THE ADVANTAGES AND DISADVANTAGES OF BEING POLYPLOID

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Abstract | Polyploids — organisms that have multiple sets of chromosomes — are common in certain plant and animal taxa, and can be surprisingly stable. The evidence that has emerged from genome analyses also indicates that many other eukaryotic genomes have a polyploid ancestry, suggesting that both humans and most other eukaryotes have either benefited from or endured polyploidy. Studies of polyploids soon after their formation have revealed genetic and epigenetic interactions between redundant genes. These interactions can be related to the phenotypes and evolutionary fates of polyploids. Here, I consider the advantages and challenges of polyploidy, and its evolutionary potential.

NEOPOLYPLOID
A polyploid that has been produced by artificially inducing chromosome doubling.

DIPLOIDIZATION
Gradual conversion from polyploidy to diploidy through genetic changes that differentiate duplicated loci.

SUBFUNCTIONALIZATION
Retention by duplicated genes of different components of the original common function.

NEOFUNCTIONALIZATION
Acquisition of novel function by a duplicated gene.

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Polyploidy is the heritable condition of possessing more than two complete sets of chromosomes. Most polyploids have an even number of sets of chromosomes, with four being the most common (tetraploidy). Polyploids are very common among plants and common among fish and amphibians, and are usually fit and well adapted. Indeed, the study of eukaryotic genomes is providing surprising proof of the evolutionary potential of polyploids: many sequenced genomes display the signature of polyploidy ancestry[1–8]. This indicates that polyploidy can bestow long-term evolutionary flexibility, instead of freezing species in a static state that is enforced by gene redundancy, as was originally proposed[9].

In fact, polyploidy can be advantageous. On the basis of the phenotypic and molecular characterization of NEOPOLYPLOIDS, it has been inferred that after polyploids form they pass through a bottleneck of instability[10–13], before becoming adapted and joining the evolutionary fray as efficient competitors of their diploid relatives. Adapted polyploids that avoid extinction enter an evolutionary trajectory of DIPLOIDIZATION, during which genomic redundancy is reduced[14,15]. Duplicated genes can be lost, retained or maintained as duplicates, often undergoing SUBFUNCTIONALIZATION and NEOFUNCTIONALIZATION[16,17]. Bioinformatic and theoretical analyses indicate that these processes are often not random and that the function and properties of the encoded protein affect the outcome[18–25]. By providing duplicated genes, polyploidization might fuel long-term diversification and evolutionary success.

In this review I discuss possible advantages of polyploidy and constraints on polyploid formation that are indicated by either experimental evidence or theoretical considerations. Becoming and remaining polyploid changes the organization and function of the genome at both genetic and EPIGENETIC levels. For example, in addition to the creation of gene redundancy, polyploidy causes nuclear enlargement and increases the complexity of the processes that are involved in managing and partitioning chromosomes during cell division. Perhaps the most striking evidence of change comes from the discovery of epigenetic remodelling, which leads to both the activation and suppression of gene expression. Although some of these changes are potentially advantageous, many cause instability of the neopolyploids and might be disruptive. This review of how polyploids form and adapt will not only be useful to readers who are interested in the evolutionary role of polyploids. It will also be useful to those who are interested in how parental gene interactions can lead to HETEROSIS or DYSGENESIS,

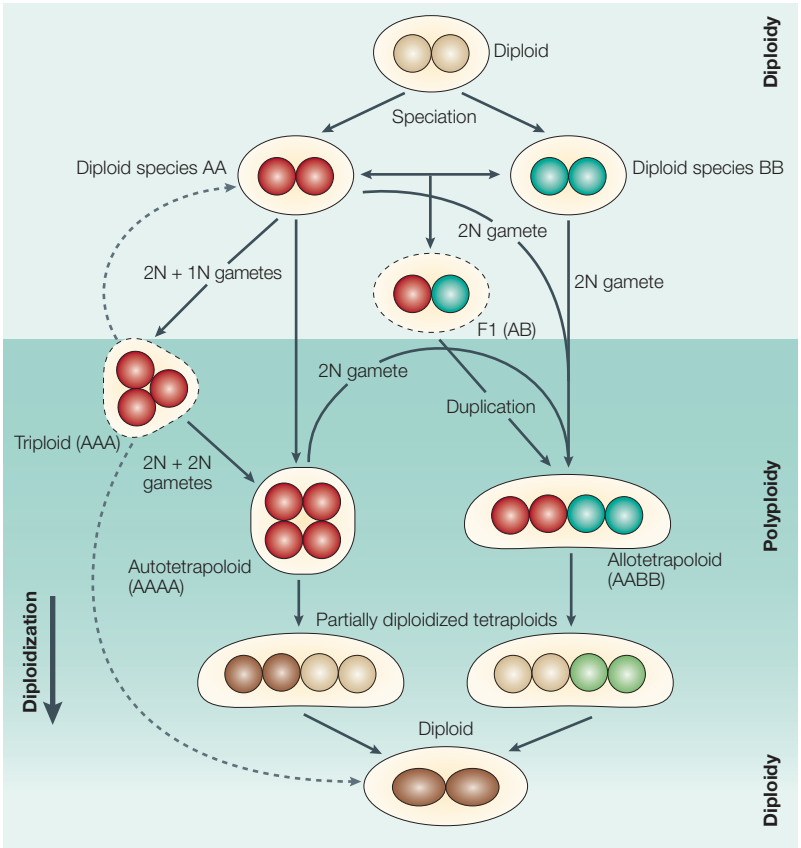


Figure 1 | **Evolutionary alternation of diploidy and polyploidy.** The figure shows the possible paths that result in the sudden transition from diploidy to polyploidy and the gradual transition from polyploidy to diploidy. The hybridization events that result in ALLOPOLYPLOIDY are also illustrated. For simplicity, not all possible paths are drawn. Triploids, for example, are shown contributing to autotetraploids but they can also contribute to allopolyploids. For each ploidy form, the haploid genome is represented by a coloured circle or oval inside the beige-filled nuclear shape. Genomes that are illustrated by ovals reflect the increased gene number that results from the retention and subfunctionalization of duplicates during diploidization. Circles or ovals of different colours represent diverged genomes. Highly unstable ploidy forms have dashed nuclear contours. A and B represent genome types and N is the gametic chromosome number.

in how epigenetic patterns are maintained or altered, and in how structural features of genomic organization affect cellular functions.

The mechanics of polyploidy

Formation and incidence of polyploidy. Polyploids are divided into categories depending on their chromosomal composition and their manner of formation. Polyploids arise when a rare mitotic or meiotic catastrophe causes the formation of gametes that have more than one set of chromosomes (FIG. 1). Diploid gametes, which arise infrequently, typically fuse with haploid ones and produce triploid zygotes, which are unstable and can either be sterile or contribute to further polyploid gametes, depending on the species[26]. The fusion of diploid gametes leads to tetraploid zygotes, which are potentially stable.

There is a basic distinction between autopolyploids and allopolyploids. Both have multiple sets of chromosomes, but in the former these are of the same type and have the same origin, whereas in the latter both the type and the origin are different. This is because autopolyploidy results from a mutation in chromosome number whereas allopolyploidy results from concurrent hybridization and mutations in chromosome number. The total number of chromosome sets is indicated by the prefix; for example, tri- (3), tetra- (4), penta- (5), hexa- (6) and octa- (8).

Meiotic pairing arrangements vary between ploidy types (FIG. 2). In particular, the increased complexity of pairing can cause the deletion or addition of chromosomes from the balanced complement that is expected in the gametes (FIG. 2b). The division between autopolyploids and allopolyploids is not absolute. The chromosome sets of allopolyploids differ proportionally to the divergence of the parental genomes: the closer the parents, the more similar the resulting allopolyploid is to an autopolyploid. This potential ambiguity between classes of polyploidy has been addressed by proposing a third class, segmental allopolyploidy[27], but the utility of this category is questionable.

An immediate consequence of polyploidy is the change in gametic and filial frequencies. Mendel's rules of segregation and assortment still apply but there is an increase in the number of "factors" to consider. This produces ratios that are different from those that were observed by Mendel in diploid peas[12] (FIG. 3).

Polyploids form at relatively high frequency (1 per 100,000) in flowering plants[26]. Because of this high formation rate and the polyploidy tolerance of plants, stable polyploidy is common in plants. The frequent occurrence of stable polyploidy in fish and frogs[28] indicates that the formation of polyploids is also possible in animals, even if it is only stable in certain animal taxa. Indeed, higher vertebrates do not tolerate polyploidy, but 10% of spontaneous abortions in humans are due to polyploidy[29].

Advantages of polyploidy

There are three documented or obvious advantages of becoming polyploid. The first two, heterosis and gene redundancy, are the result of gene duplication, whereas the mechanistic connection to polyploidy of the third, asexual reproduction, is unclear. Heterosis causes polyploids to be more vigorous than their diploid progenitors, whereas gene redundancy shields polyploids from the deleterious effect of mutations. Asexual reproduction enables polyploids to reproduce in the absence of sexual mates.

Heterosis. Polyploids take advantage of heterosis in at least three ways. One involves the fixing of divergent parental genomes in allopolyploids. Whereas heterozygosity and heterosis decay in the progeny of a diploid F1 hybrid (at each generation half the heterozygous loci become homozygous), the enforced pairing of homologous chromosomes in allopolyploids prevents intergenomic recombination, effectively maintaining the same level of heterozygosity through the generations (FIGS 2,3). Heterosis can also be exploited at the 1N (haploid) stages of polyploid plants (gametes and gametophytes, consisting of pollen and egg sac)[30,31]

EPIGENETIC
A mitotically stable change in gene expression that depends not on a change in DNA sequence, but on covalent modifications of DNA or chromatin proteins such as histones.

HETEROSIS
The increase in performance displayed by hybrids compared with their inbred parents. Because performance can be a subjective trait (for example, age of reproduction), a more precise definition is non-additive inheritance in which a trait in the F1 transgresses both parental values.

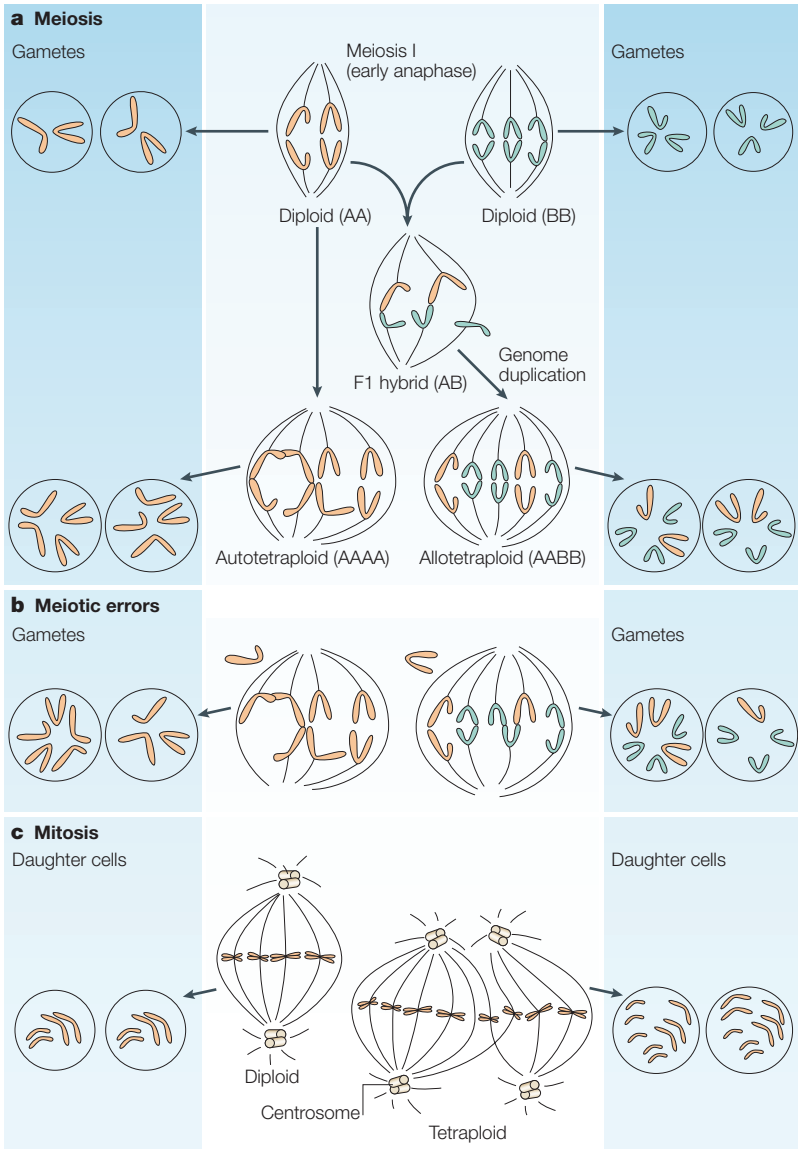


Figure 2 | **Polyploid formation and ensuing meiotic and mitotic irregularities.** The figure illustrates the chromosomal composition and behaviour of diploids and derived polyploids at different developmental times in meiosis (**a**,**b**) and mitosis (**c**). **a** | Early anaphase of meiosis I with separating chromosome pairs (central panel) and gametes (side panels) of diploid ancestors (top) and their autotetraploids and allotetraploids (bottom). The pairing of homologous chromosomes is defective in the F1 hybrid because of divergence in the structure and number of chromosomes. Pairing is restored by genome duplication; this produces an allotetraploid, in which the two HOMEOLOGOUS chromosome sets pair independently. In the autotetraploid, pairing involves frequent MULTIVALENTS, as four chromosomes of each type are present. **b** | Two examples of meiotic irregularities, resulting in laggard chromosomes (left) and ANEUPLOID gametes (right). For simplicity, the two-chromatid structure of each anaphase chromosome is not shown in panels **a** and **b**. Instead, two illustrative gametes are shown per meiosis. **c** | In animal cells the CENTROSOME number responds to the increase in genome size by forming multiple spindles that result in unbalanced mitotic products.

and might also be exploited in animals (by sperm and eggs), as postmeiotic expression of certain genes is seen in both taxa[32–34].

The mechanism that is responsible for the third advantage of heterosis is unknown. Autopolyploid hybrids show stronger heterosis than the corresponding diploid hybrids, and autopolyploid inbreds show stronger INBREEDING DEPRESSION than diploid inbreds[35–38]. These conclusions are based on a limited number of studies, so additional ones are needed that compare hybrids that are derived from verified, highly inbred parental genomes. The increased vigour of polyploid hybrids (as opposed to polyploid inbreds) is probably responsible for the widespread, but unfounded, belief that polyploids are larger than the corresponding diploids. Inbred polyploids are in fact smaller[12] than or similar in size (A. Madlung, B. Dilkes and L.C., unpublished observations) to inbred diploids.

Gene redundancy. An advantage conferred by gene redundancy is the masking of recessive alleles by dominant wild-type alleles. This effect can act at two life stages, the first of which is the gametophytic, haploid stage. Although this form of the organism has reduced complexity, its function requires the activity of many genes[39,40]. This requirement exposes pollen and egg sac to the action of lethal and deleterious loss-of-function mutations. By contrast, in the diploid gametophytes of polyploid organisms deleterious recessives can be masked by wild-type alleles. In the second, 2N phase, polyploidy can reduce the incidence of homozygous recessives[41,42]: whereas diploid *Aa* heterozygotes produce 1/4 *aa* homozygotes, *AAaa* autopolyploids produce between 1/36 and 1/22 *aaaa* homozygotes, and *AaAa* allopolyploids produce 1/16 *aaaa* homozygotes[12] (FIG. 3). The protective effect of polyploidy against deleterious recessive mutations and GENOTOXICITY might be important when isolated and severely bottlenecked populations are forced to inbreed, at a time when the purging of deleterious alleles is made difficult by the reduced number of breeding individuals.

Another advantage conferred by gene redundancy is the ability to diversify gene function by altering redundant copies of important or essential genes. In diploids, such an ability is conditional on the occurrence of a rare segmental duplication event. In polyploids, on the other hand, all genes have a duplicated copy that is available for evolutionary experimentation[14,16,43,44].

Loss of self-incompatibility and gain of asexual reproduction. Polyploidy can affect sexuality in ways that provide selective advantages. One way is by disrupting certain self-incompatibility systems, allowing self-fertilization[45]. The molecular basis of this response is unclear. In allopolyploids of *A. thaliana* it might result from interactions between the parental genomes[10,46]. In the autopolyploid, *Petunia hybrida*, it could result from interallelic interactions in the 2X pollen (where X is the normal chromosome number)[47]. Another way is by favouring the onset of asexual reproduction, which is associated with polyploidy in both animals and plants (BOX 1).

In summary, the advantages of polyploidy are caused by the ability to make better use of heterozygosity, the buffering effect of gene redundancy on mutations and, in certain cases the facilitation of reproduction through self-fertilization or asexual means.

DYSGENESIS
Sterility or other deleterious trait of an F1 hybrid that results from incompatibilities between parental genomes.

Disadvantages of polyploidy

There are several disadvantages of polyploidy, both documented and theoretical. They include the disrupting effects of nuclear and cell enlargement, the propensity of polyploid mitosis and meiosis to produce aneuploid cells and the epigenetic instability that results in transgressive (non-additive) gene regulation.

Changes in cellular architecture, and regulatory implications. Increasing the genomic content of an organism usually increases cell volume[48,49], with a consequent change in the relationship between the tridimensional and bidimensional components of the cell. An example of crucial components having different dimensional properties is provided by chromatin and the nuclear envelope. According to the relationship between volume and surface of a sphere, doubling the genome is expected to double the volume that is occupied by chromatin, but cause only a 1.6-fold increase in the nuclear envelope surface. This difference, although apparently modest, can change the stoichiometry of the interaction between components of chromatin that are located at the nuclear periphery and envelope-bound proteins. The tridimensional organization of chromosomes in the nucleus involves the peripheral positioning of telomeric and centromeric heterochromatin (see, for example, REFS 50–52). Lamins, which form a fibrous network that lines the inside of the nuclear envelope, interact with heterochromatin and have a function that is vital to the cell. This is demonstrated by the phenotypic effects of laminar abnormalities on human health[53]. In addition, many heterochromatin components[54] and at least one lamin[55] exhibit dosage sensitivity. Therefore, differential growth of the internal versus surface components of the nucleus might cause dosage imbalance, which would have regulatory repercussions. The plant orthologues of lamins are not known, although there are candidate proteins with orthologous functions[56,57].

An increase in the amount of DNA and, consequently, in cell volume can be advantageous for cells that have high metabolic rates[58]. Most organisms, from bacteria[59,60] to eukaryotes[61,62], can also modulate the amount of DNA in their nucleus by undergoing DNA ENDOREDUPLICATION, which leads to larger, ENDOPOLYPLOID cells. Therefore, endopolyploidy provides an effective solution to the problem of producing cells with different volumes in response to developmental needs. However, it is important to distinguish heritable polyploidy from developmental endopolyploidy, as the two states are not equivalent (BOX 2).

In nematodes, body size is related to cell size and polyploidy[63,64], but this is an exception. Notably, comparison of inbred diploid and polyploid *A. thaliana* (A. Madlung, B. Dilkes and L.C., unpublished observations), salamanders[65] and mice[66] indicates that the larger cells of polyploids do not necessarily result in larger bodies. Instead, a developmental mechanism regulates organ growth to compensate for cell size.

Difficulties in mitosis. Polyploidy is a problem for the normal completion of mitosis and meiosis. Autotetraploid yeast shows an increased mitotic loss of chromosomes, which results in aneuploid cells[11]. Difficulties in mitosis can arise from spindle irregularities. For example, in animal cells, tetraploidy triggers the G1-tetraploidy checkpoint, a response that is mediated by p53 (REF. 67). If this checkpoint is bypassed after the four S phase centrosomes are present, unless they are clustered in a two-by-two bipolar arrangement, the results are multipolar spindles, the chaotic segregation of chromatids and the production of aneuploid cells[68] (FIG. 2c). Sensitivity of mitotic chromosomal segregation to ploidy also occurs in wild-type yeast[11,69], where it is further demonstrated by the ploidy-conditional

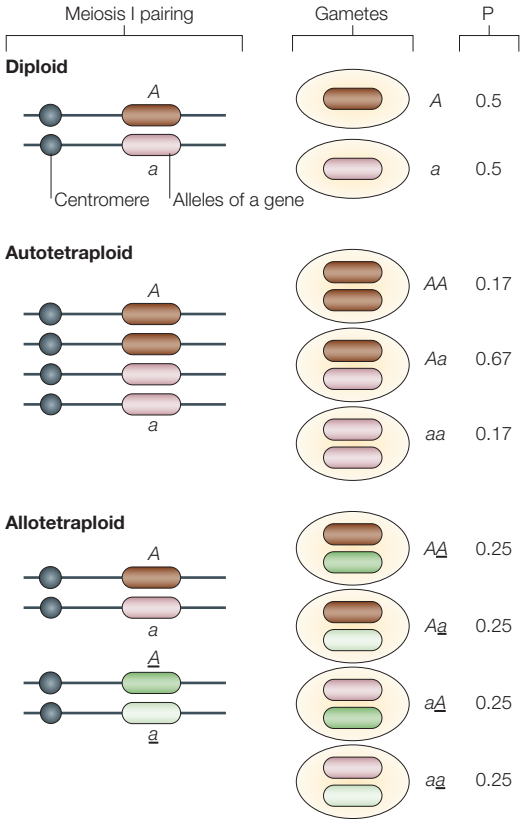


Figure 3 | **Contrasting patterns of inheritance in diploids and polyploids.** The figure illustrates meiotic arrangements and gametic output in a diploid, an autotetraploid and an allotetraploid. For simplicity, the two chromatids that make up each chromosome are not shown. A diploid heterozygote *Aa* (*A*, dark red; *a*, pink) produces two types of gamete in equal proportion (P). Polyploids produce multiple types of gamete but the pattern differs according to the type of polyploidy. An autotetraploid with genotype *AAaa* produces three types of gamete in ratios that vary according to the distance of the locus from the centromere (black circle): unlinked loci assort as 8 chromatids whereas centromere-linked loci (shown here) assort as 4 chromosomes. An allotetraploid with genotype *AaAa* (*A*, dark red; *a*, pink; *A*, dark green; *a*, light green) produces four types of gamete. Note that in the allotetraploid each genome contributes to the gametes according to diploidy rules (see FIG. 2a for the meiotic pairing pattern that occurs in this case).

ALLOPOLYPLOID
A polyploid that is generated through hybridization and thus combines different types of chromosome sets; by contrast, an autopolyploid arises through the multiplication of the same chromosome set.

HOMEOLOGOUS
Duplicated genes or chromosomes that are derived from different parental species and are related by ancestry.

MULTIVALENT
Meiotic association of more than two chromosomes, resulting in synapsis and recombination between partners.

ANEUPLOIDY
The property of having a chromosome number that is not an exact multiple of X.

CENTROSOME
The microtubule-organizing centre that divides to organize the two poles of the mitotic spindle and directs assembly of the cytoskeleton, so controlling cell division, motility and shape.

INBREEDING DEPRESSION
The loss of vigour and fitness that is observed when genome-wide heterozygosity is decreased by inbreeding.

GENOTOXICITY
The action of chemical, physical and biological agents that damage DNA.

ENDOREDUPLICATION
Successive rounds of DNA replication without cytokinesis.

ENDOPOLYPLOIDY
The property of cells in certain developmental stages of an organism of having more chromatid sets or, less frequently, more chromosome sets than the germ line.

Box 1 | **Polyploidy and sexuality**

Polyploidy is associated with the formation of APOMICTIC species. In many sexually reproducing plant species, apomictic derivatives arise from the action of a few, usually dominant genes, but these derivatives are also polyploid[116–123]. In triploid apomictic species, apomixis might be selected because it allows the organism to bypass the triploid block, a sterile condition that results from the difficulty of producing euploid gametes through triploid meiosis[120,122,124] (but see BOX 3 for examples of fertile triploids). Tetraploids are also often associated with apomixis[116,125]. Polyploidy might be required in the formation of apomictic species because a diploid or aneuploid gamete is necessary for the transmission of genes that cause apomixis.

At least three non-mutually exclusive mechanisms have been proposed to account for this property. First, genes for apomixis might be linked to recessive lethals[126,127]. Second, genes for apomixis might be linked to loci that are subject to SEGREGATION DISTORTION in haploid (and not diploid) gametes, as was proposed for a dominant gene that causes apomixis in *Tripsacum dactyloides* (gama grass, a relative of maize)[128]. A distorter locus (for example, a recessive lethal) would cause loss of the apomictic gene in haploid gametes. Third, in the case of triploid species, genes for apomixis might be located on chromosomes that are inefficient at pairing. If pairing occurs between two chromosomes out of a set of three, the one that is left out will either be lost or will segregate with one of the previously paired ones, and will never be inherited by a haploid gamete[127]. The behaviour that leads to segregation distortion or to inefficient pairing might result from the tight linkage of apomixis genes to heterochromatic regions, such as the large heterochromatic blocks that are present in supernumerary chromosomes[129,130]. Heterochromatic expansion and the interaction of heterochromatin with centromeric proteins can lead to segregation distortion[131,132].

Sexuality is believed to be a condition necessary for evolutionary flexibility, and apomictic species have been considered to be evolutionary dead ends. Interestingly, however, apomixis is not necessarily terminal because apomictic species can revert to sexuality[122,123,133]. Intermittent apomixis might provide a selective advantage by ensuring survival in times when sexual mates are scarce[116]. Finally, unisexual or asexual reproduction (such as the development of embryos from unfertilized eggs) is associated with polyploidy — often triploidy — in animals[28]. Therefore, polyploidy might facilitate the spread of a species by avoiding the need for sexual mates.

lethality of mutations in the gene that encodes the microtubule-associated protein, BIK1 (REF. 70). BIK1 is required for normal cytoskeletal function and its loss has no major consequences in diploids but results in mitotic lethality in tetraploids.

There is little information on the mitotic stability of polyploid plant cells. Organization of the plant mitotic spindle does not depend on centrosomes and, although knowledge of the dynamics of mitotic spindle formation is emerging[71,72], the response of such a system to polyploidy is unknown. Interestingly, multiple spindles have been reported in autotetraploid plant meiosis (see, for example, REF. 73). Even if aneuploid cells are formed from polyploid ones, it is possible, given the PLASTICITY of plant development, that aneuploid cells grow more slowly and are overtaken by the preferential proliferation of surrounding EUPLOID cells.

In conclusion, although the susceptibility of autopolyploids to the mitotic production of aneuploids might vary from taxon to taxon, the available data indicate the existence of a considerable risk of aneuploidy.

Difficulties in meiosis: autopolyploids. Meiosis that involves three or more sets of chromosomes can produce aneuploids, with the frequency and manner of aneuploid production depending on the type of polyploidy. Here, I consider autotetraploidy, autotriploidy and allotetraploidy. Autopolyploids have the potential to form MULTIVALENTS at meiotic metaphase I. The resolution of a tetravalent at anaphase I is more difficult than the resolution of a bivalent[12], as a tetravalent can produce abnormal segregation patterns such as '3:1' or '2:1 plus one laggard' (FIG. 2b). For this reason, it is believed that bivalent pairing is an adaptation that stabilizes polyploids[74,75]. It has been suggested, nevertheless, that a transition to bivalent pairing is not necessary and that an efficient resolution of tetravalents can be achieved by unknown mechanisms that favour a 2:2 segregation[12,76]. Whatever their nature, the mechanisms that are required to normalize autotetraploid meiosis have an important role in adaptation because neoautotetraploids frequently produce aneuploids. For example, several studies found that 30–40% of the progeny of autotetraploid maize is aneuploid[77–80].

Another type of meiotic crisis arises in triploids and pentaploids. These are formed from the union of gametes of different ploidy, such as 1X and 2X. In triploids, trivalents cannot be resolved into balanced products, and random segregation of multiple chromosome types produces mostly aneuploid gametes. Depending on the species, aneuploid gametes (or gametophytes) and the resulting zygotes vary in viability (BOX 3).

Difficulties in meiosis: allotetraploids. The last case of meiotic instability considered here involves allotetraploids. The formation of bivalents is a requirement for stable meiosis in allotetraploids because intergenomic recombination compromises the maintenance of the two parental chromosomal complements (FIG. 2). In allopolyploids, pairing between homologous chromosomes is enforced by genetic mechanisms. In allohexaploid wheat, a gene called *pairing homeologous 1* is required for the avoidance of homeologous pairing

APOMICTIC
Species that produce embryos from maternal tissues, bypassing normal meiosis and sexual fusion of egg and sperm.

SEGREGATION DISTORTION
Departure from the expected gametic ratio of alleles that is observed in the progeny of a cross, usually caused by preferential loss of certain chromosomes during gametogenesis (meiotic drive) or by selection on gametes and zygotes.

PLASTICITY
The ability of the same genotype to change and adapt its phenotype in response to different environmental conditions.

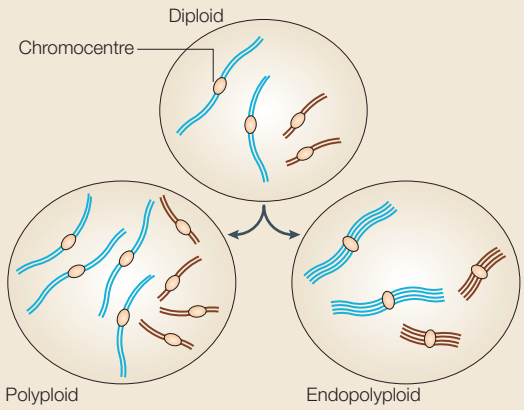
EUPLOID
An organism or cell that has a balanced set of chromosomes.

Box 2 | **Endopolyploidy**

Many organisms have some endopolyploid cells, which are defined by having a genome content that is greater than the germ line and which usually results from cycles of DNA replication in the absence of mitosis[134]. Commonly, endopolyploidy involves the side-by-side (polytenic) replication of chromosomes, resulting in the centromeric and pericentromeric DNA of sister chromatids being associated with a single, distinct heterochromatin region, known as the chromocentre (see figure)[50,134,135]. This results in a structural difference between polyploid and endopolyploid cells. For example, endopolyploid nuclei of *Arabidopsis thaliana* (with both gametic and haploid chromosome number equal to five) have the ten chromocentres that are expected in diploidy[51]. An exception is the tapetum (a nutritive tissue in anthers), in which 20 or more chromocentres are visible because polyploidy originates from nuclear restitution (nuclear fusion that occurs after telophase[136,137]).

Diploid, polyploid and endopolyploid nuclei are compared in the figure, which illustrates the chromosomal constitution in G2 phase (after replication) of a diploid with two chromosome types (long and short), and of the derived autotetraploid and an endopolyploid cell that is derived from the diploid. Both the polyploid and endopolyploid cells have a genome content of eight times that in haploids. The centromeric regions and the derived chromocentres are represented by ovals.

In endopolyploid cells, the association of centromeric DNA of a chromosome type is often more intimate than mere juxtaposition of dsDNA strands: heterochromatin can be underreplicated, resulting in branched structures in which many euchromatic arms emanate from a few strands of heterochromatin[135,138,139]. On the other hand, true polyploids (in contrast to endopolyploid cells, which arise during development) have distinct chromocentres for all of their chromosomes and therefore provide a different environment for their heterochromatic DNA (see figure). Another difference is between the genetic make up of polyploid and endopolyploid cells: polyploid cells inherit multiple chromosomes and can therefore have levels of heterozygosity that are equal to the number of chromosomes of a particular type. For example, a tetraploid cell can have the genotype $A_1A_2A_3A_4$, whereas an endopolyploid cell is confined to the allelic diversity that is present in the original zygote. An additional difference is that diploids enter endopolyploidy only during differentiation but maintain diploidy in their meristematic cells (the plant equivalent of stem cells), whereas true polyploids are polyploid in all their cells. In conclusion, the endoreduplicated state of diploid cells is not directly equivalent to true polyploidy, although it has some of the same effects, such as increased cell size.



and is believed to have resulted from an adaptation to polyploidy[81,82]. A related system might exist in allotetraploid *Brassica* species[83].

To summarize, aneuploid gametes can be produced by polyploid meiosis, although their frequency varies between species and according to polyploidy type. The mitotic and meiotic difficulties discussed in the previous sections indicate that there is a causal relationship between polyploidy and aneuploidy. Eupolyploids produce frequent aneuploids, which in turn can produce euploids[80,84]. The possibility of the occurrence of aneuploidy is relevant because it can trigger a syndrome of epigenetic and genomic instability (see below)[85,86]. Aneuploidy can cause epigenetic changes because of the sensitivity of chromatin regulatory pathways to the dosage of genes that encode regulatory factors[54,87].

Regulatory changes in gene expression. Changes in gene expression are listed here among the disadvantages of polyploidy, although this interpretation is not clear-cut. Changes in regulatory networks and output pathways are thought to be deleterious because we assume that the parental expression patterns were optimized under selection. On the other hand, changes in gene expression are likely to contribute to heterosis and can provide variation that might allow adaptation to novel conditions. On balance, I assume that the deleterious effects of regulatory changes are greater and therefore list them in this section on disadvantages.

There are several possible causes for changes in gene expression in polyploids. An increase in the copy number of all chromosomes affects all genes equally and should result in a uniform increase in gene expression. However, it is possible that some genes deviate from this assumption because they respond to regulating factors that do not change proportionally with ploidy. Additionally, polyploidy changes the structural relationship between certain cellular components and alters the progress of mitosis and meiosis, as described above. These effects might modify gene expression through reversible regulation or through persistent epigenetic resetting. Here, I consider regulatory changes in gene expression separately from epigenetic changes (which are covered in the following section), although the division between the two is largely artificial. The possibility of

ploidy-dependent regulatory changes is best evaluated in autopolyploids because the added hybridity of allopolyploids introduces confounding factors that are independent of ploidy.

Surprisingly, only a few studies have examined the effects of ploidy on gene regulation. The first such study measured the mRNA levels per genome for 18 genes in 1X, 2X, 3X and 4X maize[88]. Expression of most genes increased with ploidy, but some genes showed an inverse relationship to ploidy or an unexpected deviation in haploid and triploid tissues (here defined as the odd-ploidy response). For example, *sucrose synthase* showed the expected expression response (that is, directly proportional to genome dosage) in 2X and 4X tissues. However, its expression was 3 and 6 times higher, respectively, in 1X and 3X tissues. Two other genes showed a similar, but less extreme trend. So, 3 out of 18 genes displayed an odd-ploidy response, indicating that ~10% of the genes are sensitive to odd-ploidy.

This finding has considerable implications for our understanding of triploidy and aneuploidy, and, possibly, for the regulation of haploid stages such as gametophytes and gametes. The odd-ploidy effect is difficult to explain, yet a similar property is seen in B CHROMOSOMES, which affect the host phenotype differentially depending on whether their copy number is odd or even, with odd numbers having deleterious consequences[89,90].

Another study, that focused on *Saccharomyces cerevisiae* and used gene microarrays, measured gene regulation in 1X, 2X, 3X and 4X cells[91]. Ploidy-dependent regulation was found for 17 genes, of which 10 were ploidy-induced and 7 were ploidy-repressed. However, the odd-ploidy expression pattern that was described in maize for *sucrose synthase* was not described. Because the criteria that were set in the yeast search emphasized simple direct or inverse relationships between expression and ploidy, they might have eliminated from consideration genes whose regulation was sensitive to odd-ploidy. It would be interesting to re-examine those data for odd-ploidy responses.

A comparison between the proteomes of diploids and autopolyploids of *Brassica* species did not reveal any qualitative or quantitative differences[92]. However, the analysis of proteins is typically less sensitive than that of mRNA, and changes in low-abundance proteins could have been missed.

In summary, the consequences of autopolyploidization on gene expression have not been sufficiently clarified in animals or plants. Consistent with the sampling by Guo *et al.*[88], a relatively small fraction of plant genes should have readily measureable changes in diploid–tetraploid comparisons, and another fraction should respond to odd-ploidy. Similar changes might occur in animals. In all these analyses, it will be important to compare genotypically matched diploid, triploid and tetraploid individuals and to rule out spurious effects, such as random changes caused by the destabilizing effects of treatments that are used to induce tetraploidy.

Epigenetic instability. The many instances and possible causes of epigenetic instability in polyploids have recently been described[93]. The epigenetic resetting of ploidy-sensitive loci should be, on balance, more often deleterious than advantageous, as it is likely to perturb the regulatory adaptations that were selected in the parents. Here, I compare epigenetic instability in autopolyploids and allopolyploids, and consider the evidence for different causal contributions in the two systems.

Box 3 | Viability of gametes and zygotes arising from autotriploids and autopentaploids

The aneuploid gametes that are generated from autotriploid and autopentaploid plants, and their resulting zygotes, vary in viability. For example, triploid hybrid watermelon is bred because it produces seedless fruits whereas *Datura stramonium* (thorn apple) triploids produce some seeds. The progeny of the latter are diploids and near-diploid aneuploids, although the meiotic products that are made by the triploids span the whole range of aneuploidy types[140–142]. However, the gametophytes and seed of most of these fail at various times during development.

On the other hand, triploids of other species such as spinach[143], *Chaemerium angustifolium*[144] and *Arabidopsis thaliana*[84,145] can be considerably fertile. This degree of fertility, which is still lower than that displayed by diploids and adapted tetraploids, and the ability to produce progeny of different ploidies, give these triploids a potential bridge role — that is, to serve as intermediates in the formation of tetraploids, and as gene conduits between diploids and tetraploids[26]. The potential for such roles has been demonstrated in triploids of *A. thaliana*, which produce progeny that range from diploids to tetraploids, although most are aneuploids[84,145]. Remarkably, the aneuploids are also fertile, and by the sixth to eighth selfing generation the progeny consists of 1/3 tetraploids and 2/3 diploids, although a minority of aneuploids can still be found[84].

It is not known what causes different triploid species to vary in fertility, and also presumably in tolerance to aneuploidy. In the *A. thaliana* example, one of the triploids was produced by crossing a natural diploid accession, Col-0, to a natural autotetraploid accession, Wa-1. Polyploidy-dependent selection for Wa-1 alleles was demonstrated at one chromosomal region by mapping the triploid progeny[84], and it indicated the presence of genetic variation for ploidy responses. Variation was also evident in the progeny of different triploids of *A. thaliana*[84]. This work shows how triploids might function as bridges between ploidy types. In such a role, they mediate the formation of tetraploids from diploids or facilitate gene flow between diploids and tetraploids. Interestingly, triploidy can also be a long-term strategy, either through the production of asexual progeny (BOX 2) or through sexual reproduction in which a specialized meiosis produces haploid sperm and diploid eggs (in frogs[146]), or diploid sperm and haploid eggs (in plants[147,148]).

B CHROMOSOMES
Supernumerary chromosomes that differ from the normal complement by being dispensable, often heterochromatic and exhibiting unusual meiotic behaviour.

Epigenetic instability: autopolyploids. In autopolyploids, where instability is likely to be connected directly or indirectly to genome doubling, there are few instances of documented instability. A remarkable example was found when epigenetic effects at a transgenic locus were compared between diploid and tetraploid *A. thaliana*[94,95]. The transgene (*R*) was subject to silencing in polyploids and the silenced epigenetic state (*r*) was stably inherited in both diploids and tetraploids. Nevertheless, whereas a diploid heterozygote (*Rr*) produced the expected equal ratios of silenced alleles (*r*) and active alleles (*R*), a tetraploid heterozygote (*RRrr*) produced gametes that were predominantly *r*, violating the Mendelian rule of allelic segregation (FIG. 4). The data are consistent with transmission of the silenced epigenetic state from the *r* to the *R* allele, a phenomenon that is reminiscent of paramutation. Although the behaviour of this locus indicates an effect of ploidy on chromatin remodelling, it is unclear how widespread related phenomena are in autopolyploids and what feature of autopolyploidy is responsible for this observation. The locus in which the transgene inserted does not display any obvious hallmark of epigenetic instability according to the sequence of the standard Columbia ACCESSION Col-0. However, one cannot rule out the possibility that the Zurich accession used in this study has a different HAPLOTYPE at this locus. Instability could also have been triggered by duplication of the strong 35S promoter from cauliflower mosaic virus in the transgene[96].

Evidence for epigenetic instability was also provided in two other papers. These reported that some, but not all, of the genes that were undergoing epigenetic regulatory changes in allopolyploids of *A. thaliana* were also likely to display regulatory alterations in the autopolyploid parental strain. These changes involved the silencing or activation of genes, including the activation of a DNA transposon of the Spm/CACTA family[97,98]. However, the generality of these changes could not be determined because multiple independent autopolyploids were not examined. Therefore, it could not be ruled out that autopolyploidization had caused a GENOMIC SHOCK that changed the epigenetic regulation of random targets. A parallel analysis of DNA methylation and transcription in diploids and autotetraploids might help to identify remodelled loci. However, it will be important to examine multiple independent autotetraploids to ascertain the commonality and relevance to ploidy of the observed changes.

Epigenetic instability: allopolyploids. Extensive evidence for epigenetic remodelling is available in allopolyploids, for which structural genomic (see, for example, REFS 99,100) and expression changes have been reported for many systems. Studies that connect these changes to DNA methylation changes (the hallmark of epigenetic remodelling in plants) have been done in *A. thaliana* and wheat. These two neoallopolyploids were formed in different ways. In *A. thaliana*, neoallopolyploids were produced by crossing two autotetraploid parents, whereas in wheat they were produced by crossing diploid parents and duplicating the chromosomes of the F1 hybrids. In *A. thaliana*, epigenetically regulated genes were identified by comparing the autotetraploid parents to the allopolyploid progeny[10,97,98,101], whereas in wheat the comparison was between the diploid parents, the diploid hybrid and the allopolyploid progeny[100,102–104]. In *A. thaliana*, genes that are altered by epigenetic regulation must respond to the transition from autopolyploidy to allopolyploidy, and 2–2.5% of the genes were estimated to have undergone regulatory changes. In wheat, altered genes could have responded to both hybridization and polyploidization, and a similar number of genes was estimated to have undergone regulatory changes. In wheat, changes were induced predominantly by hybridization and not by polyploidy since the diploid hybrid displayed most of the changes that were also observed in the allopolyploid. Similar results were found when comparing methylation patterns in the parents, the diploid F1 hybrids and an allopolyploid of the *Spartina* genus of grass (see below)[105].

The interaction between parental genomes was highlighted by a microarray study that examined the regulation of 26,000 genes in the *Arabidopsis* genus neoallopolyploids[106]. This study detected a transcriptome divergence between the progenitors of more than 15%, due to genes that were highly expressed in *A. thaliana* and not in *Arabidopsis arenosa*, or vice versa. The expression of approximately 5% of the genes diverged from the mid-parent value in two independently derived synthetic allotetraploids, indicating non-additive gene regulation after interspecific hybridization. Remarkably, most non-additively expressed genes in the allotetraploids also differed in expression levels between the parents, indicating that divergence in

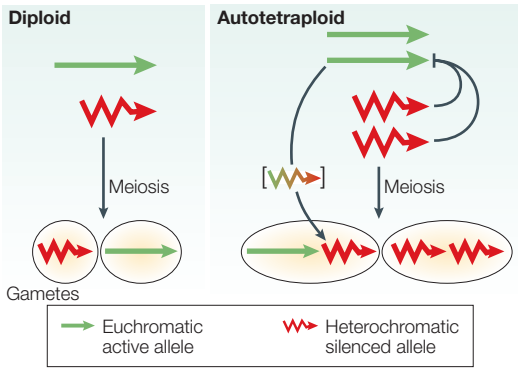


Figure 4 | **Ploidy-dependent paramutation.** Paramutation describes allelic interactions in which an epigenetic state is transmitted from a suppressed allele to an active allele, resulting in Mendelian ratios being biased towards the inheritance of loss-of-function, silenced alleles. The figure illustrates an epigenetically silenced transgenic locus — described by Mittelsten-Scheid *et al.*[94,95] — that has a polyploidy-dependent ability to convert an active locus to an inactive one. By contrast, the same locus was incapable of affecting the active allele in a diploid. The model that these authors describe is based on the assumption that the conversion from the active to inactive state occurs during meiosis. For simplicity only two gamete types are shown for the autotetraploid.

ACCESSION
A strain of a species, usually classified from the geographical site of isolation. In the *Arabidopsis* genus it is also known as an ecotype.

HAPLOTYPE
Allelic composition over a contiguous chromosome stretch.

GENOMIC SHOCK
The concomitant and widespread misregulation and activation of suppressed heterochromatic elements, leading to genomic remodelling.

regulation contributes to the non-additive response. Significantly, more than 65% of the non-additively expressed genes in the allotetraploids were repressed, and more than 94% of the repressed genes in the allotetraploids matched the genes that were expressed at higher levels in *A. thaliana* than in *A. arenosa*. On the basis of previous studies on the same hybrid lines, at least some of these changes are likely to be epigenetically induced[10,97]. However, it is possible that others changes have simpler regulatory causes, such as the dominance of negative regulatory elements from *A. arenosa*. Taken together these results indicate that the instability syndrome of neoallopolyploids should be attributed primarily to parental regulatory divergence and intergenomic incompatibilities.

Another possible cause of epigenetic remodelling is aneuploidy, which can arise frequently in neopolyploids (see above). Aneuploidy could act in two ways. The first is by altering the dosage of factors that are encoded by chromosomes that have greater or fewer than the expected number of copies. Dosage imbalance of chromatin regulatory factors, for example, might alter regulatory patterns of chromosomes[87] and even alter imprinting patterns. The second way in which aneuploidy might act is through the exposure of unpaired chromatin regions to epigenetic remodelling mechanisms. The susceptibility of meiotically unpaired DNA to silencing was first reported for *Neurospora crassa*, but it seems to be a general phenomenon[107–110]. In both cases, once a chromosomal region has become imprinted it would be inherited stably even when the cause of imprinting is removed. Therefore, some of the epigenetic instability that is observed in polyploids might result from aneuploidy. For example, do pairing phenomena affect the oddploidy responses that are observed in maize haploids and triploids[88]? Notably, the chromosomes in the haploid and triploid maize tissue that was used in this investigation had not experienced meiosis and could not have inherited epigenetic states from the "meiotic silencing of unpaired DNA"[88] (see below).

Evolutionary potential of polyploids

What is the effect of the widespread epigenetic changes that are observed in neoallopolypoids on the evolutionary potential of these species? At first sight, the epigenetic phenomena seem to be deleterious because of their disruptive effects on regulatory patterns that are established by selection. However, they might instead increase diversity and plasticity, as well as increasing heterosis, and therefore contribute to the adaptive potential of polyploids[2,105]. One example of rapid and superior adaptation is provided by the widespread dispersal of the invasive, recently formed allopolyploid, *Spartina anglica,* which contrasts with the relatively non-invasive nature of the parental species, which are presumed to be autopolyploids. However, it is not known whether the success of this species can be attributed to the fixed heterosis that is derived from allopolyploidy or to the increased variability that results from epigenetic remodelling.

Another example of the rapid adaptation of polyploids to new niches is provided by the study of arctic flora. Phylogenetic analyses indicate that arctic allopolyploids form frequently and have been particularly efficient at invading newly deglaciated areas, probably because their genomes confer hybrid vigour and buffer against the effects of inbreeding[111]. Allopolyploidy might also provide selective advantages in the context of parasites. For example, allopolyploids of the frog *Xenopus laevis* can arise in response to selective pressures from flatworm parasites, to which resistance can be gained through interspecific hybridization[112].

To take advantage of the adaptive traits that are conferred by allopolyploidy, a fertility barrier must often be overcome. Neoallopolyploids of the *Arabidopsis* genus are extremely variable in phenotype, but they also have poor fertility[10,98]. The molecular basis of this sterility is unknown. If it has an epigenetic basis, as might result from the differential imprinting of genes that are expressed in the ENDOSPERM, the outcome of altered epigenetic regulation would be deleterious. This sterility bottleneck must have been rapidly bypassed to produce the fertile and successful natural allopolyploid *Arabidopsis suecica*, whose establishment might have been favoured by marked environmental changes that are associated with glaciation[113]. Interestingly, some of the epigenetic changes that are seen in the neoallopolyploids also occur in the respective natural species[97], although their effect on phenotype and their adaptive potential are unclear.

The epigenetic marking of genes could affect their evolutionary potential. An interesting observation in cotton shows how rapidly established regulatory changes can have a role in long-term evolutionary events that involve duplicate genes. By comparing the expression of homeologous genes in different tissues, it was found that one copy could become silenced in selected tissues, resulting in subfunctionalization of the duplicates[16,114]. It is not known whether these changes reflect a preferential interaction of normal, cell-specific regulatory factors with the *cis*-regulatory regions of one parent, or whether tissue-specific epigenetic regulation that is triggered by alloploidy targets one gene preferentially. Regardless of the mechanism, uniparental expression in alternate tissue types favours both the maintenance of the duplicates as well as additional changes that optimize the function of each duplicate gene in selected cell types.

Outlook

Recent studies have provided an interesting insight into the regulatory and genomic consequences of polyploidy. Together with the emerging evidence of ancestral duplication through polyploidization in model plant, fungus and animal species, knowledge of these consequences has stimulated thinking on the relationship between early polyploidization events, success of the polyploid, and the long-term fate of the new species. Mutation is bound to eliminate gene duplicates unless there is selection for their maintenance. Such selective pressures include dosage requirements that keep members

ENDOSPERM
A fertilization-derived, triploid nutritive tissue that is found in the seeds of flowering plants.

of protein complexes fixed in their optimal stoichiometric ratios[87,115], regulatory or epigenetic interactions that lead to expression-dependent subfunctionalization, and heterotic interactions that affect fitness. All these forces act immediately after polyploidization and have protracted effects, so elucidating them is important to understanding the evolution of polyploids. However, we still need to understand the different regulatory consequences of autopolyploidy versus allopolyploidy and the effect of aneuploidy on polyploids. Finally, we need to identify which adaptations might facilitate the transition from diploidy to polyploidy. Together, this should allow the integration of the diploidy–polyploidy cycle into an evolutionary model for eukaryotes.

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