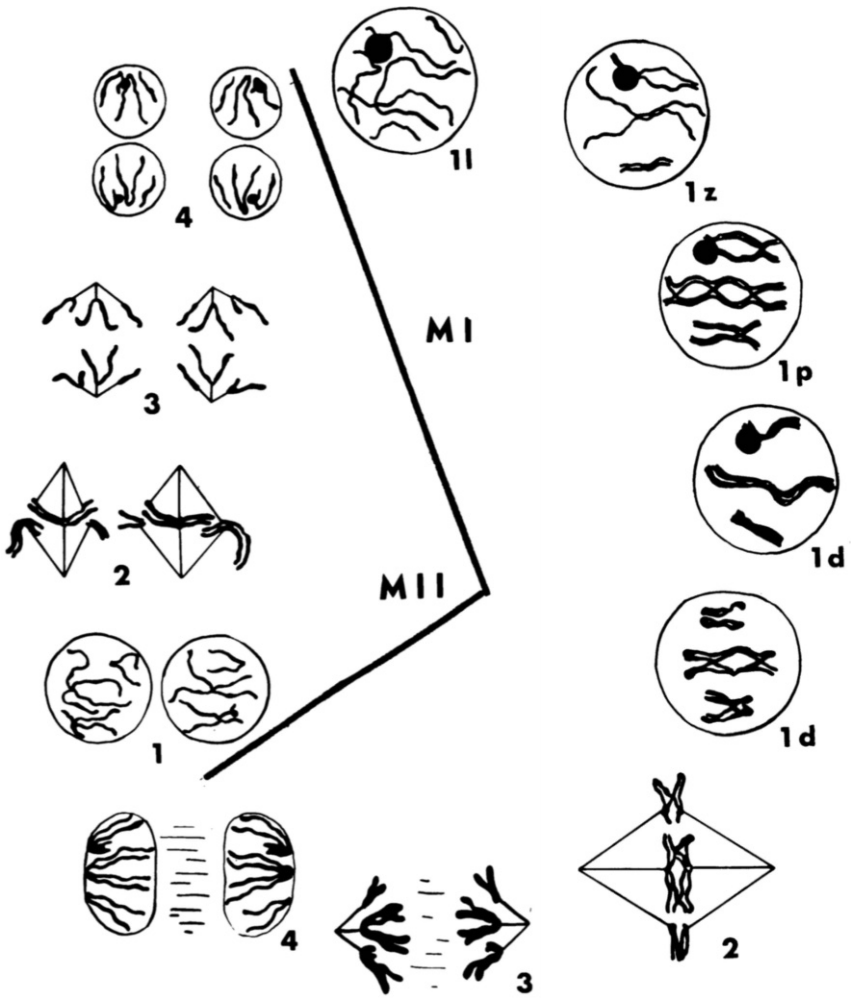

Fig. 19—Stages of meiosis illustrated by a microspore mother cell of a monocot. Meiosis I (MI): Prophase consists of the leptotene (1*l*), zygotene (1z), pachytene (1p), diplotene (1d), and diakinesis (1d) stage. At metaphase I (2), nuclear membrane and nucleolus have disappeared, the chromosomes are lined up on the equatorial plate, and spindle fibers have formed. During anaphase I (3), sets of bivalents move to opposite poles. At telophase I (4), each set of bivalents is surrounded by a newly-formed nuclear membrane. Meiosis II (MII): Prophase II (1), metaphase II (2), anaphase II (3), and telophase II (4) are a part of the second division that results in a tetrad of cells.

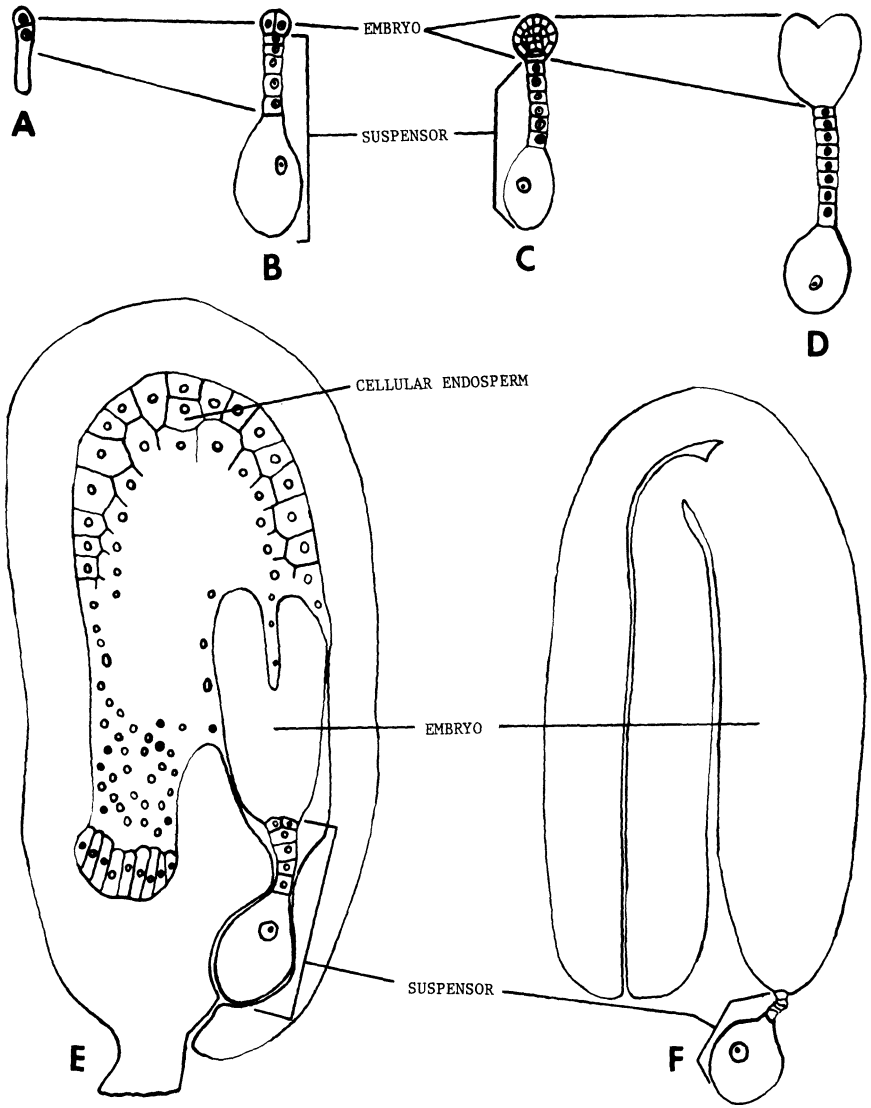

Fig. 20—Stages of embryo development in a dicot, based on *Capsella bursa-pastoris*, a crucifer. All stages are drawn to the same scale. A. First division of zygote to produce first cell of embryo and first cell of suspensor. B. Four-celled embryo with mature suspensor. C. Globe stage of embryo. D. Heart stage of embryo (cellular detail omitted). E. Torpedo stage of embryo set in developing seed. Note acellular milk stage of endosperm and peripheral development of cellular endosperm. F. Mature embryo folded on itself. Note start of collapse of the suspensor, the remnant of which will be destroyed during germination.

e) diakinesis. The chromosomes are at their shortest, and the nuclear wall and nucleolus disappear.

The second stage is metaphase I, during which the shortened chromosomes, still attached at points of crossing-over, line up within the cell in the central plane called the equatorial plate. Spindle fibers are formed.

During anaphase I, the chromosome pairs separate from each other and begin to move toward opposite ends of the cell. Each chromosome appears to be pulled by one or more threadlike spindle fibers attached at various places along its length.

When the two sets of chromosomes reach opposite ends of the cell, telophase occurs and, in most megaspore mother cells, a cell plate forms and two cells result. The interval between meiosis I and II is usually brief, and the chromosomes remain fairly short. In some plants there may be little or no interval between the two.

Meiosis II does not involve crossing-over. The two chromatids of each chromosome separate and the new cells formed are haploid. The stages of meiosis II are prophase (not subdivided), metaphase, anaphase, and telophase. At the end of telophase, cell plates are again deposited.

Determining how long meiosis lasts is technically difficult. Only a small number of plants have been studied, but these are mostly cultivated species (Bennett, 1977). Meiosis lasts about as long in the microspore mother cells of the anther as in megaspore mother cells of the ovules. The range among the approximately 40 species that have been studied is 1 to 17 days. For example, meiosis takes 24 to 51 hours in the cereal grasses (e.g., wheat, barley) that have been studied.

It should be re-emphasized that meiosis occurs only in two special kinds of cells, microspore mother cells in the anther and the megaspore mother cell in the ovule. Meiosis is the process by which matching chromosomes pair, exchange genetic material, and separate. After fertilization, two sets of chromosomes, one from the pollen and one from the egg, will again be together in all cells of the embryo. The straightforward outline presented here does not reflect the many possible complicating factors involved in meiosis following hybridization.

IV. POLLINATION AND POLLEN-STIGMA INTERACTION

A. Pollination Mechanisms in Cultivated Plants

Late in anther development, the four locules become two because of the disintegration of separating cell layers. When pollen is ready to be shed, drying and shrinking causes one or more slits to form on the sides of the anthers. More rarely, such as in potato, pores open at the tip and release pollen in saltshaker fashion. Pollen is commonly carried by insects or wind to cross-pollinate another flower. Some species are self-pollinated. Still other plants have both types of pollination, with self-pollination occurring either as a response to normal seasonal changes or adverse weather.

Many cross-pollinated species are adapted to insect pollen carriers. McGregor (1976) wrote a practical book dealing with insect-pollinated plants. It covers insect pollination in about 150 species of monocots and dicots.

B. Pollen-Stigma Interaction

Stigmas of cross-pollinated flowers may collect pollen from many different plants. Several mechanisms have evolved to screen out unwanted pollen and stimulate growth of desired pollen (Heslop-Harrison, 1975a).

Disintegrating tapetal cells from the parent plant may deposit an oily, proteinaceous coating on pollen. The proteins in this substance may later act as a signal to be recognized by protein on the stigma surface. Mustard is a well-studied example of this mechanism. Proteins also may be incorporated within the intine layer of the pollen wall below the pores or furrows during pollen development. When pollen with such protein lands in stigmatic fluid, hydration of the intine releases them to act in recognition. The difference between exine and intine protein is that the protein coating on the exine is from the parent plant, whereas the intine proteins are produced by the pollen grain.

Stigmas also may have recognition proteins. These can occur as a proteinaceous cover in the so-called dry stigmas (e.g., grasses, mustard, or sunflower), or they may be present in the stigmatic fluid secreted by wet stigmas (e.g., potato). A recent comprehensive review and survey of types of stigmas was made by Y. Heslop-Harrison and Shivanna (1977). They identify additional subtypes under the dry and wet categories.

Interaction between pollen and stigma proteins often governs pollen germination and pollen tube penetration into the style. The details of what happens on the stigma in both compatible and incompatible crosses differ somewhat among the species studied. Incompatible pollen may swell but not germinate; it may form a short tube which does not penetrate the stigma; or, there may be a slight penetration which is halted by a callose barrier formed in the stigma epidermis.

A protein incompatibility reaction occurs between the stigma and foreign pollen, and much is known about it. A still unknown mechanism controls pollen inhibition in species that are self-incompatible, i.e., plants in which pollen will not germinate on the stigma of the same flower, or perhaps not even on stigmas of other flowers on the same plant. A detailed discussion of the genetic systems involved in incompatibility reactions was presented by Heslop-Harrison (1975b).

In some species, pollen-pistil interaction occurs in the style, where pollen tubes secrete enzymes which interact with proteins secreted by the cells lining the style. Incompatible tubes stop growing because of adverse chemical reactions or because they are simply unable to take up nutrients provided by the style. Hybridization in a few such species has been accomplished by removing the style and replacing the stigma on the stub, thereby giving the pollen tube only a short path to the ovule (Maheshwari, 1950).

A third location of pollen-pistil interaction is at the micropyle, where secretion from the ovule has been reported in several plants. The secretion has been reported to attract compatible tubes, while foreign tubes grow randomly in the ovary until their food reserves are gone.

Some plants have no barriers to foreign pollen. Foreign pollen tubes can enter and cause destruction of the embryo sac. This is inefficient because such ovules do not produce seeds. The prevention of this waste has doubtless been a factor in the evolution of more elaborate barriers closer to, or on, the stigmatic surface.

V. COMPATIBLE POLLEN GERMINATION AND TUBE GROWTH

When the miniscule pollen grains are exposed to air, they become extremely dehydrated. When a grain lands on a compatible stigma, it takes up water and begins to swell immediately. In seconds, the intine expands and the wall-borne enzymes and proteins are released. Continued water absorption and renewed metabolic activity in the pollen grain allows the pollen tube to emerge and grow. Some of the pollen enzymes mediate absorption of nutrients, and others digest stigma cell walls, allowing the tube to pass between cells. A pollen tube never enters cells until it reaches the embryo sac.

The style can accommodate pollen tube growth in two ways. In general, dicots tend to have solid styles, with a column of transmitting tissue consisting of cells with a thick, nutrient-rich wall which is digested by the tube as it grows between these cells (Sassen, 1974). In cotton, the pollen tube-style interaction has been studied in detail (Jensen, 1972).

Monocots tend to have a hollow style opening directly onto the stigma, with only the stigma cells as a barrier. The pollen tube grows in a nutrient fluid secreted by cells lining the inner surface of such a style. Lily is the most intensively studied representative of this type (Mascarenhas, 1975). The grasses are a conspicuous exception, having solid styles, each with a strand of well-defined transmitting tissue. The pollen tube in grasses, however, must first grow between cells of the long, feathery stigma for a considerable distance before reaching the style.

The pollen tube has an ordinary cell wall which is thin and rapidly growing at its tip. Protoplasm is concentrated in the tip and is sealed off from older parts of the tube by occasional callose plugs. The tube cell nucleus may lead or lag behind the generative cell. The generative cell, in two-celled pollen, divides to become two sperm cells before the pollen tube reaches the embryo sac.

When the pollen tube enters the ovary, it may be guided mechanically to the micropyle of the ovule by a continuation of the stylar secretory tissue, by a cuplike outgrowth of the ovule which guides the tube, or by secretion of a substance through the micropyle, which acts as a chemical guide. The exact mechanism differs among plants. In some plants, there may be no special mechanism, and tubes appear to grow randomly, striking a micropyle only by accident. In flowers with fused ovaries but separate styles, as in carrot, there may be a horizontal plate of transmitting tissue at the base of the styles to allow random growth into any of the ovaries.

The micropyle may be a distinct opening or it may appear closed by an overlapping of inner and outer integuments (e.g., cotton, some legumes). A carbohydrate-containing fluid secreted by the nucellus may help to open a micropyle with overlapping integuments and attract the pollen tube. Such a secretion has been described in the grass *Paspalum.*

After the tube passes through the micropyle, it must grow through a certain number of nucellar layers to reach the embryo sac. At one extreme is sunflower, in which the synergids protrude through the nucellus and are immediately available at the micropyle. At the other extreme are such plants as

cotton, corn, and grape, with an embryo sac deep in the nucellus. Other plants, such as tomato or potato, have fewer nucellar layers. The swelling of the embryo sac during development usually causes destruction of at least some nucellar layers. The tube grows between nucellar cells and digests their cell wall.

Elapsed time from pollen germination to pollen tube entry into the micropyle varies from minutes to months, but 6 to 25 hours is probably most common among cultivated plants. In soybean, it takes 8 to 10 hours. In some cereal grasses, such as wheat, less than 1 hour may elapse (Bennett, 1973). Woody plants tend to be slower, and the process can take weeks or months in some trees.

VI. PENETRATION OF EMBRYO SAC AND FERTILIZATION

After penetrating the micropyle and nucellus, the pollen tube enters the embryo sac by means of enzymatic digestion of the embryo sac wall. The condition of the embryo sac cells at the time of pollen tube entry varies with the species: 1) antipodals may be unchanged, degenerated, or actively proliferating more cells; 2) polar nuclei may have fused or may still be separate; 3) synergids may be unchanged or one may have degenerated.

Pollen tubes have been reported to enter the embryo sac at different places, but they most commonly grow into the conspicuous cell wall proliferation (filiform apparatus) of one of the synergids. All electron microscope studies have reported that the tube enters the degenerating synergid (Mogensen, 1978). In the synergid cell, the tip of the pollen tube ruptures and the cytoplasm and sperm cells flow out. Rupturing of the pollen tube may be caused by osmotic conditions within the synergid cell. The details remain elusive as to how each sperm cell travels to either the egg cell or polar nuclei, gets into the cell and nucleus, and mingles its chromosomes with those of the female. Cotton, barley, and wheat have been studied most carefully (Jensen, 1972; Bennett, 1973). It is known that one sperm and the polar nuclei fuse before union of sperm and egg nuclei. In sunflower, for example, fusion occurs 2 to 3 hours earlier, and in wheat 10 to 15 hours earlier.

The sperm cell has a thin envelope of cytoplasm around the nucleus which is devoid of plastids. Cytoplasmic inheritance could occur if the sperm cell cytoplasm mingled with egg cytoplasm at the time of fertilization. There are reports that cytoplasm does enter the egg cell, but other reports that only the sperm nucleus enters. Even in species where fertilization can be predicted within a few minutes, it is nearly impossible to obtain correctly oriented preparations at just the right instant. Jensen (1972) and Kapil and Bhatnagar (1975) reported recent information on fertilization.

Polyspermy is penetration of an embryo sac by more than one pollen tube, or the occurrence of more than two sperm cells per tube, both resulting in multiple sperms entering an embryo sac. This has been observed in about 55 dicot and monocot species (Vigfusson, 1970), chiefly cultivated plants, and it may be a fairly common event according to some workers. All

reports are from cross-pollinated species, however. Penetration of an egg cell and fertilization by more than one sperm has been rarely reported, and Vigfusson feels that this has not yet been shown unambiguously.

VII. ENDOSPERM

Endosperm provides nutrition for the embryo. Endosperm is initiated by the fusion of one of the sperm nuclei with the two polar nuclei, resulting in a triploid primary endosperm nucleus in the cytoplasm of the central cell of the embryo sac. (This requirement of two sperms for sexual reproduction in angiosperms is unique.) The usual triple fusion occurs earlier than fertilization of the egg, and the endosperm begins growing before the embryo. Endosperm may develop in at least four ways, of which three occur in cultivated plants.

The most common way is for the endosperm nuclei to divide initially without cell walls being laid down. This milk stage of the endosperm is most spectacular in the coconut and is well known in corn, but it also occurs in other cereal grasses, legumes, and most other cultivated plants. Walls form later, starting at the periphery of the embryo sac, resulting eventually in a cellular endosperm (Fig. 20E). In later divisions, a cell wall is laid down after each nuclear division.

A few plants have cellular endosperm from the beginning (e.g., tobacco). Another rather small group, including some grasses and the maples, have only a non-cellular liquid endosperm in which cell walls never develop.

The outermost endosperm layer is often distinguished as the aleurone layer because it contains protein bodies called aleurone grains. This layer is developed to various degrees in both dicots and monocots. It is well developed in cereal grasses and in lettuce, and has been intensively studied there with respect to its influence on stimulating germination.

It is not uncommon for the endosperm to form saclike or filamentous outgrowths which extend into adjacent maternal tissue. Such outgrowths are thought to increase nutrient absorption. They are known in over 50 families, including some cultivated legumes.

Endosperm cells are triploid at first because of the fusion of three nuclei. Later, endosperm cells may become even more polyploid. Endosperm in onion may reach 12n and in corn 1,000n. Even higher levels have been reported from certain wild species. High endosperm ploidy often involves unusual variations of mitosis.

One can speculate as to why the triploid condition stimulates endosperm formation. One theory postulates hybrid vigor and states that this mechanism allows the endosperm to compete successfully for available nutrients with the diploid cells surrounding the embryo sac. Another theory is that the male chromosomes in the endosperm act as an intermediary between the embryo and the surrounding diploid maternal tissue. Brink and Cooper (1947) discuss these and other theories in detail.

In sugarbeet, spinach, amaranths, and other members of the Chenopodiaceae and Amaranthaceae, the nucellus persists and becomes filled with food reserves. This tissue, called perisperm, is diploid and occurs in addition to endosperm. The embryo presumably is able to absorb both of these reserves.

VIII. THE EMBRYO

The fertilized egg (zygote) is the first cell of the embryo. Fertilization usually occurs after endosperm initiation, and the zygote remains undivided until at least a small amount of endosperm has been produced. In barley, the first division of the zygote occurs about 9 hours after the first endosperm division, when eight endosperm nuclei are already present.

The first cell wall usually forms a basal cell toward the micropyle and a terminal cell away from the micropyle. The basal cell usually forms a multicellular suspensor which does not become part of the mature embryo. In lettuce, however, part of the suspensor does contribute cells to the primary root. Most commonly, the suspensor is a short filament of cells, with the tip cell sometimes swollen and polyploid, as in mustard and the garden bean (Fig. 20). In soybean, the suspensor is merely a rudiment of three to four cells, whereas in garden pea, chickpea, and some other legumes the suspensor becomes unusually massive.

The suspensor was previously thought to act as a ramrod to push the embryo further into the nutritive endosperm. More recent studies on some of the mustards and beans suggest that in these plants it may be more important in absorbing and transferring nutrients to the embryo during early stages of development; the suspensor may even secrete enzymes that control certain aspects of embryo growth. The suspensor is prominent early, but it soon ceases growth and is inconspicuous when the embryo is mature (Figs. 20 and 21).

The terminal cell produced by the first division of the zygote will become the embryo after passing through several stages. In some plants, daughter cells of the basal cell also contribute to the embryo. Continued mitotic divisions result first in a sphere of up to several hundred cells (globe stage). Toward the end of this stage the first indications of internal differentiation are seen. Up to this time, the pattern of development is similar among dicots and monocots (Figs. 20A to C and 21A).

In dicots, the globe stage is followed by the heart stage, in which two lateral multicellular wings arise which will become the pair of cotyledons (Fig. 20D). The cotyledons are the first or seed leaves of the plant. As the cotyledons grow, the embryo axis (hypocotyl-radicle axis) elongates and a torpedo stage is recognized (Fig. 20E). Further development varies with the species. An epicotyl (rudimentary main shoot) may develop and additional embryonic leaves may form (e.g., pea, soybean), or an epicotyl may be lacking in the mature seed (e.g., cotton, potato, sugarbeet). The mature embryo may be straight (e.g., lettuce) or, more commonly, coiled or folded (e.g. cotton, tomato (Fig. 20F). There may be endosperm around the mature embryo, as in potato and tomato, or all endosperm may have been absorbed and stored in the cotyledons, as in cultivated legumes.

The major distinction between dicot and monocot embryo development is that in monocots only one cotyledon forms. The developing cotyledon assumes a terminal position quite early, so that the embryo shoot tip appears to be lateral (Figs. 21B to D). The single cotyledon in many monocots remains within the seed coat, slowly absorbing endosperm during germination. In onion, only the tip of the cotyledon remains within the seed; the rest of it elongates and arches above ground, where it becomes green and photosynthetic. Except for the cotyledonary difference, embryo

and endosperm development are remarkably similar in most dicots and monocots.

The grass embryo, however, is different from that of most other monocots. There is disagreement about what is a cotyledon in grasses, but the scutellum behaves as if it were one, remaining wholly within the seed to absorb endosperm and transfer it to the seedling. The epicotyl of the embryo axis is ensheathed by the tubular coleoptile, often interpreted as the first leaf after the cotyledon. The radicle is ensheathed by the protective coleorhiza (Fig. 21E). Because of several differences in structure and germination behavior, there is controversy about the interpretation of parts of the grass embryo (Rost and Lersten, 1973).

The integuments enclosing the nucellus enlarge and change their cellular features as the embryo develops. Both integuments may contribute to the seed coat, or only one may do so. The nucellus ordinarily becomes crushed and disappears (Fig. 20E), but in some plants it remains as a paper-thin layer just below the seed coat (e.g., peanut). In grasses, the seed coat

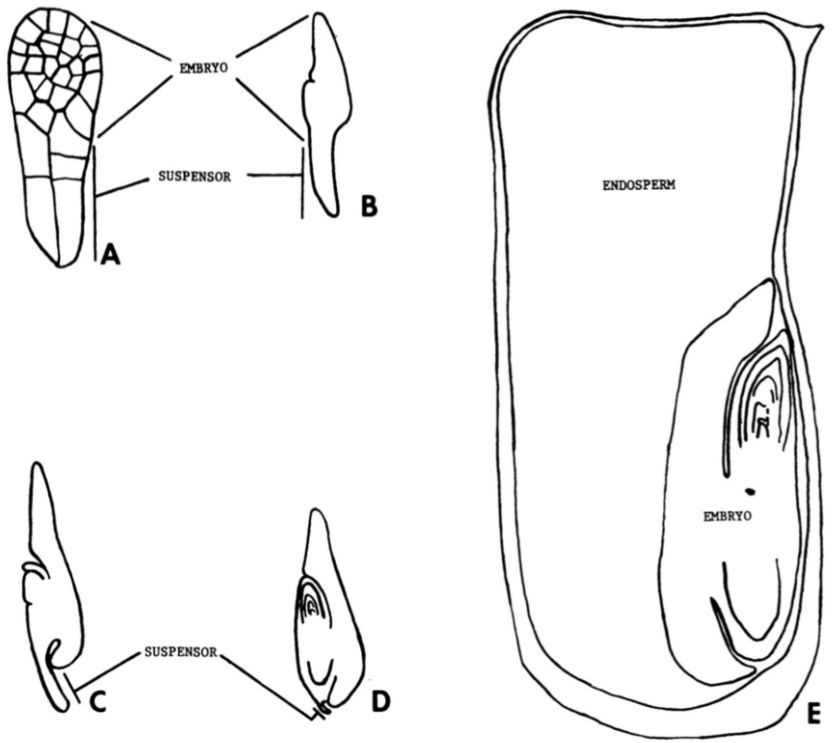

Fig. 21—Stages of embryo development in corn, illustrating general monocot features and special features of grasses. Stages are not all drawn to the same scale. A. Globe stage (6 days after fertilization) approximately equivalent to Fig. 20C. Suspensor not sharply delimited from embryo. B. Young cotyledon stage (12 days old) showing laterally displaced shoot tip and large suspensor. There is no greatly enlarged cell at tip of suspensor. C. Later stage (20 days old) showing early coleoptile development. D. Embryo 35 days old with embryo axis and several young leaves. Note relative size of suspensor. E. Mature (45-day-old) embryo in the caryopsis. No trace remains of suspensor.

becomes tightly appressed to the ovary wall (Fig. 21E) so that a separate seed coat cannot be easily distinguished (Rost and Lersten, 1973). The details of seed coat structure and development vary so much that individual species cannot be considered here.

IX. APOMIXIS AND MALE STERILITY

A. Apomixis

Apomixis, from two Greek words meaning "without mixing," is a general term for several ways of non-sexual reproduction which involve some or most of the apparatus of sexual reproduction. An embryo sac usually forms, sometimes in normal fashion, from the haploid megaspore, in other plants from the unreduced diploid megaspore mother cell or even from another diploid cell of the nucellus. Within this embryo sac, the egg cell and polar nuclei usually form an embryo and endosperm without fertilization. In some types of apomixis, a pollen tube and sperm cells are required to stimulate development, but do not fuse with the nucleus. Apomixis is dealt with in detail in Chapter 3.

B. Male Sterility

It has been known for a long time that in some plants pollen may be aborted, stamens malformed, or stamens may be lacking entirely. This occurs sporadically in wild species, and if it confers an advantage because it ensures cross-pollination, such plants may be selected for and the species may become dioecious. This seems to occur frequently in angiosperms because, while only 5% of the flowering plant genera are dioecious, they are scattered among 75% of the families (Crowe, 1964).

When stamen or pollen failure is noted among plants of cultivated species, such plants can often be selected and propagated for breeding purposes because they have become obligate outbreeders whose progeny cannot be the result of self-pollination. The practical benefits of having such male-sterile plants in otherwise male-fertile species has stimulated much research into both the application and the underlying mechanism of male sterility.

The male sterile condition can be the result of genes which are inherited according to ordinary Mendelian laws. Such genetic male sterility is well-known and is used by breeders in several crop species. In cytoplasmic male sterility, there are factors in the maternal cytoplasm which induce sterility. A combination of cytoplasmic and genetic factors is known to control sterility in still other lines. A knowledge of which category a particular male-sterile fits is necessary for one to know how to restore fertility, and also to indicate how predictable and stable the male sterile condition will be. Practical application of male sterility for plant breeding and commercial hybrid seed production is discussed in Chapters 4 and 8. A detailed review of the genetic aspects is in Frankel and Galun (1977).

Whatever the type of sterility, the visible evidence in the flower is failure of normal stamen or pollen development. In different species, or in vari-

ous male sterile lines within a species, the stage of development at which stamen or pollen abortion occurs is variable. The stage at which abortion occurs may also be affected by day length, temperature, and other field conditions.

If stamens develop and microspore mother cells are produced, abortion may occur at any stage up to the mature microspores (Frankel and Galun, 1977; Lasey and Lersten, 1972). Most abortions occur at about the tetrad stage, a smaller number at or near meiosis or closer to microspore maturation.

There are several hypotheses as to what causes abortion. The tapetum in some dicots and monocots has been observed to swell and expand inwards, seemingly crushing the microspores. Failure of the tapetum to secrete substances at the right time has also been implicated. Amino acid deficiencies and differences in mitochondria have been proposed as possible causes of pollen abortion in some grasses. Finding answers has proved difficult, but much research is in progress. Discovering the reasons for abortion is desirable before we can intelligently attempt to predictably induce male sterility.

REFERENCES

Bennett, M. D. 1973. Cell development in the anther, the ovule and the young seed of *Triticum aestivum* L. var. Chinese Spring. Phil. Trans. (Cambridge Univ.), Ser. B. 266:39–81.

———. 1977. The time and duration of meiosis. Phil. Trans. Roy. Soc. London, Ser. B, 277:201–226.

Brink, R. A., and D. C. Cooper. 1947. The endosperm in seed development. Bot. Rev. 13:423–541.

Crowe, L. K. 1964. The evolution of outbreeding in plants. I. The angiosperms. Heredity 19:435–457.

Davis, G. L. 1966. Systematic embryology of the angiosperms. John Wiley and Sons, New York.

Frankel, R., and E. Galun. 1977. Pollination mechanisms, reproduction and plant breeding. Springer-Verlag, Berlin.

Hayward, H. E. 1938. The structure of economic plants. The Macmillan Co., New York.

Heslop-Harrison, J. (ed.). 1971. Pollen: development and physiology. Butterworths, London.

———. 1972. Sexuality of angiosperms. p. 133–291. *In* F. C. Steward (ed.) Plant physiology, a treatise. Vol. VI C. Academic Press, N.Y.

———. 1975a. Male gametophyte selection and the pollen-stigma interaction. p. 177–190. *In* D. L. Mulcahy (ed.) Gamete competition in plants and animals. North-Holland Publ. Co., Amsterdam.

———. 1975b. Incompatibility and the pollen-stigma interaction. Ann. Rev. Plt. Physiol. 27:403–425.

Heslop-Harrison, Y., and K. R. Shivanna. 1977. The receptive surface of the angiosperm stigma. Ann. Bot. 41:1233–1258.

Jensen, W. A. 1972. The embryo sac and fertilization in angiosperms. University of Hawaii—Harold L. Lyon Arboretum lecture no. 3. Univ. of Hawaii, Honolulu.

Kapil, R. N., and A. K. Bhatnagar. 1975. A fresh look at the process of double fertilization in angiosperms. Phytomorphology 25:334–368.

Laser, K. D., and N. R. Lersten. 1972. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. Bot. Rev. 38:425–454.

Linskens, H. F. (ed.). 1974. Fertilization in higher plants. North-Holland and American Elsevier Publ. Co., Amsterdam.

Lloyd, D. G., and C. J. Webb. 1977. Secondary sex characters in plants. Bot. Rev. 43:177–216.

Maheshwari, P. 1950. An introduction to the embryology of angiosperms. McGraw-Hill Book Co., N.Y.

Mascarenhas, J. P. 1975. The biochemistry of angiosperm pollen development. Bot. Rev. 41:259–314.

McGregor, S. E. 1976. Insect pollination of cultivated crop plants. USDA Handbook No. 496.

Mogensen, H. L. 1978. Pollen tube-synergid interactions in *Proboscidea louisianica* (Martiniaceae). Am. J. Bot. 65:953–964.

Palser, B. F. 1975. The bases of angiosperm phylogeny: embryology. Ann. Mo. Bot. Gard. 62:621–646.

Rost, T. L., and N. R. Lersten. 1973. A synopsis and selected bibliography of grass caryopsis anatomy and fine structure. Iowa State J. Res. 48:47–87.

Sassen, M. M. A. 1974. The stylar transmitting tissue. Acta Bot. Neerl. 23:99–108.

Vasil, I. K. 1973. The new biology of pollen. Naturwissenschaften 60:247–253.

Vigfusson, E. 1970. On polyspermy in the sunflower. Hereditas 64:1–52.