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PART OF A SPECIAL ISSUE ON POLYPLOIDY IN ECOLOGY AND EVOLUTION

REVIEW

**Polyploidy and interspecific hybridisation: partners
for adaptation, speciation and evolution in plants**

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ABSTRACT

- **Background** Polyploidy or whole genome duplication is now recognized as being present in almost all lineages of higher plants, with multiple rounds of polyploidy occurring in most extant species. The ancient evolutionary events have been identified through genome sequence analysis, while recent hybridisation events are found in about half of the world's crops and wild species. Building from this new paradigm for understanding plant evolution, the papers in this Special Issue address questions about polyploidy in ecology, adaptation, reproduction and speciation of wild and cultivated plants from diverse ecosystems. Other papers, including this article, consider genomic aspects of polyploidy.
- **Approaches** Discovery of the evolutionary consequences of new, evolutionarily recent, and ancient polyploidy requires a range of approaches. Large scale studies of both single species, and whole ecosystems, with hundreds to tens of thousands of individuals, sometimes involving 'garden' or transplant experiments are important for studying adaptation. Molecular studies of genomes are needed to measure diversity in genotypes, showing ancestors, the nature and number of polyploidy and backcross events that have occurred, and allowing analysis of gene expression and transposable element activation. Speciation events and the impact of reticulate evolution, require comprehensive phylogenetic analyses and can be assisted by resynthesis of hybrids. In this Special Issue, we include studies ranging in scope from experimental and genomic, through ecological to more theoretical.
- **Conclusions** The success of polyploidy, displacing the diploid ancestors of almost all plants, is well illustrated by the huge angiosperm diversity that is assumed to originate from recurrent polyploidisation events. Strikingly, polyploidisation often occurred prior to or simultaneously with major evolutionary transitions and adaptive radiation of

species, supporting that concept that polyploidy plays a predominant role in bursts of adaptive speciation. Polyploidy results in immediate genetic redundancy and represents, with the emergence of new gene functions, an important source of novelty. Along with recombination, gene mutation, transposon activity and chromosomal rearrangement, polyploidy and whole genome duplication act as a driver of evolution and divergence in plant behaviour and gene function, enabling diversification, speciation and hence plant evolution.

Key words: Polyploidy, hybrids, ecology, adaptation, evolution, genomics, chromosomes, speciation, whole genome duplication (WGD), crops, weeds, phylogeny, bryophytes, angiosperms

INTRODUCTION

About half of all higher plant species are recognizable as evolutionarily recent polyploids, where multiple whole genomes or sets of chromosomes have come together from close ancestors (Soltis *et al.*, 2015). Additionally, over evolutionary time, all flowering plants have at least one polyploidy event, also known as a whole genome duplication (WGD), in their ancestry, from before the divergence of gymnosperms and angiosperms, the ζ (zeta) event (see Fig. 1 and references therein). Angiosperms, including *Amborella* and the basal angiosperms (i.e. ANA, for Amborellales, Nymphaeales, Austrobaileyales) that are sisters to all the other angiosperms, have a second polyploidy event in their lineage (ϵ , epsilon; *Amborella* Genome Project, 2013). Analyses of whole genome sequences in the last decade have identified additional, and often multiple, polyploidy events in the ancestry of every eudicot and monocot where the genomes have been sequenced (summarized in Fig. 1). Notably, the near-universal occurrence of multiple polyploidy events (Wendel, 2015) during plant evolution is in contrast to most groups of animals in both recent and long-term evolutionary history (e.g. Hoffmann *et al.*, 2012), marking a significant divergence between evolutionary mechanisms in the two kingdoms.

With respect to its omnipresence along the evolutionary history of higher plants, polyploidy has been the subject of numerous reviews with emphasis on the genetic and genomic consequences of WGDs (Soltis *et al.*, 2016). The present article has two main objectives: firstly, overviewing the papers in this Special Issue “Polyploidy in Ecology and Evolution”, and secondly to discuss complementary polyploidy-related topics covered. In the Special Issue, we made the choice to consider not simply the occurrence of polyploidy (or WGD) in plants (including the bryophyte *Sphagnum*), but also provide an overview of the consequences of polyploidy in adaptation, speciation and evolution in plants: the relationships between polyploidy and stressful environmental conditions have suggested a major role of polyploidy in

adaptation. This has been extensively analysed for cultivated plants, and we review this topic, in the context of concepts related to papers in the Special Issue, many of which present research in the novel area of polyploidy in natural plant populations. With the number of polyploidy events being revealed in plant evolution, the study of its evolutionary significance on wild plant species at the population scale, considering both evolutionarily ancient (deep) phylogenies and recent polyploids (some below the level of species), is now underway. The Special Issue articles consider the success and diversity found in polyploids from the ecological and evolutionary points of view, including developmental and genetic studies. Several papers deal with the relationships of polyploidy with plant reticulate evolution (i.e. natural hybridisation), while others bear on the origin and formation of neopolyploids. Some papers discuss the relationships between allopolyploidy and reproductive systems, two major processes driving angiosperm diversification, and other papers highlight the link between polyploidy and adaptation, in a biogeographical context. Finally, a review dedicated to the impact of transposable elements on polyploidy plant genomes gives consideration to the molecular basis of genomic conflicts, particularly present in genome duplications with hybrid origins. We have specifically developed this renewed interest for the study of polyploidy in plants with new avenues of investigations dedicated to the epigenetic consequences of polyploidy and their role for plasticity and adaptation in plants.

OCCURRENCE AND DETECTION OF POLYPLOIDY IN PLANTS

Plant evolution and polyploidy

Since the historical Greek and Roman cultures, more than two millennia of research on plant diversity and relationships have established a robust phylogeny, placing all plants in a few monophyletic groups (as exemplified with angiosperms that constitute now a single monophyletic group), most recently based on evidence from DNA sequences (APG IV, 2016).

Novel interpretations and new discoveries in the fossil record have been important for identifying innovations and divergences within plants (e.g. Matsunaga and Tomescu, 2016; Gondolfo and Hemsén, 2017) giving extra strength to modern phylogenies. Polyploidy is a confounding factor in phylogenetic analysis, in particular when it involves interspecific hybridisation at the origins of the genome duplication. The resulting duplicated gene copies may lead to incongruence among multiple gene phylogenies, and the reticulate evolutionary patterns that mean lineages – typically at the level of genus or tribe and below – will be defined as multiple monophyletic groups (e.g. Fortune *et al.*, 2007). Understanding the evolutionary and biological processes in their past diversification and considering future evolutionary pathways is a major research objective, notably addressed in the articles presented here.

Polyploidy and genome rearrangements, complementing mutation and recombination, are major evolutionary events, having the potential to lead to genetic and reproductive isolation. Most of the nodes within the phylogeny of plants (Fig. 1) represent disruptive changes in evolution, with vasculature, seeds and flowers representing successive evolutionary innovations. Previous Special Issues of *Annals of Botany* have stressed the major role of pollination biology and co-evolution with pollinators in the ecological diversification of flowering plants (e.g. Van der Niet *et al.*, 2014; Friis *et al.*, 2006). Another important feature of angiosperms is their broad metabolic performance and their remarkable photosynthetic efficiencies based on carbon- and water-use capacities, thanks to morphological innovations in leaves, wood and roots, which have enabled colonizing a large diversity of habitats (Field and Arens, 2007). Angiosperm diversity can also be linked to the impact of the rounds of polyploidy (WGD) duplicating all the genes (Tank *et al.*, 2015). As well as potentially giving reproductive isolation, polyploidy leads to multiplying the number of genes and incorporating redundancy in function, enabling new genetic variability that may be acted upon by natural selection for evolution and adaptation. Indeed, allopolyploidy (associated with interspecific hybridisation,

see below) has long been considered as a source of the “most important amendment to Darwin and Wallace’s account of evolution” (Haldane, 1959).

Detection of polyploidy

Extant polyploid plants can be detected by counting chromosome numbers in cytological metaphase preparations made from a group of related accessions (or species, as shown in Fig. 2). Where chromosome numbers in related plants are multiples (or sums) of others, the plant is likely to be a recent polyploid, and many species have a ploidy series with a base chromosome number x , including diploids ($2x$) and polyploids such as triploids ($3x$), tetraploids ($4x$) and polyploids with even higher ploidies (see Heslop-Harrison, 2017). Equally, DNA content of the accessions can be measured by flow cytometry or Feulgen densitometry, and will show an additive series from the ancestral diploids to multiples in the polyploids (Kron *et al.*, 2007). Nevertheless, DNA content and chromosome number are not always directly correlated to ploidy levels. For instance, karyotype evolution by chromosome fission and fusion has significantly marked the evolutionary history of diverse plant lineages, making thus difficult to infer ploidy levels from cytogenetic data (e.g. in *Crocus* – Harpke *et al.*, 2015). Where species have diverged before coming together in a polyploid, the use of total genomic DNA as a probe for *in situ* hybridisation has been valuable to both identify the occurrence of a polyploid, and assist with identification of the ancestral species (e.g. Hunt *et al.*, 2014; Patel *et al.*, 2011). Within many species and species complexes, polyploid series can be present and are described as cytotypes (in this Special Issue e.g. Čertner *et al.*, 2017; Paule *et al.*, 2017; or see Taketa *et al.*, 1999) or when polyploidy events are older, as different species (*Hordeum*; Cuadrado *et al.*, 2017).

Two main types of recent polyploidy can be distinguished. Autopolyploidy defines duplication of one genome within one species, which results in homologous chromosome sets

in the cell (diploid AA doubles to become the autotetraploid AAAA). Allopolyploidy defines WGD associated with the merger of two or more divergent genomes in a single nucleus following interspecific hybridisation, resulting in homoeologous chromosome sets in the cell ($AA \times BB \rightarrow AABB$). In practice, auto- and allopolyploidy are not entirely separate, since this terminology depends on the taxonomic definition of a species and the scope of a designated 'genome'. Higher polyploids may be combinations as results of their evolutionary histories that have been marked by recurrent WGDs including both auto- and allopolyploidy events, thus giving the 'auto-allo-polyploid' designation (usually summarized as polyploid). For ancient WGD events, where the ancestral diploid species (singular or plural) are unknown and DNA sequences are diverged, auto- or allopolyploidy, as depicted in Figure 1, cannot be distinguished.

For the detection of ancient polyploid events, whole genome sequencing coupled with comparative genomics has proved to be remarkably efficient; this is particularly well illustrated by the *Amborella* genome project (2013). Typically, following assembly of the DNA sequence, all-frame protein translations are made to produce a full set of gene predictions. Structural comparisons on the set of gene predictions then allow the identification of paralogous gene pairs used to reconstruct ancient WGDs. Indeed, even over very long evolutionary periods, regions of synteny – the same list and order of genes – and of similar, paralogous genes (thus corresponding to syntenic blocks) are readily identified on different chromosomes, enabling the detection of multiple ancient whole genome duplication events (see Fig. 1 and its references).

HOW SUCCESSFUL ARE POLYPLOIDS?

Evolutionary success of polyploidy

Before the recognition of the ubiquitous ancient polyploidy or whole genome duplication events in plants, polyploids were widely considered as being present in many lineages. The long-term compared to short-term evolutionary success of polyploids, whether in crops or wild species, was a significant question (Soltis *et al.*, 2014; Arrigo and Barker, 2012). Mayrose *et al.* (2011) consider that “polyploidy is most often an evolutionary dead end” with respect to neopolyploids and their associated low diversification, and thus speciation rates, in comparison to diploids. Nevertheless, polyploids do represent longer-term evolutionary successes; the WGD events at their origins may have generated the necessary genetic diversity that has been under positive selection for long periods, and then associated with phenotypic novelty, adaptability and higher fitness. Of course, the role of subsequent genome evolution (mutation, chromosome rearrangement, transposable element mobilisation) in contributing to this genetic diversity has also to be considered. Interestingly, the polyploidy events that have marked the evolutionary history of the green plant lineage occurred prior to or simultaneously with major evolutionary transitions and adaptive radiation of species, suggesting a role of polyploidy in adaptive speciation (De Bodt *et al.*, 2005). This is particularly well illustrated with the survival and proliferation of polyploid plant lineages during the Cretaceous-Tertiary (K-T) mass extinction event (Fawcett *et al.*, 2009; Fig. 1). The occurrence of polyploidy in response to stressful conditions has been notably analysed in the context of plant domestication that represents conditions of strong selection pressure for adaptation to human cultivation.

Polyploidy and plant domestication

Many crop species have been identified as polyploids through the presence of polyploid series in chromosome number (e.g. Salman-Minkov *et al.*, 2016) and through generation of hybrids (e.g. Renny-Byfield and Wendel, 2014), and many crops are relatively recent polyploids (Leitch and Leitch, 2008). This has led to the hypothesis of some relationship between intensive selection, typically accompanying plant domestication, and polyploidy. Salman-Minkov *et al.* (2016) considered that polyploidy followed by domestication was a key feature in early selection of crops and showed that domesticated plants have gone through more polyploidy events than their wild relatives. Nevertheless, it can be noted that the advantages of polyploidy under domestication are not overwhelming: the top three crops in the world, all cereal grains, are wheat (a modern hexaploid $2n=6x=42$; Fig. 2C), rice (diploid, $2n=2x=14$), and maize (a paleotetraploid, $2n=4x=20$). With the strict polyploid selection criteria of Salman-Minkov *et al.* (2016), 30% of crops were polyploid compared to 24% of wild species.

Induced or post-domestication polyploids represent a small proportion of crops (reviewed by Sattler *et al.*, 2016). As well as the genetic buffering and increased number of gene alleles associated with the increase in cell size, a major consequence of polyploidy may be the global increase of organ size (including leaves, stems, flowers, stigmas, tubercles) of the polyploid compared to its diploid counterpart; it is thus generally considered that polyploidy may increase fruit size as well as seed size, valuable traits for crop breeding. Such a consequence also illustrates one of the major advantages of allopolyploids that is their 'doubled interspecific hybrid' composition, which directly results in fixing heterozygosity at the origin of heterosis, or hybrid vigour (Comai, 2005). Wu *et al.* (2012) stated that there has been little systematic evaluation of fruit from induced polyploids in comparison with diploids, and that there was only little evidence for larger fruits. However, their detailed work in kiwifruit (*Actinidia chinensis*) showed that colchicine-induced autotetraploids were some 50% larger

than their diploid progenitors, and that the increase was stable during vegetative propagation. In addition, specific work on wheat polyploids clearly demonstrated that seeds from synthetic hexaploid wheats were significantly larger than seeds from either progenitor, tetraploid or diploid (Kenan-Eichler *et al.*, 2011). Thus, one can suggest that the consequences of polyploidy extend to developmental effects (see also Münzbergová, 2017).

Further investigations of the consequences of genome duplication in relation with adaptation abilities in natural populations from various plant lineages should provide a valuable insight into the important evolutionary process of polyploidy, as reported by the articles presented in this Special Issue.

POLYPLOIDY IN ECOLOGY AND EVOLUTION

Polyploidy, diversity and reticulate evolution

Understanding the components of the evolutionary success of polyploidy has generated important research activity in the last twenty years. Autopolyploidy has received relatively little attention by evolutionary biologists, not least since diploids and autopolyploids can be considered as one species (sometimes subject to redefinition as, for example, in *Brachypodium* by Catalan *et al.*, 2012); indeed, it has been estimated that the evolutionary advantages of allopolyploidy are largely superior (Parisod *et al.*, 2010b). Evolutionary advantages of allopolyploidy can be associated with merger of differentiated genomes (i.e. via interspecific hybridisation) with consequent genomic restructuring and rebalancing of gene expression, giving phenotypic changes (Parisod *et al.*, 2010b; Hegarty and Hiscock, 2008; Tayalé and Parisod, 2013; Mutti *et al.*, 2017). How large is the role of reticulate evolution in the evolutionary success of flowering plants (reviewed by Soltis and Soltis, 2009), and should polyploidy be considered, following interspecific hybridisation, as the major driver of plant evolution and speciation? Hybridisation has been proposed as a direct driver of WGD, but the

causal relationships between both processes are far from clear, and autopolyploidisation has certainly contributed to plant diversification (Soltis *et al.*, 2007; Buggs *et al.*, 2009; Barker *et al.*, 2016).

Diploid plants, including hybrids, produce unreduced gametes at relatively high rates, which participate in the formation of many polyploid lineages (Ramsey and Schemske, 2002; Otto, 2007). These lineages are often reproductively isolated from their progenitors, giving rise to new species or cultivars (Čertner *et al.*, 2017 see below; and e.g. in *Crocus*, Orgaard *et al.*, 1995, Alsayeid *et al.*, 2015; in *Mimulus*, Sweigart *et al.*, 2008; Vallejo-Marín *et al.*, 2015). Interploid fitness loss might not be universal though, because polyploid plant species with odd chromosome numbers are widespread (Otto, 2007) and triploids can often have a role of bridges between diploid and tetraploid species (Ramsey and Schemske, 1998) often exploited in breeding (Heslop-Harrison and Schwarzacher, 2007). The role of hybridisation in creating high genetic variability by producing new genotypes at the onset of potential speciation events is a question that has been recurrently addressed, notably by two papers in this Special Issue.

In the first paper from Yan *et al.* (2017), the role of hybridisation in diversifying and structuring natural populations is particularly well illustrated within the genus *Rhododendron*, where interspecific hybridisation is frequent. By analysing twenty-four *Rhododendron* populations including fifteen independent hybrid swarms, the authors have addressed the question of the extent of hybridisation in population structure and differentiation (Yan *et al.*, 2017). Using microsatellite and chloroplast DNA sequencing, the authors demonstrate that hybridisation events are complex and highly variable from one population to another, and result mainly in introgressions. The recurrent backcrosses lead to new allele combinations, generating genetic diversity for new variants among populations and potentially driving speciation. Secondly, Landrein *et al.* (2017) investigated the genetic diversity among five *Abelia* species (Caprifoliaceae) originating from China, by analyzing wild taxa and horticultural varieties, to

identify the origins of the genetic diversity, including that exploited in breeding programmes. Their study was based on phylogenetic reconstruction using nuclear and chloroplast markers and molecular genotyping for genetic diversity and population structure analysis. They demonstrate that the *Abelia* genus has been subjected to recurrent hybridisation and introgression, at the origins of allopatric speciation events. Their results demonstrate the value of exploiting hybridisation, which, in association with random recombination, may produce interesting allele combinations for ornamental breeding purposes.

A third paper of the Special Issue also reports the study of the relationships between interspecific hybridisation and genetic diversity, but includes the genome duplication process by analysing and comparing the allopolyploids. Cuadrado *et al.* (2017) demonstrate the role of allopolyploidy in diversifying *Hordeum* (a genus with nearly half of the taxa that are polyploids, and where some species such as *Hordeum murinum* are represented by morphologically indistinguishable diploid and autopolyploid cytotypes, Taketa *et al.*, 1999), by analysing the hybrid genome composition of two closely related allotetraploid species using molecular cytogenetics. Even closely related genomes in the allopolyploid harbour high genomic diversity, enabling colonization of different geographical areas. The evolutionary divergence of *H. secalinum* and *H. capense* and their current intra-genome diversity may be thus related to the differential genomic and chromosomal modifications encountered by the two parental genomes that merged during the allopolyploidisation event.

Outside the flowering plants, polyploidy has been important in the evolution of mosses (Shaw *et al.*, 2016 and Fig. 1), where it is associated to hermaphroditism (Crawford *et al.*, 2009) and more generally to changes in mating systems (Jesson *et al.*, 2011). With respect to the high level of fixed heterozygosity observed for numerous moss species, a preponderant role for allopolyploidy in bryophyte diversification has been suggested. This allopolyploidy-based evolutionary process is particularly exemplified in *Sphagnum* (Ricca and Shaw, 2010), which

is the model of the study reported in this Special Issue. Karlin and Smouse (2017) investigated the allelic diversity of the peat moss *Sphagnum ×falcatulum*, a double allopolyploid species originating from the combination of three monoploid genomes from three ancestral species (i.e. it is an allo-allo-triploid). They used SSR genotyping on a large sample of *S. ×falcatulum* gametophytes (in comparison to the immediate progenitors, i.e. the monoploid genomes) from different populations widespread in the Holantarctic. Most of the genetic diversity is captured directly from the three ancestral monoploid genomes, and multiple origins of *S. ×falcatulum* are likely (i.e. recurrent allopolyploidisation). Allopolyploidy may thus be the single most important factor in generating the genetic diversity allowing this peat moss to colonize the Holantarctic region so successfully.

Neopolyploid formation: a chromosomal point of view

Polyploids are usually formed from hybridisation of taxonomically close species, where the gene content, sequences and often chromosomal organisation, if not the repetitive DNA (e.g. Fig. 2), are similar. New hybrids in the first or early generations, including both spontaneous hybrids and those made in a research or plant breeding context, are classified as neopolyploids. In nature, some species complexes with high phenotypic diversity and many microspecies of various ploidies give complex taxonomic problems (e.g. *Rhododendron* discussed above; *Taraxacum*, Majeský *et al.*, 2012; *Rubus*, Heslop-Harrison, 1968). The taxonomic challenges and diversity in populations mean that studies with large numbers of individuals and molecular markers are required.

The first advantage of allopolyploids (AABB) in comparison to homoploid hybrids (AB) is the immediate recovering of homologous chromosome pairs as a prerequisite to regular meiosis, and therefore potentially restoring fertility (at least partial) in the resulting hybrid genotypes, thanks to chromosome set doubling. Nevertheless, polyploids usually require also a

tight genetic control of crossing-over formation and distribution to minimize aneuploidy and to ensure further fertility and genome stability, and thus the establishment and success of neopolyploids (Jenczewski and Alix, 2004; Grandont *et al.*, 2013). Three papers in this Special Issue focus on neopolyploids, dealing with the extent of the formation of polyploid cytotypes in natural conditions, which mainly involve the production of unreduced gametes from diploids (as mentioned above) and the immediate consequences of polyploidisation that can be identified using synthetic polyploids.

In their analysis of a collection of more than 800 samples of dogroses (*Rosa* sect. *Caninae*), Herklotz and Ritz (2017) addressed the question of the extent of natural interspecific hybridisation between the subsections *Caninae* and *Rubigineae*, finding populations had a mixture of hybridogenic and non-hybridogenic individuals. Dogroses comprise many allopolyploid species (4x, 5x, 6x) notably thanks to a unique asymmetric meiosis process with only two sets of chromosomes forming bivalents that results in haploid pollen grain but a polyploid egg cell (Ritz and Wissemann, 2011). The authors demonstrate that polyploids are more frequent in *Rubigineae* hybrids in relation with their higher capacity in producing unreduced gametes than in *Caninae*, representing a major bias between reciprocal crosses. However, despite their viability and abundance, it seems that neopolyploid hybrids, in contrast to non-hybrids, do not spread between localities. In a large scale spatio-temporal study in central Europe, Čertner *et al.* (2017) used flow cytometry data on more than 11,000 individuals, as well as *ex situ* germination data, to study the cytotypic structure and dynamics of a contact zone between diploid and tetraploid populations of the annual herb *Tripleurospermum inodorum* (Asteraceae). In spite of an apparent substantial amount of gene flow between the two cytotypes, and the presence of fertile triploids that could serve as bridges to new polyploids (see above), alternative cytotypes are rare. Newly formed tetraploids were extremely rare and were thus not able to establish, suggesting that successful tetraploids probably result from one or a

few rare and ancient polyploidisation events – echoing the theory developed by Mayrose *et al.* (2011). Together, these studies show the importance of mixed-ploidy populations in generation of cytotype variation, with population structure (in sympatry) that may help maintain a resource of genetic diversity conferring at least long-term adaptive advantages.

Neopolyploids, and in particular synthetic polyploids, are the most appropriate plant material to analyse and further understand the direct consequences of polyploidisation during speciation. Münzbergová (2017) addresses this question on the model *Vicia cracca* that comprises both diploid and autotetraploid cytotypes. By analyzing and comparing natural diploids, natural tetraploids and synthetic tetraploids from four different populations, the author demonstrates the direct impact of polyploidy on morphological traits (e.g. seed weight, plant height, stomata size). This study also highlights the necessity to analyse multiple populations of polyploids to provide robust conclusions about the impact of polyploidisation that may be variable with respect to the diversity of the populations at the origins of the new polyploids. Finally, the author performed a comparative study between the synthetic tetraploids and their diploid offsprings to evaluate the impact of the colchicine treatment at the origin of the synthetic polyploids: only the analysis of the third and subsequent generations of synthetic polyploids may allow to reveal the consequences of polyploidisation *per se*. Such conclusions should be beneficial to the study of ‘old’ polyploid species (e.g. oilseed rape, cotton, wheat, tobacco) for which the true diploid progenitors are not available anymore and the synthetic polyploids are the unique plant material useful to model and understand their formation.

Polyploidy and reproductive systems

Reproduction, whether sexual, apomictic or vegetative, is central to the success of polyploids. Notably, the near-universal occurrence of multiple polyploidy events in plant evolution is in complete contrast to the animal kingdom in both recent and long-term

evolutionary history, where most analyses have found only one or two early whole genome duplication events, such as the R1 and R2 WGDs in the vertebrate stem lineage followed by another WGD in early fish (e.g. Hoffmann *et al.*, 2012); these WGDs are generally viewed to have provided the genetic diversity for many innovative vertebrate or fish specific characteristics and fueled their burst-like evolution. Recent polyploids are unusual, but have been found in individual species or lineages, for example, fish, amphibians, crabs or insects (Mable *et al.*, 2011; Kenny *et al.*, 2016). Differences between plant and animal reproduction have been proposed for the differential polyploid formation rate (Coyne and Orr, 2004; Husband *et al.*, 2013; Otto and Whitton, 2000). Few animals, with the exception of protozoa and a few insect species, have the possibility of apomictic or the equivalent of vegetative reproduction. Even with mechanisms to ensure correct pairing of chromosomes in a polyploid (e.g. Sepsi *et al.*, 2017), the widespread occurrence of sex chromosomes in animals may also limit fertility in polyploid animals (Collares-Pereira *et al.*, 2013). An evolutionary association between reproductive modes and polyploidy has been studied for decades (e.g. Stebbins, 1950) through two main aspects: the occurrence and rate of asexual vs. sexual reproduction on one side, and the transitions in mating systems (i.e. self-compatibility or sexual dimorphism) on the other side. When correlations have been detected, it has usually proven difficult to disentangle the causes and consequences of the different processes. As Ashman *et al.* (2013) showed, the observed association between sex dimorphism (e.g. dioecy) and polyploidy can be explained by direct or indirect effects of one process on the other, since evolutionary transitions usually occur simultaneously, and different factors can affect these transitions in different clades (Glick *et al.*, 2016). The same questions hold for the association between self-fertilization and ploidy, since polyploidy usually correlates with self-compatibility (Barringer, 2007; Husband *et al.*, 2008; Robertson *et al.*, 2011; Alix *et al.*, 2008) even if exceptions have been reported (e.g. in the *Fragaria* genus; Liston *et al.*, 2014). Thus, the evolutionary link between mating system

and ploidy has been fairly well documented, but the association of polyploidy with asexual reproduction (beyond the odd-ploidies such as 3x or 5x), and particularly clonal reproduction (i.e. vegetative growth in plants), has been less studied, particularly in wild species. Three articles of this special issue deal with this question.

Herben *et al.* (2017) report association of vegetative reproduction with polyploidy in a large, phylogenetically broad, sample of 900 European angiosperm species to infer macro-evolutionary patterns of both traits, and examine their temporal trends. As hypothesized, they detect a signal of correlated evolution between polyploidy and vegetative growth, mostly mediated by increased distance of spread. This is nicely confirmed by experimental 'garden' data, which show that diploids rely more on seed reproduction whereas polyploids rely more on vegetative spread. They also show that vegetative reproduction may often evolve before polyploidisation and could then enhance the rates of polyploid speciation.

The evolutionary association between asexual production of seeds (i.e. apomixis) and polyploidy has been studied frequently, not least because gametophytic apomixis and polyploidisation share the same characteristic feature, the production of unreduced gametes (Ramsey and Schemske, 1998; Whitton *et al.*, 2008; Husband *et al.*, 2013; Majeský *et al.*, 2012). Here, two papers study the dynamics of apomixis in a context of hybridisation and polyploidy. In the first paper, Uhrinová *et al.* (2017) assess the genetic relationships between parental and hybrid species in the genus *Sorbus*, which is known for exhibiting apomictic microspecies produced by hybridisation (Robertson *et al.*, 2010). Three parental species, one a tetraploid species (*S. chamaemespilus*) and two mainly diploid cytotypes (*S. aria* and *S. aucuparia*), seem to have produced a set of genetically distinct polyploid microspecies through interspecific hybridisation. These polyploid species seem at least partially reproductively isolated and exhibit a low population genetic diversity compared to the parental species populations as well as a high rate of clonality. If this low diversity results from predominant

apomixis, this would be another confirmation of the evolutionary link between apomixis and polyploid speciation through hybridisation, at least in the genus *Sorbus*. Similarly, ancient polyploid apomicts of the subgenus *Rubus* studied by Šarhanová *et al.* (2017) seem to have been maintained in central Europe without relying on their facultative sexuality but rather on hybridisation with a sexual species (mainly ser. *Glandulosi*). This process may regularly produce successful apomictic polyploid lineages but with low genetic polymorphism.

Polyploidy and ecological divergence

Over long evolutionary times, at least among angiosperms, new polyploid species have driven their diploid ancestors to extinction at several points (Fig. 1). However, open questions remain: Are most polyploid lineages evolutionary dead ends? Have particular events, such as changing climate, enabled polyploids to out-compete their diploid ancestors? Are any evolutionary recent (perhaps the last 5 million years) polyploids more successful than the equivalent diploids? Several authors have considered related questions about plant genome evolution (Mayrose *et al.*, 2011; Arrigo and Barker, 2012; Soltis *et al.*, 2014; Vanneste *et al.*, 2014; Mayrose *et al.*, 2015), some reflecting the biogeographical patterns of polyploid species and their relationship to ecological adaptation (Madlung, 2013; e.g. Herklotz and Ritz, 2017). It has been postulated for a long time that polyploidisation might be associated with particular and large species ranges and/or extreme habitats (Stebbins 1950; Otto and Whitton, 2000; Levin, 2002; Husband *et al.*, 2013; Weiss-Schneeweiss *et al.*, 2013). Potentially contrasting results have been obtained from biogeographical surveys of polyploid and diploid species (e.g. Martin and Husband, 2009; Pandit *et al.*, 2011). However, even environmental correlations (e.g. polyploidy associated with higher latitudes or particular climatic niches) do not necessarily imply ecological adaptation as an explanatory factor, since other processes such as demography (i.e. dispersal variance and genetic drift) can produce the same patterns.

Experimental designs such as reciprocal transplants can provide compelling evidence of adaptation of polyploids (e.g. Ramsey, 2011; Herben *et al.*, 2017), although local biogeographical studies of sister or closely-related species can provide useful insights into the patterns of ecological divergence of polyploid and diploid species (e.g. Laport *et al.*, 2016). In this Special Issue, Paule *et al.* (2017) assess the degree of ecological divergence of polyploid species from the bromeliad *Fosterella* in Andean mountains, in relation to historical biogeographical processes. Polyploids seem to occupy slightly divergent climatic niches but patterns of cytotype geographical distribution suggest a historical parapatric differentiation of polyploid species that has not necessarily been caused by ecological adaptation. Both processes are potentially at the origin of polyploid range shifts in this southern American species complex. This is also probably the case for the Mediterranean grass *Anthoxanthum* studied by Chumová *et al.* (2017). They show that several diversification events might have taken place since the Miocene from a diploid ancestor, by recurrent range expansions possibly linked to climatic niche differentiation.

THE GENOMIC CONFLICTS AWAKEN: POLYPLOIDY AS A PEACEKEEPER?

Apart from the presence of ancient or recent polyploidy, and the wide variation in chromosome number, the large variation in size of plant genomes is an enigma. Measured as the 1C or unreplicated haploid genome size, the range known is from 63 Mb for *Genlisea margaretae* to 149,000 Mb for *Paris japonica* (Bennett and Leitch, 2011). The “C-value paradox” after work of Swift (1950) notes that there are few correlations between apparent organism characteristics and genome size (e.g. Freeling *et al.*, 2015). For example, Krahulcová *et al.* (2017) show no significant correlation between DNA content and seed size in nine *Aesculus* species. However, a correlation between plant genome size and content of transposable elements (TEs) has been observed (Ågren and Wright, 2011; Negi *et al.*, 2016;

Pearce *et al.*, 1996), with LTR-retrotransposons being the most abundant source of genome size variation in many lineages (Bennetzen and Wang, 2014; Biscotti *et al.*, 2015). In addition to polyploidy, TE activity (proliferation and/or elimination; Vitte and Panaud, 2005) is associated with genomic recombination and contributes to shape plant genomes by copy number increase (e.g. Santos *et al.*, 2015), gene disruption (e.g. Tam *et al.*, 2007) or co-mobilizing of other sequences such as those associated with reproductive incompatibility (Alix *et al.*, 2008).

Here, Vicient and Casacuberta (2017) review the major contribution of TEs to plant genome diversity and evolution as well as to gene expression variation, focusing mainly on LTR-retrotransposons. In relation to their capacity to move across the genome and their close association with unequal and illegitimate recombination, TEs (notably LTR-retrotransposons) may generate a large variety of structural mutations that can be beneficial – even if they are more commonly detrimental or at least neutral. Such positive mutational TE insertions have represented interesting genomic targets for selection during plant domestication and crop breeding (for reviews Lisch, 2013; Vitte *et al.*, 2014). Transposable elements are usually targeted efficiently by epigenetic marks (including DNA methylation and post-translational modifications of histones) for ensuring their tight control to avoid any anarchical activation and transposition across the genome (Mirouze and Vitte, 2014). Stress conditions are assumed to reactivate TEs because of the extensive epigenetic remodeling of the genome they might trigger; interspecific hybridisation and polyploidy can act as such stresses (an implication of McClintock, 1984; reviewed by Parisod *et al.*, 2010a). Vicient and Casacuberta (2017) thus emphasize the close relationships between polyploidy and TE activity and the involvement of TEs in gene regulation in response to the epigenetic modifications associated to polyploidisation. The authors highlight the impact of mediated DNA methylation changes at TE insertion sites on the functional regulation of neighbouring genes, as found, for instance, in

Arabidopsis in response to biotic stress (Downen *et al.*, 2012) and in rice following polyploidisation (Zhang *et al.*, 2015).

The major TE silencing mechanism in plants is the biogenesis of small non coding RNAs, known as small (or short) interfering RNAs (siRNAs), with populations of 21-nucleotide-long (21-nt) siRNAs and mainly 24-nt siRNAs that control TEs by TGS (i.e. transcriptional gene silencing) through RNA-directed DNA methylation, RdDM (Lewsey *et al.*, 2016). Only a few studies have dealt with the immediate impact of polyploidy on the biogenesis of small RNA populations (Ha *et al.*, 2009; Kenan-Eichler *et al.*, 2011; Martinez Palacios, 2014; Zhang *et al.*, 2015), but they all reported major changes in expression profiles of 24-nt siRNAs. The responses of small RNAs to (allo)polyploidy, which also remind of the more general dynamics of small RNAs depicted in response to hybridisation, have progressively led to the idea of a major role of TE-derived siRNAs in the process of hybrid incompatibility (reviewed by Ng *et al.*, 2012). At the onset of the hybrid or the neoallopolyploid formation is the necessity to overcome hybrid failure, which ranges from early seed inviability to hybrid sterility. Similar to parental imprinting in animals, genome reprogramming occurs in plants, but specifically in vegetative cells, with the loss of DNA methylation at the origin of transposable element reactivation, resulting in the production of novel TE-derived siRNAs (Slotkin *et al.*, 2009). If parental TE sequences differ substantially, often the case during interspecific hybridisation, TE-derived siRNAs inherited from each parent represent key factors to control the reactivated TEs, and their efficiency in repressing TEs may determine the outcome of the interspecific cross (Martienssen, 2010).

Reproductive isolation, and particularly post-zygotic isolation, between plant and animal species is thought to often result from genomic conflicts involving selfish genetic elements (Presgraves, 2010; Rieseberg and Blackman, 2010; Ågren, 2013). The view of polyploidy, and especially allopolyploidy, as a "peacekeeper" in genomic conflicts when two

divergent genomes are brought together in hybrids is not new (Rieseberg, 2001; Tayalé and Parisod, 2013), but it can also be viewed as generating conflicts that will have to be resolved in a more complex way (Comai, 2005; Jones and Pašakinskienė, 2005). These genomic conflicts can have multiple origins such as TE-mediated silencing misregulation or segregation distorters — e.g. female meiotic centromere drive or supernumerary chromosome drive (Jones and Pašakinskienė, 2005), and there are different ways by which allopolyploidisation can buffer their effects. First, when they are at the origin of epistatic autosomal Bateson-Dobzhansky-Muller (BDM) hybrid incompatibilities, they will be on average recessive and expressed mainly in F₂ and subsequent generations. A direct consequence of polyploidisation will be to avoid expression of this kind of hybrid defect because recessive BDM factors will almost never be homozygous, provided that the recombination rate between homoeologous chromosomes is low. A related effect of this lack of recombination will be the proper segregation of chromosomes in allopolyploids compared to homoploid hybrids (Tayalé and Parisod, 2013). Second, hybrid incompatibilities due to divergent gene expression (e.g. for parentally imprinted genes) or subfunctionalisation of duplicate genes will have potentially no effect in polyploids. Third, polyploidisation could facilitate the occurrence of apomixis by involving low recombination, heterochromatic regions behaving as selfish genetic elements, such as supernumerary chromosomes (Roche *et al.*, 2001; Comai 2005), and providing in turn an advantage for propagating the new species in the first generations. Finally, diploid hybrid defects as well as dosage-related problems specific to polyploidisation will be overcome, usually in later generations, by gene expression remodelling and/or chromosomal rearrangements and gene loss (Chen and Yu, 2013). The modulation of gene regulation, including genome dominance in polyploids (Woodhouse *et al.*, 2014), is probably important for the success or evolutionary failure of a new hybrid.

The epigenetic control of genomic conflicts determining the viability of hybrids opens new avenues of investigation, which include that of the evolutionary significance of epigenetic regulation in the success of interspecific hybridisation and allopolyploidy and the differential control of multiple genomes.

CONCLUSION AND PERSPECTIVES

Polyploidy is arguably the major feature of plant genome evolution, doubling the number of copies of each gene with each whole genome duplication event. Over evolutionary time, polyploids have displaced all their diploid ancestors several times in multiple independent lineages, suggesting a strong selective advantage. These multiple polyploid events have only been detected thanks to widespread large-scale whole genome sequencing since 2000, and comparative genomic analyses continue to reveal the recurrence and extent of WGD along the evolutionary history of plant lineages (Fig. 1). The "-omics" era has allowed understanding of the evolutionary history of lineages (e.g. Douglas *et al.*, 2015; Roux and Pannell, 2015; Wendel, 2015; Woodhouse *et al.*, 2014; Barker *et al.*, 2016). Several of the most important angiosperm groups include a WGD event that is detected soon after the Cretaceous-Tertiary (KT) extinction event (Fig. 1); one can speculate that their genetic structure enabled these polyploids to thrive. One can ask if new polyploids may once again have advantages during the global events including widespread extinction (see Parmesan and Hanley, 2016) now being detected, including climate change. Plants have genetic mechanisms to overcome the challenges of polyploidy, in particular co-regulation of multiple, similar or identical copies of genes, and the adoption of vegetative or apomictic reproduction, or restitution of diploid behaviour during chromosome pairing and recombination at meiosis. Now, we can identify the impact of polyploidy, and associated hybridity, on speciation, regulatory mechanisms at the gene expression level, and examine its impact on plant populations, as reported in this Special Issue.

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REFERENCES

- Ågren JA. 2013.** Selfish genes and plant speciation. *Evolutionary Biology* **40**: 439–449.
- Ågren JA, Wright SI. 2011.** Co-evolution between transposable elements and their hosts: a major factor in genome size evolution? *Chromosome Research* **19**: 777-786.
- Alix K, Joets J, Ryder CD, Moore J, Barker GC, Bailey JP, King GJ, Heslop-Harrison JS. 2008.** The CACTA transposon *Bot1* played a major role in *Brassica* genome divergence and gene proliferation. *The Plant Journal* **56**: 1030–1044.
- Alsayed N, Fernández JA, Schwarzacher T, Heslop-Harrison JS. 2015.** Diversity and relationships of *Crocus sativus* and its relatives analysed by Inter Retroelement Amplified Polymorphism (IRAP). *Annals of Botany* **116**: 359-368.
- Amborella Genome Project. 2013.** The *Amborella* Genome and the Evolution of Flowering Plants. *Science* **342**: 1241089.
- APG IV. 2016.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181**: 1–20.
- Arrigo N, Barker MS. 2012.** Rarely successful polyploids and their legacy in plant genomes. *Current Opinion in Plant Biology* **15**: 140-146.

- Ashman TL, Kwok A, Husband BC. 2013.** Revisiting the dioecy-polyploidy association: alternate pathways and research opportunities. *Cytogenetic and Genome Research* **140**: 241–255.
- Barker MS, Husband BC, Pires JC. 2016.** Spreading wine and flying high: the evolutionary importance of polyploidy after a century of study. *American Journal of Botany* **103**: 1139-1145.
- Barringer BC. 2007.** Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* **94**: 1527–1533.
- Beike AK, von Stackelberg M, Schallenberg-Rüdinger M, Hanke ST, Follo M, Quandt D, McDaniel SF, Reski R, Tan BC, Rensing SA. 2014.** Molecular evidence for convergent evolution and allopolyploid speciation within the *Physcomitrium-Physcomitrella* species complex. *BMC Evolutionary Biology* **14**: 158.
- Bell CD, Soltis DE, Soltis PS. 2010.** The age and diversification of the angiosperms re-revisited. *American Journal of Botany* **97**: 1296–1303.
- Bennett MD, Leitch IJ. 2011.** Nuclear DNA amounts in angiosperms: targets, trends and tomorrow. *Annals of Botany* **107**: 467-590.
- Bennetzen JL, Wang H. 2014.** The contributions of transposable elements to the structure, function, and evolution of plant genomes. *Annual Review of Plant Biology* **65**: 505-530.
- Biscotti MA, Olmo E, Heslop-Harrison JS. 2015.** Repetitive DNA in eukaryotic genomes. *Chromosome Research* **23**: 415-420.
- Bombarely A, Moser M, Amrad A et al. 2016.** Insight into the evolution of the Solanaceae from the parental genomes of *Petunia hybrida*. *Nature Plants* **2**: 16074.
- Bowers JE, Chapman BA, Rong J, Paterson AH. 2003.** Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* **422**: 433-438.

- Buggs RJA, Soltis PS, Soltis DE. 2009.** Does hybridization between divergent progenitors drive whole-genome duplication? *Molecular Ecology* **18**: 3334–3339.
- Cascales-Miñana B, Cleal CJ, Gerrienne P. 2016.** Is Darwin's 'Abominable Mystery' still a mystery today? *Cretaceous Research* **61**: 256–262.
- Catalan P, Muller J, Hasterock R, et al., 2012.** Evolution and taxonomic split of the model grass *Brachypodium distachyon*. *Annals of Botany* **109**: 385-405.
- Čertner M, Fenclová E, Kúr P et al. 2017.** Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. *Annals of Botany* **This issue**.
- Chen ZJ, Yu HH. 2013.** Genetic and epigenetic mechanisms for polyploidy and hybridity. In: Chen ZJ, Birchler JA, eds. *Polyploid and Hybrid Genomics*. Wiley-Blackwell, New York. 335–354.
- Chumová Z, Závěská E, Mandáková T, Krak K, Trávníček P. 2017.** The Mediterranean: the cradle of *Anthoxanthum* (Poaceae) diploid diversity. *Annals of Botany* **This issue**.
- Collares-Pereira MJ, Matos I, Morgado-Santos M, Coelho MM. 2013.** Natural pathways towards polyploidy in animals: the *Squalius alburnoides* fish complex as a model system to study genome size and genome reorganization in polyploids. *Cytogenetic and Genome Research* **140**: 97-116.
- Comai L. 2005.** The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* **6**: 836–846.
- Contento A, Heslop-Harrison JS, Schwarzacher T. 2005.** Diversity of a major repetitive DNA sequence in diploid and polyploid Triticeae. *Cytogenetic and Genome Research* **109**: 34-42.
- Coyne JA, Orr HA. 2004.** *Speciation*. Sinauer Associates Inc., Sunderland, MA, USA.

- Crawford M, Jesson LK, Garnock-Jones PJ. 2009.** Correlated evolution of sexual system and life history traits in mosses. *Evolution* **63**: 1129–1142.
- Cuadrado A, de Bustos A, Jouve N. 2017.** On the allopolyploid origin and genome structure of the closely related species *Hordeum secalinum* and *Hordeum capense* inferred by molecular karyotyping. *Annals of Botany* **This issue**.
- De Bodt S, Maere S, Van de Peer Y. 2005.** Genome duplication and the origin of angiosperms. *Trends in Ecology and Evolution* **20**: 591-597.
- D'Hont A, Denoeud F, Aury JM et al. 2012.** The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature* **488**: 213-217.
- Douglas GM, Gos G, Steige KA et al. 2015.** Hybrid origins and the earliest stages of diploidization in the highly successful recent polyploid *Capsella bursa-pastoris*. *Proceedings of the National Academy of Sciences of the USA* **112**: 2806–2811.
- Downen RH, Pelizzola M, Schmitz RJ, Lister R, Downen JM, Nery JR, Dixon JE, Ecker JR. 2012.** Widespread dynamic DNA methylation in response to biotic stress. *Proceedings of the National Academy of Sciences of the United States of America* **109**: E2183–E2191.
- Fawcett JA, Maerea S, Van de Peer Y. 2009.** Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction event. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 5737-5742.
- Field TS, Arens NC. 2007.** The ecophysiology of early angiosperms. *Plant, Cell and Environment* **30**: 291–309.
- Fortune PM, Schierenbeck KA, Ainouche AK, Jacquemin J, Wendel JF, Ainouche ML. 2007.** Evolutionary dynamics of *Waxy* and the origin of hexaploid *Spartina* species (Poaceae). *Molecular Phylogenetics and Evolution* **43**: 1040-1055.

- Freeling M, Xu J, Woodhouse M, Lisch D. 2015.** A solution to the C-value paradox and the function of junk DNA: the genome balance hypothesis. *Molecular Plant* **8**: 899-910.
- Friis EM, Raunsgaard Pedersen K, Crane PR. 2006.** Cretaceous angiosperm flowers: Innovation and evolution in plant reproduction. *Palaeogeography, Palaeoclimatology, Palaeoecology* **232**: 251–293.
- Gandolfo MA, Hermsen EJ. 2017.** *Ceratopetalum* (Cunoniaceae) fruits of Australasian affinity from the early Eocene Laguna del Hunco flora, Patagonia, Argentina. *Annals of Botany* **119**: 507-516.
- Glick L, Sabath N, Ashman TL, Goldberg E, Mayrose I. 2016.** Polyploidy and sexual system in angiosperms: Is there an association? *American Journal of Botany* **103**: 1223–1235.
- Grandont L, Jenczewski E, Lloyd A. 2013.** Meiosis and its deviations in plants. *Cytogenetic and Genome Research* **140**: 171-184.
- Ha M, Lu J, Tian L, Ramachandran V, Kasschau KD, Chapman EJ, Carrington JC, Chen X, Wang XJ, Chen ZJ. 2009.** Small RNAs serve as genetic buffer against genomic shock in *Arabidopsis* interspecific hybrids and allopolyploids. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 17835-17840.
- Haldane, JBS. 1959.** The theory of natural selection to-day. *Nature* **183**: 710-713.
- Harpke D, Carta A, Tomović G, V Randelović, Blattner FR, Peruzzi L. 2015.** Phylogeny, karyotype evolution and taxonomy of *Crocus* series *Verni* (Iridaceae). *Plant Systematics and Evolution* **301**: 309–325.
- Hegarty MJ, Hiscock SJ. 2008.** Genomic clues to the evolutionary success of polyploid plants. *Current Biology* **18**: R435–R444.
- Herben T, Suda J, Klimešová J. 2017.** Polyploid species rely on vegetative reproduction more than diploids: a re-examination of the old hypothesis. *Annals of Botany* **This issue**.

- Herklotz V, Ritz CM. 2017.** Multiple and asymmetrical origin of polyploidy dog rose hybrids (*Rosa* L. sect. *Caninae* (DC.) Ser.) involving unreduced gametes. *Annals of Botany* **This issue.**
- Heslop-Harrison JS. 2017.** Polyploidy. *Reference Module in Life Sciences (LIFE)*. Elsevier. in proof.
- Heslop-Harrison JS, Schwarzacher T. 2007.** Domestication, genomics and the future for banana. *Annals of Botany* **100**:1073-1084.
- Heslop-Harrison Y. 1968.** *Rubus*. In Flora Europaea. Vol. 2.(Eds TG Tutin, VH Heywood, NA Burges, DM Moore, DH Valentine, SM Walters, DA Webb) pp. 7–25. Cambridge.
- Hoffmann FG, Opazo JC, Storz JF. 2012.** Whole-Genome Duplications Spurred the Functional Diversification of the Globin Gene Superfamily in Vertebrates. *Molecular Biology and Evolution* **29**: 303-312.
- Hunt HV, Badakshi F, Romanova O, Howe CJ, Jones M, Heslop-Harrison JS. 2014.** Reticulate evolution in *Panicum* (Poaceae): the origin of tetraploid broomcorn millet, *P. miliaceum*. *Journal of Experimental Botany* **65**: 3165-3175.
- Husband BC, Baldwin SJ, Suda J. 2013.** The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. In: Leitch IJ, Greilhuber J, Dolezel J, Wendel JF, eds. Plant genome diversity. 2: Physical structure, behaviour and evolution of plant genomes. Springer Verlag, The Netherlands, 255–276.
- Husband BC, Ozimec B, Martin SL, Pollock L. 2008.** Mating consequences of polyploid evolution in flowering plants: current trends and insights from synthetic polyploids. *International Journal of Plant Sciences* **169**: 195–206.
- Jenczewski E, Alix K. 2004.** From diploids to allopolyploids: the emergence of efficient pairing control genes in plants. *Critical Reviews in Plant Sciences* **23**: 21–45.

- Jesson LK, Cavanagh AP, Perley DS. 2011.** Polyploidy influences sexual system and mating patterns in the moss *Atrichum undulatum sensu lato*. *Annals of Botany* **107**: 135–143.
- Jiao Y, Wickett NJ, Ayyampalayam S et al. 2011.** Ancestral polyploidy in seed plants and angiosperms. *Nature* **473**: 97-102.
- Jones N, Pašakinskienė I. 2005.** Genome conflict in the gramineae. *New Phytologist* **165**: 391–410.
- Karlin EF, Smouse PE. 2017.** Allo-allo-triploid *Sphagnum* \times *falcatulum*: single individuals contain most of the Holantarctic diversity for ancestrally indicative markers. *Annals of Botany* **This issue**.
- Kenan-Eichler M, Leshkowitz D, Tal L, Noor E, Melamed-Bessudo C, Feldman M, Levy AA. 2011.** Wheat hybridization and polyploidization results in deregulation of small RNAs. *Genetics* **188**: 263-272.
- Kenny NJ, Chan KW, Nong W, Qu Z, Maeso I, Yip HY, Chan TF, Kwan HS, Holland PWH, Chu KH, Hui JHL. 2016.** Ancestral whole-genome duplication in the marine chelicerate horseshoe crabs. *Heredity* **116**: 190–199.
- Krahulcová A, Trávníček P, Krahulec F, Rejmánek M. 2017.** Small genomes and large seeds: chromosome numbers, genome size and seed mass in diploid *Aesculus* species (Sapindaceae). *Annals of Botany* **119**: 957-964.
- Kron P, Suda J, Husband BC. 2007.** Applications of flow cytometry to evolutionary and population biology. *Annual Review of Ecology, Evolution, and Systematics* **38**: 847–876.
- Landrein S, Buerki S, Wang H-F, Clarkson JJ. 2017.** Untangling the reticulate history of species complexes and horticultural breeds in *Abelia* (Caprifoliaceae). *Annals of Botany* **This issue**.

- Laport RG, Minckley RL, Ramsey J. 2016.** Ecological distributions, phenological isolation, and genetic structure in sympatric and parapatric populations of the *Larrea tridentata* polyploid complex. *American Journal of Botany* **103**: 1358–1374.
- Leitch AR, Leitch IJ. 2008.** Genomic plasticity and the diversity of polyploid plants. *Science* **320**: 481-483.
- Leliaert F, Smith DR, Moreau H, Herron MD, Verbruggen H, Delwiche CF, De Clerck O. 2012.** Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences* **31**: 1–46.
- Leliaert F, Verbruggen H, Zechman FW. 2011.** Into the deep: New discoveries at the base of the green plant phylogeny. *BioEssays* **33**: 683-692
- Levin DA. 2002.** The role of chromosomal change in plant evolution. Oxford University Press, New York.
- Lewsey MG, Hardcastle TJ, Melnyk CW, Molnar A, Valli A, Urich MA, Nery JR, Baulcombe DC, Ecker JR. 2016.** Mobile small RNAs regulate genome-wide DNA methylation. *Proceedings of the National Academy of Sciences of the United States of America* **113**: E801-E810.
- Li Z, Baniaga AE, Sessa EB, Scascitelli M, Graham SW, Rieseberg LH, Barker MS. 2015.** Early genome duplications in conifers and other seed plants. *Science Advances* **1**: e1501084
- Li Z, Defoort J, Tasdighian S, Maere S, Van de Peer Y, De Smet R. 2016.** Gene duplicability of core genes is highly consistent across all angiosperms. *The Plant Cell* **28**: 326–344.
- Lisch D. 2013.** How important are transposons for plant evolution? *Nature Reviews Genetics* **14**: 49-61.
- Liston A, Cronn R, Ashman T-L. 2014.** *Fragaria*: a genus with deep historical roots and ripe for evolutionary and ecological insights. *American Journal of Botany* **101**: 1686 – 1699.

- Lu Y, Ran J-H, Guo D-M, Yang Z-Y, Wang X-Q. 2014.** Phylogeny and divergence times of gymnosperms inferred from single-copy nuclear genes. *PLoS ONE* **9**: e107679.
- Mable BK, Alexandrou MA, Taylor MI. 2011.** Genome duplication in amphibians and fish: an extended synthesis. *Journal of Zoology* **284**: 151–182.
- Madlung A. 2013.** Polyploidy and its effect on evolutionary success: old questions revisited with new tools. *Heredity* **110**: 99–104.
- Majeský L, Vašut RJ, Kitner M, Trávníček B. 2012.** The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts. *PLoS One* **7**:e41868.
- Martienssen RA. 2010.** Heterochromatin, small RNA and post-fertilization dysgenesis in allopolyploid and interploid hybrids of *Arabidopsis*. *New Phytologist* **186**: 46-53.
- Martin SL, Husband BC. 2009.** Influence of phylogeny and ploidy on species ranges of North American angiosperms. *Journal of Ecology* **97**: 913–922.
- Martinez Palacios P. 2014.** *Response of non-coding components of the genome – transposable elements and small non-coding RNAs – to a new allopolyploidisation event: the genome of oilseed rape (Brassica napus) as a model of study.* PhD Thesis, Université Paris Sud, GQE – Le Moulon, Gif-sur-Yvette, France.
- Matsunaga KK, Tomescu AM. 2016.** Root evolution at the base of the lycophyte clade: insights from an Early Devonian lycophyte. *Annals of Botany* **117**: 585-598.
- Mayrose I, Zhan SH, Rothfels CJ, Arrigo N, Barker MS, Rieseberg LH, Otto SP. 2015.** Methods for studying polyploidy diversification and the dead end hypothesis: a reply to Soltis *et al.* (2014). *New Phytologist* **206**: 27-35.
- Mayrose I, Zhan SH, Rothfels CJ, Magnuson-Ford K, Barker MS, Rieseberg LH, Otto SP. 2011.** Recently formed polyploid plants diversify at lower rates. *Science* **333**: 1257.

- McClintock B. 1984.** The significance of responses of the genome to challenge. *Science* **226**: 792-801.
- Mirouze M, Vitte C. 2014.** Transposable elements, a treasure trove to decipher epigenetic variation: insights from *Arabidopsis* and crop epigenomes. *Journal of Experimental Botany* **65**: 2801–2812.
- Moore MJ, Bell CD, Soltis PS, Soltis DE. 2007.** Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 19363–19368.
- Münzbergová Z. 2017.** Consequences of polyploidization vary between populations and their estimate is affected by colchicine application even in the second generation. *Annals of Botany* **This issue**.
- Mutti JS, Bhullar RK, Gill KS. 2017.** Evolution of gene expression balance among homeologs of natural polyploids. *G3: Genes, Genomes, Genetics* **7**: 1225-1237.
- Negi P, Rai AN, Suprasanna P. 2016.** Moving through the stressed genome: emerging regulatory roles for transposons in plant stress response. *Frontiers in Plant Science* **7**: 1448.
- Ng DW-K, Lu J, Chen ZJ. 2012.** Big roles for small RNAs in polyploidy, hybrid vigor, and hybrid incompatibility. *Current Opinion in Plant Biology* **15**: 154-161.
- Orgaard M, Jacobsen N, Heslop-Harrison JS. 1995.** The hybrid origin of two cultivars of *Crocus* (Iridaceae) analysed by molecular cytogenetics including genomic Southern and *in situ* hybridization. *Annals of Botany* **76**: 253-262.
- Otto SP, Whitton J. 2000.** Polyploid incidence and evolution. *Annual Review of Genetics* **34**: 401–437.
- Otto SP. 2007.** The evolutionary consequences of polyploidy. *Cell* **131**: 452–462.

- Parmesan C, Hanley ME. 2016.** Plants and climate change: complexities and surprises. *Annals of Botany* **116**: 849-864.
- Pandit MK, Pockock MJO, Kunin WE. 2011.** Ploidy influences rarity and invasiveness in plants. *Journal of Ecology* **99**: 1108–1115.
- Parisod C, Alix K, Just J, Petit M, Sarilar V, Mhiri C, Ainouche M, Chalhoub B, Grandbastien M-A. 2010a.** Impact of transposable elements on the organization and function of allopolyploid genomes. *New Phytologist* **186**: 37-45.
- Parisod C, Holderegger R, Brochmann C. 2010b.** Evolutionary consequences of autopolyploidy. *New Phytologist* **186**: 5-17.
- Patel D, Power JB, Anthony P, Badakshi F, Pat Heslop-Harrison JS, Davey MR. 2011.** Somatic hybrid plants of *Nicotiana* × *sanderae* (+) *N. debneyi* with fungal resistance to *Peronospora tabacina*. *Annals of Botany* **108**(5): 809-819.
- Paule J, Wagner ND, Weising K, Zizka G. 2017.** Ecological range shift in the polyploid members of the South American genus *Fosterella* (Bromeliaceae). *Annals of Botany* **This issue**.
- Pearce SR, Harrison G, Wilkinson M, Li D, Heslop-Harrison JS, Flavell AJ, Kumar A. 1996.** The *Ty1-copia* group retrotransposons in *Vicia* species: copy number, sequence heterogeneity and chromosomal localisation. *Molecular and General Genetics* **250**: 305-315.
- Presgraves DC. 2010.** The molecular evolutionary basis of species formation. *Nature Reviews Genetics* **11**: 175–180.
- Ramsey J, Schemske DW. 1998.** Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29**: 467–501.
- Ramsey J, Schemske DW. 2002.** Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* **33**: 589–639.

- Ramsey J. 2011.** Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences of the USA* **108**: 7096–7101.
- Renny-Byfield S, Wendel JF. 2014.** Doubling down on genomes: polyploidy and crop plants. *American Journal of Botany* **101**: 1711 – 1725.
- Ricca M, Shaw AJ. 2010.** Allopolyploidy and homoploid hybridization in the *Sphagnum subsecundum* complex (Sphagnaceae: Bryophyta). *Biological Journal of the Linnean Society* **99**: 135–151.
- Rieseberg LH. 2001.** Polyploid evolution: keeping the peace at genomic reunions. *Current Biology* **11**: R925–R928.
- Rieseberg LH, Blackman BK. 2010.** Speciation genes in plants. *Annals of Botany* **106**: 439–456.
- Ritz CM, Wissemann V. 2011.** Microsatellite analyses of artificial and spontaneous dogrose hybrids reveal the hybridogenic origin of *Rosa micrantha* by the contribution of unreduced gametes. *Journal of Heredity* **102**: 217-227.
- Robertson A, Rich TCG, Allen AM et al. 2010.** Hybridization and polyploidy as drivers of continuing evolution and speciation in *Sorbus*. *Molecular Ecology* **19**: 1675–1690.
- Robertson K, Goldberg EE, Igić B. 2011.** Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae. *Evolution* **65**: 139–155.
- Roche D, Hanna WW, Ozias-Akins P. 2001.** Is supernumerary chromatin involved in gametophytic apomixis of polyploid plants? *Sexual Plant Reproduction* **13**: 343–349.
- Roux C, Pannell JR. 2015.** Inferring the mode of origin of polyploid species from next-generation sequence data. *Molecular Ecology* **24**: 1047–1059.
- Salman-Minkov A, Sabath N, Mayrose I. 2016.** Whole-genome duplication as a key factor in crop domestication. *Nature Plants* **2**: 1-4.

- Santos FC, Guyot R, Valle CB do, Chiari L, Techio VH, Heslop-Harrison P, Vanzela ALL. 2015.** Chromosomal distribution and evolution of abundant retrotransposons in plants: gypsy elements in diploid and polyploidy *Brachiaria* forage grasses. *Chromosome Research* **23**: 571-582.
- Šarhanová P, Sharbel TF, Sochor M, Vašut RJ, Dančák M, Trávníček B. 2017.** Hybridization drives evolution of apomicts in *Rubus* subgenus *Rubus* – evidence from microsatellite markers. *Annals of Botany* **This issue**.
- Sattler MC, Carvalho CR, Clarindo WR. 2016.** The polyploidy and its key role in plant breeding. *Planta* **243**: 281-296.
- Scott AD, Stenz NW, Ingvarsson PK, Baum DA. 2016.** Whole genome duplication in coast redwood (*Sequoia sempervirens*) and its implications for explaining the rarity of polyploidy in conifers. *New Phytologist* **211**:186-193.
- Sepsi A, Higgins JD, Heslop-Harrison JS, Schwarzacher T. 2017.** CENH3 morphogenesis reveals dynamic centromere associations during synaptonemal complex formation and the progression through male meiosis in hexaploid wheat. *The Plant Journal* **89**: 235-249.
- Shaw AJ, Devos N, Liu Y, Cox CJ, Goffinet B, Flatberg KI, Shaw B. 2016.** Organellar phylogenomics of an emerging model system: *Sphagnum* (peatmoss). *Annals of Botany* **118**: 185-196.
- Slotkin RK, Vaughn M, Borges F, Tanurdzic M, Becker JD, Feijo JA, Martienssen RA. 2009.** Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* **136**: 461-472.
- Soltis PS, Marchant DB, Van de Peer Y, Soltis DE. 2015.** Polyploidy and genome evolution in plants. *Current Opinion in Genetics and Development* **35**: 119–125.

- Soltis DE, Segovia-Salcedo MC, Jordon-Thaden I, Majure L, Miles NM, Mavrodiev EV, Mei W, Cortez MB, Soltis PS, Gitzendanner MA. 2014.** Are polyploids really evolutionary dead-ends (again)? A critical reappraisal of Mayrose *et al.* (2011). *New Phytologist* 202: 1105–1117.
- Soltis PS, Soltis DE. 2009.** The role of hybridization in plant speciation. *Annual Review of Plant Biology* 60: 561-588.
- Soltis DE, Soltis PS, Schemske DW *et al.* 2007.** Autopolyploidy in angiosperms: have we grossly underestimated the number of species? *Taxon* 56: 13–30.
- Soltis DE, Visger CJ, Marchant DB, Soltis PS. 2016.** Polyploidy: Pitfalls and paths to a paradigm. *American Journal of Botany* 103: 1146-1166.
- Stebbins GL. 1950.** Variation and evolution in plants. Columbia University Press, New York.
- Sweigart AL, Martin NH, Willis JH. 2008.** Patterns of nucleotide variation and reproductive isolation between a *Mimulus* allotetraploid and its progenitor species. *Molecular Ecology* 17: 2089–2100.
- Swift H. 1950.** The constancy of desoxyribose nucleic acid in plant nuclei. *Proceedings of the National Academy of Sciences of the USA* 36: 643–654.
- Taketa S, Harrison G, Heslop-Harrison JS. 1999.** Comparative physical mapping of the 5S and 18S-25S rDNA in nine wild *Hordeum* species and cytotypes. *Theoretical and Applied Genetics* 98: 1-9.
- Tam SM, Causse M, Garchery C, Burck H, Mhiri C, Grandbastien MA. 2007.** The distribution of copia-type retrotransposons and the evolutionary history of tomato and related wild species. *Journal of Evolutionary Biology* 20:1056-1072.
- Tank DC, Eastman JM, Pennell MW, Soltis PS, Soltis DE, Hinchliff CE, Brown JW, Sessa EB, Harmon LJ. 2015.** Nested radiations and the pulse of angiosperm diversification:

- increased diversification rates often follow whole genome duplications. *New Phytologist* **207**: 454–467.
- Tayalé A, Parisod C. 2013.** Natural pathways to polyploidy in plants and consequences for genome reorganization. *Cytogenetic and Genome Research* **140**: 79-96.
- Uhrinová V, Zozomová-Lihová J, Bernátová D, Paule J, Paule L, Gömöry D. 2017.** Origin and genetic differentiation of pink-flowered *Sorbus* hybrids in the Western Carpathians. *Annals of Botany* **This issue**.
- Vallejo-Marín M, Buggs RJA, Cooley AM, Puzey JR. 2015.** Speciation by genome duplication: repeated origins and genomic composition of the recently formed allopolyploid species *Mimulus peregrinus*. *Evolution* **69**: 1487–1500.
- Van der Niet T, Peakall R, Johnson SD. 2014.** Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany* **113**: 199-212.
- Vanneste K, Baele G, Maere S, Van de Peer Y. 2014.** Analysis of 41 plant genomes supports a wave of successful genome duplications in association with the Cretaceous–Paleogene boundary. *Genome Research* **24**: 1334–1347.
- Vicient CM, Casacuberta JM. 2017.** Impact of transposable elements on polyploid plant genomes. *Annals of Botany* **This issue**.
- Vitte C, Fustier M-A, Alix K, Tenailon MI. 2014.** The bright side of transposons in crop evolution. *Briefings in functional genomics* **13**: 276-295.
- Vitte C, Panaud O. 2005.** LTR retrotransposons and flowering plant genome size: emergence of the increase/decrease model. *Cytogenetic and Genome Research* **110**: 91-107.
- Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM. 2013.** Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* **140**: 137–150.

- Wendel JF. 2015.** The wondrous cycles of polyploidy in plants. *American Journal of Botany* **102**: 1753–1756.
- Whitton J, Sears CJ, Baack EJ, Otto SP. 2008.** The dynamic nature of apomixis in the angiosperms. *International Journal of Plant Sciences* **169**: 169–182.
- Woodhouse MR, Cheng F, Pires JC, Lisch D, Freeling M, Wang X. 2014.** Origin, inheritance, and gene regulatory consequences of genome dominance in polyploids. *Proceedings of the National Academy of Sciences* **111**: 5283-5288.
- Wu JH, Ferguson AR, Murray BG, Jia Y, Datson PM, Zhang J. 2012.** Induced polyploidy dramatically increases the size and alters the shape of fruit in *Actinidia chinensis*. *Annals of Botany* **109**: 169-179.
- Yan L-J, Burgess KS, Milne R, Fu C-N, Li D-Z, Gao L-M. 2017.** Asymmetric natural hybridization varies among hybrid swarms between two diploid *Rhododendron* species. *Annals of Botany* **This issue**.
- Zhang J, Liua Y, Xia E-H, Yao Q-Y, Liu X-D, Gao L-Z. 2015.** Autotetraploid rice methylome analysis reveals methylation variation of transposable elements and their effects on gene expression. *Proceedings of the National Academy of Sciences of the United States of America* **112**: E7022–E7029.

LEGENDS TO FIGURES

Figure 1. Simplified phylogeny of the green plant lineage focusing on the occurrence of WGD (Whole Genome Duplication) events. Polyploidy events (yellow diamonds) refer to either single or multiple rounds of WGD (i.e. duplication or triplication) and are labelled where applicable (Greek letters; see references below). Complete genome sequences have clearly established that WGD has remarkably shaped the evolutionary history of angiosperms compared to the other major clades of green plants. Estimates for the age of angiosperms have suggested the range of 167-199 million years ago (Mya) (Bell *et al.*, 2010). Then rapid radiations responsible for the extant angiosperm diversity occurred after the early diversification of Mesangiospermae 139-156 Mya (Moore *et al.*, 2007; Bell *et al.*, 2010) with a burst of diversification specific of the Cretaceous, less than 125 Mya (age of the earliest angiosperm macrofossil; Cascales-Miñana *et al.*, 2016).

Early divergence times are from Bell *et al.* (2010) and Leliaert *et al.* (2012); for angiosperms from Fawcett *et al.* (2009), Jiao *et al.* (2011) and Li *et al.* (2016) and for gymnosperms from Lu *et al.* (2014). Dashed lines indicate imprecise timing or approximate representation of lineage divergence.

WGD events are from Jiao *et al.* (2011); Leliaert *et al.* (2011); d'Hont *et al.* (2012); Beike *et al.* (2014); Renny-Byfield and Wendel (2014); Li *et al.* (2015; 2016); Scott *et al.* (2016); Shaw *et al.* (2016) Bombarely *et al.* (2016). See corresponding publications for precise estimates of time divergence and occurrence of WGD.

AGF: hypothetical ancestral green flagellate; ANA: basal angiosperms including Amborellales, Nymphaeales, Austrobaileyales; following a standardized method, Greek letters are used to name polyploidy events along the phylogenetic tree, starting from the α (alpha) and β (beta) events that have been identified in the *Arabidopsis* genome (Bowers *et al.*, 2003).

Figure 2. Metaphase chromosomes of diploid, tetraploid and hexaploid wheats stained with the DNA stain DAPI (cyan) and showing fluorescent *in situ* hybridisation signal (magenta) from the 120 bp tandemly repeated (pSc119.2) DNA family common to many Triticeae species (see Contento *et al.*, 2005). This repeat family originated before the split of rye, barely, wheat and other grasses in the tribe, but has been amplified differentially in the different species. It forms large blocks at subtelomeric and intercalary chromosomal regions in the B genome wheats, both seen in the seven chromosome pairs in the diploid (A), tetraploid (B) and hexaploid (C), but has only few sites in about half of the A and D genome chromosomes with weak single subtelomeric foci (B,C).

A: *Aegilops speltoides* ($2n=2x=14$, genome constitution B'B'); B: *Triticum durum* ($2n=4x=28$, AABB); C: *T. aestivum* ($2n=6x=28$, AABBDD). Bar 10 μm .

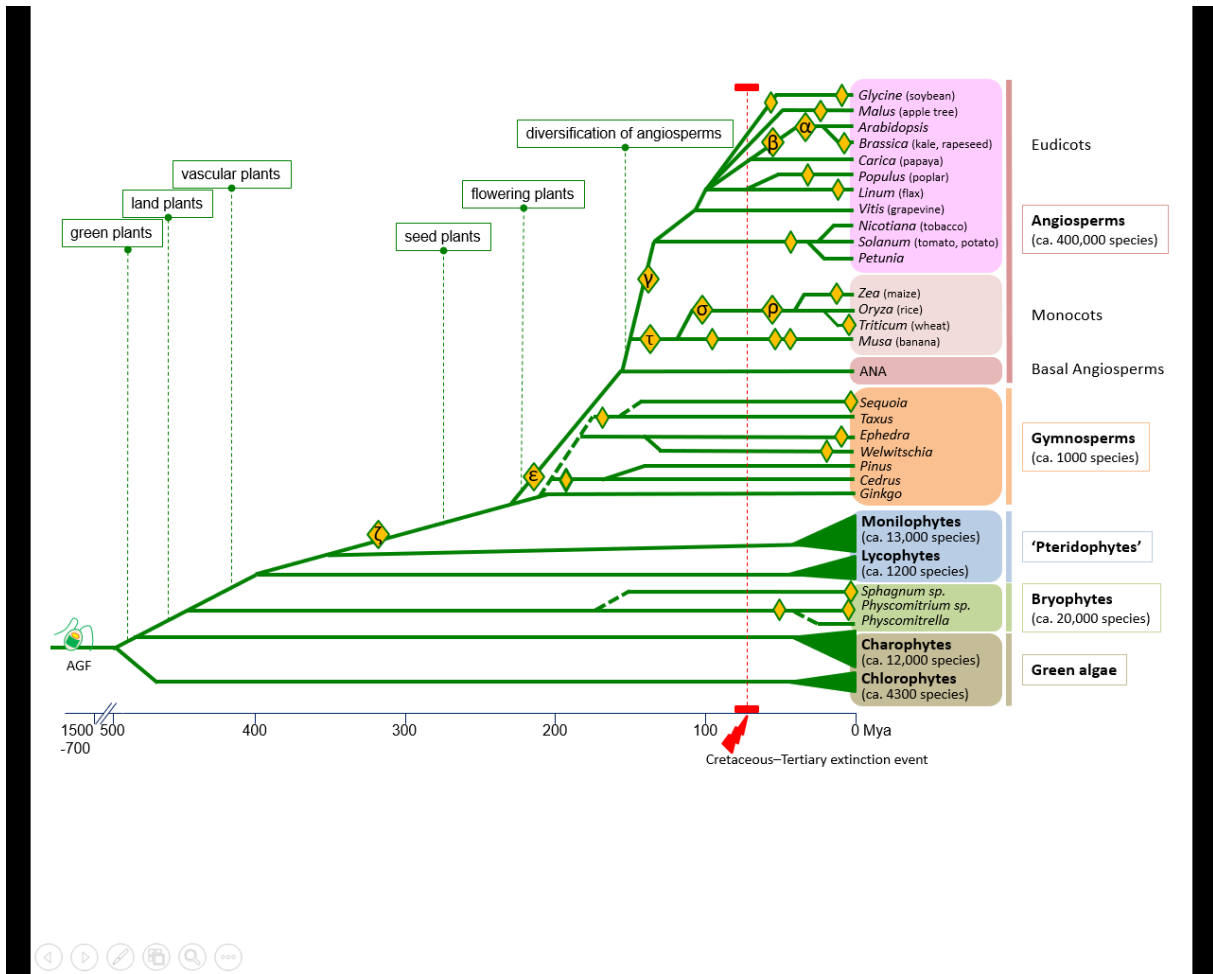


Figure 1. Alix et al. 2017. Polyploidy. *Annals of Botany* 120: 183-194.

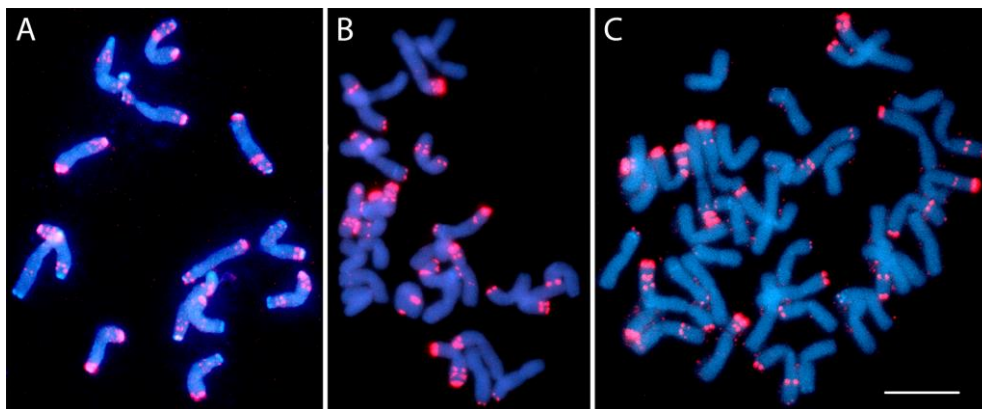


Figure 2. Alix et al. 2017. Polyploidy. *Annals of Botany* 120: 183-194.