The Ovule and the Embryo Sac

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INTRODUCTION

The plant life cycle is characterized by the alternation of generations between a diploid sporophyte and a haploid gametophyte. The sporophyte functions to produce spores, which then develop into gametophytes. The differentiated gametophytes in turn produce either the male gametes (sperm) or female gametes (egg cells). In contrast to lower plant species, in which the gametophyte is the dominant, free-living generation (see Cove and Knight, 1993, this issue), gametophytes of angiosperms are smaller and less complex than the sporophyte and are formed within specialized organs of the flower. The male gametophyte (pollen or microgametophyte) develops within the anther, whereas the female gametophyte (embryo sac or megagametophyte) is a product of the ovule. Sexual reproduction requires the delivery of the sperm nuclei, via the pollen, to the embryo sac, where fertilization occurs and the new diploid sporophyte is formed (see Dumas and Mogensen, 1993, this issue).

These unique features of the angiosperm reproductive process illustrate one of the most fundamental problems in plant biology. That is, how does the transition from sporophytic to gametophytic development occur within the context of the flower? How do the gametophytes interact with each other and the maternal plant to produce a seed? A wealth of information has been obtained regarding sexual reproduction in plants by genetic and molecular analyses of pollen development (Mascarenhas, 1990, 1993, this issue) and pollen–stigma/style interactions (Cornish et al., 1988; Nasrallah and Nasrallah, 1989, 1993, this issue; Newbigin et al., 1993, this issue). By comparison, less is known about the regulation of female gametophyte development and the relationship of the embryo sac to the ovule before and after fertilization.

The ovule is the source of the megagametophyte and the progenitor of the seed. Specification of the megasporocyte, production of a functional megaspore (megasporogenesis), formation of the embryo sac (megagametogenesis), and embryogenesis all occur within the ovule. Because embryo sacs, unlike pollen, maintain physical contact with the parent sporophyte throughout their development, this association of the female gametophyte and the sporophyte provides an opportunity to examine interactions between cells, tissues, and genomes.

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This review examines aspects of angiosperm ovule and embryo sac development, including a representative sample of histological and ultrastructural analyses, and addresses some of the current molecular genetic approaches being taken to understand ovule and embryo sac morphogenesis and function.

OVULE AND EMBRYO SAC DEVELOPMENT

Ovule Development

Although ovule morphologies show considerable diversity, the ovule illustrated in Figure 1 is representative of the most frequently observed form (Bouman, 1984). Ovules are specialized structures, derived from the placenta of the ovary wall, that produce the megasporocyte and are the site of embryo sac formation, fertilization, and embryogenesis (see also Gasser and Robinson-Beers, 1993, this issue). The ovule consists of three basic structures: a nucellus, one or two integuments, and a funiculus.

The nucellus (Figure 1A) is derived from the apical portion of the ovule primordium and functions as the megasporangium. That is, the nucellus produces the megasporocyte, which will undergo meiosis to form the megaspores. Shortly after ovule initiation, a single subdermal nucellar cell enlarges and displays a prominent nucleus (Figure 1A). This cell represents the archesporium, or spore-bearing tissue, and typically occupies a position directly below the apex of the nucellus. Progeny obtained from periclinal chimeras show that the megasporocyte is derived from the subdermal (L2) histogenic layer (Satina, 1945). The single cell of the archesporium may function directly as the megasporocyte, or it may undergo one mitotic division to produce a megasporocyte and a somatic cell (Bouman, 1984). The archesporium is not always represented by a single archesporial cell. Some species, including soybean and Brassica campestris, have a multicellular archesporium containing several archesporial cells, only one of which gives rise to the megagametophyte (Kennell and Horner, 1985b; Sumner and Van Caeseele, 1988). Factors that control the development of the archesporium and determine the identity of the megasporocyte have not been identified. The location
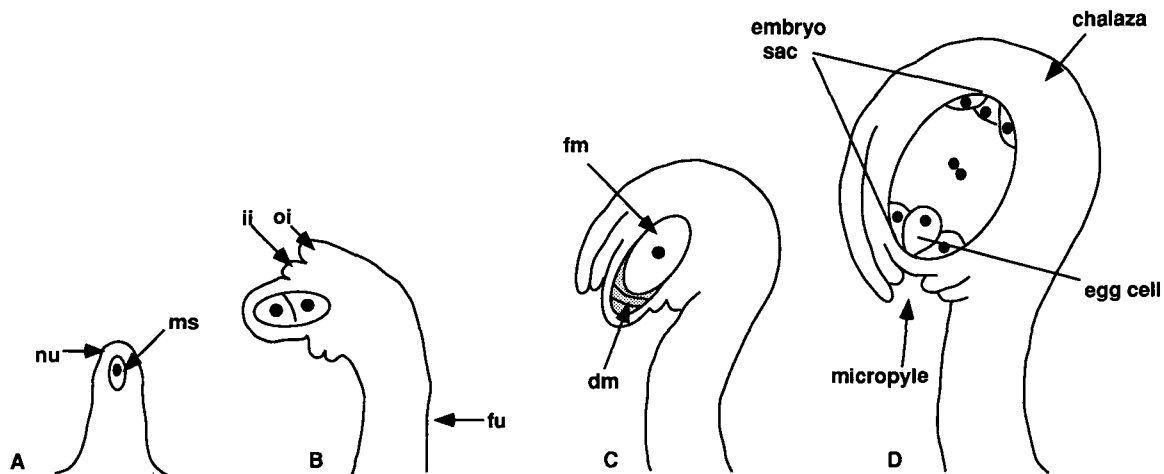
Figure 1. Ovule Development.

Stages are shown for an anatropous ovule with *Polygonum*-type embryo sac development. For more details, see text.

(A) Ovule shortly after initiation, showing a single megasporocyte (ms). nu, nucellus.

(B) Ovule after both integuments have been initiated. At this time, the megasporocyte has undergone the first meiotic division. The axis of the nucellus is transiently perpendicular to the axis of the funiculus (fu). ii, inner integument; oi, outer integument.

(C) Ovule after meiosis. The functional megaspore (fm) at the chalazal end has expanded, and the nonfunctional megaspores are degenerated. The axis of the nucellus is now parallel to the funiculus due to unequal growth, primarily of the integuments. dm, degenerate megaspores.

(D) Ovule after megagametogenesis. The mature embryo sac contains seven cells and eight nuclei.

of the megasporocyte, directly below the apex of the nucellus, suggests that position may be an important factor in megasporocyte specification.

The integuments are initiated at the base of the nucellus during megasporogenesis (Figure 1B). The inner integument is most often dermal (L1) in origin, whereas the outer integument is usually derived from both dermal and subdermal layers (Bouman, 1984). The two integuments are considered to have distinct evolutionary origins (Stebbins, 1974). Periclinal divisions in the integuments generate an increase in the number of cell layers, whereas anticlinal divisions and cell elongation are responsible for growth parallel to the nucellus. As the embryo sac develops, the integuments continue to enlarge, typically overgrowing the nucellus (Figure 1C). The amount of ovule curvature varies with the extent of differential growth of the integuments and funiculus; the degree of curvature forms a basis for classification of ovule morphology. Thus, the mature anatropous ovule shows extensive curvature such that the long axis of the nucellus is parallel to the axis of the funiculus (Figure 1D).

In ~65% of the species examined, most of the nucellus degenerates before the embryo sac reaches maturity (Takhtadzhian, 1991). The embryo sac is then in direct contact with the inner integument. In these situations, the innermost cell layer of the inner integument may differentiate into a unique cell layer termed the endothelium. Radial cell expansion, endopolyploidy, and prominent nuclei are observed in the endothelial cells and also the anther tapetum, which is thought to be involved in secretion and nutrition of the pollen (Bhandari,

1984; see Goldberg et al., 1993, this issue). Maheshwari (1950) speculated that the cytological features shared between the endothelium and tapetum could indicate a similar function for both tissues. In species in which the nucellus does not degenerate, the inner integument does not differentiate an endothelium, and the embryo sac may receive nutrients from the nucellus directly.

The ovule is connected to the ovary wall by the funiculus, a stalklike structure extending from the lowermost part of the chalaza to the placenta (Figure 1B). Usually, a single vascular strand runs through the funiculus from the placenta terminating at the base of the embryo sac. The mature ovule displays a polarity with respect to the axis determined by the location of the chalaza and micropyle. Esau (1977) defined the chalaza as the region extending from the base of the integuments to the point of attachment of the funiculus (Figure 1D). The micropyle is located at the point where the integuments terminate and is the site where pollen tubes enter the ovule (Figure 1D). The possible significance of this ovular polarity in embryo sac development is discussed below.

Embryo Sac Development

The process of embryo sac development can be divided into two stages: megasporogenesis and megagametogenesis. In general, during megasporogenesis, the megasporocyte undergoes meiosis and four megaspore nuclei are produced. Subsequent mitotic divisions, nuclear migration, and cytokinesis

during megagametogenesis produce the mature embryo sac. Considerable diversity in the pattern of embryo sac development is found among plant species. Figure 2 shows a small representative sample of some of the modes of embryo sac development that have been observed (see also Russell, 1993, this issue). Haig (1990) proposed a model for embryo sac development whereby different patterns could be generated by variations in meiosis, cytokinesis, and the timing and number of mitotic divisions.

Development of the *Polygonum*-Type Embryo Sac

The *Polygonum*-type pattern illustrated in Figure 2 is the most commonly observed form of embryo sac development. Approximately 70% of the species examined, including Arabidopsis and maize, have this form of embryo sac (Russell, 1978; Mansfield et al., 1990; Webb and Gunning, 1990). *Polygonum*-type embryo sacs originate from a single chalazally located

megaspore that undergoes three successive mitotic divisions. During the first meiotic division, the spindle is oriented parallel to the micropylar–chalazal axis of the nucellus. Wall formation occurs perpendicular to this axis, creating a dyad of megaspores. Frequently, the dyad cell closest to the micropyle degenerates without undergoing a second meiotic division (Maheshwari, 1950; Sumner and Van Caeseele, 1988). After the second meiotic division, another transverse wall is made, resulting in a linear arrangement of four megaspores. The megaspore closest to the chalaza enlarges before undergoing mitosis. The three nonfunctional megaspores degenerate and are eventually crushed by the expanding functional megaspore. Tetrahedral arrangements of megaspores have also been observed in Arabidopsis (Webb and Gunning, 1990), and T-shaped tetrads have been seen in maize (Russell, 1978). The linear array is, however, most common.

Callose, a β-1,3-glucan, is thought to function in the selection of a functional megaspore (Rodkiewicz, 1970). During megasporogenesis, callose accumulates first in the cell walls

Figure 2. Patterns of Embryo Sac Development.

Some examples of patterns of embryo sac development are illustrated schematically. Representative genera are indicated in parentheses. Comprehensive descriptions of the variation and complexity of embryo sac development can be found in several reviews (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990).

of the megasporocyte and then in the megaspore walls. After meiosis, callose walls become thinner or absent in the functional megaspore. The presence of callose in the walls of the nonfunctional megaspores probably ensures that only the functional megaspore receives nutrients from the nucellus. The pattern of callose deposition is variable, reflecting the pattern of megasporogenesis. For example, in *Oenothera*, a monosporic species, callose is thinner at the micropylar end of the ovule, where the functional megaspore is located (Rodkiewicz, 1970). In tetrasporic species (Figure 2), meiosis occurs without cytokinesis, and callose does not accumulate in the walls of the single tetranucleate megaspore.

The female gametophyte is generated from the functional megaspore via a process termed megagametogenesis. In *Polygonum*-type embryo sacs, the functional megaspore at the chalazal end enlarges prior to the first of three free nuclear divisions. After the first mitosis, the two nuclei migrate to opposite poles and the smaller vacuoles coalesce into a large central vacuole. Cass et al. (1985) suggested that formation of this central vacuole plays an important role in positioning the nuclei before subsequent mitotic divisions. Each of the two nuclei then divides two more times, resulting in an eight-celled coenocytic megagametophyte. Wall formation, nuclear migration, and differentiation follow, forming a mature embryo sac (Cass et al., 1985).

Cellular Anatomy of the Mature Embryo Sac

As shown in Figure 3, the *Polygonum*-type embryo sac has one egg cell, two synergids, three antipodal cells, and a central cell that contains two nuclei. These cells comprise four groups that function in fertilization, embryogenesis, and nutrition of the embryo sac and embryo.

Egg Cell

The egg cell is located at the micropylar end of the embryo sac and ultimately fuses with a sperm nucleus to produce a zygote. The egg cell lies adjacent to the two synergids, separated from them by either partial cell walls or the plasmalemma alone. The distribution of cytoplasm within the egg cell is highly polarized, due to the presence of a large vacuole at the micropylar end that restricts the nucleus and most of the cytoplasm to the chalazal end (Jensen, 1965a; Schulz and Jensen, 1968b; Cass et al., 1985; Sumner and Van Caeseele, 1989).

Synergids

The synergids, which are located on either side of the egg cell, play an important role in fertilization (Jensen, 1965a; Schulz and Jensen, 1968a; see Russell, 1993, this issue). The pollen tube discharges its contents into one of the synergids prior to incorporation of the sperm nuclei into the egg and central cells.
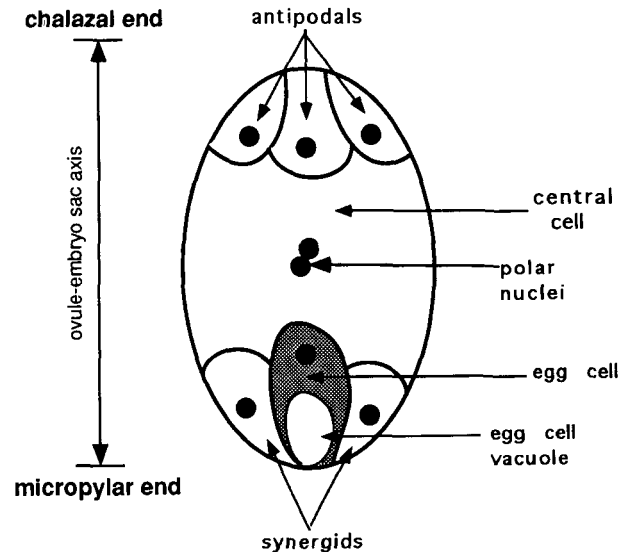

Figure 3. Organization of the Embryo Sac.

The orientation of the embryo sac with respect to the chalazal–micropylar axis of the ovule is indicated by the vertical arrow on the left. The egg apparatus, including the egg cell and synergids, is located at the micropylar end, where the pollen tube enters the embryo sac. The central cell contains two nuclei. Three antipodal cells are located at the chalazal end of the embryo sac. The egg cell is actually adjacent to, rather than between, the two synergids. Note the position of the vacuole in the egg cell.

Central Cell

Positioned in the center of the embryo sac, this cell contains two nuclei, a large vacuole, and many cytoplasmic organelles. The polar nuclei originate at both the micropylar and chalazal ends of the coenocytic megagametophyte and migrate to the center after cellularization. The polar nuclei may partially fuse with each other before they are fertilized by a single sperm nucleus, generating the triploid primary endosperm nucleus (Cass et al., 1985). The mature endosperm will provide nutrients for the developing embryo or seedling (see Lopes and Larkins, 1993, this issue).

Antipodal Cells

Three antipodal cells are located opposite the egg at the chalazal end of the embryo sac. No specific function during reproduction has been attributed to the antipodals, but they are thought to be involved in the import of nutrients to the embryo sac (Diboll, 1968).

Cytological characteristics of cells within the embryo sac as well as cytochemical localization of proteins, starches, lipids, and nucleic acids have been used to assess the physiological state of the embryo sac and suggest relative rates of metabolic

activity. For example, the presence of numerous ribosomes and mitochondria in the synergids, central cells, and antipodals suggests a high metabolic activity. By contrast, the egg cell has fewer ribosomes, plastids, and other organelles and appears to be relatively quiescent (Schulz and Jensen, 1968a, 1968b; Mansfield et al., 1990).

At the present time, there is little data available about the regulation of ovule and embryo sac development and the temporal and spatial patterns of gene expression in the embryo sac. However, this subject is an area of considerable interest and speculation. For example, to what extent do the genetic programs of the female gametophyte and the sporophyte overlap? At what point during embryo sac development are gametophyte-specific genes expressed? Are there regulatory factors inherited by the megaspores that influence the development of the embryo sac? Molecular and genetic approaches to study ovule and embryo sac development will hopefully begin to address these and other important questions.

Alternative Modes of Embryo Sac Development—Apomixis

In sexual plant species, meiosis is the prelude to embryo sac development; however, female gametophytes can develop in the absence of normal meiosis. In asexual or apomictic species, by contrast, diploid embryo sacs can develop as a result of aberrations in meiosis during megasporogenesis or directly from nonarchesporial cells of the ovule (see Nogler, 1984; Koltunow, 1993, this issue). Thus, embryo sac development can be uncoupled from meiosis. Furthermore, formation of embryo sacs from nucellar cells other than the megasporocyte suggests that competence to form embryo sacs is not restricted to the megasporocyte. Elucidation of mechanisms of apomixis will contribute to our understanding of sexual embryo sac development because similar regulatory mechanisms are likely to function in both modes of reproduction.

Embryo Sac Development Occurs along Chalazal-Micropylar Ovule Axis

Megaspore development and elaboration of the embryo sac occur along the chalazal–micropylar axis of the ovule. The polarity of the developing megagametophyte reflects the ovular polarity and suggests that the ovule plays a role in the selection of a functional megaspore and in the organization of the embryo sac (Willemse, 1981).

Polarity in the megasporocyte and megaspores is expressed in the asymmetric distribution of cellular organelles and plasmodesmata. Plasmodesmata appear more frequently, and plastids, mitochondria, and other organelles accumulate preferentially, at the chalazal end of the megasporocyte. Consequently, most of the organelles are inherited by the chalazal-most cell after the first meiotic division (Schulz and Jensen, 1971; Russell, 1978). These observations suggest that processes involved in megaspore selection may begin before meiosis. Asynchronous or incomplete second meiotic divisions in the micropylar cell after the dyad stage indicate that differences in the megaspores may be established early in megasporogenesis (Kaplan, 1969; Russell, 1978). After meiosis, plastids are preferentially distributed at the micropylar end of the functional megaspore, and plasmodesmata are usually observed only between the functional megaspore and the nucellus (Willemse and Bednara, 1979; Wilms, 1980; Cass et al., 1985). Thus, the functional megaspore inherits a richer cytoplasm and is situated so that it preferentially receives nutrients from the maternal tissues. These observations suggest that the position of megaspores within the ovule is important in the selection of the functional megaspore.

The polarity observed during megasporogenesis and early megagametogenesis may have important consequences for the development of the embryo sac. The arrangement of microtubules, as detected by immunofluorescence, indicates a role for the cytoskeleton in distribution and positioning of cytoplasmic contents during megasporogenesis and megagametogenesis (Huang et al., 1990; Webb and Gunning, 1990). Possibly, asymmetric distribution of morphogenetic determinants is mediated by the cytoskeleton, as is the case in other organisms (Hill and Strome, 1990; Yisraeli et al., 1990; Pokrywka and Stephenson, 1991). The final organization of the embryo sac may reflect the establishment of heterogeneities in the cytoplasm during megasporogenesis that are enhanced during megagametogenesis. It is possible that positional information, perhaps in the form of gradients, could be inherited from the megasporocyte. Alternatively, the ovule may provide cues via intercellular contacts throughout embryo sac development.

Although the nature of specific nutritional or hormonal interactions between the ovule and embryo sac are not known, Willemse (1981) considered the presence of intercellular contacts between the developing megagametophyte and the ovule to be important for establishing a polar nutrient flow. Synergids are frequently observed to have elaborate wall projections, the filiform apparatus that extends into the nucellus. The filiform apparatus may provide a mechanism for nutrient flow from the ovule to the embryo sac (Jensen, 1965a; Schulz and Jensen, 1968a). On the other side of the embryo sac, wall projections extend from the antipodal cells into the chalazal nucellus and may represent another site of metabolite flow from the nucellus to the embryo sac (Cass et al., 1986; Mansfield and Briarty, 1990). In some species, cell wall projections are also found between the central cell and the inner integuments; however, in other species, a cutinized wall can be found in this region (Cass et al., 1985; Sumner and Van Caeseele, 1990). The distribution of metabolite transfer sites along the embryo sac may be significant because differences in the cellular anatomy and the accumulation and mobilization of starch reserves of the nucellus and integuments also suggest a directional flow of metabolites into the embryo sac (Jensen, 1965b; Mogensen, 1973; Willemse and Bednara, 1979; Wilms, 1980). Perhaps

differences in the nutritional status at the two ends of the embryo sac influence the development and positioning of the egg and synergids.

Mechanical interactions within the ovule may also affect embryo sac development. Lintilhac (1974) proposed a model for ovule and embryo sac development in which mechanical interactions between the nucellus and integuments influence development of the megaspore and embryo sac. In this model, the architecture of the integuments and nucellus produces a region within the nucellus that is relatively free of stresses. The functional megaspore occupies this region and may thus be in a better position than the other megaspores to develop and grow without physical constraints.

In the mature embryo sac, the most notable expression of polarity is found in the egg cell and early embryo. The micropylar portion of the egg cell is occupied by a vacuole, and the nucleus, cytoplasm, and most of the organelles are located at the chalazal end (Willemse and van Went, 1984). Following fertilization, this polarity is also observed in the zygote (Mansfield and Briarty, 1990; see West and Harada, 1993, this issue). Schulz (1968b) proposed that the asymmetric distribution of organelles and cytoplasm following the first division of the zygote may have consequences for early stages of embryogenesis. Recently, embryonic pattern defects in Arabidopsis have been correlated with abnormal divisions of the zygote (Mayer et al., 1993), suggesting that defects in apical–basal patterning in these embryos may be due to abnormal partitioning of determinants in the zygote.

GENETIC AND MOLECULAR ANALYSIS OF OVULE AND EMBRYO SAC DEVELOPMENT

Mutations affecting development of the ovule and female gametophyte have been identified by screening for plants with reduced fertility. Mutations that affect embryo sac development may be expressed in the parent sporophyte or in the gametophyte itself. Each class can be identified genetically by its mode of inheritance, as described below.

Sporophytic Mutants

Mutations in genes expressed in the parent sporophyte will cause all embryo sacs within an ovary of a homozygous plant to exhibit the defect. In the case of a recessive mutation, 25% of the progeny from selfed heterozygous plants will display the sterile phenotype. Defects in fertility that are specific to the female are distinguished from male steriles by reciprocal crosses with wild-type plants. That is, mutations that abolish or reduce female fertility can be transmitted and recovered through the pollen. Recently, several female-sterile mutations have been identified among populations of Arabidopsis mutagenized with ethylmethane sulfonate (Robinson-Beers et al., 1992; Gasser and Robinson-Beers, 1993, this issue) and T-DNA

(G. W. Haughn and R. L. Fischer, unpublished results). These mutants, which are described below, illustrate the utility of a genetic approach for understanding how the ovule develops and interacts with the developing embryo sac.

Representative female-sterile mutants of Arabidopsis include short integuments-1 (sin1; Robinson-Beers et al., 1992), and as shown in Figure 4, ovule mutant-3 (ovm3), bell-1 (bel1), and ovule mutant-2 (ovm2). Each of these mutants has a defect in a discrete aspect of ovule and embryo sac development, such as initiation of the integuments, development of the nucellus, megasporogenesis, and megagametogenesis. For example, both integuments are absent in ovm3 mutant ovules (compare Figures 4A and 4D). Meiosis is initiated normally in ovm3 ovules; however, all products of meiosis degenerate, and functional megaspores are not formed. Thus, this mutation appears to affect early events in both ovule and embryo sac development. Similarly, meiosis occurs in sin1 ovules, but normal tetrads and embryo sacs are not observed (Robinson-Beers et al., 1992). Unlike ovm3 ovules, both integuments of sin1 ovules are present; however, their morphology is aberrant due to abnormal cell divisions.

Another mutation, bel1, is characterized by the absence of the inner integument and the abnormal development of the outer integument (Robinson-Beers et al., 1992; Modrusan et al., 1993; compare Figures 4B and 4E). Functional megaspores are formed and megagametogenesis is initiated, but a functional embryo sac is not produced. Thus, the bel1 mutation appears to affect early events in ovule development and later aspects of megagametogenesis. Finally, both integuments are present in mature ovm2 ovules; however, the space normally occupied by the embryo sac appears to be filled with nucellar cells (compare Figures 4C and 4F). Functional megaspores and binucleate megagametophytes have been observed in ovm2 mutant ovules. Taken together, these female-sterile mutations of Arabidopsis represent a preliminary genetic dissection of the pathways that regulate ovule and embryo sac development.

In addition to identifying genes controlling ovule and embryo sac morphogenesis, female-sterile mutants of Arabidopsis may eventually help define interactions between the ovule and the embryo sac. Abnormal megasporogenesis in ovm3 and sin1 ovules and aborted megagametogenesis in bel1 and ovm2 ovules show that embryo sac formation is dependent upon normal ovule development. Perhaps these mutations disrupt some interactions between the ovule and developing embryo sac. Another possibility is that defects in mutant megaspores and embryo sacs reflect their derivation from abnormal ovule tissue. As additional ovule mutations are discovered, we will likely gain new information about the development of each of the tissues of the ovule and their effects on embryo sac development.

Other sporophytic mutations that result in sterility have been shown to disrupt meiosis and affect both male and female gametophytes (Kennell and Horner, 1985a; Benavante et al., 1989; Golubovskaya et al., 1992). For example, meiotic mutants in maize (mei) have been shown to affect many aspects
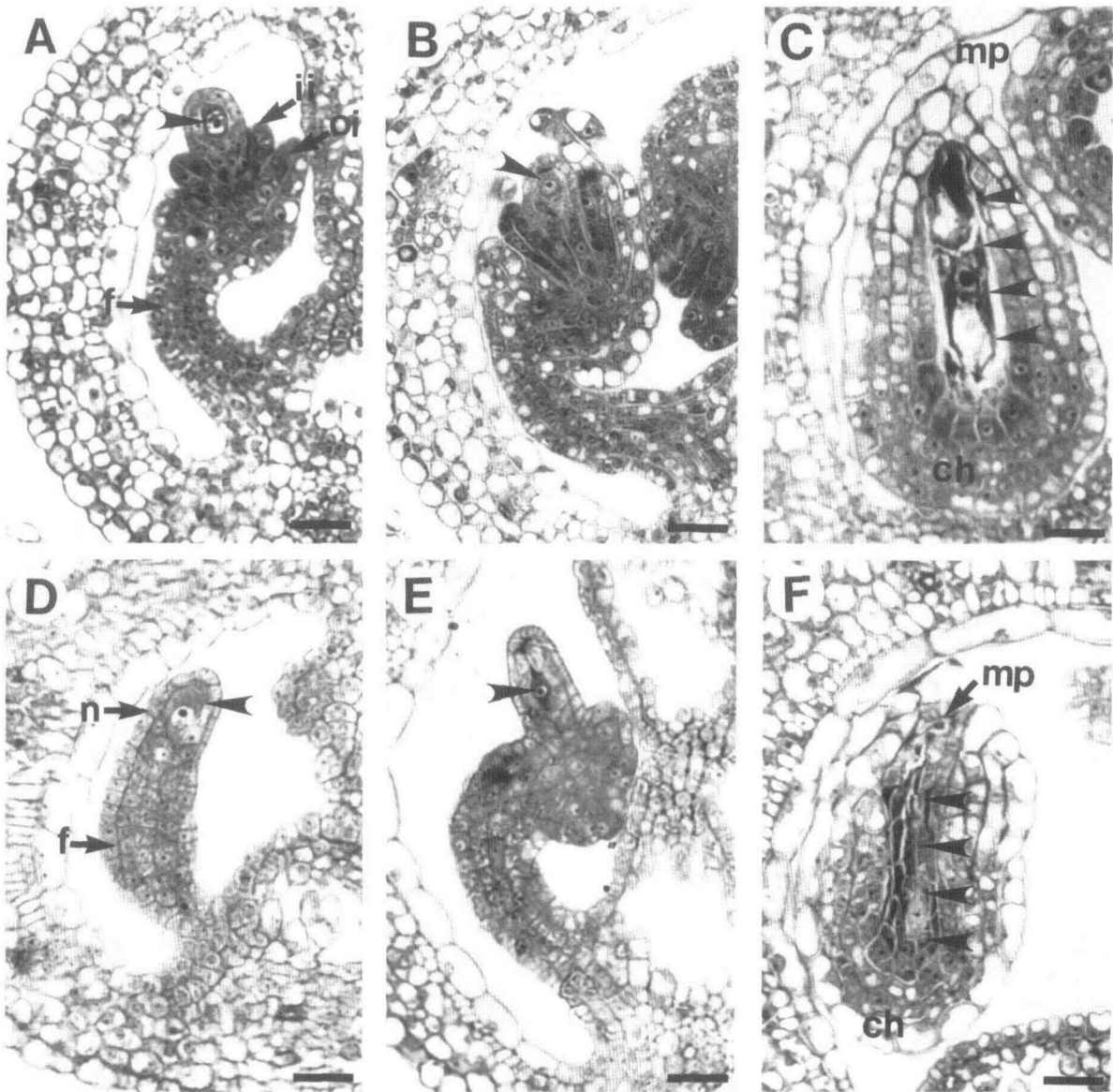
Figure 4. Ovules of Mutant and Wild-Type Arabidopsis.

(A) Wild-type ovule after both inner (ii) and outer (oi) integuments are initiated. The megasporocyte is indicated by the arrowhead. The ovule is connected to the placental wall by the funiculus (f). Bar = 10 μm.

(B) Wild-type ovule showing enlarged functional megaspore (arrowhead). Bar = 10 μm.

(C) Wild-type ovule. Arrowheads are pointing to a portion of the mature embryo sac. For orientation, the micropylar (mp) and chalazal (ch) ends are labeled. Bar = 10 μm.

(D) *ovm3* mutant ovule at same stage as the wild-type ovule in **(A)** showing the megasporocyte (arrowhead) and funiculus. n, nucellus. Integument primordia are absent from the region between the nucellus and the funiculus. Bar = 10 μm.

(E) *bel1* mutant ovule at same stage as the wild-type ovule in **(B)**. The functional megaspore (arrowhead) has not yet expanded. The inner integument is absent and the outer integument is abnormal. Bar = 10 μm.

(F) *ovm2* mutant ovule at same stage as the wild-type ovule in **(C)**. Arrowheads indicate the area normally occupied by the embryo sac. The micropylar and chalazal ends are labeled; the orientation is roughly equivalent to that of the wild-type ovule in **(C)**. Bar = 10 μm.

of meiosis, including initiation of meiosis, control of meiotic divisions, pairing of homologous chromosomes, and mitotic divisions within the gametophytes (Golubovskaya, 1989). Although meiotic mutations disrupt megasporogenesis and, therefore, alter the development of the megagametophyte, ovule morphogenesis is generally not affected.

Defects in meiosis can result in the formation of diploid embryo sacs. For example, the *dyad* mutant of *Datura* blocks meiosis II, creating a dyad of unreduced megaspores (Satina and Blakeslee, 1935). The chalazally oriented cell undergoes normal megagametogenesis, but the resulting embryo sac is diploid. Some triploid embryos were reportedly obtained from crosses of mutant females to wild-type males, suggesting that mutant embryo sacs were capable of producing sexually derived seed. These results, and studies of apomictic embryo sacs, indicate that the haploid state is not required for the development of a functional megagametophyte.

Gametophytic Mutants

Only a few gametophtyic mutations affecting the embryo sac have been described in detail. This class of mutations is determined by the haploid genotype of the embryo sac. The *indeterminate gametophyte* (*ig*) mutation of maize has pleiotropic effects on embryo sac development but primarily affects the number of mitotic divisions (Lin, 1981). The *ig* mutation is associated with the occurrence of multiple functional egg cells and embyros within an embryo sac and with the formation of defective kernels. Concordance of endosperm and embryo genotypes indicates that mutant embryo sacs are the products of a single megaspore (Kermicle, 1971).

Another mutation with gametophytic expression, *Gf*, was described in Arabidopsis (Rédei, 1964). This mutation shows reduced transmission through the female gametophyte of genes linked to *Gf*. Rédei (1964) observed multiple archesporial cells and their derivative megaspores as well as twin embryo sacs in sectioned ovules. He suggested that abnormal segregation of markers linked to *Gf* indicated that mutant megaspores are less viable than wild-type megaspores. Alternatively, the *Gf* mutation may be linked to a chromosomal rearrangement at that locus (A. Ray, personal communication).

The low frequency with which female gametophyte mutations have been described to date most likely reflects the technical complexity of identifying the mutations rather than the actual number of genes involved. In maize, heterozygous plants that contain deletions frequently display a 50% reduction in pollen viability (Patterson, 1978). Most of these deletions also do not transmit through the female gametophyte (Coe et al., 1988). This suggests that a similar number of genes may be required for pollen and embryo sac development. Mutations that affect development of the gametophytes can be propagated in maize if the mutation is complemented in the pollen by an independently segregating segmental translocation (Birchler and Levin, 1991; E. Vollbrecht, personal

communication). Gametophytic mutations can then be uncovered in either the pollen or the embryo sac in order to assess their effects on gametophyte development.

Genetic analysis of ovule and embryo sac mutants promises to offer new insight into reproductive processes in angiosperms. Construction and analysis of double and triple mutant combinations among these mutants can be used to define genetic interactions and pathways of ovule and embryo sac development.

Molecular Analysis

Combined molecular and genetic approaches have been successful in addressing a number of problems in plant development. A variety of methods have been described for other systems that can also be used to clone genes defined by mutations that affect ovule and gametophyte development (Herman and Marks, 1989; Coen et al., 1990; Strauss and Ausubel, 1990; Arondel et al., 1992). Examination of DNA sequences, predicted protein structures, and temporal and spatial patterns of gene expression, along with the genetic and developmental analyses of mutants, will help define the function of these genes during ovule and embryo sac development. In situ localization of mRNAs and proteins will be particularly helpful in distinguishing classes of genes expressed in sporophyte, gametophyte, or both.

Molecular analysis of embryo sac–specific gene expression has been slow to develop, in part due to the intractability of the tissue. Acquisition of sufficient material for nucleic acid or protein purification is extremely labor intensive given the size of the megagametophyte and its association with the ovule. New polymerase chain reaction (PCR)–based methods obviate the need for large amounts of tissue by selective amplification of small amounts of mRNA, so that isolated embryo sacs and even egg cells (Wagner et al., 1988; Huang and Russell, 1989) could be utilized as a source of material for cDNA libraries. In the future, genes expressed specifically in ovules and embryo sacs may be identified through differential or subtractive hybridization screens (Stinson et al., 1978; Sommer et al., 1990) or by PCR amplification and differential displays of mRNA profiles, as described by Liang and Pardee (1992). In addition to providing information about the temporal and spatial patterns of gene expression in embryo sacs and ovules, cloned genes can be used as cell or tissue-specific markers.

Several mRNAs and proteins known to be expressed in ovules can be used as markers for ovule development and may be useful in interpreting mutant phenotypes. For example, expression of a pistil-specific tomato mRNA, pMON9608, was localized by in situ hybridization to the integument (Gasser et al., 1989). mRNAs for *AGAMOUS* (*AG*) and *APETALA3*, two Arabidopsis floral homeotic genes encoding putative transcription factors, are found in the endothelium and integuments, respectively (Bowman et al., 1991; Jack et al., 1992). Similarly, the *AG*-like genes *AGL1* and *AGL2* are expressed in the ovules

of Arabidopsis (Ma et al., 1991). In addition, certain arabino-galactan protein epitopes show differential patterns of immunolocalization in ovules and megagametophytes (Pennell and Roberts, 1990; Pennell et al., 1991). No specific roles for any of these proteins during ovule or embryo sac development have been shown.

FUTURE PROSPECTS

An integrated approach to the study of the angiosperm female gametophyte offers new insight into mechanisms of sexual reproduction in higher plants. Genetic, molecular, and physiological approaches are being utilized to address questions raised by morphological studies. These strategies promise to yield new information regarding regulation of ovule and embryo sac development, interactions between the ovule and embryo sac, and the biology of the female gametophyte.

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REFERENCES

Arondel, V., Lemieux, B., Hwang, I., Gibson, S., Goodman, H., and Somerville, C. (1992). Map-based cloning of a gene controlling omega-3 fatty acid desaturation in Arabidopsis. Science **258**, 1353–1355.

Benavante, R.S., Skorupska, H., Palmer, R.G., and Shoemaker, R. (1989). Embryo sac development in the cv. ks male sterile, female sterile line of soybean (Glycine max). Am. J. Bot. **76**, 1756–1768.

Bhandari, N.N. (1984). The microsporangium. In Embryology of Angiosperms, B.M. Johri, ed (New York: Springer-Verlag), pp. 53–122.

Birchler, J.A., and Levin, D.M. (1991). Directed synthesis of a segmental chromosome transposition: An approach to the study of chromosomes lethal to the gametophyte generation of maize. Genetics **127**, 609–618.

Bouman, F. (1984). The ovule. In Embryology of Angiosperms, B.M. Johri, ed (Berlin: Springer-Verlag), pp. 123–158.

Bowman, J.L., Drews, G.N., and Meyerowitz, E.M. (1991). Expression of the Arabidopsis floral homeotic gene AGAMOUS is restricted to specific cell types late in flower development. Plant Cell **3**, 749–758.

Cass, D.D., Peteya, D.J., and Robertson, B.L. (1985). Megagametophyte development in Hordeum vulgare. 1. Early megagametogenesis and the nature of cell wall formation. Can. J. Bot. **63**, 2164–2171.

Cass, D.D., Peteya, D.J., and Robertson, B.L. (1986). Megagametophyte development in Hordeum vulgare. 2. Later stages of wall

development and morphological aspects of megagametophyte cell differentiation. Can. J. Bot. **64**, 2327–2336.

Coe, E.H., Jr., Neuffer, M.G., and Hosington, D.A. (1988). The genetics of corn. In Corn and Corn Improvement, 3rd ed., G.E. Sprague and J.W. Dudley, eds (Madison, WI: American Society of Agronomy), pp. 81–258.

Coen, E., Romero, J., Doyle, S., Elliot, R., Murphey, G., and Carpenter, R. (1990). floricaula: A homeotic gene required for flower development in Antirrhinum majus. Cell **63**, 1311–1322.

Cornish, E., Pettitt, J., and Clarke, A. (1988). Self-incompatibility genes in flowering plants. In Temporal and Spatial Regulation of Plant Genes, D.P.S. Verma and R.B. Goldberg, eds (New York: Springer-Verlag), pp. 117–130.

Cove, D.J., and Knight, C.D. (1993). The moss Physcomitrella patens, a model system with potential for the study of plant reproduction. Plant Cell **5**, 1483–1488.

Diboll, A.G. (1968). Fine structural development of the megagametophyte of Zea mays following fertilization. Am. J. Bot. **55**, 787–806.

Dumas, C., and Mogensen, H.L. (1993). Gametes and fertilization: Maize as a model system for experimental embryogenesis in flowering plants. Plant Cell **5**, 1337–1348.

Esau, K. (1977). Anatomy of Seed Plants (New York: John Wiley and Sons).

Gasser, C.S., and Robinson-Beers, K. (1993). Pistil development. Plant Cell **5**, 1231–1239.

Gasser, C.S., Budelier, K.A., Smith, A.G., Shah, D.M., and Fraley, R.T. (1989). Isolation of tissue-specific cDNAs from tomato pistils. Plant Cell **1**, 15–24.

Goldberg, R.B., Beals, T.P., and Sanders, P.M. (1993). Anther development: Basic principles and practical applications. Plant Cell **5**, 1217–1229.

Golubovskaya, I.N. (1989). Meiosis in maize: mei genes and conception of genetic control of meiosis. Adv. Genet. **26**, 149–193.

Golubovskaya, I.N., Avaklinka, N., and Sheridan, W. (1992). Effects of several meiotic mutants on female meiosis in maize. Dev. Genet. **13**, 411–424.

Haig, D. (1990). New perspectives on the angiosperm female gametophyte. Bot. Rev. **56**, 236–274.

Herman, P.L., and Marks, M.D. (1989). Trichome development in Arabidopsis thaliana. II. Isolation and complementation of the GLABROUS1 gene. Plant Cell **1**, 1051–1055.

Hill, D., and Strome, S. (1990). Brief cytocyhalasin-induced disruption of microfilaments during a critical interval in 1-cell C. elegans embryos alters the partitioning of developmental instructions to the 2-cell embryo. Development **108**, 159–172.

Huang, B.-Q., and Russell, S.D. (1989). Isolation of fixed and viable eggs, central cells and embryo sacs from ovules of Plumbago zeylanica. Plant Physiol. **90**, 9–12.

Huang, B.-Q., Russell, S.D., Strout, G.W., and Mao, L.-J. (1990). Organization of isolated embryo sacs and eggs of Plumbago zeylanica (Plumbaginaceae) before and after fertilization. Am. J. Bot. **77**, 1401–1410.

Jack, T., Brockman, L., and Meyerowitz, E. (1992). The homeotic gene APETALA3 of Arabidopsis thaliana encodes a MADS box and is expressed in petals and stamens. Cell **68**, 683–697.

Jensen, W.A. (1965a). The ultrastructure and histochemistry of the synergids of cotton. Am. J. Bot. **52**, 238–256.

Jensen, W.A. (1965b). The composition and ultrastructure of the nucellus in cotton. J. Ultrastruct. Res. **13**, 112–128.

Kaplan, D.R. (1969). Sporogenesis and gametogenesis in Downingia (Campanulaceae; Lobeliodeae). Bull. Torrey Bot. Club **96**, 418–434.

Kennell, J.C., and Horner, H.T. (1985a). Influence of the soybean male-sterile gene (ms1) on the development of the female gametophyte. Can. J. Genet. Cytol. **27**, 200–209.

Kennell, J.C., and Horner, H.T. (1985b). Megasporogenesis and megagametogenesis in soybean, Glycine max. Am. J. Bot. **72**, 1553-1564.

Kermicle, J.L. (1971). Pleiotropic effects on seed development of the indeterminate gametophyte gene in maize. Am. J. Bot. **58**, 1–7.

Koltunow, A.M. (1993). Apomixis: Embryo sacs and embryos formed without meiosis or fertilization in ovules. Plant Cell **5**, 1425–1437.

Liang, P., and Pardee, A. (1992). Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science **257**, 967–971.

Lin, B.-Y. (1981). Megagametogenetic alterations associated with the indeterminate gametophyte (ig) mutation in maize. Rev. Brasil. Biol. **41**, 557–563.

Lintilhac, P. (1974). Differentiation, organogenesis and the tectonics of cell wall orientation. II. Separation of stresses in a two-dimensional model. Am. J. Bot. **61**, 135–140.

Lopes, M.A., and Larkins, B.A. (1993). Endosperm origin, development, and function. Plant Cell **5**, 1383–1399.

Ma, H., Yanofsky, M.F., and Meyerowitz, E.M. (1991). AGL1-AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes. Genes Dev. **5**, 484–495.

Maheshwari, P. (1950). An Introduction to the Embryology of Angiosperms (New York: McGraw-Hill).

Mansfield, S., and Briarty, S. (1990). Early embryogenesis in Arabidopsis thaliana. II. The developing embryo. Can. J. Bot. **69**, 461–476.

Mansfield, S.G., Briarty, L.G., and Erni, S. (1990). Early embryogenesis in Arabidopsis thaliana. I. The mature embryo sac. Can. J. Bot. **69**, 447–460.

Mascarenhas, J.P. (1990). Gene activity during pollen development. Annu. Rev. Plant Physiol. Plant Mol. Biol. **41**, 317–338.

Mascarenhas, J.P. (1993). Molecular mechanisms of pollen tube growth and differentiation. Plant Cell **5**, 1303–1314.

Mayer, U., Buttner, G., and Jürgens, G. (1993). Apical-basal pattern formation in the Arabidopsis embryo: Studies on the role of the gnom gene. Development **117**, 149–162.

Modrusan, Z., Reiser, L., Fischer, R.L., and Haughn, G.W. (1993). Bel1 mutants are defective in ovule development. In Arabidopsis: An Atlas of Morphology and Development, J. Bowman, ed (New York: Springer-Verlag), in press.

Mogensen, H.L. (1973). Some histochemical, ultrastructural and nutritional aspects of the ovule of Quercus gambelii. Am. J. Bot. **60**, 48–54.

Nasrallah, J.B., and Nasrallah, M.E. (1989). The molecular genetics of self-incompatability in Brassica. Annu. Rev. Genet. **23**, 121–139.

Nasrallah, J.B., and Nasrallah, M.E. (1993). Pollen–stigma signaling in the sporophytic self-incompatibility response. Plant Cell **5**, 1325–1335.

Newbigin, E., Anderson, M.A., and Clarke, A.E. (1993). Gametophytic self-incompatibility systems. Plant Cell **5**, 1315–1324.

Nogler, G.A. (1984). Gametophytic apomixis. In Embryology of Angiosperms, B.M. Johri, ed (Berlin: Springer-Verlag), pp. 475–518.

Patterson, E. (1978). Properties and uses of duplicate deficient chromosome compliments in maize. In Maize Breeding and Genetics, D. Walden, ed (New York: John Wiley and Sons), pp. 693–710.

Pennell, R.I., and Roberts, K. (1990). Sexual development in the pea is presaged by altered expression of an arabinogalactan protein. Nature **344**, 547–549.

Pennell, R.I., Janniche, L., Kjellbom, P., Scofield, G.N., Peart, J.M., and Roberts, K. (1991). Developmental regulation of a plasma membrane arabinogalactan protein epitope in oilseed rape flowers. Plant Cell **3**, 1317–1326.

Pokrywka, N., and Stephenson, E. (1991). Microtubules mediate the localization of bicoid RNA during Drosophila oogenesis. Development **113**, 55–66.

Rédei, G. (1964). Non-mendelian megagametogenesis in Arabidopsis. Genetics **51**, 857–872.

Robinson-Beers, K., Pruitt, R.E., and Gasser, C.S. (1992). Ovule development in wild-type Arabidopsis and two female-sterile mutants. Plant Cell **4**, 1237–1249.

Rodkiewicz, B. (1970). Callose in cell walls during megasporogenesis in angiosperms. Planta **93**, 39–47.

Russell, S.D. (1978). Fine structure of megagametophyte development in Zea mays. Can. J. Bot. **57**, 1093–1110.

Russell, S.D. (1993). The egg cell: Development and role in fertilization and early embryogenesis. Plant Cell **5**, 1349–1359.

Satina, S. (1945). Periclinal chimeras in Datura in relation to the development and structure of the ovule. Am. J. Bot. **32**, 72–81.

Satina, S., and Blakeslee, A.F. (1935). Cytological effects of a gene in Datura which causes dyad formation in sporogenesis. Bot. Gaz. **96**, 521–532.

Schulz, P., and Jensen, W.A. (1971). Capsella embryogenesis: The chalazal proliferating tissue. J. Cell. Sci. **8**, 201–227.

Schulz, R., and Jensen, W.A. (1968a). Capsella embryogenesis: The synergids before and after fertilization. Am. J. Bot. **55**, 541–552.

Schulz, R., and Jensen, W.A. (1968b). Capsella embryogenesis: The egg, zygote and young embryo. Am. J. Bot. **55**, 807–819.

Sommer, H., Beltrán, J.-P., Huijser, P., Pape, H., Lönnig, W.-E., Saedler, H., and Schwarz-Sommer, Z. (1990). Deficiens, a homeotic gene involved in the control of flower morphogenesis in Antirrhinum majus: The protein shows homology to transcription factors. EMBO J. **9**, 605–613.

Stebbins, G.L. (1974). Flowering Plants: Evolution Above the Species Level (Cambridge, MA: Harvard University Press).

Stinson, J., Eisenberg, A., Willing, R., Pe, M., Hanson, D., and Mascarenhas, J. (1978). Genes expressed in the male gametophyte of flowering plants. Plant Physiol. **83**, 442–447.

Strauss, D., and Ausubel, F. (1990). Genomic subtraction for cloning DNA corresponding to deletion mutations. Proc. Natl. Acad. Sci. USA **87**, 1889–1893.

Sumner, M.J., and Van Caeseele, L. (1988). Ovule development in Brassica campestris. A light microscope study. Can. J. Bot. **66**, 2459–2469.

Sumner, M.J., and Van Caeseele, L. (1989). The ultrastructure and cytochemistry of the egg apparatus of Brassica campestris. Can. J. Bot. **67**, 177–190.

Sumner, M.J., and Van Caeseele, L. (1990). The development of the central cell of Brassica campestris prior to fertilization. Can. J. Bot. **68**, 2553–2563.

Takhtadzhian, A. (1991). Evolutionary Trends in Flowering Plants (New York: Columbia University Press).

Wagner, V.T., Song, Y., Matthys-Rochon, E., and Dumas, C. (1988). The isolated embryo sac of *Zea mays*: Structural and ultrastructural observations. In Sexual Reproduction in Higher Plants, M. Cresti, P. Gori, and E. Pacini, eds (Berlin: Springer-Verlag), pp. 125–130.

Webb, M.C., and Gunning, B.E.S. (1990). Embryo sac development in *Arabidopsis thaliana*: Megasporogenesis, including the microtubular cytoskeleton. Sex. Plant Reprod. **3**, 244–256.

West, M.A.L., and Harada, J.J. (1993). Embryogenesis in higher plants: An overview. Plant Cell **5**, 1361–1369.

Willemse, M.T.M. (1981). Polarity during megasporogenesis and megagametogenesis. Phytomorphology **31**, 124–134.

Willemse, M.T.M., and Bednara, J. (1979). Polarity during megasporogenesis in Gasteria verrucosa. Phytomorphology **29**, 156–165.

Willemse, M.T.M., and van Went, J.L. (1984). The female gametophyte. In Embryology of Angiosperms, B.M. Johri, ed (Berlin: Springer-Verlag), pp. 159–196.

Wilms, H.J. (1980). Development and composition of the spinach ovule. Acta Bot. Neer. **29**, 243–260.

Yisraeli, J., Sokol, S., and Melton, D. (1990). A two step model for the localization of maternal mRNA in *Xenopus* oocytes: Involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. Development **108**, 289–298.