Developmental genetics of gametophytic apomixis

Daniel Grimanelli, Olivier Leblanc, Enrico Perotti and Ueli Grossniklaus

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)[1,2]. 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[3–6]. 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 the aim of transferring apomixis to, or inducing apomixis in, their crop relatives. These studies have revealed an intriguing paradox: although the developmental mechanisms underlying apomixis are complex, most genetic analyses suggest that apomixis is inherited as a simple mendelian trait, and results from one or a few mutations that affect the normal course of sexual reproduction[3–6].

The growing popularity of apomixis research has brought developmental biologists into the field. Their perception is that apomixis is not a true novelty in plant development, but rather has evolved through the rearrangement of the subprograms that constitute a normal sexual pathway[4,7]. Understanding apomixis will probably depend on gaining a broader understanding of how these programs are regulated in sexual plants, and investigations of sexual development are making important contributions to apomixis research. In this review, we have attempted to summarize these recent contributions, and in particular to show how they might help to revise current models of the genetic control of apomixis.

Developmental aspects of apomixis

There has been independent evolution of a remarkable diversity of seemingly unrelated apomictic mechanisms[3–6]. 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[3–6].

Gametophytic apomixis requires profound modifications of the processes that govern sexuality. Whereas in animals gametes differentiate directly

Daniel Grimanelli*
Olivier Leblanc
Enrico Perotti
IRD-CIMMYT, Apomixis Project, carretera Mexico Veracruz, Km 45.5, El Batan, Texcoco, Ed Mexico, Mexico.
*e-mail: DGrimanelli@cgiar.org

Ueli Grossniklaus
Dept of Plant Development, Institute of Plant Biology, Zollikerstrasse 107, University of Zürich, CH-8008 Zürich, Switzerland

Glossary

Apomixis: Asexual reproduction through seeds, often referred to as agamospermy.
Adventitious embryony: A subcategory of apomixis, in which the embryo differentiates directly from a somatic cell in the ovule, without formation of a megagametophyte; often referred to as sporophytic apomixis. The sexual pathway remains functional and the apomicitic embryo relies on sexually produced endosperm. Therefore, the seeds can contain apomicitic and sexual embryos.
Gametophytic apomixis: The second subcategory of apomixis comprising diplosporous and aposporous apomixis, but excluding adventitious embryony. In gametophytic apomixis, the megagametophyte (embryo sac) arises from an unreduced spore.
Diplosporous apomixis: The megagametophyte develops from an unreduced megaspore.
Aposporous apomixis: The megagametophyte develops from a somatic cell within the ovule.
Apomeiosis: Generic term describing the failure of meiosis, via diplospory or apospory, in gametophytic apomicts.
Pseudogamous apomicts: Apomictic plants in which endosperm development depends on the fertilization of the central cell while the embryo develops parthenogenetically.
Autonomous apomicts: Apomictic plants in which both the embryo and the endosperm develop without fertilization, through parthenogenesis.
Parthenogenesis: Activation of embryo (or endosperm) development in the absence of fertilization.

http://tig.trends.com 0168-9525/01/$ – see front matter PII: S0168-9525(01)02454-4

Box 1. Sexual reproduction in higher plants

The lifecycle of higher plants (Fig. Ia) 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.

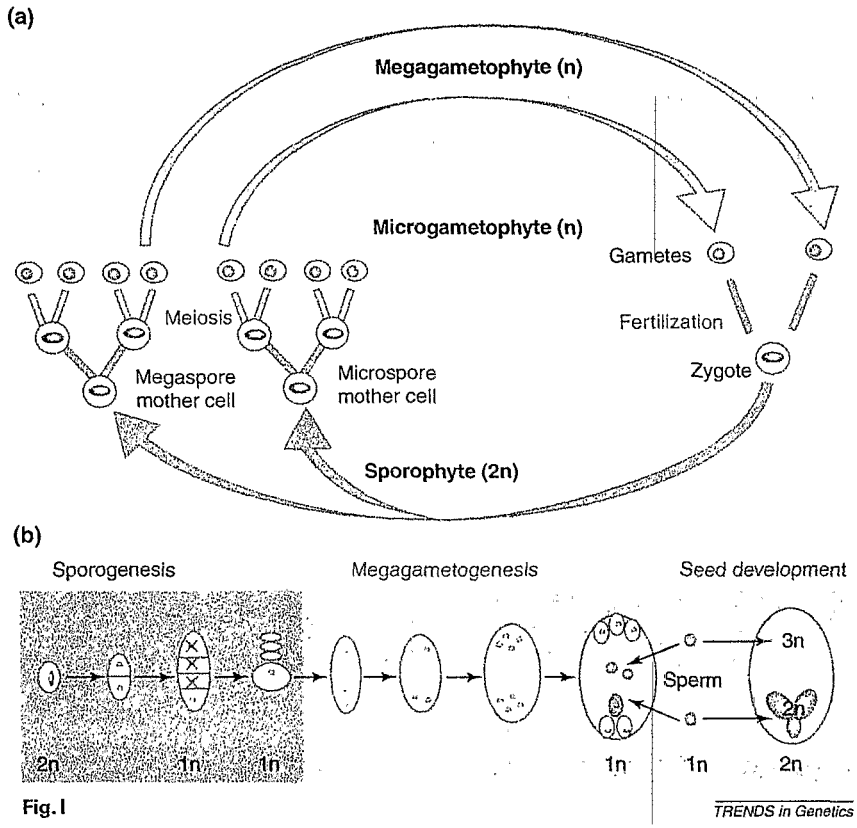

Fig. I

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[8], 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 APOMEIOSIS);
- 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

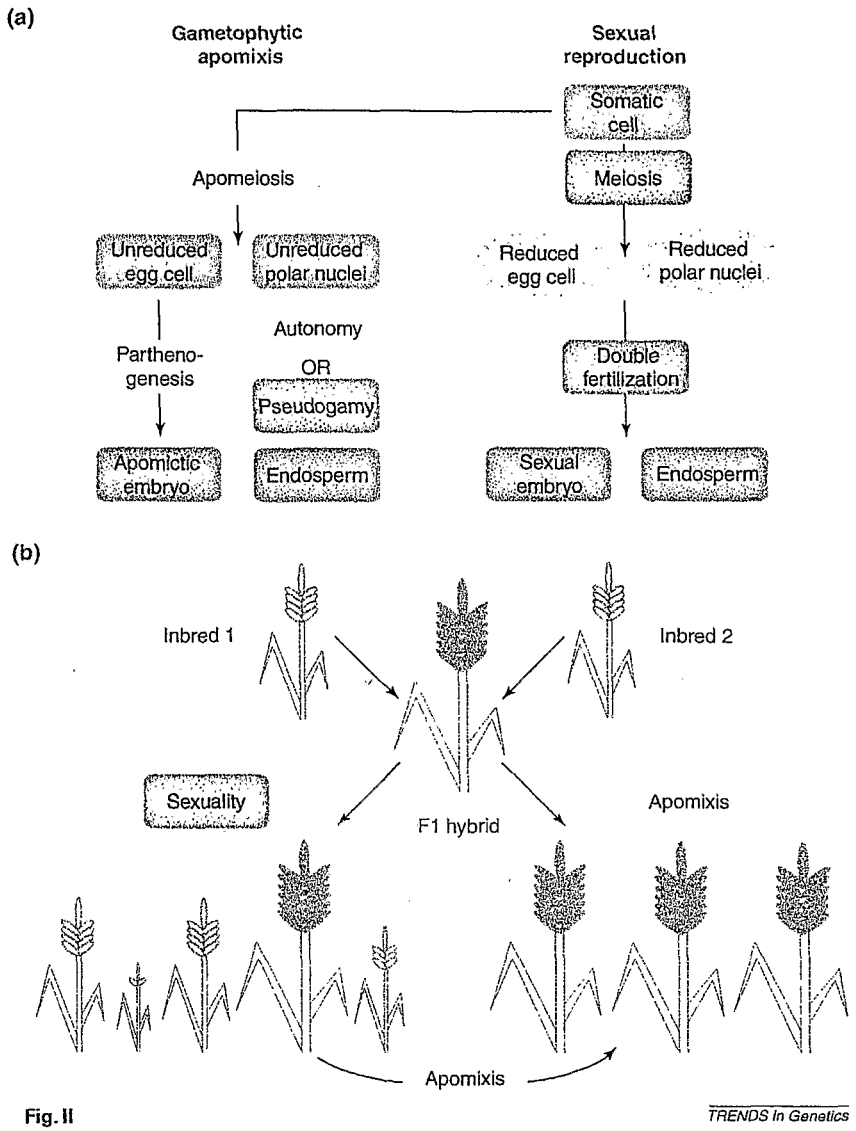

Fig. II

Apomixis can be viewed as a short-circuited sexual pathway, where meiosis and fertilization are initiated before completion of the previous developmental step. Fig. IIa 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-circuiting' of sexual pathways, where gamete formation occurs without meiosis and embryogenesis without fertilization[4]. 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[7]. 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[3–6]. 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: DIPLOSPOROUS or APOSPOROUS (Fig. III). In diplosporous 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).

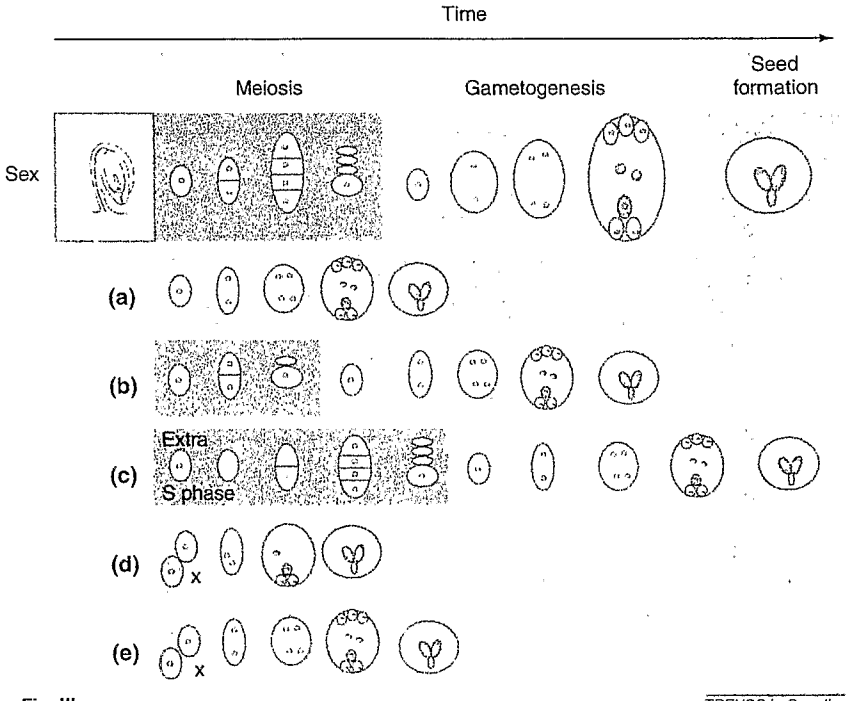

Fig. III

Many variations have been observed in both diplosporous and aposporous pathways. In the *Antennaria* type of diplospory (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 apospory. 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 diplosporous 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 trait[3–6]. 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 apomeiosis[3–6]. Recent reports in another dicotyledonous plant, *Erigeron annuus*[9] 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 species[11–13]. 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 *Tripsacum*[14], *Bracchiaria*[15] and *Paspalum*[16], three forage grasses, have shown the genomic regions that regulate apomixis to be distinct

(i.e. nonhomologous) in those species. This means that apomixis, in its various forms, probably arose in different grass species through the action of different genetic loci.

Master regulators or complex loci?

Apomixis in these different species, although relying on different loci, is always inherited in a simple manner, as one or two mendelian traits at most. The presence of such apparent simplicity in several different systems implies that plant sexual pathways can be 'short-circuited' towards apomixis relatively easily, by means of simple alterations of single loci, and at many different points in the pathway with similar effects.

A possible explanation is that the loci correspond to master regulatory factors. Peacock proposed that apomixis might result from the ectopic expression of the transcriptional cascade that induces embryo sac development[17]. Expressed normally after meiosis and megaspore differentiation, the cascade's expression in the early germ cell or in somatic cells would lead to embryo sac formation before meiosis. A global inducer of gametogenesis or embryogenesis has yet to be identified in plants, but in budding yeast most of the postmeiotic events depend on the expression of Ndt80, a meiosis-specific transcription factor[18,19]. When ectopically expressed in vegetative cells, Ndt80 induces the expression of almost the entire set of sporulation genes. The ectopic or heterochronic expression of a master regulatory gene such as Ndt80 in an unreduced plant cell, or before meiosis, could cause apomeiosis in a sexual plant. However, no homologs of Ndt80 have yet been reported in higher eukaryotes.

In contrast to this simple control mechanism, recent reports indicate that the 'apomixis genes' might be rather complex loci. In tetraploid *Tripsacum dactyloides*, a wild relative of maize[14], apomeiosis segregates as a dominant mendelian trait. Linkage maps for the segment that controls apomeiosis in tetraploid apomicts were used to compare the same linkage group in diploid sexual plants. The data show that recombination is strongly suppressed in the segment that controls apomeiosis, which therefore behaves as a single genetic unit. This unit represents 40 cM on the map developed for sexual *Tripsacum*; this block probably contains several hundred genes, of which an unknown number participate in apomixis. Similar results were found in unrelated species. In *Pennisetum squamulatum*[20,21], *Paspalum simplex*[16], and *Erigeron annus*[9], the trait was mapped to a segment showing no recombination. Although these data do not indicate that apomixis relies on complex genetic control, they show that a simple genetic control cannot be inferred from segregation analyses.

So, how many genes control apomixis? The question remains open, but our understanding of sexual reproduction has increased in recent years, offering some hypotheses to explain two aspects of apomixis: embryo development and endosperm development.

Apomixis and genomic imprinting

Apomixis is quite frequent in flowering plants[3–6]. 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 development[23–25], 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 observation[26]. 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 expression[27,28]. 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 apomixis[3–6]. 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 *Taraxacum*) 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 apomict 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[29]. 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[3,6]. In apomicts, the requirement is relaxed[30,31] or met by modifications of gametogenesis or fertilization[3,6,34]. 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[3–6]. Apomictic *Tripsacum* and *Paspalum* are apparently relatively immune to dosage deviations[30,31]. 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.[32] 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[33]. 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[34]. The three genes of the *FIS* (*FERTILIZATION-INDEPENDENT SEED*) class, *FIS2* (Ref. 35), *FIE* (*FERTILIZATION-INDEPENDENT ENDOSPERM*)[36] and *MEA* (*MEDEA*)[37], repress endosperm formation in the absence of fertilization. Molecular cloning showed that the genes of the *FIS* class share structural and functional similarities with factors thought to control higher-order chromatin structure in animals: both *MEA* and *FIE* encode polycomb group (PcG) proteins, and *FIS2* encodes a zinc finger protein, some of which are involved in PcG complex formation[34]. 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[38], 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[39–42]. 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[3], 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[43]. 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[44–46], 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).

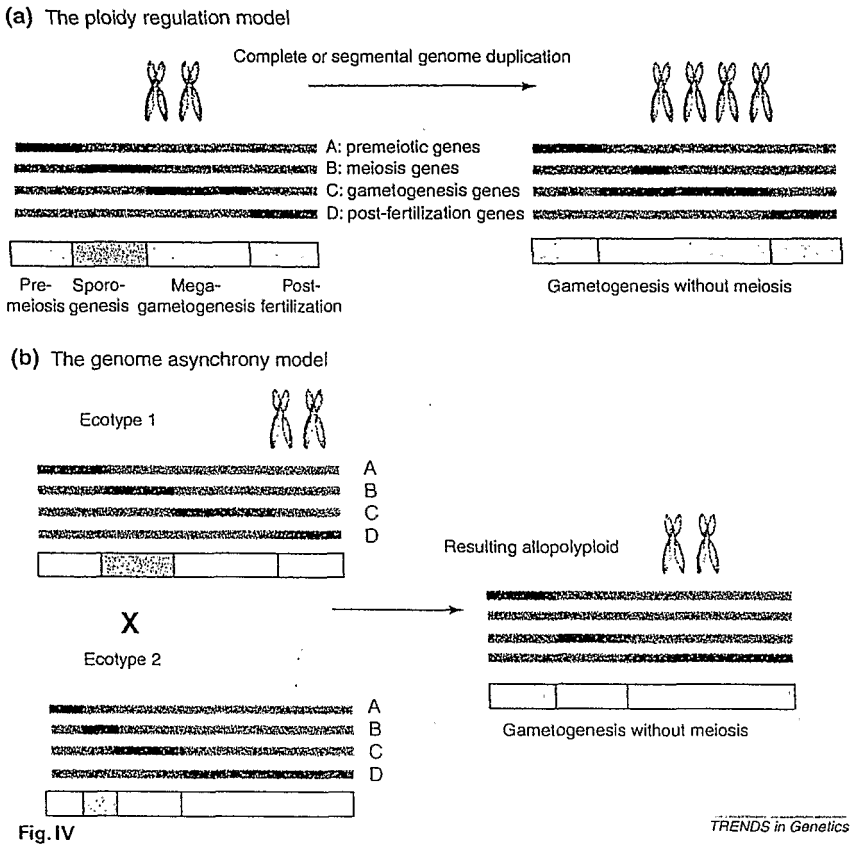

Fig. IV

The ploidy-induced apomixis hypothesis postulates that alleles of some reproductive genes are differentially expressed depending on ploidy levels. A rise in ploidy would therefore induce a modification of the expression profile of those genes. Fig. IVa shows theoretical expression profiles over time of genes with a specific role in regulating premeiotic events (gene A), meiosis (gene B), gametogenesis (gene C) and postfertilization events (gene D). The red profiles indicate the window of gene expression. In the event of ploidy-induced apomixis, partial or complete genome duplication would deregulate meiosis-specific profiles, or induce ectopic expression of gametogenesis genes, resulting in apomeiosis.

The 'genome asynchrony' hypothesis postulates that two ecotypes with divergent reproductive behavior are brought together by hybridization. In Fig. IVb, ecotype 2 has a much shorter meiosis than the ecotype 1. In the resulting allopolyploid, asynchronous expression of meiosis and gametogenesis-specific genes leads to the ectopic expression of gametogenesis, without meiosis.

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 dependent[47] (Box 4). Similarly, the 'genome asynchrony hypothesis' put forward by Carman[48] 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 megasporogenesis, 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[7]? Several recent studies have shown that polyploidy directly influences gene expression in, for example, yeast[49], maize[50] and *Arabidopsis*[51]. 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 fertility[51]. 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 cycle[49]. The maize data[50] 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.

Acknowledgements
We apologize to our colleagues whose publications we failed to quote owing to space limitations. We thank Dave Polland for help with editing, and Markus Affolter, Maggy Collinge, Francoise Jardinaud and Pierre Dubreuil for comments on the manuscript.

References

1 Grossniklaus, U. and Schneitz, K. (1998) Genetic and molecular control of ovule development and megagametogenesis. *Seminars in Cell & Devl. Biol.* 9, 227–238
2 Yang, W.C. and Sundaresan, V. (2000) Genetics of gametophyte biogenesis in *Arabidopsis*. *Curr. Opin. Plant Biol.* 3, 53–57.
3 Nogler, G.A. (1984) Gametophytic apomixis. In *Embryology of Angiosperms* (Johri, B.M., ed.), pp. 475–518, Springer-Verlag
4 Koltunow, A.M. (1993) Apomixis: embryo sac and embryos formed without meiosis or fertilization in ovules. *Plant Cell* 5, 1425–1437
5 Richards, A.J. (1997) *Plant Breeding Systems* (2nd edn), Chapman & Hall
6 Savidan, Y. (2000) Apomixis, genetics and breeding. *Plant Breed. Rev.* 18, 13–86
7 Grossniklaus, U. (2001) From sexuality to apomixis: molecular and genetic approaches. In *The Flowering of Apomixis: From Mechanisms to Genetic Engineering* (Savidan, Y. *et al.*, eds), CIMMYT, Mexico
8 Berger, F. (1999) Endosperm development. *Curr. Opin. Plant Biol.* 2, 28–32
9 Noyes, R.D. and Rieseberg, L.H. (2000) Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. *Genetics* 155, 379–390
10 Van Dijk, P.J. *et al.* (1999) Crosses between sexual and apomictic dandelions (*Taraxacum*). II. The breakdown of apomixis. *Heredity* 83, 715–721
11 Bennetzen, J.L. and Freeling, M. (1993) Grasses as a single genetic system: genome composition, collinearity and compatibility. *Trends Genet.* 9, 259–261
12 Bennetzen, J.L. (2000) Comparative sequence analysis of plant nuclear genomes: microcolinearity and its many exceptions. *Plant Cell* 12, 1021–1029
13 Keller, B. and Feuillet, C. (2000) Colinearity and gene density in grass genomes. *Trends Plant Sci.* 5, 246–251
14 Grimanelli, D. *et al.* (1998) Diplosporous apomixis in tetraploid *Tripsacum*: one gene or several genes? *Heredity* 80, 33–39
15 Pessino, S.C. *et al.* (1997) Identification of a maize linkage group related to apomixis in *Brachiaria*. *Theor. Appl. Genet.* 94: 439–444
16 Pupilli, F. *et al.* The chromosome segment related to apomixis in *Paspalum simplex* is homoeologous to the telomeric region of the long arm of rice chromosome 12. *Mol. Breed.* (in press)
17 Peacock, W.J. (1992) Genetic engineering and mutagenesis for apomixis in rice. *Apomixis Newsl.* 4, 3–7
18 Chu, S. and Herskowitz, I. (1998) Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. *Mol. Cell* 1, 685–695
19 Chu, S. *et al.* (1998) The transcriptional program of sporulation in budding-yeast. *Science* 282, 699–705
20 Ozias-Akins, P. *et al.* (1998) Tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. *Proc. Natl Acad. Sci. U. S. A.* 28, 5127–5132
21 Roche, D. *et al.* (1999) An apospory-specific genomic region is conserved between buffelgrass (*Cenchrus siliaris* L.) and *Pennisetum squamulatum* Fresen. *Plant J.* 19, 203–208
22 Reik, W. and Walter, J. (2001) Genomic imprinting: parental influence on the genome. *Nat. Rev. Genet.* 2, 21–32
23 Tilghman, S.M. (1999) The sins of the fathers and mothers: genomic imprinting in mammalian development. *Cell* 96, 185–193
24 Barton, S.C. *et al.* (1984) Role of paternal and maternal genomes in mouse development. *Nature* 311, 374–376
25 McGrath, J. and Solter, D. (1984) Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 37, 179–183
26 Vielle-Calzada, J.P. *et al.* (2000) Delayed activation of the paternal genome during seed development. *Nature* 404, 91–94
27 Ogas, J. *et al.* (1999) PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in Arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.* 96, 13839–13844
28 Lotan, T. *et al.* (1998) Arabidopsis LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. *Cell* 93, 1195–1205
29 Birchler, J.A. (1993) Dosage analysis of maize endosperm development. *Annu. Rev. Genet.* 27, 181–204
30 Grimanelli, D. *et al.* (1997) Dosage effects in the endosperm of diplosporous apomictic *Tripsacum*. *Sex. Plant Reprod.* 10, 279–282
31 Quarin, C.L. (1999) Effect of pollen source and pollen ploidy on endosperm formation and seed set in pseudogamous apomictic *Paspalum notatum*. *Sex. Plant Reprod.* 11, 331–335
32 Scott, R.J. *et al.* (1999) Parent-of-origin effects on seed development in *Arabidopsis thaliana*. *Development* 125, 3329–3341
33 Morgan, R.N. *et al.* (1998) Seed set in an apomictic BC3 pearl millet. *J. Plant Sci.* 159, 89–97
34 Grossniklaus, U. *et al.* (2001) Genomic imprinting and seed development: endosperm formation with and without sex. *Curr. Opin. Plant Biol.* 4, 21–27
35 Luo, M. *et al.* (1999) Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. U. S. A.* 96, 296–301
36 Ohad, N. *et al.* (1999) Mutations in FIE, a WD polycomb group gene, allow endosperm development without fertilization. *Plant Cell* 11, 407–416
37 Grossniklaus, U. *et al.* (1998) Maternal control of embryogenesis by MEDEA, a polycomb group gene in *Arabidopsis*. *Science* 280, 446–450
38 Vinkenoog, R. *et al.* (2000) Hypomethylation promotes autonomous endosperm development and rescues postfertilization lethality in *fie* mutants. *Plant Cell* 12, 2271–2282
39 Kinoshita, T. *et al.* (1999) Imprinting of the MEDEA polycomb gene in the *Arabidopsis* endosperm. *Plant Cell* 11, 1945–1952
40 Vielle-Calzada, J.P. *et al.* (1999) Maintenance of genomic imprinting at the *Arabidopsis* medea locus requires zygotic DDM1 activity. *Genes Dev.* 13, 2971–2982
41 Luo, M. *et al.* (2000) Expression and parent-of-origin effects for FIS2, MEA, and FIE in the endosperm and embryo of developing *Arabidopsis* seeds. *Proc. Natl. Acad. Sci. U. S. A.* 97, 10637–10742
42 Yadegari, R. *et al.* (2000) Mutations in the *FIE* and *MEA* genes that encode interacting *Polycomb* proteins cause parent-of-origin effects on seed development by distinct mechanisms. *Plant Cell* 12, 2367–2382
43 The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408, 796–815
44 Grimanelli, D. *et al.* (1998) Non-Medelian transmission of apomixis in maize–*Tripsacum* hybrids caused by a transmission ratio distortion. *Heredity* 80, 40–44
45 Tas, I.C. and van Dijk, P.J. (1999) Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. *Heredity* 83, 707–714
46 Bicknell, R.A. *et al.* (2000) Monogenic inheritance of apomixis in two *Hieracium* species with distinct developmental mechanisms. *Heredity* 84, 228–237
47 Quarin, C.L. *et al.* (2001) A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. *Sex. Plant Reprod.* 13, 243–249
48 Carman, J. (1997) Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol. J. Linnean Soc.* 61, 51–94
49 Galitsky, T. *et al.* (1999) Ploidy regulation of gene expression. *Science* 9, 251–254
50 Guo, M. *et al.* (1996) Dosage effects on gene expression in a maize ploidy series. *Genetics* 142, 1349–1355
51 Comai, L. *et al.* (2000) Phenotypic instability and rapid gene silencing in newly formed *Arabidopsis* allotetraploids. *Plant Cell* 12, 1551–1568