The angiosperm female gametophyte: No longer the forgotten generation

Vladimir Brukhin*, Mark D. Curtis* and Ueli Grossniklaus#
Institute of Plant Biology and Zürich-Basel Plant Science Center, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

The plant life cycle alternates between the diploid sporophyte (spore-producing organism) and the haploid gametophyte (gamete-producing organism). The female gametophyte of flowering plants develops within the ovule, a specialized structure within the ovary, which gives rise to the seed after fertilization. The female gametophyte, or embryo sac, contains a small number of clonally derived cell types and serves as a model for the study of many fundamental processes crucial to development. The female gametophyte develops coordinately with the sporophytic tissues of the ovule, providing an ideal system to study axis determination, pattern formation, cell–cell communication, and the role of cell lineage and position in cell specification and differentiation. Despite the important role played by the gametophyte in reproduction, few studies have focused attention on the molecular or genetic aspects of female gametophyte development and function. Consequently, the gametophyte was termed “the forgotten generation”1. In this paper we illustrate the developmental and structural diversity of gametophytes, studied to a large extent by the Indian school influenced by P. Maheshwari, and review new insights gained by genetic and molecular studies.

Keywords: Angiosperm embryology, Arabidopsis, cell specification, developmental genetics, embryo sac.

Reduction of the gametophyte during land plant evolution

The appearance of alternating generations in the plant life cycle occurred early in the evolution of plants. In many lower plants, the gametophytes are the dominant free-living generation, while in higher plants, the dominant generation is the sporophyte. This distinction has classically been used to separate lower plants from higher plants. In many algae, for example, the predominant phase of the life cycle is the haploid gametophyte, and the diploid sporophytic phase is only transient2.

As plants became adapted to life on land, the sporophytic phase became dominant – with the exception of the bryophytes, in which the predominant generation remains gametophytic and reliant on wet land habitats. In the lower vascular plants, such as ferns, the sporophyte is clearly dominant, producing sporangia in clusters (sori) on frond-like leaves. At maturity, these sporangia release spores, which, if they settle on a damp surface, germinate to produce a prothallus or gametophyte. The prothallus produces chlorophyll and grows independently of the sporophyte. It forms male (antheridia) and/or female (archegonia) reproductive organs, which produce male and female gametes, respectively. Since the sperm cells are motile, water is a requirement for fertilization.

As the land plants evolved to produce seed, the gametophytic phase became further reduced and more deeply embedded in reproductive structures of the plant, relying more on the sporophyte and less on water. In higher plants this reduction culminates in the production of the male gametophyte, or microgametophyte (i.e. the pollen grain). The male gametophyte is typically a 3-celled structure, comprising two sperm cells ensconced within a vegetative cell3,4. The microgametophytes are derived from a diploid microspore mother cell that undergoes meiosis to produce four haploid microspores. Each haploid microspore undergoes two mitotic divisions. The first division produces a large vegetative cell (the primary function of which is to deliver the sperm cells to the female gametophyte or embryo sac) and a smaller generative cell3,4. The generative cell then undergoes a second mitotic division to produce two sperm cells (for review see ref. 8).

The morphology of the female gametophyte, and the means by which it is fertilized, are defining features that separate the angiosperms from the gymnosperms9. In gymnosperms, the female gametophyte is much larger than that of angiosperms, consisting of several hundreds or even thousands of cells with several archegonia enclosed within jacket cells9. The large size of the female gametophyte means that in gymnosperms there is no requirement for endosperm to nourish the developing embryo. The so-called ‘endosperm’ of the gymnosperms is the haploid gametophyte and, although typically two sperm cells are produced, only one sperm participates in the fertilization of the egg while the other degenerates.

In angiosperms, the female gametophyte, or megagametophyte, develops within the ovule, protected by the nucellus and the integuments of the ovule. The structure and organization of the female gametophyte varies between angiosperms, the most common form being the Polygonum type, which is present in 70% of all angiosperms4.
mitotic division cycles and nuclear migrations  cellularization and cell specification  embryo sac maturation  post-fertilization development

Figure 1. a–d. Schematic representation of the development of a Polygonum type female gametophyte. a. Three free nuclear divisions occur in a syngamy to form an 8-nucleate female gametophyte. One nucleus from each pole (the polar nuclei) migrates and will eventually be enclosed by the central cell. b. Cellularization forms the typical 7-celled, 8-nucleate female gametophyte with two synergids (blue), one egg cell (red), a bi-nucleate central cell (green) and three antipodal cells (yellow). c. Before fertilization, the female gametophytes fully differentiates: the two polar nuclei fuse to form the homo-diploid secondary endosperm nucleus, the antipodals undergo programmed cell death and one of the synergids degenerates as the pollen tube arrives. d. During double fertilization one sperm cell fuses with the egg cell to form the diploid zygote (purple), while the second sperm fertilizes the central cell to form the triploid endosperm (dark green). The primary endosperm nucleus divides prior to the zygote in a syncytium.

Figure 2 a–c. Selected examples illustrating the structural diversity of angiosperm female gametophytes. a. Most common 7-celled 8-nucleate Polygonum type embryo sac with an egg apparatus, consisting of an egg cell and two synergids at the micropylar pole (top), two polar nuclei in the centre, and three antipodes at the chalazal pole (adapted from 82). b. Penaea type embryo sac of Acalypha indica: a 16-nucleate embryo sac, with 8 free nuclei in the centre and four groups of two cells at the periphery (adapted from 23). c. Embryo sacs of the Plumbaginella type, where synergids are missing (top), the antipodal cell is triploid, and the central cell nucleus is tetraploid (adapted from 24).

The Polygonum type female gametophyte results from precisely choreographed megasporogenesis and megagametogenesis. During megasporogenesis, a diploid megaspore mother cell (MMC) undergoes meiosis to produce four haploid megaspores, one of which survives while the other three degenerate. Then, during megagametogenesis, the surviving functional megaspore undergoes three rounds of mitosis, producing an 8-nucleate syngamy (Figure 1). Cellularization follows to produce seven cells belonging to four different cell types: an egg cell and two synergids (the so-called egg apparatus, located at the micropylar pole of the embryo sac), a bi-nucleate central cell, and three antipodals (located at the chalazal pole of the embryo sac). Despite the small number of cells and minute size, the female gametophyte is a central structure in angiosperm reproduction. It is involved in pollen tube guidance and reception, fertilization, egg activation, and the maternal control of seed development (for review see refs 10, 11). When the pollen tube reaches the embryo sac, the two sperm cells within are released into the degenerating synergid. One sperm cell then migrates to the central cell and, after plasmogamy, the nuclei fuse to generate a triploid nucleus. The other sperm cell migrates to the egg cell, with which it fuses to produce the diploid zygote. The triploid central cell gives rise to the endosperm and the diploid egg cell gives rise to the embryo. Viable seed formation depends on this double fertilization event, and the coordinated development of the embryo, the endosperm, and the maternal seed coat of sporophytic origin, to produce a mature seed harbouring the next sporophytic generation.

Structural diversity among angiosperm female gametophytes

Although the general features described above have been used to define the angiosperms and the gymnosperms, they do not clearly identify the evolutionary relationships within these phyla. Difficulties arise due to the evolution of different types of female gametophytes within the angiosperms. These differences can result from the number of functional megaspore nuclei that take part in the formation of the embryo sac (i.e. monosporic, bisporic, or
tetrasporic). There are two types of monosporic female gametophytes, the Polygonum type, which produces an 8-nucleate embryo sac12-15 (Figures 1 and 2a), and the Oenothera type, which produces a 4-nucleate embryo sac, present only in the family Onagraceae4,12,16,17. Unlike the Polygonum type, just two mitotic nuclear divisions occur in the Oenothera type embryo sac. In the development of a Polygonum type embryo sac the functional megaspore nucleus undergoes an initial mitotic division. The two daughter nuclei then migrate to opposite poles of the syzygium, thereby establishing the proximal–distal axis and polarity of the embryo sac (Figure 1). A large vacuole forms between the two nuclei, which increases in size during embryo sac development. Each of the nuclei undergoes two further mitotic divisions, prior to cellularization. During cellularization, the three nuclei at the micropylar pole give rise to the two synergids and the egg cell, and the three nuclei at the chalazal pole form the three antipodal cells. The remaining nucleus – one at each pole (polar nuclei) – migrates towards the center and often fuse (karyogamy), producing the homo-diploid nucleus of the central cell (Figures 1 and 2a). In the region where synergids, egg cell and central cell meet, the cell wall is discontinuous18. This is probably necessary to allow plasmogamy with the sperm cells during fertilization. In the final stages of embryo sac development, the three antipodal cells usually degenerate (Figure 1), but in some species, e.g. Zea mays, they proliferate19. In Oenothera the micropylar megaspore develops into the embryo sac. After two mitotic divisions, one of the nuclei cellularizes to form the egg cell, two nuclei form the synergids, and one constitutes the single polar nucleus. The Oenothera type embryo sac is monopolar, since all the nuclei are situated at the micropylar pole of the developing embryo sac4. It is interesting to note that, in some apogamic complexes, these two types of embryo sac coexist: e.g. the sexual forms of Panicum maximum produce a bipolar 8-nucleate female gametophyte, whereas the apomictic forms, which reproduce asexually through seeds, have a monopolar, 4-nucleate embryo sac20,21.

Bisporic embryo sacs are derived from two megaspore nuclei, which are not separated by cytokinesis and undergo two mitotic divisions each, to form an 8-nucleate, 7-celled mature bipolar embryo sac, the most common of which is the Allium type. Further variation in the development of the female gametophyte is observed when all four megaspores participate in the formation of the embryo sac. For example, Peperomia type embryo sacs are 16-nucleate, consisting of an egg and a synergid cell at the micropylar pole, and a large secondary endosperm nucleus, formed by the fusion of 8 of the remaining nuclei, whilst the other 6 nuclei are cut off as antipodal22. In Penaeus type embryo sacs, the 16 nuclei form four distinct groups, one at each pole, and one at either side of the embryo sac. One nucleus from each quartet remains free (i.e. 4 polar nuclei) and moves towards the center of the embryo sac, while the other three cellularize. Generally, one cell in the group of three cells at the micropylar end is the functional egg cell. Embryo sacs of Acalypha indica, although similar to Penaeus type embryo sacs, do not follow the same arrangement. Here, the 16 nuclei are arranged into four distinct groups at the four poles. However, 2 nuclei of each quartet remain free and migrate to the center of the embryo sac, while the others become organized into cells. This produces four distinct groups of two cells each at the periphery and eight free nuclei in the center23 (Figure 2 b). Some tetraspore embryo sacs do not produce 16 nuclei. In the Plumbagella type, for example, the 4 megaspores migrate, one to the micropylar end, the remaining three to the chalazal end, where they fuse to form a triploid nucleus. These two nuclei divide producing two haploid micropylar nuclei and two triploid chalazal nuclei. The haploid nucleus nearest to the micropylar end becomes organized into the egg and the triploid nucleus nearest the chalazal end forms a single antipodal cell. The remaining two nuclei fuse to form a tetraploid secondary endosperm nucleus and no synergid cells are produced24 (Figure 2 c).

The enormous variety of developmental pathways that lead to embryo sac formation provides a basis for studying the evolution of these developmental programs. The continued characterization of genes that play key roles in embryo sac development and function, together with cytological data, will provide insights into the evolution of the various developmental pathways.

Genetic identification of gametophytically acting genes

The Polygonum type embryo sac is the most common and has been studied most extensively. Yet, despite our intimate knowledge of its morphology, very little is known about the molecular genetic mechanisms that control its development. The inaccessibility and small size of the female gametophyte have possibly hampered progress and enthusiasm for identifying the molecular mechanisms in the past. Although the Polygonum type female gametophyte contains only seven cells, these cells perform specific functions essential to reproduction. Mutational analysis of the female gametophyte will allow the dissection of these functions and their underlying developmental pathways. The isolation of new gametophytic mutants, resulting from large-scale insertional mutagenesis projects (reviewed in ref. 25), as well as the establishment of novel tools to analyse single cells at the molecular level (reviewed in ref. 26), offer unique opportunities to dissect the molecular mechanisms involved in gametophyte development and function.

Polygonum type embryo sac development in wild-type Arabidopsis has been very well described18,27-33 and has been divided into seven morphologically distinct stages, FG1 to FG7 (ref. 33). But even in Arabidopsis early reports...
of gametophytic mutants were rare. In fact, until the mid-1990s, only the gametophytic factorI (gfl1) mutant had been described\textsuperscript{33}. Over the last few years a large number of gametophytic mutants have been isolated in Arabidopsis and their molecular analysis is underway\textsuperscript{33,35-39}. This has become possible thanks to the development of tools for the production of insertional mutants and for their rapid molecular analysis.

Mendel's laws apply to traits expressed in the diploid sporophyte, where a recessive mutation will segregate in a 3:1 ratio in the F2 generation. By contrast, mutations in genes that affect the haploid gametophyte prevent their transmission through the egg and/or the sperm, resulting in a distorted, non-Mendelian segregation ratio\textsuperscript{40}. A mutation that affects both gametophytes and is fully penetrant will not be transmitted to the next generation at all. However, partially penetrant mutations, or mutations affecting only one sex, can be transmitted to subsequent generations and recovered as heterozygotes. Gametophytic mutants are difficult to identify because half of the pollen and half of the embryo sacs carry a wild-type allele such that the plants appear fertile. While male gametophytic mutants produce enough wild-type pollen to fertilize all ovules, female gametophytic mutants are expected to be semi-sterile because half of the ovules will not form seed. A half-filled fruit, however, looks normal and it has to be opened to evaluate the reduction in seed set. Because most plants have reduced fertility after a chemical mutagenesis treatment, chemical mutagenesis followed by screening for semi-sterility is not an appropriate method by which to identify female gametophytic mutants. A new era in gametophyte genetics began with the introduction of protocols for insertional mutagenesis. These methods did not cause a general reduction in fertility and allowed segregation ratios to be determined by the simple analysis of marker gene selection (present on the insertional element, i.e. T-DNA or transposons).

The two hallmarks of gametophytic mutations, segregation ratio distortion and reduced fertility, can be used to identify such mutants from collections of insertions. A departure from the Mendelian segregation ratio of 3:1 indicates that either the homozygous zygotes (segregation ratio of 2:1) or the haploid gametophytes are lethal (Figure 3). If both gametophytes are recovered and the progeny of plants is analyzed for segregation ratio distortion\textsuperscript{35,37,42,44}. Only true gametophytic mutants show segregation ratio distortion, whereas all other causes of a reduction in fertility, such as partially penetrant sporophytic mutants, poor environmental conditions, or reciprocal translocations, do not cause a deviation from normal Mendelian segregation.

Instead of using a marked insertional mutagen, chromosomes carrying multiple markers can be used to identify gametophytic mutants because linked markers will also show some degree of segregation ratio distortion\textsuperscript{44,45}. However, in addition to carrying a marker for direct assessment, insertional mutagens have the advantage that they 'tag' the mutations so that genomic sequences flanking the insertion can be obtained by simple PCR-based methods (see for instance, refs 43, 44). But insertional mutagens can cause chromosomal rearrangements, which can hamper the analysis of such mutants\textsuperscript{43,46}.

Figure 3a,b. Strategy of the two-step screen for identifying gametophytic mutants. a, In the first step, siliques are assessed for a reduction in seed set. In the case of a plant heterozygous for a gametophytic lethal mutation, about half of the ovules remain small and do not develop into a seed. In plants heterozygous for a zygotic embryo lethal mutation, about 25% of the seeds abort, whereas a gametophytic maternal effect mutation would show 50% seed abortion, which can be assayed in green or dry siliques. b, In a second step, the segregation ratio of the insertion is analysed using the dominant marker contained within it. If the insertion has no effect on transmission, it will segregate in a ratio of 3:1, if it causes embryonic lethality 2:1, and if it causes female gametophyte lethality 1:1.
Gametophytic mutants affect all stages of gametophyte development

A large number of cellular processes are required for the development and function of the angiosperm female gametophyte. Consequently, gametophytic mutants are recovered at a high frequency in large-scale screens\textsuperscript{35-37,47,48} (VB & UG, unpublished). Most mutants have been isolated from \textit{Arabidopsis}, but a few have also been described in maize (Table 1). Female gametophyte development can be divided into the following steps: (i) megaspore specification, (ii) initiation of megagametogenesis, (iii) mitotic progression, (iv) establishment of gametophyte polarity, (v) migration of polar nuclei, (vi) fusion of polar nuclei (karyogamy), (vii) cellularization, (viii) antipodal cell death, and (ix) degeneration of the synergid. The mature female gametophyte plays an important role in several developmental processes: (i) pollen tube guidance, (ii) pollen tube reception, (iii) sperm migration, (iv) plasmagamy during double fertilization, (v) karyogamy of male and female pronuclei, and (vi) maternal effects on early embryo and endosperm development. The gametophytic mutants isolated to date are scarce in one or more of these processes and have been classified according to the primary lesion they cause in the developmental pathway (Table 1).

\textbf{Mitotic class}

Most gametophytic mutants fall into the mitotic class. This class includes all mutants with a lesion in the initiation or regulation of any of the three mitotic divisions that occur during megagametogenesis (from early FG5 to late FG5). The mutants are characterized by an unusual number of nuclei, or an aberrant distribution of nuclei in the developing embryo sac\textsuperscript{35,37,47,48}.

An interesting example of this class of mutant, which illustrates how variable expressivity of a mutation influences the degree of segregation ratio distortion and seed sterility, is the mutant \textit{nomega}. In the \textit{nomega} mutant the embryo sac is arrested at the 2-nucleate stage, and although this is a gametophytic mutant and 50\% of the ovules should contain mutant embryo sacs, only 30\% of ovules were aborted\textsuperscript{49}. In fact, variable expressivity of the phenotype appears to be surprisingly common among gametophytic mutants\textsuperscript{50}. This may be due to the fact that large populations of individual gametophytes are analyzed and, due to their haploid nature, they are more susceptible to genetic and environmental modifiers.

The \textit{NAMEGA} gene product has high homology to the APC6/CDC16 subunit of the anaphase-promoting complex and is involved in post-metaphase events (separation of chromosomes and cytokinesis). This gene is disrupted by a \textit{Ds} insertion, so that the mutant allele is unlikely to produce a functional protein. Despite this mutation, however, some female gametophytes can develop into mature 7-celled embryo sacs. In these embryo sacs, either the APC/C complex remains active in the absence of the NOMEGA protein, or sufficient NOMEGA protein remains in the

\textbf{Table 1. Types of the established female gametophytic mutations in Arabidopsis}

\begin{tabular}{|l|l|l|}
\hline
\textbf{Mutant class} & \textbf{Mutant name} & \textbf{References} \\
\hline
Mitotic & gf1 & 34, 33 \\
 & ig* & 67, 68, 83 \\
 & pr1 & 69 \\
 & hdd & 35 \\
 & gfo4, gfo5 & 70 \\
 & ada, rsa & 41 \\
 & fem2, fem3, fem5, fem9, fem16, fem18, fem26, fem29, fem31, fem33, fem38 & 47 \\
 & lma, ana & 36 \\
 & chi1 & 71, 72 \\
 & apc2 & 73 \\
 & nomega & 49 \\
 & rb1 & 74 \\
 & agp18 & 75 \\
 & sna1 & 76 \\
 & eda1-eda23 & 48 \\
 & kalpalo (kao), castile (alk) & (Brucklin, unpublished) \\
\hline
Karyogamy & gfo2, gfo3, gfo7 & 70, 37 \\
 & pri, nan & 36 \\
 & eda24-edu41 & 48 \\
 & amon (amm), api5 (aps) & (Brucklin, unpublished) \\
\hline
Cellularization & gfo2, gfo3, fem4, fem6, fem8, fem11, fem13, fem15 & 37, 47 \\
 & jum, wlg, nja, dam & 47 \\
 & 36 \\
\hline
Degeneration & fem1 & 37 \\
 & fem14 & 47 \\
 & cgfo2 & 47 \\
 & nus & 36 \\
 & yarilo (yar) & (Brucklin, unpublished) \\
\hline
Fertilization & fer & 36, 53 \\
 & Sra & 54 \\
 & une1-une18 & 48 \\
 & znue1* & 77 \\
\hline
Maternal-effect & fie & 38 \\
 & fis1 (mea), fis2, fis3 (fie) & 57 \\
 & mea & 44 \\
 & mel1* & 78 \\
 & pri & 79 \\
 & cap1, cap2 & 38 \\
 & ctrl1 & 47 \\
 & zal, ash, aya, kem & 36 \\
 & dme & 80 \\
 & bga & 62 \\
 & mee1-mee70 & 48 \\
 & lpoa2 & 81 \\
 & dilil1a (did) & (Brucklin, unpublished) \\
\hline
\end{tabular}

*These are mutants in Zea mays; all others were described in Arabidopsis thaliana.
cytoplasm, which is derived from the heterozygote MMC, allowing the three rounds of mitosis. The APC/C complex functions as an E3 ubiquitin ligase in the ubiquitin-mediated proteolysis pathway, which controls several key steps in the cell cycle. In rnege mutant embryo sacs, cyclin B is not degraded, and this probably results in the arrest of cell division at telophase.

**Karyogamy class**

The karyogamy class of mutants affects the fusion of polar nuclei. For example, in the gfa2 mutant, the polar nuclei migrate, but fail to fuse. This defect is accompanied by a delay in antipodal cell degeneration and a defect in synergid degeneration (features of another class of mutants)\(^7\). The GFA2 gene encodes a J-domain-containing protein, the ortholog of which functions in budding yeast as a chaperone in the mitochondrial matrix. This mutation provides evidence that nuclear fusion may be facilitated by mitochondrial factors and that the processes of megaspore and antipodal cell death are distinct from synergid degeneration and may be somehow linked to mitochondrial functions.

**Cellularization class**

This class of embryo sac mutants exhibit defects in cellularization, cell polarity and cell shape\(^3\). In wild-type megagametophytes, cellularization begins immediately, following the third mitosis in stages FG5 to FG7 (ref. 33). In mature embryo sacs, the egg cell is pear-shaped and highly polarized, with the nucleus and cytoplasm at the chalazal end of the cell with the remaining space filled by a large vacuole. The synergids are also polarized, however, the orientation is opposite to that of the egg cell, with the cytoplasm and nucleus at the micropylar end of these cells. The gametophytic mutant fem4, is a good example of a mutant affecting the shape and position of these cells\(^7\). Here, the egg cell is not pear-shaped and the synergid cells show altered polarity and shape, with their nuclei oriented towards the chalazal end of the cells.

The cytological characterizations of hadad (hdd)\(^5\) and of deletions in Zeu mays\(^4\) have shown that cellularization and mitotic progression are not coupled to each other. In these mutants, which arrest during the mitotic divisions and thus belong to the mitotic class, cell membranes can form even though all cell division cycles have not been completed. These findings suggest that the developmental programs controlling nuclear division and cellularization are independent.

**Degenerative class**

The degenerative class of embryo sac mutants shows defects in the degeneration of the three non-functional megaspore cells, and/or the synergids and/or the antipodal cells. This class includes the gfa2 mutation that affects synergid cell degeneration, as described earlier\(^7\), and may result simply from their failure to attract pollen tubes. Many other mutants show degenerating embryo sacs, but this may often be a secondary consequence of the failure to complete megagametogenesis. In the case of gfa2 and nantosutta (nan), both degeneration and a failure in the fusion of polar nuclei are observed\(^6\). In nan embryo sacs, the first abnormality observed is an enlargement and distortion of the nucleolus in polar nuclei, which may or may not fuse.

**Fertilization class**

The female gametophyte produces chemotactic signals that guide the growing pollen tube towards the micropyle\(^5\). One of the two synergids becomes receptive to pollen tube entry and, just before or during contact with the pollen tube, starts to disintegrate. The growth of the pollen tube arrests and the tip ruptures to release the sperm cells. After the sperm cells are released, they migrate to their female target cells and undergo plasmogamy. The mutants feronia and sirene are examples of this mutant class\(^3\). In mutant gametophytes, the pollen tubes continue to grow after entering the receptive synergid, fail to rupture and do not release the sperm cells. Frequently, numerous pollen tubes enter the embryo sac, but none affects fertilization, leading to semisterility (50% unfertilized ovules in the siliques). This evidence, together with cell ablation studies in Torenia fournieri\(^5\), shows that the synergid cells control both pollen tube guidance and reception. The absence of synergids in some classes of angiosperm embryo sacs, such as the Plumbagella type (Figure 2c), indicates that there must be alternative modes of pollen tube attraction among the angiosperms.

**Maternal-effect class**

After double fertilization the zygote develops into a diploid embryo (new sporophyte originating from the fertilized egg cell) and is nourished by the triplid endosperm (originating from the fertilized central cell). The two fertilization products are surrounded by the integuments of the ovule (tissues of sporophytic origin), which contribute to the development of the seed coat. Gametophytic mutants can also affect post-fertilization development. A mutant phenotype of this kind that depends on the genotype of the female gametophyte, but is independent of the paternal contribution, is referred to as a gametophytic maternal effect\(^10\). The mechanistic basis of maternal effects is varied and can be caused by: (i) mutations in genes that are expressed during embryo sac development, but whose products are required after fertilization for embryo and/or endosperm development, (ii) abnormal mitochondria or plastids that are usually inherited from the mother plant, (iii) alterations in gene dosage, or (iv) genomic imprinting.
The first example of such a maternal effect to be described in detail was *mea* (*mea*). Embryo and endosperm derived from a *mea* mutant embryo sac show abnormal cell proliferation, independent of the parental allele and gene dosage, leading to the formation of giant embryos and an enlarged chalazal cyst in the endosperm\(^5\). It was shown later that this was due to genomic imprinting, where only the maternally inherited allele was active after fertilization\(^5\). The MEA protein forms part of a *Polycomb* group complex that suppresses cell proliferation, not only after fertilization, but also in the absence of fertilization, preventing fertilization-independent endosperm development. Three other components of the complex are known, all of which share this interesting phenotype\(^5,44,57,62\). Whether the genes encoding other components of the *Polycomb* group complex are also regulated by genomic imprinting, or show a maternal effect because they are cytoplasmically stored, is currently unknown\(^6\).

Many gametophytic mutants show defects after fertilization. In fact, about half of the mutants described by Moore\(^46\) show post-fertilization defects at a certain frequency. Outcrossing with wild-type pollen has shown that these defects were indeed under maternal control\(^5\). A similar situation was found recently by Pagnussat *et al.*\(^48\) who also reported that half of the gametophytic mutants they isolated showed post-fertilization defects. The genes disrupted in these mutants come from diverse families that have been implicated in a wide variety of cellular functions, including protein degradation, transcriptional regulation, signal transduction and secondary metabolism\(^48\). However, many genes with unknown function have also been identified, presenting new challenges in the characterization and assignment of a function to these genes. In summary, gametophytic maternal effects seem to play a prominent role in seed development (see also ref. 64). However, with the exception of *mea*, the underlying nature of these maternal effects is still unclear\(^6\).

**Conclusion**

Over the last century, a vast amount of knowledge has been acquired about the cytology and ultrastructure of the angiosperm female gametophyte. However, our understanding of the molecular mechanisms that determine gametophyte development and how these developmental programs have evolved is not complete. During evolution several different developmental pathways have been adopted that account for the diversity of embryo sac structures. Despite these differences, the female gametophyte, in all its forms, plays a pivotal role in reproduction. Although most molecular and genetic analyses have been carried out in model organisms with a *Polygonum* type embryo sac, these studies will inevitably shed light on the processes that have led to the evolution of the other, less common, types of embryo sacs. Comparative, functional genetics will help elucidate the evolutionary relationships among the different embryo sacs providing new insights into the evolution of developmental processes.

In recent years, studies in *Arabidopsis* and maize have identified interesting genes that play a role in megagametogenesis and seed development, but a great deal is still to be learned. By taking advantage of the molecular-genetic tools available in model plants, a combination of forward- and reverse-genetics approaches can be applied to unravel the mechanisms that orchestrate the development and reproductive functions of the female gametophyte. Genetic screens directed to isolate mutants with disruptions in genes of importance to the gametophyte have already identified genes that play a role in many fundamental processes. Cytological observations show that gametophytic mutants typically have variable and incomplete phenotypes. Their defects include reduced male and female fertility, due to gametophyte lethality, the production of non-viable zygotes, and gametophytic maternal-effect seed abortion.

Understandably, the female gametophyte is physiologically very active and probably expresses thousands of genes. Certainly, the genes identified so far have diverse roles in cellular processes such as protein degradation, transcriptional regulation and signal transduction. Functional redundancy no doubt hinders the identification of some important genes that are involved in female gametophyte development and function. To identify these, second site mutagenesis or the construction of multiple mutations within single plants may be required, presenting great challenges to investigators. Strategies such as inducible gene silencing by RNA interference that specifically targets and silences gene families, could help resolve these problems\(^6,56\). New approaches such as microgenomics also hold much promise. The isolation of single cells by laser capture microdissection, used in conjunction with transcriptome analysis, will help to identify cell type-specifically expressed genes that are important for the identity and function of gametophytic cells\(^9\). Certainly, the application of such new technologies to this interesting phase of the plant life cycle will lead to new insights in the near future.

SPECIAL SECTION: EMBRYOLOGY OF FLOWERING PLANTS


ACKNOWLEDGEMENTS. Many laboratories around the world have been working to extend our understanding of the development of the female gametophyte in angiosperms. We have made every effort to record all the female gametophytic mutations in Arabidopsis described to date and apologize to any colleague(s) whose work has not been covered in this review.

1852 CURRENT SCIENCE, VOL. 89, NO. 11, 10 DECEMBER 2005