18

Female Reproductive Structures and Embryogenesis

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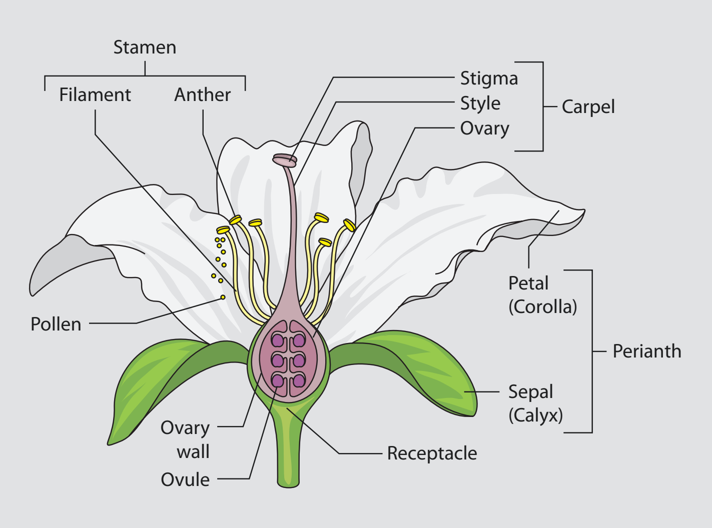
Introduction

The formation of a new seed via sexual reproduction occurs within the ovary of a flower in a series of successive steps ranging from the growth and development of an ovule to the formation of the fully mature embryo within a seed. Within this chapter, you will learn about the female reproductive structures of a flower, the formation of the embryo sac and endosperm, as well as important events including double fertilization and embryogenesis that are critical to the existence of individual angiosperms. The female structures of the flower are typically designed to enable the process of fertilization, protect the developing embryo(s), and to provide for dissemination of the species to new suitable geographical environments. All floral parts are considered to be evolutionarily modified leaves.

18.1 The Innermost Whorl of the Flower Is Typically Composed of Female Floral Parts

Female reproductive structures typically comprise the innermost whorl of floral organs in angiosperms. Primary to the design, the pistil (or carpel) has a terminal stigma, an elongated style, and a basal ovary, which in turn houses an egg cell, **central cells**, and related cells within an enclosed ovule (◘ Fig. 18.1). If only one carpel exists within an ovary, it is said to be **monocarpous**. There may be more than one ovule within an ovary and, if so, are said to be **apocarpous** if they are independent of one another or **syncarpous** if they are fused. The compilation of all carpels within the flower is collectively called the gynoecium. A pistil is a collective term for the gynoecium and, thus, may contain one or more carpels. The pistil is a modified leaf known as a megasporophyll which evolved and contains one or more ovules.



◘ Fig. 18.1 Floral structure indicating a carpel (pistil) containing apocarpous ovules. (Redrawn from Crang and Vassilyev 2003)

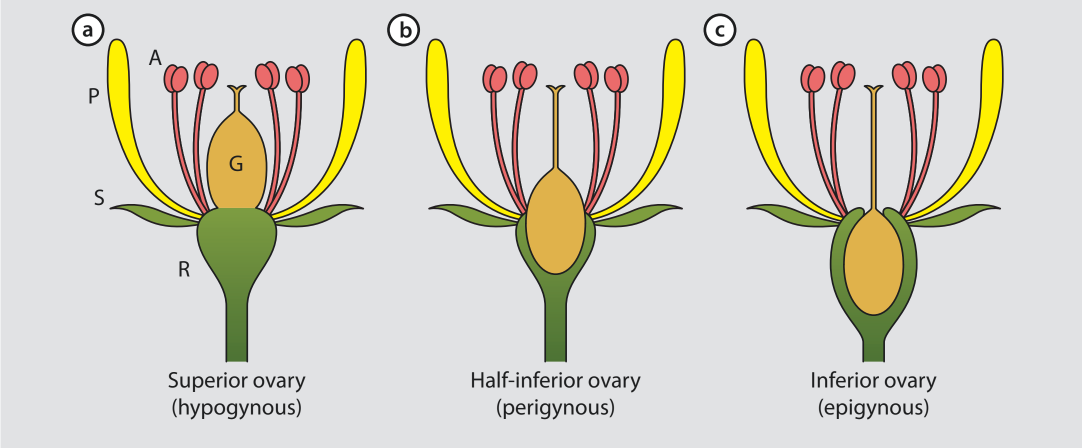
Box 18.1 Sexual Selection, Reproductive Isolation, and Speciation

The genetic bases of traits such as flowering time and flower color that contribute to pre-pollination barriers have been studied in several species and, in some cases, have been shown to be involved in preventing low-fitness hybrids. Nevertheless, the role of sexual selection as a force in plant speciation is still controversial. In sexual selection, a species produces far more pollen than ovules, making for a biased sex ratio toward males as well as a species-dependent variation in ovule number per flower. As in animals, a high level of sexual selection should speed up the evolution of traits that are responsible for pre- and post-mating hybridization between species. Thus, sexual selection is believed to be tightly linked to interspecific barriers in plant speciation. It is expected that species subject to different levels of sexual selection should differ in the selectiveness of their ovules toward male gametes. Recent studies have shown that ovules from self-incompatible *Solanum* species were more selective compared to ovules from self-compatible species. Of particular importance is that new technological advances are allowing identification of the genetic targets of selection, natural or sexual, that drive the establishment of reproductive barriers between species. As a consequence, it is believed that sexual selection can produce the establishment of interspecific reproductive barriers, leading to a unidirectional gene flow between different but closely related species. Even though the origin of reproductive barriers may be elusive, ongoing sequencing studies will foster the synthesis of complementary studies involving early new speciation with the genomics of "good species."

Reference: Lafon-Placette et al. (2016)

18.2 Ovaries Are Described Based upon Position Within a Flower and May Contain Nectaries

Three types of ovaries are recognized according to their positions with respect to the lateral organs of the flower (◘ Fig. 18.2a–c). A superior (or **hypogynous**) ovary (e.g., tulip, *Tulipa gesneriana*) is situated on the receptacle above the perianth and androecium. In flowers with an inferior (or **epigynous**) ovary (e.g., daffodil, *Narcissus pseudonarcissus*), it is positioned below the apparent points of attachments of the perianth and androecium. However, not all epigynous flowers have a hypanthium where the ovary is enclosed in the receptacle. In flowers with inferior ovaries, the lower portions of calyx, corolla, and androecium fuse into a floral tube or hypanthium, which becomes completely adnate (attached) with the whole length

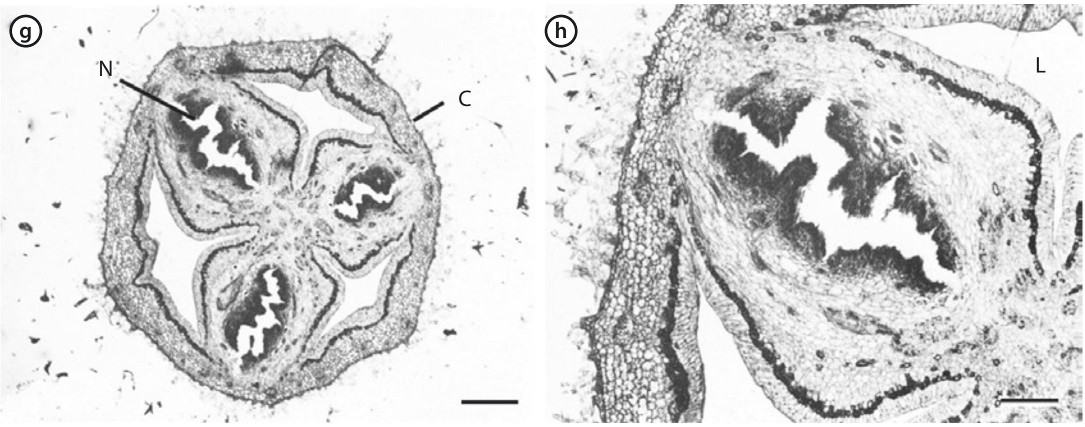


◘ **Fig. 18.2** **a**–**c** Female flowers can have one of three types of ovaries denoted by where the ovary is located relative to the sepals (calyx). *A* androecium (stamens), *P* petals, *S* sepal, *R* receptacle, and *G* gynoecium. (Redrawn from Ulf Mehlig, CC BY-SA 2.5)

◘ **Fig. 18.2** **d**–**f** A longitudinal section of a day lily (*Lilium* sp.) flower with a superior ovary. (d. natural size; e,f. magnified). The ovules and axile placentation are apparent within the hypanthium. Lily has three sepals and three petals; however, the two structures have an identical appearance, so it appears as though the flower has six petals, which is not the case. Scale bars = 1 cm in **d** and 2 mm in **e** and **f**. (**d**–**f** RR Wise)

of the ovary. In the flowers with a half-inferior (or **perigynous**) ovary (e.g., elder, *Sambucus nigra*), the hypanthium adnates (or fuses) with only the lower half of the ovary (◘ Fig. 18.2d–f).

The ovaries of many monocots contain septal nectaries that help the flower attract animal pollinators (◘ Fig. 18.2g, h). Septal nectar-



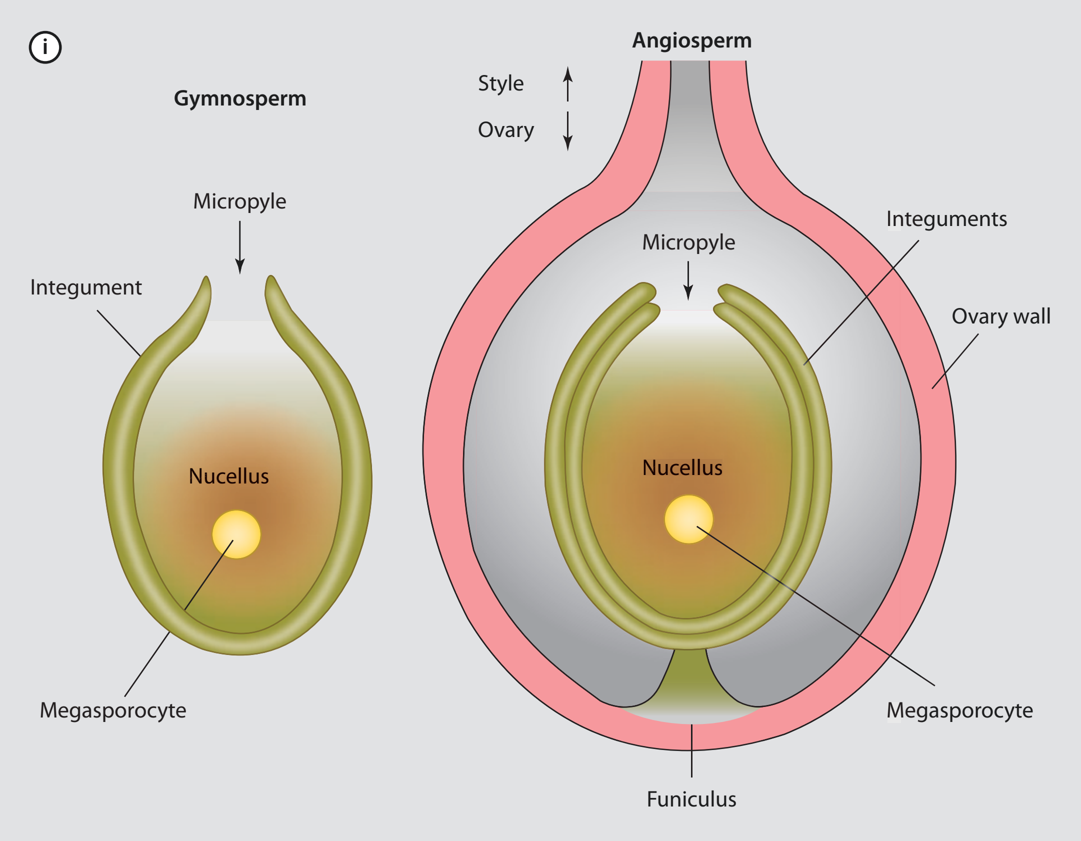
◘ **Fig. 18.2** **g**, **h** Cross-section of the ovary of black kangaroo paw (*Anigozanthos fuliginous*) showing three septal nectaries (in accordance with the number of carpels of the gynoecium). It is evident that the nectaries are formed at the interfaces of inner portions of carpels. *C* carpel wall, *N* nectary, *L* ovary locules. Scale bars = 500 µm in **g** and 200 µm in **h**. (**g**, **h** M Simpson (1993))

ies formed in the ovary wall may accompany the incomplete fusion of carpels. These nectaries represent the glandular epidermis. However, the evolution of these nectaries may parallel the formation of other forms of pollination such as "buzz-pollination" in which septal nectaries are not found. Buzz-pollination occurs in which bees and certain other insects create vibrations that obtain pollen from narrow slits of anthers and may release the pollen into non-nectary sites of the pistil. This type of pollination represents a complex adaptation of plant and insect through coevolution.

Gymnosperms, as opposed to angiosperms, are generally regarded as bearing "naked" seeds (◘ Figs. 18.2i and 18.2j). The "naked" ovules do not mean that they are without protection. In fact, the ovules are generally borne on stalks or flattened structures (megasporophylls) that cluster together to form cones. The cones do offer some protection, but not the exclusion of pollen grains from direct contact with the ovules.

18.3 Ovules Can Be Arranged Within an Ovary in a Variety of Ways

Histologically, the ovary wall at anthesis consists of homogeneous ground parenchyma and vascular bundles and is covered from the outside and inside by the epidermis and a cuticle. The sites of the ovary where the ovules are produced are known as placentae (singular, placenta). Each ovule is attached to the ovary wall by a stalk called a **funiculus**. In angiosperms, the term locule refers to the chamber within an ovary of the flower. The number of locules present in a gynoecium may be equal to or less than the number of carpels. The locules house the ovules and later the seeds. When there are fewer locules than carpels, it is because the carpels fuse during development.



◘ Fig. 18.2 **i** Illustration of gymnosperm and angiosperm ovules. Here you can also see how the gymnosperm ovule is "naked" and not enclosed by protective tissue, whereas the ovule of the angiosperm is enclosed within an ovary. Note the presence of the integuments, micropyle, nucellus, and funiculus. (public domain)

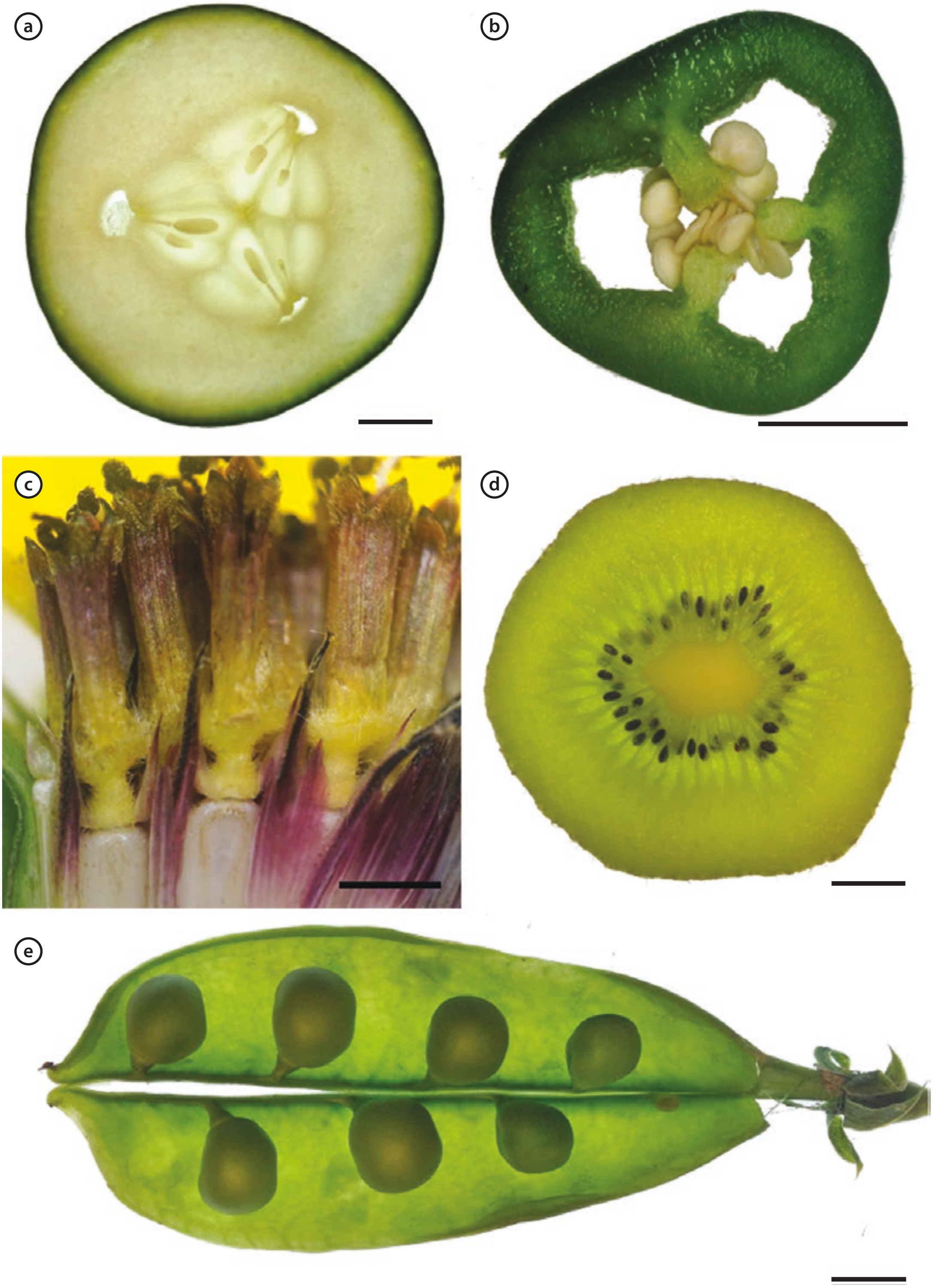
◘ Fig. 18.2 **j** A pinyon pine (*Pinus edulis*) gymnosperm cone and exposed seeds. The seeds are borne in cones on megasporophylls and are not visible until maturity. (Curtis Clark, CC BY-SA 2.5)

Five types of placentation can be distinguished with respect to the position of placentae within the ovary and the structure of the gynoecium. In **parietal placentation**, which is often found in single-locular ovaries, the placentae and ovules are formed along the peripheral parts of the ovary at the sites of fusion of the carpel(s) margins (◘ Fig. 18.3a). Parietal placentation is found in the majority of angiosperms. **Axile placentation** (or central-angular) is found in plants with multilocular ovaries (◘ Fig. 18.3b). The partitions dividing the ovaries into locules are formed by the lateral fusion of carpels along their margins, and the placentae are formed in the angles of locules. In **basal placentation**, the ovules are attached at their base and point upright (◘ Fig. 18.3c). This is common in plants such as sunflower and carnation that have multiple flowers packed in to a flat flower head. **Free-central placentation** is characteristic of one-locular gynoecia in which the inner partitions of the ovary were lysed, leaving only a central column with placentae (◘ Fig. 18.3d). Peas and beans show **marginal placentation**, in which the ovules are arranged in a linear fashion along the seam (or suture) that forms the carpel (◘ Fig. 18.3e).

Ovules, the precursors of seeds, are derived from the placenta of the ovary wall and consist of a central **nucellus** (which represents the megasporangium), where megaspores, and later an embryo sac, are produced; one or two **integuments**, which enclose the nucellus; and a supportive stalk, the funiculus, which attaches the ovule to the placenta. Usually a single vascular strand runs through the funiculus from placenta to the lower part of the ovule (◘ Fig. 18.3f). At the free end of the ovule, a small opening, the **micropyle**, is left by the integuments. The pollen tube will pass through the micropyle before entering the embryo sac. The region where the nucellus and the integuments merge is called the chalaza. In most flowering plants, a **hypostase** is differentiated in the chalazal region (basal) of the ovule, which consists of a cluster of densely cytoplasmic cells with highly refractive cell walls. The hypostase serves as a boundary to prevent further growth of the embryo sac.

In ovules such as smartweed (*Polygonum coriarium*), the ovule consists of three main parts immediately before fertilization: (1) an outer integument, (2) an inner integument, and (3) a large multicellular nucellus (◘ Fig. 18.3g). The nucellus contains an embryo sac comprised of seven cells which include the egg and two synergids (representing the egg apparatus in the micropylar region of the sac), the central cell, and, in the chalazal region, three antipodals. Beneath the embryo sac, the hypostase can be identified as a group of cells with thickened walls and dense cytoplasm. The vascular tissue which supplies the ovule (and later developing seed) ends at the basal region of the ovule. Both integuments are thin, and each consists of only two epidermises with no mesophyll.

A minority of species hold their ovules in an **orthotropous** (upright) position (◘ Fig. 18.3h). In other species, the ovules curve during development to become perpendicular to the axis of the funiculus (**hemitropous**, ◘ Fig. 18.3i) or, as is found in the majority of plants, to the ovules become completely inverted during growth



◘ **Fig. 18.3** **a**–**e** Examples of five placentation types: **a** parietal (cucumber, *Cucumis sativus*), **b** axile (jalapeno pepper, *Capsicum annum*), **c** basal (sunflower, *Helianthus* sp.), **d** free central (kiwifruit, *Actinidia deliciosa*). **e** marginal (pea, *Pisum sativum*). The funiculus can be readily observed in figures **a** and **e**. Figures **a** and **b** show fruits with three locules in contrast to fruits indicated in figures **c**–**e** with only one locule. In figure **c**, the locule is not apparent in this particular photograph. However, members of the Asteraceae form achene fruits where two carpels fuse, forming one locule that contains one seed. Scale bars = 1 cm in all images. (**a**–**e** RR Wise)

◘ Fig. 18.3 **f** The vascular strands (VS) running to each ovule (Ov) can be clearly seen in this squash (*Cucurbita* sp.) fruit. Scale bar = 1 mm. (RR Wise)

(**anatropous**, ◘ Fig. 18.3j). The number of ovules in a single ovary varies from one to a great multitude, which ultimately provides the upper limit to the number of seeds which can be formed.

18.4 Development of the Megagamete Begins with the Differentiation of the Megaspore Mother Cell and Ends with the Formation of the Megagametophyte (Embryo Sac)

The formation of the female gamete (megagamete or egg cell apparatus), which is enclosed in the megagametophyte, can be subdivided into two stages, **megasporogenesis** and **megagametogenesis**. In megasporogenesis, a diploid **megaspore mother cell** (or **megasporocyte**) undergoes meiosis to produce four haploid **megaspores**. Megagametogenesis follows in which the four haploid megaspores undergo a complex series of mitoses and nuclear fusions to produce a variety of n, $3n$, $5n$, and even $6n$ cells that make up the mature **megagametophyte**, or **egg sac**. The number and ploidy level of the cells in the mature egg sac varies among taxa. The process in lily (*Lilium* sp.) will be detailed in this section as an example. Keep in mind, however, that numerous variations exist across the plant kingdom.

An overview of megasporogenesis is given in ◘ Fig. 18.4a–c, with details provided in Fig. ◘ Fig. 18.4d-i. Megasporogenesis starts

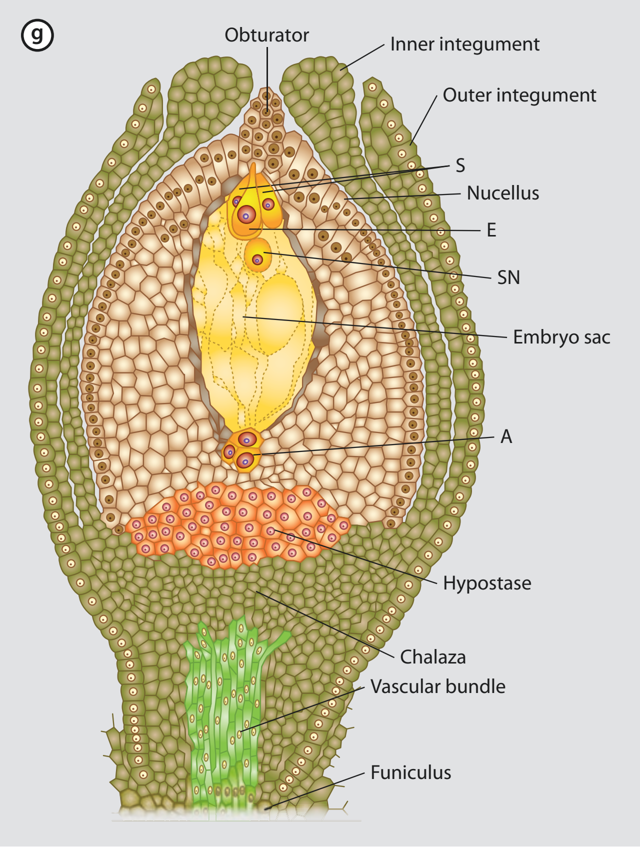


Fig. 18.3 **g** Diagram of a longitudinal section of smartweed (*Polygonum coriarium*) illustrating the orthotropous (upright) ovule immediately prior to double fertilization. *S* synergids, *E* egg cell, *SN* secondary nucleus of central cell, *A* antipodals. (Redrawn from Crang and Vassilyev 2003)

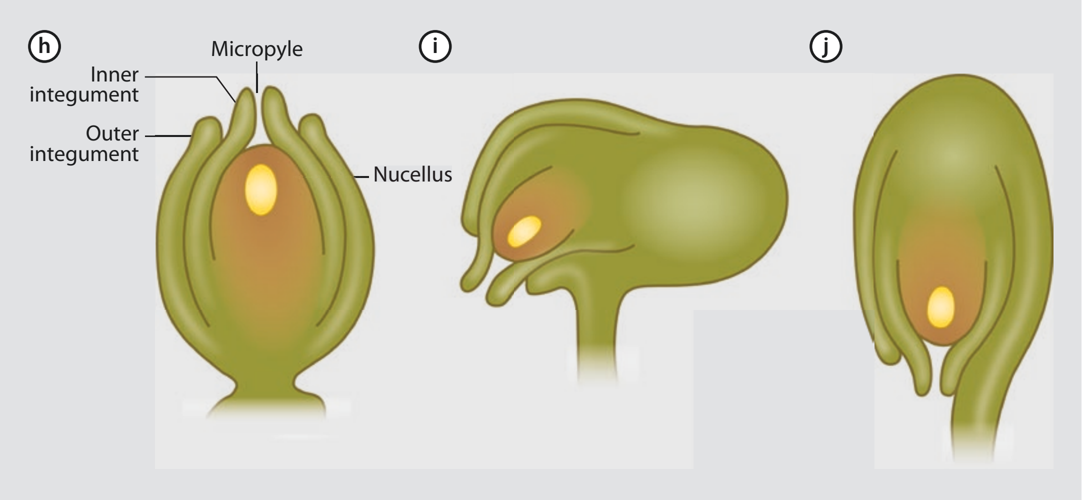
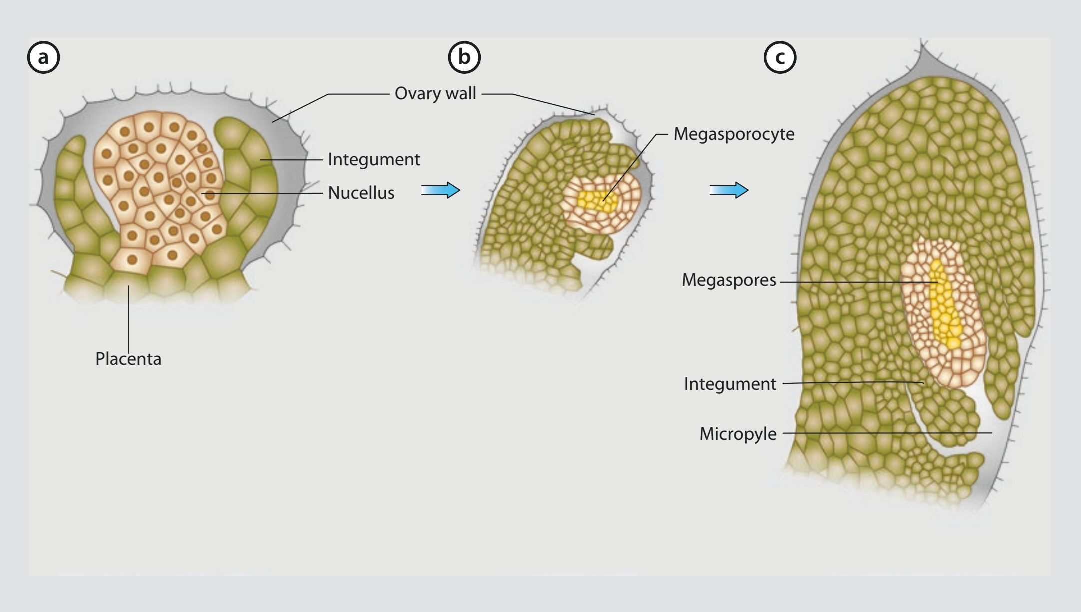


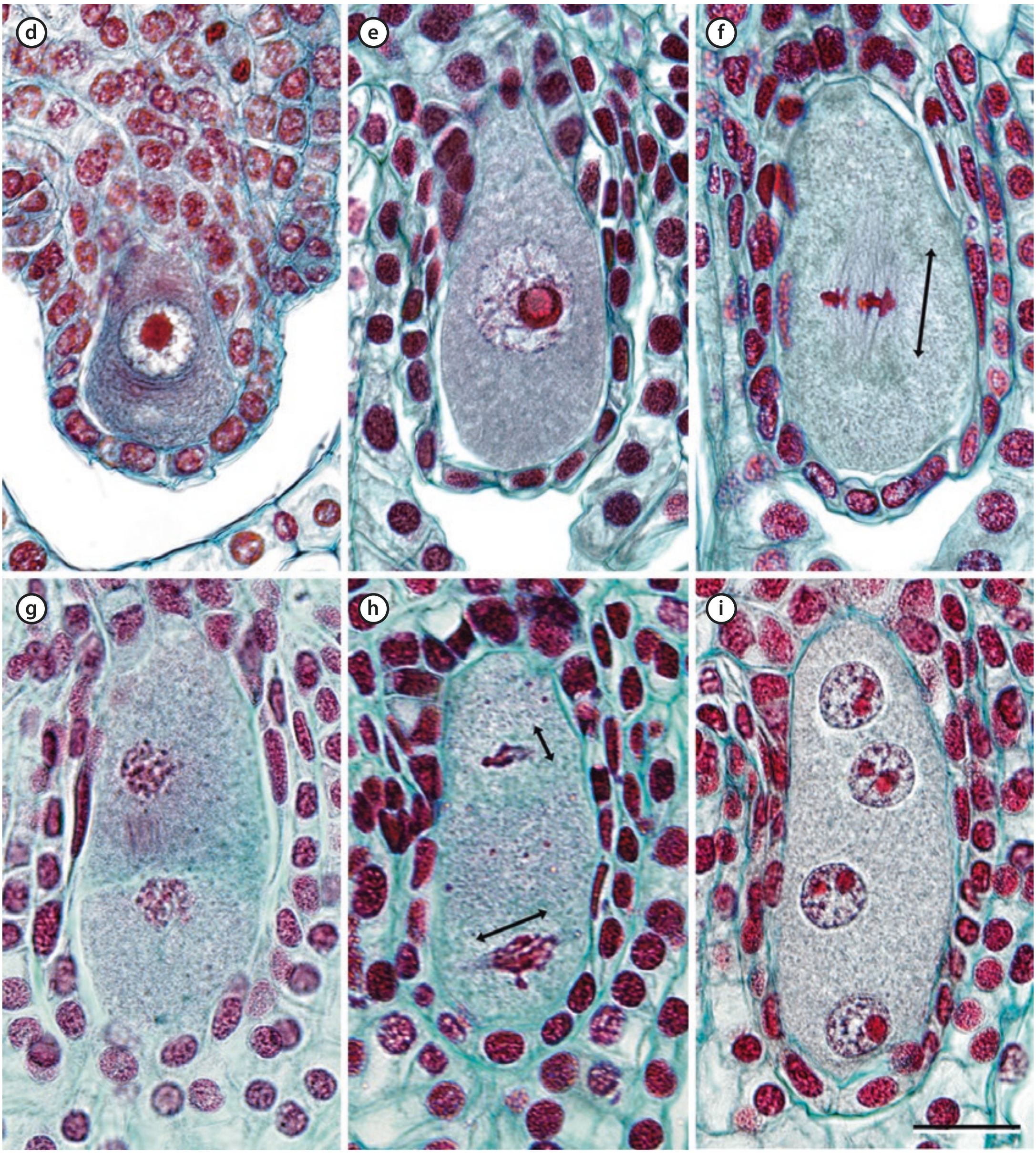
Fig. 18.3 **h**–**j** Three orientations of mature ovules, **h** orthotropous (upright), **i** hemitropous, and **j** anatropous. (**h**–**j** JM Coulter, CR Barnes and HC Cowles (1910), public domain)



◘ **Fig. 18.4** **a**–**c** In megasporogenesis a diploid megaspore mother cell (megasporocyte) undergoes meiosis to produce four haploid megaspores. This illustration portrays structural changes of an anatropous ovule of a sedge (*Carex* sp.) at three early developmental stages. Only a single ovule develops in the ovary. **a** A very young stage of development. **b** Represents the differentiation of the megasporocyte—note the change of the ovule orientation relatively to the placenta. **c** Demonstrates a postmeiotic ovule with four megaspores. The ovule is turned to such an extent that it lies near the placenta—a characteristic feature of anatropous ovules where the placenta is at the base of the ovary. (Redrawn from Crang and Vassilyev 2003)

with the differentiation of the megasporocyte in the nucellus of the young ovule. The nucellus is the central mass of diploid cells in the ovule containing the embryo sac and the developing megasporocyte. The megasporocyte is conspicuous because of its large size, dense cytoplasmic content, and prominent nucleus (◘ Fig. 18.4d). It undergoes meiosis 1 and meiosis 2, without accompanying cytokinesis, resulting in a linear array of four haploid megaspores (nuclei only), called a tetrasporic embryo sac or tetrad (◘ Figs. 18.4e–i).

Megagametogenesis then follows megasporogenesis. Megagametogenesis is a mitotic process as seen in ◘ Figs. 18.4j–o. Three of the haploid megaspores migrate to the chalazal end (◘ Figs. 18.4k) where they fuse and to form a single triploid ($3n$) mass of chromosomes (nucleus) that shares a common mitotic spindle (◘ Figs. 18.4l). The fourth haploid megaspore migrates to the micropylar end. The resulting two nuclei – one triploid and one haploid – then undergo two rounds of mitosis each (◘ Figs. 18.4m,n), resulting in four triploid nuclei at the chalazal end and four haploid nuclei at the micropylar end (◘ Figs. 18.4o). Thus, a coenocytic (lacking cell walls) eight-nucleate embryo sac is formed. Subsequent cytokinesis and cell differentiation result in a seven-cell embryo sac composed of a three-celled egg apparatus at the micropylar pole, three antipodals at the chalazal pole, and a binucleate central cell (◘ Figs. 18.4p).



■ **Fig. 18.4** **d**–**i** Stages of megasporogenesis in lily (*Lilium* sp.). Megasporogenesis is a meiotic process composed of two rounds of nuclear division without cytokinesis resulting in a tetrad of haploid nuclei. **d** The megasporocyte is a large, conspicuous cell with a single diploid nucleus. The first division (meiosis 1) proceeds from e prophase 1 to **f** metaphase 1 to **g** telophase 1 resulting in a dyad with two nuclei. The second division (meiosis 2) begins with **h** anaphase 1 and ends with **i** telophase 2. Meiosis has generated four haploid megaspore nuclei, arranged in a linear tetrad. The chalazal end is to the top of each image, and the micropylar end is to the bottom. The double-headed arrows in **f** and **h** indicate the orientation of the meiotic spindle. Scale bar in **i** = 25 µm and applies to all panels. (**d**–**i** RR Wise)

The **egg apparatus** consists of two **synergids** (sister cells) and one egg cell (■ Fig. 18.4p). In the synergids a **filiform apparatus** develops that consists of highly branched irregular wall protuberances protruding deeply inside the cells as seen with high-magnification light microscopy or electron microscopy. The filiform apparatus with its greatly extended plasmalemma surface is thought to be involved in the synthesis and secretion of

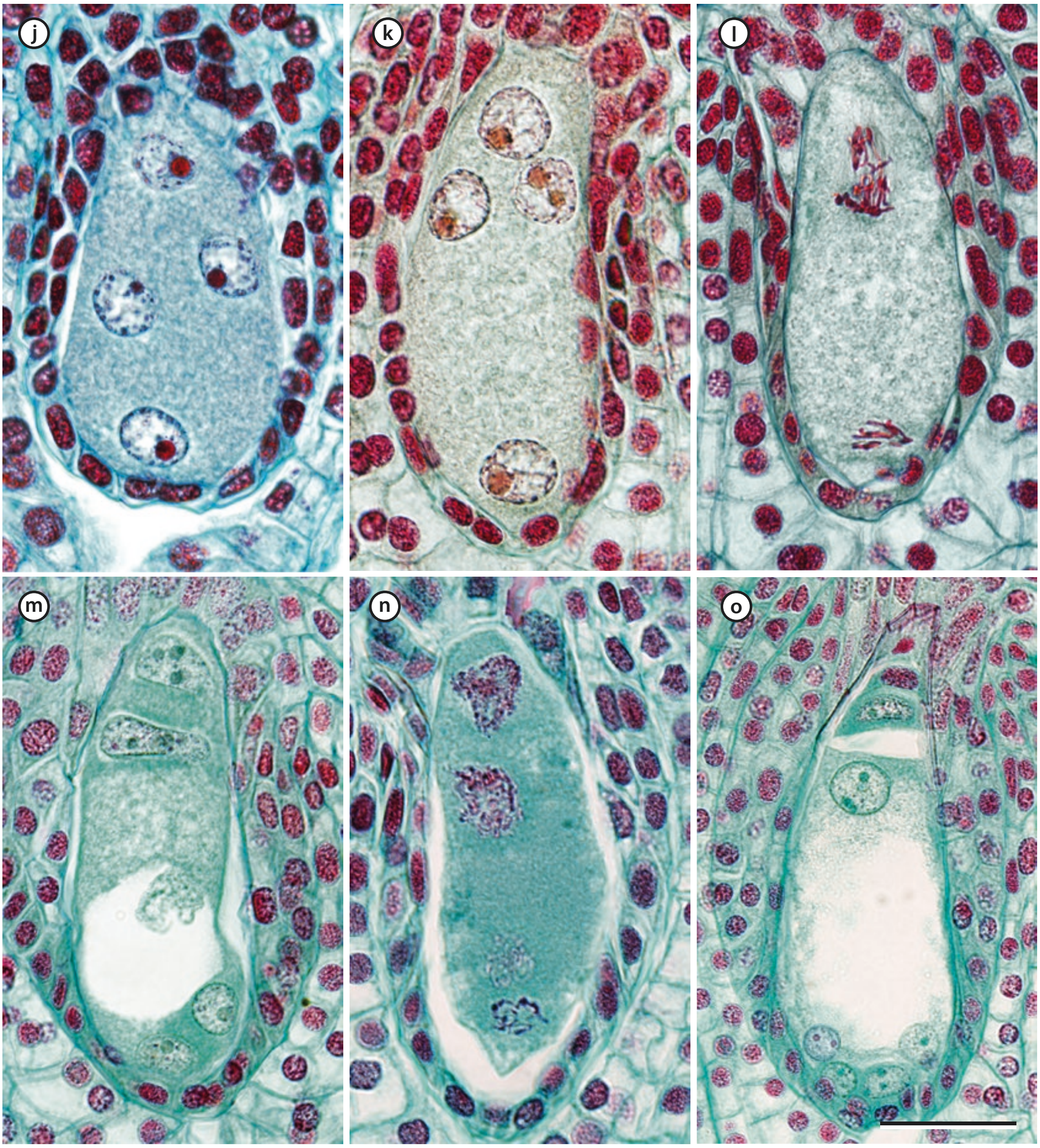


Fig. 18.4 **j**–**o** Stages of megagametogenesis in lily (*Lilium* sp.). In **j** and **k**, three of the four haploid megaspores migrate to the chalazal end and one migrates to the micropylar end. **l** The three chalazal nuclei fuse to form a single triploid nucleus. **m** Mitosis 1 generates two triploid chalazal nuclei and two haploid micropylar nuclei. **n**, **o** Mitosis 2 then generates eight nuclei (four 3*n* and four 1*n*). Scale bar in **o** = 25 µm and applies to all panels. (**j**–**o** RR Wise)

substances capable of directing pollen tube growth toward the embryo sac. In a sense, synergids may be treated as transfer cells and are further characterized by the polar distribution of cell components. Their protoplasm is concentrated in the micropylar half of the cell, while the chalazal half is highly vacuolated. The egg cell, like the synergids, also shows a specific polarity. However, in contrast to synergids, most of the protoplasm is located in the chalazal third of the cell, while the micropylar two-thirds contain a large vacuole.

The **central cell** occupies the largest portion of the embryo sac and is highly vacuolated. In the beginning, it is binucleate, and its two nuclei, along with most of the cytoplasm, are located near the egg apparatus (◘ Fig. 18.4p, q). These nuclei are called **polar nuclei**, because they are derived from groups of nuclei at the opposite poles of the eight-nucleate embryo sac. The close arrangement of polar nuclei to the egg apparatus apparently facilitates double fertilization. The polar nuclei eventually fuse with each other, but the time of fusion varies from taxon to taxon. In some plants, the fusion is completed, and the nucleus becomes diploid before fertilization. In such case, the fully mature embryo sac is seven-nucleate. But in other cases, the polar nuclei fuse only after the arrival of the sperm in the sac. The antipodals persist throughout megagametophyte development and will serve in the growth of the endosperm.

Together with the developing embryo sac, the nucellus also changes. The majority of angiosperms possess ovules in which most of the nucellus degenerates before the embryo sac reaches maturity, subsequently leaving the mature embryo sac in direct contact with the inner integument. In other cases, the inner epidermis of the integument that borders the embryo sac frequently differentiates into a specific tissue, the **endothelium** (◘ Fig. 18.4q). It consists of radially elongated cells rich in cytoplasmic content. In ovules where the nucellus is abundant, it expands by cell division during embryo sac development, and the mature embryo sac is surrounded by a massive nucellus. A general summary of the process from megasporogenesis to megagametogenesis is illustrated in ◘ Fig. 18.4r.

18.5 Pollination Is Followed by Germination of the Pollen Grain and Pollen Tube Growth

After a pollen grain reaches a stigma (whether via wind, insect, bird, etc.), it germinates, and, if it is compatible with the stigma, it forms a pollen tube capable of transporting the male gametes to their sites of fertilization. The surface of the stigma creates an

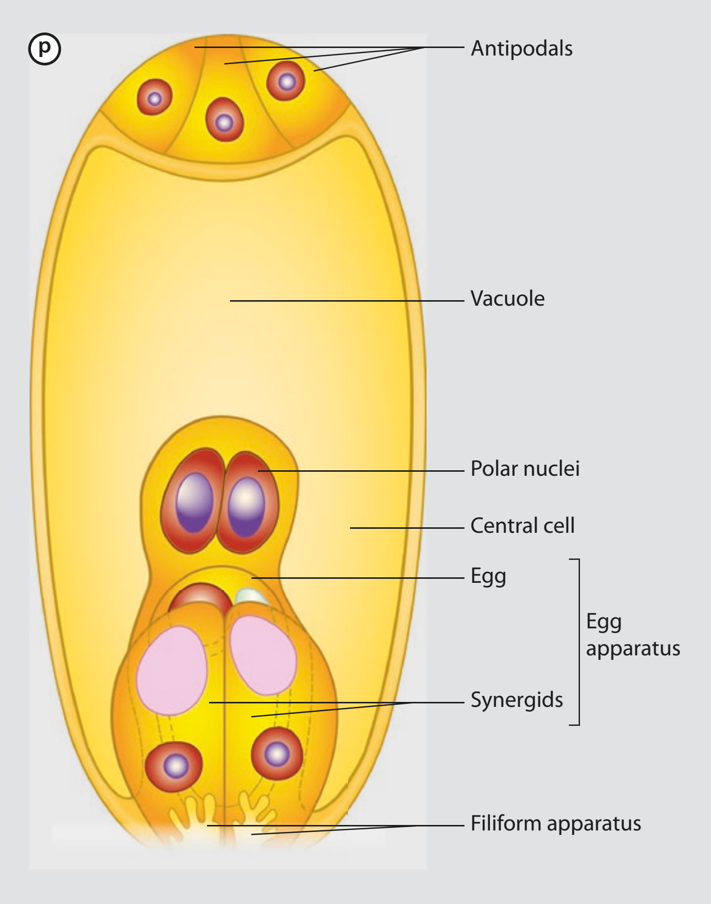
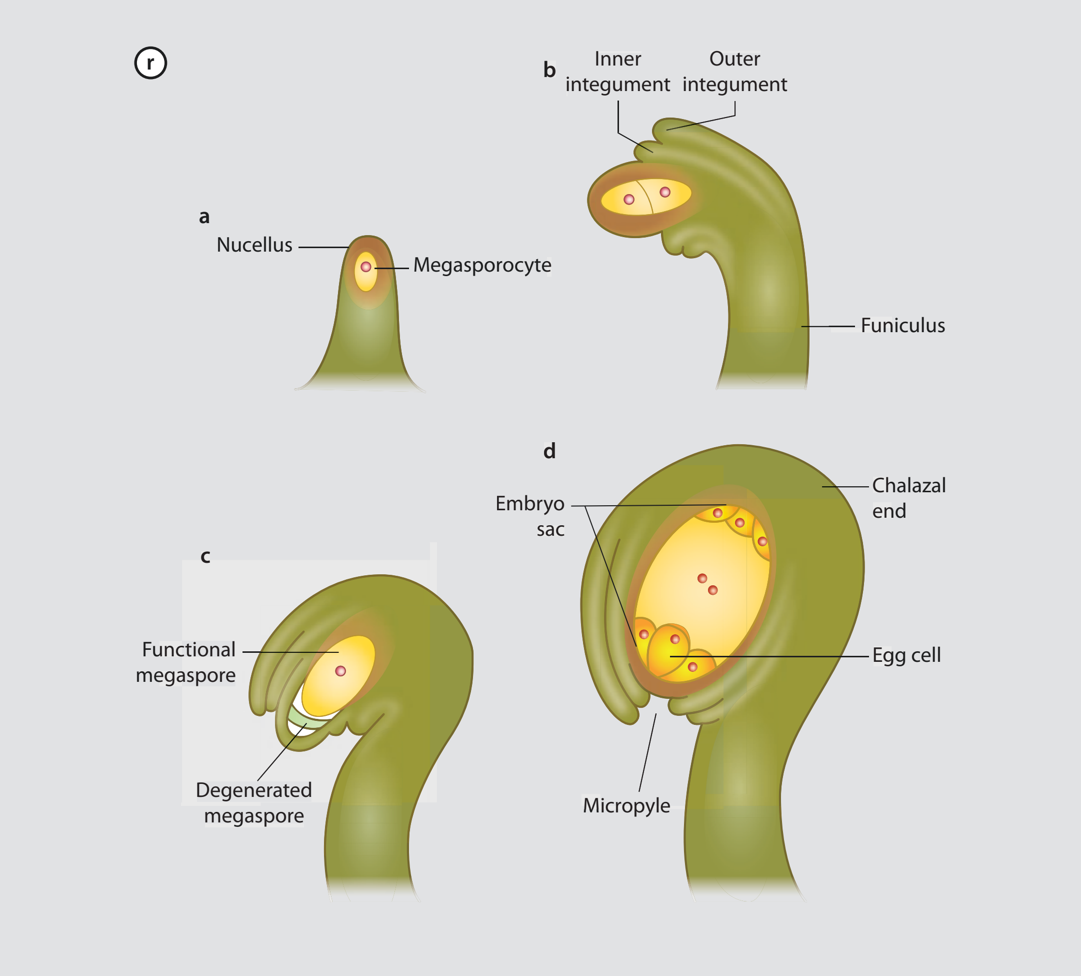


Fig. 18.4 **p** Embryo sac illustrating the highly ordered cellular arrangement that is characteristic of the majority of plants. The micropyle is at the bottom. Note the filiform apparatus in the synergids and the arrangement of the vacuoles and nuclei in synergids and egg cell. In synergids the nuclei are oriented toward the micropyle, and vacuoles are toward the chalazal end of the cells, whereas in the egg, the nucleus and most of the cytoplasm is at the chalazal end. (Redrawn from Crang and Vassilyev 2003)

optimal physiological condition for compatible pollen grains to germinate. Both the stigma and the pollen grain coatings are involved in the process of recognition that allows pollen grains to germinate and produce successful pollen tubes only in compatible combinations. In *Brassica* (as in many other plants.), two- to three-celled hairs are formed on the stigmatic surface of the carpel (Fig. 18.5a–c). The hairs secrete viscid substances that are involved in the recognition of compatible pollen. If they fit together with the substances emanating from the pollen grain wall, pollen grains germinate and produce normal filament-like pollen tubes that will grow through the style.

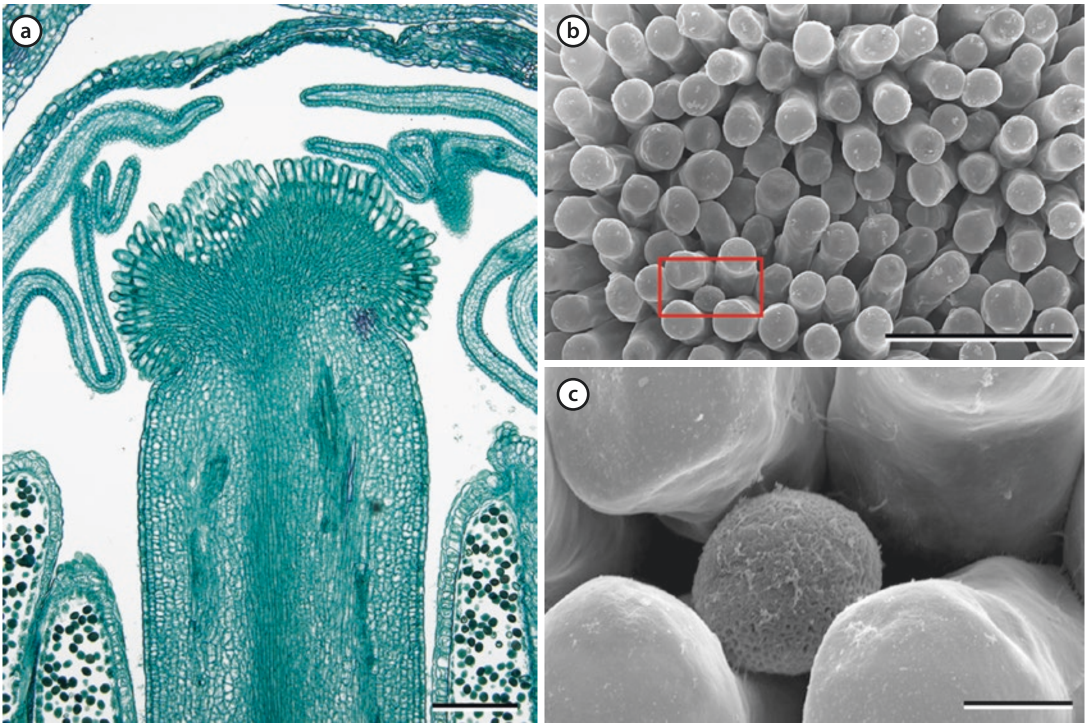
◘ **Fig. 18.4** **q** In rosinweed (*Silphium* sp., Asteraceae), the fully mature and ready to be doubly fertilized embryo sac is seven-celled and eight-nucleate. The densely cytoplasmic columnar cells surrounding the embryo sac comprise the endothelium (En). The three antipodals (A) at the chalazal end are prominent with darkly staining nuclei. Only the lower polar nucleus (P) is visible in this section. The egg cell (EC) holds the sixth nucleus, and the two synergids (Sy) account for the final two, to make eight. The filiform apparatus (FA) is at the micropylar end. Scale bar = 50 µm. (RR Wise)

The style contains a stylar transmitting tissue (a.k.a. stigmatoid tissue), specialized for conducting the growing pollen tubes from the stigma toward the ovules (◘ Fig. 18.5d, e). In styles having an open canal, the transmitting tissue lines the canal and consists of one layer of glandular cells. In solid styles, characteristic of the majority of flowering plants, the transmitting tissue forms one or more strands of elongated densely cytoplasmic cells embedded in the central ground parenchyma. The pollen tubes grow downward to the ovary through



■ **Fig. 18.4** **r** Schematic summary diagram representing the development of an anatropous ovule from the megasporocyte to the megagametophyte (embryo sac), which is characteristic of the majority of plants. Note how the orientation of the ovule changes with respect to the placenta during development. **a** Representation of an ovule shortly after initiation, showing a single megasporocyte. Note the lack of the inner and outer integuments. **b** An ovule after both integuments have started to develop. The megasporocyte has passed the first meiotic division. The axis of the nucellus is transiently at 90° to the axis of the funiculus. **c** A post-meiotic ovule. The functional megaspore at the chalazal end has expanded, and the nonfunctional megaspores have degenerated. The axis of the nucellus is now parallel to the funiculus due to unequal growth of the integuments. **d** The ovule after megagametogenesis within the megagametophyte. The mature embryo sac contains seven cells and eight nuclei. (Redrawn from Reiser and Fischer, 1993)

the thickened walls of transmitting cells which secrete wall-degrading enzymes. In hollow styles, the pollen tubes grow toward the ovary in contact with the glandular cells lining the stylar canal. This pollen transmitting tissue consists of one layer of glandular cells, which secrete various hydrophilous and lipid substances involved in nourishment and guidance of the pollen tubes as well as numerous enzymes released upon the growth of pollen tubes (■ Fig. 18.5d, e).



◘ **Fig. 18.5** **a**–**c** Sectional view of the stigma of *Brassica oleracea* showing a dense mat of hairs, which is the receptive surface for the pollen grains. **b** Surface view of a stigma from a flower of the lyre-leaved sand cress (*Arabidopsis lyrata*). The red box indicates a pollen grain trapped between the stigmatic hairs. **c** A close-up view of the pollen grain indicated in ◘ Fig. 18.5b. Scale bars = 500 µm in **a**, 100 µm in **b** and 10 µm in **c**. (**a**–**c** RR Wise)

18.6 Double Fertilization Results in a Triploid Endosperm and a Diploid Zygote

Double fertilization follows pollination and begins when the pollen tube, which has grown to and through the micropyle, discharges the two sperm cells into one of the synergids. Each will fertilize a cell, hence the term "double fertilization." One of the two male gametes (the haploid sperm cells) from the pollen tube fertilizes the haploid egg cell, and the other haploid nucleus fertilizes the diploid (or triploid, in the case of lily) central cell nucleus. The now diploid fertilized egg is called a **zygote** . It will give rise to the embryo and the suspensor (◘ Fig. 18.6a–c). A common feature of angiosperm embryos is that their apical basal axes are aligned according to the chalazal-micropyle axis, suggesting an orienting influence of the surrounding maternal tissues. The suspensor conveys nutrients to the growing embryo and pushes it into the lumen of the endosperm.

The typically triploid or tetraploid central cell resulting from double fertilization will become the endosperm, a nutritive tissue for the embryo. Two main types of endosperm development occur,

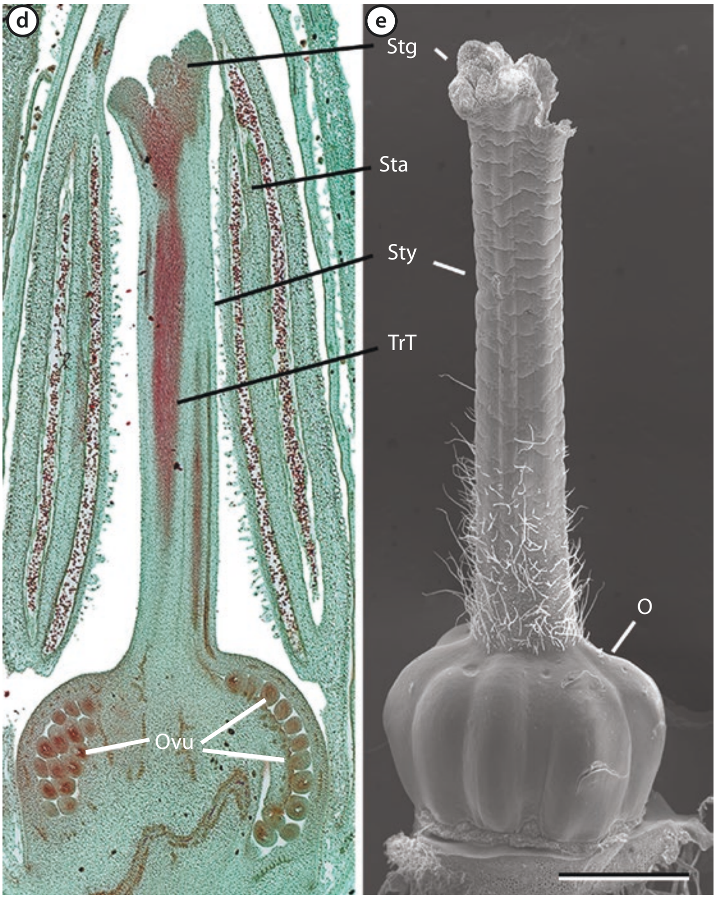


Fig. 18.5 **d** Bright-field light micrograph and **e** SEM of pistils from tomato (*Solanum lycopersicum*). *O* ovary, *Ovu* ovules, *Sta* stamen, *Stg* stigma, *Sty* style, *TrT* transmitting tissue. Scale bar in **e** = 500 µm and applies to both panels. (**d**, **e** RR Wise)

cellular and nuclear. In a **cellular endosperm**, the cell wall formation begins with the first mitosis and continues as long as endosperm is growing. In a **nuclear endosperm**, the nuclei undergo "free-nuclear division" meaning that mitoses are not accompanied by cytokinesis. In this case, cell wall formation begins only at an advanced stage of endosperm growth. Fertilization to form the endosperm is a significant evolutionary adaptation in angiosperms as plants efficiently provision for offspring only when the embryo is present.

Embryogenesis generally occurs in a series of steps from zygote to the mature embryo. These steps can be divided into three basic phases: (1) postfertilization-proembryo phase, (2) globular-cordate transition, and (3) organ expansion and maturation. Within the following section, we will focus on the embryogenesis of shepherd's purse (*Capsella bursa-pastoris*), one of the most intensely studied dicot plants for embryonic development.

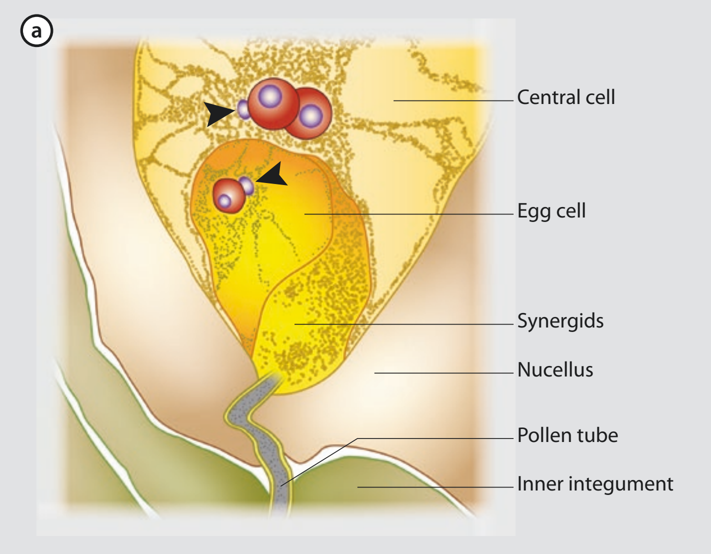


Fig. 18.6 **a** A typical illustration of the double fertilization process. The pollen tube enters through the micropyle and one of the synergids discharges its contents. Then the sperm nuclei (*arrows*) traverse the synergid and one fertilizes the egg cell to form the diploid zygote. The other sperm nucleus enters the central cell where it fuses with the two polar nuclei to form the triploid endosperm. (Redrawn from Reiser and Fischer, 1993)

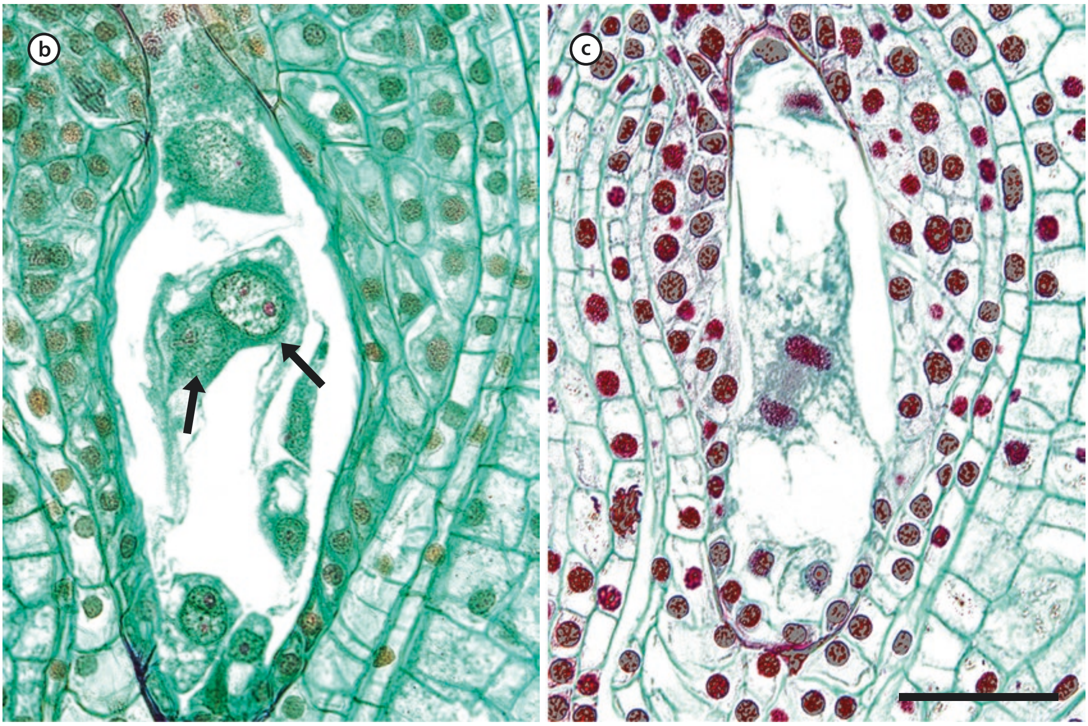
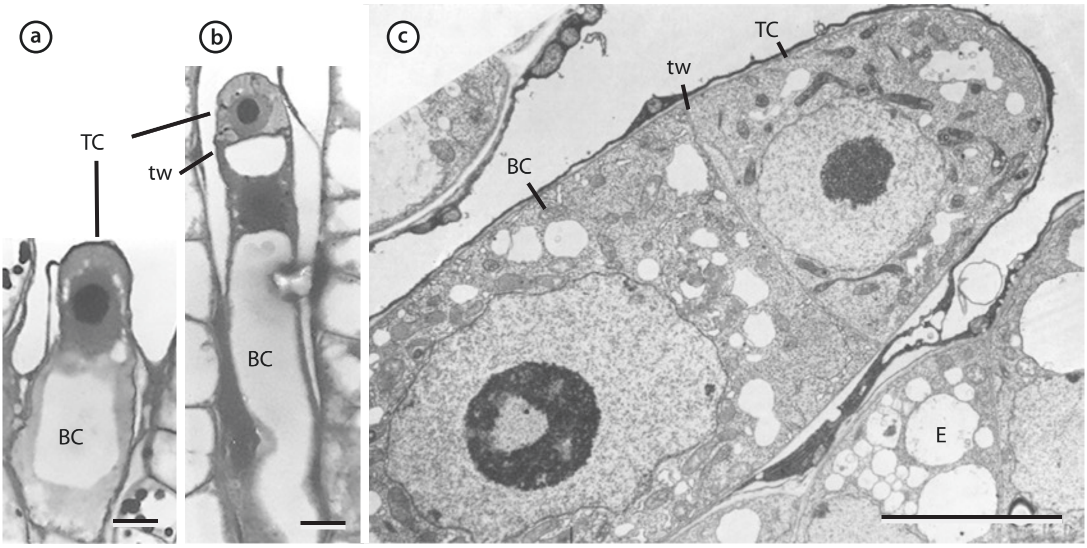


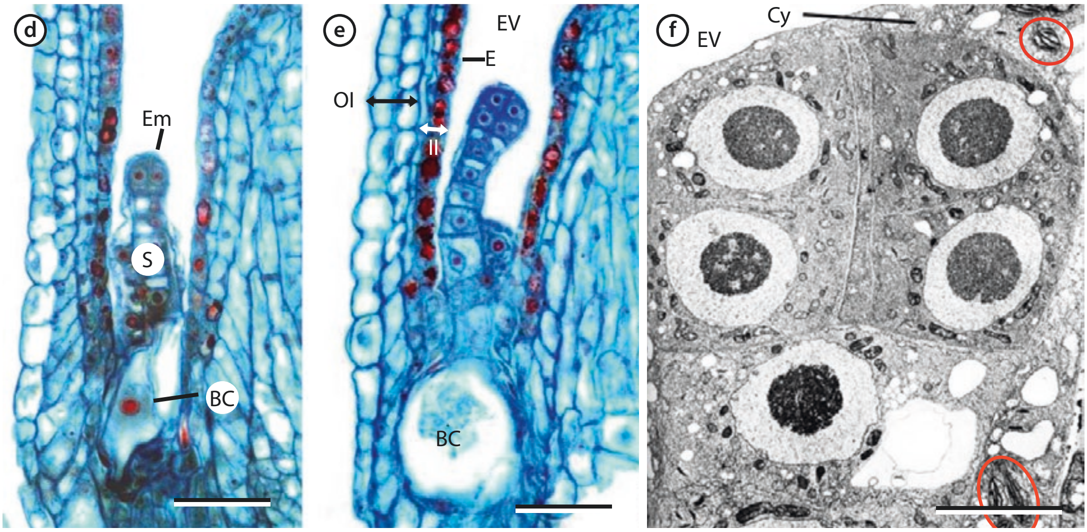
Fig. 18.6 **b** Double fertilization in Lily (*Lilium* sp.). The two polar nuclei are indicated with arrows. **c** The resulting tetraploid nucleus proceeds to divide to produce the endosperm. Scale bar in **c** = 50 µm and applies to both panels. (**b**, **c** RR Wise)

18.7 Postfertilization-to-Proembryo Phase Leads to the Formation of an Eight-Celled Embryo and Suspensor Whose Development Is Controlled by Circuit Elements

The postfertilization-proembryo phase of embryogenesis begins with the zygote and continues on to the eight-celled embryo. Embryogenesis starts with the unequal transverse division of the zygote that gives rise to a two-celled proembryo that consists of a large basal cell and a small terminal cell (◘ Fig. 18.7a). These cells differ in their ultrastructure as the terminal cell is rich in polymorphic electron-dense leucoplasts, and the basal cell is poorly vacuolated and contains electron-transparent ellipsoid plastids (◘ Fig. 18.7b, c). The proembryo protrudes into the highly vacuolated endosperm. In *Capsella*, the terminal cell undergoes longitudinal division to give rise to the embryo proper. The basal cell divides twice transversely, and the linear **suspensor** is initiated. Its cells also divide transversely. As a result, the embryo is pushed into the endosperm. During the eight-celled stage of the embryo or "octant," the suspensor completes cell divisions, and the nuclei, with their prominent and active nucleoli, occupy a large portion of the cells (◘ Fig. 18.7d–f). Thus, at the end of the first phase, we see the differentiation of the terminal and basal cells, as well as the formation of the embryo and suspensor.



◘ Fig. 18.7 **a**–**c** Early proembryo phase in shepherd's purse (*Capsella bursa*). **a** Longitudinal section through the polarized zygote within a large micropylar vacuole. **b** The two-celled proembryo. Look for the small densely cytoplasmic terminal cell (TC), the large vacuolated basal cell (BC) of the suspensor, and the transverse wall (tw) between them. **c** Transmission electron micrograph of a two-celled proembryo showing the terminal cell, the apical portion of the basal cell, and endosperm (E). Scale bars = 10 µm in **a** and **b** and 5 µm in **c**. (**a**–**c** R Schulz and WA Jensen 1968a)

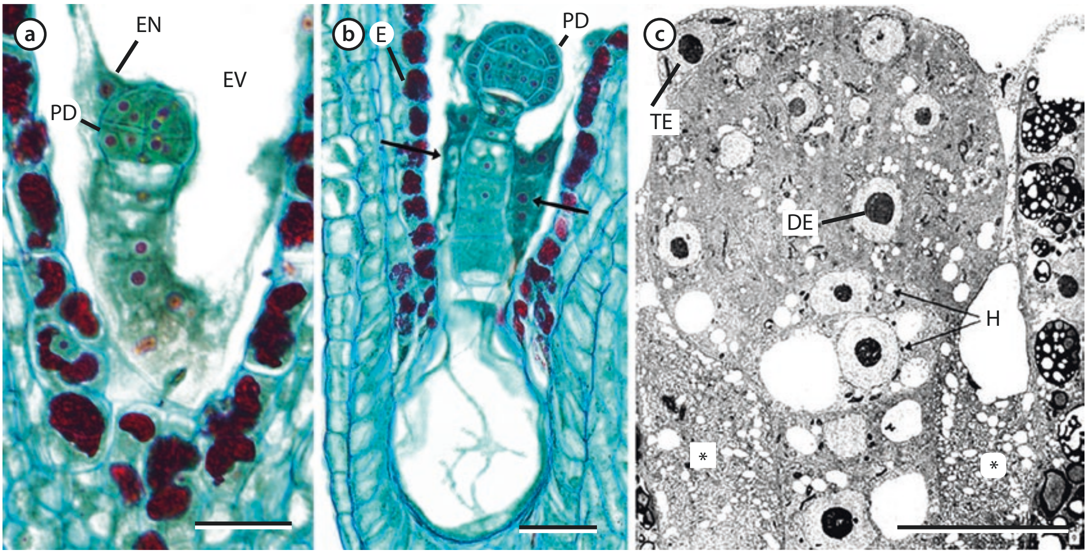


◘ **Fig. 18.7** **d**–**f** Proembryo to octant transition in *Capsella*. **d** A two-celled embryo (Em), a five-celled suspensor (S), and a large basal cell (BC) during *Capsella* embryogenesis. **e** The eight-celled or octant stage of development of embryo. Only four cells can be seen in this view since the other four are in another section of the embryo. The suspensor has grown into a long filament of ten cells. Note outer (OI) and inner (II) integuments, precursors of seed coat, endothelium (E), and basal cell (BC). **f** The axile portion of an octant embryo seen with electron microscopy. Note how the embryo protrudes deeply in the endosperm vacuole and is separated from it by very thin layer of endosperm cytoplasm (Cy). The endosperm contains chloroplasts (red ellipses) and is green. Scale bars = 20 µm in **d** and **e** and 10 µm in **f**. (**d**–**f** R Schulz and WA Jensen 1968b)

18.8 Early Embryo-to-Heart Transition Leads to the Specification of the Basic Body Plan Within the Embryo

The globular-heart transition begins with the 16-celled embryo and continues to the cordate (heart-shaped) embryo. At the 16-celled embryo stage, the outer cells of the embryo are formed from ordered divisions, both periclinal and anticlinal. These cells constitute the beginning of the protoderm (a.k.a. embryoderm), the precursor of the epidermis (◘ Fig. 18.8a). At the globular stage of development, the embryo is surrounded by endosperm cytoplasm, the **protoderm** is clearly demarcated and, in the central region of the embryo, vertical divisions have delineated the procambium. The upper suspensor cell divides and produces a hypophysis, the uppermost cell protruding into the embryo. The **hypophysis** is the only cell of the suspensor that later becomes part of the embryo. Its derivatives take part in the formation of the radicle and root cap of the mature embryo (◘ Fig. 18.8b, c).

The cordate embryo stage is identified by a characteristic heart shape. During this stage, the formation of organs within the embryo begins with the cotyledons that appear as two protrusions in the



■ **Fig. 18.8** **a**–**c** Early embryo-to-globular stage transition in *Capsella*. **a** At the 16-celled embryo stage, demarcation of the protoderm (PD) occurs. The endosperm vacuole (EV) and an endosperm-free nucleus (EN) are marked. **b**. *Capsella* has a characteristic nuclear type of endosperm development where the nuclei accumulate at two ends of the coenocytic multinucleate endosperm. In the micropylar end, they (together with the cytoplasm) form a "sheath" around the suspensor as shown here (*arrows*). Also visible are a portion of endosperm central vacuole (EV), endothelium of the inner integument (E), and several endosperm-free nuclei (at *arrows*). **c**. A transmission electron micrograph of the globular stage of the embryo. The cells of the protoderm, the future procambium, and ground meristem all show structural similarity. The two suspensor cells next to the embryo are derived from an unequal division of the hypophysis (H). The endosperm (*) closely envelopes the embryo. TE triploid endosperm nucleus; DE diploid embryo nucleus. Scale bars = 10 µm in **a** and **b** and 5 µm in **c**. (**a**–**c** R Schulz and WA Jensen 1968b)

apical part of the embryo. These protrusions give an embryo the cordate shape and are the result of preferential cell divisions in two areas of the embryo. Since cotyledons do not originate from the shoot apical meristem as do leaves, some anatomists do not consider them to be modified leaves. At this stage, both the endosperm and the embryo appear green in fresh specimens due to the differentiation of chloroplasts. In this stage, the basic body plan is established (■ Fig. 18.8d–f).

18.9 The Last Phase of Embryogenesis Involves Organ Expansion and Maturation

The last phase of embryogenesis involves three stages: the torpedo, walking-stick, and mature embryo. During this last phase, the embryo is named for what it resembles. Thus, the "torpedo embryo" resembles a torpedo. This stage marks the beginning of hypocotyl elongation where the hypocotyl becomes discernible and the cotyledons continue to elongate. The procambium extends from the hypocotyl into young cotyledons.

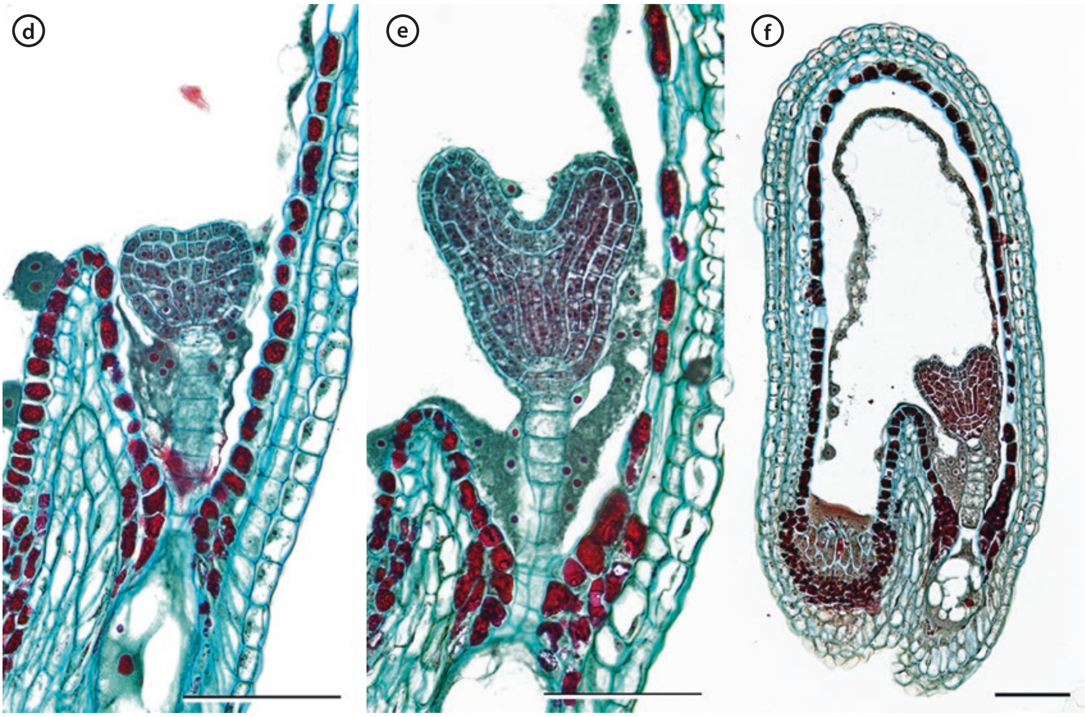
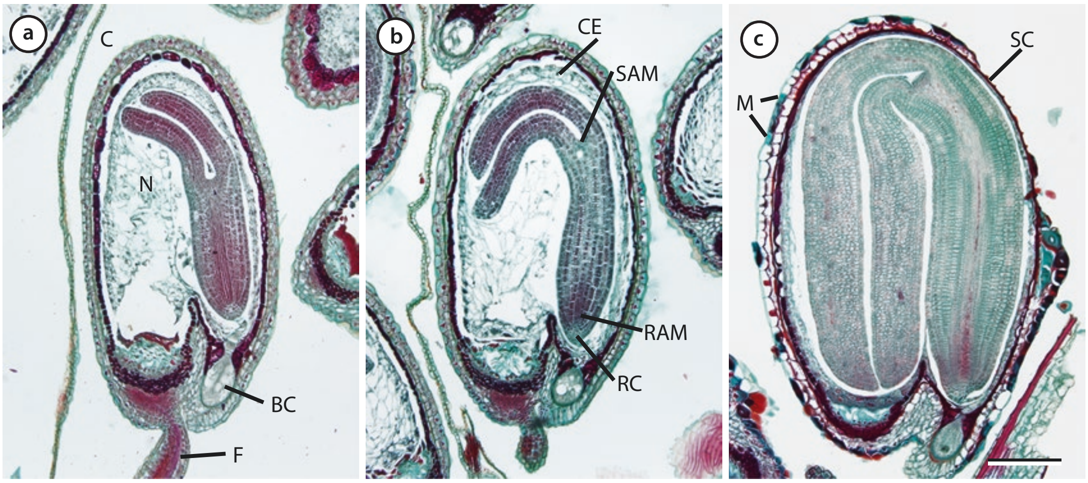


Fig. 18.8 **d**–**f** Organ specification during the globular-to-cordate shape transition in *Capsella*. **d** The cordate (heart-shaped) stage of the embryo. Identify two integuments, endothelium (inner layer of the inner integument), endosperm cytoplasm with nuclei, cotyledonary buttresses, procambium, hypophysis, and suspensor and its basal cell. **e**. A higher magnification of the heart-shaped (cordate) embryo which clearly shows its parts. **f** A low-magnification view of a longitudinal section of an ovule showing further growth of cotyledonary primordia. The bilaterally curved type of ovules in which the ovular cavity has a horse-shoe shape is characteristic of *Capsella*. Scale bars = 50 μm in all three panels. (**d**–**f** RR Wise)

More primitive plants tend to have abundant endosperm and small embryos; however, more evolutionary developed seed plants tend to have more mature and larger embryos with less endosperm. Early on, in species with abundant endosperm, the nuclei divide without cell wall formation, but later the walls are formed around the nuclei and contained protoplasmic materials. Within the developing endosperm, cell walls are formed around each nucleus so that the endosperm becomes transformed from nuclear into cellular (◘ Fig. 18.9a).

As the embryo and cotyledons continue to develop, the cotyledons bend, taking on the appearance of a walking-stick. The embryo fills a large portion of the endosperm cavity and displays cotyledon curvature, which is conditioned by the growth of the embryo in the curved ovule and embryo sac. The shoot apical meristem is recognizable between the cotyledons, and root (radicle) apical meristem is clearly demarcated from the root cap. The cells of the integuments (developing seed coat) are stretched considerably parallel with elongation of the ovule (developing seed; ◘ Fig. 18.9b).



◘ **Fig. 18.9** **a**–**c** Embryo maturation. **a** The "torpedo embryo" stage when the hypocotyl becomes discernible and cotyledons elongate. The embryo is approximately 350 µm long. **b** This walking-stick embryo (~500 µm long) is at an early-bending cotyledon stage. **c** Mature embryo within the fully formed seed. The embryo now fully fills the space and is about 900 µm long. Integuments have transformed into the seed coat. The outer layer of cells has filled with mucilage (stained blue). BC = basal cell of suspensor, C = cotyledons, CE = cellular endosperm, F = funiculus, M = mucilage, N = remnants of nucellus, RAM = root apical meristem, RC = root cap, SAM = shoot apical meristem, SC = seed coat. Scale bar in **c** = 100 µm and applies to all panels. (**a**–**c** RR Wise)

When the embryo matures, it fills the space within the seed coat. Still, the basal cell and some other cells of the suspensor are still discernible, and the funiculus can be clearly seen. In living tissue, the embryo has lost its green color. The cells of the inner integument, including the endothelium, are broken down, and the seed coat (**testa**) is formed entirely by only the dead derivatives of the two-layered outer integument. The outer layer now consists of cells filled with mucilage, and the inner layer is sclerified (◘ Fig. 18.9c). A summary of the process of eudicot embryogenesis can be seen in ◘ Fig. 18.9d.

Box 18.2 Plant Embryogenesis from Zygote to Seed

Plant embryos must establish a postembryonic structural differentiation in the shoot and root meristems of sporophytic plants as well as overcoming often severe environmental conditions during germination. The events begin within the fertilized egg cell and the early embryo. Thus, specific molecular markers are necessary in order to trace the developmental events that initiate in the early embryo but which continue into the regulatory networks with plant growth and differentiation. Auxins, such as indoleacetic acid, are involved in many plant development and transition activities and can block the transition from the globular to heart stage of the embryo and indicate that auxin asymmetries are established within the embryo-proper region of the globular stage but continue to contribute to the bilateral symmetry at the heart stage. Studies in *Arabidopsis* have identified genes that provide insight into plant development and embryogenesis.

Reference: Goldberg RB et al. (1994)

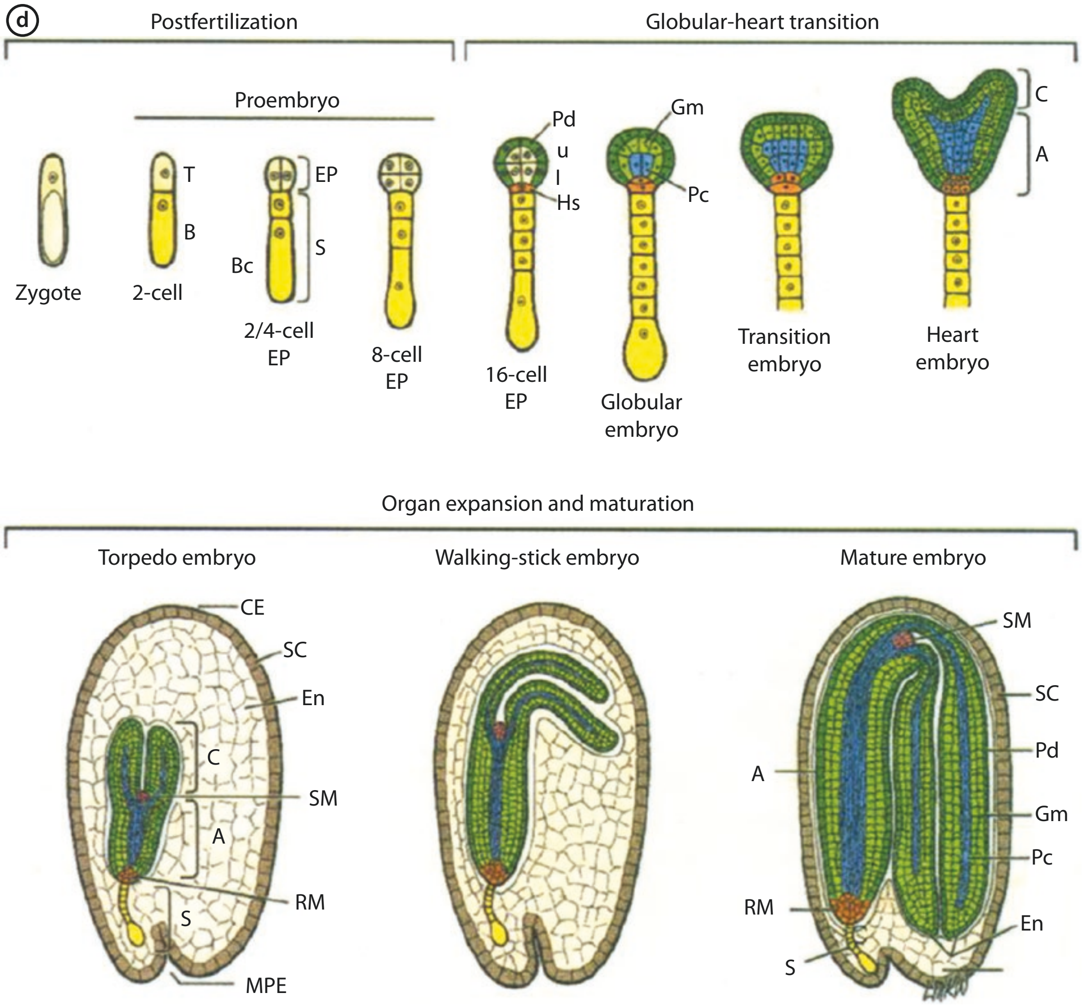


Fig. 18.9 **d** Summary of embryogenesis from zygote to mature embryo within an angiosperm such as *Capsella* or *Arabidopsis*. T terminal cell, B basal cell, EP embryo proper, S suspensor, Bc suspensor basal cell, Pd protoderm, u upper tier, Hs hypophysis, Pc procambium, Gm ground meristem, C cotyledon, A axis, MPE micropylar end, CE chalazal end, SC seed coat, En endosperm, SM shoot meristem, RM root meristem. (From Goldberg et al. 1994)

18.10 The General Pattern of Embryogenesis Differs Between Eudicots and Monocots

In monocots, early stages of embryogenesis are similar to those in eudicots, but further developmental stages are distinctly different. In monocots, the model organism for the study of embryogenesis is maize (*Zea mays*). The general pattern of monocot embryogenesis includes the zygote, proembryo, transition, coleoptile, stage 1, and succeeding mature stages. The monocot shoot apex is found, not between the bases of the two cotyledons, but occupies a lateral position to the single cotyledon as its niche. Monocots also differ from eudicots in only having one cotyledon rather than two.

Embryogenesis in maize differs from eudicots in several ways. First, the early cellular divisions in monocots are not equal. Second,

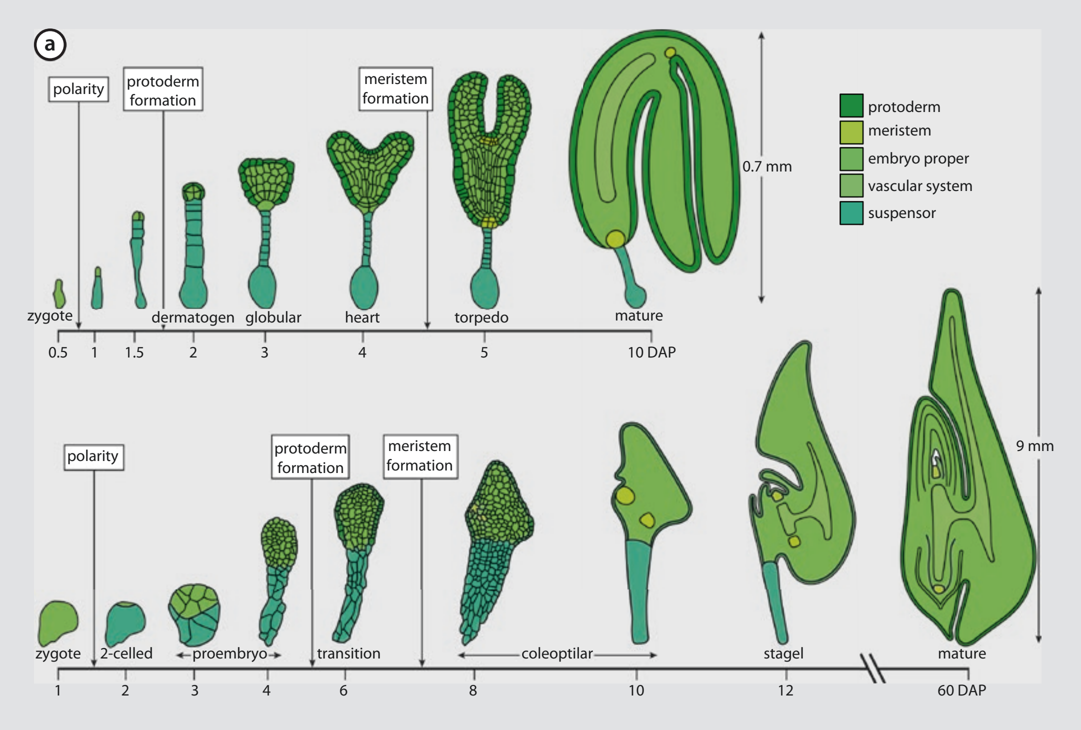


Fig. 18.10 **a** Comparison of dicot (*Arabidopsis thaliana*) and monocot (*Zea mays*) embryo development. Developmental time scale is days after pollination and will be influenced by genetics and the environment. (From Vernoud et al. 2005, open source)

the leaf primordia develop earlier in monocots in comparison to eudicots, as these structures have been noted to develop in *Arabidopsis* after entering dormancy (Vernoud et al. 2005, pg. 471). Because monocots have only one cotyledon, the axis between that apical and basal portions of the developing embryo is not as well defined in monocots as in eudicots. Third, the scutellum within the embryo of maize is functionally analogous to cotyledons of eudicots.

Differences within the environment of the developing seed exist between monocots and eudicots (Fig. 18.10a). The endosperm in *Arabidopsis* (a eudicot) is gone at seed maturity, in contrast to *Zea* (a monocot) where endosperm makes up a large part of the kernel. Both species have nuclear endosperm which is formed due to repeated nuclear divisions that are not immediately followed by cytokinesis (Fig. 18.10b).

18.11 Alternation of Generations Is Unique to Plants

Upon the formation of a zygote, a new generation begins that is diploid and considered to be the sporophyte since it produces haploid spores through the process of meiosis (megaspores and microspores). In all higher plants, the obvious organism seen is the sporophyte. The stages immediately following meiosis and leading up to the formation of the zygote represent the haploid gameto-

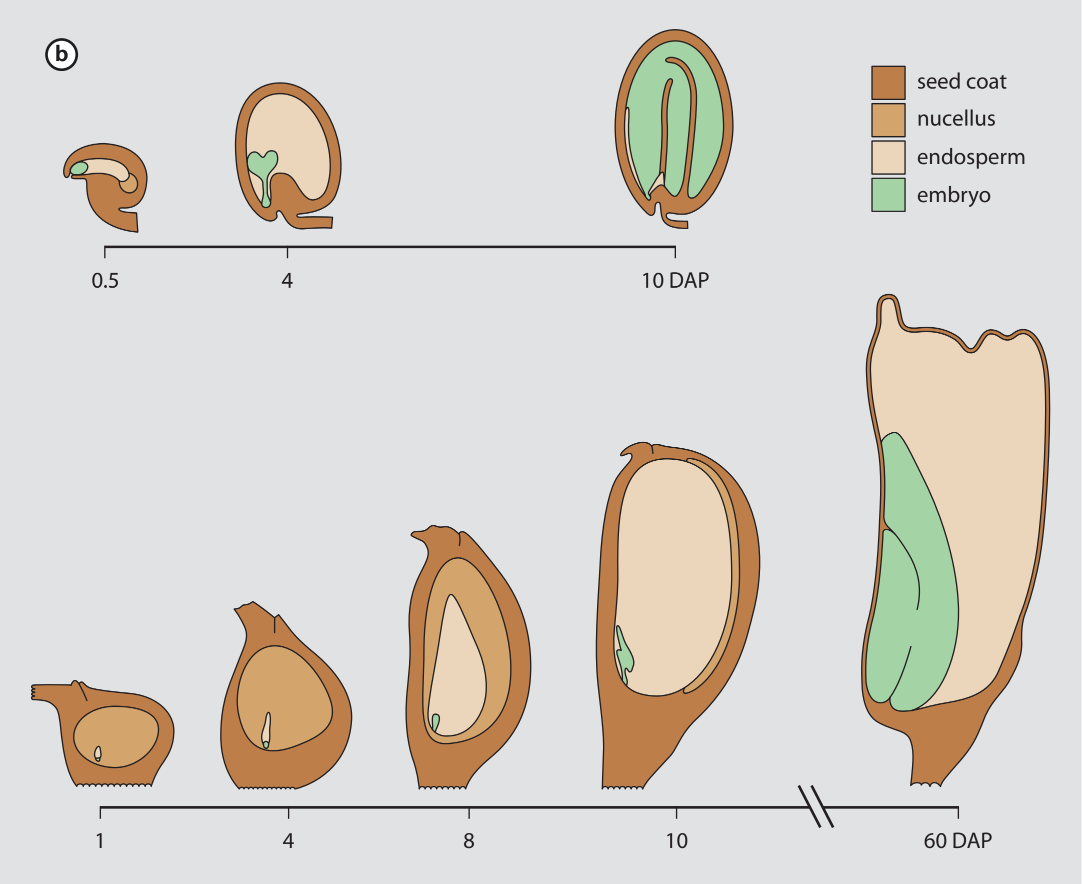


Fig. 18.10 **b** Comparison of dicot (*Arabidopsis thaliana*) and monocot (*Zea mays*) seed development. Developmental time scale is days after pollination and will be influenced by genetics and the environment. Compare relative sizes of embryos as well as endosperm during development. (From Vernoud et al. 2005, open source)

phyte stage that produces gametes by mitosis and is typically microscopic in higher plants. This shift from one phase to another in the lifetime of plants is termed the alternation of generations and is unique to plants (refer to ► Sect. 1.5).

18.12 Chapter Review

Concept Review

18.1 *The innermost whorl of the flower is typically composed of female floral parts*. The pistil (or carpel) has a terminal stigma, an elongated style, and a basal ovary, which in turn houses an egg cell, central cells, and related cells within an enclosed ovule.

18.2 *Ovaries are classified based upon their position within a flower and may contain nectaries*. Three types of ovaries are recognized according to their positions with respect to the lateral organs of the flower, superior, inferior, and half inferior. The ovaries of many monocots contain septal nectaries which represent the glandular epidermis. Gymnosperms, as opposed to angiosperms, are generally regarded as bearing “naked” seeds; the ovules are generally borne on stalks or flattened structures that cluster together to form cones.

18.3 *Ovules can be arranged within an ovary in a variety of ways*. The partitions dividing the ovaries into locules are formed by the lateral fusion of carpels along their margins, and the placentae are formed in the angles of locules. They can be represented by basal, free-central, and marginal placentation.

18.4 *Megasporogenesis begins with the differentiation of the megaspore mother cell and ends with the formation of the megagametophyte (embryo sac)*. Female gametophyte development in *Lilium* (a commonly-used example) starts with microsporogenesis. A single megasporocyte undergoes meiosis to produce four haploid megaspore nuclei. Megagametogenesis follows and involves fusion, mitosis, and cytokinesis to produce a seven-cell egg sac with a tetraploid endosperm. The egg sac is the mature female gametophyte.

18.5 *Pollination is followed by germination of the pollen grain and pollen tube growth*. Once transfer of pollen to a receptive pistil occurs, growth of a pollen tube occurs and delivers two haploid sperm to the egg cell apparatus.

18.6 *Double fertilization results in a triploid endosperm and a diploid zygote*. One sperm cell fertilizes the egg cell to generate a diploid zygote, and the other sperm cell fertilizes the two-central cell polar nuclei to generate the endosperm.

18.7 *Postfertilization-to-embryo phase leads to the formation of an eight-celled embryo and suspensor*. The eight-celled embryo is pushed into the endosperm by growth of the linear suspensor.

18.8 *Early embryo-to-heart transition leads to the specification of the basic body plan within the embryo*. At the 16-celled embryo stage, the outer cells of the embryo are formed from ordered divisions that produce the protoderm, and at a globular stage, the procambium is delineated. An upper suspensor cell, the hypophysis, forms the root cap and radicle.

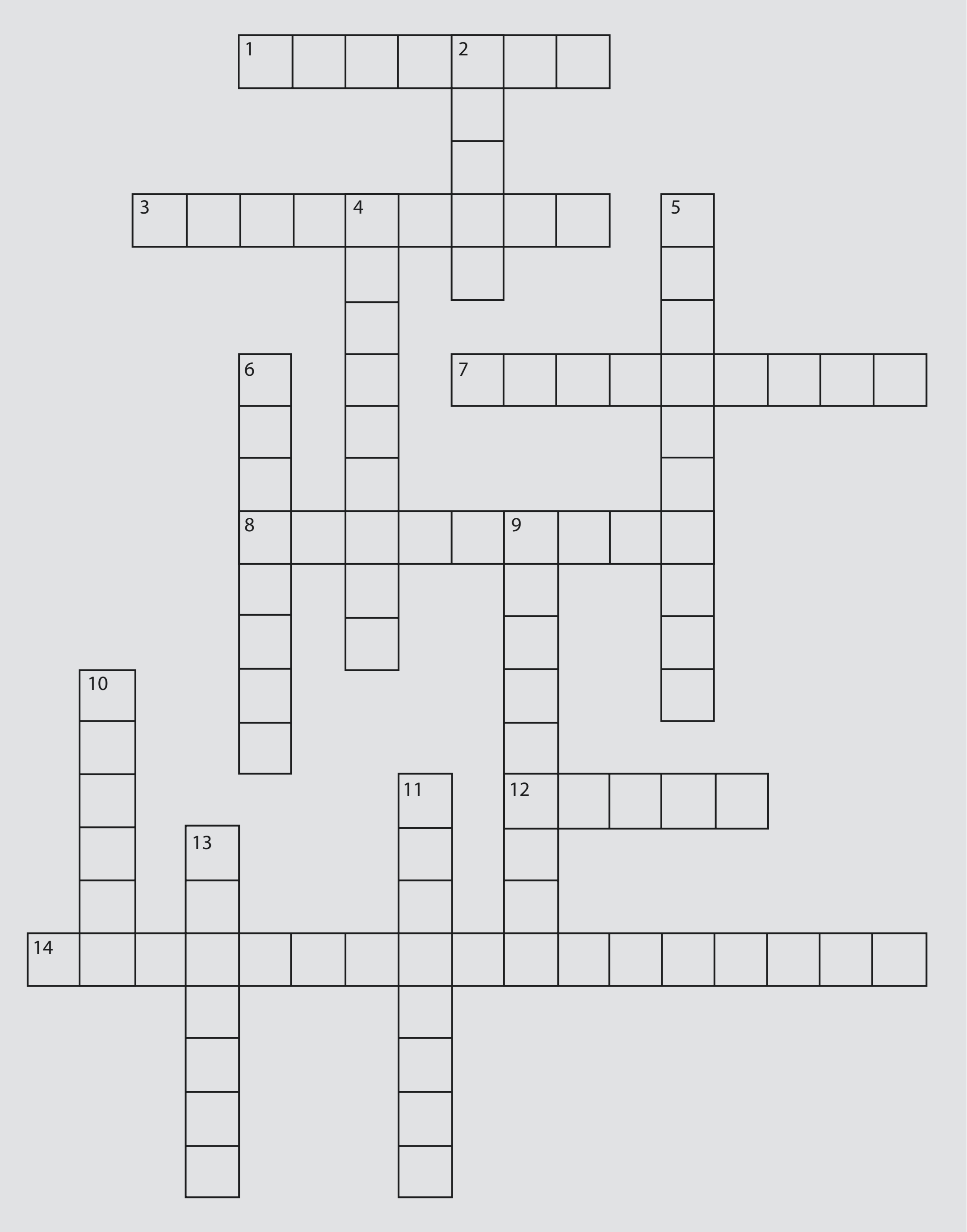
18.9 *The last phase of embryogenesis involves organ expansion and maturation*. More evolutionary developed seed plants tend to have more mature and larger embryos with less endosperm.

18.10 *The general pattern of embryogenesis differs between eudicots and monocots*. Cellular divisions in monocots are not equal, leaf primordia develop sooner in monocots, and the one cotyledon of monocots is modified as a scutellum.

18.11 *Alternation of generations is unique to plants*. Plants have a haploid phase of their life cycle, the gametophyte, and a diploid phase, the sporophyte.

Concept Connections

1. Complete the crossword puzzle with the most appropriate term:



Across

1. Heart-shaped developmental stage of the embryo
3. Stalk connecting an ovule to ovary wall
7. Uppermost cell protruding into the embryo
8. Triploid tissue that provides nutrition for the embryo
12. Placentation type where ovules are attached at base and point upright
14. Process of forming a female gamete via mitosis

Down

2. Placentation type in angiosperms with multilocular ovaries
4. Seed leaves within an embryo
5. Layers of cells around the nucellus
6. Type of ovary that has sepals, petals, and stamens attached above it
9. Consists of a large basal cell and a small terminal cell
10. Fertilization that is characteristic of angiosperms
11. Type of ovary with petals, sepals and stamens attached below it
13. Region where integuments merge with the nucellus

Concept Assessment

2. Which ovary type is characterized by having the hypanthium fusing with a portion of the ovary?
   a. inferior ovary.
   b. superior ovary.
   c. half-inferior ovary.
   d. half-superior ovary.
   e. ovary type isn't important in floral anatomy and morphology.

3. True/false: pollination is essentially the same as fertilization, and thus, these terms can be used interchangeably.
   a. true.
   b. false.

4. The antipodals play no role in egg sac development.
   a. true.
   b. false.

5. Parietal placentation within the ovary is identified when
   a. ovules are located within the center of the ovary.
   b. ovules are found within the center of the ovary, at the junction where many locules have fused.
   c. ovules are located along the edge of ovaries with several locules.
   d. ovaries are located within the center of one-locular ovaries.
   e. ovaries are located along the periphery of one-locular ovaries.

6. Megasporogenesis ends with the formation of
   a. the ovule.
   b. the nucellus.

c. the embryo sac.
d. the micropyle.
e. the megaspore mother cell.

7. The formation of the embryo sac marks the end of
a. megagametogenesis.
b. microsporogenesis.
c. megasporogenesis.
d. microgametogenesis.
e. embryogenesis.

8. What structure is thought to be involved with directing the growth of the pollen tube toward the embryo sac?
a. chalaza.
b. micropyle.
c. filiform apparatus.
d. hypocotyl.
e. synergids.

9. The formation of the endosperm in angiosperms is important because it
a. allows the plant to supply offspring with nutrients.
b. is essential for attracting pollinators to disperse pollen.
c. provides nectar rewards to potential pollinators.
d. provides compounds to the seed important for defending against predation.
e. allows for water and other nutrients to move from within the ovary to the embryo.

10. Body plan specification in *Capsella* happens during
a. fertilization.
b. the proembryo to 8-cell embryo transition.
c. the early embryo to heart transition.
d. the torpedo to walking stick transition.
e. germination.

11. Which suspensor cell will become a part of the embryo during embryogenesis?
a. hypophysis.
b. terminal cell.
c. inner integument.
d. nucellus.
e. nuclear endosperm.

▪ Concept Applications

12. What is the evolutionary significance of double fertilization?

13. What is the evolutionary significance of pollination?

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