

The Genetic Control of Apomixis: Asexual Seed Formation

Melanie L. Hand and Anna M. G. Koltunow¹

Commonwealth Scientific and Industrial Research Organization (CSIRO) Plant Industry,
Glen Osmond, South Australia 5064, Australia

ABSTRACT Apomixis (asexual seed formation) is the result of a plant gaining the ability to bypass the most fundamental aspects of sexual reproduction: meiosis and fertilization. Without the need for male fertilization, the resulting seed germinates a plant that develops as a maternal clone. This dramatic shift in reproductive process has been documented in many flowering plant species, although no major seed crops have been shown to be capable of apomixis. The ability to generate maternal clones and therefore rapidly fix desirable genotypes in crop species could accelerate agricultural breeding strategies. The potential of apomixis as a next-generation breeding technology has contributed to increasing interest in the mechanisms controlling apomixis. In this review, we discuss the progress made toward understanding the genetic and molecular control of apomixis. Research is currently focused on two fronts. One aims to identify and characterize genes causing apomixis in apomictic species that have been developed as model species. The other aims to engineer or switch the sexual seed formation pathway in non-apomictic species, to one that mimics apomixis. Here we describe the major apomictic mechanisms and update knowledge concerning the loci that control them, in addition to presenting candidate genes that may be used as tools for switching the sexual pathway to an apomictic mode of reproduction in crops.

WITHIN flowering plants (angiosperms), reproduction through seeds occurs by sexual and surprisingly, by asexual pathways. The former generates variation, while asexual seed reproduction (apomixis) produces genetically identical progeny. (See Table 1 for definitions.) The ability to produce genetically identical progeny via seed is of significant value to agriculture for its power to fix complex favorable genotypes, including the higher yields often found in F_1 hybrids. Apomixis is documented in more than 120 angiosperm genera (Carman 1997); however, no major seed crop species are apomictic, and attempts to introduce the apomixis trait to crops from apomictic relatives by cross-pollination have been largely unsuccessful (Savidan 2000). Apomixis is also of interest from developmental and evolutionary perspectives. Most natural apomicts are classified as facultative because they have the ability to reproduce both sexually or asexually. To understand the different pathways of apomictic seed formation, it is instructive to first examine the sequence of events required for sexual seed formation.

Sexual Seed Formation in Flowering Plants

Seed formation in angiosperms begins with the developmental decision to switch from a vegetative to a reproductive mode of growth. This manifests with the formation of a flower containing male (anther) and female (ovule) reproductive organs, and within these organs, diploid gamete precursor cells form (Figure 1). Many diploid male gamete precursor cells differentiate in the anther of the flower; however within the ovule, which is the progenitor of the seed, a single female gamete precursor cell differentiates. This cell is called a megaspore mother cell. Both male and female gamete precursor cells undergo meiosis followed by mitosis to form the mature multicellular male and female gamete-containing structures, known as the pollen grain and embryo sac, respectively. Figure 1 illustrates the events leading to the formation of the most common form of embryo sac, called the *Polygonum*-type, which is found in >70% of flowering plants. The diploid megaspore mother cell undergoes meiosis, forming four haploid megaspores. One of these megaspores is selected to become the functional megaspore and the others degenerate. The haploid functional megaspore then undergoes three mitotic divisions without cytokinesis, to produce a coenocyte containing eight haploid nuclei surrounded by a cell wall (stage not shown in Figure 1) (reviewed by Drews and Koltunow

Table 1 Definitions

Term	Definition
Apomixis	Plant asexual reproduction through seed. Progeny of an apomictic plant are genetically identical to the maternal plant.
Embryo sac	Multicellular female gamete-producing structure of a flowering plant. Also known as the female gametophyte.
Apomeiosis	Avoidance or failure of meiosis during the development of an embryo sac.
Diplospory	Apomeiosis pathway where a diploid embryo sac develops from the megaspore mother cell.
Apospory	Apomeiosis pathway where a diploid embryo sac develops from a somatic ovule cell that is not the megaspore mother cell, called the aposporous initial (AI) cell.
Parthenogenesis	Development of an egg found in a diplosporous or aposporous embryo sac into an embryo without fertilization.

2011). The nuclei migrate and cellularize, producing a seven-celled mature embryo sac. It contains three antipodal cells whose function is unknown, two synergid cells that have roles in attracting the male gametes, a single egg cell, which is the progenitor of the embryo in the seed, and a large central cell containing two nuclei that fuse to form a diploid precursor nucleus of the endosperm (Figure 1). Seed development initiates following double fertilization, where the male pollen tube containing two sperm cells enters the ovule. One sperm cell fuses with the egg cell stimulating divisions and pattern-forming events that give rise to the diploid embryo. The other sperm cell fuses with the diploid central cell nucleus to initiate divisions to form the nutritive triploid endosperm, which provides essential resources to the developing embryo (Berger *et al.* 2008). The ovule tissues surrounding the developing embryo and endosperm contribute to the seed coat in the mature seed.

Apomixis Mechanisms

In contrast to sexual seed formation, apomixis can occur by various mechanisms that share three common developmental components: (i) a bypass of meiosis during embryo sac formation (apomeiosis), (ii) development of an embryo independent of fertilization (a process known as parthenogenesis), and (iii) formation of viable endosperm either via fertilization-independent means or following fertilization with a sperm cell (Koltunow and Grossniklaus 2003). Derivation of the egg from a diploid maternal cell without meiotic reduction, and its subsequent fertilization-independent development into an embryo, means that the progeny derived from apomictic development are clonal and therefore genetically identical to the maternal parent.

Apomixis mechanisms are historically subdivided into two categories and classified as either gametophytic or sporophytic, based on whether the embryo develops via a gametophyte (embryo sac) or directly from diploid somatic (sporophytic) cells within the ovule (Figure 1; Nogler 1984; Koltunow 1993). During sporophytic apomixis, development of an embryo sac following the typical angiosperm sexual pathway still occurs. However, during mitosis of the functional megaspore, diploid somatic ovule cells surrounding the embryo sac differentiate and have an embryogenic cell fate. These

embryo initial cells begin mitosis forming multiple globular-shaped embryos that can develop to maturity only if the sexually derived embryo sac is fertilized, as the sexual and asexual embryos share the nutritive endosperm. Sporophytic apomixis can therefore lead to a seed containing multiple embryos (Figure 1) and is common in citrus. The sexually derived embryo may or may not mature or germinate (Koltunow *et al.* 1995; Koltunow *et al.* 1996). Sporophytic apomixis has not been extensively studied at the molecular level; however, it appears genetically complex (García *et al.* 1999). The remainder of this review focuses on gametophytic apomixis.

Gametophytic apomixis relates to mechanisms where an embryo sac is mitotically formed from a diploid cell in the ovule, bypassing meiosis. Another term for such mitotic embryo sac development is apomeiosis. Embryo development in gametophytic apomixis is fertilization independent whereas endosperm formation may or may not require fertilization (Figure 1). Apomeiotic embryo sac development is further subdivided into two types (diplospory and apospory) based upon the origin of the diploid precursor cell that ultimately gives rise to the mitotically derived embryo sac. In diplospory, the precursor is the megaspore mother cell (or a cell with an altered program that differentiates in the megaspore mother cell position) (Figure 1). This cell may enter meiosis and abort the process or it may immediately begin mitosis. Diplospory has been observed in species including *Taraxacum officinale* (dandelion), *Boechera* spp., *Erigeron annuus*, and *Tripsacum dactyloides*. By contrast, apospory involves development of the embryo sac via mitosis not from the megaspore mother cell, but from a diploid somatic cell positioned adjacent to the megaspore mother cell (Figure 1). This cell, termed the aposporous initial cell, undergoes mitosis and the nuclei cellularize. The mitotic events of diplospory and apospory may or may not make a seven-nucleate *Polygonum*-type embryo sac; however, an egg, a central cell, and synergids are typically formed (Figure 1). Depending on the species, both sexually derived and aposporous embryo sacs can coexist within the one ovule, as occurs in *Brachiaria* species (Araujo *et al.* 2000). Alternatively, development of the aposporous embryo sac may lead to the demise of the sexually derived embryo sac or pathway as occurs in aposporous *Hieracium* and *Pennisetum* species (Peel *et al.* 1997; Koltunow *et al.* 2011). Embryo development from the diploid egg formed

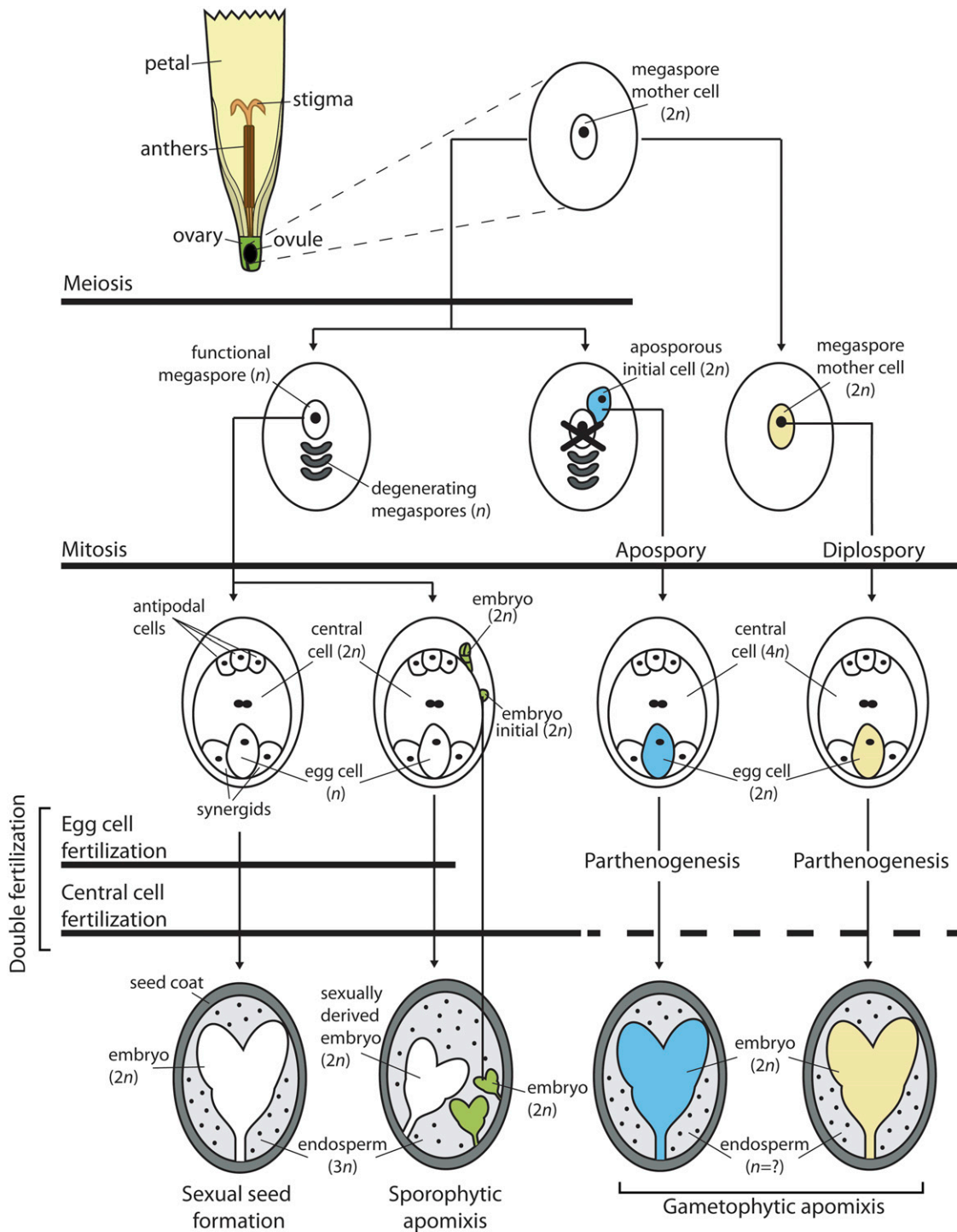


Figure 1 Mechanisms of sexual and apomictic seed development. Seed developmental processes occur within the ovule of the flower, which is depicted as a single floret typical of *Hieracium* species in this figure. This diagram compares the major differences in the seed development pathway for sexual seed formation and the apomictic mechanisms of sporophytic and gametophytic apomixis. Meiosis, mitosis, and double fertilization constitute the major components of the seed formation pathway. Arrows passing through each of these components represents the involvement of a given component within a particular pathway. In the process of gametophytic apomixis, embryo sac formation can occur via either apospory or diplospory, which are distinguished by different embryo sac precursor cells. In gametophytic apomixis, embryo formation is initiated in the absence of fertilization (parthenogenesis); however, endosperm formation can occur either with or without fertilization, which is represented by a dashed line. The relative ploidy level of cells (n) is tracked for various components throughout each pathway. The ploidy level of endosperm formed through gametophytic apomixis is variable, depends on a number of factors, and is therefore represented by a question mark (?). In the depicted apospory pathway, the sexual pathway is shown to terminate once the aposporous initial cell undergoes mitosis. Different colors track the precursor cells that form the embryo for each pathway: sexual (white), sporophytic apomixis (green), diplospory (yellow), and apospory (blue).

in aposporous and diplosporous embryo sacs occurs without fertilization and the term parthenogenesis is used to describe this process (Figure 1). Endosperm development can occur without fertilization of the central cell, although this is rare, occurring predominantly in members of the daisy family (Asteraceae). Apomicts that require fertilization to produce endosperm have disturbed maternal and paternal genome contributions (m:p) in the endosperm. For example, in such apomicts, fertilization of a tetraploid central cell may lead to a 4m:1p endosperm genome ratio, in contrast to the typical 2m:1p ratio of fully sexually reproducing species. Those apomicts that require fertilization to develop endosperm have therefore developed multiple strategies to ensure seed viability and these have been discussed in previous reviews (Koltunow and Grossniklaus 2003; Curtis and Grossniklaus 2008).

Genetics and Inheritance of Apomixis

The presence of varied apomictic mechanisms and the phylogenetic positioning of apomictic species throughout many angiosperm families together imply that apomixis has evolved independently multiple times (Carman 1997; Van Dijk and Vijverberg 2005). Genetic analyses using apomicts as pollen donors in crosses with sexual individuals as maternal parents have shown apomixis to be inherited as a dominant trait. Early genetic studies proposed that a single dominant locus controlled apomixis in most studied apomictic species. For some species, it has since been revealed that many apomixis loci exhibit suppressed recombination. Studies involving genetic analyses searching for rare recombinants in suppressed regions and gamma deletion mutagenesis have revealed that in some species, the developmental components of apomixis (meiotic avoidance, parthenogenesis, and fertilization-independent endosperm development) are controlled by independent loci. For instance, in *Taraxacum* and *Erigeron* species, two independent loci have been identified that control diplospory and parthenogenesis (Van Dijk *et al.* 1999; Noyes and Rieseberg 2000). Similarly, apospory and parthenogenesis are controlled by two independent loci in *Hypericum*, *Poa*, *Hieracium*, and *Cenchrus* species (Albertini *et al.* 2001; Catanach *et al.* 2006; Schallau *et al.* 2010; Conner *et al.* 2013). Genetic studies in *Hieracium*, which is also capable of fertilization-independent endosperm formation, have revealed that this trait can also segregate independently of the other two apomictic components (Ogawa *et al.* 2013).

Interestingly, characterized gamma deletion mutants have revealed that sexual reproduction is the default pathway in apomictic *Hieracium praealtum*. A series of deletion mutants that lack the apospory locus (called *LOSS OF APOMEIOSIS* or *LOA*) and an additional locus responsible for fertilization-independent seed development (called *LOSS OF PARTHENOGENESIS* or *LOP*) were developed. Deletion of either *LOA* or *LOP* alone sees the return of the sexual pathway for that component. For example, plants with *LOA* deleted no longer produce diploid embryo sacs via apospory as they lack apomeiosis function. Instead, the megaspore mother cell undergoes

meiosis and as aposporous initial cells are not formed, a functional haploid embryo sac develops, and the egg and central cell form an embryo and endosperm, respectively, in the absence of fertilization. Deletion of both the *LOA* and *LOP* loci results in complete reversion to sexual development (Catanach *et al.* 2006; Koltunow *et al.* 2011). Apomixis in aposporous *Hieracium* therefore seems to be superimposed on the sexual pathway, suggesting that apomixis may redirect the fate of cells with gametic potential, rather than being a detached and completely independent pathway (Koltunow *et al.* 2013). This theory is consistent with the known coexistence of apomixis and sex, suggesting that if the apomixis pathway is not initiated in an ovule, the sexual pathway remains functional. One interpretation is that apomixis may be thought of as an alteration to the spatial and temporal expression of sexual reproductive processes, where the sexual pathway is initiated at a different time and/or in a different cell. For diplospory, the megaspore mother cell appears to be specified with gametic potential; however, the sexual pathway is altered so that the events of meiosis do not initiate, or meiosis is altered so that it is not successfully completed. For apospory, an altered sexual pathway appears to be expressed in additional cells within the ovule, which results in diploid somatic cells mitotically forming an embryo sac, which is usually the role of the haploid functional megaspore in sexual reproduction. During the development of the diploid diplosporous and aposporous embryo sacs, the egg cell and central cell differentiate with a capability to directly transition into an embryo and endosperm, respectively, if seed formation is completely fertilization independent. Studies using various reproductive markers support these hypotheses (Tucker *et al.* 2003; Rodrigues *et al.* 2010).

Most apomicts are polyploid, and apomixis has previously been proposed to be a consequence of hybridization and/or genome doubling, *i.e.*, the events of polyploidization (Carman 1997). However, polyploidization alone is not sufficient to induce apomixis, as not all polyploids are apomicts. Apomixis has previously been considered as an evolutionary dead end, as it is associated with loss of the ability to generate genetic variation through recombination, which renders apomictic populations incapable of adapting to environmental change. However, the incomplete penetrance of apomixis and maintenance of a normal sexual pathway in most apomicts provides opportunity for genetic diversification and evolution via sexual recombination (Chapman *et al.* 2003; Hörandl and Hojsgaard 2012).

Characteristics of Apomixis-Related Loci

Attempts to identify apomixis loci through map-based cloning approaches have proven challenging in many apomictic species, as recombination is often suppressed around apomixis loci. This distortion of recombination frequency at apomixis loci suggests strong divergence of alleles at the apomixis-related genomic regions. In the most extreme cases, the divergence has

been associated with hemizyosity of the associated loci *e.g.*, *Hieracium* (Okada *et al.* 2011), *Pennisetum* (Akiyama *et al.* 2005), and *Paspalum* (Calderini *et al.* 2006). Some apomixis loci have also been associated with heterochromatin and/or substantial repetitive sequences. In apomictic *Pennisetum squamulatum*, the apospory-specific genomic region (ASGR) is located at a heterochromatic and nonsynaptic telomeric region of a single chromosome (Akiyama *et al.* 2004). Chromosomes carrying the ASGR in *Pennisetum* and the *LOA* locus in *Hieracium* are associated with extensive repetitive sequence and transposon-rich regions (Akiyama *et al.* 2004; Okada *et al.* 2011). Similarly, the hemizygous apomictic controlling locus (ACL) of *Paspalum*, which shows strong suppression of recombination, has undergone large-scale rearrangements due to transposable elements, when compared to a syntenic region in rice (Calderini *et al.* 2006). These commonalities of repetitive, heterochromatic regions associated with apomixis loci have led to suggestions that such a repetitive chromosomal structure may have a functional role in apomixis. For example, one hypothesis is that the repetitive sequences may act as a sink to sequester factors involved in the sexual reproductive pathway, thereby altering the expression of sexual reproductive processes, and possibly causing apomixis (Koltunow and Grossniklaus 2003). However, recent results suggest that the abundant repetitive sequences on a chromosome carrying the *Hieracium* apospory locus (*LOA*) are not required for apospory to successfully occur. In this study, progeny arising from a cross between sexual *H. pilosella* and apomictic *H. praealtum* that are recombinant for *LOA*-linked markers are missing the extensive repetitive sequence structure associated with the locus, yet still remain apomictic (Kotani *et al.* 2013). If the repetitive chromosomal structure associated with some apomixis loci is not required for function of the locus, it is possible that these structural features and allele divergence are a consequence of asexual reproduction and suppressed recombination, which may have evolved to maintain genic elements required for apomixis. The chromosomal structure and degree of recombination may reflect the evolutionary age of apomixis in a given species. For instance, those species for which the apomixis components can be separated by recombination are likely to be evolutionarily recent apomicts [*e.g.*, *Poa* (Albertini *et al.* 2001), *Taraxacum* (Vijverberg *et al.* 2010), *Hypericum* (Schallau *et al.* 2010), *Erigeron* (Noyes and Rieseberg 2000), and *Panicum* (Kaushal *et al.* 2008)]. By contrast, evolutionarily older apomicts are likely to be those characterized by apomixis associated loci found in heterochromatic regions that display suppressed recombination [*e.g.*, *Pennisetum* (Ozias-Akins *et al.* 1998), *Brachiaria* (Pessino *et al.* 1998), *Paspalum* (Labombarda *et al.* 2002), and *Tripsacum* (Grimanelli *et al.* 1998)].

Genes Linked with Apomixis Loci

Despite the suppressed recombination observed in some apomictic species, loci genetically linked to components of apomixis have been identified in various species, and sequenc-

ing of these loci has revealed a number of genes with the potential to have critical roles in apomixis.

The ASGR of *Pennisetum* was originally identified following random amplified polymorphic DNA (RAPD)-based analysis of a selection of apomictic and sexual plants, which revealed a set of apomixis-specific markers that define the ASGR (Ozias-Akins *et al.* 1998). Shotgun sequencing of *Pennisetum* and *Cenchrus* bacterial artificial chromosome (BAC) clones from the ASGR identified 40 putative protein coding regions, two of which had sequence similarity to the rice *BABY BOOM (BBM)* gene (Conner *et al.* 2008). *BBM* was originally identified in *Brassica napus* as an AP2-domain transcription factor, and overexpression in *Arabidopsis* results in embryo development from vegetative tissue (Boutillier *et al.* 2002). The ASGR-*BBM*-like genes, which are present in the ASGR of both *Pennisetum* and *Cenchrus*, are therefore candidate genes with strong potential to have a role in the induction and/or maintenance of apomixis events (Conner *et al.* 2008).

In *Hypericum*, the *HYPERICUM APOSPORY (HAPPY)* locus was identified following an amplified fragment length polymorphism (AFLP)-based screen of a selection of apomictic and sexual plants. When an AFLP, which perfectly cosegregated with apospory, was used to screen a *Hypericum* BAC library, a single clone that contained, among other genes, a ubiquitin-mediated E3 ligase was identified (Schallau *et al.* 2010). This *ARIADNE 7*-like E3 ligase (*HpARI*) has been postulated as a strong candidate for the *HAPPY* locus, as one of the four *HpARI* alleles within the tetraploid apomict is truncated compared to sexual plants. The truncated *HpARI* gene may act in a dominant-negative fashion, interacting with the three remaining intact alleles. E3 ligases are involved in ubiquitin-mediated protein degradation and are known to have a role in embryo sac development. For instance, the MATH-BTB protein MAB interacts with an E3 ubiquitin ligase component (Cullin 3a) and is required for mitotic spindle function and nuclear fate in developing maize (*Z. mays*) embryo sacs (Juranić *et al.* 2012). Alteration in expression or function of the *HpARI* E3 ligase might therefore affect embryo sac development in *Hypericum*, leading to apomixis events.

Differentially Expressed Genes Associated with Apomixis

Next-generation sequencing has fueled gene expression-based approaches in a range of apomictic species, the reasoning being that gene transcripts increased or decreased in abundance in apomicts relative to sexual individuals will provide clues to the differential transcriptional pathways involved in apomixis. If apomixis is a consequence of spatial and temporal changes in expression of sexual pathway-related genes, comparative gene expression provides a method with which to identify such genes. This approach is complicated by the nature of asexual reproduction that prevents the generation of isogenic lines that differ solely with regard to reproductive mode. Nevertheless, transcriptome comparisons between apomictic and closely related sexual individuals from reproductive

tissues at different developmental stages have uncovered pathways potentially central to apomixis including protein degradation, transcription, stress response, and cell-to-cell signaling (Albertini *et al.* 2005; Laspina *et al.* 2008; Sharbel *et al.* 2010; Silveira *et al.* 2012; Okada *et al.* 2013).

It is hoped that comparative gene expression studies in aposporous plants may reveal genes and pathways involved in processes such as cell-fate specification of the aposporous initial cell, reprogramming of a somatic cell to a germ cell fate, and degradation of sexual products (*e.g.*, the megaspores). In the aposporous grass *Brachiaria brizantha*, low-depth expressed sequence tag (EST) libraries made from ovaries of sexual and apomictic individuals revealed ESTs that show similarity to genes with known involvement in female embryo sac development (Silveira *et al.* 2012). Further characterization of some of these ESTs identified genes that are expressed in the region where aposporous initial cells appear. These genes include a helicase (*BbrizHelic*), a MADS-box transcription factor (*BbrizAGL6*), and a stress-induced protein (*BbrizSti1*), suggesting that stress-related genes may play a role in the induction of aposporous initial cells (Silveira *et al.* 2012; Guimarães *et al.* 2013). In aposporous *Hieracium praealtum*, stress-like genes are also expressed in individual aposporous initial cells and early stage embryo sacs that contain two to four nuclei (Okada *et al.* 2013). *In situ* hybridization also reveals that at least three stress-associated genes are upregulated in the aposporous initial cell, but are undetectable in sexual ovules. It is plausible that stress-associated pathways may be involved in cell-to-cell signaling during the appearance of aposporous initial cells, or possibly in the degeneration of cells derived from the sexual pathway. Components related to the ubiquitin-mediated proteasome pathway were also identified as enriched in aposporous initial cells and the early stage aposporous embryo sac when compared to other somatic ovule cells (Okada *et al.* 2013). This finding, combined with the identification of the *HpARI* E3 ubiquitin ligase within the *Hypericum* apospory locus, makes the ubiquitin-mediated pathway a candidate for involvement in aposporous initial cell initiation and growth.

Other gene expression studies in aposporous species have used cDNA-AFLPs to identify differentially expressed transcripts. In *Poa pratensis*, this led to the identification and characterization of two candidate genes: *SOMATIC EMBRYO-GENESIS RECEPTOR-LIKE KINASE* (*PpSERK*) and *APOSTART* (Albertini *et al.* 2004; Albertini *et al.* 2005). Both *PpSERK* and *APOSTART* were isolated in two copies, although further copies or alleles are present within the *P. pratensis* genome. *PpSERK* is a tyrosine kinase proposed as the switch that allows the aposporous initial cell to form and develop into an embryo sac. In apomictic *P. pratensis*, *PpSERK* is expressed in cells neighboring the megaspore mother cell, which is the region where aposporous initial cells appear (Albertini *et al.* 2005). This suggests *PpSERK* may play a role in specifying an embryo sac fate to somatic cells. *APOSTART* contains a lipid-binding START domain and is believed to have a role in meiosis. *APOSTART* may also be related to programmed cell

death and the degeneration of the nonfunctional megaspores, with one of the two isolated copies (*APOSTART1*) overexpressed in sexual lines relative to apomicts. The *Arabidopsis APOSTART1* ortholog is expressed in mature female embryo sacs and developing embryos, and the phenotype of *APOSTART1/APOSTART2* double mutants suggests that this gene has a role in embryo and seed development (Barcaccia and Albertini 2013).

Within diplosporous species, comparative gene expression studies performed during embryo sac development may identify genes that allow the megaspore mother cell to avoid or modify meiosis. Few transcriptome studies comparing diplosporous and related sexual taxa have been reported, although Selva *et al.* (2012) identified genes differentially expressed between diplosporous and sexual *Eragrostis curvula*, which include retrotransposon proteins, but no obvious candidates known to affect meiosis. Sharbel *et al.* (2010) compared gene expression between apomictic and sexual *Boechera* species across four stages of development to investigate whether a heterochronic change in gene expression is the mechanism by which a diploid embryo sac can develop from the megaspore mother cell in a sexual background. This study reported a global decrease in gene expression within the apomict compared to the sexual species during the early stages of development. While not identifying any individual candidate genes, the authors hypothesize that apomeiosis is correlated with a global downregulation of gene expression during megaspore mother cell formation.

Knowledge Gained from Sexual Species

If apomixis represents an alteration in the spatial and temporal expression of the sexual pathway and recruits genes involved in sex, it follows that conversion of the sexual pathway to an apomictic one in nonapomictic species should be possible. This assumption is supported by the characterization of sexual mutants that mimic different aspects of apomixis, thus providing candidate genes that may be involved in apomixis. Recent reviews have described many of these genes and provide comprehensive lists of genes and mutants that show apomictic phenotypes (Barcaccia and Albertini 2013; Koltunow *et al.* 2013).

Mutants that develop embryo sacs without meiosis (apomeiosis phenotype)

Mutants that display a phenotype reminiscent of diplospory involve genes that affect meiosis. For example, the maize *elongate1* mutant produces functional diploid embryo sacs by skipping meiosis II (Barrell and Grossniklaus 2005). Similarly, the *dyad* mutant of *Arabidopsis* can produce diploid embryo sacs following disrupted meiosis, where only a single equational division occurs followed by an arrest in progression (Ravi *et al.* 2008). Three recessive *Arabidopsis* mutants that have altered meiotic processes have been identified and, when combined in a triple mutant, are able to replace meiosis with mitosis, mimicking the apomeiosis phenotype. This triple mutant, called *MiMe* (*Mitosis instead of Meiosis*)

combines mutants in the *omission of second division 1 (Osd1)*, *Atspo11-1*, and *Atrec8* genes and produces viable diploid gametes that are genetically identical to their mother (D'Erfurth *et al.* 2009). The *MiMe* and *dyad* mutants have both been used in an attempt to engineer apomixis in *Arabidopsis* as a proof of concept (Marimuthu *et al.* 2011). Instead of replicating parthenogenesis, *MiMe* and *dyad* mutants were crossed with an *Arabidopsis* line that contains a manipulated centromere-specific histone (CENH3), which has previously been demonstrated to cause genome elimination (Ravi and Chan 2010). These combinations resulted in diploid progeny that were genetically identical to the maternal parent, as the paternal genome had been eliminated. While the outcome of this synthesis is the same as in apomixis, crossing is still required. Nevertheless, this elegant system clearly demonstrates the feasibility of engineering clonal propagation by seeds into sexual species.

Specification of the functional megaspore is known to be affected by both cytokinin and auxin signaling in *Arabidopsis*, suggesting that both these signaling pathways may be important in aposporous initial cell initiation. *Arabidopsis* cytokinin receptor triple mutants show disrupted embryo sac development. The absence of a functional megaspore-specific marker in mutant ovules suggests that disruption of cytokinin signaling blocks functional megaspore specification (Cheng *et al.* 2013). Similarly, the auxin efflux carrier gene *PIN-FORMED 1 (PIN1)* is necessary for embryo sac development in *Arabidopsis* (Ceccato *et al.* 2013). Moreover, inhibition of polar auxin transport in *Hieracium* also affects the timing, position, and frequency of aposporous initial cell formation (Tucker *et al.* 2012). Alterations in cytokinin and/or auxin signaling may therefore be important in specifying an embryo sac identity to aposporous initial cells of aposporous plants.

Genes with a known role in epigenetic regulation have been implicated in the regulation of ovule cell-fate specification and embryo sac development. Mutation of *ARGONAUTE* genes within sexual plants can lead to changes in the number of cells that have the capacity to initiate embryo sac development (Olmedo-Monfil *et al.* 2010; Singh *et al.* 2011). *ARGONAUTE (AGO)* proteins carry small RNAs (sRNAs) forming complexes with other proteins that cleave mRNA or lead to RNA dependent DNA methylation (Meister 2013). In *Arabidopsis*, *ago9* mutants appear to form multiple cells with the potential to mitotically form embryo sacs within a single ovule, a phenotype reminiscent of apospory (Olmedo-Monfil *et al.* 2010). Similar phenotypes are also observed in other sRNA pathway mutants including *rna-dependent rna polymerase 6 (rdr6)* and *suppressor of gene silencing 3 (sgs3)* (Olmedo-Monfil *et al.* 2010). Inactivation of DNA methyltransferases *DMT102* and *DMT103* in maize also results in apomictic-like phenotypes, including the production of diploid gametes and multiple embryo sacs within a single ovule, instead of the usual single embryo sac (Garcia-Aguilar *et al.* 2010). These results suggest that DNA methyltransferases may have a role in the cell-fate specification of embryo sac precursor cells. These apomixis-like phenotypes further suggest that differentiation

between apomictic and sexual reproduction may be epigenetically regulated via alterations in chromatin state.

In maize, mutation of *AGO104* similarly results in an apomixis-like phenotype. However, the *ago104* phenotype mimics diplospory, with a single megaspore mother cell per ovule undergoing mitosis rather than meiosis, resulting in diploid gametes (Singh *et al.* 2011). *AGO104* protein accumulates in somatic cells surrounding the megaspore mother cell, implying that a mobile signal may be responsible for repressing a somatic fate in germ cells. *AGO* genes have also been implicated in germ-cell identity in rice. For example, *MEIOSIS ARRESTED AT LEPTOTENE1 (MEL1)* is an *ARGONAUTE* gene expressed specifically in gamete precursor cells within ovules of rice and is essential for normal sporogenesis and meiosis in these cells (Nonomura *et al.* 2007).

Following the characterization of these mutants and the subsequent hypothesis that apomixis may be epigenetically regulated, approaches that compare expression of sRNAs in natural apomicts to relative sexual plants have begun, although have not yet yielded causal links between sRNAs and apomixis (Amiteye *et al.* 2011; Galla *et al.* 2013). Given the accumulation of evidence supporting the potential epigenetic regulation of apomixis obtained from sexual mutants, studies exploring differential large RNA expression combined with changes in small RNA are likely to provide important insights into the genetic regulation of apomixis.

It is possible that apomixis represents reversible, epigenetic silencing of the sexual pathway. Epigenetic regulation of apomixis is an attractive hypothesis as it potentially accounts for the facultative nature of apomixis and the ability of apomicts to revert to sexuality. In an evolutionary sense, it may be that such epigenetic changes occur following polyploidization and subsequently activate the expression of apomixis and suppression of sexual reproduction. In this scenario, all angiosperms may be considered as having “potential” for apomixis (Hörandl and Hojsgaard 2012).

Mutants capable of fertilization-independent embryo and endosperm development

Genes that may be involved in either fertilization-independent embryo or endosperm development have also been identified through sexual mutants. Interestingly, many of these genes are associated with the Polycomb-group (PcG) chromatin modeling complex. In particular, the Polycomb repressive complex 2 (PRC2) is known to have a role in suppressing seed development in the absence of fertilization. The PRC2 is conserved between plants and animals and is responsible for repressing gene expression via trimethylation of histone H3 at lysine 27 (H3K27me3). When core PRC2 genes are mutated in *Arabidopsis*, phenotypes of fertilization-independent seed development have been observed. For example, the fertilization-independent seed (FIS) PRC2 complex (FIS-PRC2) consists of the genes *MEDEA (MEA)*, *FIS2*, *FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)*, and *MULTICOPY SUPPRESSOR OF IRA1 (MSI1)*. Loss of function of any of these genes results in

fertilization-independent endosperm development and, in the case of *MSI1* mutants, parthenogenetic embryo initiation; however, viable seeds are not formed (Chaudhury *et al.* 1997; Ohad *et al.* 1999; Guitton and Berger 2005; Schmidt *et al.* 2013). The role of some of the FIS-PRC2 genes has been investigated in *Hieracium*, one of the few apomicts that develop endosperm without fertilization. Downregulation of *Hieracium FIE (HFIE)* does not result in fertilization-independent seed development in sexual plants, although it is required for fertilization-independent embryo and endosperm development in apomicts (Rodrigues *et al.* 2008). A *Hieracium* homolog of another FIS-PRC2 gene, *HMSI1*, is expressed in apomictic and sexual ovaries within *Hieracium*, but is unlikely to be linked to the *LOSS OF PARTHENOGENESIS (LOP)* locus. The authors subsequently propose that *HMSI1* may be involved in fertilization-independent seed development downstream of *LOP* activity (Rodrigues *et al.* 2010). These results demonstrate that although sexual developmental mutants provide promising gene candidates that initiate apomixis-like events, the natural apomictic pathway may be somewhat more complex with additional or alternative regulators that promote maturation of viable embryos and endosperm.

Conclusions

The genetic and molecular control of apomixis is intriguing from both developmental and evolutionary perspectives, and it attracts significant interest because of its potential value to agriculture if it can be harnessed for plant breeding. The coexistence of both apomixis and sexual reproduction within individual plants suggests that apomixis is reversibly superimposed upon the sexual pathway. Studies involving natural apomicts and sexual mutants displaying apomixis-like phenotypes have led to the identification of apomixis loci and candidate genes that may be responsible for superimposing apomixis on the sexual pathway. However, causal genes have not yet been identified from apomictic species. The possibility of apomixis being epigenetically regulated is an attractive hypothesis with growing support from studies in sexual plants where mutations in epigenetic pathways lead to apomixis-like phenotypes. Epigenetic regulation will continue to be a key focus of future studies in natural apomicts. The synthesis of apomixis in crops could also be attempted using developed tools to switch the sexual pathway to an apomictic-like route. Genes identified from natural apomicts may augment this synthetic route and perhaps ultimately allow flexibility to switch apomixis on and off for plant breeding purposes.

Acknowledgments

We thank Matthew Tucker, Jennifer Taylor, Susan Johnson, Steven Henderson, and David Rabiger for critical reading and comments on the review. Research in the Anna M. G. Koltunow laboratory is currently supported by a grant from the Science and Industry Endowment Fund.

Literature Cited

- Akiyama, Y., J. A. Conner, S. Goel, D. T. Morishige, J. E. Mullet *et al.*, 2004 High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. *Plant Physiol.* 134: 1733–1741.
- Akiyama, Y., W. W. Hanna, and P. Ozias-Akins, 2005 High-resolution physical mapping reveals that the apospory-specific genomic region (ASGR) in *Cenchrus ciliaris* is located on a heterochromatic and hemizygous region of a single chromosome. *Theor. Appl. Genet.* 111: 1042–1051.
- Albertini, E., A. Porceddu, F. Ferranti, L. Reale, G. Barcaccia *et al.*, 2001 Apospory and parthenogenesis may be uncoupled in *Poa pratensis*: a cytological investigation. *Sex. Plant Reprod.* 14: 213–217.
- Albertini, E., G. Marconi, G. Barcaccia, L. Raggi, and M. Falcinelli, 2004 Isolation of candidate genes for apomixis in *Poa pratensis* L. *Plant Mol. Biol.* 56: 879–894.
- Albertini, E., G. Marconi, L. Reale, G. Barcaccia, A. Porceddu *et al.*, 2005 SERK and APOSTART: candidate genes for apomixis in *Poa pratensis*. *Plant Physiol.* 138: 2185–2199.
- Amiteye, S., J. M. Corral, H. Vogel, and T. F. Sharbel, 2011 Analysis of conserved microRNAs in floral tissues of sexual and apomictic *Boechera* species. *BMC Genomics* 12: 500.
- Araujo, A. C. G., S. Mukhambetzhonov, M. T. Pozzobon, E. F. Santana, and V. T. C. Carneiro, 2000 Female gametophyte development in apomictic and sexual *Brachiaria brizantha* (Poaceae). *Rev. Cytol. Biol. Veg. Botan.* 23: 13–28.
- Barcaccia, G., and E. Albertini, 2013 Apomixis in plant reproduction: a novel perspective on an old dilemma. *Plant Reprod* 26: 159–179.
- Barrell, P. J., and U. Grossniklaus, 2005 Confocal microscopy of whole ovules for analysis of reproductive development: the *elongate1* mutant affects meiosis II. *Plant J.* 43: 309–320.
- Berger, F., Y. Hamamura, M. Ingouff, and T. Higashiyama, 2008 Double fertilization: caught in the act. *Trends Plant Sci.* 13: 437–443.
- Boutillier, K., R. Offringa, V. K. Sharma, H. Kieft, T. Ouellet *et al.*, 2002 Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* 14: 1737–1749.
- Calderini, O., S. B. Chang, H. De Jong, A. Busti, F. Paolucci *et al.*, 2006 Molecular cytogenetics and DNA sequence analysis of an apomixis-linked BAC in *Paspalum simplex* reveal a non pericentromere location and partial microcolinearity with rice. *Theor. Appl. Genet.* 112: 1179–1191.
- Carman, J. G., 1997 Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol. J. Linn. Soc.* 61: 51–94.
- Catanach, A. S., S. K. Erasmuson, E. Podivinsky, B. R. Jordan, and R. Bicknell, 2006 Deletion mapping of genetic regions associated with apomixis in *Hieracium*. *Proc. Natl. Acad. Sci. USA* 103: 18650–18655.
- Ceccato, L., S. Masiero, D. Sinha Roy, S. Bencivenga, I. Roig-Villanova *et al.*, 2013 Maternal control of PIN1 is required for female gametophyte development in Arabidopsis. *PLoS ONE* 8: e66148.
- Chapman, H., G. J. Houliston, B. Robson, and I. Iline, 2003 A case of reversal: the evolution and maintenance of sexuals from parthenogenetic clones in *Hieracium pilosella*. *Int. J. Plant Sci.* 164: 719–728.
- Chaudhury, A. M., L. Ming, C. Miller, S. Craig, E. S. Dennis *et al.*, 1997 Fertilization-independent seed development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 94: 4223–4228.
- Cheng, C.-Y., D. E. Mathews, G. Eric Schaller, and J. J. Kieber, 2013 Cytokinin-dependent specification of the functional megaspore in the Arabidopsis female gametophyte. *Plant J.* 73: 929–940.

- Conner, J. A., S. Goel, G. Gunawan, M. M. Cordonnier-Pratt, V. E. Johnson *et al.*, 2008 Sequence analysis of bacterial artificial chromosome clones from the apospory-specific genomic region of *Pennisetum* and *Cenchrus*. *Plant Physiol.* 147: 1396–1411.
- Conner, J. A., G. Gunawan, and P. Ozias-Akins, 2013 Recombination within the apospory specific genomic region leads to the uncoupling of apomixis components in *Cenchrus ciliaris*. *Planta* 238: 51–63.
- Curtis, M. D., and U. Grossniklaus, 2008 Molecular control of autonomous embryo and endosperm development. *Sex. Plant Reprod.* 21: 79–88.
- D'Erfurth, I., S. Jolivet, N. Froger, O. Catrice, M. Novatchkova *et al.*, 2009 Turning meiosis into mitosis. *PLoS Biol.* 7: e1000124.
- Drews, G., and A. M. Koltunow, 2011 The female gametophyte: the Arabidopsis book 9: e0155.
- Galla, G., M. Volpato, T. Sharbel, and G. Barcaccia, 2013 Computational identification of conserved microRNAs and their putative targets in the *Hypericum perforatum* L. flower transcriptome. *Plant Reprod.* 26: 209–229.
- García, R., M. J. Asíns, J. Forner, and E. A. Carbonell, 1999 Genetic analysis of apomixis in *Citrus* and *Poncirus* by molecular markers. *Theor. Appl. Genet.* 99: 511–518.
- García-Aguilar, M., C. Michaud, O. Leblanc, and D. Grimanelli, 2010 Inactivation of a DNA methylation pathway in maize reproductive organs results in apomixis-like phenotypes. *Plant Cell* 22: 3249–3267.
- Grimanelli, D., O. Leblanc, E. Espinosa, E. Perotti, D. González De León *et al.*, 1998 Mapping diplosporous apomixis in tetraploid *Tripsacum*: one gene or several genes? *Heredity* 80: 33–39.
- Guimarães, L., D. A. Dusi, S. Masiero, F. Resentini, A. M. Gomes *et al.*, 2013 *BbrizAGL6* is differentially expressed during embryo sac formation of apomictic and sexual *Brachiaria brizantha* plants. *Plant Mol. Biol. Rep.* DOI: .10.1007/s11105-013-0618-8
- Guitton, A. E., and F. Berger, 2005 Loss of function of MULTICOPY SUPPRESSOR OF IRA 1 produces nonviable parthenogenetic embryos in Arabidopsis. *Curr. Biol.* 15: 750–754.
- Hörandl, E., and D. Hojsgaard, 2012 The evolution of apomixis in angiosperms: a reappraisal. *Plant Biosyst.* 146: 681–693.
- Juranić, M., K.-o. Srilunchar, N. G. Krohn, D. Leljak-Levanić, S. Sprunck *et al.*, 2012 Germline-specific MATH-BTB substrate adaptor MAB1 regulates spindle length and nuclei identity in maize. *Plant Cell* 24: 4974–4991.
- Kaushal, P., D. R. Malaviya, A. K. Roy, S. Pathak, A. Agrawal *et al.*, 2008 Reproductive pathways of seed development in apomictic guinea grass (*Panicum maximum* Jacq.) reveal uncoupling of apomixis components. *Euphytica* 164: 81–92.
- Koltunow, A. M., 1993 Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. *Plant Cell* 5: 1425–1437.
- Koltunow, A. M., and U. Grossniklaus, 2003 Apomixis: a developmental perspective. *Annu. Rev. Plant Biol.* 54: 547–574.
- Koltunow, A. M., K. Soltys, N. Nito, and S. McClure, 1995 Anther, ovule, seed, and nucellar embryo development in *Citrus sinensis* cv. Valencia. *Can. J. Bot.* 73: 1567–1582.
- Koltunow, A. M., T. Hidaka, and S. P. Robinson, 1996 Polyembryony in *Citrus*: accumulation of seed storage proteins in seeds and in embryos cultured *in vitro*. *Plant Physiol.* 110: 599–609.
- Koltunow, A. M. G., S. D. Johnson, J. C. M. Rodrigues, T. Okada, Y. Hu *et al.*, 2011 Sexual reproduction is the default mode in apomictic *Hieracium* subgenus *Pilosella*, in which two dominant loci function to enable apomixis. *Plant J.* 66: 890–902.
- Koltunow, A. M., P. Ozias-Akins, and I. Siddiqi, 2013 Apomixis, pp. 83–110 in *Seed Genomics*, edited by P. W. Bercraft. Wiley, New York.
- Kotani, Y., S. T. Henderson, G. Suzuki, S. D. Johnson, T. Okada *et al.*, 2013 The LOSS OF APOMEIOSIS (LOA) locus in *Hieracium praealtum* can function independently of the associated large-scale repetitive chromosomal structure. *New Phytol.* DOI: .10.1111/nph.12574
- Labombarda, P., A. Busti, M. E. Caceres, F. Pupilli, and S. Arcioni, 2002 An AFLP marker tightly linked to apomixis reveals hemizygoty in a portion of the apomixis-controlling locus in *Paspalum simplex*. *Genome* 45: 513–519.
- Laspina, N. V., T. Vega, J. G. Seijo, A. M. González, L. G. Martelotto *et al.*, 2008 Gene expression analysis at the onset of aposporous apomixis in *Paspalum notatum*. *Plant Mol. Biol.* 67: 615–628.
- Marimuthu, M. P. A., S. Jolivet, M. Ravi, L. Pereira, J. N. Davda *et al.*, 2011 Synthetic clonal reproduction through seeds. *Science* 331: 876.
- Meister, G., 2013 Argonaute proteins: functional insights and emerging roles. *Nat. Rev. Genet.* 14: 447–459.
- Nogler, G., 1984 Gametophytic apomixis, pp. 475–518 in *Embryology of Angiosperms*, edited by B. M. Johri. Springer, Berlin.
- Nonomura, K. I., A. Morohoshi, M. Nakano, M. Eiguchi, A. Miyao *et al.*, 2007 A germ cell-specific gene of the ARGONAUTE family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice. *Plant Cell* 19: 2583–2594.
- Noyes, R. D., and L. H. Rieseberg, 2000 Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. *Genetics* 155: 379–390.
- Ogawa, D., S. D. Johnson, S. T. Henderson, and A. M. Koltunow, 2013 Genetic separation of autonomous endosperm formation (AutE) from two other components of apomixis in *Hieracium*. *Plant Reprod.* 26: 113–123.
- Ohad, N., R. Yadegari, L. Margossian, M. Hannon, D. Michaeli *et al.*, 1999 Mutations in FIE, a WD polycomb group gene, allow endosperm development without fertilization. *Plant Cell* 11: 407–415.
- Okada, T., K. Ito, S. D. Johnson, K. Oelkers, G. Suzuki *et al.*, 2011 Chromosomes carrying meiotic avoidance loci in three apomictic eudicot *Hieracium* subgenus *Pilosella* species share structural features with two monocot apomicts. *Plant Physiol.* 157: 1327–1341.
- Okada, T., Y. Hu, M. R. Tucker, J. M. Taylor, S. D. Johnson *et al.*, 2013 Enlarging cells initiating apomixis in *Hieracium praealtum* transition to an embryo sac program prior to entering mitosis. *Plant Physiol.* 163: 216–231.
- Olmedo-Monfil, V., N. Durán-Figueroa, M. Arteaga-Vázquez, E. Demesa-Arévalo, D. Autran *et al.*, 2010 Control of female gamete formation by a small RNA pathway in *Arabidopsis*. *Nature* 464: 628–632.
- Ozias-Akins, P., D. Roche, and W. W. Hanna, 1998 Tight clustering and hemizygoty of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. *Proc. Natl. Acad. Sci. USA* 95: 5127–5132.
- Peel, M. D., J. G. Carman, and O. Leblanc, 1997 Megasporocyte callose in apomictic buffelgrass, Kentucky bluegrass, *Pennisetum squamulatum* Fresen, *Tripsacum* L., and weeping lovegrass. *Crop Sci.* 37: 724–732.
- Pessino, S. C., C. Evans, J. P. A. Ortiz, I. Armstead, C. B. Do Valle *et al.*, 1998 A genetic map of the apospory-region in *Brachiaria* hybrids: identification of two markers closely associated with the trait. *Hereditas* 128: 153–158.
- Ravi, M., and S. W. L. Chan, 2010 Haploid plants produced by centromere-mediated genome elimination. *Nature* 464: 615–618.
- Ravi, M., M. P. A. Marimuthu, and I. Siddiqi, 2008 Gamete formation without meiosis in *Arabidopsis*. *Nature* 451: 1121–1124.
- Rodrigues, J. C. M., M. R. Tucker, S. D. Johnson, M. Hrmova, and A. M. G. Koltunow, 2008 Sexual and apomictic seed formation

- in *Hieracium* requires the plant polycomb-group gene *FERTILIZATION INDEPENDENT ENDOSPERM*. *Plant Cell* 20: 2372–2386.
- Rodrigues, J. C. M., T. Okada, S. D. Johnson, and A. M. Koltunow, 2010 A MULTICOPY SUPPRESSOR OF IRA1 (MSI1) homologue is not associated with the switch to autonomous seed development in apomictic (asexual) *Hieracium* plants. *Plant Sci.* 179: 590–597.
- Savidan, Y., 2000 Apomixis: Genetics and Breeding, pp. 13–86 in *Plant Breeding Reviews*, edited by J. Janick. Wiley, New York.
- Schallau, A., F. Arzenton, A. J. Johnston, U. Hähnel, D. Koszegi *et al.*, 2010 Identification and genetic analysis of the APOSPORY locus in *Hypericum perforatum* L. *Plant J.* 62: 773–784.
- Schmidt, A., H. J. P. Wöhrmann, M. T. Raissig, J. Arand, J. Gheyselinck *et al.*, 2013 The *Polycomb* group protein MEDEA and the DNA methyltransferase MET1 interact to repress autonomous endosperm development in *Arabidopsis*. *Plant J.* 73: 776–787.
- Selva, J. P., S. Pessino, M. Meier, and V. Echenique, 2012 Identification of candidate genes related to polyploidy and/or apomixis in *Eragrostis curvula*. *Am. J. Plant Sci.* 3: 403–416.
- Sharbel, T. F., M. L. Voigt, J. M. Corral, G. Galla, J. Kumlehn *et al.*, 2010 Apomictic and sexual ovules of *Boechera* display heterochronic global gene expression patterns. *Plant Cell* 22: 655–671.
- Silveira, E. D., L. A. Guimarães, D. M. A. de Dusi, F. R. da Silva, N. F. Martins *et al.*, 2012 Expressed sequence-tag analysis of ovaries of *Brachiaria brizantha* reveals genes associated with the early steps of embryo sac differentiation of apomictic plants. *Plant Cell Rep.* 31: 403–416.
- Singh, M., S. Goel, R. B. Meeley, C. Dantec, H. Parrinello *et al.*, 2011 Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein. *Plant Cell* 23: 443–458.
- Tucker, M. R., A. C. G. Araujo, N. A. Paech, V. Hecht, E. D. L. Schmidt *et al.*, 2003 Sexual and apomictic reproduction in *Hieracium* subgenus *Pilosella* are closely interrelated developmental pathways. *Plant Cell* 15: 1524–1537.
- Tucker, M. R., T. Okada, S. D. Johnson, F. Takaiwa, and A. M. G. Koltunow, 2012 Sporophytic ovule tissues modulate the initiation and progression of apomixis in *Hieracium*. *J. Exp. Bot.* 63: 3229–3241.
- Van Dijk, P., and K. Vijverberg, 2005 The significance of apomixis in the evolution of the angiosperms: a reappraisal, pp. 101–116 in *Plant Species-Level Systematics: New Perspectives on Pattern and Process*, edited by F. Bakker, L. Chatrou, B. Gravendeel, and P. Pelsner. Gantner Verlag, Ruggell, Lichtenstein.
- Van Dijk, P. J., I. C. Q. Tas, M. Falque, and T. Bakx-Schotman, 1999 Crosses between sexual and apomictic dandelions (*Taraxacum*). II. The breakdown of apomixis. *Heredity* 83: 715–721.
- Vijverberg, K., S. Milanovic-Ivanovic, T. Bakx-Schotman, and P. J. Van Dijk, 2010 Genetic fine-mapping of DIPLOSPOROUS in *Taraxacum* (dandelion; Asteraceae) indicates a duplicated DIP-gene. *BMC Plant Biol.* 10: 154.

Communicating editor: J. Rine