Commentary

Polyspermy in apomictic *Crataegus*: yes and no

In most angiosperms, seed development involves paternal and maternal gametes: one sperm fertilizes the egg to produce the embryo, while the other fuses with the central cell to generate endosperm. Apomictic plants do not follow this rule. Instead the embryo develops from an unreduced, unfertilized egg, whilst, with a few exceptions, the endosperm is sexual but, importantly, develops from an unreduced central cell. Gametic non-reduction is a core component of apomixis, as this enables the production of maternal clones via seed from the unfertilized egg. An unreduced central cell, however, causes a serious problem for many pseudogamous apomicts. Flowering plants are very particular about the parental genome composition of the endosperm, with the vast majority having a 2:1 maternal to paternal (2m:1p) ratio—two polar nuclei to one sperm (Fig. 1). Deviation from this ‘balance’ can result in abnormal development of the endosperm and death of the seed (Lin, 1984; Haig & Westoby, 1991). The production of unreduced male gametes would avoid this problem, but most apomicts generate reduced sperm. This gametic ploidy asymmetry results in the so-called ‘endosperm-balance problem’: put simply, how do apomicts avoid the disruptive effects of a 4m:1p ratio in the endosperm? There is no one answer: apomicts have evolved a variety of tricks to tackle the problem (Nogler, 1984). The paper by Talent & Dickinson in this issue of *New Phytologist* (pp. 231–249) reports on arguably the most interesting of these—achieving a 2:1 ratio in the endosperm by virtue of fertilization of the unreduced central cell by both reduced sperm. Whilst multiple fertilization or polyspermy works for hawthorn (*Crataegus*) and medlar (*Mespilus*), the plants used in the study by Talent & Dickinson, and occurs in a few other species, what implications are there for apomicts that approach the endosperm-balance problem in radically different ways, and for sexual plants where single fertilization of the central cell is the rule?

‘In plants, an overdose of one or other parental genome causes an imbalance in imprinted gene expression levels, resulting in aberrant development of endosperm and death of the seed.’

![Fig. 1 Parental genome composition of embryo and endosperm in sexual plants and apomictic *Crataegus* spp.](image-url)

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*Crataegus* spp. (4x)

Dispermy (25%)

- 4x
- 2m:1p
- 4x
- 12x
- 2x
- 4x

Monospermy (60%)

- 4x
- 10x
- 4m:1p
- 4x
- 2x
- 4x

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Single or double fertilization? Measuring embryo and endosperm ploidy

Apomixis in the *Crataegus* (hawthorn) and *Mespilus* (medlar) genera follows a familiar pattern – diploids are sexual and the polyploids (3x and 4x) are apomictic. The authors were interested in three questions relating to how strictly plants at these various ploidy levels adhere to expectations of behaviour: (1) do diploids always have triploid endosperms; (2) is the endosperm of apomicts ever asexual (autonomous); and (3) do the eggs of apomicts always avoid fertilization? Although not explicitly stated, an important fourth question was how *Crataegus* deals with the endosperm-balance problem. Measuring the ploidy of both the embryo and the endosperm was therefore the principal technical challenge to addressing these questions. Ploidy analysis using flow cytometry is well established and routine for certain tissues, such as leaves. Hence, determining the ploidy of the various experimental plants was straightforward, but how is the ploidy of embryo and endosperm in the same seed obtained? Mature seeds of most species, including those of dicots, contain some endosperm; in *Arabidopsis thaliana* this is the single cell layer of the aleurone, whilst the endosperm of *Zea mays* consists of tens of thousands of cells – this can be exploited to infer the origins of both zygotes (Matzk *et al*., 2000). The endosperm of mature *Crataegus* seeds consists of 15 cell layers. Although the maternal seed coat could not be separated from the endosperm, the authors found that cytometry of the separated embryo and the conjoined endosperm–seed coat in sexual diploids gave the expected ploidy levels – diploid and triploid, respectively.

These experiments provided both the means to conduct the remainder of the analysis and an answer to the authors’ first question – diploids appear to have normal double fertilization, resulting in a triploid endosperm, suggesting that there is no case for a preadaptation of the endosperm facilitating evolution of apomixis in this genus.

Some very familiar apomicts such as *Taraxacum officinale* (dandelion) are completely asexual, producing both embryo and endosperm without fertilization. Exclusion of pollinators from the flowers of male sterile *T. officinale* apomicts does not prevent seed production. Talent & Dickinson searched for evidence of this autonomous type apomixis in *Crataegus* by analysing triploid male sterile plants under conditions that also excluded pollinators and found almost no seed set. Whilst this argues against autonomous apomixis, the authors were aware that in the autonomous apomict *Hieracium* pollination is nevertheless required for seed development (Bicknell *et al*., 2003). The endosperms of ‘open-pollinated’ triploids were therefore analysed for the 3x or 6x ploidy levels that would reveal autonomy, but no such ploidy levels were found. The same result was obtained for the tetraploid apomicts, suggesting that *Crataegus* is strictly pseudogamous.

Striking a balance

Although flowering plants easily tolerate polyploidy (Leitch & Bennett, 1997), crosses between individuals of different ploidies often result in abnormal seed development followed by abortion. The role of the endosperm in seed development, combined with the phenotypes observed, strongly suggests that endosperm failure is the primary cause of seed abortion following interploidy crosses (Haig & Westoby, 1991; Scott *et al*., 1998). The most widely accepted explanation is that normal endosperm development requires a 2m : 1p ratio because of imprinted gene expression (reviewed by Haig & Westoby, 1991; Birchler, 1993). Imprinted genes represent a small subset of genes that are expressed unparentally within the developing endosperm; evidence from *A. thaliana* and *Z. mays*, and from mammals where imprinting also occurs, indicates that imprinted genes frequently regulate aspects of growth and development (Spielman *et al*., 2001). In plants, an overdose of one or other parental genome causes an imbalance in imprinted gene expression levels, resulting in aberrant development of endosperm and death of the seed.

In order to avoid such imprinting problems, some apomicts have evolved various modifications to restore the normal balance. One involves changes to the structure of the female gametophyte: in *Panicum maximum* the embryo sac contains only four nuclei, so that there is only a single unreduced polar nucleus, and therefore fertilization produces a 2m : 1p ratio (Nogler, 1984). Other species modify fertilization behaviour. In *Dichantium annulatum* the unreduced polar nuclei remain separate and either one degenerates or both are fertilized by one sperm each, resulting in two primary endosperm nuclei with a 2m : 1p constitution (Reddy & D’Cruz, 1968). In *Ranunculus auricomus* both sperm fertilize the unreduced central cell (Rutishauser, 1954), again resulting in a 2m : 1p ratio. There are also apomictic species in which the endosperm develops with a ratio other than 2m : 1p. In *Tripsacum dactyloides* a single sperm fuses with an unreduced central cell to produce a 4m : 1p endosperm (Brown & Emery, 1958). The most extreme examples, however, are autonomous apomicts such as *Taraxacum* and *Hieracium*, which have lost the normal requirement for paternal contribution to the endosperm, and may have dismantled the imprinting system altogether (Vinkenoog & Scott, 2001).

What strategy is employed by *Crataegus*? Analysis of embryos and endosperms from tetraploid apomicts found that the majority of seeds with 4x embryos were associated with 10x endosperms, but a substantial minority were associated with 12x endosperms. Given that the likely maternal contribution is 8x (two unreduced polar nuclei) the 10x and 12x endosperms would result from single and double fertilization of the central cell by reduced (2x) sperm, respectively (Fig. 1). Thus *Crataegus* falls into two camps: the *Tripsacum* (tolerate a 4m : 1p imbalance) and the *Ranunculus* (double fertilization of the central cell). Why would *Crataegus*...
Polyspermy and the ‘spare’ sperm problem

Polyspermy in higher plants is generally regarded as rare because pollen tube guidance and repulsion systems prevent multiple pollen tubes penetrating the ovule (Shimizu & Okada, 2000) and sperm is actively transported to their respective targets within the embryo sac (Weterings & Russell, 2004). There is some evidence from maize (Z. mays) that the egg operates an animal-style polyspermy barrier, but no data exist for the central cell. The behaviour of Crataegus and R. auricomus would appear to represent a significant departure from the norm. These apomicts demonstrate that multiple fertilization of the central cell occurs and is viable. This might not be expected given that in animals polyspermy leads to death of the embryo. However, the demise of the embryo is not a consequence of genomic imbalance, but results from the fragmentation of the chromosome complement by supernumerary mitotic spindles. These arise because spindles are organized around centrioles, small organelles that are carried by the sperm and not the egg: multiple fertilization delivers extra centrioles that organize the competing spindles. Plants do not have centrioles and therefore polyspermy is permissible.

Assuming that there is no specific barrier to polyspermy, it is possible that multiple fertilization in polyploidy species is a fortunate side effect of the availability of the spare sperm. However, there are two problems with this. First, most endosperms in Crataegus are produced by single fertilization and, secondly, species that switch to a four-celled embryo sac or normally tolerate a 4:1 ratio must somehow restrict fertilization. Collectively, apomicts therefore contain species in which polyspermy is positive and others in which it is negative. This duality generates an obvious question. Have some apomicts evolved a polyspermy barrier to avoid the spare sperm problem, or have others dismantled a polyspermy barrier inherited from their sexual ancestors to benefit from the spare sperm? Addressing this issue will require some effort to discover the default situation – i.e. whether sexual plants block central cell polyspermy.

What about mechanisms that might facilitate polyspermy where this is appropriate? One interesting idea is that the egg and central cell normally attract sperm using a hormonal signal – in apomicts the parthenogenetic egg does not produce the signal, which diverts both sperm to the central cell. Figuring out the mechanism is certainly interesting. However, what is truly perplexing is how a single apomictic Crataegus species indulges simultaneously in central cell monospermy and dispermy, to produce 10x and 12x endosperms. The authors suggest that the timing of the fusion of polar nuclei may generate two types of embryo sac – one requiring single and the other double fertilization to produce a viable endosperm. But how are endosperm parental ratios of 2m:1p (12x) and 4m:1p (10x) both tolerated? Perhaps Crataegus has a relatively relaxed imprinting system? In the inbreeding plant A. thaliana, both 2m:1p and 4m:1p are also tolerated, although maternal excess results in a substantial reduction in seed mass (Scott et al., 1998). Crossing experiments between diploids and autotetraploids might shed some light on this intriguing problem.

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