Flowers and Male Reproductive Structures

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Introduction

A flower may be treated as a highly shortened shoot that is modified for sexual reproduction within angiosperms (*syn.* flowering plants). It is a characteristic system of reproductive organs in which two basic processes of sexual reproduction, meiosis and the fusion of male and female gametes, occur resulting in the production of a new generation, the **embryo**. Floral sterile parts, e.g., **sepals** and **petals**, as well as stamens (male structures) and sometimes pistil (female structures) are homologous to leaves. In this chapter, the first focus will be on the basic floral anatomy of the flower. Emphasis on the male anatomy of angiosperms will then be covered with female reproductive structures following in ► Chap. 18.

17.1 Flowers Possess Parts Arranged in Whorls That Vary Within and Among Species and Are Supported by a Receptacle

Flower anatomy is as diverse as the members of the angiosperm clade. A conceptualized flower will be discussed in this section, with the proviso that there are literally hundreds of variations on the basic theme shown.

Floral parts are typically borne in whorls (circular patterns) on the axis of the flower stalk known as the **receptacle**—a modified stem. From the outside, moving to the center, the three main whorls of floral organs are the perianth ("surrounding flower," sum of sepals and petals), the androecium ("male household," sum of all the stamens), and the gynoecium ("female household," sum of all the **carpels**) (◘ Fig. 17.1a).

Flowers may or may not have all three whorls. Perfect flowers have both an androecium and a gynoecium. Imperfect flowers have one or the other and are termed **pistillate** if they contain female reproductive structures or **staminate** if only male reproductive structures. Monecious (meaning "one household") plants have both male and female reproductive structures on the same plant. The flowers may be perfect or imperfect. Examples of monecious angiosperms with imperfect flowers include squash (Cucurbitaceae) and the grasses (Poaceae). Dioecious ("two households") plants have some individuals with only staminate flowers and some with only pistillate flowers, such as the honey locust (*Gleditsia triacanthos*). Obviously, all of the flowers on a dioecious plant are imperfect.

In contrast, most conifers (gymnosperms) are monoecious with unisexual reproductive structures appearing on the same plant. For example, most plants within the pine family (Pinaceae) are examples of monoecious gymnosperms, since their individuals contain both male and female cones (◘ Fig. 17.1b–e) and lack flowers. For comparison, ginkgo (*Ginkgo biloba*) is an example of a dioecious gymnosperm, since individuals have either male or female reproductive structures, but not both types. Having reproductive structures that contain only male or female reproductive structures is one way plants can avoid the negative impacts of inbreeding.

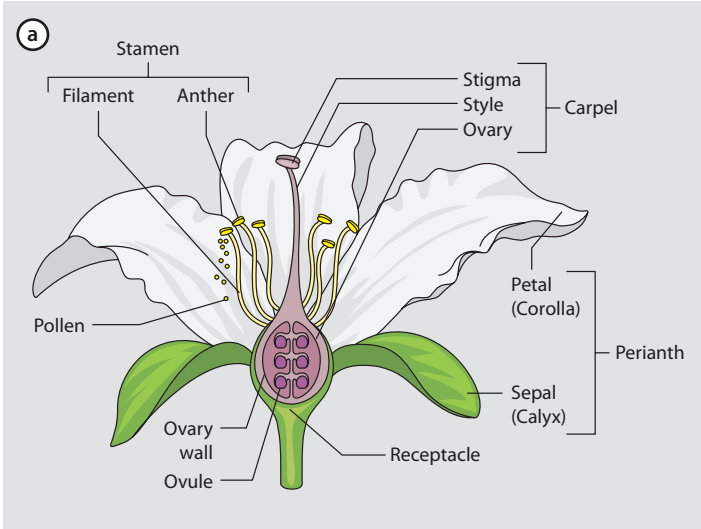


Fig. 17.1 **a** Conceptualized eudicot flower. The flower sits on a receptacle. The perianth includes the sepals and petals. The androecium is the sum of all the stamens, each of which is composed of a filament topped by an anther. Pollen cell production takes place inside the anther. The gynoecium is the sum of all the pistils, which are made of a stigma, style, and ovary. Finally, the ovary is surrounded by an ovary wall and individual egg-containing ovules that project into an inner space called the locule. (Redrawn from Crang and Vassilyev 2003)

Sepals collectively form the **calyx**, which represents the outermost whorl of the flower. Sepals serve to protect the developing flower from desiccation and herbivory. Although they are often green, they contribute little to the overall carbon economy in most plants. The next internal whorl is the **corolla** and is composed of petals. The role of petals in most plants is pollinator attraction, hence the bright colors. Together, the calyx and corolla make up the **perianth**. The sepals and petals are more or less similar to foliage leaves in their basic anatomy with a homogenous mesophyll and have veins enclosed between the adaxial and abaxial epidermises. In some species, such as tulips (*Tulipa* sp.), the sepals and petals are indistinguishable in their appearance and are thereby collectively designated as **tepals** (Fig. 17.1f). The perianth may also have stomata, trichomes, and/or various types of inner secretory cells much like typical vegetative leaves.

Petal color varies from taxon to taxon and often is important in attracting pollinators. Pigments are characteristically contained within chromoplasts (carotenoids) or vacuoles (anthocyanins). With carotenoids that normally may show red, yellow, or orange colors (Fig. 17.1g), their genetic suppression often gives rise to white petals which then result from the predominance of intercellular spaces in the petal mesophyll and are often associated with attracting pollinators that become active at dusk or during nighttime, such as moths. In many cases, the epidermal cells of petals (or tepals) differ from vegetative leaves by having softer cell walls with cellular papilla that protrude (Fig. 17.1h).

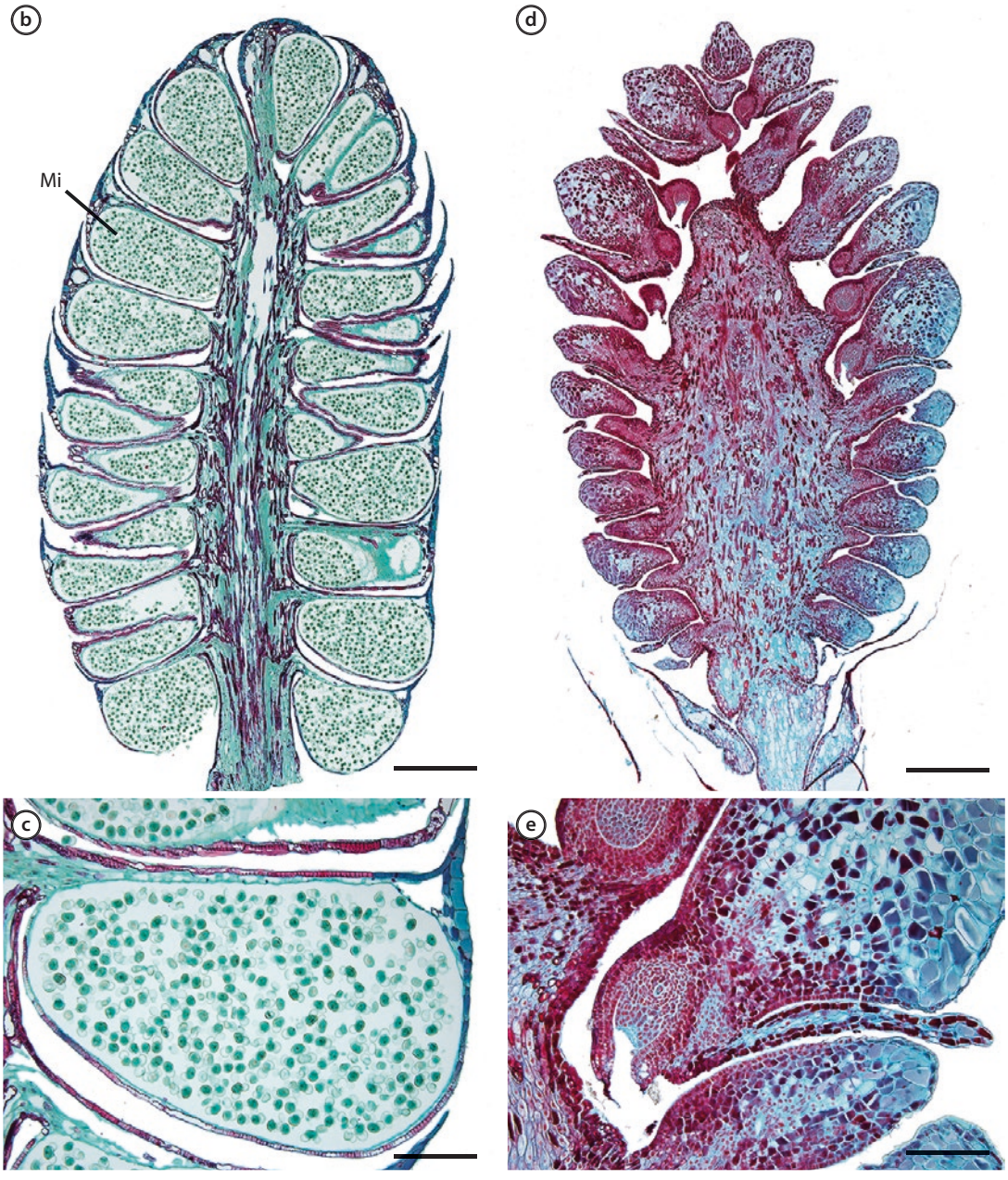


Fig. 17.1 **b**–**e** Longitudinal sections of pine (*Pinus* sp.) male and female reproductive structures. **b** A male (staminate) cone with a large microsporangium (Mi) subtending each microsporophyll. The developing pollen grains can be seen in **c**. **d** A female (pistillate) cone showing bare ovules on scalelike bracts termed megasporophylls (i.e., ovuliferous scale). Scale bars = 500 μm in **b** and **d** and 200 μm in **c** and **e**. (**b**–**e** RR Wise)

The position of the ovary relative to the surrounding floral parts is of relevance to the taxonomic status of the species such that if the whorls are attached at the top of the ovary, leaving it beneath, the ovary is termed **inferior** or epigynous (Fig. 17.1i). If the whorls are attached midway, the position is **half inferior** or perigynous (Fig. 17.1j). If the ovary is above the whorls, it is termed **superior** or hypogynous (Fig. 17.1k).

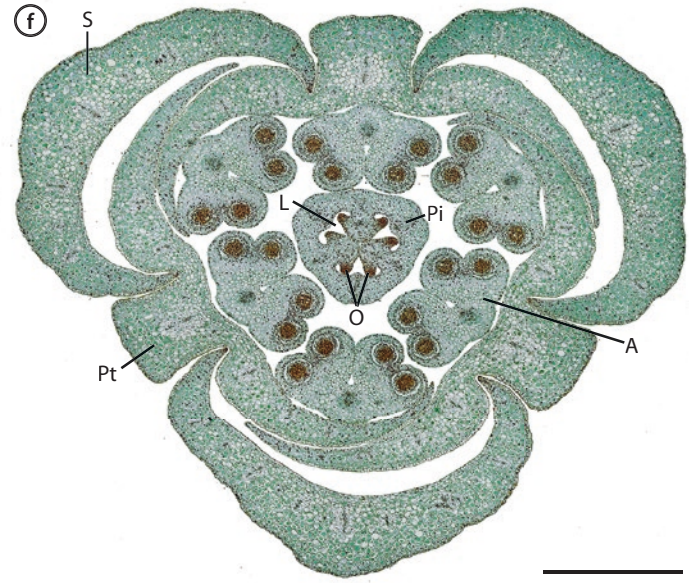


Fig. 17.1 **f** Cross-section of a perfect flower from lily (*Lilium* sp.). The flower was sectioned at a level that shows six tepals (three sepals (S) plus three petals (Pt)), six anthers (A), and one pistil (Pi) with three locules. Each locule (L) in the pistil has two ovules (O). Scale bar = 0.5 mm. (RR Wise)

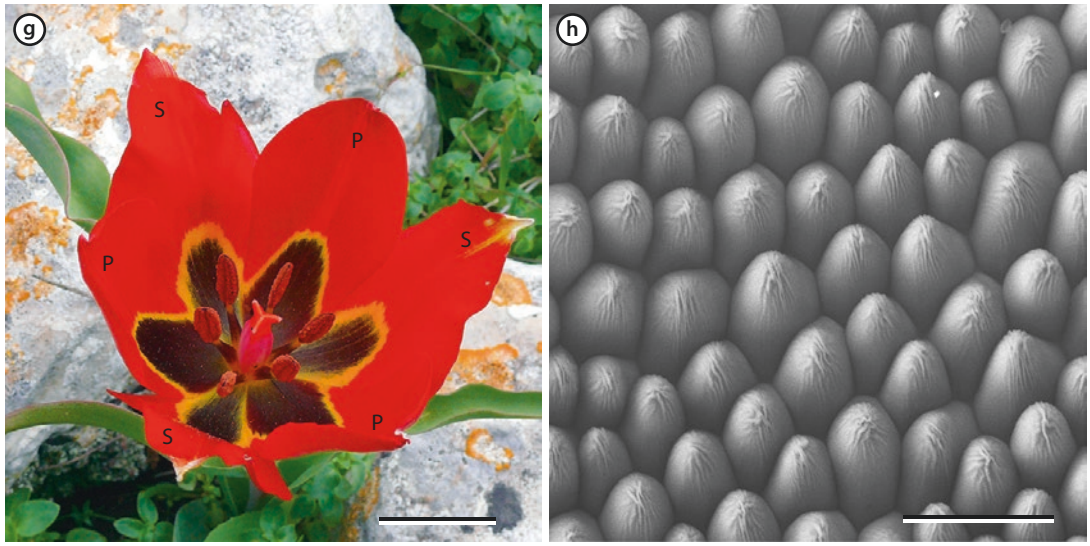


Fig. 17.1 **g** In this tulip (*Tulipa* sp.) flower, the sepals (S) lie to the outside of the petals (P). Because they are almost identical in structure and function, they are collectively called tepals. This arrangement is common in many monocotyledons (Zachi Evenor and MathKnight, CC BY 3.0). **h** Scanning electron microscopy of an adaxial petal surface of a rose (*Rosa* sp.) petal. Each epidermal cell forms a protuberance, the **papilla**, which gives the petals a soft texture and a "velvety" appearance. Scale bars = 2 cm in **g** and 50 μm in **h**. (**g** Zachi Evenor and MathKnight, CC BY 3.0; **h** RR Wise)

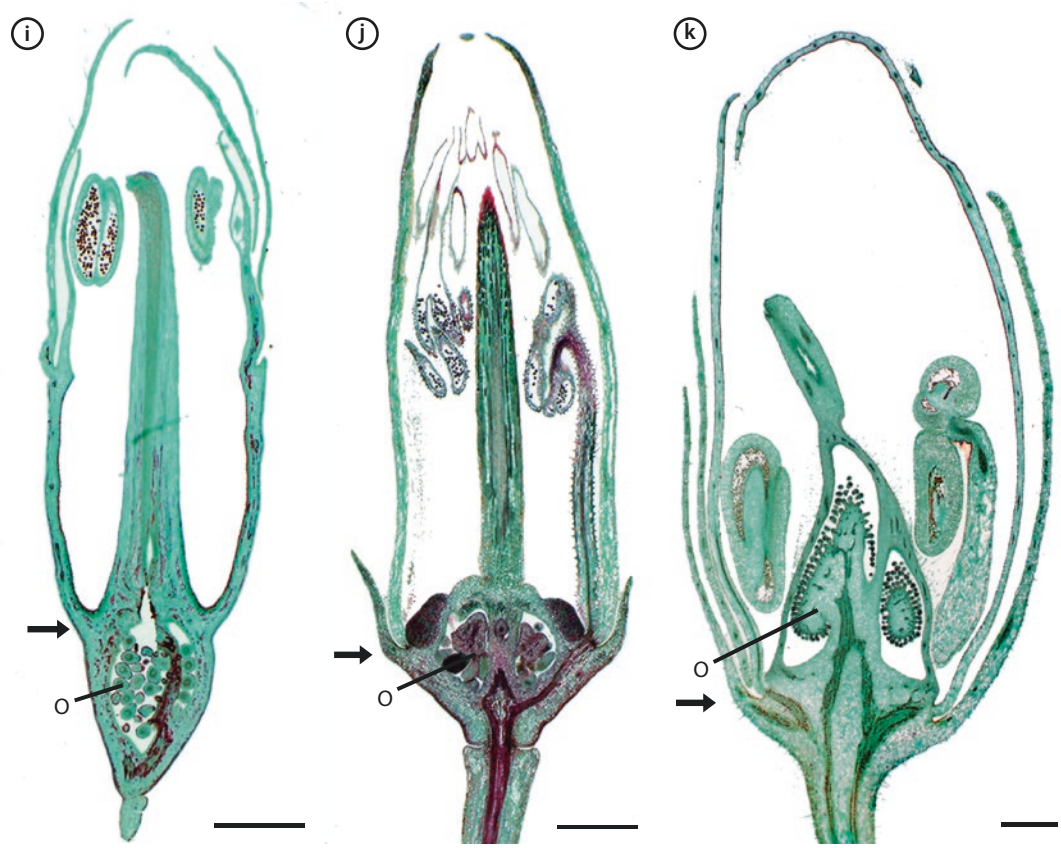


Fig. 17.1 **i** Inferior flower from black currant (*Ribes americanum*). **j** Half inferior flower from cranberry (*Vaccinium macrocarpon*). **k** Superior flower from purple foxglove (*Digitalis purpurea*). The insertion of the perianth (*arrow*) in relation to the ovary (O) is shown. Scale bars = 0.5 mm in all panels. (**i**–**k** RR Wise)

Box 17.1 What Did the Oldest Flower Look Like?

Angiosperms are the family of flowering plants that utilize flowers for sexual reproduction resulting in the production of fruits to promote seed dispersal. Fossil evidence of the earliest angiosperms goes back into the Cretaceous, about 130 million years ago; however, the common ancestor of angiosperms is thought to date back from about 250–140 mya, and no fossil evidence is known from this time period (Magallon et al. 2015). To better understand what the first flower may look like, scientists used molecular genetic analysis coupled with anatomical features from fossil data to model what the first flower may have looked like. So, what did the first flower look like? The model hypothesizes that the first flower was radially symmetrical and bisexual. The proposed floral structure was complex and composed of multiple whorls each of tepals, stamens, and carpels. This study by Sauquet and colleagues (2017) does provide a basis for further research in the morphology of the earliest flowers which is an important area in the field of evolutionary plant biology. (Figure redrawn from Sauquet et al. (2017)) (Fig. 17.1l).

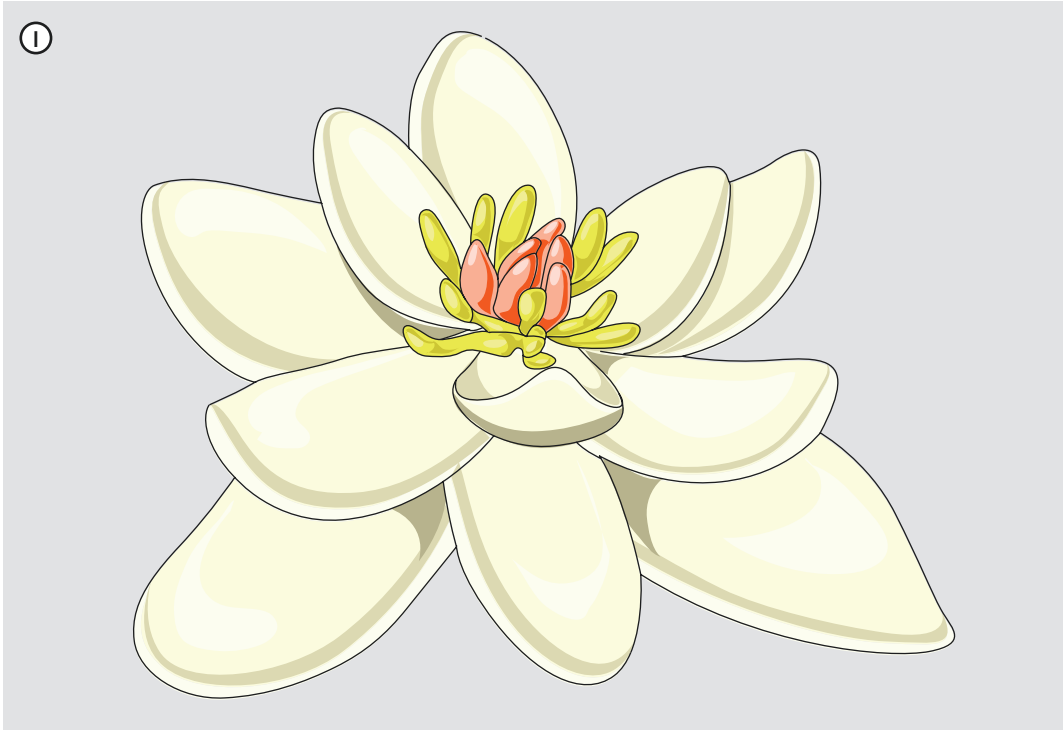


Fig. 17.1 | Model of earliest flower based upon molecular and fossil evidence. (References: Magallon et al. 2015; Sauquet et al. 2017)

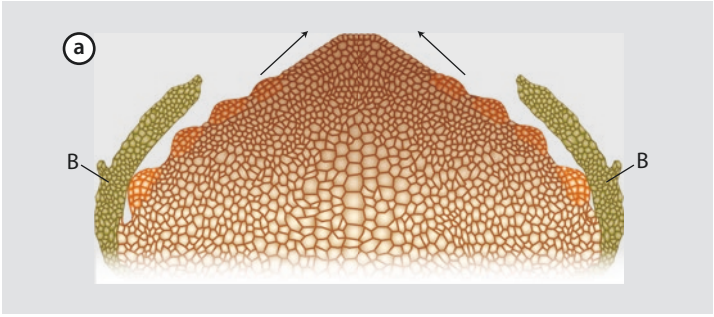
17.2 Floral Development Starts with Increasing Cell Divisions in Apical Meristems and Initiating Organs in an Acropetal Sequence

Reproductive development of a plant begins with the transition of its shoot apical meristem from producing leaves to producing inflorescence branches, floral bracts, and flowers. The organs of the flower are initiated as protrusions on the floral apex in the following sequence: bracts, calyx, corolla, androecium, and gynoecium. One of the most obvious signs of transition from vegetative to floral apex is the rise in mitotic activity of cells.

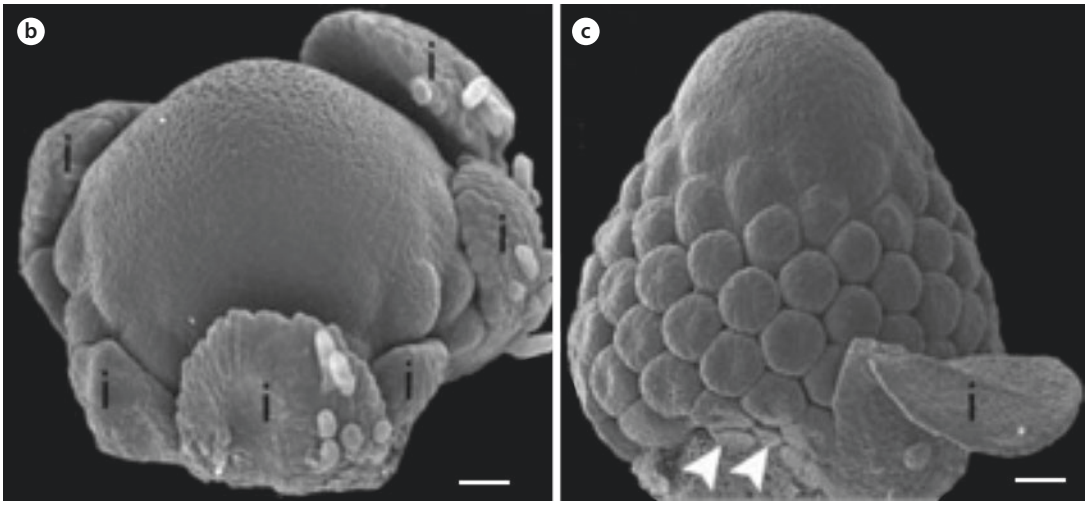
Unlike the cells of the shoot apical meristems, a characteristic feature of the floral apical meristem is its **determinate growth** when all of its cells are eventually differentiated into the floral organs. Floral ontogeny has become a new source of characters for identifying phylogenetic affinities among plants.

The organs of a flower develop in an **acropetal** sequence from the base to the apex of the floral meristem. Thus, the organs of the flower are initiated as cylindrical protrusions at the periphery of the floral apical meristem. Acropetal development is apparent when comparing the development of the petal and stamen primordia relative to each other, as well as to the carpel primordia.

The acropetal sequence of development is also noted in the floral apex of inflorescences in many angiosperms. Thus, flowers developing at the base of the inflorescence mature before those in the center. Examples that follow include head stages from *Calendula officinalis* (◘ Fig. 17.2a) and *Anacyclus homogamos* (◘ Fig. 17.2b, c). In the examples that follow, notice the changes in the structures in the developing inflorescence as well as development occurring in acropetal sequence.



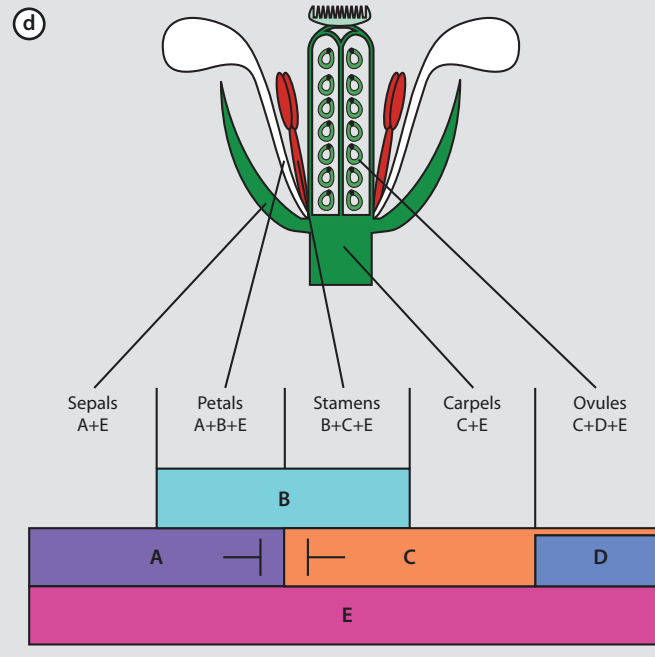
◘ **Fig. 17.2** **a** A diagram showing the initiation and early developmental stages of the inflorescence, the head, as would be found in English marigold (*Calendula officinalis*). The entire developing inflorescence would be wrapped in green bracts **B** to protect the floral primordia, indicated as orange bumps on the surface of the developing head. The two arrows indicate the direction of initiation of floral primordia with the youngest flowers in the center of the head; maturation is in the opposite direction. (Redrawn from Crang and Vassilyev 2003)



◘ **Fig. 17.2** **b**, **c** SEM micrographs of the pattern of flower initiation and early development of the inflorescence, the head, of *Anacyclus* (*Anacyclus homogamos*). **b** is a side view of a head with involucral bracts initiating helically. **c** shows a later successive developmental stage of head. Scale bar = 40 μm. (Illustration from: Bello et al. 2013)

Box 17.2 The ABCs of Floral Development: Genes Regulate Flower Development

Floral development progresses in a structured pattern that is controlled by a handful of genes (◘ Fig. 17.2d). In this basic model of *Arabidopsis* floral development, a suite of genes, "A" genes, interact to ultimately form the calyx, the outermost whorl. "A" and "B" genes interact to form the petals and stamens. "B" and "C" genes interact to form the petals and stamen. "C" genes are important for carpel and ovule development. Other genes also play a role in floral development. A group of "G" genes are involved with ovule development, and "E" genes are important for all aspects of floral development. The array of interacting genes is responsible for converting leaves into the floral parts. Changes in the genes responsible for floral development lead to differences in expression of the proteins and thus floral parts. For example, the "A" genes apply to *Arabidopsis* and close relatives and less so to other angiosperms. Not all angiosperms have perfect flowers and thus will have differences in the expression of interacting genes. Thus, the expression of genes that give rise to flowers of plants related to apples with showy flower petals will be very different than genes that lead to the formation of flowers in grasses.



◘ **Fig. 17.2** **d** Model of the ABCs of floral development. Each uppercase letter and corresponding color code designate a suite of genes that are important for development of flowers in *Arabidopsis*. Individual genes are identified within colored circles. In the rings, "A" genes determine sepals, "A" and "B" genes determine petals, "B" and "C" genes produce stamens, "C" genes alone determine carpels, and C and D genes produce carpels. "E" genes are required throughout for normal development. (Modified from Litt and Kramer 2010)

17.3 Male Reproductive Structures Give Rise to Pollen Within the Anther

Stamens are the male reproductive structures of angiosperms, and are made of pollen-containing **anthers** that are located on the tips of stalks called **filaments**. They comprise the third whorl within a flower, between the second (petals) and fourth whorls (carpels). The male reproductive structures are involved in the production of pollen grains, which give rise to male gametes, i.e., sperm cells or simply sperms. Pollen grains (the male gametophyte) develop and mature inside the anthers located at the terminal portion of the filament (◘ Fig. 17.3a, b). Pollen dispersal is facilitated by wind, animals, or, in some cases, water. After a **pollen grain** reaches a stigma, it germinates and, if it is compatible with the stigma, it forms a pollen tube capable of growing to the egg cells in the ovule(s). The surface of the stigma creates an optimal physiological condition for compatible pollen grains to germinate inasmuch as sugary secretions along with a variety of attractants and enzymes are released along the surface. 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 in compatible combinations (see ► Chap. 18).

In its earliest stage, the anther consists of a uniform mass of meristematic cells. Soon, in this homogeneous meristem, four separate groups of cells, the sporogenous tissue, by the process of meiosis, become discernible. The sporogenous tissue is composed of numerous **microspore mother cells**. Thus, unlike the nucellus (megasporangium) where a single megaspore mother cell and then single megagametophyte (embryo sac) are formed (refer to

◘ **Fig. 17.3** **a** A view of a lily (*Lilium* sp.) flower with a prominent style and shorter stamens bearing the anthers. The anthers have dehisced to release pollen grains (orange-red pigmented), giving the anthers a fluffy appearance. (S Lyons-Sobaski)

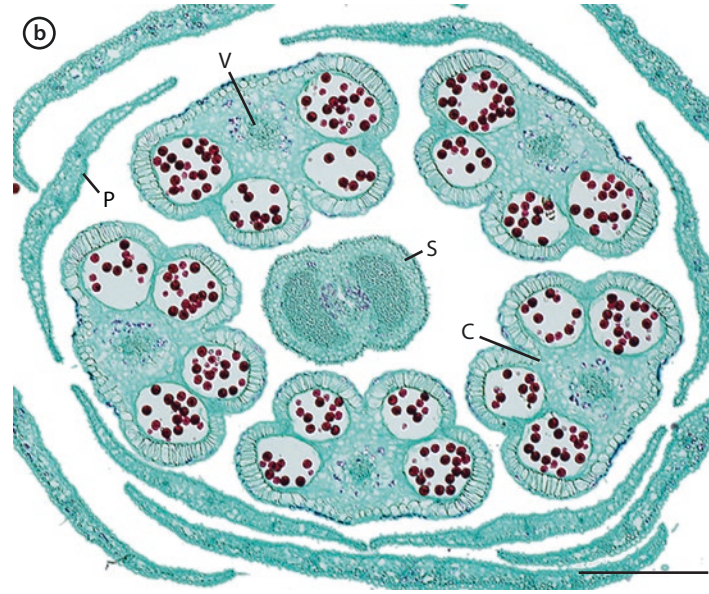
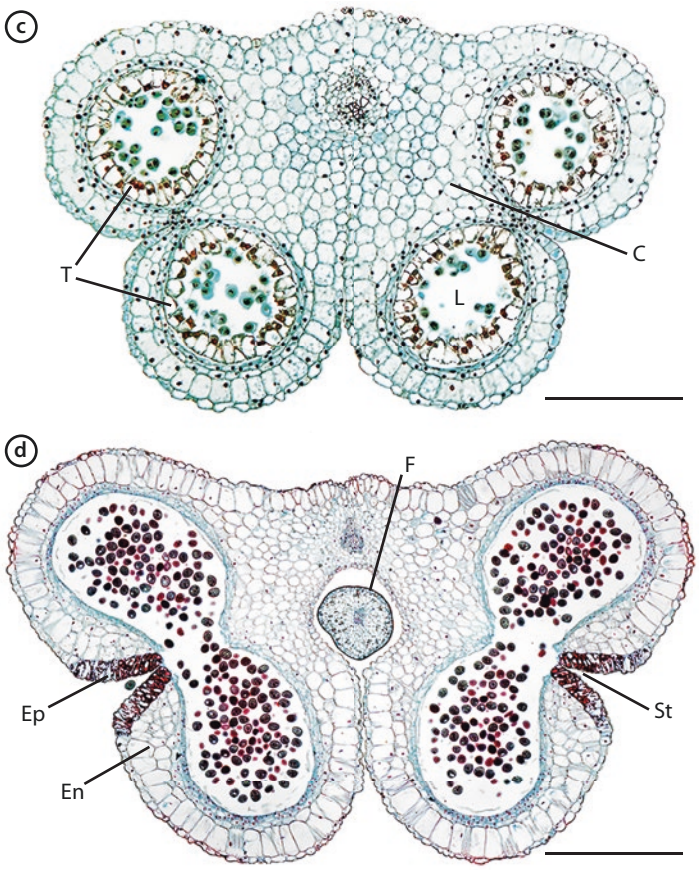


Fig. 17.3 **b** Cross-section through the upper part of the flower bud of gooseberry (*Ribes americanum*) showing petals (P) to the exterior, the central style (S), and five anthers, each with four pollen sacs (microsporangia) bearing pollen grains. Each anther has a connective (C) and a vascular bundle (V). Scale bar = 250 µm. (RR Wise)

► Chap. 18), sporogenous tissue is composed of many microspore mother cells surrounded by tapetum microspores, and then numerous **microgametophytes** (pollen grains) develop within a pollen sac (the **microsporangium**). The tapetum is a layer of cells that line the locules of the anthers, providing nutrition to the developing pollen.

The anther usually has two longitudinal lobes that are united by a band of parenchyma cells called the **connective** tissue (Fig. 17.3c), which is a continuation of the filament. Each anther lobe contains two longitudinal **pollen sacs** (or locules) within which the pollen grains are produced. A single vascular band extends from the filament into the center of the connective and continues into the anther.

At the final stage of pollen grain maturation, and immediately before **anthesis** (the time of maturation of the male and female organs of the flower), the filament undergoes rapid elongation resulting in the disruption of the tracheary elements in the vascular bundle and, therefore, the cessation of the water supply to the anther, and thus, the process of anther and pollen desiccation commences. Just before anther maturation, conspicuous secondary wall thickenings are deposited on the anticlinal and inner cell walls of the subepidermal cell layer of the anthers, often termed as the **endothecium** cells (Fig. 17.3d). These cells line the cavity of an anther and secrete materials for maturation of pollen grains.



Figs. 17.3 **c** Cross-section of an immature lily (*Lilium* sp.) anther showing region of the connective (C) as well as four locules (L). The thick callosic walls of the microspore mother cells stain light green. Note the thick tapetal (T) layer. **d** A cross-section of a mature lily anther showing the filament (F). Mature pollen grains are stained red, and the septum between neighboring locules has broken down. Epidermal (Ep) and endothecium cells (En) near the stomium (St) have multiplied and thickened in preparation for anther anthesis and pollen dehiscence. The tapetum and other anther wall layers are greatly reduced in size and thickness and appear as a thin internal lining of the endothecium. Scale bar = 500 μm in both panels. (**c**, **d** RR Wise)

They are densely cytoplasmic with little or no vacuoles. To facilitate pollen dispersal, each of the two anther lobes splits by a longitudinal slit called a **stomium**. The thickenings of the endothecium cells cause tangential shrinkage during anther desiccation, leading to the rupture and outward bending of the anther wall at the stomium; in other words, the drying of the anther causes it to dehisce and release pollen (Fig. 17.3d). The endothecium cells do not develop secondary thickenings in the region of the future stomium, thereby providing for the specific location and orientation of the slit for dehiscence and release of mature pollen grains.

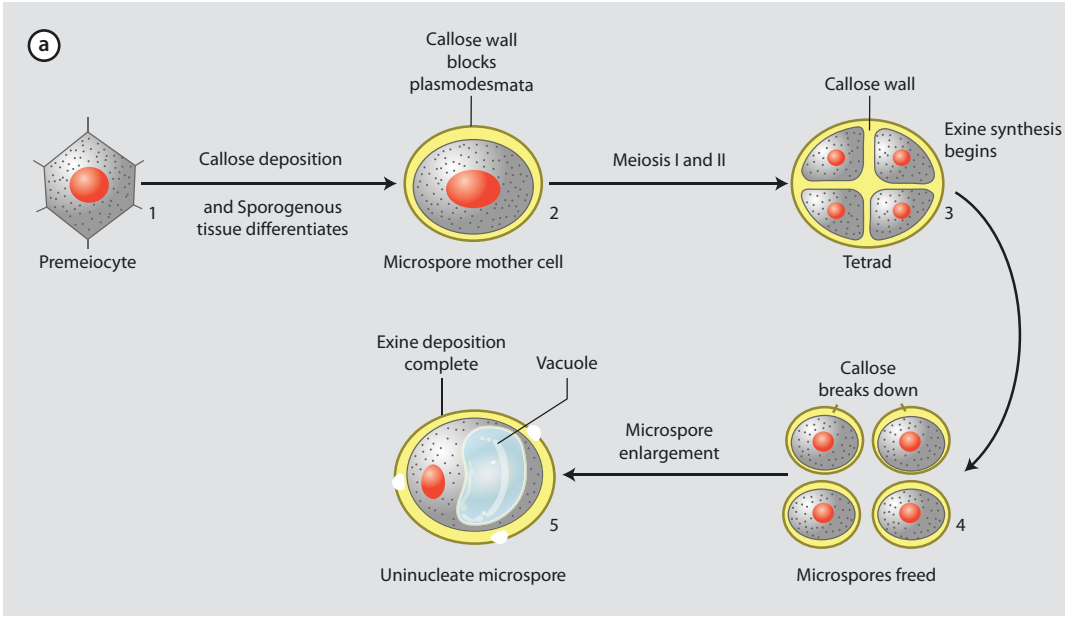
17.4 Pollen Grain Formation Begins with Microsporogenesis Followed by Microgametogenesis

The process of pollen grain formation includes **microsporogenesis** and microgametogenesis. During microsporogenesis, **microspore mother cells** (or microsporocytes) are formed in the sporogenous tissue of the anther. These cells undergo meiosis to produce tetrads of haploid, uninucleate microspores.

Microgametogenesis comprises the subsequent mitoses and development of microspores into microgametophytes. The microgametophytes in turn produce male gametes (or **sperm cells**). During microgametogenesis, microspores divide mitotically once (or less frequently twice) and become the male gametophytes or **microgametophyte** (pollen grains). In this section, microsporogenesis and microgametogenesis will be covered in detail.

17.4.1 Microsporogenesis Is the Formation of Microspore Mother Cells

The process of microsporogenesis may be subdivided into five phases (◘ Fig. 17.4a) leading to the formation of uninucleate haploid **microspores**. The first phase begins with the differentiation of compact sporogenous tissue with thin cellulosic walls and numerous plasmodesmatal connections between microspore mother cells. In the second phase, each microspore mother cell is isolated by a callosic wall in which the plasmodesmata become



◘ **Fig. 17.4** **a** The development of microspores occurs in five phases. Numbers within illustration correspond to each phase. (Redrawn from Crang and Vassilyev 2003)

blocked. The conversion from tissue structure to individual cells that undergo meiosis I and II also occurs within this second phase (◘ Fig. 17.4b–g). The third phase is noted by the formation of tetrads, four young haploid microspores that become encapsulated in the **callosic wall**. **Exine** (a highly sculptured and decay-resistant outer wall layer of pollen) synthesis in the pollen wall begins providing rigidity to the pollen grains. The fourth phase is recognized by the dissolution of callose and the release of microspores from tetrads. The fifth phase is marked by the vacuolation and enlargement of microspores with the completion of exine deposition and pollen wall development.

17.4.2 Microgametogenesis Results in the Development of the Microgametophyte: The Pollen Grain

During the formation of microgametes (or male gametes or sperm cells) in angiosperms, microspores undergo two successive mitoses that lead to the formation of the microgametophyte (male gametophyte), the pollen grain (◘ Fig. 17.4h). The first division occurs within the anther. The second division follows pollination, forms two sperm cells, and occurs within the pollen tube. This second division may occur following pollination, but in many species, it occurs prior to being shed from the anther, giving rise to a three-nucleate condition of a pollen grain. On rare occasions, both mitoses will occur inside the microspore. Following pollination, the two sperm cells may be directly involved in the process of double fertilization (refer to ► Sect. 17.7 and ► Chap. 18), with the formation of a diploid zygote and triploid endosperm (◘ Fig. 17.4i–l).

Microgametogenesis can be subdivided into four general phases. The haploid vacuolated microspore represents phase 1 which undergoes unequal mitosis giving rise to a two-celled immature pollen grain. This immature microgametophyte consists of a **vegetative cell** and a small lens-shaped **generative cell** appressed to the microspore wall, giving rise to phase 2. The vegetative cell occupies the major portion of the former microspore. In fact, it has been shown that the generative cell occupies only about 1/20 of the volume of the newly formed pollen grain. The vegetative cell undergoes de-vacuolation in phase 2 and will accumulate large amounts of starch, oil, and reserve protein by phase 4. At phase 3, the generative cell separates from the **intine** and moves to a position where it assumes a spindle-shaped form and becomes completely enveloped by the vegetative cell, thus becoming a cell within a cell prior to forming sperm cells (Yu and Russell 1992). Phase 4 is represented by the mature pollen grain (also designated as the microgametophyte) which is ready for pollination.

In phase 4, the polysaccharide cell wall of the generative cell in most species has dissolved, and the two cells (generative and vegetative) make contact with each other by their plasmalemmas

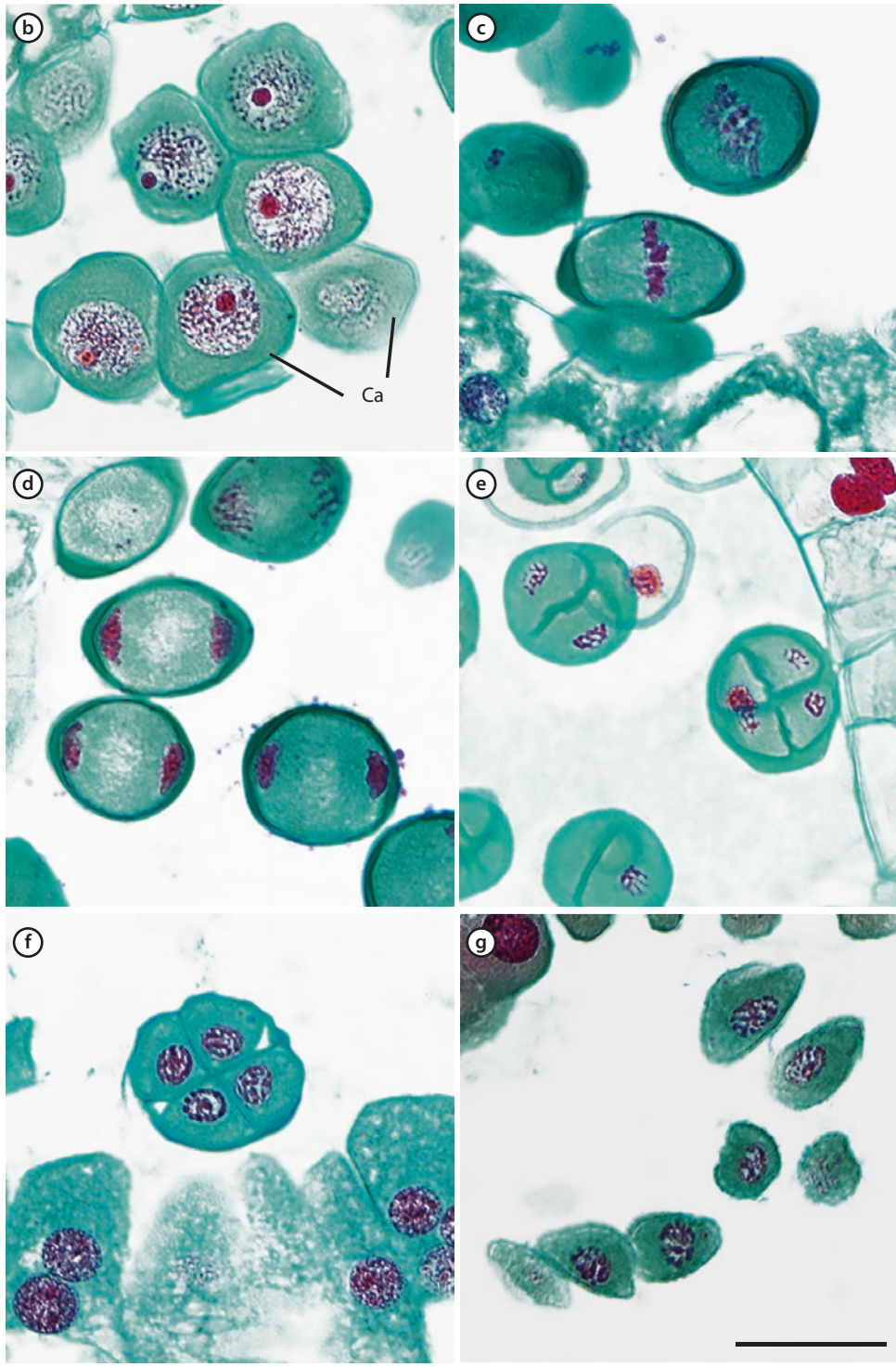


Fig. 17.4 **b**–**g** Phases of meiosis I and II during microsporogenesis in *Lilium* anthers from the premeiocyte (stage 1) to the uninucleate microspore (stage 5). **b** Early prophase I: the microspore mother cells have a thick wall of callose (Ca). **c** Metaphase I: chromosomes align at the equator. **d** Telophase I: chromosomes migrate to the cell poles; meiosis II will follow. **e** Telophase II. **f** Pollen tetrad: the four resulting haploid microspores are held together by the callosic wall, which will soon break down. **g** Uninucleate microspores: each tetrad releases four uninucleate microspores which will next proceed through microgametogenesis as described in the following section. Scale bar in **g** = 25 μm and applies to all panels. (**b**–**g** RR Wise)

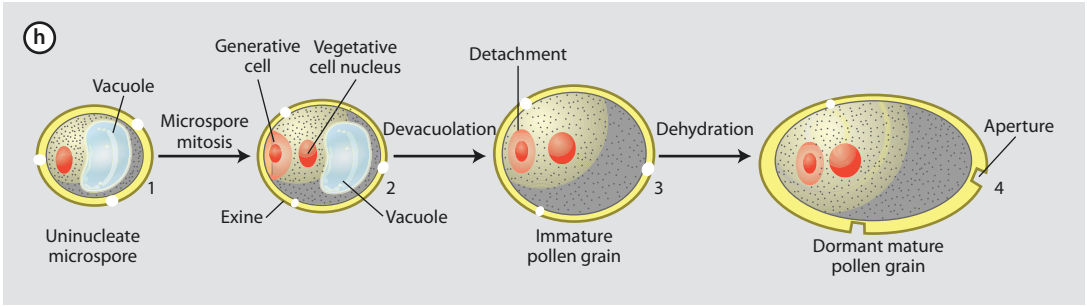


Fig. 17.4 **h** Diagram of the four phases of angiosperm microgametogenesis to form the microgametophyte. Numbers within the illustration correspond to each of four phases. (Redrawn from Crang and Vassilyev 2003)

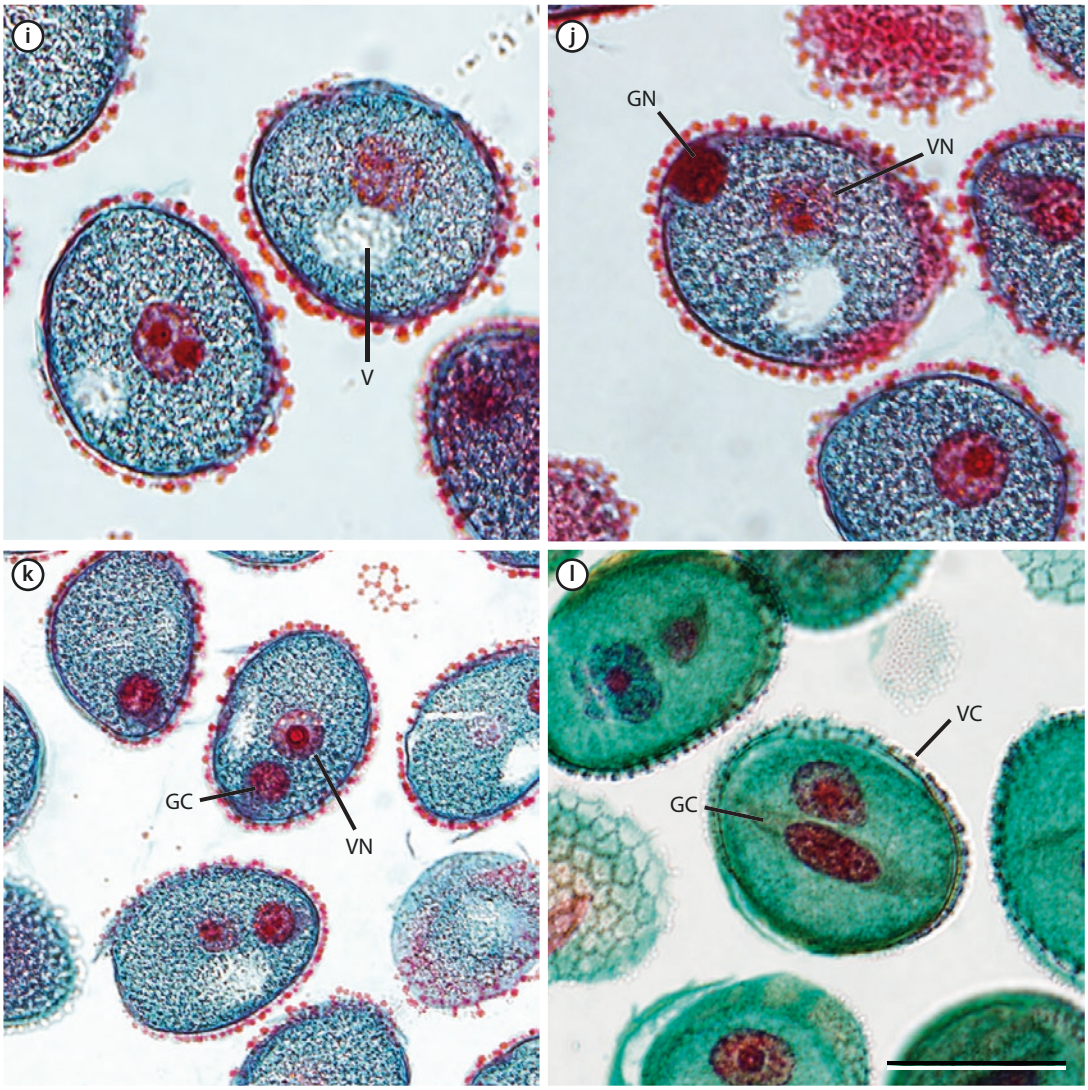


Fig. 17.4 **i** Uninucleate microspore: the cell has a single haploid nucleus (red) and a vacuole (V). **j** Developing generative cell: the nucleus has undergone one mitotic division, producing a vegetative cell nucleus (VN) and a generative cell nucleus (GN), which is attached to the intine layer. **k** Immature pollen grain: the generative cell nucleus detaches from the intine and cytokinesis is initiated. **l** Mature pollen grain: the generative cell (GC) has matured. It is housed entirely within the vegetative cell (VC) and does not form its own cell wall. Scale bar in **l** = 25 μm and applies to all panels. (**i**–**l** RR Wise)

only. The lack of a wall around the generative cell will facilitate its movement in the pollen tube following pollination. During phase 4, desiccation and transition to a dormant state occurs. At this time, the generative cell has no plastids, few mitochondria, and little cytoplasm. The pollen grain is ready to be transferred to the stigma.

Following phase 4 but prior to pollination, some species' pollen grains are binucleate, in contrast to those that are trinucleate. If a single mitotic division occurred during microgametogenesis, the mature pollen grain consists of a large vegetative cell (developing a future pollen tube) and a small generative cell (sperm mother cell). In plants with bicellular pollen grains, the generative cell divides to form two sperm cells in the pollen tube *after* germination of the grain on the stigma. In plants with tricellular pollen grains, the generative cell divides and produces two sperms during maturation of a grain, but before pollen tube emergence. The two sperm cells allow for double fertilization of the angiosperm **embryo sac**.

As the pollen grain continues to form, a cellulosic cell wall is deposited between the generative and vegetative cells during phase 2 of microgametogenesis. If pollination occurs, this cell wall will dissolve when the generative cell moves out of the pollen grain wall as the pollen tube becomes established.

17.4.3 The Tapetum Provides Nutrition and Substances to Form the Exine

The sporogenous tissue is delineated from the anther wall by a single layer of cells designated as the **tapetum**. The cells of the tapetum enlarge and develop a complex ultrastructure, which indicates that they become very active metabolically. At the time of meiotic division of microsporocytes (◘ Fig. 17.4a stages 2 to 3), the nuclei of the tapetum cells also divide, but mitotically. However, mitosis is not followed by cytokinesis, and tapetal cells subsequently become binucleate (◘ Fig. 17.4m).

Two types of tapetum may be distinguished according to subsequent development of the cells—a **secretory tapetum** and the **periplasmodial tapetum**. The cells of the secretory tapetum remain intact and persist in situ through microgametogenesis, whereas in the periplasmodial tapetum, the cell walls break down and the protoplasts intrude into the pollen sac eventually forming a coenocytic (multinucleate) plasmodium around the developing microspores and early pollen grains. The tapetum is involved in the nourishment of the microsporocytes and pollen grains and in the synthesis and deposition of **sporopollenin** and other wall materials onto the surface of the developing exine. The tapetal cells also synthesize and secrete **callase**, an enzyme responsible for the dissolution of callose around the microspore tetrads. Before an anther matures, the tapetum degenerates, and its remains are deposited on the pollen grain surface.

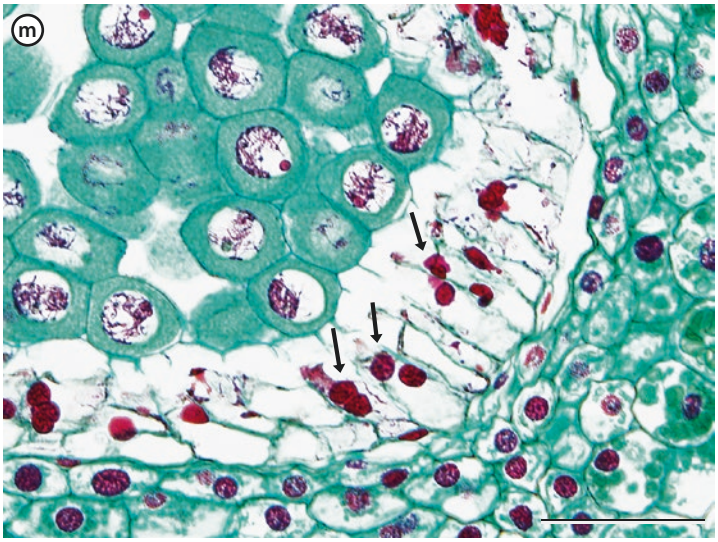


Fig. 17.4 **m** Lily anther. The enlarged cells of the tapetum, some with two nuclei (*arrows*), lie between the microspore mother cells to the top left and the connective tissue to the bottom right. Scale bar = 50 μm. (RR Wise)

17.5 The Structure of Pollen Grain Cell Walls Changes During Development from Microspore to Macrogametophyte, and Callose Is Deposited and Then Sporopollenin and Adhesives

Successive changes in the structure and composition of their cell walls during development are characteristic of pollen grains. First, the amorphous polysaccharide callose is deposited in meiocytes to the inside of very thin cellulosic cell walls and is also laid down after meiosis around each microspore of the tetrad during microsporogenesis. During callosic wall formation, all of the plasmodesmatal connections are lost, and the cells become isolated from the maternal sporophyte.

Prior to exine establishment, a thin layer of non-sporopollenin material, the primordial exine (or primexine), forms on the outer surface of the spore plasmalemma. The primexine serves a template for exine patterning. While still within the tetrad and encapsulated by callose, the **exine** begins to be deposited (Fig. 17.5a).

The exine is largely composed of sporopollenin, a complex organic hydrophobic biopolymer that is highly resistant to degradation (Dominguez et al. 1999). The tapetum contributes to the deposition of sporopollenin onto the outer surface of the exine. Upon the establishment of a first layer of sporopollenin that starts to form the young exine, dissolution of the callosic wall begins during microsporogenesis.

The rate of deposition of sporopollenin increases after the liberation of microspores from the tetrad. Exine deposition is largely absent over the pore sites through which pollen tubes emerge during germination; Fig. 17.5b. Pollen grain **apertures** form early,

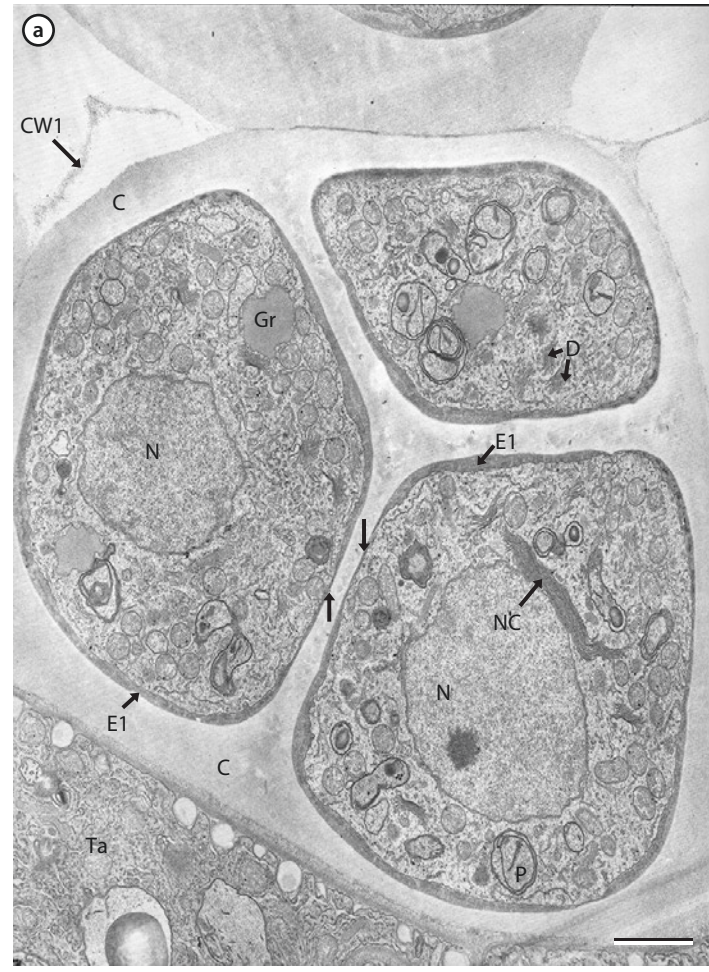


Fig. 17.5 **a** A transmission electron micrograph of a tetrad of African violet (*Saintpaulia ionantha*) during the deposition of exine. Each microspore is encapsulated in callose (C). However, the microspore wall, i.e., the sporopollenin exine (E1), is being deposited to the inside of the callosic wall. Sporopollenin can be seen on the outer surface of the exine (*arrowheads*). Note the remnants of the cellulosic cell wall (CW1) of the former pollen mother cell around the tetrad and outside of the callose layer. L leucoplast, N nucleus, T tapetum, arrows = primexine. Scale bar = 10 µm. (MC Ledbetter and KR Porter 1970)

during callosic wall deposition and before exine initiation, and leave depressions in the wall (Fig. 17.5c). The apertures may be circular or furrow-like, and the number of them varies from one in monocotyledons to three or more in dicotyledons. These apertures are important as they provide an opening for the pollen tube to emerge. They are covered with a plate termed the **operculum**. The microspores grow in circumference and, finally, an inner cellulosic cell wall, the intine, is deposited (Fig. 17.5d). The intine is similar in composition to the primary wall of typical plant cells. Mature pollen grains are enveloped with both exine and intine (Fig. 17.5e).

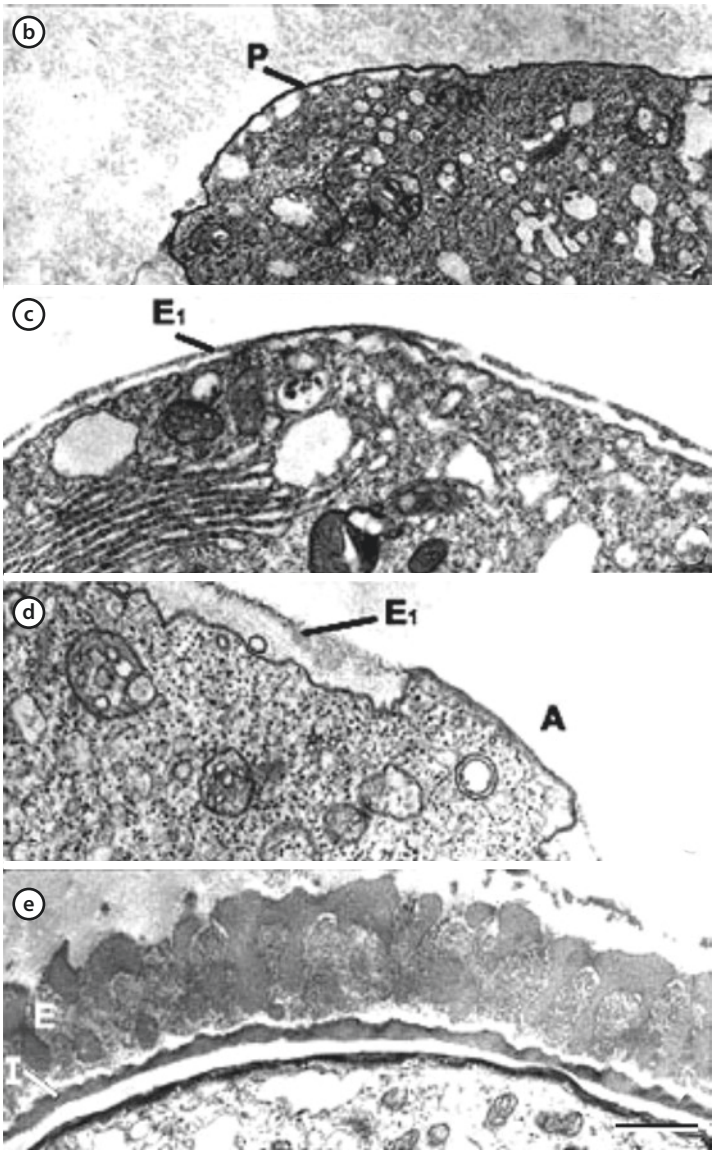


Fig. 17.5 **b–e** This series of electron micrographs captures exine initiation in white campion (*Lychnis alba)*. Panels **b**, **c**, and **d** represent early tetrad phases. In panel **b**, the primexine (P) is shown with differentiation on the microspore plasmalemma. In panel **c** the early exine (E_1) is seen. Also evident is an electron-transparent space between the plasmalemma and the exine (E_1) that is filled with sporopollenin precursors. Panel **d** portrays the future pollen grain aperture (A), which makes a depression in the callosic wall. This implies that the aperture was initiated during callosic wall deposition, i.e., before exine initiation. Panel **e** represents a nearly mature pollen wall consisting of exine (E) and intine (I). Scale bar = 5 μm. (**b–e** RC Crang)

While sporopollenin produces a tough physical- and chemical-resistant wall material in the exine, another material termed **pollenkitt**, a hydrophobic lipid, provides a sticky adhesive surface enabling pollination and adherence to the stigma of pistils prior to pollen tube growth (Pacini and Hesse 2005). Two types of sticky

pollen coat material exist in angiosperms, both produced by the anther tapetum. Pollenkitt is the most common adhesive material present around pollen grains of almost all angiosperms pollinated by animals. But in the case of the Brassicaceae, it is substituted by **tryphine**, a mixture of both hydrophobic and hydrophilic substances, and produced by the plasmodial tapetum upon its degeneration. Both pollenkitt and tryphine are produced by the tapetum. If tryphine is formed, the tapetal cell protoplasts lose their individuality at the microspore stage. If pollenkitt is formed, their contents degenerate at later stages. Cell content of pollen is totally reabsorbed, when ripe pollen is not surrounded by any gluing material. Pollenkitt becomes functional when the anther dehisces and becomes nonfunctional when the pollen rehydrates on the surfaces of the stigma following pollination.

17.6 The Surface Characteristics of Pollen Grains Are Taxon Specific

The cells within the pollen grain differ in structure, function, and ploidy from most cells in the plant body. The vegetative cell in the mature pollen grain is irregular in shape with many protrusions and invaginations. The small generative cell is immersed inside the vegetative cell and is separated from the vegetative cytoplasm only by two plasmalemmas with a very narrow space between. The generative cell within a pollen grain typically does not contain plastids, which explains why chloroplasts are often maternally inherited in most angiosperms as no "male" plastids are involved in fertilization and contained in the new embryo.

The shape and size of pollen grains varies greatly among species. The pollen grain may be spherical, ellipsoidal, threadlike, etc. The size of pollen grains also varies, from a few micrometers to as much as a quarter of a millimeter. Differences in properties associated with pollen grains are often related to the mechanism of pollination. Angiosperms that are underwater have been noted to lack apertures and have relatively thin exines in contrast to species that are found on land.

The surface of the exine is ornamented with a complex pattern of spines, netlike ridges, and/or other projections. These ornamentations vary greatly, but, at the same time, the pattern is constant in plant taxa of various ranks (taxon specific) and may be used for taxonomic purposes. From the analysis of pollen, it is possible to determine which plants have grown in a certain geographical region and during a certain geological time. Thus, scanning electron microscopy is very helpful in **palynology** (the study of plant pollen and spores from both living and fossil forms) because it shows the surface characteristics of pollen grains useful in comparative descriptions for various plant taxa, especially from past geological times. Compare the pollen grain surfaces within ◘ Fig. 17.6a–f.

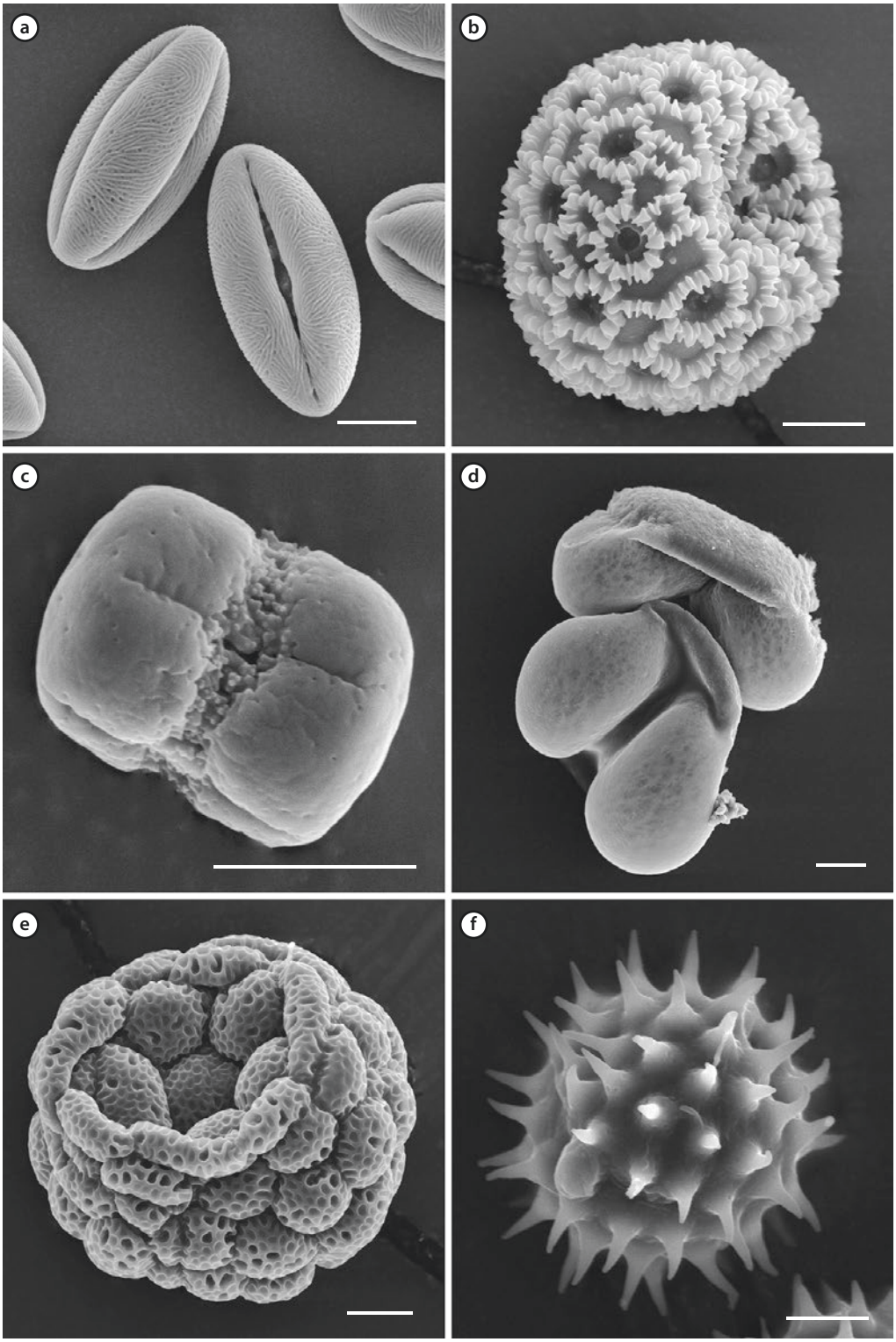


Fig. 17.6 **a–f** Scanning electron microscopy of pollen from six species. From top left to bottom right, pollen from **a** buckeye (*Aesculus glabra*), **b** Japanese spurge (*Pachysandra terminalis*), **c** Jack Frost (*Brunnera macrophylla*), **d** white pine (*Pinus strobus*), **e** catalpa (*Catalpa speciosa*), and **f** pale purple coneflower (*Echinacea pallida*). All species shown are pollinated by insects, with the exception of the **d** white pine (gymnosperm). Scale bars = 5 μm in (c) and 10 μm in all other panels. (**a–f** RR Wise)

17.7 Pollen Dispersal, Germination, and Pollen Tube Growth Precede Double Fertilization

Various substances may accumulate on the exine surface and influence pollen dispersal. In **entomophilous** (insect-pollinated) plants, pollen grains are covered with oily, sticky, and colored materials, pollenkitt or tryphine, that facilitate the attraction of insects and the adhesion and subsequent transfer of pollen. In **anemophilous** (wind-pollinated) plants, the thickness, surface sculpturing, and stickiness of the exine are all generally reduced to enhance the buoyancy of the pollen in the air (see winglike structures of white pine pollen in ◘ Fig. 17.6d).

When a pollen grain is released from the anther, it exists as an extremely reduced haploid male plant (male gametophyte) until it is carried to the stigma either by wind or insects or directly by contact of the anther with the stigma. The interaction between pollen and the pistil starts with the adhesion of pollen grains to the stigmatic surface. This is facilitated by the sticky nature of the pollen surface and an exudate on the stigmatic surface. Adhesion of pollen grains to a receptive stigma is soon followed by the uptake of water through the apertures in the exine. This imbibition of water brings about the breaking of dormancy, mobilization of food reserves, and activation of metabolism in the vegetative cell including the release of proteins from the pollen wall. These proteins participate in recognition reactions between the pollen and the stigma. In incompatible combinations, pollen germination is suppressed, or newly emerged pollen tubes fail to penetrate the stigma.

Germination occurs by the emergence of a pollen tube from one of the apertures (◘ Fig. 17.7a). In culture, two or three pollen tubes may be formed (◘ Fig. 17.6d), but only the one with the generative sperm is active for growth and fertilization. The wall of the growing pollen tube (i.e., the wall of the vegetative cell) is cellulosic and both similar to and continuous with the intine of the pollen grain. Thus, the emerging pollen tube first appears as a bulge of the intine protruding through the germination aperture since the polysaccharide tube wall is essentially a continuation of the intine (◘ Fig. 17.7a, b). The nucleus of the vegetative cell and then the sperms, in plants with three-cellular pollen grains (◘ Fig. 17.7d, h), are soon moved into the growing pollen tube by the cytoskeleton. In plants with bicellular grains (◘ Fig. 17.7a), the second division of the microgametophyte resulting in the formation of two sperm cells occurs in the pollen tube (◘ Fig. 17.7c).

After recognition and acceptance of the pollen on the stigma, the pollen tubes (carriers of the sperm cells) grow through the stigma to the stylar transmitting tissue and then down to the ovary. Such growth may be through a hollow style, along surface transmitting tissue (aka **stigmatoid tissue**; refer to ► Chap. 18, ► Fig. 18.5a) or within the papillae cell wall initially (◘ Fig. 17.7i). When the tubes reach the ovary, they grow along the surface of the placenta toward the ovules. Occasionally, more than one pollen tube may be formed by a pollen grain, but only a tube containing sperm cells continues its growth; the other is soon blocked.

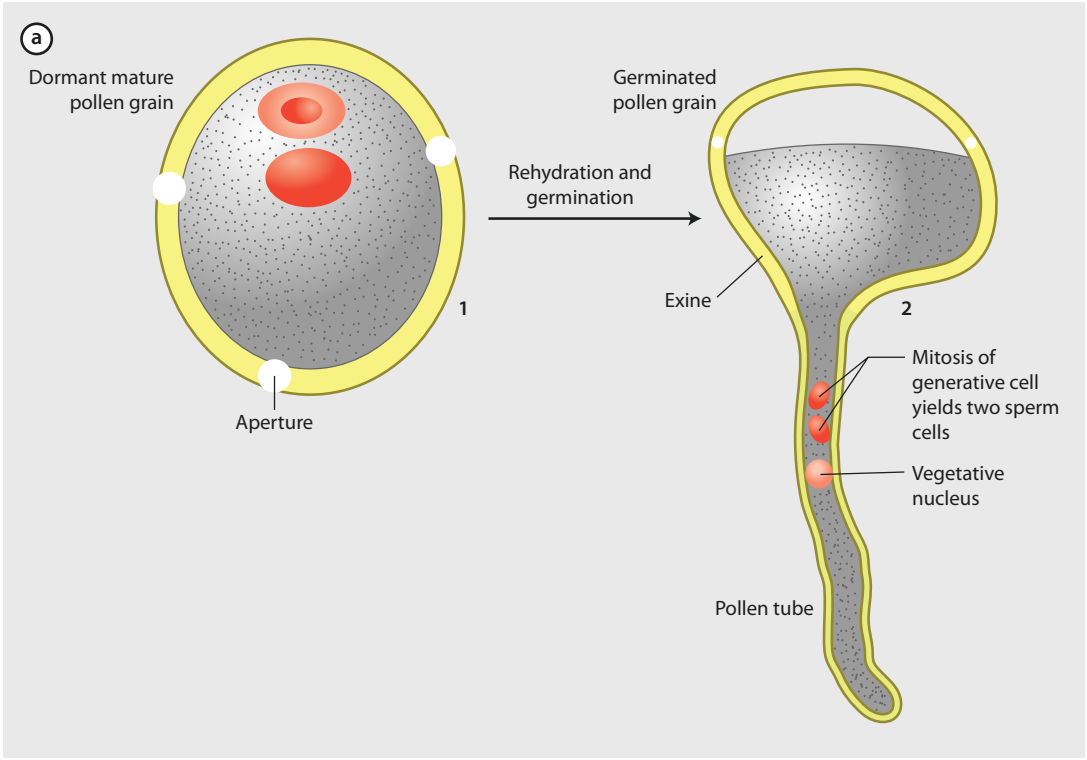


Fig. 17.7 **a** Diagrammatic representation of germination of pollen grain followed by mitosis of generative cell. After the arrival on the stigmatic surface of the pistil, the dormant pollen grain (1) adheres to a papilla, absorbs water (rehydration), and swells. The germination of the pollen grain takes place by the emergence of the pollen tube through one of the apertures (2). (Redrawn from Crang and Vassilyev 2003)

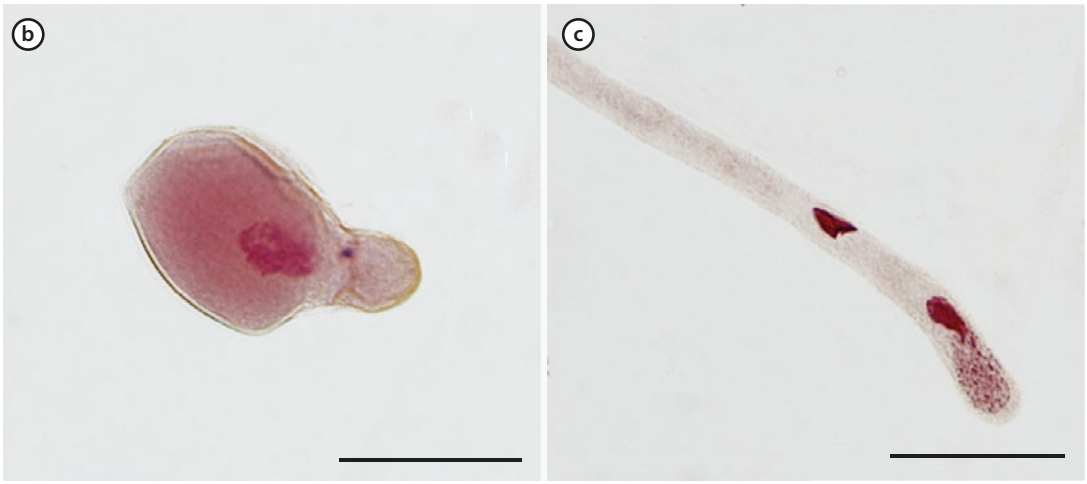


Fig. 17.7 **b**, **c** Micrographs of germinating Solomon's seal (*Polygonatum* sp.) pollen grains in vitro. Nuclei are stained in dark pink. **b** A pollen grain that has just started germinating with the vegetative nucleus at the early end of the new pollen tube. The wall of the tube is essentially an extension of the pollen grain intine. **c** Illustration shows a pollen tube with two sperms (dark red) and tube nucleus (pale pink) at tip of the tube. Scale bar = 25 µm. (**b**, **c** RR Wise)

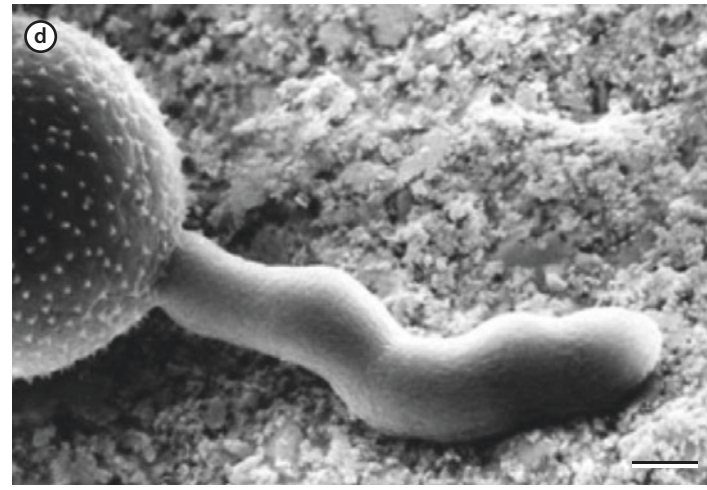


Fig. 17.7 **d** In this scanning electron microscope view, the pollen tube has grown a short distance from the pollen grain of white campion (*Lychnis alba*). The lump shown near the end of the pollen tube is most likely the site of the vegetative nucleus with the sperm cells following. Scale bar = 10 μm. (RC Crang)

The pollen tube emerges from the pollen grain by growth through the pollen aperture, which is covered by a thin layer of sporopollenin called the **operculum** (Fig. 17.7e). The pollen tube pushes against the operculum causing it to bulge (Fig. 17.7f) and eventually rupture. After pollen tube emergence, the operculum remains attached to the base of the pollen tube as an artifact of pollen germination (Fig. 17.7g).

17.8 Pollen Tube Growth Is Restricted to the Tip Region

As in other freely growing cells such as root hairs or fungal hyphae, the growth of the pollen tubes is restricted to the extreme tip (Fig. 17.8a, b). This **apical tip growth** occurs very rapidly and may reach a rate of 1 cm per hour in some plants, such as corn pollen tubes in laboratory culture. However, the actual growth zone is only a few micrometers long. This zone is filled with secretory Golgi vesicles (dictyosomes) that export the cell polysaccharides and membranes, which supply the growing wall and plasmalemma with new materials. The rate of the vesicle production has been estimated to be as high as 5000 per minute. The subapical zone is also rich in dictyosomes, the producers of secretory vesicles, as well as mitochondria and endoplasmic reticulum. Posterior to the growing tip, callose plugs form that keep the cellular contents and the generative cells growing toward the ovule (Qin et al. 2012).

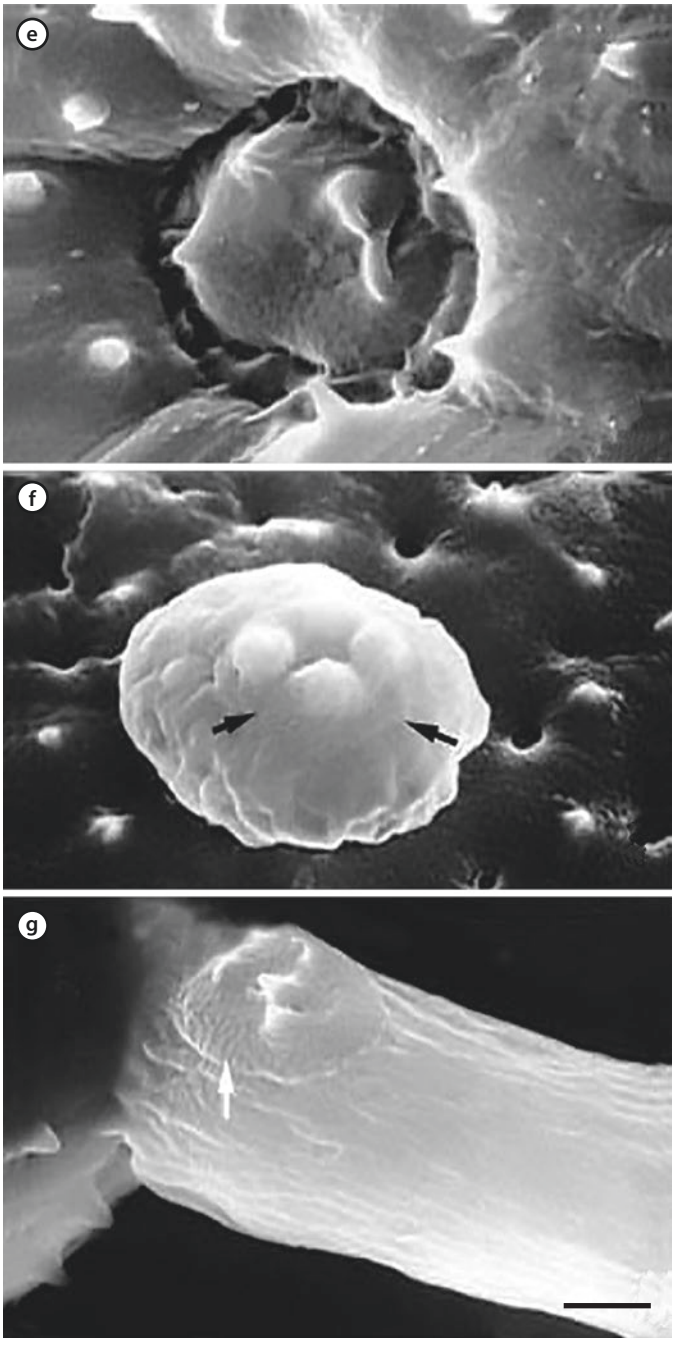


Fig. 17.7 **e**–**g** Scanning electron micrographs showing views of apertures of pollen grains in white campion (*Lychnis alba*). **e** A portion of a dry dormant pollen grain with the aperture appearing in face view as a depression in the exine. **f** A fully hydrated and activated grain immediately before pollen tube emergence. The operculum is clearly distinguishable (arrows). **g** A pollen grain with its growing pollen tube; the operculum is displaced to the side of the pollen tube. Scale bar = 1 µm and applies to all panels. (**e**–**g** RC Crang)

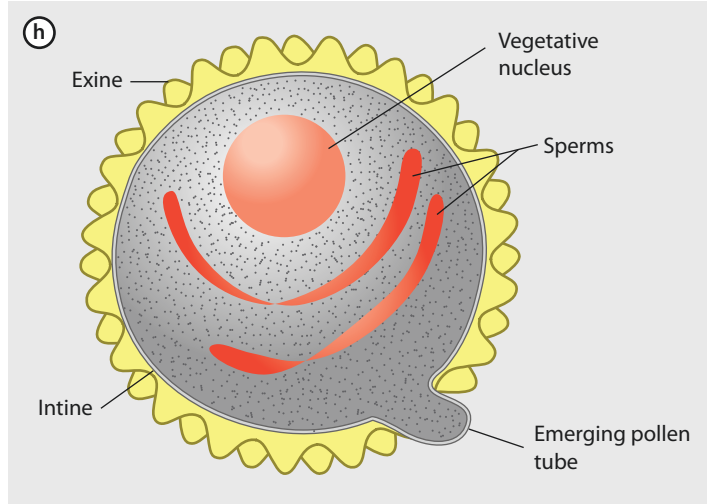


Fig. 17.7 **h** This illustration shows an early germinating tricellular pollen grain of rosinweed (*Silphium* sp.). The wall of the emerging pollen tube is in continuity with the intine. The exine is covered with sharp-pointed (i.e., echinate) spines. (Redrawn from Crang and Vassilyev 2003)

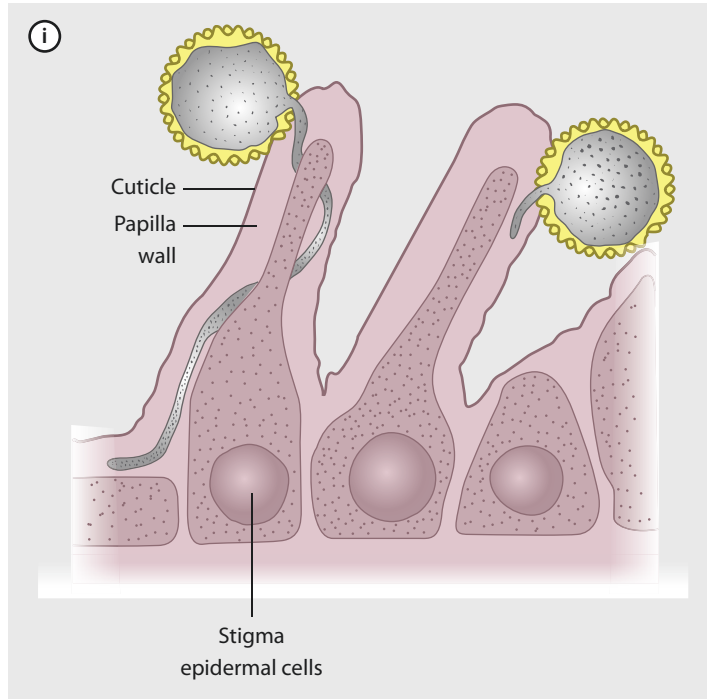


Fig. 17.7 **i** This diagram shows two pollen grains "glued" to the cell wall of a stigmatic papilla. Their pollen tubes have penetrated the cuticle (C) and grow within the papilla wall (PW) toward the stylar transmitting tissue. (Redrawn from Crang and Vassilyev 2003)

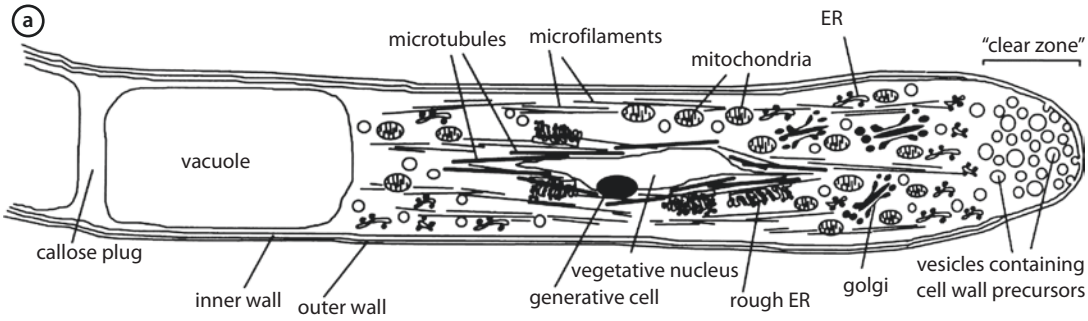


Fig. 17.8 **a** Diagram of subapical and apical tip regions of a pollen tube showing zonation. In what ways do these regions differ?. (Franklin-Tong 1999)

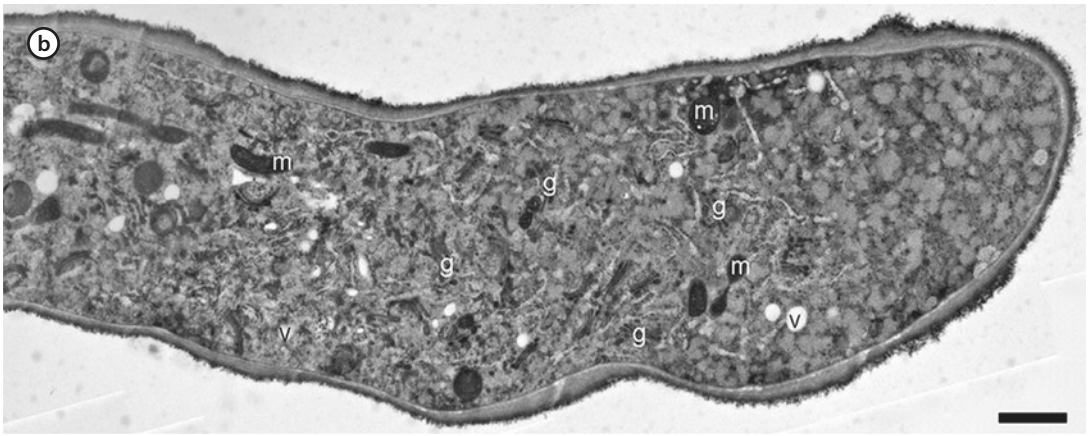


Fig. 17.8 **b** Longitudinal section of Arabidopsis (*Arabidopsis thaliana*) pollen tube apical region showing different regions of the cytoplasm. An apical cytoplasmic-clear region within 4–5 μm of the apex is full of small vesicles; large organelles are only present distally. (m mitochondria, g Golgi apparatus. Scale bar 1 μm). (Ndinyanka et al. (2017))

Similar to apically growing root hairs, pollen tubes show a distinct zonal organization (Fig. 17.8a). Usually four zones can be distinguished: (1) an apical growing zone (or clear zone); (2) a subapical zone where dictyosomes, endoplasmic reticulum, and mitochondria are concentrated; (3) a nuclear zone that includes the site of the vegetative nucleus and sperm cells; and (4) a vacuolated region.

The growing zone is filled with secretory vesicles that transport polysaccharides and membranes for the elongating cell wall and plasmalemma accordingly (Fig. 17.8a, b). These vesicles are produced by dictyosomes that are accumulated in the nongrowing subapical region. Numerous mitochondria provide energy for the active synthetic and transport processes. Endoplasmic reticulum is

the site of enzyme and membrane synthesis involved in polysaccharide production and plasmalemma growth.

It has been shown that bursts of calcium (as Ca^{2+} ions) are concentrated in the central apical stream, which are essential for the release of vesicles containing the new wall materials. Longitudinally oriented microfilament bundles of the cytoskeleton are responsible for the vigorous cytoplasmic movement characteristic of pollen tubes.

After reaching the ovule, the pollen tube enters the embryo sac through the **micropyle**. The obturator, or papillate placental cells that are formed in some plants, provide a well-defined pathway that guides the pollen tube to the tip of the ovule. It is generally assumed that the directed growth of the pollen tube occurs due to chemotropically active substances produced and secreted by the **synergid** cells. After its arrival to the embryo sac, the pollen tube enters one of the synergids through its intracellular filiform apparatus. When the pollen tube is within the synergid cytoplasm, it bursts and the vegetative nucleus and both sperm cells are discharged into the synergid. Thereafter, double fertilization occurs forming the diploid zygote and triploid endosperm (refer to Female Reproductive Structures and Embryogenesis, ► Chap. 18 for more details).

17.9 Pollen Is a Major Contributor to Seasonal Allergies

Pollen is a source of allergenic proteins that elicit the formation of a specific class of antibodies, immunoglobulin E, in sensitized humans causing "hay fever." These proteins are synthesized in the vegetative cell. Many angiosperms that have inconspicuous flowers shed pollen via the air and are a cause of allergies for many. The reaction leads to a variety of symptoms, such as sneezing, stuffy nose, and watery eyes. North American plants that are associated with seasonal allergies include many species of maple, elm, and oak that shed pollen in the spring along with many prairie plants. Massive amounts of conifer pollen produced by male cones are also especially abundant typically in the spring of the year, although generally less of an allergen than most angiosperm pollen. During late summer and early fall, at least three species of ragweed (*Ambrosia* sp.) are typically to blame. Allergy shots help the body to build resistance to pollens. Wearing a face mask, regular showering, wearing clean clothes after outdoor exposure, using recirculating air-conditioning, and not planting trees, such as catalpa, dogwood, fir, or redwoods that aggravate allergies, may help in reducing the complications (◘ Fig. 17.9).

Fig. 17.9 Ragweed (*Ambrosia* sp.) pollen is a particular bane of allergy sufferers. (With permission from Prevention CDN.NDG)

17.10 Chapter Review

Concept Review

17.1 *Flowers possess parts arranged in whorls that vary within and among species and are supported by a receptacle*. The floral parts include sepals, petals, stamens, and carpels with each species having particular floral components varying in numbers of each type from zero to many.

17.2 *Floral development starts with increasing cell divisions in apical meristems and initiating organs in an acropetal sequence*. Development from the outside inward is noted not only in the individual flower but carries on to the inflorescence.

17.3 *Male reproductive structures give rise to pollen within the anther*. A stamen includes a filament with an anther attached that contains pollen grains.

17.4 *Pollen grain formation begins with microsporogenesis followed by microgametogenesis*. Meiocytes undergo meiosis leading to the formation of four haploid microspores during microsporogenesis. Microgametogenesis follows, resulting in a multicellular pollen grain which is the microgametophyte also known as the male gametophyte.

17.5 *The structure of pollen grain cell walls changes during development from microspore to macrogametophyte, and callose is deposited and then sporopollenin and adhesives*. A thin layer of primexine is formed around the outer surface of the spore plasmalemma, followed by deposition of sporopollenin forming an exine along with adhesives.

17.6 *The physical properties of pollen grains are taxon specific*. Because of the reproductive nature of the cells within the

pollen grain, the structure and function of these cells differs markedly in structure and function from those of the rest of the plant body. Exine surfaces are often distinct among taxa, and thus, pollen grains can be helpful in identifying past, even ancient, plant communities based upon the pollen sampled from ice, soil, and/or lake beds.

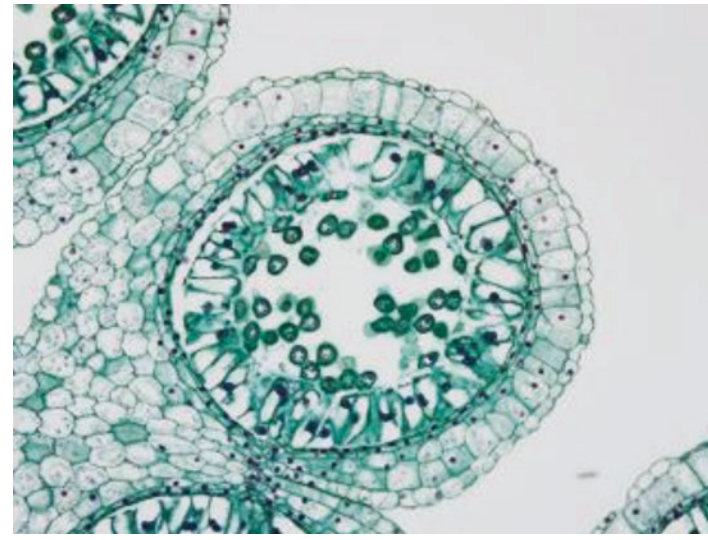
17.7 *Pollen dispersal, germination, and pollen tube growth precede double fertilization***.** The characteristics of the pollen grain surface are optimized to ensure pollen dispersal. A complicated process of pollen/stigma interaction and recognition is used to ensure that the proper pollen will germinate on the correct stigma at the right time. Germination of the pollen grain is followed by pollen tube growth through the style (which is directed by the vegetative nucleus) to the ovary to deliver the two sperm cells.

17.8 *Pollen tube growth is restricted to the tip region*. The endoplasmic reticulum, dictyosomes, and mitochondria within the pollen tube facilitate pollen tube growth via vesicles at the apical meristem of the tube cell. The synergid cells exude a chemical signal that directs the growth of the pollen tube to and through the micropyle. The pollen tube releases the two sperm cells and double fertilization follows.

17.9 *Pollen is a major contributor to seasonal allergies*. "Hay fever" is the cause of seasonal allergies as the anemophilous pollen reacts in sensitized humans.

■ Concept Connections

? 1. Below is a cross-section of a lily (*Lilium sp.*) flower bud showing an anther where the cells are undergoing late prophase of meiosis I during microsporogenesis at 100x magnification. Identify the epidermis, connective, tapetum, and developing microspores.



■ Concept Assessment

2. When flowers develop in an acropetal sequences,
   a. the stamens develop first, followed by the carpels, corolla, and calyx.
   b. the flowers develop from the inside of the flower, outward.
   c. the floral structures initially develop from the outside whorl moving sequentially inward toward the carpel.
   d. all whorls of the flower develop simultaneously.
   e. the petals are the first to develop.

3. The _____ is the structure where pollen grains are formed.
   a. anther.
   b. filament.
   c. connective.
   d. stigma.
   e. ovary.

4. Which structure is the male gametophyte?
   a. the microspore.
   b. the megagametophyte.
   c. the microgametophyte.
   d. the pollen grain.
   e. both c and d.

5. The tapetum functions mainly to:
   a. act like adhesive tape and provide a sticky substance to facilitate dispersal of pollen grains by animal vectors.
   b. shield the sun from the damaging rays of uv light.
   c. provide nutrition to the developing pollen grain, but also produce callase to degrade the callosic walls within the tetrad.
   d. enhance the effect of the dictyosomes by contributing to the formation of the cell plate.
   e. provide the anther with support.

6. Following the various divisions of the nucleus in the formation of the male gametophyte, what is the ploidy of cells within the pollen grain?
   a. haploid.
   b. diploid.
   c. triploid.
   d. tetraploid.
   e. none of the above.

7. By phase 4 of microgametogenesis, the microgametophyte may consist of:
   a. a large generative cell and a small vegetative cell.
   b. two small generative cells and a relatively large vegetative cell.
   c. two cells that are of equal size.
   d. a small generative cell and a larger vegetative cell.
   e. both b and d.

8. Apertures on the pollen grain provide:
   a. a way for the pollen grain to get oxygen.
   b. a means for transporting polysaccharides to the sperm cell(s).
   c. an opening for the pollen tube cell to grow through.
   d. drainage for excess intine to leave the structure.
   e. a path for transportation of cellular contents between cells in the pollen sac.

9. Pollen tubes grow at the apical tip of the cell in conjunction with:
   a. dictyosome secretions migrating to the tip and depositing polysaccharides for energy for the cell.
   b. callose plugs formed behind the growing apical tip funneling the cellular contents and sperm cells forward.
   c. the endoplasmic reticulum contributing to plasmalemma growth.
   d. mitochondria in the growing region providing energy.
   e. all of the above.

10. Double fertilization leads to:
   a. the formation of a haploid zygote and diploid endosperm.
   b. the formation of a haploid zygote and triploid endosperm.
   c. the formation of a diploid zygote and haploid endosperm.
   d. the formation of a diploid zygote and diploid endosperm.
   e. the formation of a diploid zygote and polyploid endosperm.

11. The role of the synergid is to:
   a. assist in the role of the endosperm in providing nutrients to growing embryo.
   b. facilitate the distribution of the contents of the elongated pollen tube to the egg cell and central cell followings its rupture.
   c. work in synergy with the other synergids in the cell to promote the growth of the potential embryo.
   d. act as a placeholder, keeping the egg cell intact.
   e. provide nutrition to the embryo.

■ Concept Applications

12. Many causes of "hay fever" stem from allergies to pollen. In North America, ragweed (*Ambrosia psilostachya*) blooms at the same time as many species of goldenrod (*Solidago* sp.). Many people feel that the cause of their allergies is the goldenrod pollen because they see the plants blooming at the same time that their allergies are problematic. How can you explain that goldenrod is not the likely cause of their immediate allergic response?

13. Consider why pollen surfaces differ among taxa.

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