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Polyploidy and genome evolution in plants

Pamela S Soltis[1,2], D Blaine Marchant[1,3], Yves Van de Peer[4,5,6] and Douglas E Soltis[1,2,3]

Plant genomes vary in size and complexity, fueled in part by processes of whole-genome duplication (WGD; polyploidy) and subsequent genome evolution. Despite repeated episodes of WGD throughout the evolutionary history of angiosperms in particular, the genomes are not uniformly large, and even plants with very small genomes carry the signatures of ancient duplication events. The processes governing the evolution of plant genomes following these ancient events are largely unknown. Here, we consider mechanisms of diploidization, evidence of genome reorganization in recently formed polyploid species, and macroevolutionary patterns of WGD in plant genomes and propose that the ongoing genomic changes observed in recent polyploids may illustrate the diploidization processes that result in ancient signatures of WGD over geological timescales.

Addresses
[1] Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA
[2] Genetics Institute, University of Florida, Gainesville, FL 32610, USA
[3] Department of Biology, University of Florida, Gainesville, FL 32611, USA
[4] Department of Plant Biotechnology and Bioinformatics, Ghent University and Department of Plant Systems Biology, VIB, B-9052, Ghent B-9052, Belgium
[5] Bioinformatics Institute Ghent, Ghent University, B-9052, Ghent B-9052, Belgium
[6] Genomics Research Institute, University of Pretoria, Pretoria 0028, South Africa

Corresponding author: Soltis, Pamela S (psoltts@flmnh.ufl.edu)

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Introduction

Plant genomes vary spectacularly in size, ranging from 0.063 Gb to 148.8 Gb, a 2400-fold difference ([1]; www.data.kew.org/cvalues). Much of this diversity results from differential expansion and loss of repeats (reviewed in [2••]), but an additional major driver shaping variation in genome size in plants is whole-genome duplication (WGD; also known as polyploidy, see Box for glossary of terms). Moreover, genome structure and gene content in plants are intimately tied to the history of WGD. We therefore contend that any understanding of plant genome structure, content, and evolution requires consideration of WGD and its consequences.

Polyploidy in plants

Polyploidy has long been considered an important mechanism of speciation in plants, particularly angiosperms (e.g., see reviews by [3,4••,5,6•,7,8•,9,10]). The frequency of polyploidy has typically been based on chromosome numbers, and estimates for major clades of green plants (Viridiplantae) have varied dramatically, ranging from very low in bryophytes to as high as 95% for ferns [6•]. For angiosperms (flowering plants) alone, estimates have ranged from 30–35% [4••] to ~70% [11], with most estimates near 50% (e.g. [5,12•,13•,14]). Genomic data for plants have demonstrated a dazzling history of repeated WGDs throughout evolutionary history. Even the small genome of *Arabidopsis thaliana* (0.157 Gb) — this small size was one of the keys to the choice of this species as a genomic model–shows signatures of ancient WGD [15,16]. All angiosperms share an ancient WGD, as do all seed plants [17]. Thus, in recent years, interpretations of plants as 'diploids' or 'polyploids' have been blurred, requiring much more nuanced vocabulary to describe plant genomes.

The origin of new species via polyploidy requires a series of seemingly low-probability events, including hybridization, unreduced gamete formation, establishment, and survival (see e.g. [18,19••,20]). Despite these apparent barriers, polyploid species are common in all floras worldwide and are particularly abundant at high latitudes and high elevations (e.g. [21,22]). The 'success' of polyploids is often attributed to the increased genetic diversity held within single polyploid individuals relative to that of their diploid progenitors (e.g. [7,8•,9,22–25]). Moreover, this genetic diversity may be manifested in novelty at the biochemical, physiological, morphological, and ecological levels, giving polyploids an advantage, at least in the short term, over their diploid parents (e.g. [26••,27]). The proximal reason behind polyploid success, therefore, may vary among species. However, if polyploidy *per se* were always a successful strategy, then plant genomes, such as that of *A. thaliana*, should show more obvious evidence of WGDs, such as high chromosome numbers, large genomes, and routinely duplicate (triplicate, quadruplicate, and so on) gene copies. Although many apparently recently formed polyploids exhibit these expected attributes of WGD,

Glossary
Allopolyploidy: polyploidy formed through the combined processes of interspecific hybridization and genome doubling.
Autopolyploidy: genome doubling that arises within a species; it may involve a single individual or crossing between individuals from genetically distinct lineages within the species.
Diploidization: the processes that return a polyploid genome to a diploid-like genome; these may include loss of duplicate genes and chromosomes, loss of repetitive DNA, gene silencing, altered chromosome pairing.
Diploidy: the state of being diploid; that is, containing two complete sets of chromosomes (or genomes).
Fractionation: the loss of one copy of a gene pair duplicated by polyploidy; losses may be random with respect to the parental genome or biased, with most/all losses from a single parental genome.
Homeolog (also homoeolog): chromosomes (and the genes they carry) that are duplicated by polyploidy.
Polyploidy: the state of having more than two complete sets of chromosomes.
Polyploidization: the process(es) of polyploid formation; this can be duplication, triplication, or higher-order multiplication of a genome.
Whole-genome duplication: the duplication of a complete genome, for example, of a diploid genome (with two copies of each chromosome) to form a tetraploid (with four copies of each chromosome); this term is sometimes used to refer to the process of duplication (i.e., polyploidization) and sometimes in reference to the state of having multiple, duplicate genomes (i.e., polyploidy).

those species, such as *A. thaliana*, that are the products of ancient WGD harbor signatures of WGD within what are generally 'diploid' genomes, based on chromosome number, genome size, and gene copy number. Certainly, processes of diploidization are at play, leading to repeated cycles of polyploidy followed by diploidization followed by polyploidy, and so on (e.g. [28–30]).

But what are these processes of diploidization, and how can they be reconciled with observations of gene family diversity, ancient signatures of WGD, and macroevolutionary patterns of polyploidization? In this paper, we will attempt to unify (i) hypothesized mechanisms of diploidization, (ii) data on genome reorganization shortly after polyploidization based on the evolutionary model, *Tragopogon*, and other recently formed polyploid species, and (iii) macroevolutionary patterns of WGD in plant genomes. We propose that the ongoing genomic changes observed in recent polyploids, such as *Tragopogon*, may illustrate the diploidization processes that result in ancient signatures of WGD over geological timescales.

Mechanisms of diploidization

Repetitive DNA sequences comprise substantial portions of plant genomes (e.g. *A. thaliana*: 15–20%; maize ~85%) and can largely influence genome size (e.g. [31,32]). Consequently, the two mechanisms by which these repetitive sequences (i.e. retrotransposons, DNA transposons, simple repeats) are lost (illegitimate recombination and unequal intra-strand homologous recombination) are the principal processes responsible for genome downsizing to a 'diploid' state. Illegitimate recombination is hypothesized to remove DNA sequences via double-stranded breaks and/or slippage during replication, whereas unequal homologous recombination occurs between two repeat sequences and results in the loss of the DNA between the repeats, as well as one of the repeats [33]. The relative importance of the two primary diploidization mechanisms appears to be species-specific in angiosperms, as the efficiency of DNA loss via either mechanism is highly variable and not phylogenetically related [33–35]. Very few studies have addressed these processes outside of flowering plants; however, recent genomic sequencing of three conifer taxa has suggested that the enormous genomes of these taxa could be due to a very low recombination rate and high homologous chromosome fidelity, removing the opportunity for genome downsizing [36], at least via these mechanisms (Figure 1).

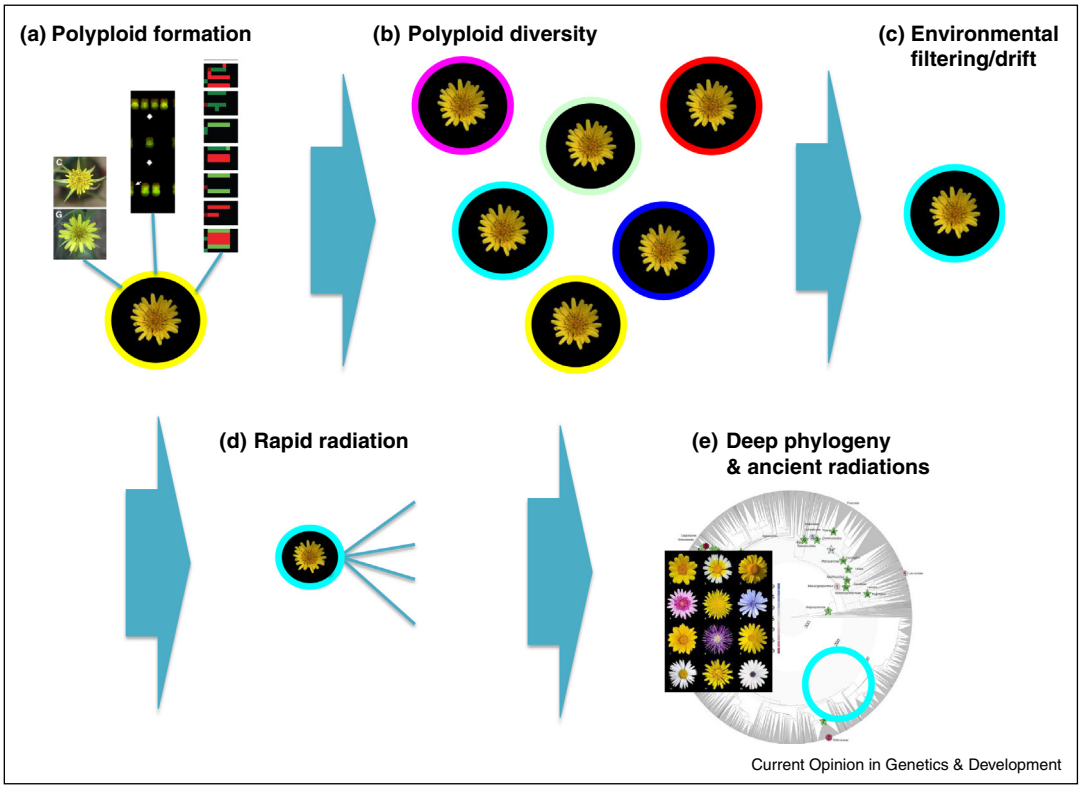
Although great strides have been made in determining the processes by which DNA content is reduced following polyploidy, few studies have addressed the second aspect of diploidization: the mechanisms by which entire chromosomes are lost. Only recently have studies of synthetic polyploids demonstrated the high prevalence of chromosomal instability immediately after genome duplication [37–40]. While aneuploidy has been found to negatively correlate with fertility in synthetic *Brassica napus* polyploids [38] and pollen viability in synthetic wheat polyploids [40], the fact that chromosome number is no longer a suitable corollary for polyploidy history (e.g., *A. thaliana* has five chromosomes and five known polyploidy events), there must be some selective force to reduce chromosome number. Polyploid systems in which multiple temporal polyploid samplings (synthetic, nascent, ancient) occur will be critical for evaluating these diploidization processes in the future.

Diploidization also includes the loss of one homeologous copy of a duplicate gene pair, a process sometimes referred to as fractionation (see also contribution by Freeling et al., [85]). Both genes and regulatory elements may be lost, with losses accumulating through time so that an ancient polyploid may have only a small number of genes retained in duplicate. These losses may be random with respect to parental genome, or losses may come from predominantly one parental genome, a process referred to as biased fractionation, the long-term result of which is a genome that resembles that of the alternative parent. Some genes are consistently returned to singleton status whereas others, such as transcription factors, are typically retained in duplicate (see below).

Genome reorganization in recent polyploids: generating novelty

The genomes of newly formed natural polyploids, as well as those of synthetic polyploids, may experience rapid homeolog loss, as well as genome restructuring post-polyploidization, and altered patterns of gene expression may set the stage for subsequent loss of duplicate gene

Figure 1



Timeline showing origin of polyploid species, generation of novelty, environmental filtering, rapid radiation from selected line, and resulting signature of paleopolyploidy. Note that diploidization processes (not pictured) may occur continuously through this timeline. **(a)** Polyploid formation (in yellow circle) and attendant processes that yield variation in, for example, inflorescence morphology, karyotype, and homeolog loss; other novelties, for example, altered gene expression, transposon activity, are not pictured. **(b)** Array of polyploid genotypes/phenotypes, represented by different colors of rings around the photograph. **(c)** A single remaining polyploid 'type' after environmental filtering or drift. **(d)** Rapid radiation from this 'successful' polyploid and resultant formation of multiple lineages that trace back to this single common ancestor. **(e)** Deep phylogeny showing the correspondence (turquoise ring) between ancient WGD and radiation, as derived in d. Other paleopolyploid events are also shown. *Source*: *Credits*: J. Tate, V. Symonds, R. Buggs, M. Chester, I. Jordon-Thaden, D. Tank *et al*. (Figure (e) modified from Tank *et al*., 2015).

copies (e.g. [30,38,41–48]). Both the extent and speed with which these diverse changes occur may vary considerably across diverse polyploid systems (reviewed in [30]).

In the recent and repeatedly formed allotetraploids *Tragopogon mirus* and *T. miscellus*, species in the sunflower family that originated in the early 20th century [49], frequent homeolog loss, subfunctionalization, and major chromosomal changes, including translocations and compensated and non-compensated aneuploidy, were detected in natural populations, as well as in synthetic lines. Transcriptomic shock was observed; hybridization and polyploidy *per se* both play important roles in these young polyploids ([50–54]; reviewed in [43]). Investigations of an older allotetraploid (*T. castellanus*) and its parents indicate that gene loss/expression changes and chromosomal alterations mirror what is seen in the recently formed *T. mirus* and *T. miscellus* and demonstrate that some of the alterations that occur immediately post-polyploidization may be retained over long evolutionary timeframes ([55]; Soltis *et al.* unpublished). In *Senecio cambrensis* (also in the sunflower family and estimated to have originated in the 1700s; see review by Hegarty [42]), transcriptome shock was also detected; hybridization altered gene expression and DNA methylation, and genome duplication resulted in an additional burst of transcriptional and epigenetic change [42]. In the grass *Spartina anglica*, which originated in the 1800s (see review by Ainouche [41]), rapid changes in gene expression were observed; hybridization played a larger role in methylation changes than polyploidy *per se* [41]. Transcriptomic shock was detected; at the transcriptomic level, both hybridization and polyploidy are important. No chromosomal changes were noted.

Synthetic lines of older, established polyploids, including *A. suecica* [44,45,56,57], *Brassica napus* [38,46], and a synthetic *Arabidopsis* hexaploid [47], have also been used to assess genomic and expression changes that arise shortly after polyploid formation. Transcriptome shock and rapid changes in expression and methylation are also observed, and the relative importance of hybridization and genome doubling seems to vary among species. The

extent of homeolog loss versus expression changes also varies. Homeolog loss and chromosomal changes are frequent in *Brassica* (as in the natural polyploids of *Tragopogon*), but expression changes predominate in other systems (e.g., *Arabidopsi*s, as in the natural polyploid, *Spartina anglica*, which has a stable karyotype).

Comparisons of synthetic and natural polyploids also indicate variation in the repeatability of evolution across independently formed polyploid lines, whether natural or synthetic. In *Tragopogon*, *Senecio*, and *A. suecica*, the consequences of polyploidy are repeated — that is, the evolutionary tape of life is replayed. However, independent origins of synthetic *Brassica napus*, a synthetic *Arabidopsis* hexaploid [47], and *Spartina* hybrids respond differently. However, many aspects of polyploidy cannot be compared across all of these systems because of large gaps in the overall data set. For example, other features of polyploid genomes that may both contribute to genomic novelty in the short term and lead to genome downsizing over the longer term — e.g., transposon activity, methylation, subfunctionalization, and proteomic diversity — are only available for a few systems [30].

Macroevolutionary patterns of genome evolution in plants

The fact that there are many recognizable polyploids of fairly recent origin, but relatively little evidence for many ancient WGD events (paleopolyploidy; at least within the same evolutionary lineage), provides an interesting paradox. Although methods and data for detecting ancient WGDs are still limited, the inferred number of such events is increasing rapidly; tetraploid cottons ($2n = 52$), for example, originated in the last one million years [58] and have an estimated 30–36-fold duplication of ancestral angiosperm genes [59]. However, even as the picture of ancient WGDs is clarified, the number of such events will likely continue to underestimate the frequency of extant polyploids. This relative paucity of paleopolyploidy may reflect undetected WGDs due to limited genomic data; however, if real, this low frequency of ancient WGD suggests that polyploidy may be an evolutionary dead end, except perhaps in specific cases. Indeed, at some time in evolution, organisms that underwent and survived WGDs must have had an adaptive advantage. Examples of ancient WGD events that have been established on the longer term are one or two WGDs early in the evolution of seed and flowering plants [17], one WGD that is ancestral to most or all of the eudicots [60,61], and one or two that occurred early in the monocot lineage [16,59]. Therefore, a question that has received much attention of late is whether these key ancient WGDs, which in many cases characterize major lineages of flowering plants, have survived by coincidence, or whether they may have originated in concert, at very specific geological times, for instance during times of major ecological or environmental upheaval, and/or periods of extinction. In this respect, one of the most striking cases is a wave of WGDs in different flowering plant lineages that seem to coincide with the Cretaceous/Paleogene (K/Pg) boundary [62,63•,64–67]. Furthermore, many of the WGDs clustered around the K/Pg extinction event are at the base of some of the largest and most successful extant plant families and other large clades. Polyploidy thus somehow appears to be correlated with plant survival through the K/Pg boundary [63•] and with species diversification in angiosperms [68].

Once polyploids are formed, they must become locally established, reproduce, and survive while adapting to different environments. These processes might ultimately lead to their long-term evolutionary success, where their descendant lineages survive for tens of millions of years. Most likely, both neutral and adaptive processes contribute to polyploid establishment under stressful conditions in the short term. The adaptive scenario is mostly based on characteristics often displayed by newly formed polyploids, such as the formation of more extreme phenotypes in the resulting hybrid populations compared with their diploid parents. Moreover, genomic instability and gene expression changes soon after polyploid formation (shown to occur in both recent natural polyploids and synthetics; above) may result in increased phenotypic variability, which might be advantageous and allow rapid adaptation to changed environments and conditions [69–72]. Other potential adaptive advantages of newly formed polyploids include the masking of deleterious recessive alleles leading to increased mutational robustness. The neutral scenario gets support from the fact that levels of unreduced gamete formation can be increased by external stimuli such as stress and a fluctuating environment [73]. Temperature in particular has a pronounced effect on unreduced gamete formation. Moreover, increased levels of unreduced pollen in the fossil record were observed in the now-extinct conifer family Cheirolepidiaceae at the Triassic–Jurassic transition, which corresponds to the fourth of the five major extinction events [74], while abnormal gymnosperm pollen [75] and lycophyte spores [76] have also been reported from the Permian–Triassic transition, corresponding to the third of the five major extinction events. Increased unreduced gamete production during times of environmental stress and/or fluctuation could thus be an important factor in explaining the apparent clustering of paleopolyploidizations at the K/Pg boundary. It could also explain why many present-day polyploids often are more abundant in stressful environments, such as the Arctic [22] or disturbed habitats [77].

Although many genes undergo homeolog loss after WGD, others, particularly regulatory and developmental genes, are retained in excess after WGD. This pattern of gene loss and retention is most likely due to dosage-balance constraints and selection against loss of individual components of completely duplicated macromolecular complexes and/or

pathways, because this would disrupt their overall stoichiometry [78–81]. Retention of dosage-sensitive duplicates thus does not provide an immediate evolutionary advantage and adaptation, but results from the fact that their loss would lead to an immediate disadvantage. In this respect, the retained regulators may be considered an evolutionary spandrel [82], which might later on facilitate evolutionary innovations and/or diversifications. Selection to maintain dosage balance eventually relaxes over time allowing functional divergence and duplicated networks to be rewired to evolve novel functionality and increase biological complexity [83•], which could help explain the vast post-WGD success observed in some of the plant clades that experienced a WGD at the K/Pg boundary.

Synthesis: linking microevolutionary processes with macroevolutionary patterns

High levels of unreduced gamete formation in natural populations of angiosperms (e.g. [19••,20]) provide a mechanism for polyploid formation and ultimately speciation via allopolyploidy (involving interspecific hybridization) and autopolyploidy (formed within a species). Both modes of formation contribute substantially to angiosperm species diversity [84]. Nascent polyploids undergo an array of genomic and expression-level processes that may result simply from the presence of multiple genomes within the same nucleus. Although resolution of this multi-genome challenge may take multiple forms (chromosomal restructuring, homeolog loss, alterations in gene expression, among others), it is clear that polyploids are not merely the additive products of their diploid progenitors. Instead, they are mosaics of parental, additive, and novel features, and even young polyploid species appear to be composed of arrays of genetically unique individuals. Moreover, diploidization processes, while returning a polyploid to a diploid-like state, do not return the polyploid to the *original* diploid state — that is, some loci are retained in duplicate, singletons may derive from one parent or the other, and shifts in gene expression (neofunctionalization and subfunctionalization) render the diploidized polyploid unique. This novelty and range of phenotypic diversity may provide polyploid species with unusual adaptive capacity, particularly in times of high environmental stress. In fact, WGD events in angiosperms are non-randomly associated with bursts in diversification [68], and these radiations tend to be marked by novelty in morphology and/or chemistry. It is intriguing indeed to consider that the processes we observe in recent polyploids may explain patterns of WGD and key innovations across macroevolutionary timescales.

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