

Evolution of gametophytic apomixis in flowering plants: an alternative model from Maloid Rosaceae

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Five figures (one multi-part) and four tables; tables 3 and 4 as supplementary material.

Abstract

Gametophytic apomixis, asexual reproduction involving megagametophytes, occurs in many flowering-plant families and as several variant mechanisms. Developmental destabilization of sexual reproduction as a result of hybridization and/or polyploidy appears to be a general trigger for its evolution, but the evidence is complicated by ploidy-level changes and hybridization occurring with facultative apomixis. The repeated origins of polyploid apomictic complexes in the palaeopolyploid Maloid Rosaceae suggest a new model of evolutionary transitions that may have wider applicability. Two conjectures are fundamental to this model: (1) that as previously suggested by Rutishauser, like many sexual flowering plants the polyploid apomicts require maternal–paternal balance in the second fertilization event that gives rise to the endosperm, and (2)

that the observed variation in endosperm ploidy levels relates less to flexibility late in development than to the known variation in developmental origin of the megagametophyte between mechanisms loosely categorized as *diplospory* and *apospory*. The model suggests explanations for the relative frequencies of apospory and diplospory, and for the wide but incomplete associations of apospory with a pollination requirement (pseudogamy) and of diplospory with autonomous development of the endosperm. It is suggested that pollination from other taxa may provide some adaptive advantage to pseudogamous *apospory*.

Keywords

Apospory, Asexual reproduction, *Crataegus*, Diplospory, Endosperm, Gametophytic apomixis, Polyploid evolution, Rosaceae

Introduction

Different authors have used varied terminologies for the developmental phenomena of apomixis in flowering plants (angiosperms), and although there is considerable agreement about terminology today, minor or quite radical differences continue (Table 1). Winkler introduced the term *apomixis* (replacing de Bary's term *apogamy*) to describe asexual methods of reproduction that lack nuclear and cell fusion, and included many forms of vegetative reproduction in his use of the term (Winkler 1908), but more recently in the context of flowering plants it is used as a synonym of *agamospermy*, i.e. asexual reproduction through seed (Crane 2001; Darlington 1937; Hörandl et al. 2007; Koltunow and Grossniklaus 2003; Rutishauser 1969; Vielle Calzada et al. 1996; Whitton et al. 2008). A hierarchical terminology based strictly on developmental processes is extremely

complex and may be impractical, especially for taxa that have not yet been sufficiently studied (Savidan 2000). The terminology used here aims for a simple overview of the processes (Table 1), and follows the tradition in which Edman's term *diplospory* is central, including Gustafsson's later work (starting with 1939), Nygren (1967), Rutishauser (1967; 1969), Nogler (1984), Asker and Jerling (1992) and many others.

With this terminology, a major category of angiosperm apomixis is gametophytic apomixis, in which a female gametophyte develops without meiotic reduction (by apomeiosis) that resembles the gametophytes involved in normal sexual reproduction (Fig. 1; Table 1), and an embryo develops from the unfertilized egg cell of this gametophyte (i.e. by parthenogenesis) or, rarely, from another cell such as a synergid (i.e. by apogamety). Gametophytic apomixis is overwhelmingly associated with polyploidy, and hybridization is also often apparent. Some triploids with gametophytic apomixis are extremely successful, but the majority of plants with gametophytic apomixis are tetraploid (Savidan 2000), and these commonly occur in complexes with related sexual diploids, and sometimes with apomictic triploids. It is the initial evolution of apomixis at these relatively low ploidy levels that I wish to consider, particularly in the Maloid Rosaceae (often called subfamily Maloideae, but most recently known as subtribe Pyrinae Dumort. in tribe Pyreae Baill.; Campbell et al. 2007).

Gametophytic apomixis occurs in many families of flowering plants, through various developmental pathways that are broadly categorized as apospory or diplospory (Table 1, Fig. 1). In Rosaceae, as in other plant families, there are reports that diplospory and apospory can occur in the same species and even in the same ovule (Czapik 1996; Koltunow and Grossniklaus 2003; Nybom 1988; Nygren 1967; Savidan 2000), including

in two Maloid genera, *Malus* (Krylova 1976 citing A. V. Konstantinov), and *Sorbus* (Jankun and Kovanda 1988; Liljefors 1953). However, it is not known whether both processes can produce a viable seed in the same individual.

It has long been suggested that hybridization causes both apomixis and chromosome doubling or chromosome increase through dispermy (Ernst 1917, 1918; Winge 1917), but the strong correlation in flowering plants between gametophytic apomixis and polyploidy suggests that these are not simply alternative strategies when chromosomes cannot fully pair for meiosis. Polyploidy can immediately induce apomixis in some cases (Quarin et al. 2001), or it can arise after hybridization and/or subsequent repatterning (Paun et al. 2006), but apomixis genes may already be present in the parental taxa in these cases. A current view is that both apospory and diplospory result originally from destabilization of sexual development, in time or in space, that occurs (most commonly) with hybridization and/or polyploidy, and that other apomixis genes act as modifiers of the basic process (Bradley et al. 2007; Carman 2001, 2007; Hörandl et al. 2007; Koltunow and Grossniklaus 2003; Rodrigues and Koltunow 2005; Spillane et al. 2001), i.e. that natural selection has been important in the evolution of apomixis, which is not just a weakening of sexual potential (Strasburger 1904) or the escape from sterility that Darlington (1939; 1958) assumed must be short-lived.

Species clusters that exhibit aposporous apomixis and others that exhibit diplosporous apomixis can occur together within a genus (Carman 2001; Fehrer et al. 2007b), and such clusters often appear at the sub-tribe level (van Dijk and Vijverberg 2005). Entire families may show principally one of the two mechanisms, e.g., Asteraceae is largely diplosporous, and Rosaceae largely aposporous. Several genera with apomixis

are included within the Maloid Rosaceae, which have a palaeopolyploid origin followed by rapid ancient radiation of genera (Campbell et al. 2007; Evans and Campbell 2002; Goldblatt 1976; Potter et al. 2007). Several genera with apomixis also occur in Poaceae subfamily Panicoideae, which, although not clearly a distinct polyploid group, is affected by ancient polyploidy and subsequent chromosome loss (Hilu 2004). The polyploid apomicts in each genus within these groups have sexual diploid ancestors, but a predisposition to produce apomictic descendants appears likely to have been conferred by ancient polyploidization. Because polyploidy has played a role in the evolution of many, perhaps most angiosperms (Ramsey and Schemske 1998), it is possible that all apomicts have palaeopolyploid ancestry (Roche et al. 2001). Capture of apomixis genes via now-obscure hybridization cannot yet be entirely ruled out, but here I disregard the gene-capture hypothesis and consider the likely possibility that apospory has arisen repeatedly *de novo* in the Maloideae. The resulting model of evolution should point to experiments to clarify this question.

With gametophytic apomixis, the pollen often functions normally. Pollination may be required for (fruit or) seed set, which is pseudogamy (following Focke's terminology (1881)). The pollination requirement may involve fertilizing the central cell as in sexual reproduction, which is centrogamy (in Solntzeva's terminology (2003)), to give rise to the endosperm, a nutritive tissue for the seed. Pseudogamous gametophytic apospory is common in the Maloid Rosaceae, and we have shown that in the genus *Crataegus* the pollen probably always fertilizes the central cell (Talent and Dickinson 2007b). In many plants with pseudogamous gametophytic apomixis, including *Crataegus*, ploidy-level increases can occur if the meiotically unreduced egg cell is fertilized by one of the two

sperm from the pollen grain (Clausen 1961; Nogler 1984; Stebbins 1941, p. 513; Talent and Dickinson 2007a). When this occurs in (partly) apomictic plants, the barriers to interspecies matings are sometimes less stringent than in matings between sexual species (Clausen 1961; Nogler 1984), so that ploidy-level increase and hybridization occur together. The hybridization picture is further complicated by “wider crosses between polyploids than between diploids” (Harlan and de Wet 1975), and matings between sexual diploids and either apomictic or sexual polyploids that produce polyploid apomicts (Fehrer et al. 2007a; Naumova et al. 1999; Talent and Dickinson 2007a). Hybridization and ploidy-level changes can probably occur repeatedly in the ancestry of any given apomict. Thus, the observation that many apomicts are derived from hybridization (Ernst 1918) does not support (Harlan and Wet 1975) the common conjecture (Camp 1942a; Carman 2007; Stebbins 1941, 1950, 1980; Stebbins and Babcock 1939) that all of them arose from initial diploid–diploid hybridization with subsequent ploidy-level increases. Nor, as Winkler saw (1920, as cited in Ernst 1921), is it appropriate to survey polyploid apomicts for hybrid ancestry, which was advocated by Ernst (1918) and has been suggested (Whitton et al. 2008) as a means of determining whether hybridization is “the causal link between apomixis and polyploidy”. The genera of Maloid Rosaceae radiated an estimated 48–50 million years ago (Campbell et al. 2007), and a simple correlation between present-day apomixis and present-day allopolyploidy is unlikely to reveal very much about the origins of apomixis.

It has long been suggested (Fagerlind 1944) that apomixis in diploids would be an important evolutionary component that leads to higher ploidy levels. However, diploid plants with a high rate of apomixis are very rare (reviewed by Savidan 2000), and those

that have been well studied contain supernumerary chromosomes or genomic regions (Calderini et al. 2006; Dobeš et al. 2006; Roche et al. 2001), possibly indicating hybrid or polyploid origin. On the other hand, apomeiotic megagametophytes occur moderately frequently in the multicellular archaesporium of otherwise sexual diploids of Rosaceae (Davis 1966; Jankun and Kovanda 1988; Krylova 1976). Here I build on the available evidence of apomixis and of embryological variability in Maloid Rosaceae, recent data primarily from the genus *Crataegus* (Talent and Dickinson 2007a, b), and some other tenable hypotheses, to build a model of evolutionary transitions. It is concluded that a particular type of gametophytic apomixis may have greater potential for gene flow between ploidy levels and also some adaptive benefits that contribute to building successful apomictic complexes. The resulting evolutionary model offers explanations for some long-observed patterns, including the relative frequencies of apospory and diplospory and their frequent associations respectively with pseudogamy/centrogamy (pollination and fertilization of the endosperm) and with autonomous endosperm development.

Evolutionary models

Gametic imprinting and the origins of polyploid apomicts

Polyploidy can result either from somatic doubling or from unreduced gametes. As with many other plant groups (Harlan and de Wet 1975; Ramsey and Schemske 1998), both auto- and allopolyploids occur in *Crataegus* (Lo 2008; Talent and Dickinson 2005).

Allopolyploids could arise immediately from unreduced gametes, or from diploid–diploid hybridization followed by polyploidization. A third possibility, of crossing between auto-tetraploids, is emphasized by Harlan and de Wet (1975) but does not appear to be

important in *Crataegus* where apomixis is often nearly obligate (Talent and Dickinson 2007a). A model proposed by Camp (1942a) for the evolution of apomixis in (North American) *Crataegus* emphasizes initial diploid–diploid hybridization, meiotically unreduced gametes, and selection for apomixis among otherwise nearly sterile triploids (Fig. 2). A similar model has been tested and is well accepted for some other plant groups (Hörandl 2006), but in *Crataegus* we have not been able to find diploid–diploid hybrids among the numerous sexual diploid and apomictic triploid and tetraploid North American species (Talent and Dickinson 2005; Talent and Dickinson 2007c). Thus, the hybridization that is evident to taxonomists (Camp 1942b; Eggleston 1910; Palmer 1932, 1943; Phipps 2005) might not result in frequent or persistent diploid hybrids.

Camp reasoned that diploid hybrids give rise to triploids because “in other groups such hybrids are notorious for the production of at least some unreduced gametes. Therefore fusion of unreduced and reduced gametes could result in triploid individuals” (Camp 1942a). Karpechenko’s hybridization experiments with *Raphanus sativus* × *Brassica oleracea* (1928) had demonstrated that failure of meiosis in largely sterile diploid hybrids could produce both male and female unreduced gametes, and hence polyploid offspring. However, it is noteworthy that the diploid *Raphanus–Brassica* hybrids produced tetraploid offspring from pollination among themselves, and triploid offspring when planted with (diploid) *Raphanus sativus* but not when planted with (diploid) *Brassica oleracea*. This asymmetric mating might possibly be an example of the *triploid-block effect* that is known in many flowering-plant families to destroy triploid embryos, particularly autotriploids (Brink and Cooper 1947a, b; Katsiosis et al. 1995; Kermicle and Alleman 1990; Kihara and Nishiyama 1932; Lin 1984; Müntzing 1933; von

Wangenheim 1961). Rutishauser (1967) noted incompatibilities in pseudogamous apomictic *Ranunculus* consistent with a triploid-block effect. Thus, the evolution of polyploids from unreduced gametes is not necessarily as straightforward as Camp (1942a) stated.

The triploid-block effect, as well as preventing triploidy, also has a role in preventing wide hybridizations at the diploid level through a requirement for balance between the parental genomes in the fertilization event that leads to the endosperm tissue of the seed (Gutierrez-Marcos et al. 2003; Haig and Westoby 1991). The developmental effects of parental imbalance vary in intensity, from complete seed failure to abnormal but viable development of the endosperm, and differ according to the direction of the cross-pollination. Gametic imprinting is involved, whereby the alleles of certain genes inherited from one or the other parent are silenced at important early stages of seed development, but the process is still not fully understood in flowering plants (Autran et al. 2005; Choi et al. 2002; Kiyosue et al. 1999; Vinkenoog and Scott 2001; Vinkenoog et al. 2003). In *Arabidopsis thaliana*, where the imprinted genes have been best studied, the effect is relatively weak but serves to prevent $2x-6x$ crosses (Scott et al. 1998).

Imprintable genes activated on the maternal side appear to be under the control of polycomb *FIS* (*FERTILIZATION INDEPENDENT SEED*) genes and *DEMETER* (*DME*) (Scott and Spielman 2004).

A triploid-block effect involving developmental failure of the endosperm is widespread in eudicots and in monocots (Table 2), and it has been suggested (for both plants and mammals) that major change in the balance of imprinted genes is an important speciation mechanism (Haig and Westoby 1991; Varmuza 1993). When an endosperm-

balance requirement prevents hybridization, plant breeders can sometimes produce triploid hybrids by replacing one parent by its autotetraploid (Carputo et al. 1999; Håkansson and Ellerström 1950; Lin 1984), and this has been referred to as halving or doubling the *effective ploidy* or *endosperm-balance number* (Brink and Cooper 1947a, b; Carputo et al. 1999; Jennings et al. 1967; Johnston et al. 1980). Haig and Westoby's evolutionary model (Haig and Westoby 1989, 1991) predicts that many genes of small effect would be involved, but Hawkes & Jackson (1992) found in *Solanum* that the success or total failure of a cross may be genotype dependent, which would indicate that mutations of large effect can occur, which presumably either multiply the endosperm-balance number, or produce a nearly equivalent effect. Thus, a genus like *Crataegus* that has numerous triploids (Longley 1924; Talent and Dickinson 2005) might be displaying the effects of allopatric diversification and subsequent sympatry similar to that seen in *Solanum* (Hawkes and Jackson 1992). With this scenario, diploid–diploid hybridization of particular North American species that differ in endosperm balance number would produce triploid offspring via unreduced gametes rather than diploid offspring. Evidence for the endosperm-balance requirement that produced that effect would be difficult to find unless the original hybridizing species pairs were examined, but there are data to suggest that a weak endosperm-balance requirement is retained in some apomictic *Crataegus* (Talent and Dickinson 2007a), as in *Ranunculus* (Rutishauser 1967).

Apomixis and the endosperm

Endosperm development is an important aspect of apomixis that until recently has received relatively little attention (Czapik 1996; Nogler 1984; Savidan 2000). The central cell of the majority of flowering-plant species is binucleate (Huang and Russell 1992), so

the ratio of parental genomes contributing to the endosperm in sexual plants is 2 maternal : 1 paternal (Fig. 3). Many, and perhaps most, sexual angiosperms require this 2:1 ratio (Brink and Cooper 1947a, b; Katsiosis et al. 1995; Kermicle and Alleman 1990; Lin 1984; Vinkenoog and Scott 2001; Vinkenoog et al. 2003; von Wangenheim 1961), which relates to the triploid-block effect (above). Centrogamous apomicts (discussed above) require fertilization of the central-cell to form the endosperm, as in sexual reproduction (Nogler 1984). In these cases, the megagametophyte is meiotically unreduced but the pollen usually undergoes normal meiosis, and the post-fertilization genome balance in the endosperm would therefore not match the 2:1 ratio (Haig and Westoby 1991). This apparent problem is bypassed in some apomicts, notably many Asteraceae, where endosperm development is autonomous, without fertilization (Nogler 1984), or the endosperm may be irrelevant to nutrition of the seed (Cooper and Brink 1949). Two other mechanisms have been noted that change the parental genome ratio to match that in sexual plants: some apomictic Poaceae form megagametophytes with one rather than two central-cell nuclei (Brown and Emery 1958; Savidan 2000; Warmke 1954), while in *Ranunculus* (Ranunculaceae; Nogler 1984) and *Crataegus* (Rosaceae, Pyrinae; Talent and Dickinson 2007b), both sperm from the pollen grain can contribute to the endosperm. But an “unbalanced” endosperm has been seen in *Crataegus* (Talent and Dickinson 2007b), in *Paspalum* and *Tripsacum* (Poaceae, Panicoideae; Brown and Emery 1958; Quarin 1999), and in a minority of the seeds sampled from *Ranunculus* (Ranunculaceae; Rutishauser 1954).

A majority of seeds sampled from apomictic *Crataegus* showed an unbalanced endosperm (Table 3). Also, experimental crosses between different ploidy levels are

often successful (Table 4), including crosses where the male parent is an apomictic tetraploid and the female parent is a sexual diploid. Thus, the endosperm-balance requirement might appear to have been lost by the sexual diploids, even before apomixis evolved in polyploid *Crataegus*, and it has been suggested (Grimanelli et al. 1997; Quarin 1999) that pseudogamous apomixis cannot evolve until the endosperm-balance requirement is relaxed or removed. However, although larger studies are required, there is some suggestive evidence that a degree of balance between maternal and paternal gametes is still required in *Crataegus*: a sample of intra-taxon pollinations of diploids produced strictly triploid endosperm, and the strongly apomictic tetraploids that produced primarily 10x and 12x endosperm with intrataxon pollination formed seeds with 10x but not with 9x endosperm when pollinated from diploids (Table 3). There is also good evidence that an endosperm-balance requirement exists in subfamily Rosoideae of Rosaceae (Table 2). I therefore suggest that the apparently flexible endosperm behaviour in *Crataegus* results, at least partly, from earlier variability in developmental processes and that a degree of endosperm-balance constraint *is* retained with apomixis and polyploidy.

Types of gametophytic apomixis: apospory and diplospory

Gametophytic apomixis in flowering plants produces an embryo within an apomeiotic megagametophyte. The developmental processes involved in apomeiosis are not yet well understood at a genetic level, but developmental categories are commonly broadly distinguished according to which of two cell types gives rise to the megagametophyte (Fig. 1; Table 1). With this terminology, a diplosporous megagametophyte develops either from the megaspore mother cell (MMC) or from a cell in the equivalent position

that has not necessarily differentiated as an MMC. An aposporous megagametophyte develops from cells somewhat distant from the MMC in the nucellus or the inner integument of the ovule.

A complex terminology exists for subtypes of diplospory (Crane 2001; Gustafsson 1946; Nogler 1984; Savidan 2000) according to whether all cell divisions leading to the megagametophyte are truly mitotic or at what stage meiosis fails (Table 1). The mitosis/meiosis distinction might be important genetically, but, as Nogler has pointed out (1984), the differences between the principal types of diplospory might be due only to a difference in the strength of a single [hormonal/signaling] factor.

The terminology of apomeiosis (Fig. 1) is confused for plants like the Rosaceae that have a multicellular archaesporium (Campbell et al. 1985; Gustafsson 1946; Hjelmqvist 1957; Liljefors 1953; Maheshwari 1950). The development of the archaesporium involves different numbers of mitoses at different positions, and the developmental history of individual cells can differ in ways that have rarely been tracked in detail. In sexual reproduction in the Rosaceae, usually only a single MMC produces the sexual megagametophyte, but in several genera of Maloid Rosaceae, including *Malus*, *Sorbus*, and *Cotoneaster*, the MMC will sometimes degenerate to be replaced by one or more secondary MMCs that differentiate from other cells of the archaesporium, undergo meiosis, and give rise to megagametophytes (Davis 1966; Hjelmqvist 1962). Some authors therefore consider that diplospory occurs anywhere in the archaesporium, and that apospory occurs only outside that region (e.g., Christen 1950; Eriksen and Fredrikson 2000; Jankun and Kovanda 1988; Krylova 1976). Others restrict the term *diplospory* to megagametophytes that originate from the single megaspore mother cell

(e.g., Asker and Jerling 1992; Hjelmqvist 1962; Liljefors 1953; Nogler 1984). Multiple aposporous initials make embryological observation difficult, but it is known that both apospory and diplospory can occur in the same species, or even in the same ovule, and these reports include species of Maloid and other Rosaceae (Czapik 1996; Jankun and Kovanda 1988; Koltunow and Grossniklaus 2003; Nygren 1967; Savidan 2000). Multiple aposporous initials are frequently reported in *Crataegus*, and the process has been simply called apospory (Dickinson 1983; Dickinson and Phipps 1986; Muniyamma and Phipps 1979a, b, 1984b; Smith and Phipps 1988). Diplospory has been reported in a single triploid, where a single initial cell of the archaesprium was involved, presumably the MMC (Muniyamma and Phipps 1984a).

The terminological uncertainty in Rosaceae underscores a lack of knowledge about the relationships between apospory and diplospory that nonetheless are clearly similar processes, and might be viewed as stages along a continuum (Fig. 1). I will use the terms *apospory* and *diplospory* in a theoretical sense (Table 1), as labels for subtly different sequences, combinations, or levels of gene expression that produce two categories of megagametophytes that behave differently after fertilization, a difference based on gametic imprinting. Possibly, several different classes of gametophytes occur, and these terms may apply to extremes of post-fertilization behaviour. The detailed studies of development and gene expression that might directly support this distinction do not yet exist.

An hypothesis concerning endosperm-balance number

The ploidy levels of endosperm in *Crataegus* samples showed apomictic polyploids without the same constraints for endosperm formation as sexual diploids (Table 3),

usually with a relatively high ratio of maternal to paternal genome copies in the endosperm (e.g., the 4:1 ratio in most seeds from tetraploids). A second notable feature was the readiness with which the diploids, triploids, and tetraploids hybridized. A relaxation or complete lack of an endosperm-balance requirement is a satisfactory explanation for both phenomena. However, I wish to consider the possibility that diploid and tetraploid *Crataegus* can interbreed because the endosperm-balance number of certain tetraploids is “halved”, as has occurred in other plant families (Carputo et al. 1999; Ehlenfeldt and Ortiz 1995). Parental imprinting in *Arabidopsis* has been shown to involve silenced genes (Autran et al. 2005; Scott and Spielman 2004; Vinkenoog and Scott 2001; Vinkenoog et al. 2003), and therefore a halving of the endosperm-balance number could presumably occur through the loss or permanent silencing of some imprintable genes or through some other mechanism that makes these loci unavailable for activation.

If this type of gene loss or gene silencing has occurred, then one possibility is that the duplicate copies of the imprintable endosperm-related genes in triploids and tetraploids are affected. Genomic repatterning occurs in hybrids and in polyploids (Chen 2007; Grant 1981; Koltunow and Grossniklaus 2003; Otto and Whitton 2000; Rapp and Wendel 2005; Spillane et al. 2001), and more specifically, in neopolyploids one copy of duplicate genes may show biased expression or silencing (Adams et al. 2003; Adams and Wendel 2004; Cronn et al. 1999; Martelotto et al. 2005). A comment about polyploid *Solanum* (Hawkes and Jackson 1992) is therefore intriguing: from a small amount of evidence, it appeared that in autotetraploid potatoes the endosperm-balance number was twice that of the diploid parent, but in allotetraploids it was half the sum of those of the

diploid parents. Given the probable widespread allopolyploidy in *Crataegus* (Talent and Dickinson 2005), allopolyploidy therefore appears to be a possible trigger for a reduction in the endosperm-balance number that could account for the crossing ability of diploids and polyploids.

The endosperm-balance-number hypothesis for *Crataegus* as just stated could satisfactorily explain the observations that diploids and tetraploids were able to hybridize through their sexual gametophytes and that triploids were able to accept pollen from either diploids or tetraploids, but it does not explain the high maternal ratio in the endosperm of apomicts (Table 3). The models below explore the extent to which these two components might be related.

Model 1: loss of constraint in high-ploid endosperm, a previous model

Quarin's model for *Paspalum* in Poaceae subfamily Panicoideae (Quarin 1999) is that endosperm with high ploidy levels (maternal contribution from the binucleate central cell $6x$ or more, as in triploids and tetraploids) loses the endosperm-balance constraint, and thus apospory is successful only in polyploids. This model could explain the rarity or absence of apomictic diploid *Crataegus*, but not the formation of diploid–tetraploid hybrids, which requires a separate reduction of the endosperm-balance number of polyploids, as discussed above. Unlike model 2 below, it does not suggest any reason for the repeated origins of apomixis in different genera of the allopolyploid clade (Rosaceae subtribe Pyrinae) that includes *Crataegus*. Quarin's model was recently challenged by indications that apomixis occurs in diploid *Paspalum*, but, for unknown reasons, is rarely expressed (Siena et al. 2008).

Model 2: replicated genes show various behaviours

This second model uses the fact that the Maloid Rosaceae are an ancient polyploid clade (Evans and Campbell 2002; Goldblatt 1976; Potter et al. 2007), and therefore two distinct sets of imprintable genes from the parents of the original polyploid would have existed at one time (Fig. 3). A behavioural difference between the genes inherited from each of the parents of the ancient polyploid is compatible with what is known about epigenetic changes subsequent to hybridization and polyploidy (Chen 2007; Rapp and Wendel 2005), but it is necessary for what follows to assume that these genes have not been lost but can be activated during or after meiosis (Fig. 3b, not 3a). If one set of the ancient replicated imprintable genes cannot be activated as a result of *apospory* (Fig. 4a), then a 2:1 ratio of maternal to paternal imprinted genes would be retained in the endosperm, the same ratio as with sexual reproduction.

In the triploids studied, pollen meiosis is unsuccessful, but pollen from diploids or tetraploids can produce apomictic seed (Table 4); this might occur because the level of imprinting in the central cell of triploids is intermediate between that of diploids and tetraploids, and the balance requirement is not absolute. However, the ability of diploids and tetraploids to interbreed with one another can be explained by halving the endosperm-balance number of tetraploids relative to diploids (as discussed above). Therefore, it is proposed that in recent triploids and tetraploids the number of imprintable genes that can be activated is the same as in diploids (the tetraploid case is shown in Fig. 4b). It is not necessarily the case that all diploids can interbreed with all tetraploids, but only that many compatible combinations exist.

Thus, two separate reductions in the number of functional imprintable genes are proposed, one that results from ancient allopolyploidy, and one that results from recent (allo)polyploidy. The alteration due to ancient polyploidy is that some gene copies resist imprinting in *aposporous* gametophytes but undergo imprinting in sexual and *diplosporous* gametophytes. The alteration due to recent (allo)polyploidy is reduction of the endosperm balance number as described above in the section “An hypothesis concerning endosperm-balance number”.

Model 3: imprinting does not occur in one central-cell nucleus during apospory

This third model is not directly related to gene-duplication in polyploid clades such as the Maloid Rosaceae (subtribe Pyrinae) and Panicoid Poaceae (subfamily Panicoideae), but involves developmental changes that are possibly due to gene duplication (and might also result from other mutations). It comes from considering the morphology of the gametophytes of some Panicoideae, which in the same plant can produce sexual gametophytes with an eight-nucleate *Polygonum*-type morphology (and a binucleate central cell), and *Eragrostis-Panicum*-type diplosporous gametophytes that have only four nuclei and (usually) four cells (two synergids, one egg cell, and a central cell with a single nucleus; Nogler 1984; Savidan 2000; Warmke 1954). In the development of these gametophytes, the spindle of the first of two mitotic divisions is oriented crossways rather than longitudinally as in the first of the three divisions that form the sexual gametophytes or the aposporous gametophytes of other plants (Nogler 1984). This suggests that it is the first of the three mitotic divisions that has been lost in *Eragrostis-Panicum*-type development, and that a reversion has occurred to a simpler condition where the gametophyte contains a single modular quartet rather than two modules and eight nuclei

(c.f. the modular duplication hypothesis in the evolution of the Angiosperm gametophyte; Friedman and Williams 2004; Friedman et al. 2008).

I hypothesize that, somewhat parallel to the loss of a modular quartet in diplosporous Panicoideae, the gametophyte of *aposporous Crataegus* has a partial loss of function in one of the quartets (Fig. 4c). This might result from heterochrony (c.f. Friedman et al. 2008) such that the determination that imprinting will occur takes place after the first mitotic division in aposporous gametophytes. In *Arabidopsis*, the *DEMETER (DME)* gene has been implicated in maternally activating imprinted genes (Scott and Spielman 2004), and *DME* is expressed in the central-cell nuclei before they fuse (Choi et al. 2002). The proposed partial loss of function might take the form that a *DME* homologue is not activated in the central-cell nucleus of one quartet of *aposporous Crataegus* (Fig. 4c).

Extending the models to diplospory

Models 2 and 3 above, and potentially some modification of model 1 that has yet to be proposed, involve a partial loss of imprinting during apomeiosis, and they are compatible with a (separate) reduction in the endosperm-balance number of polyploids. A particularly powerful addition can be made to these models by supposing that both *apospory* and *diplospory* routinely occur in diploid and polyploid *Crataegus*, and that *diplospory* involves a similar level of imprinting as regular meiosis. If this is true (and if an endosperm-balance requirement exists) then, for example in a tetraploid, the meiotically unreduced gametophyte should succeed only if it forms 12x endosperm (Fig. 4d).

Meiotically unreduced pollen could produce a 12x endosperm, or alternatively both sperm of reduced pollen must be diverted to the central cell. There is some evidence from

inter-ploidy pollinations that both sperm from the pollen can be diverted to the endosperm in *Crataegus* (Talent and Dickinson 2007a), as has been demonstrated in *Arabidopsis* (Spielman et al. 2003) and has been suggested for other apomictic flowering plants (Bashaw and Hanna 1990; Rutishauser 1954; Savidan 2000).

Discussion

The proposed new models (models 2 and 3) are equivalent in what they indicate about the evolutionary spread of apomixis in Maloid Rosaceae; only the details of megagametophyte development and endosperm formation differ. Henceforth they are considered as a single model. Thus, diploid hybrids are not a necessary step in the evolution of apomixis, but triploids, and most likely allo-triploids, would be important (Fig. 5), and this seems to fit well with the high frequency of triploids among North American *Crataegus* (Talent and Dickinson 2005). European *Crataegus* includes many diploid hybrids, but it is not clear that apomixis is as prevalent among European species. However, the new models also suggest that extensive triploidy might have evolved later than apomixis (Fig. 5); that in a genus where apomixis is evolving, triploids could be an important component, but not necessarily numerically common. Thus, in apomictic genera that are likely to be *aposporous*, and have numerous tetraploids and few triploids, the triploids might repay detailed study. Such genera include *Ranunculus auricomus* (Rutishauser 1967), some but not all Panicoid grasses (Naumova et al. 1999), and some but not all Maloid Rosaceae (Talent and Dickinson 2007c). Because of the difficulty of distinguishing diplospory from apospory, some genera currently considered to be diplosporous with numerous tetraploids and rare triploids might also fit this pattern.

The evidence that unreduced egg cells of apomictic *Crataegus* can be fertilized (Table 4) suggests that polyploids have been formed from apomeiotic gametophytes in diploids, although this process may no longer play an important part in ploidy-level transitions in the genus (Fig. 5). A more common route to ploidy-level changes involves triploid offspring from diploids that received pollen from apomictic tetraploids (Table 4), and alleles that canalize apomixis are presumably transferred to further triploids in this way. Parthenogenesis can also create dihaploids (Table 4), and if such seeds are viable, this would provide another route for apomixis alleles to be transferred to diploids.

With the models presented above, *apospory* and *diplospory* would both occur as developmental anomalies, but the requirements for endosperm development would differ. The terms *diplospory* and *apospory* are used here in genetic or functional senses, and there is no reason to suppose that structural differences could be observed in the megagametophyte. With *apospory* in a diploid, only one of the two meiotically reduced sperm would be required for endosperm development (Fig. 4, a–c show a similar situation in a tetraploid), and if the second sperm was able to fertilize the egg cell, then a triploid embryo would be produced. With *diplospory*, it is suggested that endosperm development requires either two meiotically reduced sperm, in which case fertilization of the egg cell would be impossible (Fig. 4, d shows a similar situation in a tetraploid), or one meiotically unreduced sperm, in which case any fertilization of the egg cell of the diploid would produce a tetraploid. If meiotically reduced pollen is produced, as is commonly the case in apomictic plants (Nogler 1984), it would be more likely to reach a stigma than unreduced pollen. Thus, the new model predicts that polyploid derivatives

from diploid *apospory* are likely to be triploid, but from diploid *diplospory* are relatively rare and most likely to be tetraploid.

The predictions that *diplospory* and *apospory* would lead to different ploidy levels in the progeny profoundly affect the evolution of a fully functional system of apomixis in polyploids. Because tetraploids are much more likely than triploids to be sexually fertile, triploids may provide an important component of the selection for fully functional apomixis (Camp 1942a; Darlington 1939, 1958). This might explain the observation (Nogler 1984) that apospory is much more common than diplospory in the entire Rosaceae family. It is difficult to be sure, however, whether the observed prevalence of structural apospory, which is complicated by the terminological confusion between diplospory and apospory in Rosaceae, reflects a prevalence of *apospory* in the sense that is used here. However, another suggestion that the two senses of the word might correspond quite closely comes from the fact that among angiosperm families generally, diplospory and apospory seem to be about equally common (they are reported in 21 angiosperm families each; Carman 1997). This is even though, to quote Savidan (2000), “It might be expected that meiotic mutants whose phenotype is associated with a failure of meiosis could be found in a wide range of species and families, and that consequently the diplosporous type of apomixis would be more wide-spread than apospory.” Savidan’s argument is strengthened by recent evidence from *Arabidopsis thaliana* where a single-gene mutation confers diplospory (Ravi et al. 2008) and viable endosperm is still possible because of the weak endosperm-balance requirement in that species (Scott et al. 1998).

More specifically, if pseudogamous *diplospory* is less capable of producing triploids than is pseudogamous *apospory*, then this might explain the general (but incomplete)

association of pseudogamy/centrogamy with apospory and of autonomous endosperm with diplospory (summarized by Nogler 1984; Nygren 1967). *Diplospory* may simply be less likely to succeed until it can come together with a mutation that confers autonomous endosperm or replaces the nutritive function of the endosperm in the seed. In support of that conjecture, genes important to diplospory and autonomous endosperm have been resolved to separate loci in *Taraxacum* and in *Erigeron* (Asteraceae ; Noyes et al. 2007; Tas and Van Dijk 1999; van Dijk et al. 1999).

Some specific implications of the models

Seed failure in *Crataegus*

The seed set of apomictic *Crataegus* is often very low, with around 20% of the pistils of pollinated flowers producing a seed (Dickinson 1983; Macklin 2001; Smith and Phipps 1988; Talent and Dickinson 2007a, b). If, as suggested above, both *apospory* and *diplospory* coexist in *Crataegus*, and if the fertilization requirements of the two types of gametophytes differ, then failure of the endosperm would occur whenever the central cell was fertilized by an inappropriate number of sperm. A DNA measurement from a seed that was failing for this reason would, unfortunately, be indistinguishable from a DNA measurement from a healthy seed. Measurements of DNA from failing seeds have not differed from measurements from healthy seed (Talent and Dickinson 2007a), and may thus be consistent with this scenario, but further investigation is needed.

Winter chilling

Endosperm fertilization appears to be obligate in *Crataegus* (Talent and Dickinson 2007b), and just possibly might provide an additional adaptive benefit to pseudogamous *apospory* relative to autonomous *diplospory*. One possibility involves what Haskell

(1960) has termed “pseudogamous heterosis”, i.e. heterosis effects due to an “out-crossed” endosperm that can improve seedling vigour. However, more specifically, the genus *Sorbus* appears to parallel *Crataegus* very closely (reviewed in Talent and Dickinson 2007c), and apomictic *Sorbus forrestii* showed a seed-phenotype difference with self-pollination relative to pollination from a different species (McAllister and Gillham 1980). Excised embryos from cross-pollination were unable to germinate without chilling, and the authors concluded that the paternal contribution to the endosperm determined the mobility to the embryo of dormancy factors produced in the endosperm that determine the chilling requirement. Thus, fertilization of the endosperm might be allowing apomicts to borrow endosperm adaptations to winter conditions from locally adapted forms, notably from congeneric diploids. The genetic basis of seed dormancy is not yet understood, and it is unclear how closely winter-chilling requirements correlate with climate (Barton and Crocker 1948; Finkelstein et al. 2008); germination of *Crataegus* seed is affected by endocarp characteristics as well as a chilling requirement (Bujarska-Borkowska 2007). However, detailed studies of distributions, climate, and pollination in pseudogamous apomictic *Crataegus* and *Sorbus* might answer the question of whether endosperm fertilization is a component of the long-standing puzzle, the apparently greater adaptability of polyploid/hybrid/self-compatible/apomictic species to climate variation, particularly to cold conditions (Bayer 1998; Brochmann et al. 2004; Hörandl 2006; Richards 1986; Stebbins 1980; Thompson and Lumaret 1992; van Dijk 2003).

Alternative explanations for the rarity of apomixis in diploid Rosaceae

Polyploidization can increase the expression of apomixis (Nassar 2006; Quarin and Hanna 1980; Quarin et al. 2001), but is not always essential. In Maloid Rosaceae, diploid apomicts have been found in *Sorbus eximia* (Pyreae; Jankun and Kovanda 1988).

Apomeiosis occurs as a rare phenomenon in otherwise sexual diploid *Crataegus* (Dickinson 1983) and can produce mature apomictic seeds (Table 3). An initial genetic tendency towards apomixis is expected to increase with selection for reproductive fitness, because of the “cost of meiosis” (Barton and Charlesworth 1998; Charlesworth 1980; Marshall and Brown 1981; Noirot 1993), but this has not occurred in diploids of Rosaceae or generally among diploid flowering plants.

Genetic models to explain the rarity of diploid apomixis in Asteraceae, Poaceae, and Ranunculaceae include theoretical gene-dosages that would not occur in diploids (Mogie 1992; Noirot 1993), better tolerance of supernumerary DNA in polyploids (Roche et al. 2001), and the gametophyte-expressed lethal model (Nogler 1982, 1984; Richards 1996), and the segregation-distorter model (Grimanelli et al. 1998) which hold that apomixis genes cannot be transferred (via pollen) in the monoploid or homozygous conditions (although they can be transferred to diploid plants that arise through parthenogenesis of a meiotically reduced egg cell). Also in Asteraceae, Bicknell et al. (2000) deduced that selection acted against diploid zygotes rather than against haploid gametes. Archetti’s theoretical model (2004) predicts that homozygosity increases, exposing recessive deleterious alleles, but this applies to meiotic diplospory with crossing-over, and not to mitotic diplospory or apospory.

The new model presented above involve selection in polyploids for traits that enhance apomixis, but they are not the only possible such models. Total or near-total sterility in triploids is not necessarily required for alleles related to apomixis to have a strong selective advantage. The minority-cytotype exclusion principle of Levin (1975) predicts that a newly established polyploid primarily receives pollen from plants of the different (ancestral) ploidy level, or its own pollen that is likely to be either incompatible or to carry recessive deleterious alleles. Under such conditions, autonomous endosperm and apomixis would both be favoured.

An alternative explanation for the scarcity of apomictic diploids in Rosaceae is an immediate result of polyploidy, and relates to pollen self-incompatibility. Modeling of aposporous apomixis in Poaceae (Noirot et al. 1997) demonstrated that self-compatible pollen is necessary for the maintenance of a population of pseudogamous apomicts. Although diploid Rosaceae generally have self-incompatible pollen, neopolyploids probably always have 50% or more self-compatible pollen (Crane and Lewis 1942; de Nettancourt 2001; Entani et al. 1999; Grant 1981; Lewis 1949; Ramsey and Schemske 2002; Ridout et al. 2005; Takayama and Isogai 2005; Yamane et al. 2003). Thus, self-compatibility may be sufficient to explain the greater frequency of apomixis among polyploid Rosaceae (but it is apparently not known whether the apomictic diploid *Sorbus eximia* (Jankun and Kovanda 1988) is self-compatible).

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Supplementary material is available as follows:

Table 3: Endosperm ploidy levels from *Crataegus* pollinations (summary of data from Talent and Dickinson 2007a, b)

Table 4: The origins of seed embryos of each ploidy level (summary of data from Talent and Dickinson 2007a, b).

Figure legends

Fig. 1: The apomixis continuum in flowering plants.

Gametophytic apomixis, which encompasses diplospory and apospory, is part of a continuum of developmental processes that are closely associated genetically (Koltunow et al. 2000). Sexual reproduction and adventitious embryony (= sporophytic apomixis) can be viewed as the extremes of this continuum (Ernst 1918; Naumova 1993). The term *sexual* is used here in the sense that is most common in apomixis research, to indicate that meiosis and fertilization occurred, rather than to indicate only that fertilization occurred. Diplospory and apospory are commonly distinguished by whether megagametophytes are derived from the megaspore mother cell (MMC) or from other cells (Asker and Jerling 1992; Nogler 1984). In that terminology, apospory can occur in the archaesporium, nucellus, or inner integument, but because Rosaceae have a multicellular archaesporium, some authors define diplospory as occurring in any cell of the archaesporium (see text for details). Here the terms *diplospory* and *apospory* are used in a functional (genetic) sense that might not correspond exactly to the structural sense (Table 1). Rosaceae and a few other families can form secondary MMC's after breakdown of the primary MMC (Davis 1966).

Fig. 2: The previous model of the evolution of apomixis in *Crataegus*.

The previous model of the evolution of apomixis in *Crataegus* (Camp 1942a) is similar to models accepted for some other plant groups (Hörandl 2006). Diploid–diploid hybrids produce a higher proportion of meiotically unreduced gametes than their parental species, and this leads to triploids. Apomixis alleles confer a selective advantage among the

largely sterile triploids. Tetraploids derived from the triploids could be either apomictic or sexual. Closed outline = sexual reproduction; broken outline = apomictic reproduction.

Fig. 3: Imprinting with double fertilization in a sexual diploid angiosperm with allopolyploid origin (based on a diagram by Vinkenoog and Scott 2001).

(a) This case assumes that duplicate copies of the imprintable genes have been retained since the ancestral allo-polyploidy of Maloid Rosaceae, but that the second copies cannot be expressed during or after meiosis.

(b) This case assumes that duplicate copies of the imprintable genes are expressed after meiosis. Model 2 (see text for details) assumes that this situation applies in the ancestrally (allo)polyploid Maloid Rosaceae.

m = imprintable genes that repress endosperm growth if activated in the central cell of the megagametophyte and/or in the endosperm; p = imprintable genes that promote endosperm growth if activated in the male gamete and/or in the endosperm; M = imprintable genes that are active in the central cell of the megagametophyte and/or in the endosperm; P = imprintable genes that are active in the sperm and/or in the endosperm; () = silenced imprintable genes (the default state before imprinting, that is maintained by imprinting); { } = duplicate copies of imprintable genes that are due to the allopolyploid origin of this diploid.

Fig. 4: Models of imprinting in tetraploid *Crataegus*

(a) Model 2, before revision, the tetraploid case: imprinting in a tetraploid *aposporous* megagametophyte with 10x endosperm. In this model (which assumes that Fig. 3b describes the diploid case), the duplicate copies of imprintable genes that are due to allopolyploidy are silent in the aposporous initials and their derivatives. The maternal to

paternal ratio of the active forms of the imprinted genes in the endosperm is 2:1, although the genome ratio is 4:1.

(b) Model 2, revised, the tetraploid case. Cross-pollination experiments in *Crataegus* show that diploids and tetraploids can interbreed, and it is therefore proposed that in recent triploids and tetraploids the number of imprintable genes that can be activated is the same as in diploids. See text for details.

(c) Model 3, *apospory*: imprinting in a tetraploid megagametophyte with 10x endosperm. In this model, the mechanism of imprinting with *apospory* does not occur (or is incomplete) in one of the two central-cell nuclei (see text for details). Maternal activation of imprintable genes involves all four copies in one of the central-cell nuclei, and none of them in the other nucleus. As with Model 2, the maternal to paternal ratio of the active forms of the imprinted genes in the endosperm is 2:1.

(d) Extension of Model 2 to *diplospory* (Model 3 would be similar). In this model, *diplospory* activates silenced genes as effectively as full meiosis. If both sperm fertilize the central cell, then 12x endosperm is formed and the maternal to paternal ratio of the active forms of the imprinted genes in the endosperm would be maintained at 2:1.

m = imprintable genes that repress endosperm growth if activated in the central cell of the megagametophyte and/or in the endosperm; p = imprintable genes that promote endosperm growth if activated in the male gamete and/or in the endosperm; $\{ \}$ = duplicate copies of imprintable genes that are due to the allopolyploid origin of the diploid ancestors of this polyploid; $()$ = silenced imprintable genes (the default state before imprinting, that is maintained by imprinting); M = imprintable genes that are

active in the central cell of the megagametophyte and/or in the endosperm; P = imprintable genes that are active in the sperm and/or in the endosperm.

Fig. 5: A new model of the evolution of apomixis in *Crataegus*.

This model requires rare apomixis to produce viable seed, potentially with a fertilized embryo, in (at least) one diploid species. As discussed in the text, allopolyploids are possibly more likely to be able to interbreed with diploids, although autopolyploids are also shown here. Closed outline = sexual reproduction; broken outline = apomictic reproduction. Broken arrow: transitions not observed.

Table 1: Overview of the terminology used here for apomixis in flowering plants

ADVENTITIOUS EMBRYONY

A type of AGAMOSPERMY in which embryos develop from cells of the nucellus or integument. It may or may not require a fertilized sexual embryo sac to provide endosperm. It was also called *adventive embryony*, and in earlier times misleadingly called simply *nucellarembryony*. Its development shows considerable similarity to AOSPORY, although a multicellular gametophyte is not formed (Ernst 1918; Naumova 1993).

AGAMOSPERMY

A term introduced by Täckholm (1922) to cover the three mechanisms of asexual seed production “Apogamie, Aposporie und Nuzellarembryonie”, i.e. DIPLOSPORY, AOSPORY, and ADVENTITIOUS EMBRYONY. The term is used to cover the full development of a seed, i.e. including PARTHENOGENESIS and endosperm development.

ANDROGENESIS

The development of an embryo with a sperm nucleus in an egg cell whose female nucleus is non-functional. Also called MALE PARTHENOGENESIS, and *androclinesis*. Arguably a type of AGAMOSPERMY but not considered here (Naumova 1993; Solntzeva 2003).

APOGAMETY

Development of an embryo from a cell of the gametophyte other than a gamete (Renner 1916, p. 348), but also used (e.g., Nygren 1967) to include any cell other than the egg, and thus including central-cell embryos. See also PARTHENOGENESIS and APOGAMY.

APOGAMY

Originally defined by de Bary (1878) based on the behaviour of ferns, then generalized to other plant groups but also narrowed in meaning by subsequent authors. Replaced by Winkler with the term APOMIXIS (1908), which he explicitly generalized. See also APOGAMETY.

APOMEIOSIS

The loss of meiotic reduction (Renner 1916, p. 351), a component process of GAMETOPHYTIC APOMIXIS. Intended here to include the *Allium* type of DIPLOSPORY. See also AOSPORY, DIPLOSPORY.

APOMIXIS

Winkler (1908) defined this as the replacement of sexual reproduction by another asexual reproductive process without concatenated nuclear and cell fusion (“Ersatz der geschlechtlichen Fortpflanzung durch einen anderen, ungeschlechtlichen, nicht mit Kern- und Zellverschmelzung verbundenen Vermehrungsprozess”). He included vegetative processes that replace seed production. Edman notably used a narrow interpretation of sexual reproduction and a narrow definition of apomixis (1931), which was equivalent to PARTHENOGENESIS as that word is used here. Many authors restrict the term to AGAMOSPERMY (see text for details). The term is used here, and by most authors, to cover the full development of a seed, i.e. including PARTHENOGENESIS and endosperm development. See also NON-RECURRENT APOMIXIS.

AOSPORY

Usually defined as the production of a gametophyte, bypassing SPORE formation (Bower 1887; Vines 1878). In angiosperms a practical definition has it as a subset of gametophytic apomixis in which the megagametophyte arises from an unreduced somatic cell of the nucellus (e.g., Nogler 1984). This is often expanded to include *generative apospory* which originates in the megaspore mother cell or other archaesporial tissue distinguished from *somatic apospory* which originates in other tissue (e.g., Maheshwari 1950; Mogie 1992). No attempt is made here to functionally divide apospory from DIPLOSPORY as it is argued that we do not yet sufficiently understand gametic imprinting, which might profoundly affect the behaviour of the gametophytes resulting from some but not all of the processes in these categories.

AUTOMIXIS

Fertilization of the egg cell by another cell of the megagametophyte. Not in accord with Winkler’s definition of APOMIXIS, and not considered here.

CENTROGAMY

A subset of PSEUDOGAMY in which the endosperm requires fertilization but the embryo develops by PARTHENOGENESIS (Solntzeva 2003).

DIPLOSPORY

This term was defined by Edman (e.g., 1931) for the type of APOMEIOSIS in which the initial cell of the gametophyte originates in archaesporial tissue. Characterization is difficult, notably in species with a multicellular archaesporium (see text for details). Sometimes divided according to whether the first cell division is meiotic, meiosis-like, or mitotic (e.g., by Fagerlind 1940) into *diplospory*, *semi-apospory*, and *generative apospory*, and distinguished from *somatic apospory* (for which see AOSPORY). The following simplified classification (based on Crane 2001; Rutishauser 1969) considers only major structural processes:

***Allium odorum*–*A. nutans* type:**

Endomitosis; reduction division of autobivalents (separates former sister chromosomes); degeneration of micropylar cell of dyad; three rounds of mitotic division give an 8-nucleate, 7-celled embryo sac.

***Taraxacum* type:**

Nuclear restitution; equational division of meiosis; degeneration of micropylar cell of dyad; three rounds of mitotic division give an 8-nucleate, 7-celled embryo sac.

***Ixeris* type:**

Nuclear restitution; three rounds of nuclear division without wall formation; wall formation gives an 8-nucleate, 7-celled embryo sac.

***Blumea*–*Elymus* types:**

Approximately mitotic division; (degeneration of micropylar cell of dyad); three rounds of mitotic division give an 8-nucleate, 7-celled embryo sac.

***Antennaria*–*Hieracium* types:**

Three rounds of mitotic division give an 8-nucleate, 7-celled embryo sac.

***Eragrostis*–*Panicum* types:**

Two rounds of mitotic division give a 4-nucleate embryo sac, with either three or four cells.

GAMETOPHYTIC APOMIXIS

A broader term than APOMEIOSIS (Nogler 1984) that covers the lack of meiotic reduction and also later developments (PARTHENOGENESIS, endosperm development).

HEMIGAMY

Equivalently *semigamy*, and sometimes called PSEUDOGAMY. The sperm enters a meiotically reduced egg cell and induces PARTHENOGENESIS, often in combination with somatic doubling. Arguably a type of AGAMOSPERMY but not considered here (Solntzeva 1978).

MALE PARTHENOGENESIS

See ANDROGENESIS.

NON-RECURRENT APOMIXIS

Originally (Maheshwari 1950) used for seeds with embryos from reduced PARTHENOGENESIS. Although not in accord with Winkler's definition of APOMIXIS, fertilization of an unreduced egg cell, which Rutishauser termed B_{III} hybridization (e.g. 1967; 1969) has also been called a form of non-recurrent apomixis (Mazzucato 1996).

NUCELLAREMBRYONY

See ADVENTITIOUS EMBRYONY.

PARTHENOGENESIS

Development of an embryo from an unfertilized egg cell, whether reduced or unreduced or somatically doubled. An old term from zoology, for references see Nygren (1967), that has a history of varied uses in botany (discussed, e.g. by Gustafsson 1946). See also APOGAMETY. For *male parthenogenesis* see ANDROGENESIS.

PSEUDOGAMY

Seed development requiring pollination although the embryo has no paternal inheritance (Focke 1881), a very general term. The meaning is commonly restricted to cases where the endosperm requires fertilization but the embryo develops by PARTHENOGENESIS, for which see CENTROGAMY. This is notably different from Naumova's use of the term (1993), for which see HEMIGAMY.

SPORE

Defined by Battaglia (1955) as “an immediate product of meiosis (regular or irregular) that directly develops into a gametophyte.” However, Nogler has argued (1984) that the position of the initial cell is a more practical basis for terminology than the distinction between meiosis and mitosis, and for questions arising from that see APOSPORY.

Table 2: Endosperm-balance requirements in Angiosperms.

Plant families and genera for which an endosperm-balance requirement is lethal to embryos in inter-ploidy crosses.

Family	Subfamily	Genera
Balsaminaceae		<i>Impatiens</i> L. (Arisumi 1982)
Brassicaceae		<i>Brassica</i> L. (Håkansson 1956), <i>Rorippa</i> Scop. (Jennings et al. 1967), <i>Sinapis</i> L. (Håkansson 1956)
Cucurbitaceae		<i>Cucumis</i> L. (Ortiz and Ehlenfeldt 1992), <i>Citrullus</i> Forssk. (Kihara 1951)
Ericaceae		<i>Vaccinium</i> L. (Ortiz and Ehlenfeldt 1992)
Fabaceae		
	Papilionoideae	<i>Glycine</i> Willd., <i>Lotus</i> L., <i>Phaseolus</i> L., <i>Trifolium</i> L. (Ortiz and Ehlenfeldt 1992)
Lamiaceae		<i>Galeopsis</i> L. (Håkansson 1952)
Onagraceae		<i>Oenothera</i> L. (von Wangenheim 1962)
Poaceae		
	Pooideae	<i>Avena</i> L. (Kihara and Nishiyama 1932), <i>Dactylis</i> L. (Ortiz and Ehlenfeldt 1992), <i>Hordeum</i> L. (Håkansson 1953), <i>Secale</i> L. (Håkansson and Ellerström 1950), <i>Triticum</i> L. (Ortiz and Ehlenfeldt 1992)
	Bambusoideae	<i>Oryza</i> L. (Ortiz and Ehlenfeldt 1992)
	Panicoideae	<i>Zea</i> L. (Lin 1984)
Primulaceae		<i>Primula</i> L. (Jennings et al. 1967)
Rosaceae		
	Rosoideae	<i>Potentilla</i> L. (Rutishauser 1961), <i>Rubus</i> L. (Jennings et al. 1967; Topham 1970)
Rubiaceae		<i>Galium</i> (Fagerlind 1937)
Solanaceae		<i>Datura</i> L., <i>Lycopersicon</i> Mill., <i>Petunia</i> Juss., <i>Solanum</i> L. (Ortiz and Ehlenfeldt 1992)

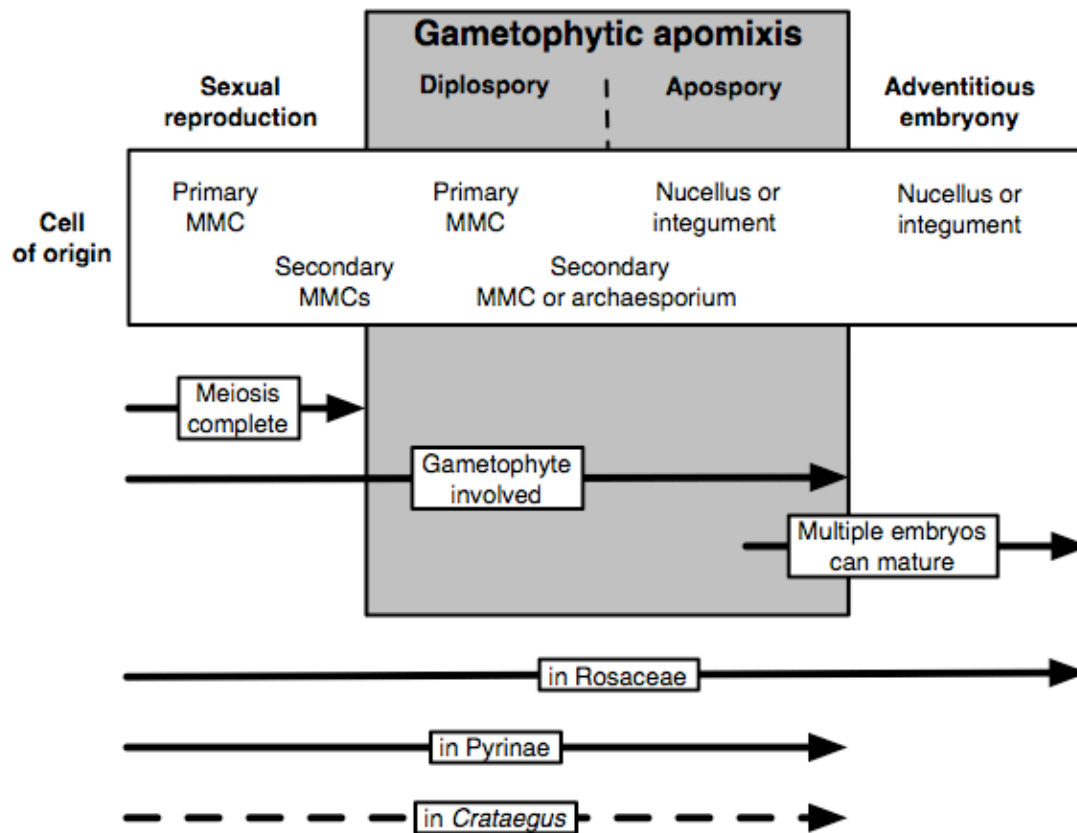


Figure 1

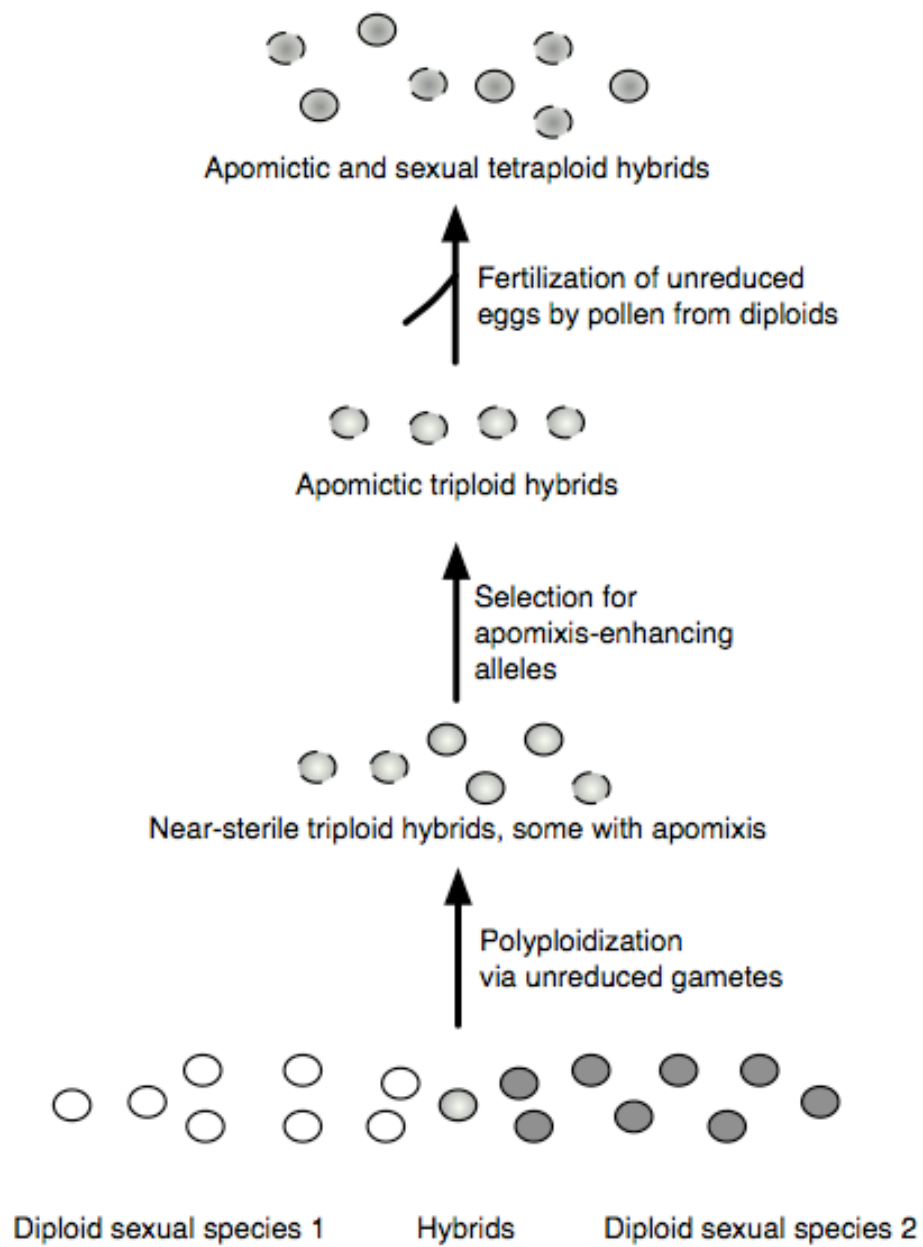


Figure 2

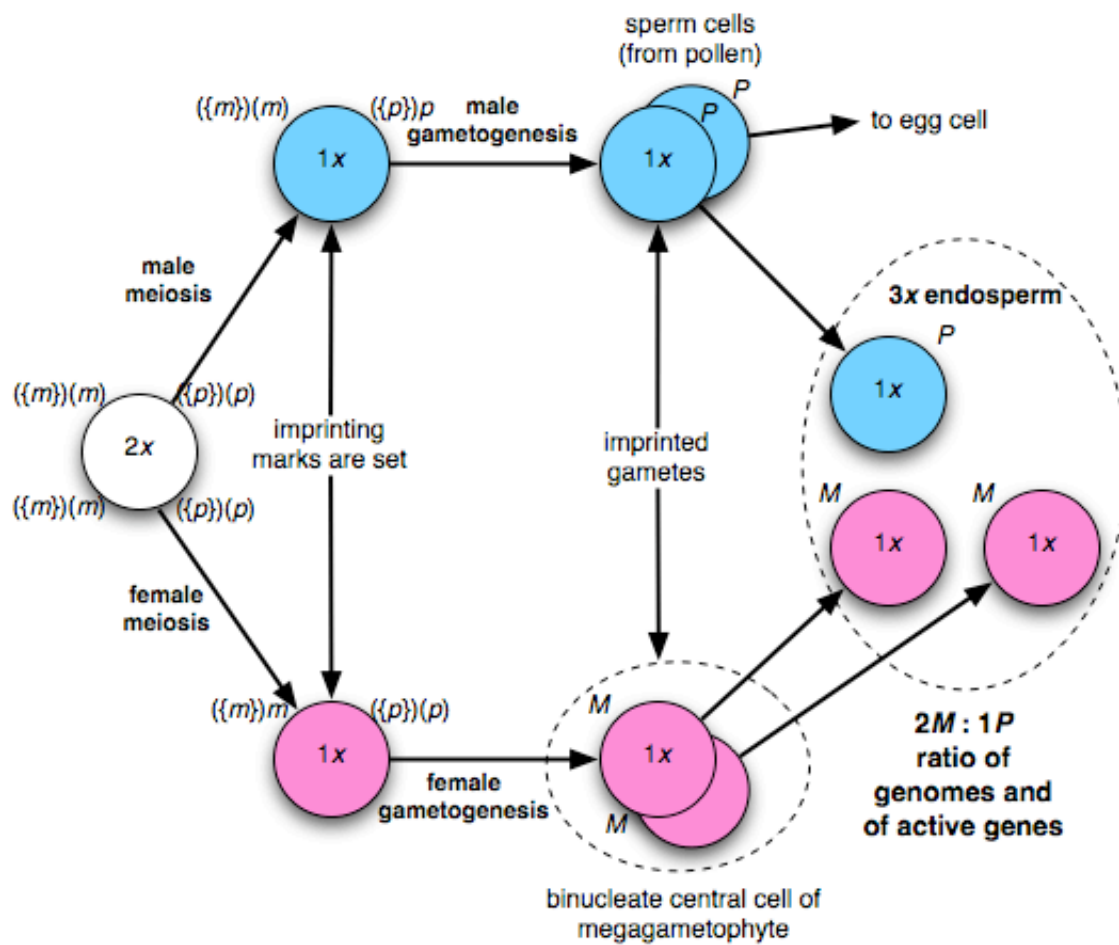


Figure 3a

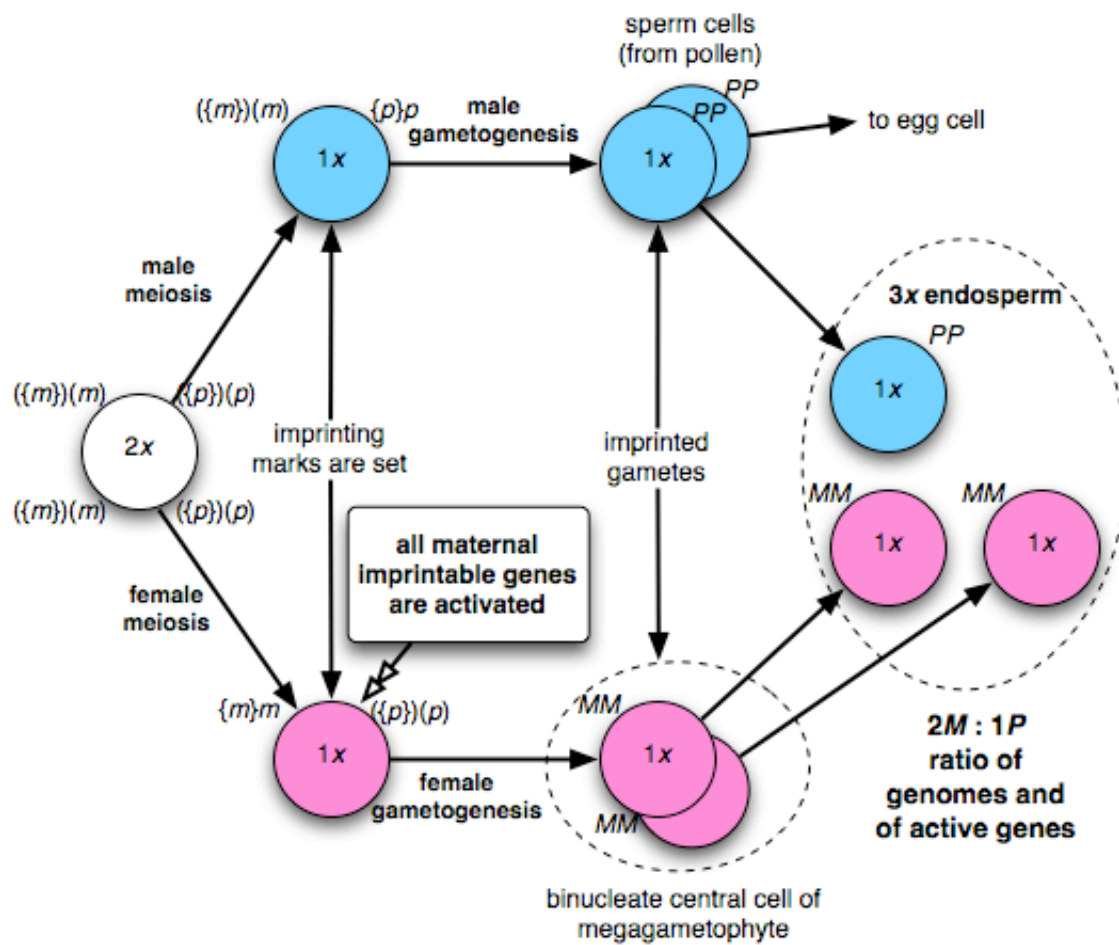


Figure 3b

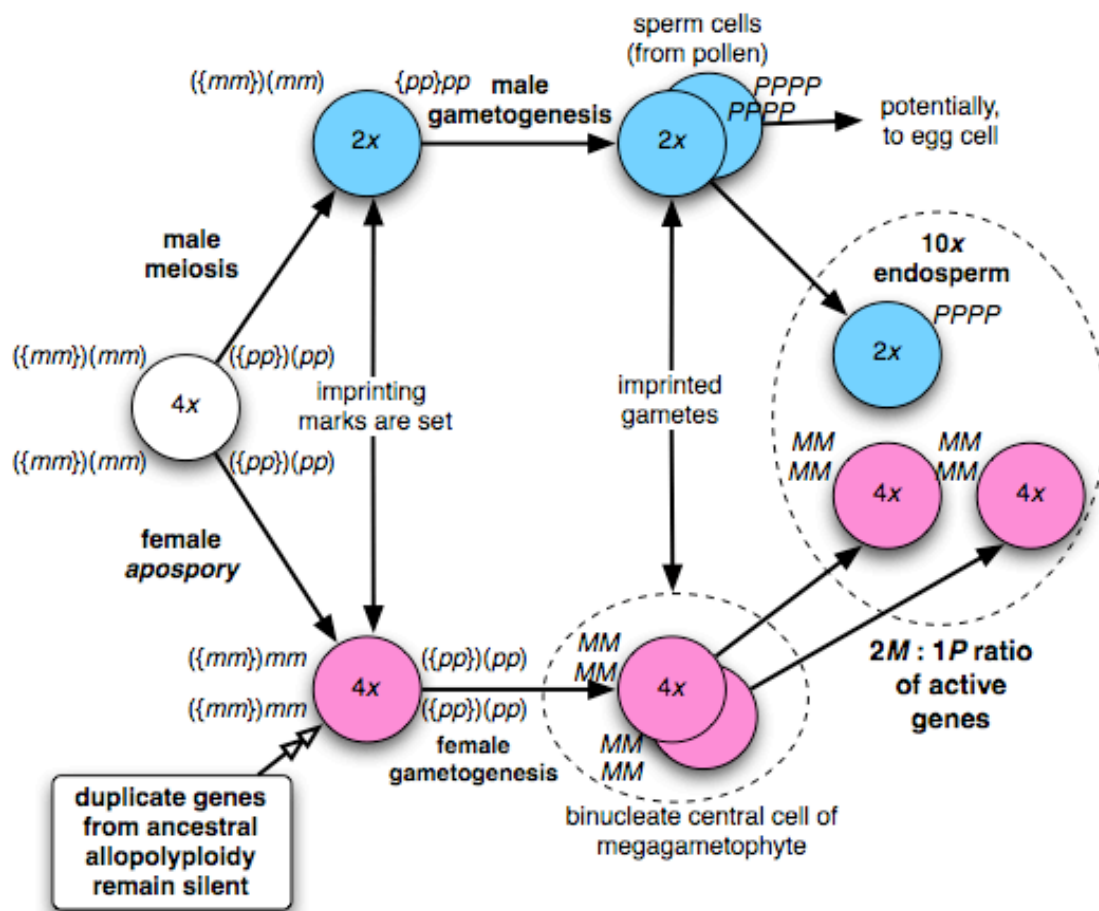


Figure 4a

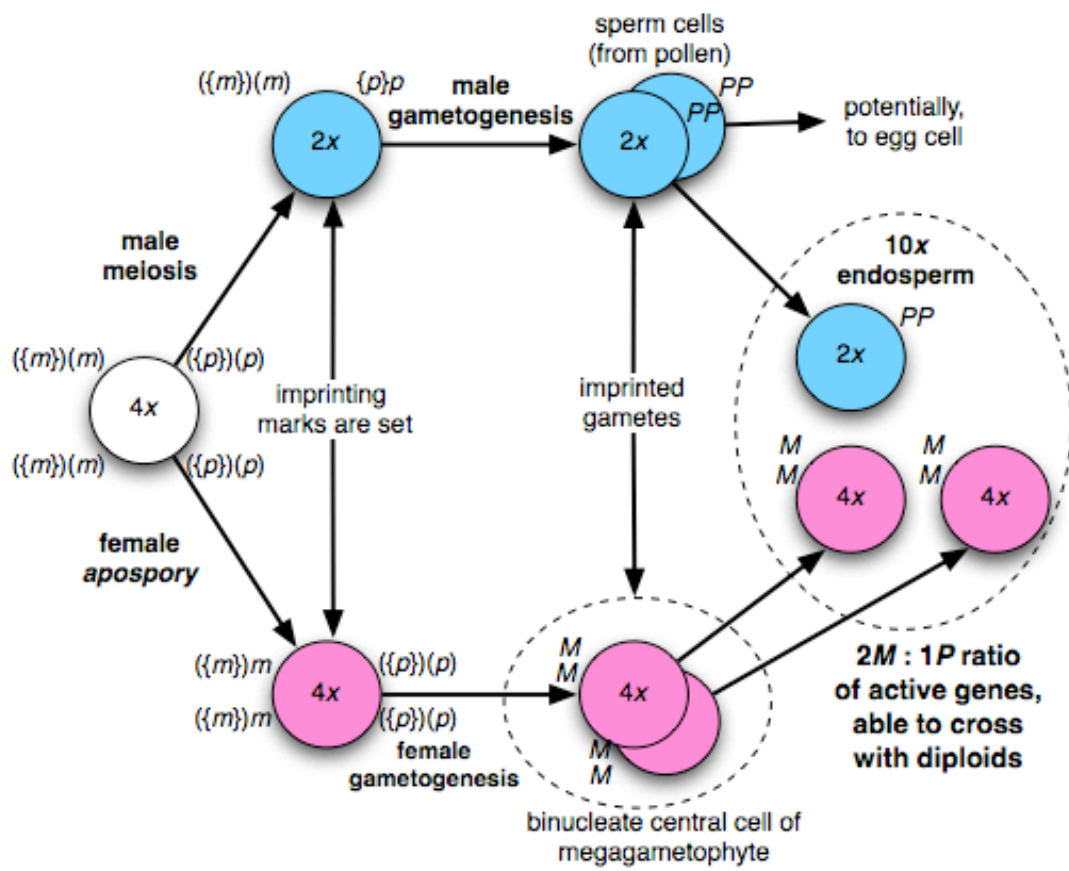


Figure 4b

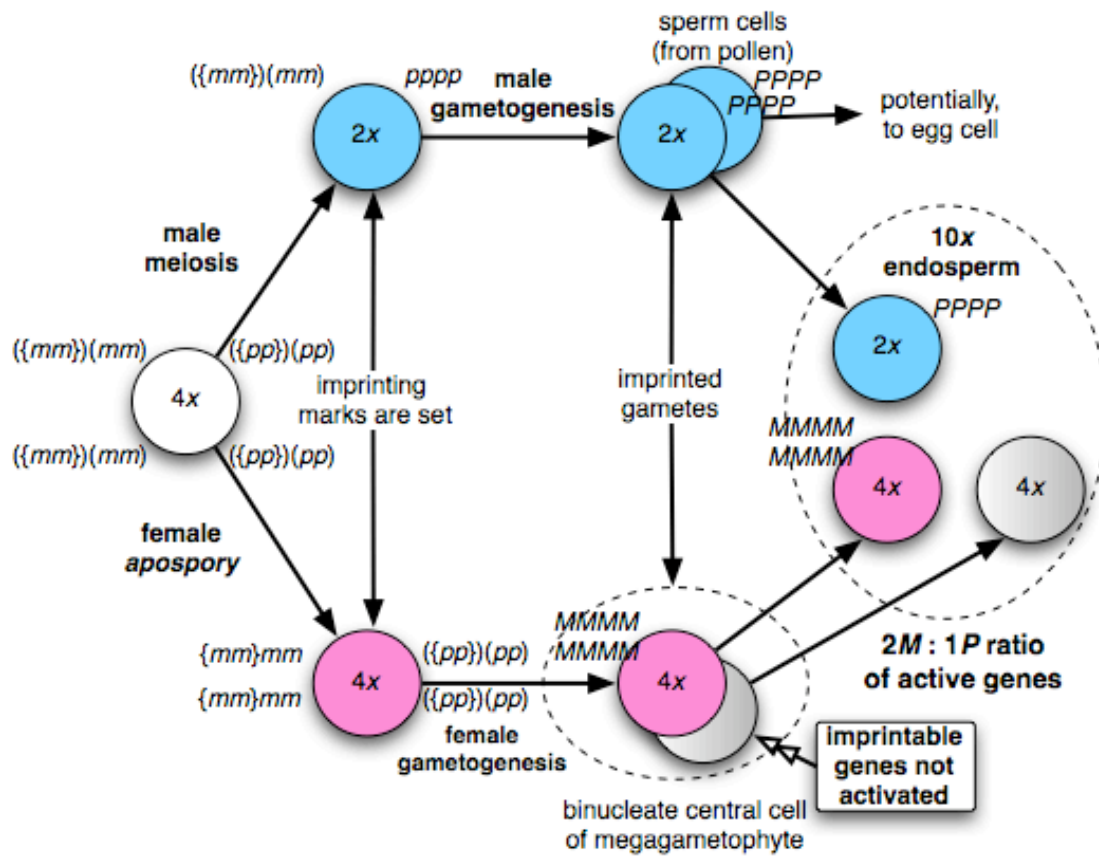


Figure 4c

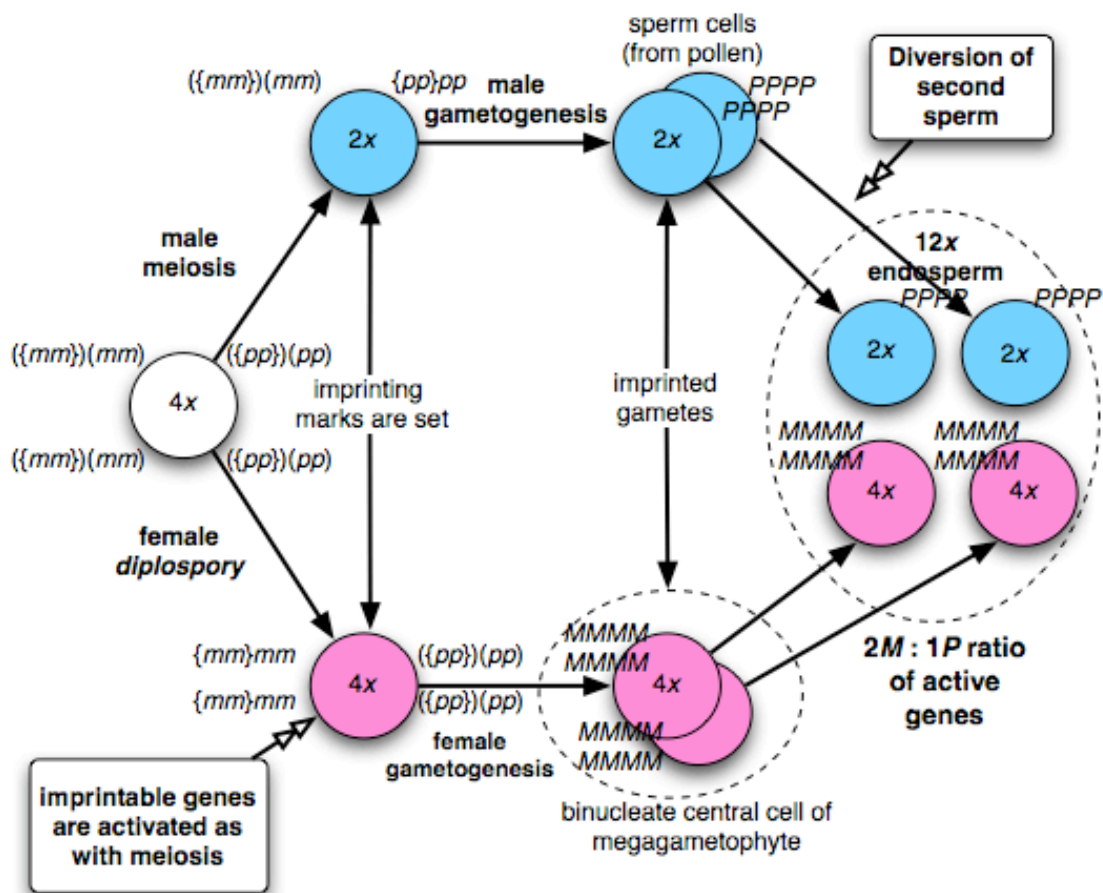


Figure 4d

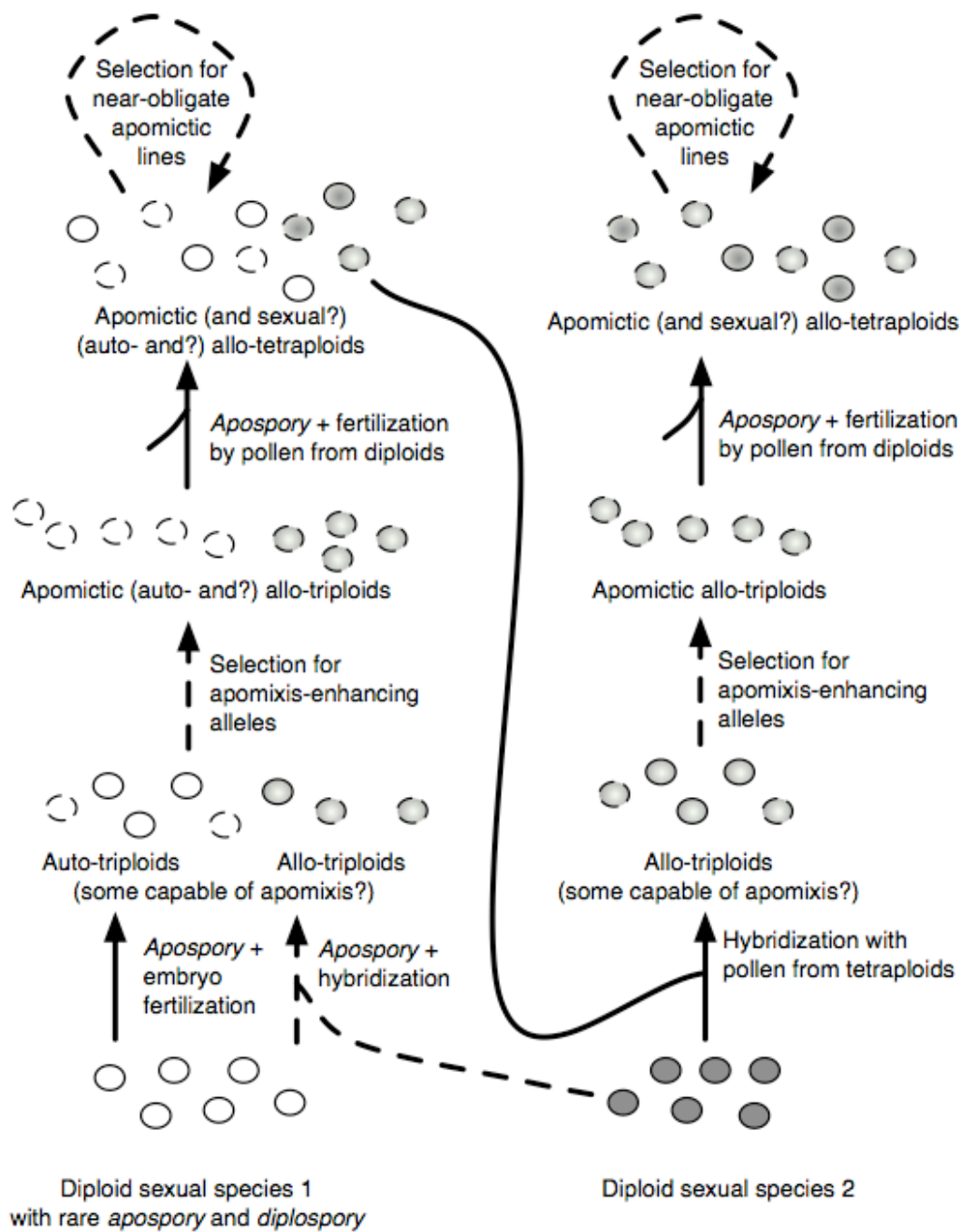


Figure 5