Critical Reviews in Plant Sciences, 25:199–214, 2006

ISSN: 0735-2689 print / 1549-7836 online
DOI: 10.1080/07352680600563926

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Apomixis: Developmental Characteristics and Genetics

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Table of Contents

Asexual reproduction through seeds, or apomixis, is widespread in angiosperms, although does not happen frequently. It occurs in no major crop plant, but its deployment in major crops would afford advantages for breeding and maintenance of hybrid genotypes. Deployment is still a long-term goal, however, since the genetic mechanisms underlying apomixis in nature have not been determined nor has the isolation of apomictic mutants in sexual plants been achieved. Nevertheless, an increasing intensity of research toward these goals over the last decade has greatly expanded our knowledge of genome structure and gene expression in naturally occurring apomicts and female gametophyte development in sexual plants. A common working hypothesis is that apomixis is a "deregulation" of sexual processes and is increasingly supported by gene expression data. Nevertheless, the search for a unique trigger that initiates apomictic development still cannot be disqualified. Further characterization of female gametophyte-related genes and genomes of apomicts and model sexual

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plants will be fruitful for identifying overlaps in developmental networks.

Keywords adventitious embryony, apospory, asexual reproduction, diplospory, embryo sac, female gametophyte

I. INTRODUCTION

A. Definition

In a classic review on apomixis in angiosperms (Nogler, 1984a), apomixis is defined as "asexual (agamic) reproduction by seeds, i.e., agamospermy." As was also noted, the alternative to apomixis is amphimixis, or sexual reproduction. Apomixis, in the context of this review, will be confined to the angiosperms. The major developmental events for both apomixis and amphimixis occur in the ovule, the precedent to the seed. Amphimixis in angiosperms requires an alternation of sporophytic and gametophytic generations where the gametophytes (female gametophyte or embryo sac and male gametophyte or pollen) are dependent on the sporophytic tissues for development. The gametophytes have half the chromosome number of the sporophyte because a reductional division (meiosis) occurs during spore formation. The fusion of female and male gametes (egg and sperm) restores the diploid chromosome number in the embryo (incipient sporophyte). The absence of chromosome reduction in the pathway to embryo development is one defining feature of apomixis. The floral structures recognizable during this sequence of developmental events are listed in Figure 1. The practical significance of apomixis is that it can fix heterosis—progeny are genetically identical to their mother, and the potential impact on agriculture has been widely discussed (Hanna, 1995; Hanna and Bashaw, 1987; Savidan, 2000). This review will present the current state of knowledge on developmental characteristics and genetics of apomixis that occurs naturally in flowering plants.

B. Identifying Apomicts

In order to accurately and thoroughly phenotype an individual for mode of reproduction, it is necessary to cytologically examine the developmental events that occur in the ovule as well as characterize the genetic variability/uniformity of offspring from the individual. Cytological study can involve microscopic observation of paraffin- or resin-embedded, sectioned material or cleared organs. Organ clearing is a more facile technique than sectioning, but must be optimized for the species and tissue being studied, particularly with regard to the refractive index of the clearing solution. Numerous protocols are available for clearing ovules (Crane, 2001). Grass ovules typically are cleared in methyl salicylate after fixation for 24 h in (FAA) formalin: acetic acid: ethyl alcohol and storage in 70% ethanol (Young *et al*., 1979). Examples of meiotic and ameiotic ovules cleared in methyl salicylate and observed under differential interference contrast optics are shown in Figure 2.

Progeny analysis is necessary to establish the frequency of functional apomixis or amphimixis. Several approaches can yield useful data. The simplest is to examine the variability/uniformity of progeny from an open-pollinated individual. A near-obligate apomictic plant will produce very uniform progeny. Since apomicts typically are highly heterozygous, either self- or open-pollination would result in some "offtype" progeny should sexual reproduction also be occurring. A more quantitative method to estimate the degree of apomixis versus

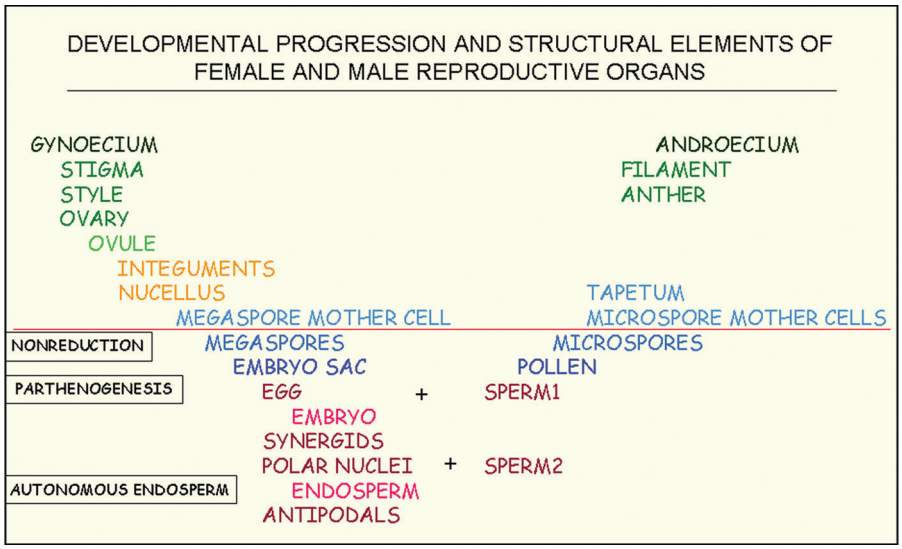

FIG. 1. The gynoecium (female) and androecium (male) parts of the plant are floral organs that house the sporophyte-dependent gametophytes. The sequential development of distinct structures during sexual reproduction is shown along with the points (boxes) where gametophytic apomixis alters the characteristics of certain structures. Cells in structures above the horizontal line are diploid while those below vary in ploidy level depending on the operation of components of apomictic or sexual reproduction.

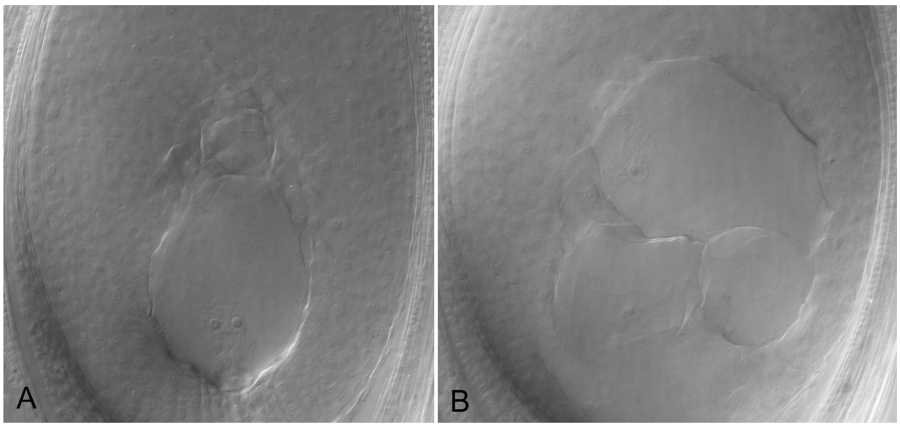

FIG. 2. Ovules of *Pennisetum* hybrids cleared in methyl salicylate and viewed with differential interference contrast. Left panel: meiotic embryo sac from amphimictic plant—note antipodal cells at chalazal end of embryo sac. Right panel: multiple aposporous embryo sacs in ovule from apomictic plant—note lack of antipodal cells.

amphimixis is to cross an apomict with pollen from a male parent that has a homozygous, dominant, readily scorable phenotypic marker. One convenient marker gene is for red or purple color where progeny that result from sexual reproduction show pigmentation in vegetative and/or reproductive organs (Devos *et al*., 2005). Green progeny are not products of fertilization by the test-cross parent and most likely would have been derived through apomixis.

Another method to obtain an estimate of apomictic versus amphimictic reproduction is the flow cytometric seed screen (FCSS) (Matzk *et al*., 2000). The seed screen analyzes stained nuclei from seeds, i.e., embryo and endosperm nuclei, using flow cytometry. A comparison of the relative DNA contents in embryo and endosperm nuclei allows conclusions to be drawn regarding the nuclear origin of these two tissues. For example, 2C and 3C primary peaks for a seed would indicate sexual reproduction. An egg (n) + sperm (n) fusion would have resulted in a 2n embryo whose cells would pass through G1 (2C DNA content) and G2 (4C DNA content) phases of the cell cycle. The endosperm would have been formed from fusion of two polar nuclei [n + n] with a sperm, and endosperm cells also would pass through G1 (3C DNA content) and G2 (6C DNA content) phases of the cell cycle. Since most cells are in the G1 phase of the cell cycle at any point in time, the 2C and 3C peaks predominate. The extent of variation in apomicts that can be identified using this method is described below.

II. DEVELOPMENTAL FEATURES OF APOMIXIS—THREE ESSENTIAL COMPONENTS

A. Apomeiosis and Adventitious Embryony

The brief definition of apomixis describes an end result, but the developmental events that lead to this result can vary extensively. Apomixis can be broadly divided into the following three types: adventitious embryony, a sporophytic type, or diplospory and apospory, two gametophytic (apomeiotic) types. Previous reviews have thoroughly described the morphology and distribution of these types of apomixis (Carman, 1997; Koltunow, 1993; Nogler, 1984a). There is considerable variation in the timing of developmental events within an ovule undergoing apomictic and sometimes concurrent amphimictic processes. This variation has led to the classification of numerous subtypes of gametophytic apomixis (Nogler, 1984a; Asker and Jerling, 1992; Savidan, 2000; Crane, 2001), but these subtypes will not be presented in detail in the current review.

Sporophytic differs from gametophytic apomixis in that no alternation of generations intervenes prior to embryo development. The embryo forms directly from a somatic cell in the ovule, typically a nucellar, but sometimes an integumentary cell. Since seed development also requires a nutritive endosperm, and endosperm can only develop during the gametophytic phase of the life cycle, amphimixis is concurrent with apomixis in sporophytic apomicts. The simultaneous occurrence of adventitious embryos and reduced embryo sacs allows for endosperm to develop after fertilization of the central cell in the reduced embryo sac. The endosperm can thus nourish either an adventitious embryo or the zygotic embryo when one out-competes the other, or both may develop to relative maturity resulting in polyembryony.

Gametophytic apomixis can completely replace amphimixis in an "obligate" apomict, although it is unlikely that completely obligate apomicts actually exist (Asker and Jerling, 1992). Within the ovule of a gametophytic apomict, the megaspore mother cell is either arrested in its development or does not enter or complete meiosis. Without meiosis, haploid megaspores cannot develop and all cells of the unfertilized ovule retain the somatic chromosome number. Diplospory results when the megaspore mother cell or one of its diploid daughter cells develops into an unreduced female gametophyte. Considerable variation in megaspore mother cell development prior to diplosporous embryo sac initiation can occur and has led to

the classification of multiple types of diplospory (Crane, 2001). The most common are the *Antennaria* type, also known as mitotic diplospory where the megaspore mother cell directly undergoes mitosis, and the *Taraxacum* type with a restitution of the somatic chromosome number at meiosis I (Nogler, 1984a). In apospory, the origin of the unreduced female gametophyte is a somatic cell, leading to the possibility that both amphimictic and apomictic processes could occur in the same ovule in facultative apomicts. Such situations are relatively rare, however, and one process usually outcompetes the other. The competitiveness of sexual versus apomictic development probably depends in part on heterochronic shifts in reproductive events. The plasticity of these events within and between apomictic genotypes has been documented in *Tripsacum* accessions and hybrids with maize (Grimanelli *et al*., 2003) as well *Hieracium* genotypes (Koltunow *et al*., 2000). A similar conclusion was reached from study of these diplosporous and aposporous taxa—apomixis is most likely a deregulation of the sexual process (Koltunow and Grossniklaus, 2003). However, examples are known where the absence of apomeiotic events in an ovule of an apomict does not restore successful sexual reproduction (Nogler, 1984a; Roche *et al*., 2001a), suggesting that there is a specific factor required at least for initiation of apomeiosis if not for the downstream events of embryo sac development.

B. Parthenogenesis

Parthenogenesis is the development of an unfertilized gamete into a plant. In amphimicts as well as apomicts, two sperm are released from the pollen tube that penetrates a synergid in an embryo sac (Figure 1); however, the fate of the sperm in an apomict differs from that in an amphimict. Typically, no sperm fertilizes the egg because it develops parthenogenetically (Figure 1). In exceptional cases, an unreduced egg can be fertilized resulting in a hybrid embryo with an elevated ploidy level. When meiosis occurs in an apomict, allowing chromosome reduction, it typically is followed by syngamy between egg and sperm; however, the egg may occasionally develop parthenogenetically to produce a haploid (or polyhaploid) embryo. Possible nuclear states of embryo and endosperm are shown in Table 1.

Embryo development from an unreduced egg can be precocious in an apomict (Nogler, 1984a). Precocity is particularly pronounced in *Tripsacum dactyloides* (Grimanelli *et al*., 2005). Interestingly, out of 5534 transcriptional units surveyed with maize microarrays, no gene expression differences were observed between unfertilized maize ovules and unfertilized, apomictic maize-*Tripsacum* hybrid ovules even though the embryo had begun to develop prior to pollination in the apomictic hybrid (Grimanelli *et al*., 2005). A significant difference in the proportion of polyadenylated mRNA was found between the two genotypes, however, suggesting that mRNA maturation may play a greater role in embryo initiation than transcription, and that the maternal to zygotic transition, in terms of altered transcriptional profile, does not occur until after the initial zygotic divisions.

Even in *Pennisetum ciliare* where parthenogenetic embryo development is not considered to be precocious, ultrastructural examination revealed a complete cell wall around the egg cell of apomictic *P. ciliare* prior to penetration of the embryo sac by a pollen tube (Vielle *et al*., 1995). This pattern differed in the chromosomally reduced egg where exposed membrane remained at the chalazal end. In this case, precocious cell wall development in the apomeiotic egg may prevent fertilization. Indeed, more 2n + n hybrids were recovered when pollination took place 2 to 3 days prior to anthesis instead of at anthesis (Burson *et al*., 2002).

One interesting model for parthenogenesis in plants is the "Salmon" system in wheat (Matzk, 1996). "Salmon" contains a wheat-rye translocation in its nucleus that, when in a particular cytoplasmic background, will result in male sterility and haploid parthenogenesis. While the Salmon system is not apomictic, it does provide useful isogenic lines for molecular studies including the construction of egg-cell-specific cDNA libraries (Kumlehn *et al*., 2001).

C. Fertilization and Endosperm Development

The central cell must be fertilized in many apomicts in order for endosperm to develop (Table 1). This phenomenon is known as pseudogamy. Some apomicts, e.g., many in the Asteraceae, do

TABLE 1

Combinations of reduced (Red) or unreduced (NonRed) nuclei due to fertilization (Fert), or lack of recombination due to autonomous development (Parth), that can lead to different ploidy states in embryo or endosperm cells. The first or [] nuclear state derives from the egg (embryo) or polar nuclei (endosperm) while the subsequent ones are of sperm origin

	Red/Fert	NonRed/Fert	Red/Parth	NonRed/Parth
Embryo	n + n n + 2n	2n + n 2n + 2n	n + 0	2n + 0
Endosperm	[n + n] + n [n + n] + 2n	[2n + 2n] + n [2n + 2n] + 2n [2n + 2n] + n + n 2n + n	[n + n] + 0	[2n + 2n] + 0

not require fertilization of the central cell and the endosperm develops autonomously. The maternal to paternal genome contribution to the endosperm and its relationship to the ploidy level of the embryo have a strong effect on seed viability in amphimicts (Haig and Westoby, 1991; Vinkenoog and Scott, 2001). Maize has been particularly well studied in this regard and was shown to have normal endosperm development only when the maternal to paternal genome ratio in the endosperm was 2m:1p (Lin, 1984). Apomicts either are more tolerant of variation in maternal:paternal genome ratios or have adaptations that restore such ratios. For example, apomictic genotypes of *Tripsacum dactyloides* produce [2n (4x) + 2n (4x)] + 1n (2x) endosperm, i.e., two unreduced polar nuclei fuse with a reduced sperm to generate a 8m:2p ratio in normally developing endosperm (Grimanelli *et al*., 1997). An adaptation to avoid endosperm imbalance is displayed by apomictic *Pennisetum* species as well as other panicoid apomicts that develop four-nucleate embryo sacs. Four-nucleate embryo sacs typically have a uninucleate central cell that is fertilized to create 2n + n endosperm or reconstitute a 2m:1p genome ratio (Ozias-Akins *et al*., 2003). A change in this developmental pattern occurs when apomixis is introgressed into sexual *P. glaucum* from an apomictic relative: the frequency of uninucleate central cells drops dramatically in favor of binucleate central cells and is correlated with a reduction in seed set (Morgan *et al*., 1998). Other patterns of endosperm balance displayed and tolerated by apomictic species have been previously reviewed (Savidan, 2000; Koltunow and Grossniklaus, 2003).

III. APOMICTIC GENOMES

A. Evolution/Phylogenetic Distribution

Apomixis is widely distributed among angiosperm families where both sporophytic and gametophytic types have been reported among monocots, eudicots, and magnoliids (Carman, 1997; Table 2). Apomixis clearly originated multiple times during flowering plant evolution. It appears to be most widespread in the grass (Poaceae) and sunflower (Asteraceae) families although its frequency could be due, in part, to the intensity with which reproduction has been studied in these families, both of which contain crop species. However, Solanaceae also contains many crop species (e.g., tomato, potato, tobacco), but apomixis is relatively infrequent. Both sporophytic and gametophytic types of apomixis often are present in the same plant family as are the diplosporic and aposporic forms of gametophytic apomixis. The occurrence of multiple types of apomixis in a family, genus, species, and sometimes individual genotype could be interpreted as a result of overlapping or intersecting developmental pathways that are subject to genotype x environment effects. Evidence is accumulating that apomixis likely is a consequence of deregulation of sexual reproduction, although the point of deregulation can vary and may be responsible for the different developmental phenotypes (Koltunow and Grossniklaus, 2003).

Since apomixis and amphimixis are not mutually exclusive, genetic variation can be found in agamic complexes and often is greater than would be expected by extrapolating from the frequency of aposporous or diplosporous embryo sac development. Apomixis is not a tightly controlled developmental process, but is subject to considerable plasticity with regard to fate of an egg cell and its coordinate development with the endosperm. All of the egg cell cytological fates shown in Table 1 are possible in an apomictic individual, particularly where residual sexual reproduction has been documented. Similarly, potential cytological states in the endosperm are very variable when apomixis as a whole is considered, but usually are more limited within any individual apomict. An example of reproductive diversity comes from *Hypericum perforatum* where the flow cytometric seed screen was used to document 11 different reproductive outcomes of embryo and endosperm combinations (Matzk *et al*., 2001). This extreme plasticity may be unusual, but definitely could contribute to the genetic diversity observed within or among populations or agamic complexes. Progeny from crosses between Australian populations of *H. perforatum* had an average of 2.5% meiotic embryo sac development (Mayo and Langridge, 2003), which, although seemingly low, would allow for numerous recombination events to occur over multiple generations. Whether variation in populations of apomicts is due primarily to recombination or mutation has been debated, although recent evidence from *Hieracium pilosella*, using a method of cladistic analysis called component compatibility analysis, implicated residual sexual reproduction as the primary determinant of genotypic diversity (Houliston and Chapman, 2004).

B. Genetic and Genomic Analysis

Early genetic studies have been summarized previously (Nogler, 1984a; Stebbins, 1941). The present review will focus primarily on genetic studies that have incorporated molecular tools. Other recent reviews also discuss the inheritance of apomixis (Grimanelli *et al*., 2001; Grossniklaus *et al*., 2001a; Savidan, 2000).

1. Sporophytic Apomixis

Sporophytic apomixis has been reported to be inherited simply as a dominant trait (Iwamasa *et al*., 1967; Parlevilet and Cameron, 1959). In the only mapping study published thus far on apomictic citrus, a 3:1 segregation ratio was observed for apomixis in a cross of *Citrus volkameriana* X *Poncirus trifoliata*. However, QTL mapping with molecular markers and phenotyping for nucellar embryony and polyembryony resulted in the identification of six QTL for both positive and negative effects, a more complex pattern than anticipated (Garcia *et al*., 1999). Further genetic and molecular studies in *Citrus* and its relatives, as well as other species reproducing by adventitious embryony should be forthcoming.

TABLE 2

Distribution of apomixis among flowering plants using the phylogenetic tree from the Tree of Life project (http://tolweb.org/tree?group=Angiosperms&contgroup=Spermatopsida) and data in the Appendix of Carman (1997)

Q2

Phylogenetic context				Family with respective type of apomixis		
				Gametophytic	Sporophytic	No. genera
Eudicots	Ranunculales			Ranunculaceae		1
	Proteales					nr
	Sabia					nr
	Trochodendraceae					nr
	Buxales				Buxaceae	1
		Didymeles				nr
	Core Eudicots	Gunnerales				nr
		Santalales				nr
		Saxifragales			Grossulariaceae	1
		Berberidopsidales				nr
		Vitaceae				nr
		Rosids	Fagales	Casuarinaceae		1
				Betulaceae	Betulaceae	3
			Cucurbitales	Cucurbitaceae	Cucurbitaceae	5
			Rosales	Rhamnaceae		1
				Urticaceae	Urticaceae	6
				Rosaceae	Rosaceae	12
			Fabales		Fabaceae	2
			Zygophyllales		Zygophyllaceae	1
			Oxalidales			nr
			Malpighiliales		Euphorbiaceae	3
				Malpighiaceae	Malpighiaceae	7
				Hypericaceae	Hypericaceae	3
				Ochnaceae	Ochnaceae	1
					Salicaceae	1
			Celastrales		Celastraceae	2
			Huaceae			nr
			Picramnia			nr
			Alvaradoa			nr
			Geraniales			nr
			Crossosomatales			nr
			Myrtales		Combretaceae	1
					Melastomataceae	1
				Myrtaceae	Myrtaceae	2
					Onagraceae	4
			Tapiscia sinensis			nr
			Brassicales	Brassicaceae	Brassicaceae	3
					Tropaeolaceae	1
			Malvales		Bombacaceae	3
					Dipterocarpaceae	3
				Thymelaeaceae	Thymelaeaceae	2
			Sapindales		Burseraceae	2
					Meliaceae	2
				Rutaceae	Rutaceae	11
					Anacardiaceae	1
		Dilleniaceae				nr
		Caryophyllales		Amaranthaceae	Amaranthaceae	2
					Cactaceae	5
				Plumbaginaceae		1
				Polygonaceae	Polygonaceae	1

TABLE 2
Distribution of apomixis among flowering plants using the phylogenetic tree from the Tree of Life project (http://tolweb.org/tree?group=Angiosperms&contgroup=Spermatopsida) and data in the Appendix of Carman (1997) *(Continued)*

Phylogenetic context				Family with respective type of apomixis		
				Gametophytic	Sporophytic	No. genera
		Asterids	Cornales			nr
			Ericales		Simplocaceae	1
				Cyrillaceae		1
					Myrsinaceae	1
			Solanales		Solanaceae	4
			Lamiales	Plantaginaceae		1
			Gentianales		Apocynaceae	1
			Boraginaceae	Boraginaceae	Boraginaceae	3
			Vahlia			nr
			Garryales			nr
			Oncotheca			nr
			Icacinaceae			nr
			Asterales	Asteraceae	Asteraceae	35
			Apiales		Apiaceae	1
			Dipsacales		Caprifoliaceae	1
				Adoxaceae		1
			Quintinia			nr
			Bruniaceae			nr
			Columellia			nr
			Tribeles australis			nr
			Eremosyne pectinata			nr
			Escalloniaceae			nr
			Aquifoliales			nr
	Cytinaceae					nr
	Balanophoraceae			Balanophoraceae		1
	Cynomorium					nr
Monocots	Alismatanae					nr
	Aranea			Araceae	Araceae	2
	Liliales				Smilacaceae	1
					Liliaceae	2
					Colchiceae	1
					Melanthiaceae	2
	Asparagales			Amaryllidaceae	Amaryllidaceae	3
				Alliaceae	Alliaceae	2
					Hemerocallidaceae	1
				Hyacinthaceae	Hyacinthaceae	1
					Tecophiliaceae	1
				Orchidaceae	Orchidaceae	13
					Agavaceae	1
	Pandanales					nr
	Dioscoreales			Taccaceae		1
				Burmanniaceae		1
	Arecanae					nr
	Zingiberanae					nr
	Commelinanae			Poaceae	Poaceae	38
Ceratophyllum						nr
Chloranthaceae						nr
Magnoliids	Magnoliales					nr
	Laurales				Calycanthaceae	2
	Piperales			Saururaceae		1
	Winterales					nr

nr = None reported.

2. Aposporous Apomixis

In most species, apospory appears to be simply inherited based on segregation of the trait in crosses between aposporous male parents and strictly sexual female parents. Some of these crosses have been made between biotypes within a species and others between different sexually compatible species. Since apomicts are always highly heterozygous, segregation of the trait for mode of reproduction as well as molecular markers can be observed in the F1 generation. This strategy differs from most inheritance and genetic mapping studies where inbred lines exhibiting different traits would be crossed. In some studies, both the male (apomict) and female (amphimict) maps have been constructed using data from the F1 population (Jessup *et al*., 2003; Porceddu *et al*., 2002; Pupilli *et al*., 2004).

Apospory rarely has been shown to segregate from parthenogenesis and behaves as a dominant trait, inherited in Mendelian fashion, although sometimes subject to segregation distortion. This pattern of inheritance has been observed for *Pennisetum squamulatum* (Dujardin and Hanna, 1983; Ozias-Akins *et al*., 1998); *Cenchrus ciliaris* syn. *Pennisetum ciliare* (Jessup *et al*., 2002; Sherwood *et al*., 1994), *Panicum maximum* (summarized in Savidan, 2000) (Ebina *et al*., 2005), *Brachiaria* sp. (do Valle *et al*., 1993; Miles and Escandon, 1996), *Paspalum notatum* (Martinez *et al*., 2001), *Ranunculus* sp. (Nogler, 1984b), and *Hieracium* sp. (Bicknell et al., 2000). The only clearly documented case of recombination between apospory and parthenogenesis is found in *Poa pratensis* (Albertini *et al*., 2001b). In *Poa*, parthenogenesis can be readily phenotyped in the absence of fertilization by the development of embryos after auxin treatment (Matzk, 1991). Using auxin treatment, parthenogenesis had been mapped as a single gene, qualitative trait using AFLP (amplified fragment length polymorphism) and SCAR (sequence characterized amplified region) markers (Albertini *et al*., 2001a; Barcaccia *et al*., 1998). From a similar cross of sexual and apomictic genotypes, a small number of non-parthenogenetic genotypes were observed to produce aposporous embryo sacs (Albertini *et al*., 2001b). The functionality of these embryo sacs was not tested. A more complex genetic model was postulated for *Poa pratensis* that included single, unlinked genes for apospory initiation, apospory prevention, parthenogenesis initiation, and parthenogenesis prevention as well as a megaspore development gene (Matzk *et al*., 2004). This model was predicated on the evolution of apomixis from sexual plants (Holsinger, 2000) and was supported by the observation of discrete classes of expressivity that could best be explained by the five-gene model.

As with parthenogenesis in *Poa*, apospory typically is phenotyped as a qualitative trait (i.e., an individual either has the capacity to produce aposporous embryo sacs or only meiotic embryo sacs), and not quantitatively with respect to the frequency with which aposporous embryo sacs can either be formed or function. Environment is known to affect frequency of mode of reproduction in some species (Knox, 1967), but not others (Hussey *et al*., 1991), which suggests that modifiers can play a role in the penetrance of the trait in some species; however, none of these modifiers have been mapped to date. Other evidence for modifiers comes from the reproductive phenotype of backcrosses involving an apomictic trispecific hybrid of *Pennisetum glaucum*, *P. purpureum*, and *P. squamulatum* (Dujardin and Hanna, 1984). A BC_3 plant produced >99% maternal progenies (Dujardin and Hanna, 1989), but several BC_4 plants produced only up to 89% maternal progenies (Hanna *et al*., 1993). The difference in penetrance of the trait is most likely due to modifiers because both generations carried an intact chromosome from *P. squamulatum* that was shown to be the only genomic region required for transfer of apomixis (Goel *et al*., 2003). Modifiers may also play a role in fate of embryo sac nuclei since the majority of central cells in BC_3 were binucleate compared with uninucleate central cells in *P. squamulatum* (Morgan *et al*., 1998; Ozias-Akins *et al*., 2003).

A single regulatory gene has been proposed as sufficient for the induction of apomixis (Peacock, 1992), and although simple genetic inheritance appears to support this hypothesis, molecular evidence suggests that more complex genetic control of the entire apomixis process cannot be discounted. In particular, the linkage groups typically transmitted with apospory display large blocks of non-recombining molecular markers leading to speculation that adapted gene complexes within supernumerary chromatin might be required for the function of at least certain types of apomixis (Ozias-Akins *et al*., 2003; Roche *et al*., 2001b). The association of apomixis with a non-recombining region of the genome first was described in *P. squamulatum* (Ozias-Akins *et al*., 1998). Extensive characterization of this chromosomal region using RFLPs and fluorescence in situ hybridization of apomixis-linked bacterial artificial chromosome clones has shown the region to be extremely large in size (estimated at least 50 Mbp), heterochromatic, and highly hemizygous (Akiyama *et al*., 2004; Akiyama *et al*., 2005; Goel *et al*., 2003; Ozias-Akins *et al*., 1998; Roche *et al*., 1999). A heterochromatic and hemizygous region is found in *P. squamulatum* as well as *C. ciliaris*, both polyploid apomicts, and is indicative of heteromorphism between the homologous chromosomal pairing partners apparently due to an insertion in both species combined with an inversion/translocation in *P. squamulatum*. Lack of recombination and hemizygosity have not been confined to apomictic *Pennisetum* and close relatives, but also were found in *Paspalum simplex* (Labombarda *et al*., 2002). The association of apospory with a heterochromatic region of the genome, rich in retrotransposons, as shown for *Pennisetum*, raises the intriguing possibility that chromatin structure and/or RNA interference (Lippman *et al*., 2004) could play a role in control of "apomixis" gene expression.

3. Diplosporous Apomixis

Contrary to apospory, linkage between diplosporous embryo sac development and parthenogenesis can readily be broken in at least two taxa of the Asteraceae, *Erigeron* and *Taraxacum*. In crosses of sexual diploid and apomictic triploid *Taraxacum*

genotypes, many 3x and 4x hybrids set seed in the absence of pollination as would be expected for apomictic *Taraxacum* where the endosperm develops autonomously (Tas and van Dijk, 1999). However, several 3x hybrids did not set seed when left unpollinated, although one produced exclusively 2n + n embryos when pollinated with a diploid genotype (van Dijk *et al*., 1999). This genotype was incapable of parthenogenesis. The independence between diplospory and parthenogenesis was further confirmed in a diplosporous 4x × sexual 2x cross, which produced only 5x progeny, and the DIP allele was shown to be linked to the 18S-25S rDNA locus in the reciprocal cross (van Dijk and Bakx-Schotman, 2004). Autonomous endosperm development also has been shown to segregate independently from parthenogenesis (van Dijk *et al*., 2003).

Similarly, progeny from a cross of sexual diploid and apomictic triploid genotypes of *Erigeron* showed a range of chromosome numbers that, except for the diploid state, were not strongly predictive of mode of reproduction (Noyes, 2000). Nevertheless, a single locus model for diplospory was a close fit to the data. While all parthenogenic plants were diplosporous, several diplosporous plants did not form embryos suggesting that a genetic component for parthenogenesis had been eliminated. When one such diplosporous, but nonparthenogenic triploid plant, was used as a female parent in crosses with a sexual diploid male parent, predominantly tetraploid (2n + n) progeny were recovered (Noyes, 2005). Genetic mapping of the segregating F1 population provided 4 and 12 AFLP markers linked to parthenogenesis and diplospory, respectively. Clearly, the two traits were segregating independently (Noyes and Rieseberg, 2000). Furthermore, 11 of the 12 diplospory-linked markers strictly cosegregated with each other and the trait, reminiscent of the recombinationally repressed apospory-linked region previously identified in *P. squamulatum*. Markers for parthenogenesis were not in a region of low recombination.

One diplosporous grass species, *Tripsacum dactyloides*, has been extensively studied, in particular because of its potential for transfer of apomixis to maize through traditional breeding (reviewed in Savidan, 2000). Diplospory was determined to be inherited simply in a cross of *Zea mays* × *Tripsacum dactyloides*, and five RFLP markers that had been mapped to the long arm of maize chromosome 6 were found to be strictly cosegregating with diplospory (Grimanelli *et al*., 1998; Leblanc *et al*., 1995). However, in a sexual, diploid *Tripsacum* cross, considerable recombination between the five linked markers was observed (Grimanelli *et al*., 1998). The localization of apomixis to a maize-*Tripsacum* chromosome translocation also supported the conclusion that only a single *Tripsacum* chromosome transmitted apomixis (Kindiger *et al*., 1996).

The association of apospory and diplospory with recombinationally suppressed chromosomal regions has now been observed in aposporous (*Pennisetum*, *Paspalum*) and diplosporous (*Erigeron*, *Tripsacum*) species. Surprisingly, the pattern of low recombination has been broken upon construction of an AFLP map of the diplospory region in *Taraxacum* (Vijverberg *et al*., 2004). Since diplosporous embryo sac development was inherited as a single, dominant gene (*DIP*), bulked segregant analysis was used to identify 34 AFLP markers that were linked and spanned a distance of 18.6 cM, although none was found to strictly cosegregate with diplospory. *Taraxacum* provides a singular example of recombination in a genomic region harboring genes for apomeiotic embryo sac formation.

C. Model Systems for the Study of Apomixis Aided by Comparative Genomics

The overwhelming majority of apomicts are not crop species and/or have relatively large genome sizes; therefore, molecular tools for their direct analysis are not well developed. Table 3 compares characteristics of some of the more intensely studied apomicts with their monocot or eudicot, model sexual species. The entire genome sequence now is available for one eudicot (*Arabidopsis thaliana*) (The Arabidopsis Genome Initiative, 2000) and one monocot plant (*Oryza sativa*) (International Rice Genome Sequencing Project, 2005), providing templates for genome structural comparisons. There are some criteria that are desirable for both model sexual and apomictic species (•), although other criteria are specific to apomicts (➢). Many of these criteria have been previously described (Bicknell, 2001; Bicknell and Koltunow, 2004) and are listed below:

- Easy to cultivate
- Easy to propagate
- ➢ Perennial (for maintenance of specific genotypes)
- Compact plant stature
- Short generation time
- Abundant seed set
- Small genome size
- ➢ Easy to cytologically evaluate mode of reproduction
- ➢ Easy to genetically evaluate mode of reproduction
- ➢ Highly apomictic (i.e., low level of residual sexual reproduction)
- ➢ Fertile pollen (required for genetic studies)
- ➢ Cross-compatible sexual and apomictic biotypes in the same species, preferably at the same ploidy level
- Diploid (extremely rare for apomicts)
- Transformation competent

All of the monocot apomicts are relatively large in plant stature and are likely to have low transformation efficiencies. However, since monocots and eudicots are evolutionarily distant, it would be pertinent to study apomixis in both groups of plants. Comparative genetic mapping is one method to transfer information from model species to larger genome species that have fewer molecular tools. Although genome sequence of one eudicot and one monocot is now available, the most direct application would be to closely related species. In this respect, the *Boechera holboellii* (syn. *Arabis holboellii*) complex has the greatest advantage since it is in the same family (Brassicaceae) as the first plant genome sequenced, *Arabidopsis thaliana*. This apomictic

TABLE 3
Characteristics of model apomicts and related model sexual species

Species	MOR[a]	Phylogenetic context[b]	Genome size[c] (Mbp)	Genetic/genomic resources	Generation time	No. plants per 1 m^2	Transformation	Comments
Arabidopsis thaliana	S	Core Eudicots/Rosids/Brassicales/ Brassicaceae	157	Ecotypes/Mutants/Maps Genome Sequence	<2 mo	1000	High efficiency	
Boechera holboellii	A	Core Eudicots/Rosids/Brassicales/ Brassicaceae	Unpubl	Ecotypes		10–100	Unpublished See comments	(1)
Hypericum perforatum	A	Core Eudicots/Rosids/Malpighiales/ Hypericaceae	637	Ecotypes		<10	Unpublished	
Taraxacum officinale	D	Core Eudicots/Asterids/Asterales/ Asteraceae	1250	Ecotypes/Maps/BAC library	~6 mo	10–100	Unpublished See comments	(2)
Erigeron	D	Core Eudicots/Asterids/Asterales/ Asteraceae	Unpubl		~2 yr	10–100	Unpublished	(3)
Hieracium subgenus *Pilosella*	A	Core Eudicots/Asterids/Asterales/ Asteraceae	≥1054	Mutants	3–6 mo	10–100	Bicknell and Borst, 1994	(4)
Oryza sativa	S	Monocots/Commelinanae/Poaceae	490	Ecotypes/Mutants/Maps Genome Sequence	~ 6 mo	20	Moderate efficiency	
Tripsacum dactyloides	D	Monocots/Commelinanae/Poaceae	3800	Mutants/Maps	~ 6 mo	<10	Unpublished See comments	(5)
Pennisetum squamulatum	A	Monocots/Commelinanae/Poaceae	4700	Maps/BAC library	~ 6 mo	<10	Unpublished See comments	(6)
Cenchrus ciliaris	A	Monocots/Commelinanae/Poaceae	1300	Maps/BAC library	~6 mo	<10	No stable See comments	(7)
Poa pratensis	A	Monocots/Commelinanae/Poaceae	≥4155	Maps		10–100	Ha *et al*., 2001	(8)

[a] S—sexual, A—aposporous, D-diplosporous.

[b] According to http://tolweb.org/tree?group=Angiosperms&contgroup=Spermatopsida.

[c] According to RBG Kew C-value database (http://www.rbgkew.org.uk/cval/homepage.html).

(1) Close relative of *Arabidopsis*; Some *Arabis* species have been renamed as *Boechera*; *Arabis gunnisoniana*, an apomict, has been transformed (Taskin *et al*., 2003).

(2) Segregation of diplospory and parthenogenesis; BAC library (P. van Dijk, personal communication); *Taraxacum mongoliam* transformation (Song *et al*., 1991).

(3) Segregation of diplospory and parthenogenesis.

(4) Easy to transform (Bicknell and Borst, 1994); detailed characterization of floral developmental events (Koltunow *et al*., 1998); mutants (Bicknell *et al*., 2001; Weld *et al*., 2002).

(5) Close relative of *Zea mays*; numerous publications on transformation of maize; Relatively large ovules.

(6) Genes for apomixis localized to 50% of a chromosome arm; BAC library (Roche *et al*., 2002); A sexual relative, *P. glaucum* has been transformed (Goldman *et al*., 2003 and citations therein).

(7) syn. *Pennisetum ciliare*; BAC library (Roche *et al*., 2002); transformation attempts have been published (Ross *et al*., 2000; Bhat *et al*., 2001).

(8) Segregation of apospory and parthenogenesis.

species complex is diplosporous and primarily pseudogamous (Boecher, 1951; Naumova *et al*., 2001). Apomictic *Boechera* are often triploid (2n = 21), but can also be aneuploid or diploid (Boecher, 1951), although the most recent analyses indicate that some *B. holboellii* apomicts are near-diploid aneuploids (2n = 15) containing a B chromosome rather than strict diploids (Sharbel *et al*., 2004; Sharbel *et al*., 2005). Whether the B chromosome is always associated with the apomixis phenotype remains to be tested.

Apomictic grasses also have the advantage of one sequenced genome in the family, that of *Oryza sativa*. Comparative mapping in cereals has shown that some linkage blocks can be highly conserved in gene order and content across species that diverged from one another approximately 60 million years ago (Devos and Gale, 2000; Devos, 2005). This macrosynteny observed on a genetic map scale, however, often can be interrupted by translocation of chromosomal regions from another part of the genome or intra- and inter-chromosomal duplications (Bennetzen and Ramakrishna, 2002; Bennetzen and Ma, 2003). In apomicts, comparative mapping has been a target of several projects in order to utilize the genome information from model species such as rice and maize (Grimanelli *et al*., 1998; Leblanc *et al*., 1995; Pessino *et al*., 1998; Pessino *et al*., 1997; Pupilli *et al*., 2001). The results have not demonstrated common regions of synteny associated with apomixis among multiple apomictic grass species. Grimanelli *et al*. (2001) argue that such results may indicate that apomixis has evolved independently in related species and may have a different genetic basis in each. It is not unprecedented that a single phenotype, dwarf being an example, can result from the mutation of positionally unrelated genes, although the genes may operate through the same metabolic or developmental pathway.

Comparative mapping in apomictic plants largely has been carried out with restriction fragment length polymorphism (RFLP) analysis. Early studies identified common RFLP fragments among multiple apomictic *Pennisetum* species/genotypes (Lubbers *et al*., 1994; Ozias-Akins *et al*., 1993; Roche *et al*., 1999). However, anonymous DNA clones from *P. squamulatum* that were linked with apomixis often did not hybridize to sexual *Pennisetum* species, including pearl millet (*P. glaucum*); therefore, they were not useful for comparative mapping with other grasses. More recently, limited DNA sequencing of BAC clones localized to a region of the *P. squamulatum* genome shown to be transmitted with apospory has allowed the identification of likely orthologous regions in rice based on sequence similarities (Conner *et al*., in preparation). Macrosynteny between *P. squamulatum* and rice appears to be highly interrupted although regions of microsynteny are apparent (Gualtieri et al., 2006). Other mapping efforts in apomictic grasses have taken advantage of available cDNA clones mapped in sexual species such as maize and rice. Using cDNA clones mapped in rice on a segregating population of *Paspalum simplex*, apospory was localized to a region of the genome syntenic with the long-arm telomeric region of rice chromosome 12 (Pupilli *et al*., 2001). Synteny within this region was complete between *P. simplex* and *P. malacophyllum* but partial with *P. notatum* (Pupilli *et al*., 2004). The apomixis-associated chromosome in *P. notatum* appears to have been involved in a translocation, and as a consequence, the region containing two of the five mapped rice markers falls on another chromosome in this species and can be eliminated from consideration as containing a major effect gene for apomixis.

1. Ploidy Variation

Genetic and genomic analysis of apomixis is further complicated by the polyploid nature of essentially all apomicts. Polyploidy does not appear to be required for apomixis to be expressed. There are multiple examples of polyhaploid plants that have developed from parthenogenetic reduced eggs in apomicts, and such plants, although often lacking vigor, nevertheless reproduce by apomixis (Bicknell, 1997; Dujardin and Hanna, 1986; Leblanc *et al*., 1996). One example where expression of apomixis may be dependent on ploidy level or gene dosage, perhaps not of the apospory factor itself but of an epistatic regulator, comes from *Paspalum* (Quarin *et al*., 2001). Certain diploid genotypes of *Paspalum* form vigorous stands with no sympatric occurrence of tetraploids and therefore are unlikely to have arisen as dihaploids. Nevertheless, aposporous embryo sacs can be found at a low frequency in these genotypes. This frequency increases dramatically when the chromosomes of the diploids are doubled. Aposporous embryo sacs were not observed in an induced tetraploid from a strictly sexual diploid genotype, suggesting that penetrance of the apomixis phenotype was dependent on ploidy level in this case.

In several studies, transmission of apomixis "genes" through haploid gametes appears to have been impaired, probably as a result of linked deleterious mutations associated with a typically non-recombining region of the genome or of the "apomixis factor" itself (Nogler, 1984a; Roche *et al*., 2001). An exception is found in *Hieracium* where haploid gametes were shown to be viable in a cross of sexual 4x × apomictic 3x genotypes since many apomictic triploids were recovered from n + n events; however, the diploid zygote apparently was disadvantaged in sexual 2x × apomictic 3x seeds because no apomictic diploids were produced (Bicknell *et al*., 2000). In addition to the occasional parthenogenetic development of a reduced egg of an apomict, apomixis predicts that higher ploidy levels also would arise through the occasional fertilization of unreduced eggs with either reduced or unreduced sperm. The varied combinations of reduction/nonreduction with fertilization/parthenogenesis result in a range of possible ploidy levels (Table 1) and participate in the formation of agamic complexes through diploid-tetraploid-dihaploid cycles, e.g., *Bothriochloa-Dichanthium* (de Wet, 1968; Harlan and de Wet, 1963).

IV. GENE EXPRESSION IN OVULES OF APOMICTS

In addition to structural analysis of a natural apomict genome, several groups have sought to isolate genes differentially expressed between ovules of an apomictic versus sexual genotype.

Much of this work has been implemented with differential display, which uses arbitrary primers (Liang and Pardee, 1992). One caveat of differential display stems from the highly heterozygous, and thus polymorphic at the DNA sequence level, nature of all apomicts. It is not possible to create isogenic lines that vary only in their mode of reproduction; therefore, any expressed gene identified by nucleotide sequence-based techniques would have to be verified, preferably by northern blot analysis or sequencing of corresponding cDNA clones, as differing in expression rather than just DNA sequence polymorphism. In some cases, the effect of this heterozygosity has been minimized by using pools of mRNA from different individuals, one pool from apomictic individuals and the second from sexual.

Differential display has successfully detected bands uniquely amplified in either apomictic or sexual genotypes or their respective pools (Leblanc *et al*., 1997; Pessino *et al*., 2001; Rodrigues *et al*., 2003; Vielle-Calzada *et al*., 1996). In two studies (*Brachiaria* and *Pennisetum*), mature ovules were used for RNA extraction and although differential expression was validated in one study, the cloned genes are unlikely to be responsible for the induction of apomixis (Leblanc *et al*., 1997; Vielle-Calzada *et al*., 1996). In similar work with *Paspalum notatum*, verification of differentially expressed transcripts was only carried out by RT-PCR, a technique that also would be affected by sequence polymorphisms. A cDNA library screen was used to isolate a single cDNA from *Panicum maximum* that was expressed in flowers of the apomict but not the sexual (Chen *et al*., 1999). Differential display performed on mature embryo sac stage and developing embryo and endosperm stage ovules of *Hieracium* resulted in the isolation of a DEFICIENS homolog that showed transient down-regulation in the chalazal region of ovules from apomicts (Guerin *et al*., 2000). Recently, careful staging of ovary development has led to the identification of differentially expressed transcripts in *Brachiaria* (Rodrigues *et al*., 2003) and *Poa* (Albertini *et al*., 2004). It is likely that careful staging will be critical for interpretation of results, particularly if misexpression, rather than unique gene expression, is responsible for the switch in mode of reproduction (Figure 3).

Perhaps the most thorough study using a transcript profiling approach comes from *Poa pratensis* where cDNA-AFLP analysis resulted in the isolation of fragments that were specific to carefully staged florets of either a sexual or apomictic genotype and were not present in leaves (Albertini *et al*., 2004). Out of 2248 transcript-derived fragments, 179 were qualitatively or quantitatively different between the sexual and apomictic genotypes. After sequencing and BLAST analysis, the majority of fragments showed similarity to genes expressed in reproductive organs of other species. Of eight full-length cDNA sequences cloned (*PpRAB*1-1, *PpRAB*1-2, *PpARM, PpAPK, PpSERK1, PpSERK2, APOSTART1, APOSTART2*), none was specifically expressed in apomictic or sexual genotypes but rather their expression was differentially modulated or quantitatively different, lending additional support to the hypothesis that apomixis results from a deregulated sexual pathway (Albertini *et al*., 2005; Albertini *et al*., 2004).

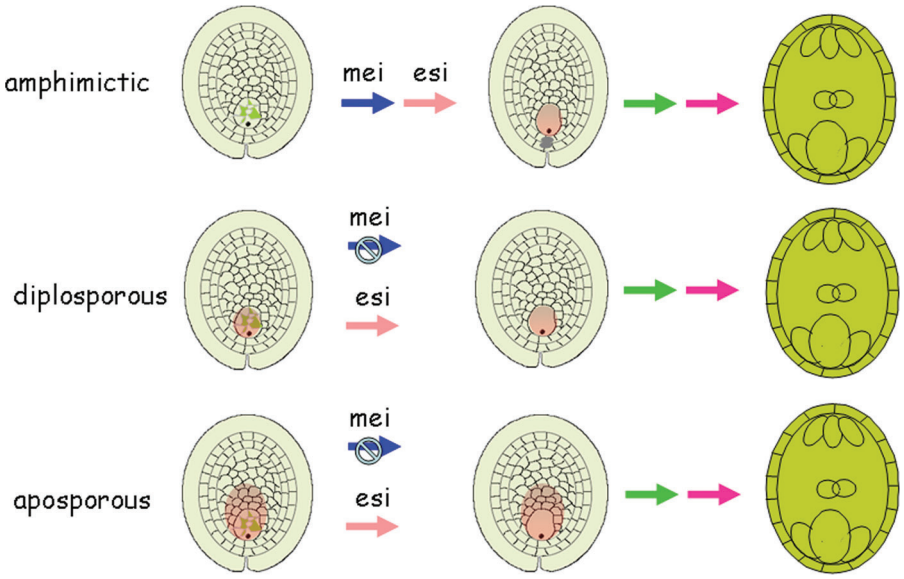

FIG. 3. A simplistic model of heterochronic changes in gene expression (pink) between amphimictic and apomictic ovules, which follows Nogler's postulate that "the physiological conditions for the activation of genes involved in the differentiation of the embryo sac, which normally prevail only in megaspores, prevail also in certain unreduced cells." An embryo sac initiation gene could be misregulated in apomicts such that earlier expression in the megaspore mother cell could prevent meiosis and lead directly to embryo sac development by mitosis. Similarly, ectopic expression in somatic nucellar cells could result in the development of aposporous embryo sacs. The resulting embryo sacs may be indistinguishable among developmental types.

V. BEYOND NATURALLY OCCURRING APOMIXIS

An alternative to determining the genetic mechanism(s) underlying apomixis in a natural apomict is to induce mutations in sexual plants that result in phenotypes displaying one or more components of apomixis (Koltunow *et al*., 1995). The developmental biology of ovules (Skinner *et al*., 2004), female gametophyte (Drews *et al*., 1998; Yadegari and Drews, 2004), and endosperm (Gehring *et al*., 2004; Olsen, 2004) has recently been reviewed, and although much of the research has focused on genes involved in sexual reproduction, it can only enhance our progress in understanding apomictic reproduction, particularly if apomixis is indeed a deregulation of the sexual pathway. This approach to study sexual plants has been very successful for isolation of mutants from *Arabidopsis* that will develop endosperm in the absence of fertilization (Gehring *et al*., 2004; Grossniklaus *et al*., 2001b), one component of the apomixis pathway. Other mutants of genes involved in sexual reproduction that may be of relevance to apomixis, such as those required for ovule identity and pattern formation (MADS box genes, *ANT, BEL1, HLL, INO, LUG*), megaspore mother cell and embryo sac development (*NZZ/SPL, eMAC1, AM1, AFD, SWI1, EL, AGP18*), embryo initiation (*SERK, LEC, BBM, WUS*), and endosperm formation (*FIS, FIE, MEA*), among others, have been described (Acosta-Garcia and Vielle-Calzada, 2004; Autran *et al*., 2005; Chaudhury *et al*., 1998; Estrada-Luna *et al*., 2002; Koltunow and Grossniklaus, 2003; Spillane *et al*., 2001).

VI. CONCLUSIONS

Apomixis is a developmental process that directly or indirectly interferes with and may replace sexual reproduction in angiosperms. Gametophytic apomixis, however, maintains an alternation of generations without participating in chromosome reduction. Since diplospory and apospory, two morphologically distinguishable forms of gametophytic apomixis, may differ in either their regulatory control or heterochronicity of gene expression, it will be important to understand the genetic mechanisms underlying both types of gametophytic apomixis in order to have the greatest success with manipulation of the trait in crop plants.

Generation of progeny that are genotypically identical to the maternal parent would have its most practical and widespread application in agriculture, particular in those crops that are cultivated as hybrids. Whether genes for apomixis can be isolated from natural apomicts and transferred to crop species, or whether apomixis can be synthesized by combining mutations for its components in a sexual plant still is an open question. Nevertheless, analysis of such isolated genes and mutations is providing valuable information for our understanding of female reproduction and could lead to other discoveries, independent of apomixis, which could increase crop productivity.

ACKNOWLEDGEMENTS

I thank Yajuan Zeng for original photographs of embryo sacs and Joann Conner for critical reading of the manuscript. USDA-NRI and NSF-PGRP have provided recent funding for apomixis research to P.O.-A..

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