

## Primer

## Gametophyte development

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Unlike animals, which produce single-celled gametes directly from meiotic products, plants have generations which alternate between the diploid sporophyte and haploid gametophyte (Figure 1A). The diploid sporophytic generation develops from the zygote, the fusion product of haploid gametes. Sporophytic cells undergo meiosis to produce haploid spores, which divide mitotically to form the multicellular gametophyte. Certain cells in the gametophyte subsequently differentiate into gametes.

Land plants are divided into four groups (Figure 1B). The most basal group, the bryophytes, including mosses and liverworts, lack vascular tissues. Plants of the second group, including ferns and horsetails, have a vasculature but lack seeds. Gymnosperms and angiosperms both produce seeds. Angiosperms produce flowers and include most of the familiar plants. Although land plants in all four groups share a common life cycle (Figure 1A), the relative sizes of, and nutritional relationship between, the sporophyte and gametophyte vary greatly among different groups of land plants. Gametophyte development also varies among these groups.

## Gametophyte development in mosses

In bryophytes, the sporophyte is minute and dependent on the relatively prominent and nutritionally independent gametophyte for resources. The moss gametophyte looks like a miniature herb, with tiny leaf-like photosynthetic organs. The gametophyte generation begins as a dormant spore, which germinates under appropriate conditions to produce filamentous and branching protonemal

tissues. These form multicellular bud-like structures, each of which develops into a leafy shoot. The mature gametophytes produce male and female sexual organs, the antheridia and archegonia, respectively. The gametophyte is often sexually distinct, and plants are either male or female.

Each antheridium has an outer layer that encloses and protects thousands of motile sperm, which swim through available external water layer to the egg. Fertilization at the base of the cylindrical archegonium produces a diploid zygote which develops into an unbranched sporophyte. The sporophyte consists of a thin stalk attached to the gametophyte, and a capsule that encloses the sporophytic meiotic cells.

In recent years, the mosses *Physcomitrella patens* and *Funaria hygrometrica* have emerged as attractive model systems for studying gene function in non-vascular plants because of the relative ease of molecular manipulation by homologous recombination. Mutants affecting gametophyte development have been isolated and their analysis should provide insights into the molecular basis of gametophyte development in mosses.

## Gametophyte development in ferns

Unlike bryophytes, in vascular plants the sporophyte generation is macroscopic but the gametophyte generation is microscopic. Fern gametophytes are free living and they require moist conditions for reproduction. Most ferns, such as *Ceratopteris richardii* — an attractive genetic model system — are homosporous, producing only one kind of spore. In isolation, a *C. richardii* spore develops into a hermaphrodite gametophyte, producing both eggs and sperm. The gametophyte becomes a male if exposed to enough of the pheromone antheridiogen during its early development. After this initial period, the gametophyte is insensitive to antheridiogen and develops into a hermaphrodite, which produces and secretes antheridiogen.

The first gametophytic division is asymmetric, producing one large and one small cell. In *C. richardii*, the large cell divides again asymmetrically to produce another small cell, which later develops into the rhizoid, a root-like structure. The small cell from the first division becomes the protonemal initial, which divides further to form a linear three-celled protonema. The middle cell then undergoes a transition in divisional plane, forming a two-dimensional structure. During hermaphrodite development, a meristem is formed in the two-dimensional plane and gives rise to the male antheridia and the female archegonia. In the male gametophyte, most cells, except the apical and basal cells, will develop into antheridia.

In *C. richardii*, each antheridium contains an outer sterile tissue and an inner spermatogeneous cell, which undergoes five rounds of mitosis to produce 32 small spermatocytes. Further differentiation of the spermatocytes generates functional sperm cells, each with a coil structure and a flagellum. In the flask-shaped archegonium, a highly cytoplasmic egg is formed near the interior base. During fertilization, the archegonium opens a channel allowing direct access of the sperm to the egg.

The differences between male and hermaphrodite gametophyte development allow screening for *C. richardii* mutants that have altered sex determination. Studies of such mutants have led to a model for sexual determination in fern gametophyte development.

## Gametophyte development in gymnosperms

In seed plants, the sporophytic generation is dominant and free-living, and the gametophytes are very small and dependent on the sporophyte for nutrients. Among gymnosperms, conifers have male and female cones on the same tree. Other gymnosperms bear male and female reproductive structures on different individuals. In all gymnosperms, the male gametophyte (pollen grain) reaches the female reproductive structure helped by wind or animals.

The cones bear scale-like male or female organs, called microsporophylls and megasporophylls, respectively. In pines, the microsporophyll has two microsporangia, sac-like structures filled with many diploid microspore mother cells, which undergo meiosis to produce haploid microspores. The megasporophyll has two ovules, each containing a single megaspore mother cell which produces four meiotic products, one of which becomes the functional megaspore. The microspore and megaspore then develop into male and female gametophytes, respectively.

In the west white pine *Pinus monticola*, male gametophyte development involves a series of asymmetric cell divisions. First, the microspore divides to produce a large central cell and a small first primary prothallial cell. The central cell divides to produce a second primary prothallial cell and an antheridial initial. Another unequal division of the antheridial initial results in a large tube cell and a small antheridial cell. The small antheridial cell divides to form a sterile cell and a generative cell, which divides later to form two sperm cells.

In the female cone of *P. monticola*, the megaspore undergoes multiple mitoses, followed by cellularization, to produce about 2000 primary prothallial cells. Three to five primary prothallial cells near the micropyle, an opening of the ovule, enlarge and develop into archegonial initial cells. Each initial cell divides and develops into an archegonium containing an egg and other supportive cells.

After pollen lands on the female cone, it germinates to produce a pollen tube that grows towards the female gametophyte, often over two consecutive growth seasons. One sperm cell from each pollen grain fertilizes a single egg, while the other degenerates. Typically, the egg cells of all archegonia of a female gametophyte are fertilized and begin embryo development, but usually only one of the embryos develops to maturity.

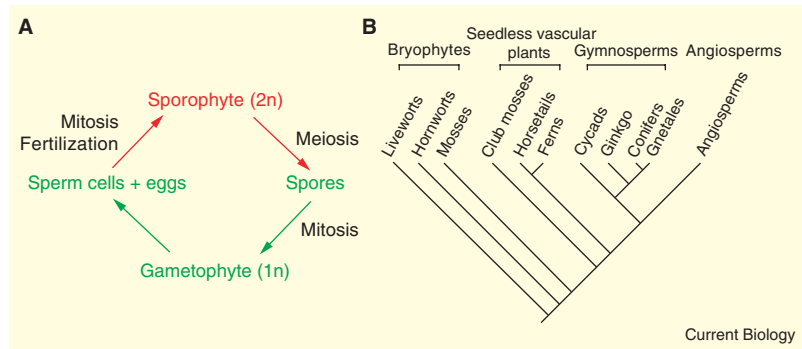


Figure 1. The life cycle and phylogenetic tree of land plants.

(A) The life cycle. From the diploid ( $2n$ ; red) sporophyte generation, sporocytes undergo meiosis and produce haploid spores ( $1n$ ; green). Mitoses then form gametophytes, which produce gametes, sperm cells and eggs. The fertilization of an egg with a sperm cell produces a zygote. (B) A phylogenetic tree of major land plants, showing the relationships of the groups of plants discussed here.

### Male gametophyte development in angiosperms

Angiosperms are defined by having seeds in the enclosing fruit derived from the ovary of a flower. The flower consists of primarily sporophytic tissues, with both male and female gametophytes which are highly reduced in size in comparison to all other land plants. Angiosperms also have the unique property of double fertilization, producing a usually triploid endosperm in addition to the embryo. The male gametophyte is formed in the anthers of the stamens, and the female gametophyte is located in the ovules within the pistil.

In the anther, four pollen sacs (locules) contain numerous microspore mother cells, each of which undergoes meiosis to produce four microspores in a tetrad (Figure 2A). The male gametophyte generation begins with the microspore. Initially, the microspore has a uniformly distributed cytoplasm with a centrally located haploid nucleus. A large vacuole later forms at the center, displacing the nucleus to the side. In many flowering plants, including the model species *Arabidopsis* and maize, two mitotic divisions occur during pollen development. The first division produces a large vegetative cell and a much smaller generative cell. The vegetative cell inherits most of the cytoplasm from the microspore cell, has a relatively loose nucleus that is active in transcription, and

completely envelops the generative cells. In contrast, the generative nucleus is more tightly organized and less active in transcription. The generative cell later undergoes a second mitosis to produce two sperm cells. Pollen development depends on the function of a surrounding sporophytic tissue called tapetum.

The pollen dehydrates during maturation; after pollination, the pollen grain rehydrates and germinates to produce a pollen tube. This tube grows towards the ovule, providing a passageway for the sperm cells to reach the female gametophyte. Because the female gametophyte is within the female reproductive organ of the sporophyte, the pollen tube must extend considerable distances — many times the size of the pollen grains — to reach the egg.

### Female gametophyte development in angiosperms

The female reproductive organ, the ovary, completely encloses one or more ovules, the location of the female gametophyte, commonly called the embryo sac. The ovule is attached to the inner surface of the ovary, with a micropyle at the distal end. Each ovule contains a single megaspore mother cell which is surrounded by integuments, the protective and nutritive layers of sporophytic cells. The megaspore mother cell undergoes meiosis to generate four haploid products. Three of the haploid products degenerate and only the one near

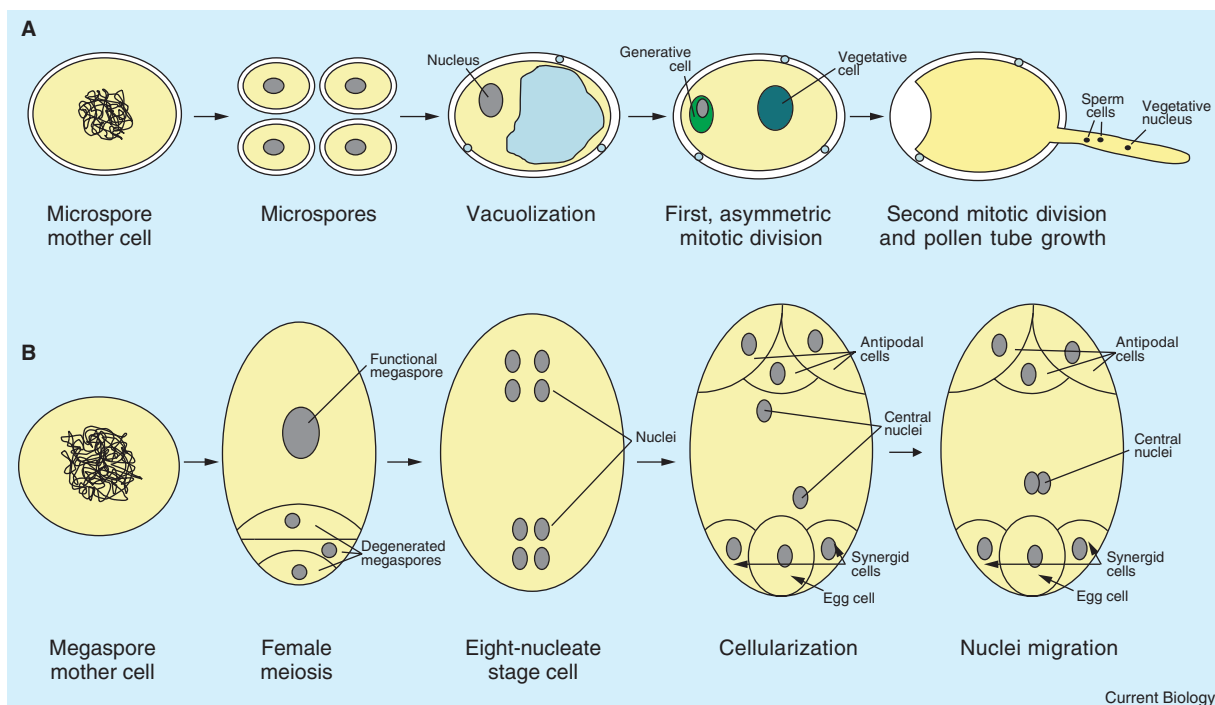


Figure 2. Male and female gametophyte development in angiosperms.

(A) Male gametophyte. Meiosis produces four microspores; vacuolization is accompanied by nuclear migration. The microspore divides asymmetrically to form a large vegetative cell and a small generative cell. The generative cell then produces two sperm cells, which later move toward the ovule through the growing pollen tube. (B) Female gametophyte. Meiosis in the megaspore mother cell produces four haploid products; one becomes the megaspore. During development, three rounds of mitosis form two clusters of four nuclei at the two ends of the female gametophyte. Cellularization generates seven cells: one egg cell and two synergid cells form the egg apparatus at the distal end, and three antipodal cells at the proximal end. In the large central cell, two nuclei migrate toward the center and fuse together.

the proximal end becomes the functional megaspore.

Depending on the pattern of subsequent cell divisions, four types of female gametophyte development have been described. In most flowering plant species, including *Arabidopsis* and maize, the megaspore undergoes a polygonum-type pathway that results in a seven-celled female gametophyte (Figure 2B). During this type of embryo sac development, three rounds of mitotic divisions occur without cytokinesis. The resulting eight nuclei are separated by a large central vacuole into two groups, each of which contains four nuclei and is located near one end of an elongated embryo sac. The embryo sac then undergoes cellularization, producing three antipodal cells at the proximal end and two synergid cells and one egg cell at the distal end. The remaining two nuclei, and much of the cytoplasm at the center, form a large central cell.

Angiosperms exhibit double fertilization. When the pollen tube reaches the distal end of the ovule, it grows into the micropyle and penetrates into one of the two synergids. The two sperm cells are released: one fuses with the egg cell to produce the zygote; the other fuses with the central cell, giving rise to the precursor of the endosperm. In a polygonum-type embryo sac, the endosperm is a triploid organ. But in the water lily *Nuphar polysepalum*, and several other basal angiosperms, the embryo sac is produced by two rounds of mitosis following meiosis, and contains four haploid cells, including the egg and central cells. Consequently, both products of the double fertilization, the zygote and the endosperm, are diploid.

Regardless of ploidy, the endosperm is a unique angiosperm organ which is an important nutritional structure that supports embryo and/or seedling development. The presence of endosperm, animal-attracting

flowers and protective fruit are all thought to have contributed to the success of angiosperms.

#### Genetic studies of gametophyte development in angiosperms

Because the angiosperm gametophytes are microscopic and dependent on the sporophyte, genetic studies usually involve mutagenesis of the sporophyte generation. Mutations that cause defects in sporophytic tissues resulting in sterility or reduced fertility are described by several reviews cited in the references and will not be discussed here. Examples of gametophytic mutations that affect gametophyte development are discussed below. Because the gametophyte is haploid, even recessive mutations in essential genes will not be transmitted to the next generation. Thus, a heterozygous sporophyte with a female gametophytic mutation is semi-sterile, because half of the embryo sacs will carry the mutant allele and fail to develop. A similar

heterozygous plant with a male gametophytic mutation is still fertile, because half of the pollen is normal and can successfully pollinate the pistil. Such mutations can be detected by their failure to transmit via the pollen.

Several mutations in *Arabidopsis* and maize have been identified that affect different stages of male gametophytic development. *Arabidopsis gemini pollen1* pollen grains show an incompletely penetrant phenotype; the mutant pollen grains exhibit equal, unequal or partial division for the first mitosis, possibly because of an inability to establish a polar nuclear position. So *GEMINI POLLEN1* may be required for proper nuclear migration before the first mitosis.

In maize mutant *gaMS-2* pollen, the first mitosis produces two similar cells; most pollen grains are blocked after the first mitosis. Maize *gaMS-1* mutant microspores develop into non-functional pollen grains with reduced sizes, usually arrested after the first mitosis. The genes *gaMS-1* and *gaMS-2* may be required for normal progression through the first mitosis.

In the *Arabidopsis* mutant *limpet pollen (lip)* pollen, the generative cell fails to migrate inward after the first mitosis. In another *Arabidopsis* mutant, *sidecar pollen (scp)*, pollen grains often contain an extra vegetative cell. The extra vegetative cell is produced before the asymmetric division, suggesting that *SCP* is an important negative regulator that prohibits the microspore from dividing symmetrically.

Several female gametophytic mutants exhibit defects at different stages. The maize *lethal ovule2* mutation results in gametophytes arrested at one-, two- and four-nucleate stages, suggesting this gene may function in regulating female nuclear division cycles. Similarly, the development of *Arabidopsis hadad* mutant embryo sacs arrests after one or two mitotic divisions, and there may be a defect in nuclear migration. In another maize mutant, *indeterminate gametophyte1 (ig)*, the polarity of the embryo sac at

the two-nucleate stage seems to be lost; in addition, the second and third rounds of mitosis are not synchronized, and sometimes only the distal nucleus undergoes the second mitosis. Furthermore, after cellularization, some distal cells undergo extra divisions, resulting in an indeterminate number of eggs and polar nuclei. In *Arabidopsis gf* and *fem* mutants, female development is arrested at different stages.

These studies suggest that many genes may be involved in regulating rounds of mitoses, cell polarity, and nuclear position during gametophyte development. Cell-cycle control and cytoskeleton function are likely important for these processes. These mutants and others should provide valuable insights into the regulation of gametophyte development. In addition, genomic projects in combination with reverse genetic tools should also be very informative.

### Conclusions

The gametophyte generation in land plants has evolved from a free-living and relatively complex organism into microscopic structures dependent on the sporophyte. The strong vasculature, sporophyte-supported gametophytes and drought-resistant pollen are likely to have contributed to the success of seed plants. The protective ovary/fruit and the triploid endosperm most likely further increased the reproductive fitness of flowering plants, making them the most successful group of plants.

Understanding the evolution of gametophytic sexual dimorphism is an important aspect in the study of gametophytes. Mosses generally produce morphologically uniform gametophytes which differ only in sexual organs, whereas ferns produce sexually dimorphic gametophytes generally from a single type of spore. The gametophytes in seed plants, both gymnosperms and angiosperms, are genetically pre-determined and structurally dimorphic from the first mitosis. Progressively determined sexual

dimorphism of reproductive structures may represent an evolutionary trend that might have provided increasing fitness to land plants.

Molecular and genetic studies have begun to reveal genes that control several aspects of gametophyte development, including sexual determination, cell polarity establishment, cell fate determination and the control of cell division. We can look forward to a rapid expansion in the knowledge of gametophyte development, with the use of genetic, genomic, and evolutionary approaches to identify both conserved and specialized components of regulatory networks.

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