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COMMENTARY

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Is supernumerary chromatin involved in gametophytic apomixis of polyploid plants?

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Abstract Gametophytic apomixis, or unreduced embryo sac development that results in asexual reproduction through seeds, occurs in several families of angiosperms and must be polyphyletic in origin. The molecular mechanisms underlying gametophytic apomixis have not been discovered and are the subject of intense investigation. A common feature of almost all apomicts is their polyploid nature. From genetic mapping studies in both monocots and dicots, there is low genetic recombination associated with a single (rarely two), dominant locus for either aposporous or diplosporous embryo sac formation. In *Pennisetum squamulatum* and *Cenchrus ciliaris*, some DNA sequences mapping to the apospory locus are unique to apomictic genotypes and apparently hemizygous. This sequence divergence at the apomixis locus could be a consequence of genome rearrangements and isolation from genetic recombination, both of which may have contributed to the definition of a chromosomal region as supernumerary. The possible involvement of supernumerary chromatin, formed as a result of interspecific hybridization, in the origin of apomixis, is explored here.

Keywords Apomixis · Genome rearrangements · Hemizygosity · Hybridization · Polyploidy · Supernumerary chromatin

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Introduction

Apomixis, or clonal propagation by seed, has been reported in approximately 330 genera of higher plants of which 126 genera from 33 out of 460 angiosperm families follow one of the two forms of gametophytic apomixis (Carman 1997). In diplospory, the unreduced embryo sac differentiates from the megaspore mother cell through mitosis or modified meiosis. In apospory, unreduced embryo sacs originate from somatic nucellar cells (Nogler 1984a). The occurrence of polyploidy is pervasive in higher plants. Stebbins (1950) estimated that polyploidy was associated with 30% to 35% of angiosperm species. Soltis and Soltis (1995) provide an even higher figure with "perhaps 50% of all angiosperms of polyploid origin". Chromosomal duplications, often taken as evidence of ancient polyploidy, are common in even the simplest of angiosperm genomes (Blanc et al. 2000; Gaut et al. 2000; Vision et al. 2000). It is remarkable, however, that gametophytic apomixis in naturally occurring genotypes is almost always linked to obvious polyploidy (Savidan 2000). In any group of related species of different ploidy levels or within the same species in which several ploidy levels are documented, the diploid plants are sexual while the polyploid could be either sexual or apomictic (Babcock and Stebbins 1938).

Recent progress in genetic and molecular analysis of apomixis

Inheritance studies and molecular genetic analyses of apomixis recently have been reviewed (Pessino et al. 1999; Savidan 2000). Savidan (1982) in *Panicum maximum* and Nogler (1984b) in *Ranunculus auricomus* were the first to report the dominant control of apospory by a single locus. In recent years this unexpected simplicity has been confirmed in *Brachiaria* (Pessino et al. 1997), *Pennisetum squamulatum* (Ozias-Akins et al. 1998), *Cenchrus ciliaris* (Sherwood et al. 1994) and *Hieracium* sp. (Bicknell et al. 2000). Linkage analysis of molecular

markers to apomixis was carried out in a population of 397 individuals segregating for apospory and sexuality generated from a cross between sexual pearl millet (*P. glaucum*) and apomictic *P. squamulatum* (Ozias-Akins et al. 1998). Bulked-segregant analysis was used to isolate apospory-linked molecular markers that resulted in 12 sequence-characterized amplified regions (SCARs) which were always linked to apospory. Ten of these *P. squamulatum*-derived markers were linked to apospory with essentially no recombination within a tight linkage group in a population produced by an intraspecific cross between apomictic and sexual buffelgrass (*Cenchrus ciliaris*) parents (Roche et al. 1999). Considering the hemizygosity of some of these markers, the lack of recombination between them, as well as the absence of physical maps for *P. squamulatum* and *C. ciliaris* to provide any indication of distance between markers, we recently opted for the concept of an apospory-specific genomic region (ASGR) to describe the apomixis locus in these species.

For the other form of gametophytic apomixis, diplospory, a simple dominant action by one locus is not always as clear. Recent work on *Tripsacum* (Kindiger et al. 1996; Grimanelli et al. 1998a) seems to indicate that the genetic control of meiotic non-reduction resides on a single chromosome. In this species the involvement of an unknown number of genes within a non-recombining section of a chromosome is hypothesized (Savidan 2000). In the Asteraceae, however, evidence continues to accumulate that supports the action of two independent loci in the regulation of functional diplospory (van Dijk et al. 1999; Noyes and Rieseberg 2000). In *Taraxacum*, triploid plants were obtained from crosses between diploid sexual individuals and triploid apomicts. Certain of the triploid progeny produced unreduced eggs that required fertilization for embryo development; thus, diplosporous embryo sac development and parthenogenesis (development of an unfertilized egg) apparently had been uncoupled by recombination (van Dijk et al. 1999). Similarly, parthenogenesis, as deduced from seed set (which, however, may be affected by other genetic factors controlling endosperm development), was shown to segregate independently of diplosporous embryo sac development in *Erigeron annuus* and to be subject to recessive-lethal gametophytic selection (Noyes and Rieseberg 2000). The locus required for formation of unreduced embryo sacs was characterized by eleven non-recombining AFLP markers considered to represent a substantial genomic region which likely follows univalent inheritance.

The robust association between polyploidy and gametophytic apomixis

Based on cytogenetic analyses, the genomic relationships within apomictic polyploids often suggest segmental allopolyploidy rather than strictly delineated allo- or auto-polyploidy (Burson 1997; Patil et al. 1961). Significant progress has been achieved in our understanding of apomixis by studies of sexual and apomictic taxa of various ploidy levels which belong to a single "agamic complex" (Babcock and Stebbins 1938), e.g., *Alchemilla* sp., *Bothriochloa-Dichanthium*, *Brachiaria* sp., *Crepis* sp., *Hieracium* sp., *Panicum* sp., *Parthenium* sp., *Paspalum* sp., *Pennisetum* sp, *Poa* sp., *Potentilla* sp., *Ranunculus* sp., *Rubus* sp., *Taraxacum* sp., and *Tripsacum* sp. (see Harlan and de Wet 1963; Nogler 1984a; Asker and Jerling 1992). Thus, Nogler (1984a) concluded that "allopolyploidy is just as typical for gametophytic apomicts as hybridity and heterozygosity". One can suggest that in many angiosperms gametophytic apomixis is a natural means to maintain completely heterozygous interspecific combinations, which would otherwise be doomed by meiotic failure and gametic instability (Harlan and de Wet 1963). In the past when investigators looked for the immediate appearance of apomixis upon interspecific hybridization, it seldom occurred, which led to the conclusion that hybridization and apomixis were not causally associated (Asker and Jerling 1992). However, it is likely that hybridization has latent effects, namely genome restructuring, which may take several generations to occur (reviewed in Wendel 2000). Similar events subsequent to interspecific or interracial hybridization may have played a role in the origin of gametophytic apomixis.

In addition to hybridization as a means to test polyploidization as a cause for apomixis, chromosome doubling by colchicine treatment of sexual diploids, conducted in groups of species for which gametophytic apomixis has been documented, did not produce tetraploid apomicts (Burton et al. 1970; Savidan 1982; Lutts et al. 1991; Leblanc et al. 1995; Salon and Earle 1998). One possible exception is the report of Quarin and Hanna (1980) where induced doubling of the chromosome number of diploid *Paspalum hexastachyum* did indeed generate tetraploid plants that produced aposporous embryo sacs as determined by cytological observation. However, no data from progeny tests were provided which would have confirmed the functionality of the aposporous embryo sacs, and diploid *P. hexastachyum* displayed quadrivalent associations at meiosis indicating that it probably is not a simple diploid (Quarin and Hanna 1980). This observation with *Paspalum* as well as recent molecular data indicating tetrasomic inheritance of the apomixis locus in three different grass genera (*Tripsacum*, *Paspalum* and *Pennisetum*) have led to a revival of the involvement of autopolyploidy in gametophytic apomixis (Pessino et al. 1999). The debate over auto- vs. allo-polyploidy continues, however, since there is not strict congruence between cytogenetic and inheritance data. Furthermore, the assumption of autotetraploidy based on current models could be misleading because tetrasomic inheritance of apospory in a complex genome, such as with the hexaploid *P. squamulatum* that has been characterized as a segmental allopolyploid (Patil et al. 1961), is insufficient evidence that apomixis has been organized by the event of autopolyploidy.

Apomixis has been documented at lower ploidy levels in plants derived from polyploid apomicts. The parthenogenetic development of rare reduced egg cells from an apomictic polyploid results in production of apomictic dihaploids. Apomictic dihaploids have been documented in *Pennisetum* (Dujardin and Hanna 1986), *Hieracium aurantiacum* (Bicknell 1997), *Ranunculus auricomus*, *Potentilla argentea* and *Panicum maximum* (see references in Bicknell 1997) and *Tripsacum* (Leblanc et al. 1996). Thus the apomictic potential could be maintained at a lower level of ploidy although it seems to prevail at a higher level of ploidy, which supports the idea that the absence of gametophytic apomixis in wild diploids "is not a problem of expression but rather of transmission constraints" (Savidan 2000).

What is the nature of the link between gametophytic apomixis and polyploidy?

One possibility is that gametophytic apomixis is a mechanism for producing polyploidy. In plants, two types of polyploidization events are recognized: somatic chromosome doubling and formation of functional 2n gametes. Spontaneous chromosome doubling in plants (i.e., somatic polyploidization) must be relatively rare in nature, as thoroughly demonstrated by Harlan and de Wet (1975). On the contrary, there are many reports of unreduced gametes leading to sexual polyploidization (Bretagnolle and Thompson 1995). In a diploid genotype in which a genomic modification leading to the formation of unreduced embryo sacs with incompletely penetrant parthenogenesis would occur, it is reasonable to assume that the probability of fusion between two unreduced gametes would be significantly enhanced. The unreduced female gamete would be a product of a novel genetic mechanism while the unreduced male gamete would remain the result of a rare restitution event. The generation of polyploid genomes, then, would be a consequence of gametophytic apomixis.

It may be relevant to note that there is no association between polyploidy and the occurrence of a non-gametophytic form of apomixis (adventitious embryony) found in *Citrus* and other genera (Asker and Jerling 1992; Richards 1996). In this case somatic embryos are directly differentiated from the nucellus or integuments with no formation of embryo sacs (Koltunow 1993). The absence of unreduced female gametes during adventitious embryony would preclude any potential event of sexual polyploidization.

The converse possibility is that gametophytic apomixis is dependent upon the polyploid state, and several hypotheses to explain this link have been proposed in the last 20 years. In the case of apospory, Savidan (1982) postulated that differences in the duration of meiosis in polyploids compared to the duration in diploids would allow a window of opportunity for the aposporous embryo sacs to develop in lines with the higher level of ploidy. A gene required for initiation of aposporous initials similar to the one hypothesized by Peacock (1992) would, consequently, be penetrant only in polyploid genotypes. This would not explain meiotic failure unless we assume that aposporous initials are negatively affecting the megaspore mother cell and/or megaspore(s).

Alternatively, a gametophyte-expressed lethal model has been suggested in which the genetic determinant for apomixis is linked to, or is itself, a recessive lethal factor (Nogler 1984b; Richards 1996). If so, apomixis existing in a simplex condition in a diploid organism would be doomed at the gametophyte stage. Alternatively, genetic load also could be deleterious to the survival of diploid zygotes rather than haploid gametes as observed in *Hieracium* (Bicknell et al. 2000). These observations may explain why apomixis would not be tolerated in diploids.

Carman (1997, 2001) suggested that apomixis is generally caused by "intergenomic" (or homeologous) heterozygosity at several to many loci, which are critical to spatiotemporal aspects of megasporogenesis and embryo sac development. In hybrids, the heterozygosity at these critical loci would cause reproductive asynchrony (apomixis). It could occur in autopolyploids but allopolyploidy would provide a better genomic background for apomixis (Carman 2001). Recent research documented that ploidy changes can quantitatively and qualitatively alter gene expression (Comai 2000; Wendel 2000). However, these hypotheses relating to alteration of, or interaction between, gene expression(s) do not involve any apomixis-linked genomic region and are by themselves difficult to reconcile with findings from mapping efforts in *Brachiaria, Tripsacum,* and *Pennisetum-Cenchrus, Erigeron,* (Pessino et al. 1997; Grimanelli et al. 1998a; Ozias-Akins et al. 1998; Roche et al. 1999; Noyes and Rieseberg 2000) in which large tracts of DNA have been linked to the trait.

Recent molecular studies have shown that rapid changes occur not only in gene expression but also in genome structure upon polyploid formation (Leitch and Bennett 1997). In angiosperms some form of genome reorganization, provoked and tolerated by polyploidization, may disturb meiosis and induce the formation of reduced embryo sacs, favoring diplosporous or aposporous differentiation of unreduced gametophytes.

Finally, we should consider the similarities between higher plants and animals with regard to the link between apomixis/parthenogenesis and polyploidy. In both kingdoms "asexual taxa are generally polyploid, whereas their sexual relatives are diploid" (Asker and Jerling 1992). Are we studying a universal genomic phenomenon that arises upon polyploidization in diverse eukaryotes? Does it involve reorganization of genomic regions with as yet unknown consequences upon transcription of genes involved in normal sexuality?

Is there a connection between gametophytic apomixis, polyploidy and supernumerary DNA?

First, supernumerary chromosomes, also called B-chromosomes, are not rare and "probably occur in all living

taxa" (Beukeboom 1994). They are usually smaller than chromosomes of the normal A-chromosome complement and do not pair with them (Jones and Rees 1982; Camacho et al. 2000). B-chromosomes are the "free" forms of supernumerary DNA and thus the most easily recognized forms. Nevertheless, supernumerary chromatin also can be present on A-chromosomes either in intercalary segments or in distal positions. For example, an interstitial location of supernumerary chromatin has been documented in rye (Nagaki et al. 1999), and numerous intercalary "knobs" have been observed in all ten chromosomes of *Zea mays* as well as terminally on an anomalous version of chromosome 10 known as *Ab10*. In the presence of *Ab10*, female meiotic drive occurs for any chromosome heterozygous for a knob, including *Ab10*. This type of supernumerary DNA and its response to meiotic drive in the presence of *Ab10* may have played a major role in the evolution of maize genome structure (Buckler et al. 1999). In insects, Basso and Lifschitz (1995) reported a B-chromosome that was alternatively free or terminally attached to the X-chromosome, and Cabrero et al. (1998) showed the presence of a supernumerary chromosome segment in a distal position of an autosome.

Over the past few years, study of the origins of supernumerary chromatin/chromosomes has been facilitated by fluorescence in situ hybridization and Southern blot analyses of genomes. In many instances, it has been shown that supernumerary genomic segments originated from the autosomal complement (Peppers et al. 1997; Nagaki et al. 1999; Brinkman et al. 2000). A simple translocation event would explain the distal position of supernumerary chromatin on an autosomal chromosome (Cabrero et al. 1998). However, more complex origins have been proposed for supernumerary chromosomes where a "B-chromosome arose as a leftover centromere from centric fusion" (Peppers et al. 1997) or when "B-chromosomes are isochromosomes that have arisen by means of centromere misdivision and chromatid nondisjunction" (Mestriner et al. 2000). In other instances, straightforward autosomal origin of supernumerary chromatin from within the genome was not supported by molecular data (Cheng et al. 2000). Horizontal transfer of supernumerary chromosomes between vegetatively incompatible fungal biotypes also has been demonstrated (He et al. 1998). Furthermore, a supernumerary chromosome already present in a given species could be transferred to another species through interspecific hybridization (Dobson and Tanouye 1998b).

In favor of the argument for the involvement of supernumerary DNA in the origin of gametophytic apomixis we offer several circumstantial facts. (1) "Free" supernumerary chromatin (i.e., B-chromosomes) has been documented in apomictic species and their close relatives, such as *Agropyron* (=*Elymus*), *Brachiaria*, *Calamagrostis*, *Panicum*, *Paspalum*, *Pennisetum*, *Poa*, and *Tripsacum* (Jones and Rees 1982), to cite genera of monocotyledons only. This observation does not suggest that an apomixis gene(s) necessarily resides on a B-chromosome, but points out the capacity for generation of supernumerary chromatin in these species. (2) In plants, parasitic (as opposed to mutualistic) supernumerary chromosomes are expected to persist mainly in cross-pollinated species and to decline in self-pollinated species (Burt and Trivers 1998). Asker and Jerling (1992) have enumerated close to 30 cultivated or domesticated apomicts. The majority of those species that reproduce by gametophytic apomixis also are associated with cross-pollinating breeding systems. (3) Supernumerary chromosomes follow a non-Mendelian mode of inheritance (Jones and Rees 1982). Their rate of transmission can be irregular with different levels of meiotic and post-meiotic drive or drag (Beukeboom 1994; Rusche et al. 1997). There are two recent reports of strong distortion in the transmission of diplospory (Grimanelli et al. 1998b) or apospory (Roche et al. 2001) through female gametes. These non-Mendelian transmissions may be tentatively explained by negative effects on meiosis or female gametophyte fitness, or by unknown phenomena of imprinting upon fertilization (Dobson and Tanouye 1998a). (4) The lack of recombination around the diplospory locus in *Tripsacum* (Grimanelli et al. 1998a) and *Erigeron annuus* (Noyes and Rieseberg 2000) as well as the partially hemizygous ASGR in both *P. squamulatum* and *C. ciliaris* (Ozias-Akins et al. 1998; Roche et al. 1999) could be explained by a supernumerary nature of those genomic regions. (5) Many B-chromosomes seem to be involved in "anomalies" of meiotic or post-meiotic events in plants (Jones and Rees 1982). As an example, B-chromosomes from *Agropyron* species were shown to reduce homologous pairing during meiosis in intergeneric crosses between *Triticum* and *Agropyron* (Chen et al. 1993). (6) Finally, in one animal species, B-chromosomes were found in polyploid individuals reproducing by pseudogamous parthenogenesis but were conspicuously absent in the diploid sexual individuals (Beukeboom et al. 1998).

We showed in two different species (*P. squamulatum* and *C. ciliaris*) that a linkage group we termed apospory-specific genomic region (ASGR) determines apospory and is characterized by the presence of a high proportion of hemizygous sequences. Furthermore, we recently demonstrated that some of the hemizygous sequences are present in several linked copies, and thus represent duplications (Roche et al. unpublished). These regions are, however, conserved between the two apomictic taxa, suggesting a monophyletic origin of the ASGR, similar to the proposed evolution of related B-chromosomes from an ancestral B-chromosome in the genus *Brachycome* (Houben et al. 1997, 1999). Could the ASGR comprise part of a supernumerary chromosomal region that evolved through a combination of mobile elements or replication-based amplification and divergence (Ananiev et al. 1998a, b), perhaps assisted by synthesis-dependent strand annealing of unrelated DNA fragments (Langdon et al. 2000), to result in a chimeric assembly of unique regions? Given the sequence conservation observed, it seems likely that such an assembly would have occurred

once and subsequently would have been transmitted through pollen by multiple hybridization events leading to speciation of *Pennisetum* and *Cenchrus* apomicts.

We do not suggest that all supernumerary DNA can induce apomixis since other genic functions, or in other cases no genic functions, have been documented for its induction (Green 1990; Schartl et al. 1994; Covert 1998). Neither do we postulate that supernumerary DNA could occur in polyploid plants only, since it frequently has been described in diploid sexual species (Jones and Rees 1982). One could argue that the presence of supernumerary DNA in an apomict might simply be due to the increased tolerance of a polyploid apomictic lineage to a larger genetic load (Beukeboom et al. 1998), which otherwise might be eliminated by successive meiotic events in a sexual lineage if drag were associated with the supernumerary chromatin. In addition to tolerance, however, we suggest that an allopolyploid genome derived from hybridization between two or more species would indeed be a fertile environment to generate supernumerary DNA from A chromosomes by hybrid disruption (McVean 1995). The appearance of B-chromosomes following interspecific hybridization has previously been demonstrated (Sapre and Deshpande 1987; Camacho et al. 2000). These B-chromosomes or other forms of supernumerary DNA, through as yet uncharacterized molecular mechanisms, could have played a role in the evolution of gametophytic apomixis.

There is growing evidence for the involvement of genome conflicts in the evolution of sex-determining mechanisms, including sex chromosome drive, cytoplasmic sex-ratio distortion and cytoplasmic male sterility (Werren and Beukeboom 1998). Evolution of B-chromosomes and other forms of supernumerary chromatin is in many respects similar to that of univalent sex chromosomes (Camacho et al. 2000). Polyploidization events in plants, especially alloploidy, should be at the origin of genome conflicts. We think it is relevant to consider these conflicts, with supernumerary chromatin-inducing potential, as possible explanations for the very strong link observed between polyploidy and gametophytic apomixis. This new hypothesis is consistent with a polyphyletic origin of apomixis in polyploids throughout several families of angiosperms.

Unfortunately, if the ASGR physically maps to supernumerary chromatin/chromosomes in *Pennisetum* sp. and *Cenchrus ciliaris*, the challenge for determining the molecular mechanisms for apomixis will be greatly amplified. Specifically, it may be very difficult to determine if there are novel expressed apomixis genes within the supernumerary DNA or direct effects of chromatin remodeling, such as have been proposed for paramutation phenomena in maize (Chandler et al. 2000), since analysis will be hampered by the overwhelming task of characterizing genes on a genomic region devoid of recombination. Such challenges have been met in similarly complex systems where a novel fusion gene recently was discovered within the *t*-complex of mouse, one of several required genes in a highly rearranged genomic region that is responsible for segregation distortion (Schimenti 2000). In our case, however, the worst-case scenario might be the discovery that non-recombining genomic regions previously described in *Tripsacum*, *Erigeron*, *Pennisetum* and *Cenchrus* are indeed not single genomic regions and would be the artifactual product of our failure in the application of Mendelian genetics to apomictic studies. There are no means through genetic analysis to distinguish between totally linked markers and unlinked markers which are always driven together, although whole chromosome maps from *Tripsacum* and *Erigeron* do not support this hypothesis. Rhoades (1942) showed that a maize line carrying *Ab10* demonstrated meiotic drive in megasporogenesis not only for *Ab10* but also for all other chromosomes heterozygous for a knob. Linkage analysis in apomicts, however, has always been dependent on genetic recombination during microsporogenesis. The occurrence of quasi-linkages could be tested by physical mapping of large-insert genomic clones known to contain apomixis-linked markers by using chromosomal fluorescence in situ hybridization.

It is also possible that a primary effect of supernumerary chromatin might be to impair female meiosis. Subsequently, initiation of aposporous embryo sacs in apospory, and mitosis or restitution meiosis in diplospory, would constitute mere default pathways. Then, in some aspects we would be revisiting Ernst's 1918 hypothesis in which the author postulated that apomixis was a default pathway in interspecific hybrids induced by poor chromosome pairing at female meiosis. A few authors already acknowledge there may be no need of particular genes for the chronologically terminal element of apomixis that is the autonomous development of an unreduced egg (Mogie 1988; Nogler 1995; Grimanelli et al. 1998a). This default scenario leads to the exciting prospect of inducing apomixis in certain genetic backgrounds by cytotoxic engineering targeted at the early meiotic stages of the megaspore mother cell.

Future perspectives

Much effort recently has been devoted to investigating the different elements of apomixis in Arabidopsis thaliana in which documentation of gametophytic apomixis is non-existent (Savidan 2000). This plant species may have an excessively "simplified" genome for such potential. Similarly, the inbreeding mating system used in most schemes of plant improvement may have inadvertently been "cleaning up" genomes of crop species to a point where apomixis is absent in most of them (Asker and Jerling 1992).

The association between polyploidy and gametophytic apomixis interjects obstacles to a finer genetic and molecular understanding of this unique natural process of cloning shared by many species of angiosperms. In polyploid organisms, genic interactions between different genomes remain obscure. Nevertheless, continued study of apomixis will provide valuable insight on the

reproductive biology, genomic organization and fine genetic tuning of polyploid plants. Further, if an association between supernumerary chromatin/chromosomes and gametophytic apomixis is shown, this discovery would establish a most spectacular biological function for genetic elements often depicted as selfish DNA devoid of genic activity.

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