Chapter 6

Applications of Molecular Genetics in Apomixis Research

Daniel Grimanelli, Joe Tohme, and Diego González-de-León

Introduction

Apomixis in higher plants refers to a wide range of mechanisms of asexual reproduction through seeds (Nogler 1984a). It is found in at least 400 wild species belonging to 35 higher plant families (Richards 1986; Asker and Jerling 1992; Carman 1997). The modalities of apomictic development in the wild are nearly as diverse as the number of species studied, but in most cases, apomictic processes completely bypass meiosis and egg cell fertilization, and produce offspring that are exact genetic replicas of the mother plant.

Two major types of gametophytic apomixis have been described, namely diplosporous apomixis and aposporous apomixis, based on the origin of the megagametophytes. In aposporous apomicts, one or more unreduced female gametophytes form mitotically from somatic nucellar cells while the legitimate sexual line generally aborts. Diplospory results from meiotic failure in megasporocytes that directly develop into mature unreduced female gametophytes through three or more mitoses. Typically, apomixis is a facultative phenomenon, and an apomictic plant usually produces both asexually (apomeiotic) and sexually derived embryos.

Apomixis, for the most part, is found in wild species. In contrast, major crop plants are sexual, with only rare exceptions such as some prominent tropical forages. This could be conceived partly as a consequence of crop domestication because the process necessarily implies that early farmers had access to variability and segregation among the wild types. In *modern* agriculture, however, the ability to fix superior genotypes through generations would offer numerous advantages. Recognition of these advantages has led to a growing interest in apomixis research, and indeed, many scientists have extolled the tremendous potential that apomixis holds for plant improvement (this volume; Jefferson and Bicknell 1996; Grossniklauss et al. 1999; Savidan 2000).

Various strategies are being considered by a growing number of research groups around the world to introduce apomixis into major food crops. The oldest efforts were directed toward the introgression of the genes for apomixis from wild species into cultivated relatives (see review by Savidan 2000). As an alternative approach, the *de novo* synthesis of apomixis in sexual plants through genetic engineering is now underway through a number of intiatives (Jefferson and Bicknell 1996; Grossniklaus et al. 1999; Luo et al. 2000).

Despite this growing interest, surprisingly little is known about the biology of apomictic plants. This is certainly the primary reason why attempts to manipulate apomixis have failed to yield useful products to date, and it is clear that harnessing the potential of apomixis will strongly depend on our ability to develop a reliable understanding of the basic features of the biological processes of apomixis and its genetic control. The emergence of powerful

tools in molecular genetics now offers new approaches to gain much needed knowledge about the regulation of apomixis. In this chapter, we discuss, in detail, potential applications of molecular genetics to apomixis research. First, we define the biological aspects of the genetics of apomicts that lend themselves to analyses using molecular genetics. A discussion about different strategies for tagging or manipulating the corresponding genes then follows.

Some Biological Aspects of Apomixis Worth Studying using Molecular Genetics

Nonreduction followed by Parthenogenesis

Decades of cytoembryological observations have yielded precise descriptions of the apomictic processes (reviewed by Crane, Chap. 3). These observations have revealed both the complexity of the developmental process of apomictic reproduction and the remarkable diversity of mechanisms leading to the generation of unreduced gametes in apomictic plants. Nevertheless, to date, we do not have a clear understanding of the genetic bases of this developmental trait. According to recent work reviewed by Sherwood (Chap. 5), apospory is probably simply inherited. Much less is known about diplospory, but the literature suggests a similar working hypothesis (Leblanc et al. 1995; Noyes and Rieseberg 2000, Bicknell et al. 2000), and works on *Taraxacum* reviewed by Mogie 1988). However, those results, both on diplospory and apospory, fail to provide information about the fine genetic control of nonreduction followed by the failure of fertilization and the induction of embryogenesis. In brief, it remains unclear whether all three events rely on distinct, but linked, genetic factors, or on a single gene controlling their successive induction as a pleiotropic effect. Both hypotheses have been defended logically, but whatever the number of genes specifically transmitted to an apomict, they should either behave genetically as a single locus, or manifest as a monomorphic trait in both sexual and apomictic ecotypes.If the genes were independent, upon segregation such mutations would rapidly be eliminated because of their low individual viability. Note that exceptions to this principle have been reported (Nogler 1984b; Asker and Jerling 1992; Kojima et al. 1994; Noyes and Riesberg 2000).

In work on *Ranunculus* species, for example, Nogler (1984b) first reported a trisomic hybrid lacking the ability for parthenogenesis, despite being highly aposporous. A similar case was described by Kojima et al. (1994) for *Allium* species, and by Noyes and Rieseberg (2000) for *Erigeron annuus*. In addition, apomictic plants usually produce "off-type" progenies, in which one of the two steps is skipped. This results in dihaploids, in which there is reduction but also parthenogenesis, or in 2n + n off-types, in which there is nonreduction but fertilization. Such cases do not necessarily require different genes, but they least entail the independent expression of the putative developmental components. Finally, it should be noted that in the case of grasses, it may not be neccesary to transfer specific genes for parthenogenesis, since it is apparently a latent ability in most of them.

Whether one or several genes are involved in apomixis, many questions remain about their mode of expression and regulation. Early results from *Panicum* (Savidan 1982) and *Ranunculus* ssp. (Nogler 1984b) indicated that although the induction of apospory is under simple genetic control, the overall apomictic behavior of these aposporous species is more quantitative as evidenced by the relative proportion of ameiotic and meiotic embryo sacs, some environmental effects, etc. Modifier effects that need to be identified include the

number of genes, their relative importance, their dominance relationships, epistatic effects, pleiotropic effects, possible allelic diversity, chromosomal localization, maternal and/or paternal effects, and environmental regulation.

Expression of Apomixis and Ploidy Levels

A remarkable aspect of apomixis is its relationship to polyploidy. Except in rare cases, apomicts are polyploids while sexuality in the same species, if known, is usually found at lower ploidy levels. It is widely accepted that some type of mechanism protects diploid sexual populations from being "invaded" by apomixis.

Three broad types of hypotheses have been proposed concerning that mechanism. One school of thought assumes that the alleles controlling apomixis could eventually be transmitted to diploid plants, but that the expression of the trait is restricted to polyploids; the penetrance of the character depending on dosage effects between the various alleles at the locus or loci controlling diplospory (Mogie 1992; Noirot 1993). Noirot, assuming a single allele *A* controlling apomeiosis in a dominant manner, proposed that not more than one copy of the *A* allele would be found among every four alleles (a ratio between *A* and *a* not to exceed 0.25). The hypothesis contradicts reported cases of apomictic triploids, trisomics, and dihaploids (a ratio of up to 0.5) (Leblanc et al. 1996; Nogler 1982). Mogie (1992) proposed a different though related dosage model for the regulation of diplospory in *Taraxacum*, in which the dominance relationship between the wild type (*a*) and mutant (*A*) alleles is determined by their relative copy numbers: avoidance of meiotic reduction occurs when the mutant allele is present in more copies than the wild type *a* allele. Mogie also assumes that the a locus plays an important role in mitosis and meiosis, thus explaining why *A* is not expressed or eliminated when transmitted in the haploid or homozygous states. Mogie's data for *Taraxacum* have been challenged recently by van Dijk et al. (2000), who proposes a more complex model for the inheritance of diplospory in this genera.

The second hypothesis (Nogler 1982) is based on the assumption that apomixis is usually not transmitted to diploids. In Nogler's work with *Ranunculus* hybrids, the *A* factor was not transmitted through haploid gametes, presumably because of a lethal effect of the allele when present under haploid conditions. Noyes and Riesberg (2000), working with *Erigeron*, proposed a more complex but related explanation, in which the absence of diplospory in diploids is best explained by both the combined effect of a recessive lethal gametophytic selection against a unique parthenogenetic-controlling locus, and univalent inheritance of the region responsible for diplospory. Related data was also obtained in *Tripsacum* (Grimanelli et al. 1998a), showing that apomeiosis was generally not transmitted via haploid gametes. In *Hieracium*, however, Bicknell et al. (2000) suggest that diplosporous apomixis can be transmitted both by diploid and haploid gametes, and that the absence of diploid apomictic progenies is caused by selection against the survival of diploid zygotes, rather than against the elimination of haploid gametes. Once more, as with the control of apomeiosis, it is hard to define a model that fits all apomicts. Whether those differences are due to experimental bias or simply to more fundamental differences in the nature of the various forms of apomixis has not yet been determined.

Carman (1997) puts forward a third general hypothesis that cites differences in rates of reproductive development between different ecotypes as being responsible for multiple reproductive anomalies, among which is

apomixis. In his hypothesis, Carman assumes that polyploidy may result in asynchronous expression among the genomes contributed to the polyploid of different genes regulating megasporogenesis and megagametogenesis, and that this asynchrony might be responsible for the apomictic phenotype. According to Carman, polyploidy, or at least the existence of multiple copies of asynchronously expressed genes, is a causal factor for the expression of apomixis.

Endosperm Development

Dosage studies, mainly in maize (reviewed by Birchler 1993), have shown that a major factor influencing endosperm development is the dosage effect between the relative contributions of the male and the female genomes in the endosperm. In maize, a genomic ratio of 2 maternal doses to 1 paternal (2m:1p) is required for normal development, and even limited perturbations around that ratio can have strong deleterious effects on endosperm development and thus on the viability of the embryo. By contrast, apomictic plants seem to develop normal embryos with a great variety of maternal and paternal contributions that can strongly differ from the 2m:1p ratio needed in many sexually reproducing plants. In *Tripsacum*, for example, the endosperm seems to develop normally, even though the ratio of genomic contributions deviates from the 2m:1p ratio (Grimanelli et al. 1997). Indeed, ratios of 2:1, 4:1, 4:2, 8:1, and 8:2 can be observed, depending on both the ploidy level of the parents and mode of reproduction. Autonomous apomicts, in which the endosperm develops without fertilization of the central cell, also provide striking evidence that some adaptation to dosage response exists in apomicts.

Surprisingly little has been published about this specific aspect of apomictic reproduction. It is clear, however, that understanding the basis of endosperm development in apomicts is a critical step toward the utilization of apomixis in food crop production. Recent publications concerning the induction of seed and endosperm development in *Arabidopsis thaliana* (Grossniklaus et al. 1998; Vielle Calzada et al. 1999; Luo et al. 2000) and the specific role of Polycomb group-like proteins in the process have intensified interest in this issue. The application of these data to the production of apomictic plants, especially monocots, remains uncertain.

The Single-Gene Model Revisited

Most likely, many genes act to insure the viable development of an apomictic embryo. Most, if not all, of those genes also play a role in the development of sexual embryos, and so should be common to both apomictic and sexual development. But one or several alleles of some of these genes, or alternatively their regulation, must be specific to the apomictic plants. The challenge here is less that of understanding the fine genetic control of apomixis (the genes acting during the apomictic process), rather than identifying the specific alleles that must be transmitted to, or altered in, sexual plants in order to induce an apomictic mode of reproduction. The identification of these alleles is important for understanding the process of apomixis and crucial for the ultimate introduction of apomixis into crops.

Despite the complexity of the developmental process of apomictic reproduction, most genetic analyses of apomixis conclude that a simple mode of inheritance is involved. Studies on *Panicum, Ranunculus Hieracium, Tripsacum, Erigeron and Brachiaria* (see earlier references) show that apomixis segregates as a single, or eventually a few dominant loci. Such conclusions, however, should be taken with caution. The cited genetic analyses have been conducted mainly by crossing apomictic and sexual genotypes within species or genera,

and they are not necessarily informative when it comes to manipulating apomixis genes beyond their respective species. Indeed, it could well be that a single mutation in those species gave rise to an apomictic genotype. This does not rule out the possibility that several other genetic factors may be required to ensure the expression of apomixis. Such factors would not necessarily be detected through classical genetic analysis, simply because of a lack of polymorphism for those characteristics, but the factors would be revealed by manipulating apomixis beyond the limits of specific species or genera. Those characteristics, necessary but not sufficient, probably would have accumulated during the evolution of those species prior to their switch from sexual to apomictic modes of reproduction.

Several observations support this hypothesis. During various attempts to transfer apomixis from wild relatives to cultivated crops, the observed transmission of apomixis through generations of backcrossing did not conform to a simple genetic model. In the case of the maize-*Tripsacum* system, genetic data show that the expression of functional apomictic reproduction depends on a complex mode of inheritance (Leblanc, personal comm.; Savidan, Chap. 11). The conditions of endosperm development in pseudogamous apomictic grasses is another strong illustration of this hypothesis. Angiosperm apomicts evolved from sexual ancestors that may have been subject to dosage effects in the endosperm, as apparently many angiosperms have to a variable degree (see Birchler 1993). This suggests that some adjustments in the mechanisms governing endosperm development might have accompanied the evolution of apomixis; because the switch from sexual to apomictic reproduction simultaneously changes the genomic ratio of the endosperm, dosage requirements would have acted as a barrier against the emergence of apomixis by preventing endosperm formation. Hence, only families in which the regulation of endosperm development had somehow been modified would have been prone to the emergence of apomixis.

By the same token, it is conceivable that different families would have been inclined to different types of apomixis. Strong supporting evidence that different species are compatible with different forms of apomixis can be found in the phylogenetic pattern of distribution of the various forms of apomixis (Richards 1986; Asker and Jerling 1992; Mogie 1992; Carman 1997). Most apomictic taxa (75%) belong to only three families: Asteraceae, Poaceae, and Rosaceae, which together comprise no more than 10% of angiosperm species. Diplospory is common among the Asteraceae, but less so among the Rosaceae and the Poaceae, while apospory is common among the Rosaceae and the Poaceae, but less so among the Asteraceae. Autonomous apomixis appears to be restricted mostly to the Asteraceae and is found only infrequently in the Poaceae and Rosaceae. Clearly, the occurrence of apomixis and the distribution of its various forms are not random (Carman 1997). This might reflect (*i*) that not all taxa are compatible with the emergence of apomixis, and (*ii*) that different taxa are not compatible with the same types of apomixis.

Hence, introducing apomixis into otherwise sexually reproducing crops may depend on more than the few genes responsible for polymorphism in modes of reproduction within agamospecies. Other factors may need to be considered, such as the endosperm, that represent necessary conditions for the successful expression of the apomictic genes per se.

Applications of Molecular Genetics to Apomixis Research

What Material?

It could be speculated that the diffusion of apomixis in crops could be achieved through the isolation and manipulation of genes from a well-chosen model system. It is worth considering, then, whether this model could be defined for apomixis research. But is there solely one "universal apomixis," despite the amazing diversity of apomictic processes? In other words, should we consider the different types that have been described in the literature as different expressions of the same genetic components, or should different sources of apomixis be studied as distinct and unrelated processes? According to Sherwood (Chap. 5), a single gene might be responsible for the induction of both diplospory and apospory. Still, apomixis has occurred in a seemingly independent fashion in various taxa during their evolution through different processes, which might also be viewed as evolutionary convergence. Although answering these questions is undoubtedly an important long-term goal, given our current knowledge, the choice of a model system for apomixis research is more a matter of technical considerations. Some of those considerations are proposed by Bicknell (Chap. 8) in this volume, and include the ability for both in vivo and in vitro culture, a short generation time, easy hybridization, the availability of related sexual and apomictic biotypes, good characterization at the genome level, and ability for transgeny. Another important consideration is that using diploid apomicts greatly simplifies genetic analyses. Furthermore, access to efficient mutagenesis procedures, including transposon mutagenesis, would provide attractive tools for functional analyses.

While no known taxon fulfills all of the above criteria, researchers working on the genetics and molecular biology of apomixis have considered two alternatives. The first alternative is to use existing apomictic species that fulfill, as much as possible, the criteria described earlier. Bicknell proposed *Hieracium* as a model system and has been developing a transposon tagging approach for aposporous apomixis in that species (Chap. 8). On the other hand, the wealth of genetic information available in the grass families, including the remarkable level of genomic synteny found in the Poaceae (Bennetzen and Freeling 1993; Ahn and Tanksley 1993; Moore et al. 1995), make apomictic grasses an attractive model because the various forms of apomixis can be compared.

The second alternative relies on generating or transferring the components of apomixis into a well-characterized, easily handled organism, such as *Arabidopsis* or maize.Three approaches for this alternative have been proposed: (*i*) the transfer of apomixis from a wild species to a related and genetically well-studied crop through sexual hybridizations, (*ii*) the *de novo* generation of apomixis in normally sexual organisms by mutagenesis and manipulation of gene expression, and (*iii*) the *de novo* generation of apomixis through wide hybridization after selection of the appropriate parental reproductive phenotypes (based on Carman's hypothesis [Carman 1997, Chap. 7]). A review and discussion of the first two approaches follow later in this chapter. A description of the third approach may be found in Chapter 7.

Most current work in apomixis research essentially focuses on the very first event in the apomixis mechanism, i.e., the failure or absence of meiosis. This is partly a consequence of the prevailing hypothesis that apomixis processes in their entirety, or at the very least, apomeiosis, might depend on a single-gene regulation. As opinions evolve regarding this regulation, more effort will be

directed toward identifying the components required for the expression of functional apomixis and dissecting their genetic basis. Most of the works presented herein deal with apomeiosis. The strategies described, however, apply to most aspects of apomictic development.

Molecular Mapping of Apomixis

The first molecular work on apomixis essentially focused on the development of molecular maps and the localization of the DNA regions that control apomixis in various organisms. Part of the interest in developing genetic maps lays in the nature of molecular markers; their Mendelian inheritance is independent of either environmental conditions, or our ability to actually observe a given phenotype. Therefore, by studying their cosegregation with any trait of interest, one can identify and characterize chromosomal regions that play a role in the expression of that trait. Once mapped, any trait can theoretically be studied or followed—regardless of its expression and with a known confidence level—by detecting and analyzing the segregation of linked molecular markers.

Chapter 10 is devoted to the genetic improvement of apomictic cultivars. Most applications of molecular maps in plant improvement are also relevant to apomicts, and comprehensive reviews on such applications are readily available. Note, however, that molecular markers are particularly valuable for studying characters that are expressed late in plant development, such as apomixis or other reproductive traits. By using DNA markers, reproductive behavior can be rapidly predicted at the seedling stage, with confidence levels that depend mainly on the linkage between the marker and the mapped gene(s). Moreover, as opposed to cytoembryological tests, molecular marker analysis is not destructive.

DNA markers linked with both apospory and diplospory have been reported for apomictic *Pennisetum, Brachiaria, Taraxacum, Tripsacum, and Erigeron* species, among others (Ozias-Akins et al. 1998; Pessino 1997; Leblanc et al. 1995; Grimanelli et al. 1998b; Noyes and Rieseberg 2000). Interestingly, these diverse reports reach common conclusions about several aspects of apomixis. Taken together, they demonstrate that apomeiosis is likely controlled by one or several genes located on a single chromosome segment. Furthermore, reports on *Tripsacum* (Grimanelli et al. 1998a) *Pennisetum* (Ozias Akins et al. 1998) and possibly *Erigeron* (Noyes and Riesberg 2000) indicate that this segment might be characterized by a very strong restriction to recombination. In *Tripsacum*, where the mapping data could be compared between apomictic and sexual accessions, this restriction to recombination appears to be apomict-specific; while in the sexual forms the mapped alleles underwent a significant rate of recombination, complete linkage was observed in the apomict for the alleles detected by the same probes. Clearly, recombination is restricted at the tetraploid (apomictic) level as opposed to the diploid (sexual) level in both *Tripsacum* and maize, as seen in their RFLP maps. In *Pennisetum*, the segment itself seems to be apomixis specific, as revealed by Southern analysis.

Because the specific chromosome segment shows a restricted level of recombination, the classical model of monogenic inheritance for apomixis probably warrants a careful review, because regardless of the number of genes involved, they behave as a single locus in segregating populations. This number of genes might be particularly important within the framework of a gene isolation program.

Cloning the Apomixis Gene(s) Using Molecular Genetics Tools

A major difficulty encountered by those interested in cloning "apomixis genes" is simply defining what they are. Introducing apomixis into crops implies that specific genes are transferred or altered and expressed in the target crops. Most likely, not all of the genes involved in the apomictic process should be targeted: most, if not all, of them should already be present and playing a role in sexually reproducing plants. The issue then is which alleles of pertinent genes must be transmitted or manipulated for the induction and successful development of apomictic embryos and seeds. To date, all efforts to tag apomixis genes, including those presented in this paper, have focused on the mechanism of nonreduction, mainly because it is an excellent indicator of apomictic development and it is probably the easiest one to score. Nevertheless, it should be remembered that apomixis is probably more complex than the simple process of nonreduction. The importance of this constraint will likely emerge when attempts are made to synthesize *de novo* apomicts in sexual organisms

"Map-based" cloning in apomictic species. Once a gene has been located on a genetic map, subsequent efforts to specify its position can ultimately lead to its isolation (for the first successful efforts in plants, see Giraudat et al. 1992; Martin et al. 1994). The recent development of powerful new approaches for physically mapping chromosome segments combined with the ability to clone large DNA fragments (Burke et al. 1987; Shizuya et al. 1992), and progress in genome sequencing techniques have created new and higher standards for positional cloning in plants. It is still a laborious and risky task outside of a few well-characterized model genomes, but the number of genes cloned in this manner are rapidly increasing. However, positional cloning for apomixis is not very promising because most, if not all, of the candidate species for a map-based cloning project are highly heterozygous tetraploids, for which little genomic characterization exists.

Furthermore, when attempting positional cloning, the first step is to identify a chromosomal region, defined by two or more molecular markers, that flanks the gene under study. The precision of the estimated position of the gene is therefore limited by the smallest measurable recombination unit, meaning one recombinant in a given mapping population. Hence, the recombination level around the apomixis gene(s) presents another significant challenge: positional cloning will prove efficient only insofar as recombination can be observed near the locus of interest. As mentioned earlier, recombination near the apomictic alleles is very likely restricted, at least in *Pennisetum* and *Tripsacum*. Consequently, the smallest recombination unit defined by two markers that encompasses the apomixis locus might well be a relatively large amount of DNA.

Transposon tagging of apomixis genes. Some model plants, such as maize, rice, tomato, *Arabidopsis*, and *Petunia* have undergone extensive genome characterization. Specific approaches are available for gene tagging these plants that might be considered for tagging apomixis gene(s), provided that components of apomixis occur in one of these organisms.

A very promising approach is that of transposon tagging. Transposable elements are short DNA sequences that have the property to transpose to more or less random locations in the genome (see Walbot 1992, for a review). They were discovered in maize, but have since been identified or introduced in very diverse organisms. They have been used in a wide range of genetic studies, and have been found to be highly effective for gene tagging and cloning.

Transposon tagging in apomicts presents some constraints, including access to transposable elements and the genetic control of the trait. To the best of our knowledge, transposon activity has not been demonstrated in apomictic species. This might be overcome by introducing functional transposable elements into apomicts, either through transformation (as in *Hieracium*, Bicknell, Chap. 8) or through hybridization with a close relative (as with maize and *Tripsacum*, Grimanelli 1997). In both cases, maize transposable elements were successfully introduced into an apomictic background, and transposable activity was demonstrated.

In our view, the main issue concerning transposon tagging of apomixis is genetic control of the trait. While this approach is efficient for phenotypes controlled by single genes, it might yield no, or disappointing, results if apomixis is genetically more complex. But taken further, it would at least provide an elegant method to determine whether apomixis is controlled by one or several genes: if a single allele controls the trait, then a single mutation should allow complete reversion to sexuality; if a more complex system is involved, then individual mutations should lead to abnormal or only partial expression of the trait.

Candidate gene approaches. Although apomixis is unknown in major crop plants or other genetically well-characterized organisms, useful information can be derived from detailed analyses of the reproduction processes of select sexual organisms. For example, genes involved in the control of ovule development, the initiation of meiosis, embryogenesis, and endosperm development have been described in various organisms, and a close look at these genes might provide useful information about the regulation of apomixis. Such genes, but not necessarily their respective alleles, might represent prospective "candidates" for the apomixis gene(s), i.e., the gene(s) that would code for identical functions as their apomicitic counterparts. The best, though not the only, candidates are the yeast genes responsible for the induction of meiosis and the meiotic mutants identified in higher plants.

Major biochemical pathways involved in the regulation of the cell cycle and meiosis appear to be relatively well conserved between distant organisms such as yeast and higher plants, and the advance of whole-genome sequencing puts provides complete catalogs of putative candidate genes. This progress offers great promise, but it is tempered by the fact that it is usually difficult to verify whether a yeast gene of known function plays a similar role in plants. One powerful way to corroborate such gene functions is the so called "reverse genetics" strategy, using either insertional mutagenesis or homologous recombination. When based on transposon or T-DNA insertions, reverse genetics (or site-specific transposon mutagenesis) implies that transposon tagging is performed to identify individuals carrying a transposon insertion in a gene of known sequence. The expected function of that given gene can then be corroborated by confirming that its disruption leads to the loss or alteration of the expected function. Powerful reverse-genetic systems are available in various plant species, including maize, *Arabidopsis*, and tomato.

A specific candidate gene strategy based on comparative mapping can also be undertaken within the grass family. The identification of orthologous genes between species (i.e., genes that diverged from a common gene at the time that the species harboring them diverged) could be used to understand the relationships between the genes responsible for various components of apomixis in apomictic plants,

and meiotic or developmental mutants that are well characterized in sexual plants. Numerous mutants are known in grasses, especially in maize (Neuffer et al. 1997), for various aspects of sexual reproduction. Furthermore, large numbers of such mutants can be generated through classical (e.g., chemical) or transposon mutagenesis. Recent results of comparative mapping among grasses (Bennetzen and Freeling 1993; Ahn and Tanksley 1993; Moore et al. 1995) demonstrate that most grasses probably share the same basic set of genes, and that the obvious differences separating the species are based on allelic variations and not on their relative gene combinations.. Therefore, we suggest that the genes whose actions produce an apomictic phenotype in some grasses almost certainly can be found in sexual species. In this instance, comparative mapping could be used to identify genes in maize or some other sexual grass that are orthologous to the apomixis genes, and then use them to isolate their counterparts in the apomictic species.

The process of identifying maize orthologs of genes responsible for apomixis involves three successive steps: (*i*) candidate genes are identified through phenotypic characterization and genetic mapping; (*ii*) promising candidates are then isolated in maize; once cloned, the isolated genes are sequenced, and the sequence information is used to clone orthologous genes in the apomicts; (*iii*) the relationship between the alleles isolated in the previous steps and the expression of apomixis is confirmed using a reverse genetic strategy in apomictic plants. For step *iii*, the construction of transposon tagging populations in apomicts are of great interest to R. Bicknell and the CIMMYT apomixis team.

Three criteria can be employed to select candidate genes: (*i*) because apomixis often affects only the female function, we propose that the gene(s) responsible for the failure of meiosis have a megasporogenesis-specific phenotype, meaning that mutants of interest should affect only the female function; (*ii*) as in diplosporous plants, the candidates should affect early stages of meiosis, ideally, the induction of meiosis; meiotic mutations acting at later stages in meiosis are probably not directly related to apomixis; and (*iii*) interesting candidates should be able to produce unreduced gametes, (thus, as in apomictic plants, the completion of unreduced gamete formation implies that the checkpoints (Hartwell and Weinert 1989), which usually act during the meiotic cell-cycle to ensure the production of normally reduced haploid gametes, failed to override abnormal behavior. With aposporous-like mutants, obvious phenotypes relate to the induction of megagametogenesis in somatic cell. Sheridan et al. (1996) describe a remarkable example of this type of mutant.

Manipulation of gene expression in model species: To date, this is probably the most widely used approach for developing apomictic cultivars, (details are discussed elsewhere in this volume). Current work centers on large-scale mutant screening in *Arabidopsis* and *Petunia* (Jefferson and Bicknell 1996; Ohad et al. 1996; Chaudurhy et al. 1997; Grossniklaus et al 1999; Luo et al. 2000). The best prospect from these approaches would be the engineering of a mode of apomixis that better meets the requirements of agricultural production than the apomixis mechanisms found in the wild (see Jefferson and Bicknell 1996, and Chap. 8). The remarkable results obtained recently with a set of mutations in Polycomb-related genes in *Arabidopsis* (Grossniklaus et al. 1998; Luo et al. 2000) are very encouraging. They demonstrate that phenotypes related to apomixis may eventually be obtained by manipulating the expression of genes involved in sexual reproduction, without reference to apomixis as seen in the wild.

Conclusions

Our understanding of the genetics of apomixis is changing rapidly, from the idea that a simple genetic system might control the whole developmental process, to a more integrated conception and sophisticated models. Part of that evolution stems from the application of molecular genetic technologies to the study of apomixis. Still, many important questions and problems remain unresolved; there is no shortage of challenges in the field of apomixis research. Many serious research efforts may only serve as preliminary and somewhat academic steps toward the long-term goal of introducing apomixis into farmers' fields. To reach the distant goal of deployment to farmers, future research should include an assessment of the social and economic impact of apomixis, and a definition of adequate deployment strategies. These critical elements will strongly influence the biological aspects of apomixis research and what "kind" of apomixis should be targeted for development and deployment.

References

Ahn, S.N., and S.D. Tanksley. 1993. Comparative linkage maps of the rice and maize genomes. *Proc Natl Acad Sci* (USA) 90: 7980–84.

Asker, S., and L. Jerling. 1992. *Apomixis in Plants*. Boca Raton, Florido: CRC Press.

Bennetzen, J.L., and M. Freeling. 1993. Grasses as a single genetic system: genome composition, collinearity and compatibility. *Trends in Genetics* 9:259–61.

Bicknell, R.A., N.K. Borstk, and A.M. Koltunow. 2000. Monogenic inheritance of apomixis in two *Hieracium* species with distinct developmental mechanisms. *Heredity*. 84 :228–37.

Chaudhury, A.M., L. Ming, C. Miller, S. Craig, E.S. Dennis, and W.J. Peacock. 1997. Fertilization-independent seed development in *Arabidopsis thaliana*. *Proc Natl Acad Sci* (USA) 94: 4223–28.

Birchler, J.A. 1993. Dosage analysis of maize endosperm development. *Annual Review of Genetics* 27: 181–204.

Burke, D.T., G.F. Clarke, and M.V. Olson. 1987. Cloning of large segments of hexogenous DNA into yeast by mean of artificial chromosome vectors. *Science* 236: 806–11.

Calzada, J.P-V., C.F. Crane, and D.M. Stelly. 1996. Apomixis: an asexual revolution. *Science* 274: 1322–23.

Carman, J.G. 1997. Asynchronous expression of duplicated genes in angiosperms may cause apomixis, bispory, tetraspory and polyembryony. *Biol. J. Linnean Soc.* 61, 51-94

Chaudhury, A.M., L. Ming, C. Miller, S. Craig, E.S. Dennis, and W.J. Peacock. 1997. Fertilization-independant seed development in Arabidopsis thaliana. *Proc. Natl. Acad. Sci.* (USA) 94:4223–28.

Giraudat, J., B.M. Hauge,C. Valon, J. Smalle, F. Parcy, and H.M. Goodman. 1992. Isolation of the *Arabidopsis AB13* gene by positional cloning. *Plant Cell* 4: 1251–61.

Grimanelli, D. 1997. *L'apomixie dans le genre Tripsacum: contrôle génétique et contraintes associées à son transfert chez les plantes cultivées*. Ph.D. dissertation, Institut National Agronomique Paris-Grignon.

Grimanelli, D., M. Hernandez, E. Peratti, and Y. Savidan. 1997. Dosage effects in the endosperm of diplosporous apomictic *Tripsacum* (Poaceae). *Sexual Plant Reproduction*10: 279-282

Grimanelli D., O. Leblanc, E. Perotti, D. González de León, and Y. Savidan.. 1998a. Non-Medelian transmission of apomixis in maize-*Tripsacum* hybrids caused by a transmission ratio distortion, *Heredity* 80: 40–44.

———. 1998b. Diplosporous apomixis in tetraploid Tripsacum: one gene or several genes? *Heredity* 80: 33–39.

Grossniklaus U, J.P. Vielle-Calzada, M.A. Hoeppner, and W.B. Gagliano. 1998. Maternal control of embryogenesis by MEDEA, a polycomb group gene in *Arabidopsis*. *Science*. 280(5362): 446–50.

Grossniklaus, U., A. Koltunow, and M. van Lookeren Campagne. 1999 A bright future for apomixis. *Trends Plant Sci.* 3: 415–16.

Jefferson, R.A., and R. Bicknell. 1996. The potential impact of apomixis: a molecular genetic approach. In B.W.S Sobral (ed.), *TheImpact of Plant Molecular Genetics*. Boston: Birkhaüser.

Kojima, A., T. Kazono, Y. Nagato, and K. Hinata. 1994. Non-parthenogenetic plants detected in Chinese Chive, a facultative apomict. *Breeding Science* 44:143–49.

Leblanc, O., D. Grimanelli, D. Gonzaléz de Léon, and Y. Savidan. 1995. Detection of the apomictic mode of reproduction in maize-Tripsacum hybrids using maize RFLP markers. *Theor. Appl. Genet.* 90: 1198-1203.

Leblanc, O., D. Grimanelli, N.I. Faridi, J. Berthaud, and Y. Savidan. 1996. Can diploid cereals reproduce through apomixis? Evidence from maize-*Tripsacum* hybrids. *Journal of Heredity* 87: 108-15.

Luo, M., P. Bilodeau, E.S. Dennis, W.J. Peacock, andA. Chaudhury. 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* (USA) 97(19):10637–42.

Ma, H. 1999. Seed Development; with or without sex ? *Current Biology* 9: 636–39.

Martin, G.B., S.H. Brommonschenkel, J. Chunwongse, A. Frary, M.W. Ganal, R. Spivey, T. Wu, E.D. Earle, and S.D. Tanksley. 1994. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science* 62:1432–36.

Mogie, M. 1988 A model for the evolution and control of generative apomixis. *Biol. J. Linn. Soc.* 35: 127–53.

———. 1992. *The evolution of asexual reproduction in plants*. London: Chapman and Hall.

Moore, G., K.M. Devos, Z. Wang,. and M.D. Gale. 1995. Grasses: line-up and form a circle. *Current Biology* 5: 738–42.

Neuffer, G.M., E.H. Coe, and S.R. Wessler. 1997. Mutants of maize. Cold Spring Harbor, New Jersey (USA): Cold Spring Harbor Laboratory Press.

Nogler, G.A. 1982. How to obtain diploid apomictic Ranunculus auricomus plants not found in the wild state. *Bot.Helvetica* 92: 13–22.

———. 1984a. Gametophytic apomixis. In B.M. Johri (ed.), *Embryology of Angiosperms*. Berlin: Springer-Verlag. Pp. 475–518.

———. 1984b. Genetics of apaspory in apomictic *Ranunculus auricomus* V. Conclusions. *Botanica Helvetica* 94: 411–22.

Noirot, M. 1993. Allelic ratios and sterility in the agamic complex of the Maximeae (Panicoideae): evolutionary role of the residual sexuality. *J. Evol. Biol.* 6:95–101.

Noyes, R.D., and R.H. Rieseberg. 2000. Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. *Genetics* 155: 379–90.

Ohad, N., L. Margossian, Y.-C. Hsu, C. Williams, P. Repetti, and R.L. Fisher. 1996. A mutation that allows endosperm development development without fertilization. *Proc. Natl. Acad. Sci.* (USA) 93: 5319–24.

Ozias-Akins, P. 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.* (USA) 95: 5127–32.

Pessino, S.C. 1997. Identification of a maize linkage group related to apomixis in Bracharia. *Theor. Appl. Genet.* 94: 439–44.

Richards, A.J. 1986. *Plant breeding systems*. London: George Allen and Unwin.

Savidan, Y. 1982. *Nature et hérédité de l'apomixie chez Panicum maximum Jacq.* Ph.D. dissertation Université of Paris XI.

———. 2000. Apomixis: Genetics and Breeding. *Plant Breeding Reviews* 18: 13–86.

Sheridan, W.F., A.A. Nadezhda, I.I. Shamrov, T.B. Batygina, and I.N. Golunovskaya. 1996. The mac gene: controlling the commitment to the meiotic pathway in maize. *Genetics* 142: 1009–20.

Shizuya, H., B. Birren, U.-J. Kim, V. Mancino, T. Slepak, Y. Tachiiri, and M. Simon. 1992. Cloning and stable maintenance of 300-kilabase-pair fragments of human DNA in Escherichia coli using a F-factor-based vector. *Pro. Natl. Acad. Sci.* (USA) 89: 8794–97.

van Dijk, P.J. 1999. Crosses between sexual and apomictic dandelions (*Taraxacum*). II. The breakdown of apomixis. *Heredity* 83: 715–21.

Vielle-Calzada J.P., J. Thomas, C. Spillane, A. Coluccio, M.A. Hoeppner, and U. Grossniklaus. 1999. Maintenance of genomic imprinting at the *Arabidopsis* medea locus requires zygotic DDM1 activity. *Genes Dev.* 13(22): 2971–82.

Wolbat, V. 1992. Strategies for mutagenesis and gene cloning using transposon tagging and T-DNA mutagenesis. *Ann Rev. Plant Physiol. Plant Mol. Biol.* 43: 49–82.

The Flowering of APOMIXIS:

From Mechanisms to Genetic Engineering

Y. Savidan, J. G. Carman, and T. Dresselhaus, Editors

Contents