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Apomixis: Embryo Sacs and Embryos Formed without Meiosis or Fertilization in Ovules

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INTRODUCTION

The term apomixis has, in the past, been used as a general term for any form of asexual reproduction in plants, including vegetative propagation. This original definition has become more restricted and now covers only those asexual reproductive processes that, paradoxically, occur in the ovule of flowering plants—the structure that has evolved to carry out female sexual reproductive functions in angiosperms (Nogler, 1984; Asker and Jerling, 1992).

Apomictic processes mimic many of the events of sexual reproduction and give rise to fertile seeds. An important difference is that the apomictic embryo is derived solely from cells in the maternal ovule tissues rather than from the fusion of male and female gametes. The fertile seeds that result from apomictic reproduction contain embryos that have, barring mutation, a genetic constitution identical to that of the female parent. At least three developmental differences also serve to distinguish apomictic embryo formation from somatic embryogenesis. First, apomictic embryo formation occurs within a differentiated structure. Second, apomictic embryos form directly from a cell located in, or close to, a gametophytic structure, without entering an intervening callus phase, which is often necessary for somatic embryogenesis (Nomura and Komamine, 1985; see also Zimmerman, 1993, this issue). Third, the pattern of embryo formation in apomictic species is often indistinguishable from that which occurs in the nearest sexual relative (Nogler, 1984), which is not always the case for somatic embryogenesis.

Apomictic processes have been observed in at least 300 plant species spanning 35 different families and are most common in the Gramineae, Compositae, Rosaceae, and Rutaceae (Richards, 1986; Hanna and Bashaw, 1987). With the exception of apple and *Citrus*, apomixis is not very common in agriculturally important crops. Because apomictically produced embryos are genetically identical to the female parent plant, they are of obvious benefit to agriculture. If apomixis can be introduced into agricultural crops, it can be an inexpensive way to perpetuate a given genotype, preserving even such characters as heterosis through successive generations via seed (Hanna and Bashaw, 1987). Apomictic plants could potentially provide a constant source of renewable seed capable of producing high-yielding food crops, an agricultural trait of great value, particularly for developing countries. Programs aimed at using conventional breeding methods to transfer apomixis to most agricultural crops have been largely unsuccessful, although pearl millet has been an exception (Dujardin and Hanna, 1989). The continuation of such organized breeding programs is important, and a greater understanding of the processes controlling apomixis at the molecular level should facilitate the transfer of apomixis into crops of economic importance.

In this review, the different forms of apomixis that occur in ovules will be discussed and compared at a developmental level with sexual reproductive processes. Current mechanistic and genetic knowledge about apomictic processes will be presented. An understanding of apomixis at the molecular level should result in the characterization of genes that control female gametophyte formation and the early events of embryo development in both apomictic and sexually reproducing plants.

THREE DISTINCT APOMICTIC PROCESSES ARE INITIATED AT DIFFERENT TIMES DURING OVULE DEVELOPMENT

In sexual reproduction, the ovule has a multifunctional role because it houses the sequential processes of female gametogenesis, fertilization, and embryo development, as shown in Figure 1. Apomictic processes overlap with all of these events; therefore, a prerequisite for understanding apomixis is a clear understanding of the sexual reproductive events that occur in the ovule. The ovule-specific developmental events of sexual reproduction presented in Figure 1 have been treated in detail in several of the articles in this issue (Reiser and Fischer, 1993; Russell, 1993).

A defined sequence of events must be completed to result in the generation of a fertile and genetically unique seed that is the end product of sexual reproduction in angiosperms. This sequence comprises the following events: megaspore mother cell differentiation from the nucellus (Figure 1D), megaspore production by meiosis (megasporogenesis), megaspore selection, embryo sac development by mitotic processes (megagametogenesis) (Figures 1F and 1G), embryo sac

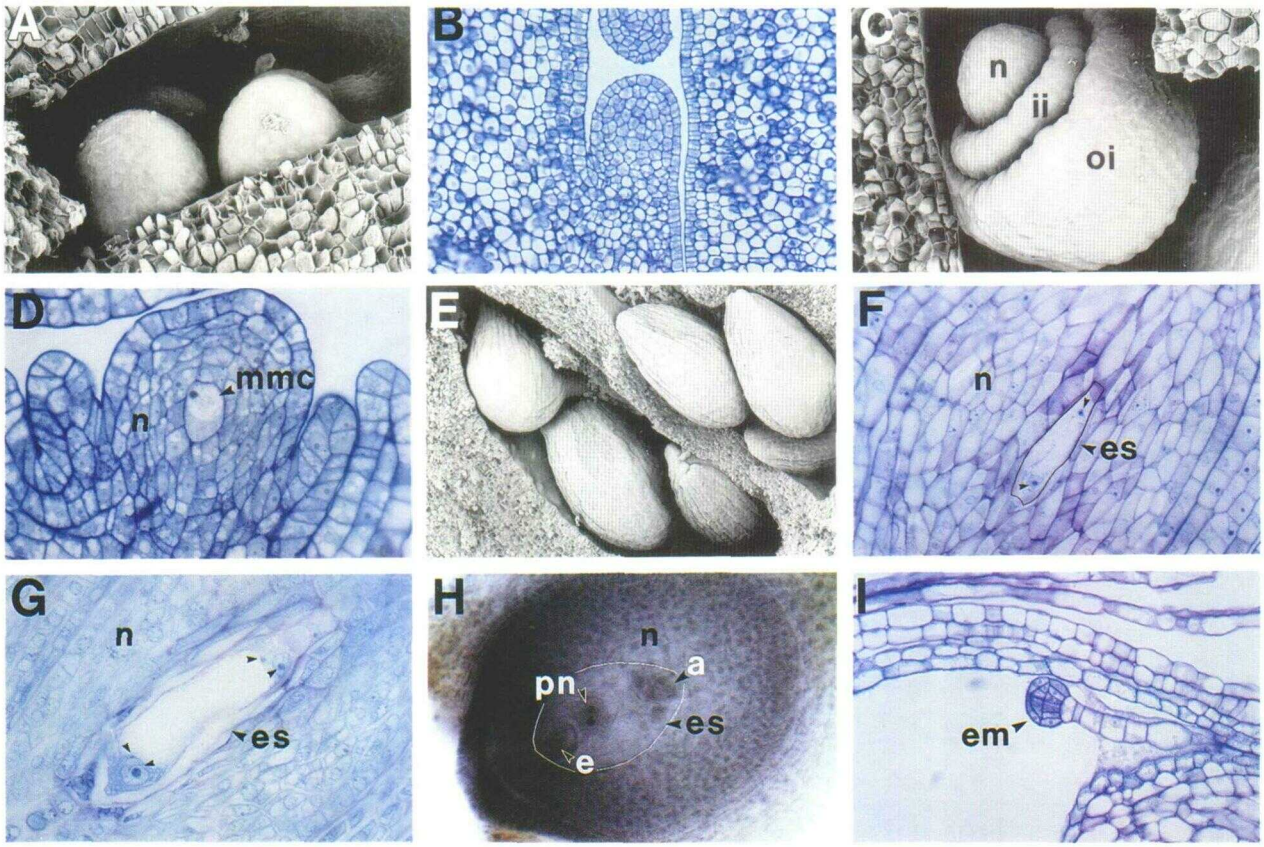

Figure 1. Ovule-Specific Sexual Reproductive Events Spanning Ovule Initiation to Embryogenesis.

(A) Scanning electron micrograph of *Citrus* ovule primordia.
(B) Section through *Citrus* ovule primordium shown in **(A)**.
(C) Scanning electron micrograph of developing *Citrus* ovule showing development of nucellus (n) and outer (oi) and inner (ii) integuments.
(D) Section through ovule shown in **(C)** showing megaspore mother cell (mmc).
(E) Scanning electron micrograph of *Citrus* ovules at the completion of integumentary development.
(F) and **(G)** Mitotic events of *Citrus* megagametophyte formation showing the embryo sac (es) at the two-nucleus stage **(F)** and the four-nucleus stage **(G)**. Arrowheads indicate the nuclei. In **(F)**, the embryo sac has been outlined in black.
(H) A mature ovule of *Paspalum dilatatum* cleared by the method of Stelly et al. (1984) showing the embryo sac (es), outlined in white, containing an egg cell (e), polar nuclei (pn), and proliferating antipodals (a).
(I) Globular embryo (em) of Arabidopsis at the 32-cell embryo proper stage.

maturation (Figure 1H), double fertilization, and endosperm and embryo formation (Figure 1I). In sexually reproducing plants, the absence or disruption of any one of these steps usually results in a cessation of the developmental program, and a viable seed is not produced.

By contrast with sexual reproduction, apomictic processes can completely omit some of the events in this sequence and still produce a fully formed, viable embryo within the confines of the ovule. Cytological studies have revealed that apomictic processes always deviate from sexual reproduction in more than one respect (Asker, 1979, 1980; Nogler, 1984; Asker and Jerling, 1992). These developmental differences occur in commonly identifiable combinations, such that apomictic processes have often been divided into three mechanisms, termed diplospory, apospory, and adventitious embryony. These mechanisms are summarized in Figure 2, where they are compared directly to the corresponding events of sexual reproduction in the Polygonum-type embryo sac, the most common embryo sac structure found in angiosperms (Willemse and van Went, 1984).

Each of the apomictic mechanisms differs in the time at which it is initiated during ovule development relative to the normal sexual pathway. Diplospory and apospory are initiated early in ovule development, at the time of megaspore mother

cell differentiation in the case of diplospory and after megaspore mother cell differentiation in apospory (Figure 2). Diplospory and apospory result in the formation of a megagametophytic structure without meiotic reduction, and the embryo develops from a cell inside this unreduced megagametophyte. Diplospory and apospory are, therefore, commonly referred to as gametophytic apomictic processes (Nogler, 1984; Asker and Jerling, 1992) (Figure 2). By contrast, adventitious embryony is initiated late in ovule development and usually occurs in mature ovules. Embryos are initiated directly from individual cells in ovule tissues that are external to a sexually derived megagametophyte (Figure 2). Therefore, adventitious embryony has been described as sporophytic apomixis (Nogler, 1984; Asker and Jerling, 1992). Most plants with gametophytic apomixis are polyploid; however, genera with adventitious embryony are commonly diploid (Asker and Jerling, 1992).

IN DIPLOSPORY, THE MEGASPORE MOTHER CELL SWITCHES FROM A SEXUAL TO AN APOMICTIC PATHWAY TO PRODUCE AN UNREDUCED EMBRYO SAC

The two types of diplospory, meiotic and mitotic, are shown in Figure 2. In meiotic diplospory, a megaspore mother cell differentiates from the nucellus and begins meiosis, but meiosis is inhibited at a particular stage by unknown mechanisms and the nucleus is restored to a form that enables mitosis to occur (Figure 2, *Taraxacum* and *Ixeris*). In mitotic diplospory, which is the more common form of diplospory, the megaspore mother cell appears to be inhibited from entering meiosis (Nogler, 1984) (Figure 2, *Antennaria*). It is possible that in some plants exhibiting mitotic diplospory, entry to meiosis does occur but is then inhibited at a very early stage. This would be evident only at the molecular level and not by cytological

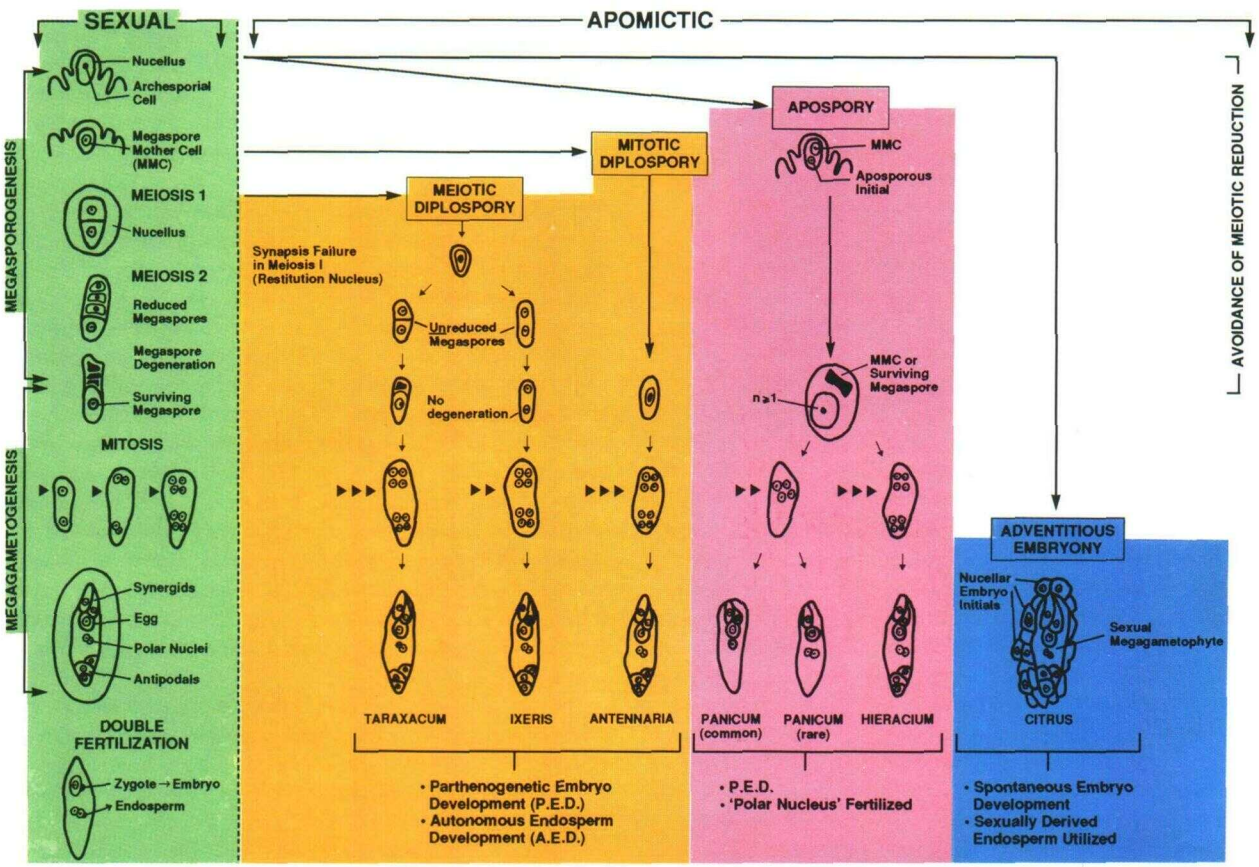

Figure 2. The Three Apomictic Mechanisms—Diplospory, Apospory, and Adventitious Embryony—Compared with the Events of Sexual Reproduction.

The information for the events of apospory and diplospory shown in this scheme was primarily obtained from Nogler (1984). Pathways common to a particular genus are shown. The rigidity of the four-nucleate embryo sac structure depicted for *Panicum* has been criticized recently by Bashaw and Hanna (1990). *Hieracium* species usually develop endosperm autonomously (Nogler, 1984).

examination. Whether it results from aberrant meiosis or from the failure of the megaspore mother cell to even enter meiosis, the unreduced cell continues development and appears to be developmentally equivalent to a megaspore: it undergoes mitosis to produce an unreduced embryo sac containing the same number of cells in the same arrangement as an embryo sac of the nearest sexual relative. Thus, in both forms of diplospory, the lack of meiosis does not impair formation of an embryo sac.

In unreduced diplosporous embryo sacs, some of the cells appear to function in a manner identical to the cells of reduced embryo sacs. For example, one cell is specified to perform a function similar to that of the egg cell of the sexual embryo sac. The apomictic embryo is, however, formed without fertilization (i.e., parthenogenetically) by unknown mechanisms in diplosporous species. Autonomous endosperm production is also common among diplosporous apomicts, particularly in the Compositae (Figure 2), and in the few apomicts in which autonomous endosperm formation has been studied, endosperm is derived from the unreduced polar nuclei (Nogler, 1984). The mechanisms that initiate autonomous endosperm formation are not understood; however, autonomously produced endosperm can have variable ploidy in diplosporous apomicts, suggesting that fusion of the polar nuclei is not a prerequisite for the initiation of mitotic activity in the endosperm (Nogler, 1984; Richards, 1986; Bashaw and Hanna, 1990; Asker and Jerling, 1992). In the few apomictic grass genuses displaying diplospory, such as *Elymus, Poa, Eragrostis*, and *Tripsacum*, pollination is necessary for endosperm production (Bashaw and Hanna, 1990). Pseudogamy is the term used to describe the selective fusion of an unreduced "polar nucleus" with a sperm nucleus from the male gametophyte in order to initiate endosperm production for fertile seed set. What is the mechanism that prevents fertilization of the unreduced egg cell in these diplosporous grasses? In both *Poa* and *Tripsacum*, it has been shown that embryo development begins before anthesis (Asker and Jerling, 1992). Mechanisms that prevent unreduced egg cell fertilization in the other diplosporous grasses need to be determined.

It is not known when the megaspore mother cell is directed onto the apomictic pathway in diplosporous plant species. If the cell that is specified to differentiate from the nucellus is programmed to undergo the apomictic route of development as it is differentiating, or even soon after it has differentiated, then the inhibition of meiosis would be an integral part of the apomictic pathway. This may be the case for those diplosporous apomicts that retain normal meiotic processes for pollen production. Some apomicts may result from general defects in meiosis; in these cases, apomictic development may be initiated by the inhibition of meiosis in the megaspore mother cell. If there is a consistent defect in meiosis, such plants would also be male sterile, and the apomictic pathway would, therefore, offer an escape from lethality because it provides a mechanism for perpetuation of the current genotype.

In diplosporous apomixis, the male gametophyte makes no genetic contribution to the embryo or, often, the endosperm. Meiotic reduction and fertilization can be absent in diplosporous apomicts, yet all other processes proceed in sequence, with no obvious disturbance to either the structural pattern of the embryo sac or the pattern of embryo development. Clearly, some of the rigid, sequential events of sexual reproduction have been uncoupled from the remaining sequence of events. Diplosporous plants must, therefore, possess alternative signals or triggers that allow both the mitotic events of embryo sac formation to proceed in the absence of meiosis and embryo and endosperm development to proceed in the absence of fertilization.

IN APOSPORY, NUMEROUS CELLS DIFFERENTIATE FROM THE NUCELLUS AND CAN FORM UNREDUCED MEGAGAMETOPHYTES

In contrast to diplosporous processes, in which the megaspore mother cell appears to be the direct progenitor of the unreduced embryo sac, aposporous embryo sacs form from additional cells that differentiate from the nucellus following megaspore mother cell differentiation (Figure 2). These cells, called aposporous initials, are shown in Figure 3A. Aposporous initials, like differentiating sexual megaspore mother cells, possess large nuclei and dense cytoplasm.

As in diplospory, aposporous initials give rise to unreduced embryo sacs by mitotic processes (Figure 3B). However, sexual and aposporous processes can coexist in one ovule, which, by definition, is not possible for diplosporous apomicts. Cytological comparisons of aposporous species have shown that one or more aposporous initial cells can differentiate from the nucellus in close proximity to cells involved in sexual reproduction at any stage of megasporogenesis or megagametogenesis and begin aposporous embryo sac formation. Aposporous embryo sacs appear to develop faster than sexual embryo sacs because they are not delayed by meiotic division. The percentage of ovules containing both sexual and aposporous embryo sacs is high in some apomictic species; however, the development of the sexual embryo sac is often terminated in many aposporous apomicts at the megaspore mother cell or megaspore stage, as shown in Figure 2, and the products of the sexual process degenerate (Nogler, 1984; Asker and Jerling, 1992).

The timing of initiation of apospory is often an indicator of whether sexual and aposporous processes will coexist in an individual ovule. In *Potentilla*, for example, it has been observed that the earlier the aposporous initials appear, the more likely that the sexual process will be inhibited. It is not clear whether the differentiation of the aposporous initials is the direct cause of the termination of sexual megagametophyte development at this early stage. If the aposporous initials differentiate late, when the formation of the sexual megagametophyte is relatively advanced, both types of embryo sac may coexist (Nogler, 1984).

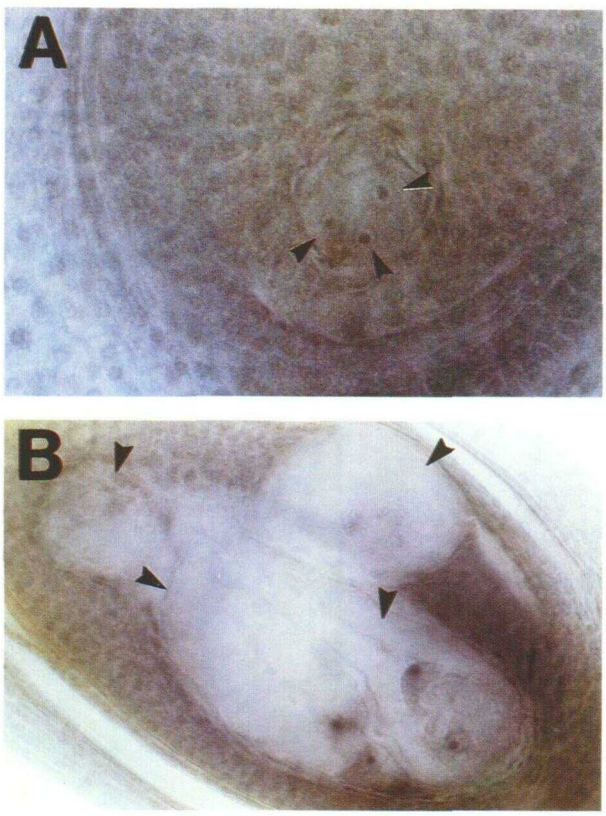

Figure 3. Aposporous Processes in Grasses.

(A) Three aposporous initial cells indicated by arrowheads in a cleared ovule of *Paspalum dilatatum*.

(B) The arrowheads indicate four aposporous embryo sacs in different planes of focus in a cleared ovule of *Bothriochloa ischeamum*. Ovules have been cleared using the method of Stelly et al. (1984).

The number of aposporous embryo sacs formed in an ovule depends on the species (Nogler, 1984; Richards, 1986; Bashaw and Hanna, 1990). If more than one forms, their orientation is random relative to the micropylar–chalazal polarity, which is strictly conserved in the sexual megagametophyte (Willemse and van Went, 1984). Aposporous embryo sacs often resemble those of the nearest sexual relative in nucleus number and arrangement (Figure 2, *Hieracium*). The Gramineae, e.g., *Panicum*, are exceptional in that their aposporous embryo sacs can contain four or even as few as two nuclei (Bashaw and Hanna, 1990) (Figure 3B), unlike the Polygonum-type embryo sac structure with proliferating antipodals that is common in sexually reproducing grasses (Figure 1H). Whatever the ultimate structure of the aposporous embryo sac, however, a single cell of the embryo sac is always specified to initiate parthenogenetic embryo development. It is not yet known whether aposporous embryo development occurs by the same mechanisms as parthenogenetic embryo formation in diplosporous apomicts.

With the exception of *Hieracium* species, autonomous production of endosperm in aposporous apomicts is rare; endosperm formation generally requires pollination (Asker, 1979, 1980; Nogler, 1984; Richards, 1986; Bashaw and Hanna, 1990; Asker and Jerling, 1992). If numerous embryo sacs exist in an ovule, the embryo sac in the most favorable position, i.e., closest to the micropylar end of the ovule, is usually the one that the pollen tube enters. If that embryo sac is sexual, double fertilization occurs. In aposporous embryo sacs, however, pseudogamy takes place and a sperm cell from the male gametophyte fuses selectively with an unreduced polar nucleus. Occasionally, an unreduced egg cell is fertilized, resulting in an alteration in the ploidy of the resulting zygote, which may or may not be viable (Asker and Jerling, 1992). Fertilization of the unreduced egg cell occurs rarely, however. In some species, this is because parthenogenetic embryogenesis has already been initiated at the time pseudogamy takes place (Nogler, 1984); alternatively, the unreduced cell may synthesize a wall that acts as a physical barrier to fertilization (Savidan, 1989; Asker and Jerling, 1992). In most aposporous species, the mechanisms that prevent fertilization of the aposporous egg cell after pollination are not known.

Aposporous processes appear to allow extra megaspore mother–like cells that are able to form unreduced megagametophytic structures by mitosis to differentiate from the nucellus. In ovules of sexually reproducing plants and diplosporous apomicts, just one cell is generally specified to give rise to an embryo sac. This suggests that, in most plants, a selective mechanism operates to limit the number of megaspore mother cells that can differentiate from the nucellus and that aposporous plants have bypassed this limitation. The observation that aposporous initials develop in a zone adjacent to the progenitor cells of a sexual megagametophyte (Nogler, 1984) suggests that either the signal that specifies the megaspore mother cell has been expanded to include cells in a greater region of the nucellus or that a restrictive control preventing wide-scale differentiation of numerous megaspore mother cells has been relaxed.

IN ADVENTITIOUS EMBRYONY, EMBRYOS DEVELOP FROM CELLS IN TISSUES EXTERNAL TO A SEXUAL EMBRYO SAC

Adventitious embryos can arise from individual cells within two different somatic tissues of the mature ovule, the nucellus and the inner integument (Lakshmanan and Ambegaokar, 1984). The nucellar form of adventitious embryony is most common and will be focused on here because insight into this process has recently been gained in *Citrus*, which is a model system for the process of nucellar embryony.

In apomictic *Citrus* species, sexual and apomictic processes occur concurrently within the same ovule. As shown in Figure 4, the egg cell and polar nuclei of the sexual embryo sac can be fertilized to produce a zygotic embryo and endosperm;

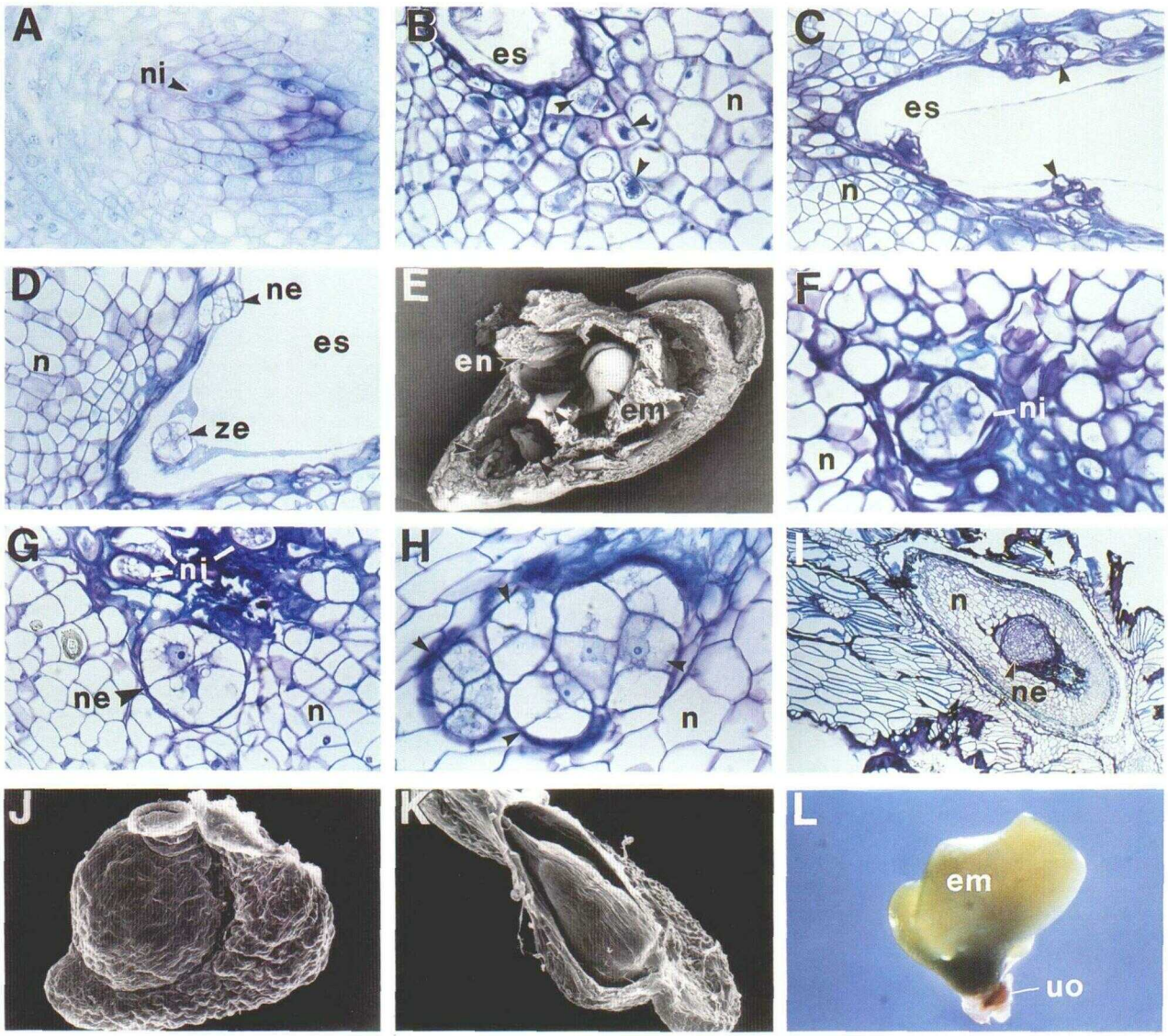

Figure 4. Adventitious Embryony in *Citrus*.

(A) to **(D)** Developmental processes in fertilized ovules of Valencia.

(A) A nucellar embryo initial (ni) in an ovule extracted from a flower prior to anthesis.

(B) Numerous nucellar initials (denoted by arrowheads) with thick callose walls surrounding a fertilized embryo sac (es). This is a cross-section through the ovule. n, nucellus.

(C) A longitudinal section through the micropylar end of a seed with an enlarged embryo sac. Thick-walled nucellar embryo initials (arrowheads) are in close proximity to the embryo sac.

(D) A longitudinal section through the micropylar end of a fertilized seed showing the zygotic embryo (ze) and a small nucellar embryo (ne), which is growing into the embryo sac.

(E) Scanning electron micrograph of a dissected *Poncirus trifoliata* seed midway through development. The endosperm (en) has been peeled away to expose numerous nucellar embryos (em) (indicated by black arrowheads) at different stages of development.

(F) to **(L)** Embryo development in unfertilized ovules of Valencia.

(F) An unfertilized ovule taken from the same fruit as **(C)**. The nucellar initial has enlarged and contains large granules, a morphology identical to that observed in initials found in the developing seed shown in **(C)**.

(G) Nucellar embryo development in unfertilized ovules. A nucellar initial cell has divided to produce a two-celled nucellar embryo. Other nucellar initials can be seen differentiating from the nucellus.

(H) At a later stage, multiple nucellar embryos are present in the nucellus.

however, nucellar embryos are initiated from the nucellar tissue in the region surrounding the developing sexual embryo sac. The nucellar cells destined to become embryos are morphologically distinguished from the surrounding nucellar cells by their large nuclei and dense cytoplasm (Kobayashi et al., 1981; Wilms et al., 1983; Bruck and Walker, 1985) (Figure 4A), a morphology reminiscent of that of aposporous initial cells and developing megaspore mother cells. The mechanisms that specify only some nucellar cells to form nucellar embryo initial cells are unknown, as is the time during ovule development that nucellar embryo initial cells are first specified.

Wakana and Uemoto (1987, 1988) observed that nucellar embryo initial cells are first apparent mainly in the nucellar cell layers surrounding the chalazal portion of the sexual embryo sac at, or soon after, anthesis (Figure 4B). More initials subsequently appear in the micropylar region and also along the length of the embryo sac. As the initials differentiate, a callose wall forms within the original primary cell wall (Wilms et al., 1983) (Figure 4B). In some *Citrus* cultivars, an average of 50 such cells can be observed per ovule (Wakana and Uemoto, 1988). After fertilization, the ovule enlarges as a result of rapid cell division in the noninitial nucellar cells in the chalazal region of the ovule. Simultaneous enlargement of the embryo sac toward the chalazal end of the ovule as a result of endosperm growth results in a clustering of the thick-walled initial cells toward the micropylar end of the embryo sac (Figure 4C) (Wakana and Uemoto, 1988).

The first division of the *Citrus* nucellar initial cells occurs around the time of the first zygotic division. Only the initial cells located in the micropylar region divide and form embryos. As the initial cells divide, they grow into the embryo sac (Figure 4D), to which they are directed by unknown mechanisms. Additional nucellar embryo initial cells continue to differentiate from the nucellus in the micropylar region of the ovule and form embryos throughout seed development (A. M. Koltunow, unpublished observations). Nucellar embryos do not have a suspensor early in their development, but a suspensor is observed after the globular stage. (Osawa, 1912; Maheshwari and Rangaswamy, 1958; Wakana and Uemoto, 1988). As the preglobular embryo begins to grow into the embryo sac, the side abutting the nucellus becomes the suspensor and the opposite end becomes the embryo proper (Wakana and Uemoto, 1988). Although suspensor development is delayed in nucellar embryos as compared to sexually derived embryos, the observation that a suspensor does form in nucellar embryos clearly indicates that fertilization is not a prerequisite for suspensor formation.

Nucellar embryo development is morphologically similar, if not identical, to the stages of sexually derived embryo development in *Citrus* (Bruck and Walker, 1985). Often, the growth of the zygotic embryo is slower compared with the more vigorous growth of the nucellar embryos, and the zygotic embryo may or may not complete development in seeds that contain multiple nucellar embryos (Frost and Soost, 1968). The end result of nucellar embryony in an ovule that has been fertilized by normal sexual processes is the production of a polyembryonic seed containing embryos at different stages of maturation, many of which can germinate to form viable seedlings (Figure 4E). Differences in maturation probably reflect differences in time of initiation of nucellar embryogenesis (A. M. Koltunow, unpublished observations) or competition for and availability of nutrients (Wakana and Uemoto, 1987, 1988).

Not all ovules within a particular *Citrus* ovary are fertilized, and nucellar embryos are also found in the chalazal region of unfertilized ovules excised from mature fruits (Figures 4I to 4K). Wakana and Uemoto (1987) found, however, that all of the nucellar embryos in unfertilized ovules were arrested at the globular and very early cotyledonary stages. We have observed embryo development in fertilized (Figures 4B to 4E) and unfertilized ovules (Figures 4F to 4K) of Valencia and have found that the initiation of apomictic embryo development occurs at a similar time in both kinds of ovule in the developing fruit, suggesting that there exists a general ovary signal for nucellar embryo development that is independent of fertilization (A. M. Koltunow, unpublished observations). If unfertilized ovules are excised from mature fruit and cultured on a simple nutrient medium lacking plant hormones (Moore, 1985), the arrested embryos can complete development. It appears that a sufficient source of nutrition is essential for the completion of nucellar embryogenesis and that this is normally supplied by the endosperm formed in the sexual embryo sac following double fertilization. Unfertilized ovules of *Citrus* varieties that are not capable of inducing nucellar initials do not produce embryos under these conditions (Moore, 1985).

Whereas initial cells surrounding an unfertilized embryo sac all seem to have the capacity to divide and form embryos within the limits of available nutrient, the fertilized embryo sac appears to have an inhibitory influence on nucellar embryogenesis in the chalazal region (Wakana and Uemoto, 1987, 1988). An obvious difference between fertilized and unfertilized

Figure 4. (continued).

(I) Continued growth of the nucellar embryos. The nucellus is still clearly evident as an organized tissue. The cells of the outer integument of the ovule have expanded in size to produce the sheet of cells to the left.

(J) Scanning electron micrograph of a desiccated unfertilized ovule taken from a mature fruit.

(K) Scanning electron micrograph of an ovule from the same source as **(J)**, but the outer integument has been slit to expose the embryo complex. The outer layer of this complex is covered with the remains of the inner integument and nucellar cells.

(L) Continuation of embryogenesis from unfertilized ovules dissected from mature fruit when cultured on simple nutrient media (Moore, 1985). The green embryo has developed from arrested embryos present in the unfertilized ovule (uo).

ovules is the presence of endosperm in all but the micropylar end of the sexual embryo sac, which is occupied by the developing embryo. The inhibition of nucellar embryo initiation in the chalazal region of fertilized *Citrus* ovules means that embryo development, be it sexual or nucellar, is primarily restricted to the micropylar region of the embryo sac. The nucellar initials in the chalazal end of the embryo sac may be inhibited by mechanisms that normally act in sexual embryo development to polarize embryo morphogenesis to the micropylar end of the embryo sac. This could be a gradient of some morphogen or even nutrient. Alternatively, Wakana and Uemoto (1988) have suggested that a component of the endosperm in the chalazal end of the sexual embryo sac of *Citrus* may act specifically to inhibit nucellar initial cell division in that region.

Taken together, the above observations of nucellar embryony show that in apomictic *Citrus*, nucellar embryos can be initiated with or without fertilization of the sexual embryo sac. This dispels the longstanding notion that fertilization of the sexual embryo sac is necessary for the initiation of embryogenesis in the surrounding nucellar cells and that nucellar embryos are initiated only in the micropylar end of the nucellus. Individual cells in the nucellus of the ovule, therefore, have the capacity to initiate an embryogenic developmental pathway without the need for the paternal genome to trigger embryo development. However, nucellar embryony in *Citrus* depends on sexual reproduction to produce endosperm for nucellar embryo growth and development and seed set. Most species with adventitious embryony require endosperm generated by pseudogamy for seed set (Asker and Jerling, 1992).

The somatic nucellar initial cell in *Citrus* is egglike in developmental potential, but, although it is adjacent to an embryo sac, it is not surrounded by a megagametophytic structure; instead, it is surrounded by other nucellar cells. This is in contrast to sexual, aposporous, and diplosporous reproduction, in which the cell that develops into the embryo is part of a megagametophyte-like structure. Thus, nucellar embryogenesis generates a sporophyte independent of an embryo sac structure. As in apospory and diplospory, the processes that stimulate the egglike nucellar initial cell to undergo embryogenesis are not known.

REGIONAL AND CELL-SPECIFIC GENE EXPRESSION IN THE NUCELLUS MAY REGULATE APOMICTIC AND SEXUAL PROCESSES IN OVULES

The nucellar tissue of the ovule is where sexual female gametophyte development and also the apomictic processes described above take place, yet little is known about this tissue and its exact role in the developmental processes of the ovule. In plants that reproduce sexually, the initiation of Polygonum-type embryo sac development is restricted to a single cell, which differentiates from the nucellus. By contrast, observations of apomictic species indicate that the nucellus of these species has a greater developmental plasticity than that of sexually reproducing plants, because numerous cells can differentiate from it and form gametophytes or embryos, and because in some cases, both apomictic and sexual reproductive structures can coexist in an ovule. This implies that individual cells in the nucellus of apomictic species can collectively express a greater variety of independent developmental programs and at different times in ovule development than those of sexual species. Nothing is yet known about the similarities and differences between genes expressed during apomictic and sexual developmental pathways. Comparative molecular analysis of genes expressed in the nucellar tissue of apomictic and sexually reproducing plants at carefully chosen times in development should indicate whether independent cell- and region-specific gene expression programs indeed operate in the nucellus during sexual and apomictic development as well as provide an indication of how different these developmental pathways are at the molecular level.

THE EXISTENCE OF APOMICTIC PROCESSES SUGGESTS THAT THE ORDER AND TIMING OF EVENTS IN SEXUAL REPRODUCTION ARE MAINTAINED BY ACTIVE MECHANISMS

What mechanisms maintain the order of the events spanning female gametogenesis and embryogenesis in sexual systems? Processes similar to cell cycle control mechanisms (Hartwell and Weinert, 1989) may ensure that ovule-specific sexual reproductive events in angiosperms are executed in a particular order. One mechanism that could act in sexually reproducing plants is a substrate–product relationship in which the early event produces a component or a structure essential for a subsequent event. It would be difficult to disrupt the order or timing of events governed by such a mechanism because each event is dependent solely on the completion of the previous event.

Alternatively, feedback controls may exist that keep the early event under surveillance, such that if that event is not completed, a signal can be sent to an active controlling system or checkpoint (Hartwell and Weinert, 1989) that acts directly or indirectly to delay or block the initiation of a later event. In this way, checkpoints could actively enforce a dependency and maintain a rigid sequential order (Hartwell and Weinert, 1989). In contrast to substrate–product dependent mechanisms, however, it should be possible to uncouple sequential events controlled by checkpoint mechanisms, for example by inducing mutations in the feedback controls that normally act on a checkpoint such that a late event is induced to proceed without the completion of an earlier one. Indeed, the experimental evidence to date suggests that the cell cycle is in fact regulated at three key points by checkpoints that are kept informed by feedback controls. Genes whose products appear to function as feedback controls have been identified by mutagenesis (Hartwell and Weinert, 1989; Nurse, 1991; Murray, 1992).

The observation that apomictic processes bypass key events in sexual reproduction yet form viable seeds suggests that it is unlikely that the events spanning embryo sac formation and embryogenesis are regulated solely by a substrate–product mechanism. The absence or disruption of meiosis in sexually reproducing plants normally results in cessation of megagametophyte development, and the lack of fertilization results in floral abscission or seedlessness. In apomictic processes, neither megagametophyte formation nor embryo formation is dependent on meiosis, and only endosperm formation may be dependent upon fertilization. Therefore, in apomicts, there appears to be an uncoupling of the sequential events normally observed in sexual reproduction so that a later event can proceed without the completion of an earlier event. This suggests that in apomicts, a feedback–checkpoint control mechanism that normally operates during sexual reproduction is disrupted. Perhaps some apomictic processes overcome the normal feedback control by presenting a checkpoint with a signal that tells the control system that an event is complete (Murray, 1992). In any event, no matter what the controlling mechanism turns out to be, it is clear that apomictic processes would have to link up with and even interact directly with whatever control system operates during sexual development to maintain the sequential order of the events that span megagametophyte formation and the initiation of embryogenesis.

MORPHOGENIC MARKERS MAY INDICATE CELLULAR COMMITMENT TO A SEXUAL OR AN APOMICTIC PATHWAY

At specific stages in sexual reproduction, a boundary of callose serves to isolate the cells involved in a particular developmental process from surrounding sporophytic cells (Bouman, 1984; Knox, 1984; Bell, 1992). Before the initiation of meiosis, a callose wall forms around each of the microspore mother cells in the anther and around the megaspore mother cell in the ovule. The reason for the physical isolation of the gametophytic progenitor cells at this stage is not clear; one possibility is that the callose wall may protect these cells from somatic differentiation signals, a role that has been postulated for the germline signal in *Xenopus* (Wylie et al., 1985). Alternatively, callose may isolate these cells from hormones or other compounds in the nucellus or tapetum that might otherwise diffuse in and disrupt meiosis (Rodkiewicz, 1970; Bouman, 1984; Carman et al., 1991).

The completion of meiosis in each of the many microspore mother cells of an individual anther generates a tetrad of microspores in a tetrahedral configuration. Each microspore is surrounded by a rigid callose wall, and these microspores remain fused until pollen shape and germination aperture sites are established. The callose is then actively digested by enzymes synthesized in the surrounding tapetal cells. Each of the separated microspores has the developmental capacity to form a pollen grain (Knox, 1984). By contrast, only one of the products of megaspore mother cell meiosis has the developmental capacity to form a megametophyte in sexual development of the Polygonum type (Rodkiewicz, 1970; Bouman, 1984). This chalazal megaspore is not surrounded by a callose wall, whereas the other three megaspores, which degenerate, are surrounded by callose walls. The principal function of callose during megasporogenesis therefore appears to be the suppression of the further development of nonfunctional megaspores by isolating them, thus ensuring that only one functional megaspore participates in megagametogenesis (Webb and Gunning, 1990).

The pattern of callose deposition in ovules of sexual species is not conserved during apomictic embryo sac formation. *Elymus* species, which reproduce by meiotic diplospory similar to the *Taraxacum* type illustrated in Figure 2, lack callose around the megaspore mother cell and in subsequent stages of apomictic "megaspore" formation (Carman et al., 1991). Megaspore mother cells of diplosporous *Tripsacum* species also lack callose (Leblanc et al., 1993). In aposporous species such as *Poa*, in which the megaspore mother cell is specified but degenerates soon after aposporous initials differentiate from the nucellus, an incomplete callose wall forms around the megaspore mother cell. Callose walls have not been observed around the aposporous cells, which is consistent with the lack of callose in functional megaspores about to undergo mitosis in sexual megagametogenesis (Willemse and Naumova, 1992). These observations may be representative of aposporous and diplosporous embryo sac formation in general, but we do not yet know whether they are universal. The deficiency in callose in megaspore mother cells among gametophytic apomicts observed to date may be a coincidental result of a more fundamental genetic lesion that causes apomixis or it may itself be the responsible lesion (Carman et al., 1991). Nevertheless, the comparison of apomictic and sexual species suggests that callose is essential for meiosis in the development of the Polygonum-type embryo sac.

By contrast, callose wall formation is conserved during embryogenesis in both sexual and apomictic developmental pathways in plants (Jensen, 1968, 1974; Natesh and Rau, 1984; Williams et al., 1984; Wakana and Uemoto, 1987, 1988). This consistent occurrence of callose suggests that it may be necessary for embryo development whether or not the embryo is derived by sexual or asexual processes.

Cell surface markers much more subtle than callose have been found that may denote lineages of reproductive cells in developing angiosperm flowers (Pennell and Roberts, 1990; Pennell et al., 1991; Bell, 1992). Reproductive and somatic cells appear to be marked by changes in arabinogalactan proteins at the outer surface of the plasma membrane (Pennell and Roberts, 1990). One such protein, detected by monoclonal antibody MAC207 (Pennell and Roberts, 1990), is present in all somatic tissues but is absent in nucellar cells of the ovule and sporogenous cells of the anther. Some of the cells lacking the MAC207-reactive epitope contain another arabinogalactan protein, which is recognized by the JIM8 monoclonal antibody (Pennell et al., 1991). This new protein becomes detectable

in maturing anthers and ovules and is found on the surfaces of the sperm cells, the egg cell, and the synergids but is absent from the membranes of the pollen grain vegetative cell, the antipodals, and the central cells. After fertilization, JIM8-reactive epitopes are present in the zygote and young embryo, but at the globular stage the epitope is detected only in the suspensor (Pennell et al., 1991). The distribution of these cell surface markers has not yet been tested in apomictic systems. If such cell surface changes prove to be reliable indicators of a commitment of a cell lineage to a specific developmental pathway, then extension of these investigations to apomictic systems may provide useful information about the molecular relationships between apomictic and sexual systems (Bell, 1992).

APOMIXIS AND SEXUALITY ARE NOT MUTUALLY EXCLUSIVE MODES OF REPRODUCTION

An additional layer of complexity to the process of apomixis is that it may be obligate or facultative. Obligate apomicts breed true and are stable for apomictic reproduction in successive generations (Asker, 1980; Bashaw and Hanna, 1990). An obligate apomict possesses the following characteristics: avoidance of reductional meiosis, avoidance of sexual fusion, spontaneous embryo development, and, with the exception of pseudogamous species, spontaneous endosperm development (Richards, 1986).

Facultative apomicts, by contrast, have the capacity to reproduce both sexually and apomictically; thus, a percentage of their ovules have sexual embryo sacs containing reduced egg cells that can be fertilized by normal sexual processes. Apomicts in which sexual and apomictic processes occur within the same ovule are also, of course, facultative apomicts. The progeny of facultative apomicts are a variable population of seedlings, some of which breed true and others of which are sexual hybrids (Asker, 1980). In evolutionary terms, facultative apomicts benefit from the possession of both reproductive mechanisms. A particular genetic combination can be fixed by apomictic reproduction to allow rapid colonization of a favorable niche. At the same time, the presence of sexual reproduction, with its ability to generate genetic diversity, allows evolution should conditions change (Richards, 1986). Overall, most apomicts have a tendency to be facultative (Nogler, 1984; Asker and Jerling, 1992).

There is evidence that the degree of apomixis can be influenced by a number of factors external to the maternal plant. The frequency at which sexual embryos occur in some apomictic species is influenced by the pollen parent (Frost and Soost, 1968; Nogler, 1984) and by photoperiod and temperature (Knox and Heslop-Harrison, 1963; Knox, 1967; Frost and Soost, 1968; Evans and Knox, 1969; McWilliam et al., 1978). In other apomictic species, the degree of sexuality is independent of these parameters (Hussey et al., 1991). Inorganic salts (Gounaris et al., 1991) and nutrient levels (Frost and Soost, 1968; Cox and Ford, 1987) also appear to modify the degree of sexuality versus apomixis. The time of initiation of apomictic processes in relation to sexual events also appears to be affected by some of the above factors (Nogler, 1984). How these parameters exert their effects to shift the balance between sexuality and apomixis is not known.

Taken together, the observations that sexual and apomictic processes can coexist within one ovule and among ovules of an individual plant indicate that mechanistically, sexuality and apomixis are not mutually exclusive modes of reproduction. Thus, apomicts do not arise simply through a combination of mutations in the sexual process. Instead, apomixis and sexuality can be simultaneous and interdependent phenomena (Nogler, 1984). It is currently thought that apomixis and sexuality exist in a state of balance in apomictic plants. In stable, obligate apomicts, this balance has been shifted so that apomictic processes somehow suppress the sexual mechanism, but in facultative species, both mechanisms coexist (Asker, 1980; Nogler, 1984).

APOMIXIS HAS A GENETIC BASIS INITIATED BY FEW GENETIC LOCI

Apomixis is not a process that is randomly stimulated by environmental and nutritional factors. Analysis of the progeny of crosses between apomictic and sexual forms has shown that the ability to reproduce apomictically is genetically determined. For example, nucellar embryony in *Citrus* appears to be controlled by a single dominant locus (Parlevliet and Cameron, 1959; Iwamasa et al., 1967).

Studies on the inheritance of apomixis have been most extensive in plants exhibiting apospory. The most recent information on the genetic control of apospory in *Pennisetum* species, *Panicum*, and *Ranunculus* suggests that in all three systems, apospory is probably controlled by a single dominant gene locus (Asker and Jerling, 1990). The trait of apospory observed in *Pennisetum squamulatum* has been introduced to a sexual species, pearl millet (Dujardin and Hanna, 1989), and the resultant apomictic line, BC_3, has a single supernumerary chromosome containing the apomictic genes from *P. squamulatum*. BC_3 is proving to be a powerful tool for the molecular analysis of apospory. The transferred chromosome can be detected by restriction fragment length polymorphisms (RFLPs), and molecular markers linked to apospory have recently been identified on the transferred chromosome (Ozias-Akins et al., 1993). Other studies that may lead to mapping of the loci responsible for apospory include RFLP analysis of *Potentilla* biotypes and *Rubus* species using DNA fingerprinting (Asker and Jerling, 1992). Whereas the molecular analysis of aposporous apomixis is beginning to get underway, evidence pertaining even to the inheritance of diplospory is limited and relatively inadequate. A recent reexamination of studies pertaining to the genetic regulation of diplospory

in *Taraxacum* by Mogie (1988) suggests that the control of female meiosis resides on a single chromosome and, probably, at a single locus.

The available data therefore suggest that only a few genes control apomixis, but the effects of these genes are profound: they direct a somatic nucellar cell to form an embryo sac without meiosis, and embryos and endosperm to form without fertilization. As yet, genes involved in apomictic reproduction have not been isolated, so the identity of apomictic gene products and their functions remain to be elucidated.

THE CONCEPT OF AN APOMIXIS GENE: MODELS FOR GENE ACTION

Any hypothesis concerning the nature of an apomictic gene product and its mode of action needs to explain satisfactorily how only a few dominant genes are necessary to elicit the formation of megagametophytes by mitosis and of embryo and endosperm without fertilization. In addition, the hypothesis needs to explain how both sexual and apomictic processes can coexist in the nucellus of a single ovule and among ovules of an individual plant.

These criteria can be satisfied if in apomictic species the products of apomictic genes are thought of as initiators of mitotic megagametophyte formation and autonomous embryo and endosperm development. Once these events are initiated in a nucellar cell, the subsequent developmental events could then proceed via the normal developmental molecular processes that operate during sexual reproduction. For example, once apomictic genes initiate nucellar embryo development and the initial cell forms and divides, the genes controlling embryo cell formation, structure formation, and embryo pattern formation are probably the same as those required for sexual embryo development. Cell-specific and/or regional expression of the apomictic and sexual genes in specific nucellar cells would determine whether sexual, apomictic, or both processes occur in an ovule. For sexual reproduction to be evident, the apomictic gene would be absent or not expressed. For facultative apomixis, once the apomictic gene was turned on in a specific nucellar cell at a specific time to initiate a particular event, the elements of the sexual machinery would then complete the event. In another nucellar cell, only the sexual events would occur unperturbed. In obligate apomicts, sexual events may need to be inhibited; various scenarios could be proposed to account for this.

A more important question to consider is whether the products of apomictic genes are new proteins with a novel initiating function not observed in sexually reproducing plants or whether they are, instead, proteins that normally function to initiate events in sexual reproduction but may have an altered activity or spatial and temporal distribution during development. Current models of apomictic gene action tend to favor the latter.

For example, in a model proposed for the genetic regulation of meiosis in diplospory, Mogie (1988) views the apomixis gene as a mutated sexual gene. According to this model, the control of the avoidance of meiotic reduction resides at a single locus, the identity of which can vary between plant lines. The affected locus contains a wild-type allele that codes for meiotic reduction and excess copies of a mutant allele that codes for its avoidance. Phenotypic expression of the mutant allele is favored in female generative cells and expression of the wild-type allele is favored in somatic cells because the locus is also important for mitosis. The dominance in expression of the two alleles is determined by their ratio and influenced by the environment. The requirement for excess copies of the mutant allele explains why diplosporous apomicts are typically polyploid. Variable degrees of expression of the mutant allele in a single plant would account for facultative apomixis. It was also suggested by Mogie (1988) that parthenogenetic initiation of embryo development might result from additional "parthenogenesis genes," other environmental stimuli, or physiological or developmental changes induced by the avoidance of meiotic reduction.

An interpretation of dominant gene action in aposporous species has been suggested by Peacock (1992). In this model, the apomictic gene product is viewed as a normal component of sexual maturation, a transcriptional activator that normally binds to the promotor(s) of the earliest acting gene(s) involved in the developmental cascade necessary for the formation of the embryo sac. In sexual systems, this putative embryo sac induction gene (*Esi*) would normally be expressed in the megaspore after meiosis. Apospory could then result from the precocious expression of that protein or its expression in a group of nucellar cells in which it would not normally be expressed in sexual systems. Each of these cells would then respond to the Esi transcription factor to produce an unreduced embryo sac. Whether or not this gene is expressed at the correct time in cells undergoing sexual gametophyte development would determine whether sexual processes would occur in aposporous plants.

What of autonomous embryo and endosperm formation? Both of the above models are vague about this. Are additional genes required to induce these aspects of apomictic processes? Possibly, but, along the lines of Peacock's (1992) idea of timing and location of expression of a key regulatory protein, it may also be that the same protein is normally required at different times in the sexual process to help initiate the mitotic events of megagametogenesis and embryo and endosperm formation. At each of these developmental stages, the key factor would interact with a repertoire of different gene sets depending on the developmental status of the cell. Ectopic expression of a regulatory gene in apomictic systems in a number of nucellar cells at different times in ovule development would also be consistent with the observations that most of the apomictic processes studied intensively to date appear to be controlled by a single dominant gene locus. Information on the types of genes expressed during sexual and

apomictic development in ovules should indicate whether apomictic processes are a result of genes specific to apomictically reproducing plants or whether apomixis reflects alterations in the spatial and temporal expression of key regulatory genes normally involved in sexual reproduction.

CONCLUSION

The existence of apomictic species that have inherent deviations from the reproductive processes maintained in their closest sexual relatives provides ideal experimental material for comparative studies at the cytological, physiological, molecular, and genetic levels to gain an accurate developmental understanding of how the complex, ovule-specific reproductive events are controlled in angiosperms.

The ovule in angiosperms is the progenitor of the seed, and as such it is a structure in which both gametophytic and sporophytic phases of the plant life cycle occur. The ovule-specific reproductive events of sexual reproduction are an ordered series of stepwise events that result in a seed having a mixed genetic constitution representing both the pollen and maternal parents. By contrast, apomictic processes generate seeds containing embryos of a purely maternal genetic constitution. This is achieved by an avoidance of meiotic reduction and embryo formation in the absence of fertilization by processes that are not yet understood. It has been suggested from genetic studies that few genes are needed to elicit the various apomictic phenotypes. Recent studies aimed at understanding the molecular basis for apomictic processes using differential and subtractive screening between apomictic and sexual species (Gustine and Sherwood, 1992; A. M. Koltunow, unpublished data) should provide an indication of the degree of similarity between the genes expressed in sexual and apomictic developmental pathways.

The models described here, however, propose that apomictic gene products are developmental initiators or regulators that normally function to initiate cascades of developmental reactions in sexually reproducing plants but that are ectopically expressed in the nucellus of apomictic plants at different times during ovule development and therefore initiate apomictic processes. In this way, apomictic processes could easily utilize the existing developmental framework required for sexual reproduction, and both types of reproductive processes could exist simultaneously in ovules, as occurs in facultative apomicts.

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