Developmental genetics of gametophytic apomixis

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Some higher plants reproduce asexually by apomixis, a natural way of cloning through seeds. Apomictic plants produce progeny that are an exact genetic replica of the mother plant. The replication is achieved through changes in the female reproductive pathway such that female gametes develop without meiosis and embryos develop without fertilization. Although apomixis is a complex developmental process, genetic evidence suggests that it might be inherited as a simple mendelian trait—a paradox that could be explained by recent data derived from apomictic species and model sexual organisms. The data suggest that apomixis might rely more on a global deregulation of sexual reproductive development than on truly new functions, and molecular mechanisms for such a global deregulation can be proposed. This new understanding has direct consequences for the engineering of apomixis in sexual crop species, an application that could have an immense impact on agriculture.

Sexual reproduction in plants entails a series of developmental steps that culminate in the formation of the seed (Box 1)\(^1\). The process is highly regulated and most aberrations result in abortion\(^2\). Nevertheless, plants have retained considerable plasticity in the possible outcomes of reproduction. One example is APOMIXIS (see Glossary), where meiosis and fertilization of the egg by male gametes are bypassed to result in the production of clonal progeny without a paternal contribution. Apomixis therefore allows perpetuation of a fixed genotype through generations\(^3\). The ability to fix indefinitely even highly complex genotypes, including high-yielding hybrids, through apomixis would have tremendous advantages in plant breeding and seed production (Box 2). Apomixis does not occur in the major crop species, but is found in many wild species. The genetics of apomixis in these species has been under scrutiny for several decades, with important contributions to apomixis research. In this review, we focus on one class of apomixis, GAMETOPHYTIC APOMIXIS, in which the embryo originates from an unreduced gamete. Reviews on a second class of apomictic development, ADVENTITIOUS EMBRYONY, in which the embryo differentiates directly from a somatic cell, can be found elsewhere\(^5\).

Gametophytic apomixis requires profound modifications of the processes that govern sexuality. Whereas in animals gametes differentiate directly
Box 1. Sexual reproduction in higher plants

The lifecycle of higher plants (Fig. la) alternates between a diploid sporophytic phase, which produces the spores through meiosis (the sporophytic phase, dark blue), and a haploid gametophytic phase (blue), which produces the gametes after several cell divisions and extensive cell differentiation. The nuclei in red are diploid (unreduced) and the nuclei in purple are haploid (reduced).

Fig. Ib shows sporogenesis and seed development in higher plants.

Megasporogenesis, or female meiosis, occurs within a specialized organ of the sporophyte, the ovule. It starts with the differentiation of the megaspore mother cell, which undergoes meiosis to produce a tetrad of megaspores, only one of which usually survives to form the functional megaspore. Microsporogenesis (male meiosis) occurs within the anthers, and produces four microspores, all of which survive. The differentiation of the spores marks the beginning of the gametophytic generation.

Both the micro- and megaspores divide mitotically to form the multicellular gametophytes: the microgametophyte (pollen grain) on the male side and the megagametophyte (embryo sac) on the female side. The mature megagametophyte is embedded in maternal sporophytic tissue of the ovule, and typically comprises seven cells. Two of those cells, the egg cell and the binucleate central cell participate in double fertilization that is a unique feature of the angiosperms. The mature microgametophyte contains two sperm cells. One fertilizes the egg, to produce the zygote and eventually the embryo. The second sperm fertilizes the central cell, producing the endosperm.

Hence, the mature seed contains three types of tissue: the maternal tissues, and two hybrid tissues of different ploidy levels—the diploid embryo and the triploid endosperm.

from the meiotic products, gametogenesis in plants is preceded by formation of multicellular gametophytes (Box 1). In the female reproductive organ (the ovule), a single cell normally becomes committed to the sexual pathway, undergoes meiosis and forms a tetrad of reduced spores. One of these spores divides mitotically to form a multicellular female gametophyte, the embryo sac, which contains the female gametes. The three other spores degenerate. In the male reproductive organs (the anthers), all meiotically produced spores undergo mitotic development to form mature male gametophytes—the pollen grains. A pollen grain usually consists of two sperm cells contained within a large vegetative cell. Fertilization involves two pairs of gametes. One sperm fuses with the egg cell to form the zygote and eventually the embryo, and the second sperm fuses with the binucleate central cell to form the endosperm. The endosperm has important nutritive and physiological functions during seed development and germination, although its relative importance varies greatly from species to species. For example, the endosperm never forms in certain orchids and is transient in Arabidopsis thaliana, but constitutes 80–90% of mature cereal seeds.

The switch from a normal sexual pathway (Box 1) to an apomictic pathway entails at least three major steps (Box 3):

- Circumvention of meiosis (a process called apomixis);
- Development of the embryo independently of fertilization (i.e. parthenogenesis);
- Formation of a functional endosperm.

These requirements are met by a variety of means in apomictic plants, and as a result the mechanisms of apomixis are numerous (Box 3), although they share common characteristics. First, most if not all apomicts are polyploid. Second, apomixis affects only the female reproductive pathway and male gametes are still produced through meiosis. Third, most
Box 2. Apomixis and its potential in agriculture

Apomixis can be viewed as a short-circuited sexual pathway, where meiosis and fertilization are initiated before completion of the previous developmental step. Fig. Ila compares the steps in sexual and apomictic development, highlighting two major elements of apomixis—apomeiosis and parthenogenesis. Endosperm formation and apomixis can be autonomous or rely on fertilization of the central cell.

Cloning is no less a hot topic in plants than it is in the animal world, and apomixis has attracted much attention from academics and commercial researchers. For the academic, apomixis offers an ideal opportunity to study an efficient natural cloning system, and investigate a wide range of biological questions, from the molecular basis of genomic imprinting to the evolutionary role of sex. However, the current wave of apomixis research is largely driven by marked interest from the private biotechnology sector and the seed industry.

Apomixis is potentially a valuable means of crop improvement, one application being hybrid seed production (Fig. IIb). In crops, the production of hybrids is complex and expensive, requiring large-scale crossing schemes under controlled conditions. Apomixis could reduce the cost of seed production, while ensuring purity. In addition, apomixis should allow farmers, especially in the developing world, to save seed from hybrid plants for the next cropping season and retain the benefits of hybrid vigor, which is normally lost in the next generation because of segregation.

Unreduced cells are denoted by rectangles, and reduced cells are denoted as ovals. Key developmental events that are affected in apomictic species are highlighted in yellow.

apomicts are facultative, in the sense that a proportion of the progeny still results from sexual reproduction. Hence, apomixis does not replace sexuality; rather, it coexists with sexual development within the same plant. Finally, the precocious initiation of a developmental step before completion of the previous one is a hallmark of apomixis; that is, apomixis corresponds to a ‘short-circuited’ sexual pathway, where gamete formation occurs without meiosis and embryogenesis without fertilization. In certain types of apomixis, the fate of cells within the ovule is altered, and somatic cells that are usually not committed to the reproductive pathway take part in apomictic reproduction (Box 3). Apomixis can be viewed, therefore, as the result of a relaxation of temporal and spatial constraints on sexual developmental processes. Asexual pathways are built by reassembling, in space and time, the elements of ‘normal’ sexual reproductive pathways.

Genetic analysis of apomixis: few genes or many genes?
Apomixis is a heritable trait, but its genetic control is unclear. How many genes control apomixis, and are those genes the same in the various forms of apomixis? Apomixis is found almost exclusively in polyploid, highly heterozygous and genetically poorly characterized species, making its genetic dissection difficult. The development of female gametes without meiosis (i.e. apomeiosis) is the element of apomixis that has attracted the most interest.
Box 3. Mechanisms of apomictic development

Gametophytic apomixis can follow one of two types of pathway: diplsporous or aposporous (Fig. III). In diplsporous pathways the gametophyte is derived from the megaspore mother cell, and the megaspores result from an aberrant or modified meiosis that restores the genome of the mother (a,b,c). The unreduced nuclei are shown in red, and reduced nuclei in purple. In aposporous pathways the megaspores are derived from somatic cells within the ovule that develop directly into a megagametophyte, bypassing meiosis (d,e).

Many variations have been observed in both diplsporous and aposporous pathways. In the Antennaria type of diplspory (a), the unreduced spore is formed without undergoing meiosis (a), whereas in the Taraxacum type, it results from the restitution of the nucleus at meiosis I (b). In the Allium type, meiosis is normal but preceded by an extra round of DNA replication before meiosis I (c). In all these examples, the egg cell forms an embryo parthenogenetically, that is, without fertilization by a male sperm. Variations also exist for aposporous. In the Panicum type, the megagametophyte is mature after only two mitoses and hence contains only four nuclei (d). In the Hieracium type, three mitoses occur, and the embryo sac contains eight nuclei, closely resembling the sexual one (e).

Another fundamental difference (not illustrated) among apomicts relates to the formation of the endosperm. In autonomous apomicts, both embryo and endosperm develop parthenogenetically. In contrast, pseudogamous apomicts still require fertilization of the central cell for the formation of a hybrid endosperm to support seed development. The mode of endosperm development does not correlate with the modality of apomeiosis: some aposporous plants have autonomous endosperm while others are pseudogamous, and the same is true for diplsporous species.

Genetic analyses conducted in tetraploid Panicum maximum, a forage grass, and Ranunculus auricomus, a dicotyledonous plant, have shown that apomeiosis is inherited as a single dominant mendelian trait4-6. This finding has often been taken as evidence for monogenic inheritance, although a mendelian trait can encompass anything from a single gene to an entire chromosome (e.g. the Y-chromosome of humans).

In the same experiments, apomeiosis and parthenogenesis were shown to co-segregate strictly, suggesting that these two components rely on the same genetic control, or that parthenogenesis is a pleiotropic consequence of apomeiosis4-6. Recent reports in another dicotyledonous plant, Erigeron annuus6 and in dandelions (Taraxacum officinale)10, however, have shown that in some species apomeiosis and parthenogenesis can segregate independently, suggesting that they probably rely on different genes. Note, however, that although apomeiosis can occur in plants in which parthenogenesis does not, the reverse is not true.

This suggests a functional relationship between the traits, in spite of their genetic independence.

The contradiction between these data and observations in Panicum and Ranunculus illustrates a characteristic of apomixis genetics: major conclusions are drawn from a few case studies and attempts to derive general principles often prove unsatisfactory. Comparative mapping studies of different forms of apomixis in various grass species helps to explain why this is so. The genome of the grasses has been remarkably stable during evolution, and gene content and gene order along the chromosomes is well conserved between homologous chromosomes, even among distantly related species11-14. Hence, comparative mapping among grasses of apomixis loci, which are always inherited as simple traits, offers a way to determine whether different apomictic processes rely on identical or different loci. Attempts to map apomixis with common molecular probes in several species, including Trisacum14, Broachiaria15 and Paspalum16, three forage grasses, have shown the genomic regions that regulate apomixis to be distinct.

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Apomixis and genomic imprinting

Apomixis is quite frequent in flowering plants. By contrast, mammals do not have apomixis-related phenomena, such as parthenogenesis, because of genomic imprinting. Genomic imprinting refers to parent-of-origin specific gene expression, and it renders maternal and paternal genomes functionally different to each other. It can affect entire genomes, chromosomes or individual loci (reviewed in Refs 22,23). In mammals, genomic imprinting renders the maternal and paternal genomes complementary for genes that are essential to embryo development22-25, and thus it ensures that both genomes are present in the zygote.

The relatively frequent occurrence of apomixis indicates that embryo development in plants must be governed by radically different rules. In particular, it suggests that the presence of a paternal genome might not be an absolute requirement for embryo development.

Vielle-Calzada and colleagues recently offered a possible explanation for this observation26. In a survey of 20 genes in Arabidopsis thaliana, no paternally derived transcripts were detectable in the developing seed (embryo or endosperm) for the first few days after fertilization. Their observation suggests that this early phase of development could be largely under maternal control, through a combination of maternal products stored in the gametes before fertilization and uniparental expression of some genes after fertilization; that is, genomic imprinting. This indicates that, in apomicts, the fundamental developmental processes of early embryo growth are essentially unchanged from those in sexual plants; in both instances, to a large extent, only the female genome is involved. Hence, the difference between apomictic and sexual seed development should lie in the mechanisms that regulate the activation of the corresponding program, not the program itself. This would support a simple inheritance model for parthenogenesis: although the program is necessarily complex, its activation might rely on a limited number of regulatory factors. This resembles the hypothesis put forward earlier for gametogenesis, and is equally hypothetical.

Nevertheless, several regulatory factors have been identified in plants that, when mutated or expressed ectopically, induce partial embryo development or embryo-specific gene expression27,28. The identification of proteins that regulate or are targets of such genes might shed light on the mechanisms of embryo initiation, and therefore on the mechanisms that are altered in apomictic plants.

Dosage effects and endosperm development in apomictic plants

Endosperm development has rarely been considered by those studying the inheritance of apomixis4,5. The endosperm is equally important to apomictic and sexual seed development, but its development differs significantly between the two pathways. In some apomicts (such as Erigeron or Truxxscum) the
endosperm develops autonomously; that is, parthenogenetically without fertilization (AUTONOMOUS APOMICTS). In others (such as Panicum, Pennisetum or Tripsacum), it still depends on fertilization of the central cell by a male sperm (PSEUDOGAMOUS APOMICTS).

Both these types of apomicts differ from their sexual counterparts in the relative contributions to the endosperm of the maternal and paternal genomes. In diploid plants, the embryo is typically diploid while the endosperm is triploid, receiving two maternal genomes and one paternal genome (2m:1p).

In apomicts, the relative genomic contributions of the maternal and paternal genomes are altered, because the central cell contains unreduced nuclei, whereas the male gametophytes are normal. For example, tetraploid autonomous apomicts produce endosperm with a 8m:0p ratio, whereas a tetraploid pseudogamous apomict produces a pentaploid endosperm with a ratio of 8m:1p.

In many plants, genome dosage is critical to seed development. In maize and probably most other cereals, normal development of the endosperm requires a maternal to paternal genome ratio of 2m:1p; deviations lead to seed abortion. This requirement has been regarded as a strong barrier to the emergence of apomixis during evolution, and to introgression of the trait in crop species.

In apomicts, the requirement is relaxed by modifications of gametogenesis or fertilization. In Panicum and many aposporous plants, the apomictic forms have a modified embryo sac, the central cell of which contains only a single unreduced polar nucleus. This nucleus is fertilized to produce an endosperm with a 2m:1p ratio. Apomictic Tribesacum and Paspalum are apparently relatively immune to dosage deviations. Note that a strict requirement for a genome ratio of 2m:1p, which is particularly pronounced in maize, might not be a general feature of sexual plants. For example, seed viability in Arabidopsis is much less susceptible to variation from the 2m:1p ratio.

But in a crop plant such as maize with marked susceptibility to dosage effects in the endosperm, inducing apomixis would probably create problems with seed viability. Indeed, lines where introgression of apomixis into sexual crops from wild relatives has been attempted typically have a high level of seed abortion. This occurs because the mechanisms responsible for relaxation of the dosage constraints were apparently not transmitted with apomeiosis and parthenogenesis. The genetic bases of dosage response in the endosperm remain unknown, but it is clear that they represent an essential aspect of the genetics of apomixis.

Initiation of endosperm development in apomicts

In sexual plants, endosperm develops in response to fertilization. Pseudogamous apomicts are similar to their sexual counterparts in that respect, and do not require specific modifications. The situation is more complex for autonomous apomixis, where the initiation of endosperm development has to rely on alternative pathways. Screens for mutants that allow fertilization-independent seed development in Arabidopsis thaliana have identified a class of mutations that partially allow autonomous endosperm development. The three genes of the FIS (FERTILIZATION-INDEPENDENT SEED) class, FIS2, FIE (FERTILIZATION-INDEPENDENT ENDSERM) and MEA, encode polycomb group (PCG) proteins, which are involved in PCG complex formation. Endosperm development in autonomous apomicts probably requires the specific inactivation of the repressive PCG complexes. Note, however, that fis class mutants allow only partial development of the endosperm.

Recent results show that autonomous endosperm development progresses further if fie is combined with genome-wide hypomethylation, indicating that the actual mechanisms operating in apomicts rely on the deregulation of a larger number of genes.

Moreover, it has been shown that MEA and possibly FIS2 are regulated by genomic imprinting; that is, only the maternally inherited alleles are expressed after fertilization. This regulation is reminiscent of the mechanisms described by Vielle-Calzada and colleagues in the embryo. Nevertheless, the FIS mutants allow partial endosperm development, but no embryo development. This is important, because it suggests that activation of the egg and activation of the central cells rely on different processes, and hence that in autonomous apomicts parthenogenetic development of the embryo occurs independently of parthenogenetic development of the endosperm.

A functional role for polyploidy

The studies that we have summarized illustrate the complexity of the regulatory pathways that need to be altered if we are to develop apomictic seeds. Overall, they suggest that apomixis might require the coordinated deregulation of several genes involved in reproduction. Polyploidy is a possible route to such deregulation.

Complete or segmental polyploidy is apparently ubiquitous in gametophytic apomicts. Rare instances of diploid apomicts have been reported, for example in Arabis holboellii, a relative of Arabidopsis, but as the recent release of the Arabidopsis genome sequence revealed, those might well be paleopolyploids. Several authors have proposed that apomixis might eventually be expressed in diploids, but that apomixis alleles are not transmitted through haploid gametes, or are lethal in diploid progenies, suggesting that the relationship between apomixis and polyploidy might be structural rather than functional. Other
Box 4. Polyploidy and apomixis

Several authors have proposed that polyploidy (complete or segmental duplication of the genome) might induce apomixis (see text). Two possible models are shown here: the ploidy regulation model (Fig. IVa) and the genome asynchrony model (Fig. IVb).

(a) The ploidy regulation model

<table>
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<tr>
<th>Complete or segmental genome duplication</th>
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<tr>
<td>A: premeiotic genes</td>
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<td>B: meiosis genes</td>
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<tr>
<td>C: gametogenesis genes</td>
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<tr>
<td>D: post-fertilization genes</td>
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Pre- Sporo- Mega- Post-
meiosis genesis gametogenesis fertilization

(b) The genome asynchrony model

Ecotype 1

A B C D

Resulting allopolyploid

X

Ecotype 2

A B C D

Gametogenesis without meiosis

Fig. IV

evidence, however, suggests a functional role for partial or complete genome duplications. In *Paspalum notatum*, for example, simple chromosome doubling of diploid plants produces apomictic autotetraploids, indicating that the alleles for apomixis are present in the diploids, and that their expression is ploidy dependent60 (Box 4).

Similarly, the ‘genome asynchrony hypothesis’ put forward by Carman69 proposes that polyploidy, when originating from genetically divergent genomes, induces apomixis. Hybridization of divergent genotypes could cause asynchrony in the expression of the regulatory genes that control reproductive programs, leading to the concurrent asynchronous expression of essentially unaltered developmental programs. For example, if embryo sac developmental programs are superimposed on meiosporogenesis, meiosis could be omitted.

Is it plausible that polyploidy per se induces the deregulation of genes crucial for reproduction, thus giving rise to apomixis in some specific situations? Several recent studies have shown that polyploidy directly influences gene expression in, for example, yeast68, maize69 and *Arabidopsis*70. In *Arabidopsis*, comparative profiling of cDNAs in two diploid species and the corresponding allotetraploid showed that up to 20 of 700 genes surveyed in the allotetraploids are eventually silenced; this results in considerable variation in morphology, flowering time and fertility71. In yeast, a genome-wide comparison of gene expression profiles between isogenic strains at different ploidy levels, from haploid to tetraploid, indicates ploidy regulation of essential genes, including key regulators of the cell cycle68. The maize data69 show, moreover, that segmental polyploidy can induce trans-effects, affecting genes that are not part of the segment of higher ploidy. Polyploidy, however, is clearly not a sufficient condition for apomixis. Indeed, polyploids account for more than 50% of angiosperm species, most of them sexual – a disproportionately large percentage when compared with apomictic species. The model remains speculative, but a trend for polyploidy to play a part in the regulation of apomixis is clearly discernable.
Conclusions
Our understanding of apomixis is changing rapidly. Until recently, it was widely accepted that apomixis was the result of a few mutations within the reproductive pathways, but the results of molecular mapping in apomicts challenge this assumption. Also, mapping shows that naturally occurring forms of apomixis have probably evolved from distinct genetic bases. Defining general principles for apomixis genetics might therefore be an impossible task. Rather, the mechanisms of apomixis are interesting because of their diversity. They show that sexual reproduction in plants has remained highly plastic, and can be altered in many different ways.

In apomicts, such alterations allow the rearrangement in time and space of the programs that constitute a sexual pathway. Therefore, understanding the regulation of apomixis will depend on a better understanding of the basic process of sexual plant reproduction, about which fundamental questions remain unanswered. What are the signals that commit a cell to the meiotic pathway? What factors control the initiation of megagametogenesis? How is the egg cell activated? What factors control the expression of imprinted genes? At the same time, the diversity of apomictic phenomena offers a unique opportunity to study and understand the plasticity of reproductive development in plants.

References