


How to Avoid Sex: The Genetic Control of Gametophytic Apomixis

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, apomorphic embryos are formed without paternal contribution. Therefore, apomorphic 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; Vieille-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; Matzk 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 Schneitz, 1998; Yang and Sundaresan, 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; Vieille-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; Matzk 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 almost 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 *Erigeron annuus* (Noyes and Rieseberg, 2000). In *Brachiaria decumbens* (Pessino et al., 1998), *Tripsacum dactyloides* (Grimanelli et al., 1998a), and

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**Figure 1.** Scheme of Sexual and Apomictic Reproduction.

Reduced stages of the life cycle are shown in shaded ovals, and unreduced stages are shown in rectangular boxes. The key developmental events that are affected or altered in apomictic species are highlighted in hexagons.
**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’Hagain 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

<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 of 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>Aaa</td>
<td>–</td>
<td>–</td>
<td>Savidan, 1982</td>
</tr>
<tr>
<td><em>Pennisetum squamulatum</em></td>
<td>Apospory</td>
<td>Poaceae</td>
<td>Aaa</td>
<td>0 cM</td>
<td>Yes</td>
<td>Ozias-Akins et al., 1998</td>
</tr>
<tr>
<td><em>Brachia 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>Eriogon 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 of 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>Eriogon 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.
(Matzk et al., 2000), which suggests that the elements of apomixis in these species are regulated by different genes. In Erigeron annuus, separate genes for diplospory (A) and parthenogenesis (P) have been mapped geneti-
cally (Noyes and Rieseberg, 2000). Apomicts in this species carry the sim-
plex genotype for both genes (Aaa and Ppp). In contrast to the diplospory A locus, no suppression of recombination was observed around the parthenogen-
esis P locus (Table 1). In light of these new findings, it is likely that coadapted
gene complexes are present in those species in which all elements of apo-
mixis cosegregate.

SEGXESIONS DISTORTION OF
APOMIXIS LOCI

As mentioned above, gametophytic apomicts are usually polyploid, whereas
related sexuals are diploid. Is gameto-
phytic apomixis incompatible with dip-
loidy? Again, the pioneering work on R.
aricomus by Nogler appears to have
general relevance. Nogler showed that
diploid offspring that developed par-
thenogenetically from reduced diploid
egg cells of tetraploid apomicts (dihap-
loids) or diploids produced through an-
other culture were able to reproduce
apomictically (Nogler, 1982). This shows
that apomixis and diploidy are not in-
compatible, a finding that has been
confirmed in several other species
(Bicknell, 1997; Kojima and Nagato,
1997). However, what matters is the or-
igin of the diploid offspring, because
zygotic diploids derived from the fusion
of haploid egg cells and haploid sperm
never reproduced apomictically in Ra-
nunculus. Nogler hypothesized that the
apospory (A) locus was recessive lethal
in the gametes. Consequently, the A lo-
cus could be transmitted via diploid ga-
metes to generate polyploid apomicts
but not via haploid gametes to gener-
ate diploid apomicts. It is also possible
that mutations closely linked to the A
locus cause haploid gametes nonfunc-
tionality. The net result is that haploid
gametes carrying the A locus do not
contribute to offspring production, re-
sulting in segregation distortion of the
A locus.

More recently, additional evidence
has been obtained for segregation dis-
tortion of apomixis loci in other plant
species, such as Tripsacum dacty-
loides, 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 transmit-
ted because of selection against hap-
loid gametes, as was observed for the
A locus in R. auricomus. The diplospory
locus A, in contrast, was not transmit-
ted because of meiotic drive. In these
triploid apomicts, the nondiplospory
chromosomes seem to pair preferen-
tially, leaving the diplospory chromo-
some as a univalent that always ends
up in a diploid pollen grain (Noyes and
Rieseberg, 2000). In Hieracium pilosel-
loides, different crossing schemes indi-
cate that apomixis can be transmitted
via both haploid and diploid gametes,
but post-zygotic lethality rather than
segregation distortion causes the ab-
ence of apomixis in diploids (Bicknell
et al., 2000).

CONCLUSIONS

Apomixis is a complex trait involving
the modification of several steps of nor-
mal sexual development. Recent re-
ports on the inheritance of apomixis
have revealed several common fea-
tures. (1) The genetic control of apo-
mixis is dominant; this is true for all
elements of apomixis studied so far.
This observation is often taken as evi-
dence 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 (re-
viewed in Carman, 2001) or simple
chromosome doubling (Quarin et al.,
2001). (2) The apomeiosis locus resides
in a recombinationally suppressed re-
dion, suggesting that even in species in
which the elements of apomixis are un-
der common control, a complex of sev-
eral coadapted genes may be present.
(3) The apomixis locus is usually asso-
ciated with gametic or zygotic lethality
or its transmission is reduced by some
other mechanism, with the result that
haploid gametes do not produce prog-
eny, thereby maintaining or driving the
polyplidization associated with apo-
mictic species.

As many reproductive mutants in
sexual species indicate, disturbances
of sexual development toward ele-
ments of apomixis can easily cause
abortion and sterility. Selection has
perfected natural apomicts, refining the
developmental modifications and inte-
grating them into a functional develop-
mental system with high fertility that is
likely controlled by coadapted gene
complexes. Although many of the fea-
tures discussed in this article pose ob-
stacles to understanding the molecular
basis of natural apomixis, a comple-
mentary approach using both sexual
and apomictic model systems bears
up promise that apomixis can even-
tually be harnessed to contribute to
sustained agricultural production.

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REFERENCES


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