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Move It on Out with MATEs

Multidrug transporters form a large class of membrane proteins present in the cells of most organisms. These proteins bind to a variety of potentially cytotoxic compounds and remove them from the cell in an ATP- or proton-dependent process (Zhelenova et al., 2000). Traditionally, multidrug transporters have been divided into four superfamilies: the ATP binding cassette (ABC) superfamily, the major facilitator superfamily, the small multidrug resistance family, and the resistance-nodulation-cell division family. Brown et al. (1999) defined a fifth family, called the multidrug and toxic compound extrusion (MATE) family of transporters. The MATE family is characterized by the presence of 12 putative transmembrane segments and by the absence of "signature sequences" specific to the other multidrug transporter superfamilies. MATE proteins are believed to function as proton-dependent efflux transporters, based on the genetic characterization of two family members, NorM from V. parahaemolyticus and its homolog YdeH from E. coli. Expression of these proteins in E. coli confers resistance to various antibiotics and antimicrobial agents that depend on the maintenance of a proton gradient across the plasma membrane. MATE genes are abundant in bacteria and plants—the Arabidopsis genome contains at least 54 MATE family members—but have not been found in mammals. Aside from NorM and YdeH, very little functional information is available on these proteins.

In this issue of The Plant Cell, Diener et al. (pages 1625-1637) describe the functional analysis of the MATE gene ALF5 in Arabidopsis. The alf5 mutant exhibits greatly inhibited formation of lateral roots when grown in commercial Bacto agar (Figure 1). Interestingly, it was found that the mutant produced roots that were indistinguishable from wild-type roots when grown in soil, in a different brand of agar, or in extensively washed Bacto agar, suggesting that the alf5 mutation caused sensitivity to a soluble contaminant present in the Bacto agar. The alf5 locus was cloned and found to contain a 29-bp deletion in a gene, ALF5, that encodes a MATE family integral membrane protein. Gene expression analysis using AFL5 fused to the β-glucuronidase reporter gene in transgenic plants indicated that the ALF5 gene is highly expressed in the root epidermis and cortex. In addition, expression of ALF5 in yeast conferred resistance to the toxic cation tetramethylammonium, supporting the conclusion that ALF5 is a functional MATE efflux transporter.

PLANTS AS “GREEN LIVERS”

Plant cells, like the cells of most organisms, are capable of removing a large number of potentially toxic compounds from the cytoplasm. In plants, these compounds are either sequestered in vacuoles or transported to the cell wall. Toxic compounds may be of xenobiotic origin or produced endogenously (e.g., phenolics, flavonoids, and phytoalexins). The bronze-colored phenotype of the Bronze2 mutation in maize, for example, is caused by the inability of the mutant to transport anthocyanin from the cytosol to the vacuoles. Bronze2 encodes a glutathione transferase (Marrs et al., 1995), and the mutant is unable to carry out conjugation of anthocyanin with glutathione, a necessary step before transport of conjugated glutathione to the vacuole. Sandermann (1992) likened plant metabolism of toxic compounds to that of the mammalian liver because of the presence and activity of cytochrome P450 monoxygenases and glutathione transferases, which resemble the two major enzyme systems of the liver. Plant cytochrome P450s and glutathione transferases are involved in the first two phases of detoxification of a number of polychlorinated and polycyclic hydrocarbons and related xenobiotic compounds as well as endogenous toxins. In phase I, cytochrome P450s prepare a substrate for phase II via hydroxylation, and phase II glutathione transferases carry out the conjugation of the hydroxylated compound to reduced glutathione. Subsequently, phase III involves the transport of the glutathione conjugate out of the cytoplasm to the vacuole or cell wall. There is good evidence that multidrug transporters of the ABC superfamily are involved in the transport of glutathione conjugates in plant cells (Rea et al., 1998; Theodoulou, 2000). Sandermann (1992) described plants as “green livers” that might act as a global sink for environmental pollutants of this nature. The presence of MATE efflux proteins in plants, which are presumed to carry out transport of lipophilic cations and related compounds that are not glutathione conjugates, broadens the scope of this concept and opens up more possibilities for plant biotechnology.

EVOLUTIONARY PLANT TRICKS

Despite the wide range of chemically and structurally distinct substrates for multidrug transporters, transporters of all five superfamilies show a preference for hydrophobic (lipophilic) cations, such as quaternary ammonium antiseptics (Stermitz et al., 2000). Lipophilic cations, such as berberine alkaloids, are
commonly produced by plants. Lewis (1999) proposed that berberine alkaloids represent a larger group of cationic toxins that fueled the evolution of microbial multidrug transporters. Interestingly, Stermitz et al. (2000) found that several plant species in the genus Berberis, which produce berberine, also produce an inhibitor of multidrug transporters, identified as 5′-methoxyhydnocarpin. Berberine exhibited relatively weak antimicrobial action, presumably because of its efflux from bacterial cells by multidrug transporters. 5′-Methoxyhydnocarpin had no antimicrobial activity alone, but it strongly potentiated the action of berberine and other toxins against the growth of Staphylococcus aureus. This finding suggests that the main function of some microbial multidrug transporters is resistance against plant-produced antimicrobial compounds.

Aside from plant–pathogen interactions, one habitat in which bacteria are likely to encounter toxic plant compounds is in root nodules. In Rhizobium etli, multidrug transporters have been found to play an important role in nodule formation of bean (Phaseolus vulgaris). RmrA encodes an R. etli multidrug efflux pump gene that is induced by flavonoids released from the roots of P. vulgaris, and mutations in this gene were found to reduce nodulation in the bean by an average of 40% (Gonzalez-Pasayo and Martinez-Romero, 2000).

Another habitat in which bacteria might commonly encounter plant toxins is in the stomachs of herbivores. In E. coli, the transcription repressor MarR binds various phenolic compounds such as salicylate and regulates the expression of two multidrug transporters to produce a more effective efflux pump system. Sulavik et al. (1995) suggested that drug resistance in E. coli is thus enhanced when the bacteria reside in an omnivore gut rich in plant antimicrobial compounds.

DIVERSITY OF FUNCTIONS FOR MULTIDRUG TRANSPORTERS

It is apparent that multidrug transporters constitute large superfamilies in plants, as in other organisms. The Arabidopsis genome contains at least 60 open reading frames for ABC transporters (Davies and Coleman, 2000). As with the less well known MATE family, the function of the majority of these genes is unknown, but characteriza-
tion of a few family members suggests a multiplicity of functions in plant growth and development within the superfamily, in addition to their role in the transport of xenobiotic compounds.

Sidler et al. (1998) showed that an Arabidopsis ABC transporter, AtPGP1, is involved in the regulation of hypocotyl elongation during photomorphogenesis. Under certain light conditions, plants overexpressing PGP1 developed longer hypocotyls, whereas plants with inhibited expression of PGP1 produced shorter hypocotyls compared with the wild type. Hypocotyl elongation in the dark was unaffected by alterations in PGP1 expression. In wild-type plants, the AtPGP1 gene was found to be expressed in the plasma membrane of root and shoot apices, and the authors proposed that AtPGP1 is involved in the transport of a signal molecule, such as a peptide hormone, from the shoot apical region.

Some mammalian ABC transporters, such as the cystic fibrosis transmembrane conductance regulator (CFTR) and the sulfonyleurea receptor (SUR), have been shown to act as ion channels and/or channel regulators. CFTR functions as an outwardly rectifying Cl– channel that also regulates other ion channels, and the SUR acts as an ATP-dependent K+ channel (Theodoulou, 2000). Some researchers have begun to look for such activity among ABC transporters in plants and have found evidence that ABC proteins may function as ion channel regulators in guard cells. Gaedeke et al. (2001) and Leonhardt et al. (1999) have investigated a slow ion channel in Arabidopsis guard cells that shows CFTR-like characteristics and that may coordinate the efflux of K+ and other ions during stomatal closure. The MATE transporter superfamily also may cover a diverse range of functions in plant growth and development. Debeaujon et al. (2001) recently reported that the TRANSPARENT TESTA12 (TT12) gene encodes another MATE family member in Arabidopsis. The function of TT12 appears to be in controlling the vacuolar sequestration of flavonoids in the seed coat (testa) endothelium. Because of their high chemical reactivity, flavonoids are toxic endogenous compounds that must be removed from the cytoplasm after their synthesis and sequestered in the vacuole or cell wall. There is evidence that they function as protectants against UV light damage, oxidative stress, and pathogen attack. The mutant seeds, lacking the function of the TT12 MATE protein, appear to be unable to transport and accumulate flavonoids in the vacuoles of the seed coat endothelium. The seeds are pale in color and also show reduced seed dormancy, supporting the idea that flavonoids play an important role in seed biology (Winkel-Shirley, 1998). Thus, it appears that we can expect a multiplicity of functions in growth and development for the many other plant MATE family members. Surprisingly, Diener et al. found a second open reading frame at the alf5 locus, LAL5, which lies immediately downstream of ALF5 and encodes a polypeptide with 83% identity to ALF5. It is not known if LAL5 is expressed, and this needs to be determined, but the authors reported that the gene appeared to be intact in the alf5 mutant. If the gene were expressed, it would appear to be functionally distinct from ALF5. The discovery of multidrug sensors that regulate the expression of some microbial multidrug transporters suggests that the main function of these transporters is the efflux of xenobiotic toxins (Lewis, 1999). At least three multidrug sensors have been identified. BmrR is a transcription factor in Bacillus subtilis that activates the expression of the multidrug transporter gene Bmr in response to binding a number of hydrophobic cations, many of which are also substrates of the Bmr protein. In S. aureus, the QacA multidrug transporter gene is repressed by QacR, and binding of QacR to various cations induces QacA expression. And in E. coli, repression of the EmrRAB transporter gene is relieved by binding of the EmrR repressor to various neutral compounds. It is interesting to speculate that multidrug sensors also will be found to control the expression of plant transporter genes such as ALF5, whose principal function appears to be the efflux of toxic compounds.

Nancy A. Eckardt
News and Reviews Editor
neckardt@aspp.org

REFERENCES


MEETING REPORT


APO2001: A Sexy Apomixer in Como

Long before promiscuity was discovered to bear significant risk, many flowering plants had partially abandoned the pleasures of their sexual life to evolve one of the most intriguing reproductive alternatives found in nature. Apomixis is an asexual method of reproduction through seed that circumvents meiosis and fertilization to culminate in the autonomous development of an embryo. Thus, unlike sexual reproduction, which yields genetically diverse progeny, apomixis produces clonal offspring. The production of clonal, genetically identical, seed bears great potential for applications in plant breeding and seed production (Hanna and Bashaw, 1987; Savidan, 1992; Koltunow et al., 1995). Apomixis has evolved several times independently from sexual ancestors and can be viewed as a modification of the sexual reproductive program. In angiosperms, sexual reproduction entails complex interactions between a variety of tissues. Female reproductive development occurs in a specialized organ, the ovule, where usually a single cell becomes committed to the reproductive pathway (the megaspore mother cell, or MMC). After meiosis, a single reduced product, the functional megaspore, divides mitotically to form the mature female gametophyte or embryo sac. The usually seven-celled embryo sac contains the egg cell and the binucleate central cell, both of which get fertilized. In the male reproductive organs, meiosis produces a tetrad of reduced spores, all of which divide mitotically to form the male gametophytes (pollen). The male gametophyte consists of two sperm cells, which are contained in a large vegetative cell that delivers the sperm cells to the female gametophyte. During double fertilization, one sperm fuses with the egg to form the zygote and the second sperm fuses with the central cell to form the endosperm.

In apomictic plants, this sexual developmental program is bypassed or deregulated at various steps (Koltunow, 1993; Grossniklaus, 2001): (1) meiosis is altered or absent to produce an unreduced female gametophyte with the full complement of maternal chromosomes (apomeiosis); (2) fertilization is avoided, producing an autonomous embryo (parthenogenesis); and (3) endosperm development is initiated autonomously or sexually; in the latter case, embryo sac development or fertilization is often modified to adjust to a different genomic context (Savidan, 2000; Grossniklaus et al., 2001). In contrast to the modified female reproductive program, pollen formation usually is unaffected in apomicts.

During the last two decades, the introduction of apomixis into sexual crops has been perceived as one of the most promising challenges faced by agricultural biotechnology. Apomixis could allow the fixation of any genotype, however complex, including that of high yielding F1 hybrids. The enormous potential of this trait was realized as early as the 1930s by Navashin and Karpenko...
(cited by Asker, 1971). Simplified apomixis breeding programs would allow an immediate fixation of any genotype and the production of self-perpetuating improved hybrids and could promise social and economic benefits that would challenge those of the Green Revolution (Vielle-Calzada et al., 1996a; Grossniklaus et al., 1998a).

The 2nd International Conference on Apomixis took place in Como, Italy, from April 24 to 28, 2001. The scientific program was organized by Lucia Co-lombo, Thomas Dresselhaus, Yves Savidan, and Rod Scott and was sponsored by the European Union, the Food and Agriculture Organization of the United Nations, the Institut de Recherche pour de Développement (IRD), the Italian National Research Center, and many private sponsors. Following on the success of the 1st International Apomixis Meeting held at College Station, Texas, in 1995, the Como conference attracted 170 participants from 27 countries. All meeting abstracts of invited speakers and poster sessions are available at http://www.apomixis.de.

Leo Beukeboom (University of Leicester, The Netherlands) gave the opening lecture on “Origin and Genetics of Parthenogenesis in Animals.” Asexual reproduction is a widespread phenomenon across the animal and plant kingdoms. There are differences in both the terminology and the mechanisms of asexual reproduction in plants and animals. Parthenogenesis (virgin birth), first observed by Bonnert in 1745, is the development of an egg without fertilization. Asexual reproduction in animals can occur either by mitotic parthenogenesis (apomixis) or by meiotic parthenogenesis (autopomixis). In contrast to the phenomenon in plants, apomixis in animals is rarely facultative, and most forms of autopomixis occur exclusively in animals. A wide range of cytological mechanisms underlie mitotic or meiotic parthenogenesis in animals. Beukeboom presented examples of asexual reproduction in freshwater flatworms (Polyclad nigra), in which B chromosomes may be associated with parthenogenetic lineages. Although much is known of the molecular mechanisms of fertilization in animals, remarkably little is known about the mechanisms of parthenogenesis.

**MECHANISMS AND EVOLUTION OF APO MIXIS**

Early studies demonstrated that apomixis is controlled genetically. Typically, a single dominant mendelian trait is associated with apomixis, although more complex modes of inheritance have been reported (see accompanying Insight article). Cytological descriptions emphasized distinctions between different apomictic mechanisms and have led to a simplified classification that is based on the origin and the location of cells initiating apomorphic development (reviewed in Koltunow, 1993). In diplospory, the MMC undergoes an aberrant meiosis or divides mitotically, producing unreduced spores that eventually form an embryo sac containing an egg cell with the full genetic complement of the mother. In apospory, a cell in the ovule other than the MMC produces the unreduced embryo sac. Anna Koltunow (Commonwealth Scientific and Industrial Research Organization, Adelaide, Australia) described the enormous variability existing in apomorphic processes. In Hieracium, apomictically derived embryo sacs usually are produced through apospory. However, several loci modify the timing of apomictic initiation, the frequency at which apomictic embryo sacs are formed, and the mode of progression of apomictic development (Koltunow et al., 1998, 2000; Bicknell et al., 2000). These findings indicate that the major locus associated with apomixis might create a competence for a variety of reproductive developmental processes in the ovule. Koltunow hypothesized that sporophytic cells in the ovule play an active role in modulating and controlling reproductive development. Transformation-induced alterations in ovule development that result in significant changes of the mode of apomixis in Hieracium add support to this hypothesis.

For Yves Savidan (IRD and Centro Internacional de Mejoramiento de Maíz y Trigo [CIMMYT], México) has cytologically analyzed the apomorphic mechanism of *Tripsacum dactyloides*, focusing on chromosome and chromatid dynamics and the characteristics of the cytoskeleton during ameiosis. A wide phenotypic variability occurs during apomorphic initiation, even within a single genotype. Many of the developmental abnormalities characteristic of apomorphic processes in *Tripsacum* have striking similarities to defects found in meiotic mutants of maize. Although these meiotic mutants have a strong impact on fertility, similar abnormalities do not compromise apomorphic seed formation. This suggests that developmental events occurring after apomorphic initiation can compensate for, and rescue, meiotic defects.

Whereas in *Tripsacum* the MMC proceeds directly to divide mitotically, in dandelions (*Taraxacum* sp) the MMC enters prophase of meiosis I without

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subsequent chromosome pairing. This attempted first division results in a restitution nucleus enclosing a complete set of chromosomes that undergoes the second meiotic division (diplospory). Hans de Jong (Wageningen University, The Netherlands) presented a detailed genetic analysis of apomixis based on interploidy crosses between sexual diploids and apomictic triploids of Taraxacum. The occurrence of recombinants in which the initiation of apomixis and the autonomous development of embryo and endosperm were uncoupled indicates that apomixis is controlled by at least three loci. A dominant trait linked to a microsatellite marker and located on one of the nucleolar organizer chromosomes appears to control diplospory. Interestingly, this marker is absent in sexual diploid progeny, suggesting that haploid pollen grains cannot transmit this trait. Emidio Albertini (University of Perugia, Italy) confirmed that apospory in *Poa pratensis* can be uncoupled from the mechanisms controlling the parthenogenetic development of embryos. Although parthenogenesis is seemingly contingent on apomeiosis, the reverse is not the case. The variability of the degree of parthenogenesis suggests that it is likely not controlled by a discrete locus and may be under complex control.

Apomixis is reported in more than 400 species belonging to 40 families. However, it is particularly prevalent within the Asteraceae, Poaceae, and Rosaceae. Focusing on the distribution and evolutionary history of apomixis, John Carman (Apomyx, Inc., Logan, UT) emphasized that only 127 of more than 14,000 genera of flowering plants contain apomorphic species, most of which have appeared during or after the Pleistocene. Carman suggests that apomicts may have arisen by wide hybridization of ancestral sexual parents having distinct phenotypic traits related to reproduction (Carman, 2001). To test this hybridization-derived floral asynchrony hypothesis, Carman’s group has documented variation of reproductive traits among sexual relatives of well-known apomicts (Tripsacum and Antennaria), finding that they are heterozygous and polygenic. Many forms of reproductive variation, including apomixis, may have arisen after hybridization of sexual ancestors with divergent reproductive traits. In many agamic complexes which are composed of individuals of varying ploidy levels, diploid genotypes usually are sexual and polyplody genotypes usually are apomictic. Tim Sharbel (Max Planck Institute for Chemical Ecology, Jena, Germany) studied the evolution of apomixis and polyplody in the *Arabis holboellii* agamic complex. Using chloroplast haplotypes identified in diploid, aneuploid, and triploid individuals, he concluded that polyplody arose repeatedly and independently within this complex (Sharbel and Mitchell-Olids, 2001). The variation in reproductive mode and population structure suggests that apomixis might have a single evolutionary origin, followed by multiple instances of phenotypic expression of this trait.

BREEDING APOMIXIS INTO SEXUAL CROPS

Among the grasses, apomixis occurs in several economically important forage genera (e.g., *Pennisetum*, *Brachiaria*, *Paspalum*, and *Poa*). At least three groups have attempted the introgression of apomixis into sexual crops via wide hybridization using backcrossing (BC) strategies combined with embryological or cytogenetic studies. For close to 20 years, Wayne Hanna (United States Department of Agriculture–Agricultural Research Service, Tifton, GA) and his colleagues have attempted the transfer of apomixis from *Pennisetum squamulatum* to pearl millet (*P. glaucum*) (Hanna et al., 1998). By screening large populations to identify partially male fertile apomictic plants, backcrossing has progressed to the BC7 generation. Apomictic tetraploid BC 7 plants have 28 or 29 chromosomes and closely resemble pearl millet. However, they form very little viable seed, a problem possibly related to the dosage sensitivity of endosperm development (Morgan et al., 1998). Seed sterility may be overcome by exploiting the genetic diversity found within the tetraploid germplasm pool.

Following the pioneering work of D.F. Petrov, who realized the potential of introgressing apomixis into maize by wide hybridization more than 50 years ago, Victor Sokolov’s group (Institute of Cytology and Genetics, Novosibirsk, Russia) attempted to transfer apomixis from *T. dactyloides* to maize using tetraploid female parents. F1 hybrids having 20 chromosomes from maize and 36 from *Tripsacum* were apomictic but male sterile. Recurrent backcrossing to male diploid or tetraploid maize resulted in apomictic genotypes invariably containing the same nine *Tripsacum* chromosomes. The absence of any additional *Tripsacum* chromosomes resulted in the loss of apomixis, suggesting polygenic control. Moreover, apomeiosis and parthenogenesis segregated and are controlled by different loci (Sokolov and Khatypova, 2001). Inspired by the work conducted in Russia, Yves Savidan launched an initiative to transfer apomixis from *Tripsacum* to maize in the early 1990s. Large population screening, flow cytometry, and genomic in situ hybridization were used to obtain BC3 plants that have 20 chromosomes from maize and 18 from *Tripsacum*. Because little sexuality was present in BC3 plants, the acquisition of subsequent BC populations was difficult (Savidan, 2000). A plant with a chromosome responsible for apomixis has yet to be found among the BC4 generation. Olivier Leblanc (IRD-CIMMYT, Mexico) indicated that the maize genome severely alters the expression of apomixis in members of these BC pop-
ulations. Specific attributes necessary for the proper expression of apomixis in maize could be related to gene dosage effects of specific modifiers or to parent-of-origin–dependent expression (i.e., genomic imprinting) of key regulatory genes that control embryo sac development and/or early seed formation.

ISOLATION OF GENES CONTROLLING APOMIXIS

Basic knowledge of the genetic and molecular regulation of female reproductive development in apomictic species remains poor. Although the inheritance of the trait has been studied in several species, efforts to isolate the genes that control apomixis are at an early stage. The development of the genetic and molecular tools necessary to establish an apomictic model system is well under way (Bicknell, 1994, 2001), and the first mutants altered in apomictic developmental pathways have been isolated. Ross Bicknell and his team (Institute for Food and Crop Research, Lincoln, New Zealand) have implemented γ-ray and insertional mutagenesis strategies in Hieracium spp. At least two mutants have been isolated that have lost the ability to form apomictic seed but still reproduce sexually, indicating that apomixis and sexuality can be uncoupled in Hieracium. In both mutants, the differentiation of aposporous initials early during ovule development is affected. Further characterization promises insights into the molecular nature of the genes involved in apomictic initiation.

Peggy Ozias-Akins and co-workers (University of Georgia, Tifton) have mapped apomixis to a single locus in both Pennisetum ciliare and Pennisetum squamulatum (Ozias-Akins et al., 1998; Roche et al., 1999), two species related to pearl millet. Detailed analysis of P. squamulatum revealed no recombination between 12 markers and the apomixis locus. Interestingly, no sequences that cross-hybridize to four of these markers are present in sexual individuals of a segregating population, suggesting that the region is hemizygous or highly divergent in the apomicts. On the basis of marker conservation between the two species, bacterial artificial chromosome clones linked to apomixis were identified, revealing a series of duplications that might explain the absence of meiotic recombination in this chromosomal region. Informative bacterial artificial chromosome clones have been physically mapped using fluorescence in situ hybridization. Fulvio Pupilli’s group (Consiglio Nazionale delle Ricerche, Perugia, Italy) has mapped the apomixis locus in Paspalum simplex using selected rice probes as restriction fragment length polymorphism anchor markers and amplified fragment length polymorphism (AFLP) markers. They confirmed that apomixis segregates as a single dominant trait and identified five rice markers tightly linked to apomixis (Pupilli et al., 2001). As in Pennisetum, the genomic region linked to apomixis shows no signs of meiotic recombination. The authors concluded that the apomixis locus is contained in a chromosomal area that is syntenic to a 15-centimorgan region on rice chromosome 12.

Genes specific to apomictic development may be identified by comparing gene expression in sexual and apomictic ovules among closely related genotypes of the same species (Vielle-Calzada et al., 1996b). Sexual and apomictic genotypes have been characterized in the genus Brachiaria. Julio Rodrigues from Vera Carneiros’s group (Embrapa, Brasilia, Brazil) reported on a differential display strategy to isolate differentially expressed transcripts during specific stages of ovary development in Brachiaria brizantha. Several dozen fragments were specific to either sexuals or apomicts and are being analyzed further. Similarly, Gianni Barcaccia (University of Padova, Italy) is investigating gene expression during flowering of mutants of alfalfa that frequently form unreduced 2n egg cells. A collection of 40 polymorphic cDNA-AFLP clones was isolated by differential display.

In a special lecture on “Approaches to Capturing Wild Apomictic Genes Combining Genetics and Genomics,” Michael Freeling (University of California, Berkeley) presented a strategy to identify regulatory regions. Nick Kaplinski, a graduate student with Freeling, developed a sliding window–type algorithm that identifies regions of conserved noncoding sequences both within and between species. Such an inverse genetics approach can be used to identify regions that are under functional selective pressure.

OVULE AND FEMALE GAMETOPHYTE DEVELOPMENT

The initiation of apomictic development occurs at early stages of ovule development, during the differentiation of meiotic or apomeiotic products. What leads to the meiotic or apomeiotic commitment of cells within the ovule? Do megaspores sense their position and communicate with other sporophytic cells? It is becoming apparent that meiosis and female gametophyte development are controlled by regulatory genes that may be deregulated in space and time in apomicts (Koltunow, 1993; Grossniklaus, 2001). In fact, many events relevant to apomixis, such as the initiation of embryo sac development, autonomous activation of the egg cell, and modified fertilization mechanisms, are functions of the developing female gametophyte. Yet, we know little about the genetic control of these events, even in sexual model species. A better understanding of the molecular mechanisms underlying embryo sac development and its interactions with sporophytic cells in the ovule
could enhance our understanding of apomixis dramatically.

Ueli Grossniklaus and his team (University of Zürich, Switzerland) use Arabidopsis as a model system to identify genes involved in megasporogenesis and embryo sac development. Using an enhancer detection strategy (Sundaresan et al., 1995), they identified mutants affected in female gametophyte development and double fertilization. Some of them may be relevant to the developmental alterations that ensure the normal endosperm formation observed in many apomicts. For example, a new signaling pathway between male and female gametophytes was identified by the feronia mutant: a wild-type pollen tube was unable to release its sperm cells into a mutant feronia embryo sac, suggesting an active role of the female gametophyte in this process. Grossniklaus and colleagues also identified genes expressed in specific cell types of the ovule and embryo sac, many of which encode regulatory proteins. Promoters controlling expression in the ovule at the site of apomictic initiation, the MMC, or the egg could be used to induce elements of apomixis by expressing candidate genes in specific cell types.

In Arabidopsis, female meiosis results in three cells that die, whereas a single megasporocyte produces the embryo sac. Wei-Cai Yang (Institute of Molecular Agrobiology, Singapore) used transposon mutagenesis (Sundaresan et al., 1995) to investigate cell fate determination among the megasporocytes. He identified a tagged mutant with embryo sacs consisting of large multinucleated cells. These cells may be derived from several surviving megaspores, indicating a defect in cell specification, or may result from aberrant cellularization events in the embryo sac. The isolation of regulatory genes implicated in cell fate determination may be related directly to the developmental alternatives associated with apomictic initiation. In that regard, the role of transcription factors implicated in the regulation of ovule development in sexual species is particularly relevant. The work of Rebecca Favaro and colleagues in Lucia Colombos’s group (University of Milan, Italy) demonstrated that the expression of a MADS box gene (AGL11) during ovule and seed formation in Arabidopsis is controlled by an intron that is crucial for determining the temporal and spatial context in which AGL11 acts.

**CELL CYCLE AND MEIOSIS**

Critical aspects of cell cycle regulation and meiosis differ between apomicts and sexuals. Unlike sexually reproducing plants, apomicts do not undergo recombination during meiosis I, and they produce unreduced female gametes. Greater understanding of the (mis)regulation of the cell cycle and meiosis will facilitate the development of systems to induce parthenogenesis and apomeiosis.

To maintain genetic stability and fertility, polyploid crops (e.g., wheat) must both pair and segregate the closely related chromosomes correctly during meiosis. In polyploids, homologous chromosomes must accurately distinguish each other from homeologous chromosomes. Peter Shaw (John Innes Centre, Norwich, UK) described how the Ph1 locus controls the specificity of somatic and meiotic chromosome association in wheat. The Ph1 locus restricts chromosome pairing and recombination to true homologs. Thus, in hexaploid wheat with Ph1 deletions, pairing and recombination can occur between homeologs and homologs. In the absence of Ph1, nonhomologously associated centromeres fail to separate at the beginning of meiosis. Shaw discussed how the Ph1 locus promotes the specificity rather than the induction of centromere association (Martinez-Perez et al., 2001).

Screens for male sterility in Arabidopsis by Hong Ma’s group (Pennsylvania State University, University Park) identified genes regulating chromosome segregation during male meiosis. Two male sterile mutants (ask1 and sds) were shown to have abnormal chromosome segregation during meiosis I. Premature homolog dissociation occurs in the sds mutant near the end of prophase I, whereas the homologs seem to remain attached at anaphase I in the ask1 mutant. The ASK1 gene is most similar to yeast SKP1, which is a subunit of the Skp1-Cullin-1-F-box (SCF) ubiquitin ligase complex. Although the yeast and human SKP1 genes regulate the mitotic cell cycle, it was not known until recently that these proteins also could be required for meiosis (Yang et al., 1999). Molecular determination of the developmental mechanisms underlying male and female meiosis is under way by Tom Gerats’ team (University of Gent, Belgium), who is undertaking cDNA-AFLP transcript profiling in Petunia. Transcript profiling techniques were applied to male meiosis, focusing on the identification of genes involved in synopsis and recombination. Reproducible cDNA-AFLP patterns were obtained using RNA from only three developing anthers. In a pilot study, ~100 (5%) differentially expressed transcripts were identified, including previously characterized meiotic genes (e.g., DMC1-like protein).

Fifty years ago, Böcher reported unreduced pollen development in the apomict A. holboellii (Böcher, 1951). Alexei Kravtchenko (Russian Academy of Sciences, Novosibirsk, Russia) conducted a cytological reexamination of unreduced pollen formation in diploid and triploid A. holboellii accessions. The first meiotic division was equal, and omission of the second division produced unreduced microspores. For both triploid and diploid plants, pollen viability was highly variable. Yet, some triploids exhibited greater than 90% pollen fertility, an advantage for apomixis research on A. holboellii.

Although many meiotic mutants have been described in plants, only six genes
involved in meiosis have been cloned. Christine Horlow (Institut National de la Recherche Agronomique, Versailles, France) showed that the Arabidopsis SWITCH1 protein is required for both sister chromatid cohesion and bivalent formation (Mercier et al., 2001). Horlow has identified a second swi1 allele (swi1-2) that affects both male and female meiosis, whereas swi1-1 was reported to be female specific (Montamayor et al., 2000). The cytological behavior of swi1-2 during metaphase in male meiocytes is intriguing: instead of the normal 5 bivalents, 20 chromatids are observed that segregate aberrantly.

Cell cycle control is a complex process that is mediated largely by protein kinases, which activate proteins with specific functions during the cell cycle. Danny Geelen (University of Gent, Belgium) reviewed current research on cell cycle progression that is under way in Dirk Inzé’s laboratory (Joubes et al., 2000; Stals et al., 2000; de Veylder et al., 2001). Transgenic studies in which the function of key cell cycle regulators is disrupted demonstrated that the cell cycle is integrated with plant development. The Inzé group has conducted an AFLP-based screening for cell cycle genes in tobacco BY-2 cells using an aphidicoline blocker. Approximately 500 cell cycle–modulated expressed sequence tags (ESTs) have been identified, and 50% of the clones isolated exhibited no significant homology with known proteins. Jim Murray (University of Cambridge, UK) focused on the roles of D-type cyclins (cycD) in modulating cell division rates that affect growth and development (Meijer and Murray, 2000). The CycD genes play an important role in deciding whether a cell enters a division cycle. Cytokinin activates cell division through the induction of CycD3 at the G1–S cell cycle transition (Riou-Khamlichi et al., 1999). Overexpression of CycD2 reduces the length of the G1 phase, causing faster cell cycling and accelerated plant development (Cockcroft et al., 2000). The constitutive overexpression of the CycD2 cyclin in the shootmeristemless Arabidopsis mutant led to a restoration of vegetative growth and long-lived plants.

**FERTILIZATION AND PARTHENOGENESIS**

Parthenogenesis is a critical element of apomixis whereby an (unreduced) egg cell initiates embryogenesis without fertilization. The molecular mechanisms that trigger parthenogenesis in apomictic plants remain largely unknown. Helmut Bäumlein (Institut für Pflanzenzogenetik und Kulturpflanzenforschung, Gatersleben, Germany) discussed embryological and molecular studies on apomeiosis in Poa pratensis and parthenogenesis in wheat that his group conducted in close cooperation with Fritz Matzk. Genetic and ploidy analysis (Matzk et al., 2000) of crosses between oblige sexual and apomictic Poa lines demonstrated that apomixis was dominant and that apomeiosis and parthenogenesis are not linked. A molecular approach based on SMART subtractive suppression hybridization identified cDNAs from (apo)meiotic stages specific to obligate sexual and apomictic lines. The “Salmon system” of wheat that his group performed in cooperation with Fritz Matzk, genetic and ploidy analysis (Matzk et al., 2000) of crosses between obligate sexual and apomictic Poa lines demonstrated that apomixis was dominant and that apomeiosis and parthenogenesis are not linked. A molecular approach based on SMART subtractive suppression hybridization identified cDNAs from (apo)meiotic stages specific to obligate sexual and apomictic lines. The “Salmon system” of wheat that his group performed in cooperation with Fritz Matzk, genetic and ploidy analysis (Matzk et al., 2000) of crosses between oblige sexual and apomictic Poa lines demonstrated that apomixis was dominant and that apomeiosis and parthenogenesis are not linked. A molecular approach based on SMART subtractive suppression hybridization identified cDNAs from (apo)meiotic stages specific to obligate sexual and apomictic lines.

In apomicts, embryogenesis occurs completely without the contribution of the paternal genome. Thomas Dresselhaus (University of Hamburg, Germany) presented data suggesting that maize differs from Arabidopsis with regard to the time of activation of the paternal genome during early embryogenesis (Vielle-Calzada et al., 2000). In maize, the switch from maternal to zygotic control of gene expression appears to occur 18 to 24 hr after fertilization. Using reproductive cells isolated in vitro, Dresselhaus and coworkers developed a procedure that allows for the isolation of mRNAs present in individual cell types of the female gametophyte or the zygote. This approach led to the identification of differentially expressed genes (Cordts et al., 2001) and is being implemented in Tripsacum, allowing comparative studies of gene expression between sexual and apomictic cells.

Jean-Emmanuel Faure (Ecole Normale Superieure, Lyon, France) presented a cytological study of Arabidopsis fertilization using confocal laser scanning microscopy (Christensen et al., 1997). Characterization of the time course of fertilization indicates that (1) synergids degenerate at ~7 hr after pollination (HAP), (2) there is a rapid change in egg cell polarity, (3) karyogamy occurs 8 to 9 HAP, and (4) the first division of the primary endosperm nucleus occurs 9 to 12 HAP. Faure’s group identified mutants that affect fertilization among 4000 γ-ray-mutagenized M1 plants. They confirmed phenotypes for a collection of 200 families containing putative early fertilization mutants. Maura Cardarelli (University La Sapienza, Rome, Italy) discussed the effects of the Agrobacterium rhizogenes rolB gene on gamete development and embryogenesis. rolB expression driven by AtDMC1 caused sterility that was likely attributable to a delay in anther dehiscence, whereas rolB expression under the control of the FBP7 promoter caused floral defects and a slight delay in anther dehiscence.

**EMBRYOGENESIS**

Despite the existence of large collections of mutants that affect plant embryogenesis, the molecular basis underlying the developmental steps leading to egg cell activation and early
embryo development remains poorly understood. Bob Goldberg (The Seed Institute and University of California, Los Angeles) discussed a genomics approach toward the understanding of plant embryogenesis. The giant embryos of scarlet runner bean allowed the microdissection and isolation of cDNAs from either the embryo proper or the suspensor. EST sequencing was used to identify differentially expressed genes in the embryo proper (2863 ESTs, 50% unique) and the suspensor (3138 ESTs, 59% unique). Approximately 10 to 12% of the ESTs exhibited no homology with genes currently in databases. On the basis of these expression data, the suspensor appears to be the major source of gibberellic acid in the developing embryo. The Seed Institute also uses Affymetrix chips for transcript profiling in Arabidopsis wild-type and mutant seed. The profiles show highly complex changes during the early steps of seed development.

Sacco de Vries (Wageningen University, The Netherlands) discussed the role of the Arabidopsis somatic embryogenesis receptor–like kinase (AtSERK1) in ovule and embryo development. SERK encodes a leucine-rich repeat transmembrane receptor kinase (Schmidt et al., 1997) that is similar to animal receptors. The use of fluorescence spectral imaging microscopy to study physical interactions in plant cells showed that AtSERK1 can oligomerize in vivo (Shah et al., 2001) and interact directly with the kinase-associated protein phosphatase (KAPP) phosphatase. Plants overexpressing AtSERK1 have an increased potential for somatic embryogenesis in culture. To investigate the potential to induce embryogenesis in plants, the de Vries team developed a multiplex marker system for testing AtSERK1–overexpressing F2 plants. Fixed heterozygosity is indicative of apomixis. Indeed, lines expressing AtSERK1 in the ovule produced progeny without a recombination event among 14 markers tested, whereas control crosses did not yield such progeny among more than 1000 F2 plants. This could be attributable to apomictic embryo initiation or suppressed recombination in these plants. Ed Schmidt (Genetwister Technologies, Wageningen, The Netherlands) focused on the family of transmembrane Receptor Kinases–like SERK (RKS) genes. There are 16 RKS members in Arabidopsis, which can be grouped into three classes. Genetwister Technologies characterizes the developmental function of the RKS family using transgenic plants, which either overexpress or cosuppress the different RKS genes.

Kim Boutillier’s presentation (Plant Research International, Wageningen, The Netherlands) focused on the engineering of adventitious embryony. Using subtractive hybridization, Boutillier isolated an embryo-expressed gene called BABY BOOM (BBM) from microspore embryo cultures of Brassica napus. BBM encodes an AP2 domain transcription factor. When expressed constitutively, it induces the spontaneous formation of somatic embryos in young seedlings. Because the BBM overexpression phenotype is restricted to seedlings, Boutillier uses tissue-specific promoters with the aim of inducing somatic embryos in ovules. Although L1 expression of BBM extends the tissue range and penetrance of somatic embryo production, it is still restricted to seedlings. BBM-expressing lines show a cytokinin overproduction phenotype, and mutants such as amp1 (Chin-Atkins et al., 1996) and other backgrounds affecting cytokinin levels enhance the BBM somatic embryo phenotype.

Gabriella Consonni (Università degli Studi di Milano, Italy) presented a characterization of the maize mutant fused leaves (fdl). The fdl mutant causes organ fusion and affects both embryo organization and seedling growth: epidermal cells of the coleoptile and first leaf, and the first and second leaves are joined by a single cell wall.

ENDOSPERM DEVELOPMENT

For agricultural applications, it is essential that endosperm development in engineered apomicts is normal. Most apomictic seed formation requires fertilization of the central cell (pseudogamy). This poses a problem for the transfer of apomixis to sexual crops because maize and likely most cereals require a ratio of maternal to paternal genomes of 2m:1p for normal endosperm development (Lin, 1984). Fertilization of an unreduced central cell with a reduced sperm will yield a 4m:1p ratio, which is expected to cause seed abortion. In contrast, natural apomicts are either insensitive to unbalanced endosperm or circumvent the problem by altering embryo sac development or the mechanism of fertilization (Grossniklaus et al., 2001).

Abed Chaudhury (Commonwealth Scientific and Industrial Research Organization, Canberra, Australia) provided an overview of ongoing research on the fis (fertilization-independent seed) class of Arabidopsis mutants. The FIS class includes MEDEA (MEA), FIS2, and FERTILIZATION-INDEPENDENT EN- DOSPERM (FIE) (Grossniklaus et al., 1998b; Luo et al., 1999; Ohad et al., 1999). Mutations in these genes lead to maternal effect seed abortion, and mutant central cells are capable of endosperm development in the absence of fertilization, a component of apomixis (reviewed in Grossniklaus et al., 2001). Chaudhury’s group made promoter::GUS fusions for all three FIS class genes, showing that the expression of MEA and FIS2 is similar but differs significantly from the FIE pattern (Luo et al., 2000). Only maternally inherited copies are expressed early in seed development, suggesting regulation by genomic imprinting. Seed abortion can be prevented if a demethylated paternal genome is introduced. This effect is independent of FIS gene activity (Luo et al., 2000). Interestingly, a demethylated
paternal genome appears to have an effect on gene expression from the maternal genome.

Robert Fischer (The Seed Institute and University of California, Berkeley) focused on the suppression of endosperm development by the FIS genes. FIE and MEA encode WD40 and suvar3-9, enhancer-of-zeste, Trithorax (SET) domain proteins of the Polycomb group. FIE and MEA proteins interact directly, like their mammalian and Drosophila homologs, which form a multiprotein complex (Luo et al., 2000; Spillane et al., 2000; Yadegari et al., 2000). Fischer reviewed data showing silencing of the paternal mea locus in the endosperm (Kinoshita et al., 1999; Vielle-Calzada et al., 1999). He discussed how these findings fit with the parental conflict theory for the evolution of genomic imprinting (Haig and Westoby, 1991) and with the observation that many apomicts require fertilization of the central cell for endosperm formation.

Problems associated with the engineering of autonomous endosperm in apomicts also were reviewed by Rod Scott (University of Bath, UK). Endosperm abortion may be caused by the nonequivalence of paternal and maternal genomes at imprinted loci. Autonomous endosperm development observed in the Arabidopsis fie mutant can be enhanced by combining it with hypomethylation (Vinkenoog et al., 2000). On the basis of the parental conflict theory (Haig and Westoby, 1991), Scott proposes that endosperm size in plants is an assay for gametic “gender” as exemplified by interploidy crosses (Scott et al., 1998). In Arabidopsis, modifications of gametic gender may be achieved through changes in the methylation profile, which either “maternalize” or “paternalize” the gametic genome, or as a result of mutants such as fie, which paternalize maternal gametes (Adams et al., 2000; Vinkenoog et al., 2000).

Fred Berger (Institut National de la Recherche Agronomique, Lyon, France) discussed the recent work of Boisnard-Lorig et al. (2001), in which a HistonE::YFP fusion protein was used to demonstrate that syncytial endosperm is divided into three distinct mitotic domains. Enhancer detection screens (Haseloff, 1999) were used to isolate GFP-based endosperm markers, such as KS117, which is expressed initially in the entire endosperm but then is restricted to the chalazal region. Using this marker, it was found that in fis class mutants endosperm polarization is disturbed (Sorensen et al., 2001). The KS117 line has been used in a γ-ray mutagenesis screen for mutants that alter the expression of KS117. Among 4000 M1 plants, 60 lines with disturbed KS117 GFP expression were identified.

José Gutierrez (University of Oxford, UK) presented the isolation of candidate imprinted genes that are expressed differentially in maize endosperm depending on parental origin. Allelic display polymerase chain reaction was used to screen for imprinted maize genes in reciprocal crosses between different inbred lines of maize. Loci that exhibit either maternal-specific or paternal-specific expression were identified at early endosperm stages (10 days after pollination; 15 candidates) and at later endosperm stages (30 days after pollination; 31 candidates). The group of Angelo Viotti (Consiglio Nazionale delle Ricercche, Milan, Italy) investigates epigenetic phenomena in maize endosperm. Some alleles of the α-z-ein and α-tubulin genes derived from specific inbred lines are subject to genomic imprinting (Lund et al., 1995a, 1995b). Two of the six α-tubulin genes displayed a correlation between DNA demethylation at the locus and increased RNA accumulation in the endosperm. α-z-ein and α-tubulin genes are hypomethylated only when transmitted through the female. Viotti’s results confirm that the imprinting status of certain z-ein and tubulin alleles in maize endosperm can be influenced by interactions of parental factors and are cross and genotype dependent (Ciceri et al., 2000).

Hilde-Gunn Opsahl-Ferstad (Agricultural University of Norway, Aas) focused on the defective kernel1 (dek1) and crinkly4 (cr4) genes that regulate cell identity in the cereal endosperm (Becraft et al., 2001; Olsen, 2001). The Olsen group is studying several of the Pioneer Hi-Bred “Trait Utility System for Maize” Mutator-induced mutants to isolate genes involved in the control of aleurone cell identity. Promoter studies of the LIPID TRANSFER PROTEIN1 (LTP1) and LTP2 upstream sequences have shown LTP2 to direct expression in cereals similar to the patterns described in dicots, whereas the LTP1 directs a different expression pattern.

ECONOMIC AND ECOLOGICAL IMPLICATIONS OF APOMIXIS

The long gestation period for the development of apomixis technology (Savidan, 2000) has allowed reflection on both economic (Jefferson, 1994; Bicknell and Bicknell, 1999) and ecological issues (van Dijk and van Damme, 2000) regarding the deployment of apomictic crops in agriculture. Apomictic varieties are being developed for a few (mainly forage grass) species. Jorge Gonzalez (Universidad Autónoma Agraria Antonio Narro, Saltillo, Mexico) presented such a breeding program for new disease-tolerant buffelgrass (Penn. ciliare) cultivars. Contiguous planting of a single cultivar of this obligate apomict over large geographic areas in the United States and Mexico since 1949 led to genetic vulnerability to blight disease. To develop new disease-resistant cultivars, Gonzalez and colleagues crossed sexual lines with the apomictic clone Zaragoza-115. Progeny testing and multilocation evaluation trials over several years led to the release of the hybrid AN-17-PS for commercial seed production last year.
Given current controversies regarding transgenic crop plants in agriculture, Peter van Dijk (Netherlands Institute of Ecology, Heteren) suggested that biosafety issues, which will undoubtedly arise (Ellstrand, 2001), should be considered well in advance of future apomixis technology deployment. He looked at the deployment of apomixis technology from an evolutionary and population genetics viewpoint (van Dijk and van Damme, 2000). Three hypothetical problems were identified with apomictic crops: (1) invasive weeds, (2) novel weeds, and (3) infectious apomixis, which might reduce genetic diversity by freezing the gene pool. Overall, van Dijk concluded that if pollen production is necessary in engineered apomictic crops, pollen flow of a dominant apomixis transgene should be prevented. The use of inducible or conditional systems to control apomixis could be a means to prevent such gene flow.

In recent years, there has been an increased industrial interest in the development of apomixis technology. Marc Albertsen (Pioneer Hi-Bred, IA) provided a well-balanced perspective on the factors that will determine how apomixis is deployed in commercial or subsistence farming. Albertsen raised the question of who will have access to proprietary apomixis technology (and the necessary supporting technologies) and under what terms. Both intellectual property management and freedom-to-operate issues will have a major bearing on which beneficiaries will have access to apomixis technology. From a strictly commercial perspective, approaches are needed that allow financial returns on technology investment and to ensure that “donated” technology is not used competitively against future apomixis technology deployment. He looked at the deployment of apomixis technology from an evolutionary and population genetics viewpoint (van Dijk and van Damme, 2000). Three hypothetical problems were identified with apomictic crops: (1) invasive weeds, (2) novel weeds, and (3) infectious apomixis, which might reduce genetic diversity by freezing the gene pool. Overall, van Dijk concluded that if pollen production is necessary in engineered apomictic crops, pollen flow of a dominant apomixis transgene should be prevented. The use of inducible or conditional systems to control apomixis could be a means to prevent such gene flow.

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Richard Jefferson (Center for the Application of Molecular Biology to International Agriculture, Canberra, Australia) continued the theme of how apomixis technology will be applied in agriculture by focusing on the challenge to deliver apomixis technology as a public good. This issue arose at a meeting funded by the Rockefeller Foundation in 1998 when the Bellagio Declaration was issued by many of the leading apomixis researchers (http://billie.btny.purdue.edu/apomixis). Jefferson focused on how current intellectual property management limits the application of enabling technologies such as apomixis to solely commercial objectives. He expressed concern that, unless the research community paid greater attention to the terms of research agreements and promoted non-exclusive licensing, a small number of multinational companies could exercise monopolistic control over apomixis technology worldwide. The current exclusionary intellectual property management of both the private and public sector are unsustainable business strategies. Many publicly funded bodies are adopting exclusive licensing models that are more suited to commercial than to public good objectives. Access to proprietary enabling technologies could be achieved by a consortium approach (enabling technology cooperative) that would develop proprietary technologies but adopt a broad nonexclusive licensing policy, allowing it to act as a clearinghouse for innovative proprietary technologies (G. Graaf, A. Bennett, B. Wright, and D. Zilberman, unpublished data; see http://www.cnr.berkeley.edu/csr/technology/ipcmech/).

CONCLUSION

Research presented at the 2nd International Apomixis Conference coalesced around several themes, some of which are new and some of which have a long but unfinished history in apomixis research. A wide range of topics—the importance of existing apomictic mechanisms and their evolutionary implications, the isolation of genes controlling apomixis, the molecular and genetic basis of ovule and female gametophyte development, and the economic and ecological implications of apomixis—were discussed during plenary talks. It is increasingly apparent that sexuality and apomixis are interrelated and that they need to be investigated simultaneously to obtain a complete understanding of plant reproduction at the developmental, ecological, and evolutionary levels. Indeed, a number of changes were evident in the content and focus of apomixis research since the 1st International Apomixis Meeting. There is now a greater acceptance that genetic and molecular approaches to study sexuality can yield insights into the regulation and components of apomixis. This was particularly evident in the number of new groups entering this field. In addition to the impact of sexual model species such as Arabidopsis and maize, it is evident that a number of apomicts (Hieracium, Taraxacum, Tripsacum, Paspalum, and A. holboellii) are emerging as models that are amenable to molecular and genetic analyses. A number of new techniques (e.g., flow cytometry, transcript profiling, differential screening methods, and enhancer detection) will increase our knowledge of apomixis in the coming years and bring us a step closer to the engineering of apomixis in sexual crops.

Charles Spillane
University of Zürich,
CH-8008 Zürich, Switzerland
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Kinoshita, T., Yadegari, R., Harada, J.J.,
MEETING REPORT


Apomixis is the natural ability of more than 400 plant species to reproduce asexually through seed (Nogler, 1984a). Sexual embryos result from the union of male and female gametes, which produces genetically varied offspring. In contrast, apomictic embryos are formed without paternal contribution. Therefore, apomictic offspring carry the full genetic constitution of the mother and form a stable clone, a feature of great value for plant breeding and seed production.

For many years, apomixis was studied only by a small group of interested botanists (Nogler, 1984a; Asker and Jerling, 1992) and visionary plant breeders (Petrov et al., 1979; Hanna and Bashaw, 1987; Savidan, 1992). However, because of its tremendous potential for agriculture, apomixis research has attracted much more attention during the last few years (Koltunow et al., 1995; Vielle-Calzada et al., 1996; Grossniklaus et al., 1998). If apomixis could be introduced into sexual crops, it would greatly simplify breeding schemes and allow the fixation of any genotype (however complex), including that of F1 hybrids. Apomixis technology could play a major role in feeding the growing population of our planet (Jefferson, 1994; Thoenissen, 2001) provided that it will be freely accessible to all users, especially resource-poor farmers in developing countries, requiring innovative approaches for technology generation, patenting, and licensing (http://billie.btny.purdue.edu/apomixis).

Current apomixis research focuses on elucidating the genetic basis and molecular mechanisms that control apomictic reproduction (see accompanying Meeting Report). Two major complementary approaches are being pursued: (1) to identify genes controlling individual elements of apomixis in well-defined sexual model species (reviewed in Grossniklaus, 2001), and (2) to unravel the genetic control of apomixis in natural apomicts (reviewed in Savidan, 2000). For nearly two decades, the genetic control of apomixis had been elucidated in very few species. Recently, however, inheritance studies for several natural apomicts have been published that shed new light on the genetic control of this important developmental process (van Dijk et al., 1999; Bicknell et al., 2000; Matzke et al., 2000; Noyes and Rieseberg, 2000; Pupilli et al., 2001; Quarin et al., 2001).
DEVELOPMENTAL ASPECTS
OF APOMIXIS

Apomixis occurs in many species from more than 40 plant families and is thought to have evolved multiple times from sexual ancestors. Therefore, it is likely that the controls of sexual and apomictic reproduction are closely interrelated. Sexual reproduction and apomixis are not mutually exclusive, and both processes can occur in parallel, as it is typical of facultative apomicts, which produce a mixture of apomictic and sexual progeny. Although the mechanisms leading to apomictic reproduction are diverse (Koltunow, 1993; Crane, 2001), they share the common feature that ancestral sexual processes are deregulated in space and time (Grossniklaus, 2001). Two distinct types of apomixis have been described (Gustafsson, 1947a, 1947b): (1) sporophytic apomixis (adventitious embryony), in which an embryo forms directly from an unreduced sporophytic cell, and (2) gametophytic apomixis, which involves the formation of an unreduced embryo sac (female gametophyte). Although no less interesting, the genetics of sporophytic apomixis has not been investigated in great detail, which is why we focus on gametophytic apomixis in this article.

Sexual reproduction involves the generation and fusion of reduced gametes (Figure 1). Female gametogenesis and double fertilization occur within the ovule, a specialized reproductive organ (reviewed in Drews et al., 1998; Grossniklaus and Schnetzl, 1998; Yang and Sundaesran, 2000). Usually a single cell within the ovule, the megaspore mother cell (MMC) becomes committed to the sexual pathway, undergoes meiosis, and forms a tetrad of four reduced spores. Only one of these will divide and ultimately form the mature embryo sac containing the female gametes. Double fertilization involves two pairs of gametic cells: the egg cell fuses with one sperm to form the embryo and give rise to the next generation, and the central cell fuses with a second sperm to form the endosperm, a nutritive tissue important for seed development and/or germination.

During gametophytic apomixis, several of these developmental steps are bypassed or altered (Figure 1; Koltunow, 1993; Vielle-Calzada et al., 1996). (1) Chromosome reduction is circumvented (apomeiosis) such that unreduced cells initiate embryo sac development. These unreduced cells can originate from an aberrant or missing meiosis of the MMC (diplospory). Alternately, the unreduced embryo sac forms directly from a cell within the ovule other than the MMC (apospory) and the sexual products degenerate. (2) The unreduced egg cell initiates embryogenesis in the absence of fertilization (parthenogenesis). (3) The central cell either develops autonomously or, in the majority of apomicts, requires fertilization to initiate development (pseudogamy). The development of normal endosperm in apomicts is important for seed viability and often requires special adaptations of embryo sac development or fertilization (reviewed in Grossniklaus et al., 2001). Recent studies suggest that some of these developmental steps are under independent genetic control, at least in some apomicts (van Dijk et al., 1999; Matz et al., 2000; Noyes and Rieseberg, 2000).

THE INHERITANCE OF APOMIXIS

Genetic studies largely depend on crosses and recombination events neither of which is easily obtained in apomicts. However, because most apomicts produce normal, reduced pollen, the inheritance of apomixis can be investigated by analyzing the segregation ratios in crosses with related sexuals. Such analyses are difficult because gametophytic apomicts are allmost without exception polyploids, causing complex modes of inheritance. An assessment of the breeding system in the hybrids requires cytological observations or, at least, time-consuming progeny tests. Variation in the expressivity of apomixis may create an additional complication. Moreover, much of the earlier work was done on the Rosaceae, which are extremely difficult to analyze because the multiple MMCs formed in sexuals make the distinction between reduced and unreduced embryo sacs difficult. As a consequence, for many years the genetics of apomixis seemed unclear, complex, and idiosyncratic. However, since the end of the 1970s, a clear general pattern in the inheritance of various types of gametophytic apomixis has emerged, first in the Ranunculaceae and Poaceae and later in the Compositae.

Using more suitable apomictic species and focusing on one element of apomixis, apomeiosis, pioneer studies by Nogler in the buttercup species Ranunculus auricomus and by Savidan in the grass Panicum maximum indicated that apospory in these two species segregated as a single dominant mendelian factor (Savidan, 1982; Nogler, 1984b, and references therein). Subsequent investigations showed that both apospory and diplospory in other species also fitted this segregation model (Table 1). Although a dominant mendelian factor can represent any genetic constitution from a single gene to an entire chromosome (e.g., mammalian sex determination), these observations often were taken as evidence for monogenic inheritance. According to this model, apomictic plants possess the simplex genotype Aaaa, carrying in addition to the dominant apomeiosis allele A several recessive alleles for sexual reproduction. Apomictic plants thus carry the potential for sexual reproduction, but in a more or less repressed state, because of the presence of the dominant apomixis factor. Limited penetrance of the apomixis factor...
explains the occurrence of facultative apomixis. The presence of recessive sexual alleles explains how a cross between two facultative apomicts can generate abundant purely sexual offspring. Although the occurrence of apomixis fits this model, the degree of apomixis often is dependent on environmental conditions (Nogler, 1984a) and/or on modifier genes (Bicknell et al., 2000). These as yet unspecified factors need further investigation in the future. Moreover, it remains to be determined whether this general model also applies to the many apomicts in the Rosaceae.

**THE APOMEIOSIS LOCUS IS LOCATED IN A RECOMBINATIONALLY SUPPRESSED REGION**

The segregation model described above has been supported and refined by the isolation of molecular markers that are linked to the presumed apomixis loci in several species (Table 1). In all cases in which it has been critically tested to date, a strong suppression of recombination around the apomeiosis locus has been found. For instance, strict cosegregation with apomeiosis of many more molecular markers than expected was found in aposporous *Pennisetum squamulatum* (Ozias-Akins et al., 1998) and diplosporous *E. annuus* (Noyes and Rieseberg, 2000). In *B. decumbens* (Pessino et al., 1998), *T. dactyloides* (Grimanelli et al., 1998a), and
INSIGHT

Paspalum simplex (Pupilli et al., 2001), comparative mapping with maize or rice markers showed a lack of recombination in the region associated with the apospory locus. Markers that were spread over a region ranging from 15 to 40 centimorgans in the sexual relatives strictly cosegregated in these apomicts. Repression of recombination could frustrate map-based cloning efforts because closely linked markers may be at great physical distances from the apomixis loci.

Because suppressed recombination occurs in both dicot and monocot species, it may be a general characteristic of apomixis loci. This could be related to their function as observed in other complex loci containing several genes involved in a common process (coadapted gene complexes), such as the heterostyly supergene in Primula (Ernst, 1936), the self-incompatibility (S) loci in Brassica (Lewis, 1962; Awadalla and Charlesworth, 1999), the mating-type locus in Chlamydomonas (Ferris and Goodenough, 1994), and the major histocompatibility locus in humans (O’HUigin et al., 2000).

Alternately, it could be an evolutionary by-product of long term asexual reproduction (Judson and Normark, 1996; Welch and Meselson, 2000). In Pennisetum species, markers that are linked to apospory in the apomicts could not be detected by hybridization in sexual relatives (Ozias-Akins et al., 1998; Roche et al., 1999), indicating that the apomicts were either hemizygous for the apomixis locus (A−−) or that the alleles were highly divergent (A a’ a’ a’), as was observed for the Brassica S locus (Boyes et al., 1997; Suzuki et al., 1999).

**ONE MASTER APOMIXIS GENE OR SEVERAL INDEPENDENT APOMIXIS GENES?**

Apomictic development deviates from the sexual pathway in apomeiosis, parthenogenesis, and often endosperm development (autonomy, altered embryo sac development, or altered fertilization). Are these elements of apomixis all controlled by a single gene or by several genes? In the pioneering studies on *R. auricomus* and *P. maximum*, parthenogenesis was strictly associated with apospory. Hence, apomixis as a whole was inherited as a single mendelian trait (Savidan, 1982; Nogler, 1984b). Similarly, in *Hieracium piloselloides*, all three elements are inherited as a single genetic trait (Bicknell et al., 2000). In these species, apomixis could be regulated by a single master regulatory gene controlling all elements or by a gene complex of several tightly linked genes that are recombinationally locked. In other species, however, crosses between sexuals and apomicts have yielded progeny combining elements of both the sexual and the apomictic developmental pathways. In *Taraxacum officinale*, hybrids were recovered that displayed diplospory and autonomous endosperm development but that lacked parthenogenesis (van Dijk et al., 1999). Such “apomixis recombinants” also have been reported in *Poa pratensis*.

![Table 1. Inheritance of Elements of Gametophytic Apomixis (Apomeiosis and Parthenogenesis) in Members of the Ranunculaceae, Poaceae, and Compositae](image)

<table>
<thead>
<tr>
<th>Species</th>
<th>Apomeiosis Type</th>
<th>Family</th>
<th>Inferred Genotype</th>
<th>Most Closely Linked Molecular Marker</th>
<th>Evidence for Suppression Recombination</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ranunculus auricomus</em></td>
<td>Apospory</td>
<td>Ranunculaceae</td>
<td>Aaaa</td>
<td>–</td>
<td>–</td>
<td>Nogler, 1984b</td>
</tr>
<tr>
<td><em>Panicum maximum</em></td>
<td>Apospory</td>
<td>Poaceae</td>
<td>Aaaa</td>
<td>–</td>
<td>–</td>
<td>Savidan, 1982</td>
</tr>
<tr>
<td><em>Pennisetum squamulatum</em></td>
<td>Apospory</td>
<td>Poaceae</td>
<td>Aaaa</td>
<td>0 cM</td>
<td>Yes</td>
<td>Ozias-Akins et al., 1998</td>
</tr>
<tr>
<td><em>Brachytrium decumbens</em></td>
<td>Apospory</td>
<td>Poaceae</td>
<td>Aaa</td>
<td>1.2 cM</td>
<td>?</td>
<td>Pessino et al., 1998</td>
</tr>
<tr>
<td><em>Paspalum simplex</em></td>
<td>Apospory</td>
<td>Poaceae</td>
<td>Aaa</td>
<td>0 cM</td>
<td>Yes</td>
<td>Pupilli et al., 2001</td>
</tr>
<tr>
<td><em>Hieracium piloselloides</em></td>
<td>Apospory</td>
<td>Compositae</td>
<td>Aaa</td>
<td>–</td>
<td>–</td>
<td>Bicknell et al., 2000</td>
</tr>
<tr>
<td><em>Hieracium aurantiacum</em></td>
<td>Apospory</td>
<td>Compositae</td>
<td>Aaa</td>
<td>–</td>
<td>–</td>
<td>Bicknell et al., 2000</td>
</tr>
<tr>
<td><em>Tripsacum dactyloides</em></td>
<td>Diplospory</td>
<td>Poaceae</td>
<td>Aaa</td>
<td>0 cM</td>
<td>Yes</td>
<td>Grimanelli et al., 1998a, 1998b</td>
</tr>
<tr>
<td><em>Erigeron annuus</em></td>
<td>Diplospory</td>
<td>Compositae</td>
<td>Aaa</td>
<td>0 cM</td>
<td>Yes</td>
<td>Noyes and Rieseberg, 2000</td>
</tr>
<tr>
<td><em>Taraxacum officinale</em></td>
<td>Diplospory</td>
<td>Compositae</td>
<td>Aaa</td>
<td>4.4 cM</td>
<td>?</td>
<td>van Dijk et al., unpublished</td>
</tr>
</tbody>
</table>

Parthenogenesis

<table>
<thead>
<tr>
<th>Species</th>
<th>Apomeiosis Type</th>
<th>Family</th>
<th>Inferred Genotype</th>
<th>Most Closely Linked Molecular Marker</th>
<th>Evidence for Suppression Recombination</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Poa pratensis</em></td>
<td>Apospory</td>
<td>Poaceae</td>
<td>Pppp</td>
<td>6.6 cM</td>
<td>?</td>
<td>Barcaccia et al., 1998</td>
</tr>
<tr>
<td><em>Erigeron annuus</em></td>
<td>Diplospory</td>
<td>Compositae</td>
<td>Ppp</td>
<td>7.3 cM</td>
<td>No</td>
<td>Noyes and Rieseberg, 2000</td>
</tr>
</tbody>
</table>

The genetic models are based on segregation analyses of sexual × apomictic crosses and in most cases supported by cosegregation of closely linked molecular markers. No distinction is made between disomic (Aa/aa) and tetrasomic (Aaaa) inheritance. The distance between the apomixis locus and the most closely linked marker is indicated in centimorgans (cM). (−), not investigated; (?) no conclusive data.
SEGREGATION DISTORTION OF APOMIXIS LOCI

As mentioned above, gametophytic apomicts are usually polyploid, whereas related sexuals are diploid. Is gametophytic apomixis incompatible with diploidy? Again, the pioneering work on *E. annuus* by Nogler appears to have general relevance. Nogler showed that diploid offspring that developed parthenogenetically from reduced diploid egg cells of tetraploid apomicts (dihaploids) or diploids produced through another culture were able to reproduce apomictically (Nogler, 1982). This shows that apomixis and diploidy are not incompatible, a finding that has been confirmed in several other species (Bicknell, 1997; Kojima and Nagato, 1997). However, what matters is the origin of the diploid offspring, because zygotic diploids derived from the fusion of haploid egg cells and haploid sperm never reproduced apomictically in *Ranunculus*. Nogler hypothesized that the apospory (*A*) locus was recessive lethal in the gametes. Consequently, the *A* locus could be transmitted via diploid gametes to generate polyploid apomicts but not via haploid gametes to generate diploid apomicts. It is also possible that mutations closely linked to the *A* locus cause haploid gamete nonfunctionality. The net result is that haploid gametes carrying the *A* locus do not contribute to offspring production, resulting in segregation distortion of the *A* locus.

More recently, additional evidence has been obtained for segregation distortion of apomixis loci in other plant species, such as *Tripsacum dactyloides*, *Pennisetum squamulatum*, and *E. annuus* (Grimanelli et al., 1998b; Ozias-Akins et al., 1998; Noyes and Rieseberg, 2000). Transmission studies of markers linked to apomixis loci in *E. annuus* indicate different causes of nontransmission: the parthenogenesis locus *P* in *E. annuus* was not transmitted because of selection against haploid gametes, as was observed for the *A* locus in *R. auricomus*. The diplospory locus *A*, in contrast, was not transmitted because of meiotic drive. In these triploid apomicts, the nondiplospory chromosomes seem to pair preferentially, leaving the diplospory chromosome as a univalent that always ends up in a diploid pollen grain (Noyes and Rieseberg, 2000). In *Hieracium piloseloides*, different crossing schemes indicate that apomixis can be transmitted via both haploid and diploid gametes, but post-zygotic lethality rather than segregation distortion causes the absence of apomixis in diploids (Bicknell et al., 2000).

CONCLUSIONS

Apomixis is a complex trait involving the modification of several steps of normal sexual development. Recent reports on the inheritance of apomixis have revealed several common features. (1) The genetic control of apomixis is dominant; this is true for all elements of apomixis studied so far. This observation is often taken as evidence for apomixis being caused by a mutated gene, but it is also compatible with the misexpression of wild-type genes playing key regulatory roles in sexual development (Koltunow, 1993; Grossniklaus, 2001). The latter view is supported by the fact that both sexual and apomictic reproduction coexist in facultative apomicts and that apomictic plants can be obtained from sexual progenitors through hybridization (reviewed in Carman, 2001) or simple chromosome doubling (Quarin et al., 2001). (2) The apomeiosis locus resides in a recombinationally suppressed region, suggesting that even in species in which the elements of apomixis are under common control, a complex of several coadapted genes may be present. (3) The apomixis locus is usually associated with gametic or zygotic lethality or its transmission is reduced by some other mechanism, with the result that haploid gametes do not produce progeny, thereby maintaining or driving the polyploidization associated with apomictic species.

As many reproductive mutants in sexual species indicate, disturbances of sexual development toward elements of apomixis can easily cause abortion and sterility. Selection has perfected natural apomicts, refining the developmental modifications and integrating them into a functional developmental system with high fertility that is likely controlled by coadapted gene complexes. Although many of the features discussed in this article pose obstacles to understanding the molecular basis of natural apomixis, a complementary approach using both sexual and apomictic model systems bears great promise that apomixis can eventually be harnessed to contribute to sustained agricultural production.

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Ueli Grossniklaus
Gian A. Nogler
Institute of Plant Biology
University of Zürich
Zollikkerstrasse 107
CH-8008 Zürich, Switzerland
grossnik@botinst.unizh.ch

Peter J. van Dijk
Netherlands Institute of Ecology
P.O. Box 40
6666 ZG Heteren, The Netherlands
pjvandijk@cto.nioo.knaw.nl

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**Move It on Out with MATEs**  
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