Chapter 7
Apomixis: The Asexual Formation of Seed

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7.1 Introduction

Apomixis is the clonal propagation of a plant through seed, avoiding the processes of meiosis and fertilization. In common with other natural forms of clonal reproduction, apomixis leads to the formation of uniform populations, and these can extend over large areas of land and persist over long periods of time (Horandl 2008). Several comprehensive reviews have been written on apomixis detailing the variation observed in known natural systems of apomixis (Crane 2001; Nogler 1984a), the genetics of the trait (Ozias-Akins and van Dijk 2007), its evolutionary and ecological implications (van Dijk 2003; van Dijk et al. 2009), the role of temporal and epigenetic factors (Koltunow and Grossniklaus 2003) and the potential that apomixis offers for increasing the rate of plant breeding for seed-propagated crops (Savidan 2000a). This chapter is intended to inform the reader of the potential of apomixis and of progress being made in apomixis research, focusing on more recent findings and contemporary approaches. A general description of the trait is given to provide context to the latter discussion, but this is deliberately broad in scope.

7.2 Why Study Apomixis?

Apomixis is not, in itself, a form of somatic manipulation. It is, however, a potentially invaluable tool for applying advances in somatic manipulation to seed-propagated crops. Most of the world's key crops, as measured by traded value, land under cultivation, and/or nutritional importance, are propagated by seed. Examples include wheat, rice, soy, maize, cotton and most timber, forage, oil and fibre crops. The reasons for using seed are compelling. Typically, seed is small, robust, contains

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storage reserves to support germination and establishment, and (in most cases) is easily produced, harvested, cleaned, transported, stored and sown. However, seed is usually also the product of sexual reproduction, specifically of the combined actions of meiosis and fertilization, and is therefore genetically diverse. The degree of this diversity varies between breeding systems. Self-fertile systems, such as wheat and rice, can be inbred to form uniform (true breeding) lines; however, they are produced at the cost of lost heterosis (hybrid vigour). On the other hand, outcrossing species tend to retain heterosis, but the progeny are variable, compromising commercial utility. Again, a breeder can reduce variation through inbreeding but at the cost of lost heterosis. In the special case of F_1 hybrid breeding, variation is reduced and heterosis is obtained, but this is achieved at a high economic cost. Cloning allows the multiplication of elite forms, but it is typically expensive and restricted to plants of high unit value such as fruit trees. The obvious exceptions to this are plants that already have an efficient form of cloning which can be used for production, such as the tubers of potato and the runners of strawberry.

As apomixis is cloning through seed, it offers the uniformity and predictability of clonal reproduction along with the economic advantages of seed production. In agriculture, it could be used to generate new hybrid varieties quickly and to perpetuate them cheaply and indefinitely through seed. As some forms of apomixis can occur without pollination, it could be used to avoid difficulties associated with cross compatibility and pollinator availability. Finally, even in vegetatively propagated plants like potatoes, apomixis may be useful in avoiding viral transfer, as very few viruses are able to transmit through true seed (Hanna 1995; Jefferson and Bicknell 1995; Koltunow et al. 1995; Savidan 2000a, b). For potato production in temperate regions, this would need to be managed as part of a high health programme as it is only possible to reach an acceptable tuber size in a short temperate growing season by using tubers as the planting material.

7.3 The Key Components of Apomixis

Apomixis is currently recorded in more than 400 taxa of flowering plants, involving 32 plant families, with a pattern of distribution that indicates it has arisen many times amongst the flowering plants (Carman 1997). It is generally agreed that in most if not all of these cases, apomixis has evolved through the modification of sexual reproduction. Evidence to support this conclusion comes from comparative studies of the structures, processes and gene expression patterns of sexual and apomictic plants (see below). Before describing the processes that underlie apomixis therefore, we have included a brief review of the current knowledge of sexual reproduction, focusing on developmental elements that either have remained constant in apomicts or have been the key steps of change permitting the evolution of apomictic development.

Most importantly, the two defining events of sexual reproduction that are not involved in apomixis are meiosis and fertilization. Clearly these are the events that give rise to the unique genotype of the sexually derived seedling and both, therefore, are avoided during the development of asexually derived embryos. That said, incomplete meiosis is a common feature of apomicts, in which the progression of meiosis is interrupted either in the first cycle (first division restitution) or in the second cycle (second division restitution) of meiotic division. Similarly, fertilization of the egg is not seen in apomictic development, but fertilization of the central cell, giving rise to the endosperm, is commonly seen (see below).

Within an angiosperm flower, the megagametophyte generation is represented by an embryo sac, typically comprising of eight meiotically reduced nuclei and seven cells. The embryo sac is surrounded by the tissues of the ovule and that in turn is encased within an ovary. The structures of the ovary, ovule and even the embryo sac differ significantly between species. Four key developmental features, however, remain constant amongst sexual flowering plant species. These features are also known to be common to all forms of apomictic seed development. First, only a single cell within the embryo sac differentiates into an egg cell and that is the only cell within the embryo sac that is fully totipotent. Many forms of apomixis involve the embryo sac (see below) and in all of these it is the egg cell that gives rise to the new embryo. Similarly, only the central cell can give rise to the endosperm and this is true in apomicts as well. Other cells within the somatic (sporophytic) plant body can be totipotent, which forms the basis of the sporophytic mechanisms of apomixis discussed below.

Second, in sexual plants the development of the angiosperm embryo requires the presence of a nutritive endosperm tissue, and that tissue is the product of fertilization. There are some exceptions to this rule. Orchids and members of the Podostemaceae lack an endosperm and both have evolved other mechanisms for nourishing the young embryo. Only two known apomicts lack an endosperm. Both are orchids (Teppner and Klein 1993; Huang et al. 2009). In all other apomicts, an endosperm is required. The endosperm tissue in apomicts may arise following fertilization (pseudogamy) or it may arise spontaneously without fertilization (autonomous endospermy), but, as in sexual species, it is always the derivative of the central cell.

Third, in flowering plants the endosperm and embryo typically have different ploidy states. Most commonly, this is 2n:3n for the embryo and endosperm respectively. Other ratios are known to occur in nature, particularly amongst apomicts, and plants are known to differ in their tolerances to different ploidy ratios (Scott et al. 1998, Berger 1999). In grasses, however, a 2n:3n ploidy balance appears to be particularly critical. Apomixis is a common trait amongst the grasses and most of the developmental mechanisms seen amongst apomicts in this group result in a 2n:3n ploidy balance (Crane 2001).

Finally, in sexual plants, embryogenesis and endosperm formation take place within a wider developmental programme of reproduction including flowering, gametogenesis, seed maturation and fruit formation. These events are tightly and coordinately regulated. As all forms of apomixis also employ the structures of the flower, seed and fruit, coordinated developmental timing is a critical parameter in the success of these mechanisms.

7.4 The Mechanisms of Apomixis

The known mechanisms of apomixis are subdivided into two primary groupings (sporophytic and gametophytic) based on the tissue involved in the formation of the clonal embryo. In the case of gametophytic types, they are then further subdivided based on the cell type giving rise to the embryo sac. As described above, mechanisms are also separately categorized with respect to whether the endosperm tissue arises spontaneously or as the result of fertilization.

7.4.1 Sporophytic Apomixis

In sporophytic apomixis, the clonal embryo arises directly from a somatic cell (or cells) of the ovule (Fig. 7.1). Citrus and mango provide good examples of this kind of apomixis (Wakana and Uemoto 1987, 1988; Litz et al. 1995). In these plants, the sexual reproductive pathway proceeds normally with the fertilization of meiotically derived egg and central cells. Somatic embryo formation occurs in the surrounding ovule tissue, normally coinciding with the early development of the sexually derived embryo. As the endosperm arises normally from the union of a 2n central cell nucleus and a 1n sperm nucleus, it has a 3n ploidy state. Both the sexually derived embryo and any clonally derived somatic embryos within the ovule have a ploidy of 2n, so a 2n:3n ploidy balance is achieved in this system. Within the developing seed of these plants, it is common for there to be more than one embryo developing (polyembryony), including one sexually derived embryo and one or more asexually derived embryos. Only one endosperm tissue is present, however, so polyembryony in sporophytic apomicts leads to competition for the limited reserves of the ovule and in particular for the reserves of the endosperm. Paradoxically, therefore, although polyembryony is a reflection of the highly embryogenic state of the ovule tissue, it can often lead to reduced seed viability.

As sporophytic apomicts generate seedlings derived both sexually and asexually, it is not uncommon for seedling populations to consist of a mixture of genetically identical (and maternal) and genetically unique (sexually derived) individuals. This combination of progeny types is referred to as “facultative apomixis.” It is particularly typical of sporophytic apomicts because a sexually derived embryo and endosperm must form in these species, but it is also seen amongst gametophytic apomicts as well (see below). It is theorized that facultative apomixis may confer some evolutionary advantages, as it provides apomicts with an opportunity to evolve in response to environmental changes while still expending most of their reproductive effort on the perpetuation of a successful clonal lineage (van Dijk 2003). To breeders of crops where sporophytic apomixis is common, however, such as mango and citrus, it can pose quite a challenge, as progeny testing is needed to determine which seedlings are clonal and which are not (López-Valenzuela et al. 1997; de Oliveira et al. 2002).

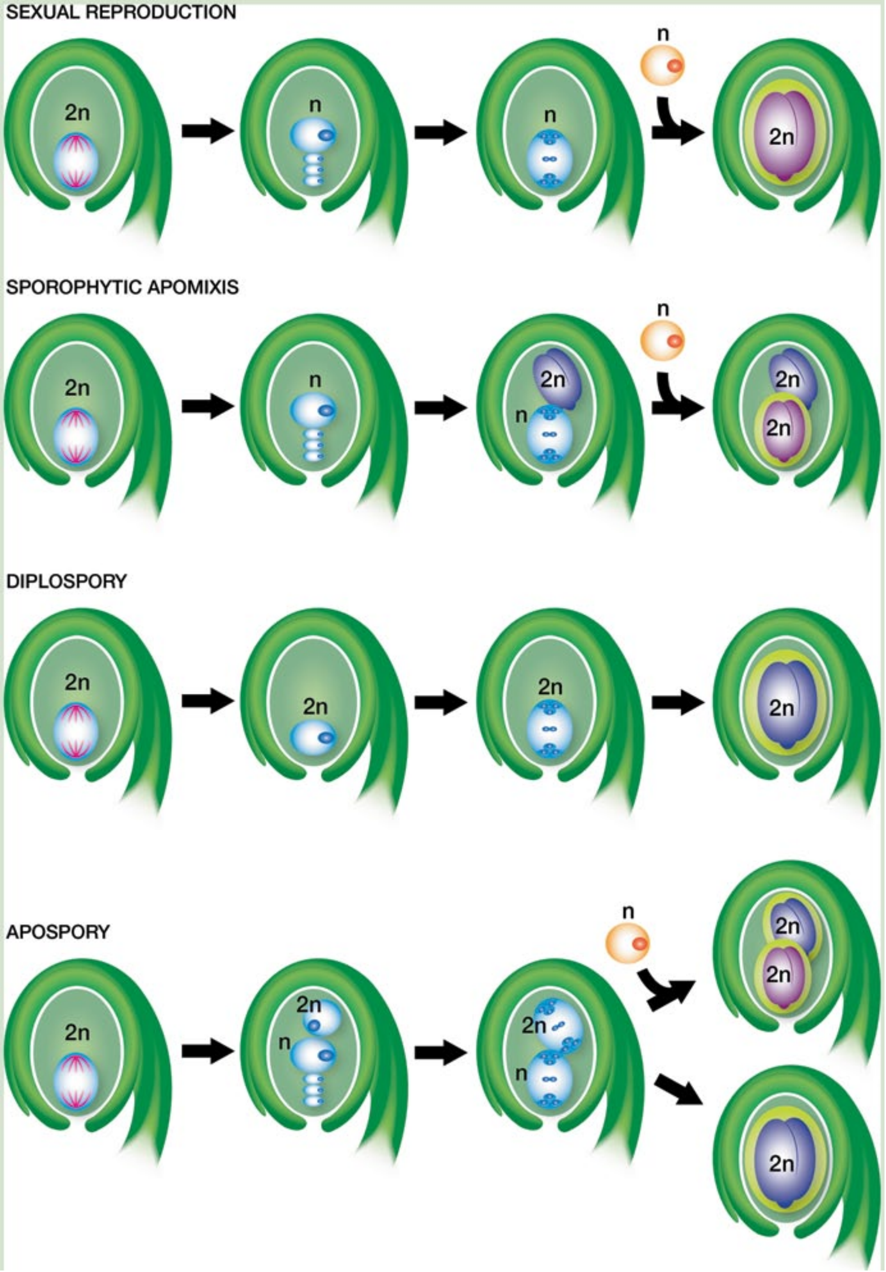

Fig. 7.1 Sexual reproduction compared with three forms of apomixis in flowering plants at four key stages of development: the initiation of meiosis, following megaspore differentiation, the differentiation of a mature embryo sac and early embryogenesis. For simplicity, only the pathway to embryo formation is depicted. Endosperm origin is not detailed but the endosperm tissue is depicted in *light green*. Sperm cells involved in the fertilization of the egg are depicted in *red*. During sporophytic apomixis and apospory, fertilization can lead to the formation of both hybrid and

7.4.2 Gametophytic Apomixis

In gametophytic apomixis, the totipotent cell giving rise to the clonal embryo is the egg. The parent tissue of the clonal progeny is, therefore, the female gametophyte. The recruitment of the egg into this developmental pathway satisfies several of the constraints discussed above: the egg is totipotent and preadapted to undergo embryogenesis, the egg occurs in the right location and at the right time during development for it to divide in concordance with the broader programme of reproductive development and finally, as the egg is the sole totipotent cell within the embryo sac, it will give rise to a single embryo for each endosperm tissue produced. The egg, however, is normally the product of meiosis, which results in it being genetically reduced. All forms of gametophytic apomixis, therefore, include a mechanism that results in the formation of an embryo sac without meiotic reduction (“apomeiosis”) coupled with a mechanism for spontaneous embryogenesis (“parthenogenesis”). Interestingly, the expression of either apomeiosis or parthenogenesis alone would confer a selective disadvantage. If a plant mutated to express apomeiosis but still required fertilization to form an embryo (i.e., did not express parthenogenesis), then unreduced eggs would combine with sperm nuclei, resulting in plants of higher ploidy. Successive generations would have successively higher ploidy and the outcome would be untenable. Conversely, if parthenogenesis arose without apomeiosis the opposite would happen, with meiotically derived, reduced egg cells spontaneously dividing to give rise to progressively lower ploidy plants. In gametophytic apomixis, therefore, there appears to be a “chicken and egg” paradox where the combination of apomeiosis and parthenogenesis establishes a tenable reproductive mechanism, but either expressed on its own would lead to reproductive failure. This may explain why almost all gametophytic apomicts are perennials and many employ other forms of vegetative reproduction, such as stolons, to sustain the life of an individual genet. In a perennial, a mutation conferring one part of gametophytic apomixis would not be immediately deleterious to the parent plant, providing more opportunity than in an annual for the other component then to arise spontaneously.

In gametophytic apomicts, meiosis is avoided by one of two different mechanisms, diplospory or apospory (Fig. 7.1). In diplospory, meiotic development progresses normally with the differentiation of the archesporial cell and entry into the first meiotic division. At some stage during meiosis, however, the normal course of reductional division is halted and the product is a megaspore with the maternal ploidy state. The timing of the disruption of meiosis is used to subdivide diplosporous apomicts further by mechanism, the details of which are well covered by several reviews (Nogler 1984a; Asker and Jerling 1992; Crane 2001). Once an unreduced

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◄ asexually derived progeny. In sporophytic apomixis, only a meiotically derived egg is present and that is the egg that is fertilized. In apospory, although both reduced and unreduced eggs are present, the sperm nucleus typically just fertilizes the meiotically derived reduced egg. During development in aposporous apomicts, however, meiotically derived structures are often lost. In this case, only asexually derived embryo sacs are present and only asexually derived embryos form. The nuclear state (n or 2n) of the cells or structures involved is noted. Where an embryo is clonal in origin it is depicted in *blue* and where it is hybrid in origin it is depicted in *purple* megaspore is formed, it undergoes gametogenesis to give rise to an unreduced embryo sac, within which an unreduced egg and unreduced polar nuclei differentiate. The spontaneous division of the egg initiates embryogenesis without fertilization (parthenogenesis). The development of the endosperm in diplosporous apomicts may be either pseudogamous or autonomous, as described previously. Sexually derived progeny can still arise in this system, and they do so when female meiosis is successfully achieved. Meiotically derived seedlings, however, are typically rare in diplosporous apomicts. For this discussion, the main feature of note in diplospory is that both the megaspore and the egg are directly involved in the formation of the clonal embryo. Only a single embryo arises in each seed, and its development is typically in synchrony with related events of seed and fruit development. Unlike sporophytic apomicts, therefore, polyembryony is uncommon in these plants.

In apospory, one or more somatic cell(s) of the ovule (or occasionally of the ovary) differentiate into a structure that is similar in form and function to the megaspore. These cells are called “aposporous initials”. As they are somatically derived, aposporous initials have the maternal genotype. Once formed, aposporous initials divide and differentiate into an embryo sac, within which an unreduced egg and unreduced polar nuclei form. The egg then undergoes parthenogenesis to initiate embryogenesis. As with diplospory, both pseudogamous and autonomous endospermy are seen amongst aposporous apomicts. In aposporous apomicts, both the meiotic pathway and the asexual pathway occur in parallel and both can contribute to the production of seedlings. Typically, one pathway predominates at the other’s expense; however, polyembryonic seeds are common in these plants. Multiple embryos can arise either from the survival of both sexually and asexually derived progeny, and/or from multiple asexually derived embryos arising from multiple aposporous initials.

7.4.3 Gametophytic Apomixis and Polyploidy

There is a highly significant association between the expression of gametophytic apomixis and the occurrence of polyploidy in plants naturally expressing the trait. Of the hundreds of taxa known to include gametophytic apomicts (Carman 1997), naturally occurring diploid apomicts have only been clearly identified in *Boechera* (Kantama et al. 2007). Müntzing (1928) reported diploid apomicts in the genus *Potentilla,* but the reproductive biology of this material is now contested (Holm and Ghatnekar 1996). Even in *Boechera,* the majority of apomictic field collections are reported to be polyploids (Schranz et al. 2005). The reason for the close association between gametophytic apomixis and polyploidy is unknown. Amongst sporophytic apomicts diploidy is common, so it does not appear that the reproductive phenomenon of apomixis induces or requires polyploidy. Diploid apomicts have been identified as weak individuals in experimental populations of some gametophytic species (Nogler 1982; Bicknell 1997; Kojima and Nagato 1997) indicating that polyploidy is not a prerequisite for the expression of gametophytic apomixis. Similarly, polyploidy has been induced in many species, yet this has very seldom resulted in apomixis of any type. Instead, it has been proposed that polyploidy may be necessary for the transmission of the trait via gametes. If true, this would, over evolutionary time, lead to its near exclusive expression in polyploid races. Nogler (1984a) working in *Ranunculus auricomus,* noted that apomixis would not transmit via a haploid gamete, yet sexual reproduction would. He proposed that a genetic element involved in apomixis was associated with a recessive gamete lethal factor, which became exposed in haploid gametes. There is now evidence of transmission bias against apomixis in *Tripsacum* (Grimanelli et al., 1998a) and *Pennisetum* (Roche et al. 2001; Jessup et al. 2002), suggesting that the ability to transmit the trait may be an important factor limiting native gametophytic apomixis to polyploid genotypes.

The role of polyploidy in the expression of apomixis is of particular interest because it represents a potential impediment to the installation of apomixis into target crop species. Many of our crop species are diploids, including some that have been explicitly identified as potential candidates for apomixis to facilitate breeding progress. Cultivated rice, in particular, is a diploid inbreeding species which is known to express significant hybrid vigour (Yu et al. 1997). Apomixis would have a profound impact on this important crop but this would ideally not require a change in the ploidy of the cultivated varieties.

7.4.4 Can Apomixis be Installed into Crop Species, and What Form of Apomixis Would Best Meet Our Needs?

Apomixis is already known to occur in a small number of crop species. Kentucky blue grass (*Poa pratensis*) is an important temperate turf and fodder grass that reproduces by gametophytic apomixis (Naumova et al. 1992). Similarly, *Brachiaria decumbens, Panicum maximum* and *Tripsacum dactyloides* are tropical forage grasses that are gametophytic apomicts (Naumova et al. 1999; Savidan 1980; Leblanc et al. 1995a). As mentioned above, sporophytic apomixis is common in citrus and in some tropical fruits such as mango and mangosteen (*Garcinia mangostana*). In commerce, however, these trees are typically reproduced by vegetative means and apomixis is not employed in the husbandry of the crop as it is in the grasses. In other cases, apomixis is not known in a crop species, but does occur in a closely related species. Attempts to transfer it by conventional introgression through crossing have been reported in maize (from *T. dactyloides*; Leblanc et al. 1995b; 1997), millet (from *Pennisetum squamulatum*; Dujardin and Hanna 1983; Roche et al. 2001), wheat (from *Elymus rectisetus*; Peel et al. 1997) and beet (from *Beta lomatogona*; Cleij et al. 1976). In each case, the trait was best expressed in polyploid F_1 progeny, and its expression fell as the percentage of the apomictic parent was reduced in subsequent backcross generations. The reason for this remains unknown.

For most crops, however, an apomictic relative is unavailable, so conventional introgression cannot be used to introduce the trait into commercially adapted germplasm. Even in cases where introgression is an option, the type of apomixis available may not be ideal for the intended purpose. Most current research, therefore, is directed at understanding the mechanisms that underlie the expression of apomixis in model species and using this information to direct a synthetic approach to engineering the trait. As apomixis appears to have arisen as a modified form of sexual reproduction, work is progressing on both sexual and apomictic model plants. Before reviewing our current understanding of the genetics of apomixis, it is valuable to consider what form of apomixis would best meet our future needs. To have maximum utility installed, apomixis should address the demands of plant breeders, seed producers, farmers and consumers alike. Seed multiplication, commercial production and downstream processing would all benefit from asexual uniformity, so apomixis would need to be highly expressed during multiplication and commercial production. Plant breeding, however, combines phases where variation is encouraged, with phases in the breeding cycle where uniformity is valued. It would, therefore, be highly advantageous for apomixis to be available in an inducible format. A plant breeder would then be able to switch it off to allow sexual reproduction to generate variation, and then switch it on to permit the clonal multiplication of desirable genotypes.

As mentioned above, many of the natural forms of apomixis lead to polyembryony, the formation of multiple embryos in a seed. This tends to reduce the viability of the seed and it can also increase the variability of the seedlings produced if some of the embryos are sexually produced while others are clonal. An ideal form of apomixis would only result in a single embryo in each seed. Of the known natural forms of apomixis, diplospory would best suit this requirement, as it involves the conversion of the meiotic apparatus to a structure that produces a single maternally derived seedling.

Finally, the formation of the endosperm is a prerequisite for seed formation in most flowering plants and this must also be addressed in any engineered mechanism of apomixis. Grasses are widely cited as potential targets for engineered apomixis, and the endosperm is a particularly prominent tissue in the grass seed. Critically, it is also a key nutritional component of cereals such as wheat, rice and maize, so the success of endosperm formation also dictates the food value of these crops. As mentioned above, the embryo/endosperm ploidy balance is a factor in the normal development of many seeds and it is one that is particularly critical in the grasses. Fortunately, apomixis occurs in many grass species, so asexual mechanisms are already known that enable endosperm formation and appropriate ploidy balance within the grass seed.

7.5 The Genetics of Apomixis

7.5.1 Mutations of Genes in Sexual Plants with Features of Apomixis

The model plant *Arabidopsis thaliana* has been mutagenized extensively and a number of mutants have been reported that appear to display aspects of apomixis.

Mutations reflecting apomeiosis include the *dyad* allele of *SWITCH1*. Plants homozygous for the recessive allele *dyad* form a high proportion of unreduced egg cells that can participate in fertilization (Ravi et al. 2008). Male meiosis is not affected, so triploid progeny are often observed when these plants are allowed to self-fertilize. Similarly, d'Erfurth et al. (2009) identified a gene, *omission of second division* (*OSD1*) that controls the entry of cells into the second meiotic division. Plants with *osd1,* a mutant form of this gene, underwent second division restitution (SDR), producing unreduced gametes.

Several other mutations appear to reflect parthenogenesis. The *LEAFY COTYLEDON* genes *LEC1, LEC2* and *FUSCA3* regulate embryogenesis, specifically the identity of the suspensor, cotyledons and other structures arising late in embryo development (Meinke et al. 1994; West et al. 1994; Lotan et al. 1998; Stone et al. 2001). The ectopic expression of *LEC1* and *LEC2* in vegetative cells induces the expression of embryo-specific genes and also leads to the development of embryo-like structures (Lotan et al. 1998; Stone et al. 2001). The chromatin-remodelling factor *PICKLE* (*PKL*) is a master regulator of *LEC1, LEC2* and *FUSCA3* (Ogas et al. 1999; Rider et al. 2003). In *pkl* mutants, embryo-like structures arise on the roots, indicating that this gene acts to repress embryogenic development throughout the body of the plant (Henderson et al. 2004). Polycomb group proteins participate in the stable repression of specific genes through chromatin remodelling. The fertilization-independent seed (FIS) genes of *Arabidopsis* are members of this group which are involved in endosperm formation and in establishing early parent-of-origin effects (Koltunow and Grossniklaus 2003). *BABY BOOM* (*BBM*) is an AP2 domain transcription factor, the ectopic expression of which induced embryo-like structures to form on the cotyledons and leaves of *Arabidopsis* (Boutilier et al. 2002). *Somatic embryogenesis receptor kinase* (SERK) was found as an upregulated transcript in carrot (*Daucus carota)* cultures induced to form somatic embryos (Schmidt et al. 1997). Interestingly, a similar transcript (PpSERK) was found to be differentially expressed between apomictic and sexual forms of the grass *Poa pratensis* (Albertini et al., 2005). Mutations that demonstrate spontaneous endosperm formation include the *fis* mutants described above, in which the endosperm over-proliferates but is typically poorly cellularized (Kinoshita et al. 1999; Sorensen 2001). *MEDICIS* is a member of this group (Guitton et al. 2004), the mutation of which leads to the formation of rudimentary embryo and endosperm-like structures in the absence of fertilization.

7.5.2 Forward Genetics with Native Apomicts

Research into the genetics of apomixis of native apomicts has largely been approached through mapping or expression analysis, followed by the targeted sequencing of associated genomic bacterial artificial chromosomes (BACs). Apomicts are often cross-compatible to sexual relatives, permitting the production of segregating populations, and apomixis loci are dominant and are often in simplex, so they are amenable to map-based cloning.

For sporophytic apomixis, *Citrus* is the best-studied system. García et al. (1999) reported the detection of six quantitative trait loci (QTL) associated with the extent of somatic embryo formation in a cross between *Poncirus trifoliate* and *Citrus volkameriana*. Recently, Nakano et al. (2008, 2012) provided an analysis of 380 kb of genomic sequence from *Citrus* that is believed to be strongly associated with the trait. The region contains 70 predicted open-reading frames with a diverse range of putative functions.

For gametophytic apomicts, a notable development was the identification of 12 markers tightly linked to the apospory-specific genomic region (ASGR) of *Pennisetum* (Ozias-Akins et al. 1998). No meiotic recombination was seen to occur between the markers and apospory. BACs carrying those markers were physically mapped to the ASGR using fluorescence *in situ* hybridization enabling the size of the region to be estimated at approximately 50 Mb. This is a very large region of nonrecombinant DNA, comparable to the human Y chromosome (59 Mb). Apospory in other grasses also maps to single dominant loci, for example *Brachiaria* (Pessino et al. 1997), *Paspalum* (Martínez et al. 2001) and *Tripsacum* (Grimanelli et al. 1998b).

Given difficulties associated with mapping genes at large nonrecombinant loci, Catanach et al. (2006) used deletion mutagenesis to identify mutations in apomixis loci in the aposporous apomict *Hieracium caespitosum*. Two loci were clearly identified, one associated with apomeiosis (the *LOA* locus) and one associated with parthenogenesis (the *LOP* locus). Amplified fragment length polymorphisms (AFLP) markers were used to form molecular maps of these loci and to confirm that they act as independently inherited, dominant, simplex factors in this plant. Inheritance studies have been reported in two other members of the Asteraceae, the diplosporous apomicts *Taraxacum* (van Dijk et al. 1999) and in *Erigeron* (Noyes and Rieseberg 2000). As in *Hieracium,* two loci appear to act independently, one controlling apomeiosis and the other controlling both parthenogenesis and autonomous endospermy. Recent evidence in *Hieracium* indicates that autonomous endospermy and parthenogenesis can be separated, suggesting that the factors involved are tightly linked rather than pleiotropic in this system (Ogawa et al. 2013).

Until recently, no native apomict was sequenced, nor were there any comprehensive resources such as transfer-DNA (T-DNA) mutant panels available. The current sequencing of *Boechera holboellii* is expected to assist in this effort and may have significant effects on the landscape of apomixis research. Apomixis in *B. holboellii* is typically diplosporous with pseudogamy, although autonomous endospermy is also observed (Naumova et al. 2001). *Boechera* is a relative of *Arabidopsis,* so it is hoped that the extensive resources associated with *Arabidopsis* will be transferable and can be used to identify factors influencing apomixis in this plant.

7.5.3 Genes at Loci That Control Apomixis: What Are the Critical Determinants?

In some apomicts, loci associated with the trait have been cloned and sequenced, revealing candidate regulatory sequences. Calderini et al. (2006) sequenced a BAC clone of approximately 129 kb linked to apomixis from *Paspalum simplex*. They found it contained two genes along with evidence of significant loss of coding capacity due to mutation and transposable element (TE) insertion. The large and nonrecombinant ASGR of *Pennisetum* has both gene-rich and gene-poor regions (Conner et al. 2008) and potentially a vast array of candidate genes. Shotgun sequencing of approximately 2.7 Mb of the ASGR from *Cenchrus ciliaris* and *Pennisetum squamulatum* revealed a total of 74 putative protein-coding regions (PPCRs). Of most interest to the authors were two distinct copies of *ASGR-BABY BOOM* (*BBM*)*-like* genes, orthologous to the *BBM* genes mentioned above. The polyembryony locus in citrus has been fully sequenced and out of a total of 70 open-reading frames, seven were found with similarity to transcription factors (Nakano et al. 2012). The authors propose that the gene or genes that control polyembryony are likely to be transcription factors, and of most interest was a MADS homolog, because of the role that MADS-box transcription factors have in development including embryogenesis.

Whilst some candidate genes for apomixis have been revealed, determining which of them are critical has remained elusive. Only a small part of the ASGR has been sequenced and the authors state that if the whole of the ASGR was sequenced, it would contain too many genes to be analyzed using a gene-by-gene approach (Conner et al. 2008). The polyembryony locus of *Citrus,* while significantly smaller, still contains a significant number of candidates. Approaches towards identifying critical genes include microsynteny, where DNA sequence is compared across related apomicts to identify highly conserved sequences, or between apomicts and their sexual relatives (Gualtieri et al. 2006), to identify key differences such as gene translocations, alterations in regulatory sequences or epigenetic features unique to the apomict. These approaches are available because of the likely single phylogenetic origins of apomixis within related species.

Reports of genes identified at apomixis loci of different species are yet to reveal any common candidate genes. For example, genes linked to apospory of *Paspalum simplex* (Calderini et al. 2006) and the ASGR (Gualtieri et al. 2006) show synteny with different regions of the rice genome, implying that different routes of evolution were taken resulting in positionally different genetic mechanisms of control. Similarly, overlap between apomixis-associated gene expression profiles of more distantly related species is yet to be reported. On the other hand, apomixis loci of apomicts of common lineage often do reflect common apomictic ancestors. The ASGR of the genera *Pennisetum* and *Cenchrus,* and the polyembryony haplotype among a range of *Citrus* species, are both notable examples.

A small number of studies have aimed to determine variations in gene expression seen in apomicts compared with their sexual counterparts. The techniques used have included differential display (Leblanc et al. 1997; Rodrigues et al. 2003) and complementary DNA (cDNA)-AFLP (Albertini et al. 2004; Polegri et al. 2010), and more recently transcriptomics using next-generation sequencing. While expression differences between related sexuals and apomicts have been revealed, it is likely that most, if not all of these differences reflect events downstream from critical signals. Findings from these studies have yet to yield expression differences that clearly reflect regulatory mechanisms underlying the trait. An interesting approach taken by Zeng et al. (2011) found only one transcript from the ASGR that was expressed in apomictic ovules, despite a significant number of genes present in this region (Conner et al. 2008). This result, however, underscores an important advance in expression analysis, where the transcriptomes of critical tissues at critical developmental time points, are used to analyze the role of genes identified at apomixis loci. Further work like this, where gene expression and gene mapping converge, is likely to continue into the foreseeable future.

7.5.4 Evolution of Apomixis and of Apomixis Loci

Models of the evolution of apomixis are currently subjects of considerable discussion in the literature. Determining the evolutionary paths taken for plants to acquire apomixis is not only of academic interest but may also assist in the identification of regulating factors and processes of apomixis, and may even provide opportunities to induce apomixis in sexual species. We have already mentioned the conundrum faced when considering how apomixis evolved, given that the modifications of sexual reproduction, apomeiosis and parthenogenesis (with or without autonomous endospermy) need to be present together for apomixis to be a viable form of reproduction. In most cases, two unlinked loci have been reported, one controlling apomeiosis and another controlling parthenogenesis (Ozias Akins and van Dijk 2007). In apomictic grasses, however, there is often a single locus that carries both apomeiosis and parthenogenesis, which may be a more evolutionarily advanced adaptation, given that both apomeiosis and parthenogenesis need to be present together. Also previously mentioned is how apomixis is often found in perennial plants that already have means of clonal reproduction (for example, through stolons), which may offer a greater opportunity for a plant to acquire all modifications of sexual reproduction separately.

While apomixis has been reported in 32 different plant families (Carman 1997), it is not uniformly distributed. Three families, the Poaceae, Asteraceae and to a lesser extent, Rosaceae, contain most-known apomictic species. This bias suggests the existence of predisposing factor(s) that made members of these families more likely to evolve apomixis. The nature of any predisposing factors is unknown but a genetic component could be speculated. For example, certain combinations of linked genes may provide suitable ancestral loci for genetic modifications for apomixis that took place in sexual ancestors. Physiological predisposing factors could include developmental plasticity such as tolerance to egg/endosperm dosage imbalances, a feature of *Hieracium,* or to the presence of heterochronies involving the timing of gene expression in key events involved in reproduction (Carman 2007). This will be covered in more detail below. It should not be overlooked that the skewed distribution of apomixis amongst a small number of families may also reflect, to some extent, a familial predisposition to ecological and evolutionary success with asexuality, which has manifested in greater representation of apomicts seen today. This raises an interesting view that apomixis or rudimentary forms of apomixis may occur or "evolve" repeatedly, only to be maladaptive to the species and the ecological niche in which it resides.

At the level of genomic structure, apomixis loci tend to have very low rates of meiotic recombination and show "scars" of their genomic isolation with gene mutation, typified by the presence of pseudogenes (Matzk et al. 2003; Calderini et al. 2006), allele sequence divergence (see van Dijk 2009 for review) and retro-element accumulation (Matzk et al. 2003; Akiyama et al. 2004; Okada et al. 2011). The ASGR has little or no recombination occurring across an estimated 50 Mb, and, as a likely consequence, appears diverged to the point of hemizygosity, while the rest of the genome is more able to recombine.

Despite the genomic isolation, the large numbers of functionally conserved predicted gene sequences at apomixis loci is striking. If apomixis utilizes or "exploits" genes normally used in sexual reproduction, the gene complement of apomixis loci might only be a small number of high-level regulators and perhaps lower-level effectors. Apomixis is almost always found in polyploids which have higher gene redundancy, and should therefore be more tolerant of mutation of genes that have no critical dosage requirements. Under these circumstances, any gene at an apomixis locus that is not required for the trait itself, may be prone to eventual loss-of-function mutation. However, counter to this argument, the roles of an apomixis locus may extend beyond the induction of apomeiosis and/or parthenogenesis; there may be genes that have roles in the maintenance of an apomixis locus through evolutionary time. Given that the success of a locus depends on that of the clones in which it resides, it may provide further redundancy, or even plasticity or functionality to ensure the fitness of the clone.

7.5.5 Apomixis as a Product of Hybridization

There is growing support for the hypothesis that apomixis may be induced by hybridization and polyploidization. Most, if not all, natural apomicts show features of being hybrids; in particular, they are usually polyploid and highly heterozygous. The potential role of hybridization in the establishment of apomixis was first postulated by Ernst (1918). Carman (2007) extended this hybridization model by postulating that apomixis is caused by the asynchronous expression of duplicated genes derived from each parent. Mistimed regulation of developmental checkpoints controlling megasporogenesis, embryo sac, embryo and endosperm formation, could lead to unreduced egg cell formation and spontaneous embryogenesis. Typically, when a wide hybrid forms between sexual species, any resulting asynchronous gene expression is under negative selective pressure and synchronization is re-established within a small number of sexual generations. However, in "fledgling apomicts" where the synchronizing fails because of epigenetic modifications of the polyploid genome, apomixis can establish then stabilize. Alternatively, sexual reproduction can be reinstated through successive generations with meiotic recombination disturbing duplicate gene asynchronies (Carman 2007). Fledgling apomicts have received little research interest, probably because of low expression of apomixis. They may, however, be valuable tools for detecting the most fundamental alterations required for apomixis to evolve, as these differences are obscured by stabilizing evolution in established apomictic species.

Evidence of asynchrony of gene expression was reported in ovules of *B. holboellii,* in which heterochrony, gene duplication and "parent of origin" effects were seen in the transcriptomic profiles associated with apomeiosis. In addition, the observation that large numbers of genes commonly occur in nonrecombinant apomixis-associated loci is consistent with the hybridization model. They presumably represent remnant linkage blocks inherited following a wide hybridization event, in which several genes of diverse functions cluster around a smaller number of key regulatory sequences that influence the expression of trait.

7.5.6 Epigenetic Regulation

Epigenetic regulation, including the role of transposable elements, is an expanding frontier of research into many biological processes, and apomixis is no exception. Given the conundrum that apomixis inhibits genetic change yet the expression of apomixis, and the life histories of apomictic species are often highly adapted, it appears that some form of genetic change is occurring at a rate that allows evolution to proceed. Epigenetic modifications can occur without meiosis or fertilization and they can lead to rapid heritable adaptations. Epigenetic modification is also particularly prevalent during hybridization and polyploidization, as discussed above.

Significant research is being undertaken on the regulatory roles of non-coding RNA in a wide range of processes, and roles in aspects of reproduction are now well established. The *Arabidopsis* protein AGONAUTE 9 (AGO9) appears to restrict female gamete formation while repressing transposable elements. Post-meiotic ovules of *ago9* mutants carry additional unreduced somatic cells that have the identity of functional megaspores, which resembles apospory (Olmedo-Monfil et al. 2010). A preliminary investigation into the roles of microRNA expression in apomixis has shown many conserved microRNAs expressed in both sexual and apomictic ovules. One microRNA target, the transcription factor squamosa promoter binding protein like SPL11, is significantly upregulated in *Boechera* stage 2 ovules at the time of megaspore mother cell (MMC) development (Amitey et al. 2011).

7.6 Synthetic Forms of Apomixis

Do we already know enough to attempt the synthesis of apomixis in a model system? As noted above, Ravi et al. (2008) reported that plants homozygous for *dyad* produced a high proportion of unreduced egg cells which could be fertilized. Similarly, d'Erfurth et al. (2009) noted that plants homozygous for *osd1* underwent second division restitution (SDR), producing unreduced gametes. Chromosome recombination and chromatid segregation, however, occur in the first meiotic division, so the gametes formed on their *osd1/osd1* plants did not share the maternal genotype. However, by combining *osd1*with mutations blocking recombination (*Atrec8*) and chromatid segregation (*Atspo11*), they were able to generate a genotype that produced unreduced maternal gametes, effectively synthesizing a form of apomeiosis in *Arabidopsis* (d'Erfurth et al. 2009). Plants with this genotype were named mitosis instead of meiosis (*MiMe*).

Ravi and Chan et al. (2010) constructed a chimeric sequence combining the core of the *Arabidopsis* centromeric histone protein CENH3, the amino terminal tail domain of histone H3.3 and the green fluorescent protein (GFP) reporter sequence. This was referred to as the GFP-tailswap construct. When introduced into a plant homozygous for *cenH3,* a recessive mutant allele of CENH3, none of the nuclear DNA from that modified plant transferred to its progeny. Due to problems with male fertility, this cross was conducted with the modified plant as the maternal parent. Seedlings formed following crosses with wild-type male pollen parents were, therefore, predominantly haploid and entirely paternal in origin. This demonstrated uniparental transmission through the egg—an outcome analogous to parthenogenesis. In this case, it was the male contribution that was conducted and the female genome that was lost. Male parthenogenesis is known to occur very rarely in native systems, the most well-studied example being the formation of patrogenic progeny in the conifer *Cupressus dupreziana* (Pichot et al. 2008).

Combining these findings, Marimuthu et al. (2011) recently demonstrated that when *Arabidopsis* plants with either the *dyad* or *the MiMe* triple mutation were crossed to plants with the GFP-tailswap construct and *cenH3* mutation, a significant proportion of the progeny were strictly maternal in origin. The fertility of these crosses was low and both parents needed to have specific mutant backgrounds, but this result does provide an example of how synthetic apomixis might be achieved.

7.7 Conclusion

Apomixis offers the potential to significantly increase the rate of genetic gain achievable in seed-propagated plants by allowing a breeder to produce true breeding lines with unique, selected, genetic combinations. The desirable genotype might include somatically derived mutations, transgenes and/or hybrid vigour. Research into apomixis is progressing on several fronts. The developmental genetics of *Arabidopsis* has contributed significantly and it is now clear that the regulation of apomixis is very similar to that of sexual reproduction. It appears in most cases to represent a derived form of sex, and this transition has occurred on several occasions in flowering plants. Advances in sequencing, expression analysis, histological imaging and bioinformatics are all contributing to our understanding, most notably in native apomictic systems, which are now gaining in prominence in the apomixis literature. Revealing the determinants of apomixis, however, remains elusive, and research in the future will focus on the convergence of genetic mapping, genomic sequencing and transcriptomics, and also on the role of epigenetic regulation. Investigating the evolution of apomixis is of great interest, and "fledgling apomicts", those that show apomixis or aspects of apomixis following recent hybridization and/or polyploidization events, may provide new clues on the key events that characterize the transition of plants from sexuality to apomixis. Alternatively, the convergence of this knowledge may result in a synthetic form of the trait which may, or may not, reflect the native systems currently known. In closing, therefore, it now seems reasonable to predict that apomixis will be part of the tool kit available to future breeders and propagators of our crop plants and that this will have a significant impact on the genetic advance and practice of agriculture in the coming decades.

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