Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Post-polyploid diploidization and diversification through dysploid changes

Terezie Mandáková and Martin A Lysak

Whole-genome duplications are widespread across land plant phylogenies and particularly frequent in ferns and angiosperms. Genome duplications spurred the evolution of key innovations associated with diversification in many angiosperm clades and lineages. Such diversifications are not initiated by genome doubling *per se*. Rather, differentiation of the primary polyploid populations through a range of processes results in post-polyploid genome diploidization. Structural diploidization gradually reverts the polyploid genome to one functionally diploid-like through chromosomal rearrangements which frequently result in dysploid changes. Dysploidies may lead to reproductive isolation among post-polyploid offspring and significantly contribute to speciation and cladogenetic events.

Address
CEITEC – Central European Institute of Technology, Masaryk University, 62500 Brno, Czech Republic

Corresponding author: Lysak, Martin A (martin.lysak@ceitec.muni.cz)

Current Opinion in Plant Biology 2018, **42**:55–65

This review comes from a themed issue on **Genome studies and molecular genetics**

Edited by **Yves Van de Peer**

https://doi.org/10.1016/j.pbi.2018.03.001

Successive rounds of polyploidization and post-polyploid diploidization

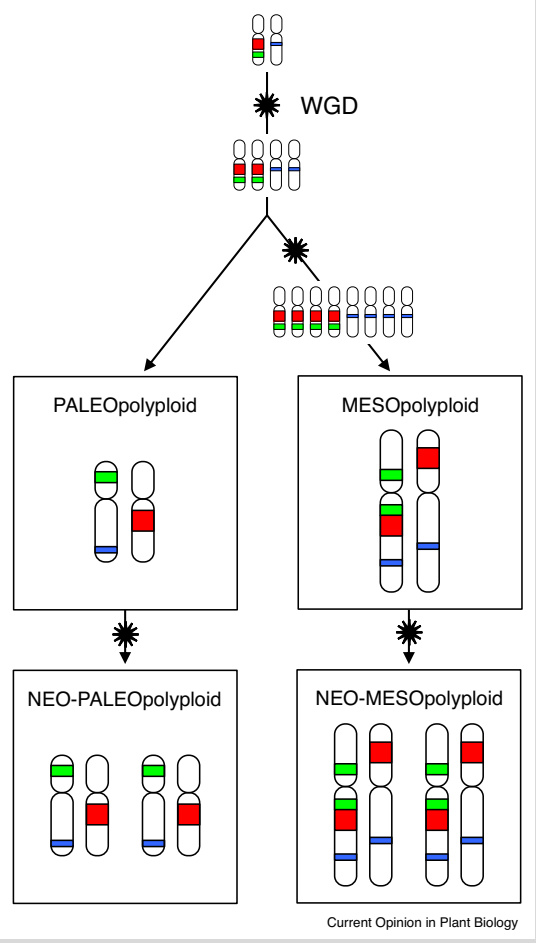
The explosion in genome sequencing projects has revealed that evolution of land plants was, to a large degree, shaped by multiple rounds of polyploidization or whole-genome duplication (WGD) events that significantly contributed to speciation and diversification in several lineages (e.g. [1–4,5•,6,7•]). Since publication of the *Arabidopsis thaliana* genome sequence, establishing its paleotetraploid nature [8], an increasing number of independent lineage-specific WGD events of different ages has been identified (e.g. [9–11,12,13•,14]). Extant angiosperm genomes range in complexity from those that have undergone only a few WGDs (e.g. *Amborella*, grapevine) to others that experienced genome multiplications of 128× (sugar cane), 144× (cotton) or even 288× (canola). Thus the contemporary paradigm of angiosperm genome evolution is based on cyclic alternation of polyploidization and diploidization processes, with each subsequent WGD being superimposed on the diploidized genomes from earlier rounds of polyploidy. Depending on the time which has elapsed since a WGD and the diploidization rate, WGD events can be broadly classified as neopolyploid, mesopolyploid and paleopolyploid [15,16] (Box 1).

Despite the substantial disadvantages of polyploidy, including the increased cost of genome replication, propensity of polyploid mitosis and meiosis to produce aneuploid cells, and epigenetic instability [17,18], paleogenomic data indicate that repeated cycles of polyploidization have generated evolutionarily advantageous genetic diversity [1,3,4,6,19]. Numerous papers and reviews have discussed the importance of ancient WGDs in increasing genome complexity and establishing 'a genomic playground' that allows new mutations to appear and become fixed (gene sub-functionalization and/or neofunctionalization), contributing to physiological and morphological innovations (e.g. [4,6,19–21]), colonization of new continents, habitats or ecological niches (e.g. [22,23••]), and survival of major cataclysms [3,24]; the 'evolutionary dominance' of angiosperms over gymnosperms may also be attributable to ancient polyploidy [25].

A polyploidization event is not itself likely to have initiated many of the diversifications documented across the angiosperm phylogeny; rather it was post-polyploid diploidization (PPD) that generated important genetic and taxonomic diversity in paleopolyploid and mesopolyploid lineages. The complex process of PPD, encompassing a variety of evolutionary modifications transforming a polyploid genome into a *quasi*-diploid one, is a largely overlooked and under-studied topic [26]. PPD is associated with a wide range of processes, such as genome downsizing, subgenome-specific fractionation (including biased gene retention/loss and gene sub-/neofunctionalization), modulation of gene expression, activation of transposable elements (TE) and epigenetic reprogramming (e.g. [4,26•,27,28,29•,30–33]). At the chromosomal level, PPD is mediated by inter-subgenome (homeologous) recombination and illegitimate recombination between TEs leading to structural chromosomal changes including reductions of chromosome number — post-polyploid descending dysploidy [16,23••,32,34–37,38••,39,40].

Box 1 Cytogenomic features of post-polyploid genome diploidization.

Depending on the time which has elapsed since WGD and on the rate of diploidization processes, polyploidization events can be classified as neopolyploid, mesopolyploid and paleopolyploid. Neopolyploids are the most recently formed polyploids (e.g. *Arabidopsis suecica*), which are characterized by additive genome size and chromosome number, duplicated 'single copy' genome regions and genes, largely intact and usually easily distinguishable (sub)genomes, and often by parental species still being extant. Within a given phylogenetic clade and with the passage of time, neopolyploids turn into mesopolyploids and subsequently into paleopolyploids due to progressive genome diploidization. Mesopolyploid genomes (e.g. *Brassica rapa*) exhibit diploidized genomes up to *quasi*-diploid complements with very low chromosome numbers, diploid-like meiosis and often biased subgenome fractionation. In mesopolyploids, parental subgenomes are discernible not only by bioinformatic analyses but also by comparative (cyto)genetic and phylogenetic approaches. Paleopolyploid genomes (e.g., *A. thaliana*) are usually characterized by highly diploidized genomes with a *quasi*-diploid number of linkage groups, diploid-like meiosis and often biased subgenome fractionation. With the exception of some slowly evolving paleopolyploids (e.g., *Vitis vinifera* [9]), the long-lasting and extensive genome restructuring in this category leads to assimilation of parental subgenomes. The footprints of paleopolyploidization can only be revealed by bioinformatic searches for orthologous and paralogous sequences, whereas (cyto)genetic and phylogenetic approaches fail to identify the subgenome structures.



In this review we will discuss the role of chromosomal rearrangements (CR), mainly those mediating descending dysploidy, in PPD and their potential significance for plant speciation and diversification.

Post-polyploid diploidization by descending dysploidy

Descending dysploidy, one of the most crucial routes of diploidization, means evolutionarily fixed decreases in base chromosome number (x). Descending dysploidy acting on polyploid genomes has been termed polyploid drop [41] and is traditionally viewed as one of the mechanisms that turn polyploids into functional diploids. Currently available comparative genomic data on large angiosperm families, such as grasses [42], crucifers (e.g. [38••]), Asteraceae [13•] and Solanaceae [12], suggest that post-polyploid descending dysploidies are much more frequent than the converse, ascending dysploidy, that is, evolutionary increases in base chromosome number usually mediated by breakage at centromeres (centric fission).

Multiple base chromosome numbers resulting from PPD

Several genera and higher-order taxa of angiosperms exhibit variations in base chromosome numbers. This is characteristic of several genera, tribes or subfamilies of Asteraceae, Brassicaceae, Cyperaceae, Fabaceae, Poaceae and Rosaceae [12,13•,34,35,38••,43–46]. In Cyperaceae (sedges), the extensive variation in chromosome number is underlined by the nature of holocentric chromosomes [44], but the mechanisms generating base number variation in taxa with monocentric chromosomes remained unclear until recently. Recent technical advances in genome analysis have pinpointed independent diploidizations as the prime causative agent of chromosome number variation in many polybasic plant groups (i.e. taxa with multiple base numbers). Analysis of genome evolution in mesopolyploid lineages is particularly enlightening because the relatively recent occurrences of these WGDs allow us to reconstruct genome evolution in polybasic taxa. The genus *Brassica* and tribe Brassiceae (Brassicaceae) were the first taxa where numerical variation was identified, by comparative cytogenetic analysis, as resulting from multiple diploidizations of the mesohexaploid ancestor(s) [34,35]. Recently, Mandáková and colleagues [38••] combined comparative cytogenomic and transcriptome analysis to reveal that at least eleven (22%) out of 49 tribes of Brassicaceae [47] diversified after a WGD or whole-genome triplication (WGT) event. Almost all the mesopolyploid tribes are polybasic, with diploid-like chromosome numbers predominating [38••].

All the above-mentioned studies strongly support the hypothesis that chromosome number variation in polybasic angiosperm genera or higher-order taxa represents a

consequence of WGDs and subsequent diploidizing descending dysploidies. Similarly, multiple base chromosome numbers can serve as a proxy for paleopolyploid or mesopolyploid WGDs followed by PPD. This points to an important conclusion: chromosome number alone is not a reliable indicator of a taxon's ploidy level and its evolutionary past [37]. Progressive diploidization conceals the polyploid nature of *bona fide* diploid genomes because descending dysploidy frequently reverts the number of linkage groups to the same (or even a lower) number as in the diploid ancestor (e.g. maize and sorghum both have $n = 10$ despite a WGD having occurred only in the maize ancestor [42]). Now we realize that many plant species traditionally regarded as diploids are actually diploidized paleopolyploid or mesopolyploid, and that clade-specific WGD events in land plants are probably far commoner than previously thought (e.g. [13•,38••]).

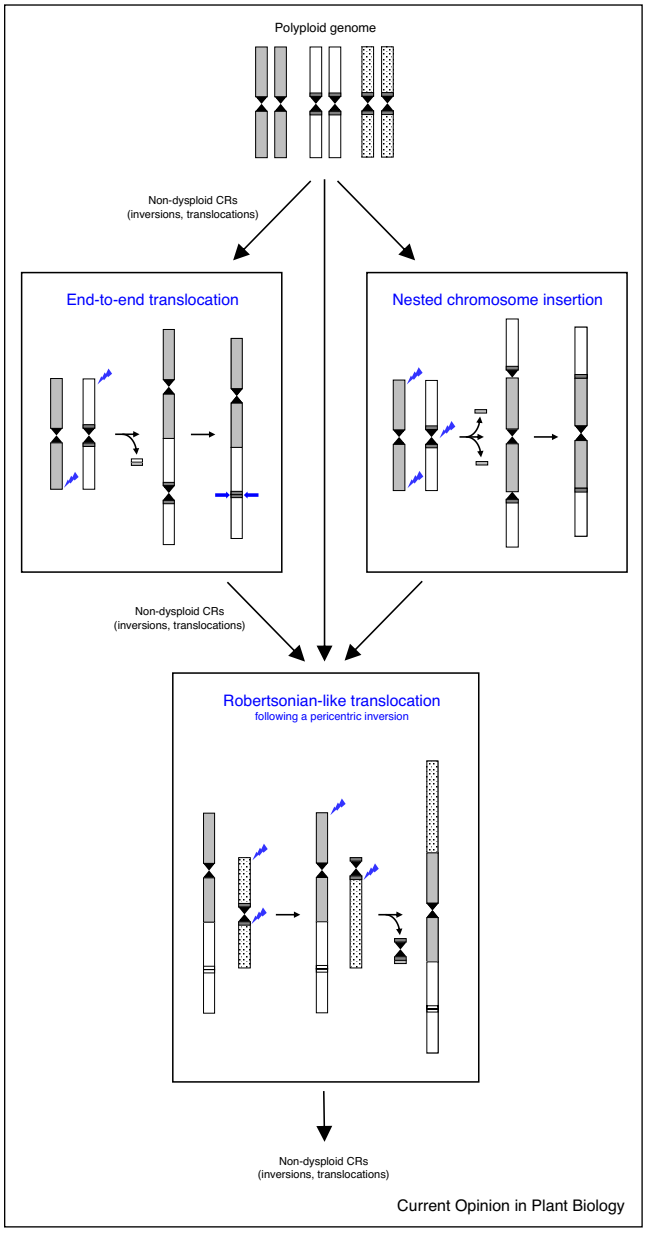
However, as the identification of younger WGDs and whole-genome analysis of polybasic taxa are in their infancy, it would be premature to conclude that PPD represents a one-way ticket allowing polyploid genomes to turn into *quasi*-diploid ones via descending dysploidy. It is quite plausible that ascending dysploidy based on centric fissions is a prominent mechanism in some families, such as orchids [48,49]. Moreover, hybridization between two genomes having different chromosome numbers, with or without subsequent dysploidy, may give rise to new base chromosome numbers (e.g. [45,50]). Fertilization of euploid gametes by aneuploid ones ($n + 1$ or $n - 1$) may result in chromosome numbers mimicking the outcome of ascending or descending dysploidy [51•,52]. Similarly, aneuploid variants generated due to post-polyploid instability of neopolyploids (e.g. [53,54]) may become evolutionarily fixed. Thus, although we propose that PPD via dysploid changes is frequent in some — perhaps many — plant families, its importance in generating chromosome number variation needs to be corroborated.

Mechanisms underlying post-polyploid descending dysploidy

Translocations among non-homologous and homeologous chromosomes form the mechanistic basis of descending dysploidy. Three whole-chromosome/arm translocation-based processes have been identified as possible mechanisms reducing base chromosome numbers during PPD (Figure 1): end-to-end translocation (EET), nested chromosome insertion (NCI) and Robertsonian or Robertsonian-like translocation. Naturally, chromosomal diploidization can be accompanied by various non-dysploid CRs, such as inversions, reciprocal translocations, deletions and duplications [55].

EETs result from two double-strand breaks (DSB) at terminal regions of two different chromosomes followed

Figure 1



The proposed hierarchy of chromosomal rearrangements (CRs) underlying post-polyploid structural diploidization including descending dysploidy in angiosperms. The initial phase of descending dysploidy may be dominated by whole-chromosome translocations, that is, end-to-end translocations (EET) and nested chromosome insertions (NCI), or by Robertsonian and Robertsonian-like reciprocal translocations. EET between two non-homologous chromosomes is mediated by two DSBs at chromosome ends and results in the formation of a dicentric chromosome. In order to stabilize a dicentric translocation chromosome one of the two centromeres has to become inactive and/or be removed. NCI are termed translocation events when an 'insertion' chromosome becomes translocated into the (peri)centromere of a 'recipient' chromosome; at least three DSBs are needed for NCI. Descending dysploidy through a Robertsonian-like translocation assumes recombination between a telo/acrocentric and a chromosome of any type, with DSBs at the (peri)centromere of the long arm of the telo/

by recombination tandemly merging the two chromosomes. Due to the, usually considerable, distance between the two centromeres on the translocation chromosome, one of the centromeres must become inactive or eliminated to avoid segregation problems which affect dicentric chromosomes [55,56]. The molecular mechanism of centromere inactivation remains elusive; it presumably includes epigenetic silencing and/or recombinational sequence removal [56]. EET events can be inferred from retention of synteny blocks corresponding to a whole ancestral chromosome without an active centromere. EETs seem to be rare in grasses [42,57]; however, compelling evidence for descending dysploidy via EET has been obtained for mesopolyploid Brassicaceae clades [16,23••,36,37,38••]. In crucifers, EETs are thought to be the initial mechanism for descending dysploidy, followed by Roberstonian-like CRs [23••].

NCI merges two non-homologous chromosomes by an illusive insertion of one ('insertion') chromosome into or near the centromere of the second ('recipient') chromosome. At least three DSBs are needed to ensure translocation of the insertion chromosome between the arms of the recipient chromosome. For as yet unclear reasons, NCIs represent the predominant mechanism for descending dysploidy in grasses [42,46,58], whereas they have only been rarely documented in other families (crucifers [36,59], legumes [60], poplar/Salicaceae [61]).

A Robertsonian translocation transforms two telocentric or acrocentric chromosomes into one (sub)metacentric chromosome [62]. Alternatively, descending dysploidy occurs through translocation events involving one DSB close to the centromere of the long arm of a telocentric or acrocentric chromosome (frequently formed by pericentric inversions) and one within a (sub)telomeric region of any type of chromosome (Robertsonian-like translocation [63]). More extensive descending dysploidies in crucifer lineages, and probably in other angiosperm families, were mediated by pericentric inversions and Robertsonian-like translocations which occurred after the initial EET phase [16,23••,36,37].

Biased chromosomal fractionation

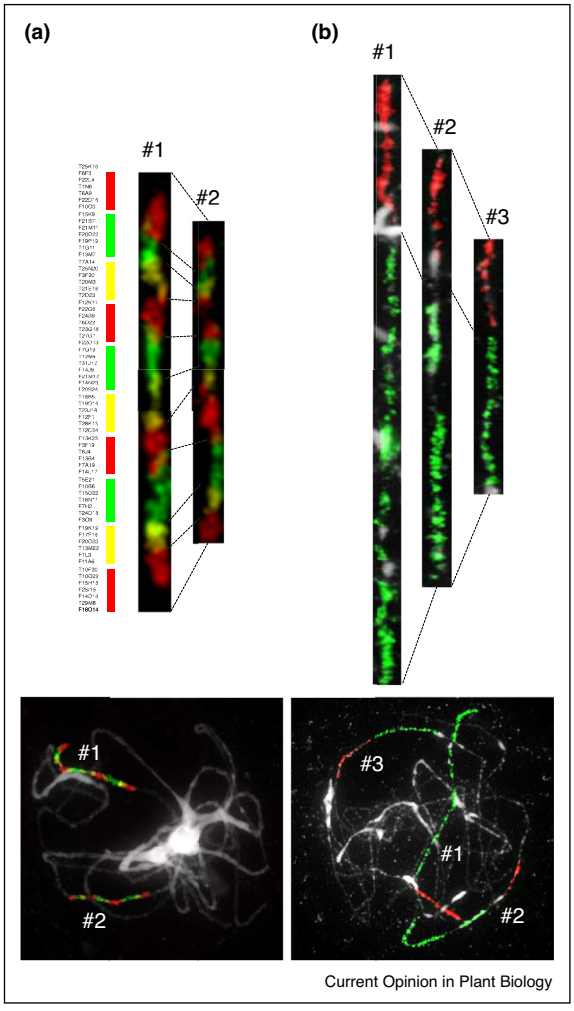
It has been repeatedly suggested that paralogous genes of one subgenome in a diploidized polyploid genome are preferentially retained and exhibit higher gene expression levels relative to the other (more fractionated) subgenome(s). This phenomenon, termed biased gene fractionation [64], has been widely observed across angiosperm lineages [29•,38••,39,40,64–67]. At the chromosomal level, differential subgenome fractionation was first observed in mesopolyploid *Brassica* genomes [34,35] and later documented by comparative chromosome painting in other crucifer mesopolyploids [16,23••,38••,40,68,69] (Figure 2). Biased subgenome fractionation is manifested as different physical lengths of homeologous chromosomal segments and a corresponding reduction in fluorescence intensity of the (smaller) segments identified by FISH with specific DNA probes. Since cytogenetic subgenome differentiation has been observed only in mesopolyploid taxa which have undergone extensive and long-term PPD [38••], the phenomenon is unlikely to be related to primary differences between the parental (allo)genomes; rather it reflects biased fractionation at the genic level [64].

Different rates of post-polyploid descending dysploidy

Genome reshuffling and decreases in chromosome number do not proceed with the same tempo and intensity along all clades descending from a single WGD event. The extent and rate of PPD should be positively correlated with the level of genome duplication and the time elapsed since a WGD event. Hexaploids should contain more templates not only for homeologous recombination but also for illegitimate recombination between TEs [e.g. LTRs serving as ectopic homologous templates for DSB (mis-)repair] than tetraploid genomes. Indeed, this relationship was found to hold true for most mesopolyploid events across the Brassicaceae. Mesohexaploid clades showed on average more advanced descending dysploidies than mesotetraploid ones [38••]. This correlation may, however, be related not only to genome redundancy, but in some instances also to a greater age and longer diploidization process in hexaploids, which are usually formed by two successive hybridization/polyploidization events. Moreover, as progressive diploidization inevitably decreases the primary genome redundancy of polyploid genomes, any new CRs can be deleterious and compromise fitness [32]. This risk is apparently lower in higher-level polyploid genomes and therefore the toleration of mis-repair and thus degree of genome shuffling can be somewhat higher in hexaploids than in tetraploids. Intuitively, ancient autopolyploid genomes should manifest more extensive PPD than allopolyploid ones. The lack of subgenome dominance and biased fractionation in banana ($n = 11$), poplar ($n = 18$) and soybean ($n = 20$) [29•,70,71] suggests that these genomes may have originated by autopolyploidizations. However, the relatively high chromosome numbers in these plants do not indicate that autopolyploid genomes should be more prone to diploidizing descending dysploidy than allopolyploid

(Figure 1 Legend Continued) acrocentric and any chromosome end of the second chromosome. The terminal centromere position of the acro/telocentric may result from a preceding pericentric inversion on an originally metacentric chromosome. Robertsonian-like translocation produces a large translocation chromosome, comprising most of the four original chromosome arms, and a small minichromosome formed chiefly of the telo/acrocentric's centromere. The minichromosome is meiotically, and, if very small, also mitotically unstable and hence becomes eliminated. Structural diploidization is also accompanied by various non-dysploid chromosomal rearrangements (CR), such as inversions, reciprocal translocations, deletions and duplications.

Figure 2



Cytogenetic evidence for biased subgenome fractionation during post-polyploid diploidization. Fine-scale comparative chromosome painting of ancestral genomic block A [85] with *A. thaliana* BAC clones in pachytene complements of two Brassicaceae mesopolyploid genomes. **(a)** The mesotetraploid *Biscutella laevigata* ($n = 9$ [40]), **(b)** the mesohexaploid *Leavenworthia alabamica* ($n = 11$ [69]). The individual paralogous copies differ in length and fluorescence intensity (the longest and brighest copy is always labeled as #1). The painted regions of pachytene chromosomes were straightened using Image J [94].

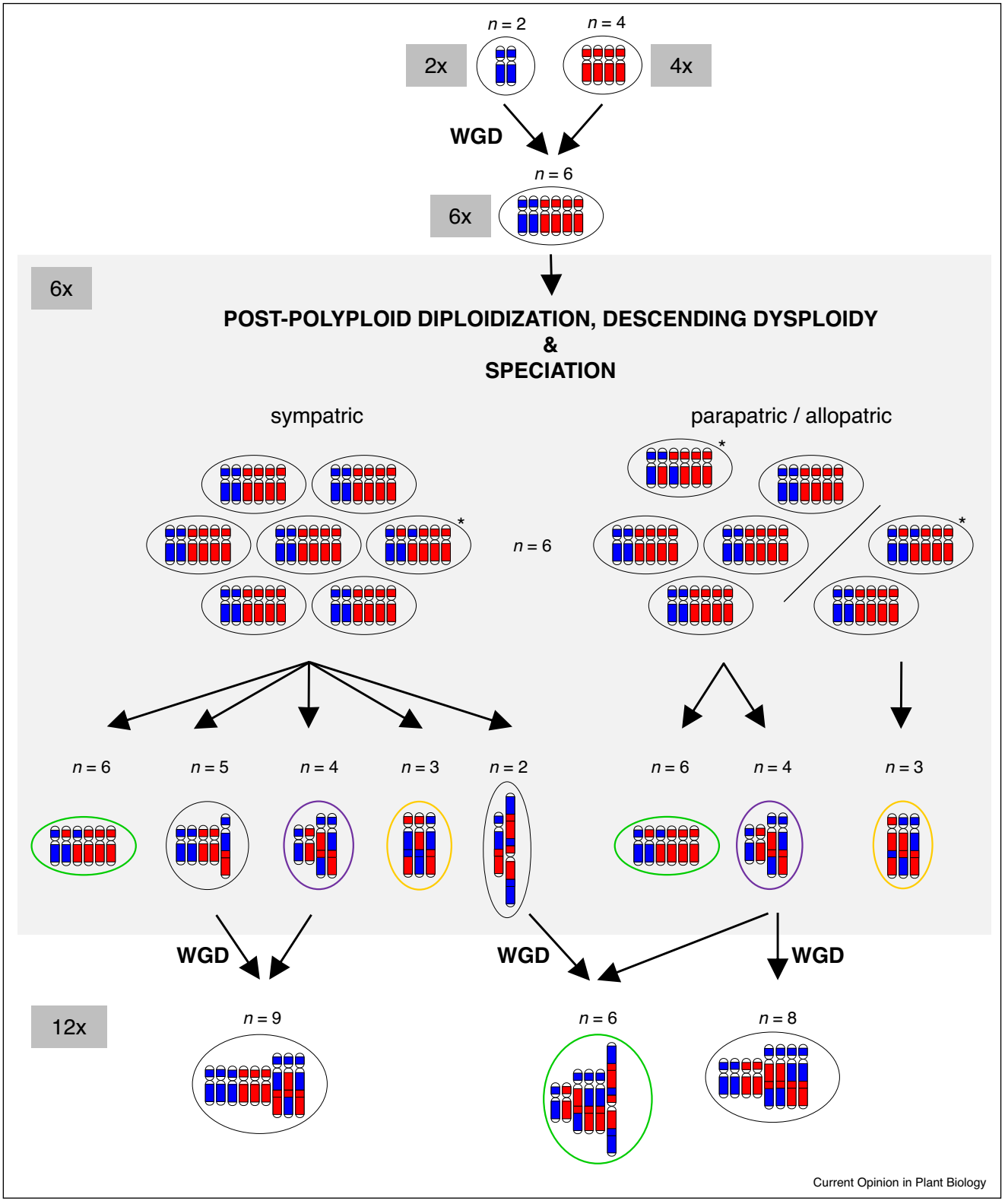
ones. The autopolyploid soybean genome ($n = 20$), despite exhibiting a much larger number of chromosomal rearrangements than the allotetraploid maize genome ($n = 10$), has twice the number of linkage groups than maize [71]. More data are required to elucidate the relationship between types of polyploidization and character of PPD.

Variable extant chromosome numbers and genomic differences, that is, the unequal progression of diploidization among diploidized polyploid genomes, point to differential rates of PPD including potential delays in PPD ('lag') and alternation of periods of genome reshuffling and relative genome stasis. In crucifers, PPD of the α-paleotetraploid produced a diploidized $n = 8$ genome placed at the base of the Lineage I/Clade A super-tribe, radiating some 23 million years ago (mya) [72]. Whereas the diploidized paleotetraploid genome remained structurally unchanged in several Camelineae taxa, a 1.6-fold descending dysploidy ($n = 8 \rightarrow n = 5$) resulted in the origin of five chromosomes of *Arabidopsis thaliana* [63] after c. 17 mya of genomic stasis (as *A. thaliana* originated c. 6 mya, [72]). For mesopolyploid crucifer clades, Mandáková *et al.* [23••] demonstrated that the allotetraploid ancestor of the Microlepidieae with ($n = 15$) probably experienced three different routes of PPD that led to least ($n = 12$), medium-level ($n = 10$) and most diploidized ($n = 4$–7) genomes.

Differential rates of descending dysploidy are influenced by, among other factors, life histories and mating systems. Descending dysploidies are thought to proceed faster in annuals than in perennial and woody plants [62]. Herbaceous plants show, on average, higher rates of molecular evolution (e.g. nucleotide substitution rates) than trees and shrubs with longer generation times [73–76]. Genomes of several woody angiosperms and gymnosperms, including grape [9], walnut [76], conifers [11,77], Pyreae (e.g. apple [45]), and the willow family [61,70], are characterized by slow rates of PPD manifested as absence of, or very moderate, descending dysploidy. The higher number of generations in annuals is also associated with a higher probability of DSB misrepair potentially generating CRs, including those mediating descending dysploidy. A lower number of chromosomes, smaller genome and faster life cycle can confer a valuable competitive advantage on diploidized ephemeral and annual plants in adapting to seasonally-available habitats. However, life history should not be treated as a universal proxy for accelerated or decreased rate of descending dysploidy. Despite the annual lifestyle of rice, its genome exhibits a slow rate of descending dysploidy compared to other members of the grass family (e.g. [42]).

Like other CRs, newly arising dysploid karyotypes are underdominant within the progenitor population and reduce fitness in the heterozygous condition. Structural heterozygotes can be fixed or turned to homozygotes due to self-fertilization [78]. Because selfing is often found to be associated with annual life cycles (e.g. [79]), chromosomal dysploidy is believed to become more easily fixed in annual selfers. Besides selfing, apomictic reproduction can be advantageous in fixing newly arising karyotype variants. Hoerandl and Hojsgaard [80] highlighted the advantage of (facultative) apomixis first in overcoming potential meiotic problems in polyploid ancestors and later in fixing new genotypes formed by PPD. However,

Figure 3



Speciation and diversification driven by post-polyploid diploidization (PPD) via descending dysploidy. The origin of a hypothetical paleohexaploid ancestor ($n = 3\times = 6$) followed by speciation events and cladogenesis as a consequence of parallel PPDs. Diploidization, here operating mainly through descending dysploidy and other non-dysploid CRs (in genomes labeled by an asterisk), proceeds with different rates and efficacies

apomicts may revert to full sexuality during PPD and a new WGD can re-initiate a shift to apomixis [15,80].

Structural diploidization and dysploid changes may enforce speciation and cladogenesis

The origin of chromosome number variation accompanying PPD can be correlated with speciation events, adaptive radiations and cladogenesis. However, many radiations are difficult to interpret due to uncertainty over whether dysploid changes facilitated speciation or karyotype reshuffling occurred after the speciation event (s). Despite these impediments, recent advances in inference of genome duplications and their phylogenetic placement have furnished convincing evidence that WGDs occurred before large radiations across the angiosperms [1,4,5•,7•,9,24,80]. Such WGD events were frequently, though probably not always, followed by a phylogenetic split at the base of a lineage into a large diversified crown-group clade and smaller, much less diversified sister clade [5•,81••]. The observation that post-polyploid diversification of the crown group frequently commenced million of years after the corresponding WGD (e.g. [7•,81••]) was reflected in the WGD Radiation Lag-Time Model [81••]. The lag between a WGD and subsequent diversification suggests that the polyploid ancestor genome, or better, its populations, must undergo some 'adjustment' — the process of genome diploidization.

We propose that diploidization, including large-scale genome alterations and descending dysploidy, can trigger speciation and cladogenesis [7•,23••,26•,38••]. As described in the preceding section (*Different rates of post-polyploid descending dysploidy*), chromosome 'fusions' may be associated with adaptive advantages, including shortening of DNA replication, cell cycle and meiosis, altered gene expression, formation of supergenes (i.e. tightly linked genes), and reduced recombination between locally adapted alleles [82•]. By analyzing 15 angiosperm clades, Escudero *et al.* [83] found that dysploid transitions often co-occurred with polyploidy and that dysploidy may have persisted longer evolutionarily than polyploidy, hence dysploidies may have played an important role in angiosperm cladogenesis. While large-scale CRs do not necessarily change the number of linkage groups even over extensive periods of time, ongoing genome-wide homeologous recombination promotes dysploid changes in the diploidizing polyploid offspring. PPD acting with differing intensities on individuals or whole populations of the primary polyploid may generate genetically variable progenies with reproductive barriers, eventually resulting in speciation and cladogenetic events (Figure 3). The picture can be even more complex if polyploids originate recurrently and polytopically [84].

Impact of post-polyploid dysploid change on diversification of large angiosperm families

With the steadily increasing number of newly identified WGD events across land plant phylogenies, the association between ancient genome duplications, diversification and diploidizing descending dysploidies becomes more evident. Here we discuss the issue for some particularly species-rich and/or relatively well researched angiosperm families.

Whereas in crucifers (Brassicaceae) and grasses (Poaceae), the two angiosperm families with the most comprehensive information on karyotype evolution, the trend of diploidization of paleopolyploid and mesopolyploid genomes via descending dysploidy is illustrated by a number of studies ([23••,42,85,86••,87 and references therein]), the patterns in the largest vascular plant family, the Orchidaceae (c. 28 000 species and 736 genera [88]), are much less well resolved. The three sequenced orchid genomes have quite high chromosome numbers ($2n = 38$ and $2n = 68$), suggesting polyploid origins. Zhang *et al.* [14] identified a WGD pre-dating the early divergence of the orchid family, which could have been instrumental in orchid diversification. A 20-fold variation in chromosome numbers ($n = 6$ to $n = 120$ [89,90]) suggests that many more clade-specific WGDs and diploidizations occurred during orchid evolution (see also [91]). Strikingly different chromosome numbers, sizes and morphologies between orchid subfamilies and tribes indicate that ascending as well as descending dysploidies followed WGDs in orchids [48,49,91].

The Asteraceae (Compositae) is the second largest family of vascular plants, harboring some 24 700 species [88]. Asteraceae are well known for their enormous karyological variation, with more than 180 different mitotic chromosome counts known and chromosome numbers ranging from $n = 2$ to c. $n = 216$ [92]. Recently, by analyzing transcriptome sequences of 73 species from 18 tribes in six Asteraceae subfamilies, Huang *et al.* [13•] identified several WGDs of different ages, including

(Figure 3 Legend Continued) alongside other processes of genetic divergence (e.g., genetic drift). Independent descending dysploidies and genome-wide molecular diploidization generate a wide range of partly or fully reproductively isolated *quasi*-diploid genomes which may turn into new species as the basis of an explosive radiation. The chromosome number diversity among the *quasi*-diploid species renders new taxa (e.g. genera) polybasic. New rounds of WGDs start the next polyploidization–diploidization cycle. Autonomous diploidization processes and additional WGDs may generate identical chromosome numbers.

several tribe-specific WGDs, placed at various nodes of the Asteraceae family tree. If the inferred WGDs and base chromosome numbers are plotted on the phylogeny, it becomes apparent that PPD in various Asteraceae clades worked with differing efficacy. For instance, early-branching tribes of the core Asteraceae possess $n = 27$, tribes of the Heliantheae alliance have $n = 17$ or 19, whereas the predominantly African tribes analyzed have reduced chromosome complements of $n = 9$ or 10 despite at least two subsequent WGD events. This and earlier studies (e.g. [93]) clearly point to the importance of polyploidization and diploidizing descending dysploidy in generating the enormous karyological and taxonomic variation of Asteraceae.

In legumes (Fabaceae; 19 500 species [88]), the current understanding of genome evolution assumes that the family descended from a γ-paleohexaploid ($n = 21$ [86••]) followed by a legume-specific WGD [43]. The Indigoferoid/Millettioid clade was proposed to comprise the ancestral paleotetraploid genomes with eleven linkage groups ($n = 11$), followed by descending dysploidy in some *Phaseolus* species ($n = 10$ [60]). PPD was more prominent in the Dalbergioid and Hologalegina clades, which were shaped by descending dysploidy toward $n = 10$ to 6 [43].

The Solanaceae (2600 species [88]) underwent the eudicot γ-WGT and a younger family-specific WGT followed by diversification associated with clade-specific PPD [12]. The chromosome number of the family's paleohexaploid ancestor was most likely $n = 12$ or higher and its subsequent evolution followed stasis or descending dysploidy in the *Nicotiana*/*Solanum* clade ($n = 12$) and reduction to either $n = 9$ or $n = 7$ in the *Calibrochoa*/*Petunia* clade.

Concluding remarks

The evolutionary role of post-polyploid diploidization is emerging from the shadow of WGDs. Although dysploid chromosomal changes have been recognized and sometimes associated with ancient polyploidization since Darlington's time, the recent advent of new phylogenomic tools has spurred renewed interest in analyzing the impact of dysploid changes. Given the vast species and chromosome number variation across land plants, more in-depth studies focusing on identifying clade-specific WGDs and reconstructing chromosomal evolution are needed. New paleogenomic data together with experimentally induced post-polyploid dysploidies should elucidate the role of dysploid changes in genome evolution and speciation.

Acknowledgements

We would like to thank Ingo Schubert for his comments on the manuscript. This work was supported by a research grant from the Czech Science Foundation (grant no. P501/12/G090) and the CEITEC 2020 (grant no. LQ1601) project.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, Depamphilis CW, Wall PK, Soltis PS: **Polyploidy and angiosperm diversification**. *Am J Bot* 2009, **96**:336-348.

2. Soltis PS, Marchant DB, Van de Peer Y, Soltis DE: **Polyploidy and genome evolution in plants**. *Curr Opin Genet Dev* 2015, **35**:119-125.

3. Vanneste K, Baele G, Maere S, Van de Peer Y: **Analysis of 41 plant genomes supports a wave of successful genome duplications in association with the Cretaceous–Paleogene boundary**. *Genome Res* 2014, **24**:1334-1347.

4. Edger PP, Heidel-Fischer HM, Bekaert M, Rota J, Glöckner G, Platts AE, Heckel DG, Der JP, Wafula EK, Tang M *et al.*: **The butterfly plant arms-race escalated by gene and genome duplications**. *Proc Natl Acad Sci U S A* 2015, **112**:8362-8366.

5. • Tank DC, Eastman JM, Pennell MW, Soltis PS, Soltis DE, Hinchliff CE, Brown JW, Sessa EB, Harmon LJ: **Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications**. *New Phytol* 2015, **207**:454-467.
Analysis of nine well-documented ancient WGD events demonstrating a non-random association between WGDs and a delayed increase in rates of diversification, as articulated by the WGD Radiation Lag-Time Model [81••].

6. Soltis PS, Soltis DE: **Ancient WGD events as drivers of key innovations in angiosperms**. *Curr Opin Plant Biol* 2016, **30**:159-165.

7. • Clark JW, Donoghue PCJ: 1: **Constraining the timing of whole genome duplication in plant evolutionary history**. *Proc Biol Sci 2017* 2017, **284** pii: 20170912.
Dating of two major WGD events shared by all seed plants (399–381 mya) and flowering plants (319–297 mya). This study supports the WGD Radiation Lag-Time Model [5,81••] and the importance of post-polyploid diploidization preceding major radiations.

8. The Arabidopsis Genome Initiative: **Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana***. *Nature* 2000, **408**:796-815.

9. Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C *et al.*: **The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla**. *Nature* 2007, **449**:463-467.

10. Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS *et al.*: **Ancestral polyploidy in seed plants and angiosperms**. *Nature* 2011, **473**:97-100.

11. Li Z, Baniaga AE, Sessa EB, Scascitelli M, Graham SW, Rieseberg LH, Barker MS: **Early genome duplications in conifers and other seed plants**. *Sci Adv* 2015, **1**:e1501084.

12. Bombarely A, Moser M, Amrad A, Bao M, Bapaume L, Barry CS, Bliek M, Boersma MR, Borghi L, Bruggmann R *et al.*: **Insight into the evolution of the Solanaceae from the parental genomes of *Petunia hybrida***. *Nat Plants* 2016, **2**:16074.

13. • Huang CH, Zhang C, Liu M, Hu Y, Gao T, Qi J, Ma H: **Multiple polyploidization events across Asteraceae with two nested events in the early history revealed by nuclear phylogenomics**. *Mol Biol Evol* 2016, **33**:2820-2835.
A most comprehensive study identifying multiple WGDs in Asteraceae. These findings to a large degree elucidate the 108-fold variation in chromosome numbers and frequent desceding dyploidies across Asteraceae [92].

14. Zhang GQ, Liu KW, Li Z, Lohaus R, Hsiao YY, Niu SC, Wang JY, Lin YC, Xu Q, Chen LJ *et al.*: **The *Apostasia* genome and the evolution of orchids**. *Nature* 2017, **549**:379-383.

15. Carman JG: **Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony**. *Biol J Linn Soc* 1997, **61**:51-94.

16. Mandáková T, Joly S, Krzywinski M, Mummenhoff K, Lysak MA: **Fast diploidization in close mesopolyploid relatives of *Arabidopsis***. *Plant Cell* 2010, **22**:2277-2290.

17. Moore G: **Meiosis in allopolyploids — the importance of 'Teflon' chromosomes**. *Trends Genet* 2002, **18**:456-463.

18. Comai L: **The advantages and disadvantages of being polyploid**. *Nat Rev Genet* 2005, **6**:836-846.

19. Soltis DE, Visger CJ, Soltis PA: **The polyploidy revolution then . . . and now: Stebbins revisited**. *Am J Bot* 2014, **101**:1-22.

20. Van de Peer Y, Maere S, Meyer A: **The evolutionary significance of ancient genome duplications**. *Nat Rev Genet* 2009, **10**:725-732.

21. Madlung A: **Polyploidy and its effect on evolutionary success: old questions revisited with new tools**. *Heredity* 2013, **110**:99-104.

22. te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubesová M, Pysek P: **The more the better? The role of polyploidy in facilitating plant invasions**. *Ann Bot* 2012, **109**:19-45.

23. •• Mandáková T, Pouch M, Harmanová K, Zhan SH, Mayrose I, Lysak MA: **Multi-speed genome diploidization and diversification after an ancient allopolyploidization**. *Mol Ecol* 2017, **26**:6445-6462.
A pioneer cytogenomic study showing that post-polyploid diploidization can proceed with different rates and efficacies, and can be associated with diverse life histories.

24. Fawcett JA, Maere S, van de Peer Y: **Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction event**. *Proc Natl Acad Sci U S A* 2009, **106**:5737-5742.

25. Leitch AR, Leitch IJ: **Ecological and genetic factors linked to contrasting genome dynamics in seed plants**. *New Phytol* 2012, **194**:629-646.

26. • Dodsworth S, Chase MW, Leitch AR: **Is post-polyploidization diploidization the key to the evolutionary success of angiosperms?** *Bot J Linn Soc* 2016, **180**:1-5.
The authors argue that the diploidization process is responsible for the lag period between WGD events and lineage diversifications in flowering plants [81••].

27. Freeling M: **Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition**. *Annu Rev Plant Biol* 2009, **60**:433-453.

28. Renny-Byfield S, Gong L, Gallagher JP, Wendel JF: **Persistence of subgenomes in paleopolyploid cotton after 60 my of evolution**. *Mol Biol Evol* 2015, **32**:1063-1071.

29. • Garsmeur O, Schnable JC, Almeida A, Jourda C, D'Hont A, Freeling M: **Two evolutionarily distinct classes of paleopolyploidy**. *Mol Biol Evol* 2014, **31**:448-454.
The authors suggest that WGDs with genome dominance and biased fractionation may result from ancient allotetraploidizations, while WGDs without genome dominance or biased fractionation may result from ancient autotetraploidies.

30. Conant GC, Birchler JA, Pires JC: **Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time**. *Curr Opin Plant Biol* 2014, **19**:91-98.

31. Zenil-Ferguson R, Ponciano JM, Burleigh JG: **Evaluating the role of genome downsizing and size thresholds from genome size distributions in angiosperms**. *Am J Bot* 2016, **103**:1175-1186.

32. Schubert I, Vu GT: **Genome stability and evolution: attempting a holistic view**. *Trends Plant Sci* 2016, **21**:749-757.

33. Vicient CM, Casacuberta JM: **Impact of transposable elements on polyploidy plant genomes**. *Ann Bot* 2017, **120**:195-207.

34. Lysak MA, Koch MA, Pecinka A, Schubert I: **Chromosome triplication found across the tribe Brassiceae**. *Genome Res* 2005, **15**:516-525.

35. Lysak MA, Cheung K, Kitschke M, Bures P: **Ancestral chromosomal blocks are triplicated in Brassiceae species with varying chromosome number and genome size**. *Plant Physiol* 2007, **145**:402-410.

36. Mandáková T, Heenan PB, Lysak MA: **Island species radiation and karyotypic stasis in *Pachycladon* allopolyploids**. *BMC Evol Biol* 2010, **29**:367.

37. Mandáková T, Gloss AD, Whiteman NK, Lysak MA: **How diploidization turned a tetraploid into a pseudotriploid**. *Am J Bot* 2016, **103**:1187-1196.

38. •• Mandáková T, Li Z, Barker MS, Lysak MA: **Diverse genome organization following 13 independent mesopolyploid events in Brassicaceae contrasts with convergent patterns of gene retention**. *Plant J* 2017, **91**:3-21.
Evidence for several clade-specific mesopolyploid WGDs across the mustard family. The WGDs were followed by post-polyploid diploidization toward diploid-like chromosome numbers. These results suggest that variation in chromosome numbers in many groups of flowering plants can result from independent diploidizing descending dysploidies.

39. Murat F, Zhang R, Guizard S, Flores R, Armero A, Pont C, Steinbach D, Quesneville H, Cooke R, Salse J: **Shared subgenome dominance following polyploidization explains grass genome evolutionary plasticity from a seven protochromosome ancestor with 16K protogenes**. *Genome Biol Evol* 2014, **6**:12-33.

40. Geiser C, Mandáková T, Arrigo N, Lysak MA, Parisod C: **Repeated whole-genome duplication, karyotype reshuffling and biased retention of stress-responding genes in Buckler Mustards**. *Plant Cell* 2016, **28**:17-27.

41. Darlington CD: *Chromosome Botany*. George Allen and Unwin; 1956.

42. Murat F, Xu JH, Tannier E, Abrouk M, Guilhot N, Pont C, Messing J, Salse J: **Ancestral grass karyotype reconstruction unravels new mechanisms of genome shuffling as a source of plant evolution**. *Genome Res* 2010, **20**:1545-1557.

43. Wang J, Sun P, Li Y, Liu Y, Yu J, Ma X, Sun S, Yang N, Xia R, Lei T: **et al.: Hierarchically aligning 10 legume genomes establishes a family-level genomics platform**. *Plant Physiol* 2017, **174**:284-300.

44. Hipp AL: **Nonuniform processes of chromosome evolution in sedges (*Carex*: Cyperaceae)**. *Evolution* 2007, **61**:2175-2194.

45. Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, Fontana P, Bhatnagar SK, Troggio M, Pruss D *et al.*: **The genome of the domesticated apple (*Malus* × *domestica* Borkh.)**. *Nat Genet* 2010, **42**:833-839.

46. International Brachypodium Initiative: **Genome sequencing and analysis of the model grass *Brachypodium distachyon***. *Nature* 2010, **463**:763-768.

47. Al-Shehbaz IA: **A generic and tribal synopsis of the Brassicaceae (Cruciferae)**. *Taxon* 2012, **61**:931-954.

48. Cox A, Abdelnour G, Bennett M, Leitch I: **Genome size and karyotype evolution in the slipper orchids (Cypripedioideae: Orchidaceae)**. *Am J Bot* 1998, **85**:681-687.

49. Moraes AP, Koehler S, Cabral JS, Gomes SS, Viccini LF, Barros F, Felix LP, Guerra M, Forni-Martins ER: **Karyotype diversity and genome size variation in Neotropical Maxillariinae orchids**. *Plant Biol* 2017, **19**:298-308.

50. McKain MR, Wickett N, Zhang Y, Ayyampalayam S, McCombie WR, Chase MW, Pires JC, dePamphilis CW, Leebens-Mack J: **Phylogenomic analysis of transcriptome data elucidates co-occurrence of a paleopolyploid event and the origin of bimodal karyotypes in Agavoideae (Asparagaceae)**. *Am J Bot* 2012, **99**:397-406.

51. • Considine MJ, Wan Y, D'Antuono MF, Zhou Q, Han M, Gao H, Wang M: **Molecular genetic features of polyploidization and**

aneuploidization reveal unique patterns for genome duplication in diploid *Malus*. *PLoS ONE* 2012, **7**:e29449.
Characterization of eupolyploidization and aneuploidization in diploid *Malus* populations using cytology and microsatellite markers. The data suggest that unreduced and aneuploid gametes can impact speciation and origin of new chromosome numbers.

52. De Storme N, Mason A: **Plant speciation through chromosome instability and ploidy change: cellular mechanisms, molecular factors and evolutionary relevance**. *Curr Plant Biol* 2014, **1**:10-33.

53. Xiong Z, Gaeta RT, Pires JC: **Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus***. *Proc Natl Acad Sci U S A* 2011, **108**:7908-7913.

54. Chester M, Gallagher JP, Symonds VV, Cruz da Silva AV, Mavrodiev EV, Leitch AR, Soltis PS, Soltis DE: **Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae)**. *Proc Natl Acad Sci U S A* 2012, **109**:1176-1181.

55. Schubert I, Lysak MA: **Interpretation of karyotype evolution should consider chromosome structural constraints**. *Trends Genet* 2011, **27**:207-216.

56. Lysak MA: **Live and let die: centromere loss during evolution of plant chromosomes**. *New Phytol* 2014, **203**:1082-1089.

57. Wang H, Bennetzen JL: **Centromere retention and loss during the descent of maize from a tetraploid ancestor**. *Proc Natl Acad Sci U S A* 2012, **109**:21004-21009.

58. Luo MC, Deal KR, Akhunov ED, Akhunova AR, Anderson OD, Anderson JA, Blake N, Clegg MT, Coleman-Derr D, Conley EJ *et al.*: **Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in Triticeae**. *Proc Natl Acad Sci U S A* 2009, **106**:15780-15785.

59. Mandáková T, Kovarík A, Zozomová-Lihová J, Shimizu-Inatsugi R, Shimizu KK, Mummenhoff K, Marhold K, Lysak MA: **The more the merrier: recent hybridization and polyploidy in *Cardamine***. *Plant Cell* 2013, **25**:3280-3295.

60. Fonsêca A, Ferraz ME, Pedrosa-Harand A: **Speeding up chromosome evolution in *Phaseolus*: multiple rearrangements associated with a one-step descending dysploidy**. *Chromosoma* 2016, **125**:413-421.

61. Hou J, Ye N, Dong Z, Lu M, Li L, Yin T: **Major chromosomal rearrangements distinguish willow and poplar after the ancestral "Salicoid" genome duplication**. *Genome Biol Evol* 2016, **8**:1868-1875.

62. Stebbins GL: *Variation and Evolution in Plants*. Columbia University Press; 1950.

63. Lysak MA, Berr A, Pecinka A, Schmidt R, McBreen K, Schubert I: **Mechanisms of chromosome number reduction in Arabidopsis thaliana and related Brassicaceae species**. *Proc Natl Acad Sci U S A* 2006, **103**:5224-5229.

64. Freeling M, Woodhouse MR, Subramaniam S, Turco G, Lisch D, Schnable JC: **Fractionation mutagenesis and similar consequences of mechanisms removing dispensable or less-expressed DNA in plants**. *Curr Opin Plant Biol* 2012, **15**:131-139.

65. Sankoff D, Zheng C, Wang B: **A model for biased fractionation after whole genome duplication**. *BMC Genomics* 2012, **Suppl. 1**:S8.

66. Schnable JC, Freeling M, Lyons E: **Genome-wide analysis of syntenic gene deletion in the grasses**. *Genome Biol Evol* 2012, **4**:265-277.

67. Renny-Byfield S, Kovarik A, Kelly LJ, Macas J, Novak P, Chase MW, Nichols RA, Pancholi MR, Grandbastien MA, Leitch AR: **Diploidization and genome size changes in allopolyploids is associated with differential dynamics of low- and high-copy sequences**. *Plant J* 2013, **74**:829-839.

68. Mandáková T, Mummenhoff K, Al-Shehbaz IA, Mucina L, Mühlhausen A, Lysak MA: **Whole-genome triplication and species radiation in the southern African tribe Heliophileae (Brassicaceae)**. *Taxon* 2012, **61**:989-1000.

69. Haudry A, Platts AE, Vello E, Hoen DR, Leclercq M, Williamson RJ, Forczek E, Joly-Lopez Z, Steffen JG, Hazzouri KM *et al.*: **An atlas of over 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions**. *Nat Genet* 2013, **45**:891-898.

70. Liu Y, Wang J, Ge W, Wang Z, Li Y, Yang N, Sun S, Zhang L, Wang X: **Two highly similar poplar paleo-subgenomes suggest an autotetraploid ancestor of Salicaceae plants**. *Front Plant Sci* 2017, **8**:571.

71. Zhao M, Zhang B, Lisch D, Ma J: **Patterns and consequences of subgenome differentiation provide insights into the nature of paleopolyploidy in plants**. *Plant Cell* 2017, **29**:2974-2994.

72. Hohmann N, Wolf EM, Lysak MA, Koch MA: **A time-calibrated road map of Brassicaceae species radiation and evolutionary history**. *Plant Cell* 2015, **27**:2770-2784.

73. Smith SA, Donoghue MJ: **Rates of molecular evolution are linked to life history in flowering plants**. *Science* 2008, **322**:86-89.

74. Soria-Hernanz DF, Fiz-Palacios O, Braverman JM, Hamilton MB: **Reconsidering the generation time hypothesis based on nuclear ribosomal ITS sequence comparison in annual and perennial angiosperms**. *BMC Evol Biol* 2008, **8**:344.

75. Yue JX, Li J, Wang D, Araki H, Tian D, Yang S: **Genome-wide investigation reveals high evolutionary rates in annual model plants**. *BMC Evol Biol* 2010, **10**:242.

76. Luo MC, You FM, Li P, Wang JR, Zhu T, Dandekar AM, Leslie CA, Aradhya M, McGuire PE, Dvorak J: **Synteny analysis in Rosids with a walnut physical map reveals slow genome evolution in long-lived woody perennials**. *BMC Genomics* 2015, **16**:707.

77. de Miguel M, Bartholomé J, Ehrenmann F, Murat F, Moriguchi Y, Uchiyama K, Ueno S, Tsumura Y, Lagraulet H, de Maria N *et al.*: **Evidence of intense chromosomal shuffling during conifer evolution**. *Genome Biol Evol* 2015, **7**:2799-2809.

78. Charlesworth B: **Evolutionary rates in partially self-fertilizing species**. *Am Nat* 1992, **140**:126-148.

79. Grant V: *Plant Speciation*. Columbia University Press; 1981.

80. Hörandl E, Hojsgaard D: **The evolution of apomixis in angiosperms: a reappraisal**. *Plant Biosyst-Int J Deal Asp Plant Biol* 2012, **146**:681-693.

81. •• Schranz ME, Mohammadin S, Edger PP: **Ancient whole genome duplications, novelty and diversification: the WGD Radiation Lag-Time Model**. *Curr Opin Plant Biol* 2012, **15**:147-153.
An important synthesis formalizing the observation that major species radiations in angiosperms often followed WGD events after a lag period of millions of years. This work stimulated growing interest in the role of post-polyploid diploidization.

82. • Guerrero RF, Kirkpatrick M: **Local adaptation and the evolution of chromosome fusions**. *Evolution* 2014, **68**:2747-2756.
Application of population genetics models to study chromosome 'fusions'. Authors conclude that chromosome fusions (i.e. descending dysploidy) may reduce recombination between locally adapted alleles.

83. Escudero M, Martín-Bravo S, Mayrose I, Fernández-Mazuecos M, Fiz-Palacios O, Hipp AL, Pimentel M, Jiménez-Mejías P, Valcárcel V, Vargas P, Luceño M: **Karyotypic changes through dysploidy persist longer over evolutionary time than polyploid changes**. *PLoS ONE* 2014, **9**:e85266.

84. Soltis DE, Soltis PS: **Polyploidy: recurrent formation and genome evolution**. *Trends Ecol Evol* 1999, **14**:348-352.

85. Lysak MA, Mandáková T, Schranz ME: **Comparative paleogenomics of crucifers: ancestral genomic blocks revisited**. *Curr Opin Plant Biol* 2016, **30**:108-115.

86. •• Murat F, Armero A, Pont C, Klopp C, Salse J: **Reconstructing the genome of the most recent common ancestor of flowering plants**. *Nat Genet* 2017, **49**:490-496.
An important comparative framework for paleogenomic studies in angiosperms. Reconstruction of the most recent common ancestor genome of modern flowering plants ($n = 15$) based on the inferred ancestral grass

karyotype (AGK, *n* = 7), ancestral monocot karyotype (AMK, *n* = 5) and ancestral eudicot karyotype (AEK, *n* = 7).

87. Salse J: **Deciphering the evolutionary interplay between subgenomes following polyploidy: a paleogenomics approach in grasses**. *Am J Bot* 2016, **103**:1167-1174.

88. Christenhusz MJ, Byng JW: **The number of known plants species in the world and its annual increase**. *Phytotaxa* 2016, **261**:201-217.

89. Felix LP, Guerra M: **Chromosome analysis in *Psygmorchis pusilla* (L.) Dodson & Dressier, the smallest chromosome number known in Orchidaceae**. *Caryologia* 1999, **52**:165-168.

90. De Assis FNM, Souza BCQ, Medeiros-Neto E, Pinheiro F, Silva AEB, Felix LP: **Karyology of the genus *Epidendrum* (Orchidaceae: Laeliinae) with emphasis on subgenus *Amphiglottium* and chromosome number variability in *Epidendrum secundum***. *Bot J Linn Soc* 2013, **172**:329-344.

91. Felix LP, Guerra M: **Basic chromosome numbers of terrestrial orchids**. *Plant Syst Evol* 2005, **254**:131-148.

92. Semple JC, Watanabe K: **A review of chromosome numbers in Asteraceae with hypotheses on chromosomal base number evolution**. *Systematics, Evolution and Biogeography of Compositae*; *Vienna: IAPT; 2013:61-72*.

93. Barker MS, Kane NC, Matvienko, Kozik MA, Michelmore RW, Knapp SJ, Rieseberg LH: **Multiple paleopolyploidizations during the evolution of the Compositae reveal parallel patterns of duplicate gene retention after millions of years**. *Mol Biol Evol* 2008, **25**:2445-2455.

94. Kocsis E, Trus BL, Steer CJ, Bisher ME, Steven AC: **Image averaging of flexible fibrous macromolecules: the clathrin triskelion has an elastic proximal segment**. *J Struct Biol* 1991, **107**:6-14.