Current Biology *18*, R435–R444, May 20, 2008 DOI 10.1016/j.cub.2008.03.043

Genomic Clues to the Evolutionary Success of Polyploid Plants

Review

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Polyploidy, or the presence of two or more diploid parental genome sets within an organism, is found to an amazing degree in higher plants. In addition, many plant species traditionally considered to be diploid have recently been demonstrated to have undergone rounds of genome duplication in the past and are now referred to as paleopolyploids. Polyploidy and interspecific hybridisation (with which it is often associated) have long been thought to be important mechanisms of rapid species formation. The widespread occurrence of polyploids, which are frequently found in habitats different from that of their diploid progenitors, would seem to indicate that polyploidy is associated with evolutionary success in terms of the ability to colonise new environmental niches. A flurry of recent genomic studies has provided fresh insights into the potential basis of the phenotypic novelty of polyploid species. Here we review current knowledge of genetic, epigenetic, and transcriptional changes associated with polyploidy in plants and assess how these changes might contribute to the evolutionary success of polyploid plants. We conclude by stressing the need for field-based experiments to determine whether genetic changes associated with polyploidy are indeed adaptive.

Introduction

Polyploidy has attracted scientific study for almost a century, since de Vries first described the *Gigas* 'mutant' of *Oenothera lamarkiana*, which was later shown to be a tetraploid [1,2]. Winge [3] subsequently observed that different chromosome numbers within genera followed an arithmetic progression of multiplication (within the Asteraceae, for example, some species are 2n = 18, others 2n = 36, 54, and so on). This led Winge to propose a hypothesis of hybridisation followed by doubling of the chromosome complement. Successful attempts to create 'synthetic' polyploids confirmed this hypothesis and helped to demonstrate potential mechanisms by which polyploidy could arise naturally [4,5]. Today, polyploidy is recognised as a major force in the evolution of new plant species [6–9], a fact attested to by the prevalence of polyploid taxa in world flora.

Estimates of the frequency of polyploidy in angiosperms range from 30–35% [3], to 80% [10], with other studies suggesting closer to 50% [8,11]. In addition, recent studies indicate that many 'diploid' species may in fact be ancient polyploids [12,13], with estimates suggesting that over 70% of angiosperms have undergone at least one round of genome duplication during the course of their evolution [14]. These estimates are based on the fraction of existing genera that display polyploidy and thus, while valuable, provide no insights into the frequency of polyploidy during the course of plant evolution. This issue was resolved by Otto and Whitton [15] who developed a means of calculating the frequency of polyploidy associated with speciation, and estimated that, at any given time, polyploidy is probably involved in 2–4% of angiosperm speciation events.

Importantly, polyploid formation can occur recurrently and independently within populations, to such an extent that multiple origins are considered the rule, rather than the exception [16,17]. This further increases the likelihood of polyploid emergence and allows for increased genetic diversity among polyploid complexes, as polyploid offspring derived from different parents of the same species can interbreed. Polyploidy provides an obvious route to the rapid emergence of new species, as differences in chromosome number will often (though not always) lead to reproductive isolation from the parental taxa or ensure that introgressants (see Table 1 for glossary of terms) are largely sterile, limiting gene flow between hybrids and their parents [18]. Once formed, the establishment of these polyploid neospecies will depend on their ability either to colonise new habitats or to persist in sympatry with the parental populations. In the latter case, newly formed polyploids will likely suffer from 'minority cytotype disadvantage' [19,20], whereby mating opportunities with other polyploids will be rare and introgression with one or both parental taxa limited. Self-fertilisation or asexual reproduction (e.g., apomixis) may enable the polyploids to persist until opportunities for ecological speciation arise, but ultimately these are limiting. Nevertheless, theoretical simulations suggest that, in species with restricted seed and pollen dispersal, polyploids can persist in sympatry with their progenitors [21,22]. In this model, reduced seed dispersal allows polyploids to establish a local majority within the wider population, while limited pollen flow decreases the likelihood of pollen from the progenitor species being more prevalent than polyploid pollen within the local cluster. Although this model requires testing in natural populations, it predicts that species with restricted pollen/seed dispersal will enable polyploid persistence, even if the frequency of inbreeding/clonal reproduction is low [21].

Polyploids may also become established if they are able to occupy new habitats where the progenitor taxa do not exist. It has been observed that polyploids are more prevalent at higher latitudes and altitudes, typically occupying ecological niches different from those of their progenitors [7,23]. Studies of arctic *Draba* species, for instance, showed that the polyploids occupied broader niches than the diploids [24]. To explain such differences in habitat preferences of diploids and polyploids, it has been suggested that increased heterozygosity (in allopolyploids) may have been important in allowing polyploid species to colonise and establish themselves in areas not favoured by their diploid progenitors [7,25]. One of the most famous examples of adaptive radiation in angiosperms is the Hawaiian silversword alliance, which is composed entirely of allopolyploids [26]. Members of the alliance are highly adapted to particular environmental niches found on the Hawaiian islands and current evidence suggests that the original colonisation occurred as a result

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Table 1. Glossary of terms.

Allopolyploid: A polyploid produced from a hybrid between two or more species and therefore possessing two or more dissimilar sets of chromosomes, e.g. allotetraploid, allohexaploid etc.

Autopolyploid: A polyploid in which chromosome sets are all derived from a single species, e.g. autotetraploid, autohexaploid etc.

Homeologous chromosomes: Duplicated forms of a single homologous chromosome, as in polyploids.

Homeologues: Identical forms of a genetic locus/allele, arising from genome duplication, i.e. polyploidy.

Homologous chromosomes: Chromosomes with the same sequence of genes/alleles.

Homoploid hybrid: A hybrid with the same number of chromosomes as its parental species.

Introgressant: A hybrid derived from introgressive hybridisation.

Introgressive hybridisation: Genetic modification of one species by another through the intermediacy of hybrids; introgression.

Polyploid: An organism with more than two chromosome sets.

QTL: Quantitative trait locus.

Sympatry, sympatric: Originating in, or occupying the same geographical location.

Transgressive segregation: A phenomenon whereby hybrids exhibit phenotypes that are extreme or novel relative to their parents.

of an allopolyploid formed between two (presumed) diploid species from the North American mainland [26]. Unfortunately, because these diploid progenitors are not present in Hawaii, it is impossible to say whether they are unable to occupy the same niches as their allopolyploid derivatives, or whether they would simply be outcompeted by them. To resolve such issues of differential fitness between polyploids and diploids, more field transplant experiments akin to those of Baack and Stanton [27] and Buggs and Pannell [28] are needed. Until then, perhaps the strongest anecdotal evidence of the colonising ability of polyploids comes from the observation that many of the world's most noxious and invasive weeds are polyploid [29].

Grant [8] defined polyploidy as the presence of three or more chromosome sets within an organism, a state which can arise in a number of ways. Polyploids are typically grouped into autopolyploids (where the genomes are from the same species; Figure 1) or allopolyploids (where interspecific hybridisation is involved; Figure 2). However, these classifications can be problematic, because autopolyploidy can involve crosses between two diverged populations of the same species with genetically distinct, but structurally similar, chromosomes ('interracial autopolyploidy'), and there are also noted instances of allopolyploidy followed by a further round of genome duplication ('autoallopolyploidy'). For the purposes of this review, we will use the broader definitions and refer to polyploidy within species as autopolyploidy and that involving interspecific hybridisation as allopolyploidy.

Of the two, it might be predicted that autopolyploidy will be the more frequent form of polyploidy, because within-species mating is more common than interspecific hybridisation [30]. However, in natural populations, it has been estimated that alloploidy is more prevalent than autoploidy [14]. Estimates such as this, however, need to be treated with caution because of the difficulties associated with identifying autopolyploids in the field [18,31]. Notwithstanding this caveat, the apparent prevalence of allopolyploids suggests that they are in some way more successful in establishing themselves than autopolyploids, perhaps because their hybrid origin allows them to more easily overcome barriers such as minority cytotype exclusion [19,20] and competition with parental species [32,33].

A number of theories have been proposed to explain why polyploids are so ubiquitous and successful — for example, increased heterozygosity, higher selfing rates, reduced inbreeding depression (due to masking of deleterious alleles) and increased genetic diversity due to multiple formations of polyploids within populations (reviewed in [34]). In addition, a number of recent molecular studies have provided fresh insights — at the genetic, transcriptional and epigenetic levels — into the potential mechanisms behind the broader phenotypic range of polyploid species. Such mechanisms range from structural alterations in the chromosomes themselves to widespread changes in gene expression resulting from epigenetic factors (e.g., chromatin modifications), each of which has the potential to modify the phenotype of a polyploid individual.

Genetic Factors Associated with Polyploidy

One of the major problems faced by diploid (homoploid) hybrid neospecies is the possibility that the different parental chromosomes will be too dissimilar to pair up correctly at meiosis, leading to hybrid sterility or, at best, a severe reduction in fertility. In the latter case, a proportion of offspring will possess recombinant karyotypes and this can lead to an increase in fertility amongst the hybrids while reinforcing reproductive isolation from the parental taxa [35]. This was proposed by Grant [8] as one mechanism by which speciation could occur — 'recombinational speciation'. Another solution to the problem of chromosome incompatibility is polyploidisation. The duplication of each parental genome immediately provides each chromosome with an identical partner, allowing for correct meiotic pairing. Many allopolyploids are therefore expected to have arisen from initially sterile hybrids with unbalanced chromosome numbers [30] or from sterile or partially fertile homoploid hybrids. This latter scenario appears to be more likely as the level of genetic divergence between the parental species increases, as shown in a recent study by Chapman and Burke [36], which found significantly greater (more than double) levels of divergence between the parents of allopolyploid taxa than between the parents of diploid hybrids.

Although polyploids do not face many of the problems associated with karyotypic divergence, rapid chromosomal rearrangements have been observed in allopolyploid as well as homoploid hybrid systems [37–42]. In homoploid hybrids, rearrangements that restore fertility by removing incompatibilities between the two parental genomes are selected for at the embryonic level. An ongoing process of recombination between parental linkage blocks then serves to reinforce reproductive isolation from the progenitor taxa and leads to the establishment of the hybrid as a new species [43]. Despite the prediction that allopolyploids will not suffer difficulties in meiotic pairing to the same extent as homoploid hybrids, chromosomal rearrangements have been observed in allopolyploid hybrids, most notably *Spartina*, *Tragopogon* and wheat [38–42]. However, it should be emphasised that current evidence suggests that chromosomal rearrangements are by no means the rule in polyploid systems, because some polyploids, in particular polyploid cottons, show no rearrangements [44].

Where chromosomal rearrangements do occur in polyploids, they may arise as a consequence of colinearity of

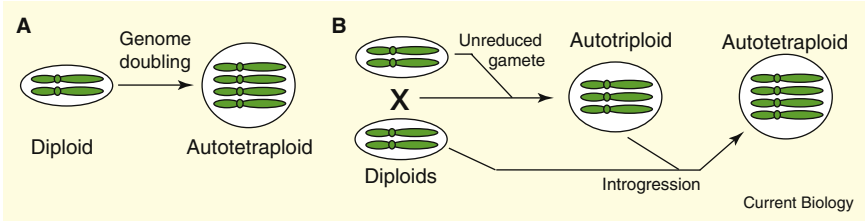

Figure 1. Autopolyploidy.

(A) Autopolyploidy resulting from spontaneous genome duplication or fusion of unreduced (2n) gametes within a single species. (B) Fusion of an unreduced (2n) gamete with a normally reduced gamete within a single species, resulting in the formation of an autotriploid. If this triploid can produce viable triploid eggs, fertilisation with a normally reduced pollen grain will yield a viable autotetraploid.

gene order between closely related parental genomes [45], leading to potential mispairing between highly homeologous chromosomes during meiosis [46]. Rearrangements within one or both parental genomes would reduce this possibility by making the two genomes non-homeologous. In a similar manner, loss of non-coding, repetitive DNA regions from one or both parental genomes — a process that has been observed in new allopolyploid wheat species [38,40] — would also serve to distinguish between the parental homeologues. These rearrangements may also be a response to changes in nuclear-cytoplasmic interactions [47,48] since the cytoplasm of a hybrid will be derived solely from one parental species and the nucleus from both. Studies using restriction fragment length-marker assays in synthetic *Brassica* polyploids showed that rearrangement events tended to occur primarily in the paternally contributed genome [49].

What are the potential mechanisms by which these rapid genomic rearrangements could occur? One theory, put forward by McClintock [50], is that hybridisation might result in widespread activation of transposable elements due to the instability of the new genome — what McClintock termed 'genome shock'. Transposon activity within the genome can increase the likelihood of chromosome breakage [51] and may result in other changes to the genome such as sequence amplification/gene duplication [52]. This has been proposed as a more likely mechanism for rapid genomic reorganisation in allopolyploids than homoploid hybrids [53] because transposon insertions into genomic regions are less deleterious in polyploids, which possess duplicated copies of every gene. Nevertheless, retrotransposon proliferation has also been reported in homoploid hybrid sunflowers [54].

Recombination between parental genomes is also a possibility in allopolyploids if the two genomes are similar enough to enable bivalent formation, as demonstrated by a study of multiple independent synthetic lines of *Brassica napus* allotetraploids [55]. This study showed few genetic changes in the initial allotetraploids, but subsequent selfed progeny displayed segregation for a number of non-reciprocal translocations between parental homeologues. These translocations occurred at each successive generation, being detected on nearly every chromosome by the fifth selfed generation. Since mispairing of the parental homeologues must occur in order for these recombinations to take place, this process may serve to distinguish between the two genomes in a similar manner to the loss of non-coding repetitive DNA in wheat allopolyploids (indeed, such translocations may be one mechanism by which this occurs).

Translating Genotypic Change to Phenotypic Change in Polyploids

How then can these changes to the genomes of new hybrids bring about novel phenotypes? In addition to the increased heterozygosity found in allopolyploid hybrids (due to possession of multiple alleles for each genetic locus), the potential also exists for novel combinations of gene regulatory factors [56]. The union of diverged regulatory networks in a hybrid has implications for both homoploid and allopolyploid individuals. In animals, for example, experiments with hybrids of *Drosophila* species showed that the activity of gene enhancers was altered as a consequence of interspecific hybridisation, leading to loss or gain of function of different enhancers within the same hybrid individual [57]. Autopolyploids may also undergo phenotypic changes resulting from alterations to gene regulation: many regulatory networks are known to be highly dosage-dependent and a duplication of the genome would immediately provide (in a tetraploid) two extra dosage levels [56]. It has been theorised that the triploid endosperm of angiosperms was such an evolutionary success for this very reason — the extra dosage level imparting a greater level of control over imprinted genes within the developing seed [58]. In addition to dosage effects, recombination between divergent chromosomes may lead to different parental regulatory elements being brought from *cis* to *trans* relative to the genes they control [59] and thus alter the expression of those genes. The widespread transposon activation proposed in McClintock's 'genomic shock' theory could also result in alterations to gene expression because transposable elements can alter the promoter activity of a gene if inserted upstream of a coding region [60,61] or can result in gene silencing due to gene disruption.

Another way in which hybridisation could produce phenotypic differences allowing niche divergence is through 'transgressive' segregation of parental alleles [62]. According to this model, adaptively important alleles (which are fixed in the same direction in the parental species) recombine in novel patterns in the hybrids. Due to complementary interactions between these genes, the hybrids can possess 'transgressive' genotypes/phenotypes which enable them to tolerate environmental conditions outside the range of either parent [62]. This tolerance can lead to a new hybrid being 'pre-adapted' for survival in novel, often extreme, habitats, thereby facilitating ecological speciation. This pre-adaptation has been elegantly demonstrated in studies of homoploid hybridisation in wild sunflowers [63], but the same mechanism is also likely to be applicable to allopolyploid hybrids.

Interspecific hybridisation between two sunflower species, *Helianthus annuus* and *H. petiolaris*, has been shown to have resulted in the formation of three distinct hybrid taxa, each of which is found in a different extreme habitat that the parental taxa cannot tolerate. Synthetic hybrids of the parental taxa were generated and were found to segregate for higher survivorship under extreme environmental conditions, similar to those in which the wild hybrid taxa are typically found [63–65]. In each case, the hybrids segregated for particular combinations of parental survivorship quantitative trait loci (QTLs) that were also identified in the wild hybrid populations [64]. In a similar manner, the translocation events observed between parental homeologues in

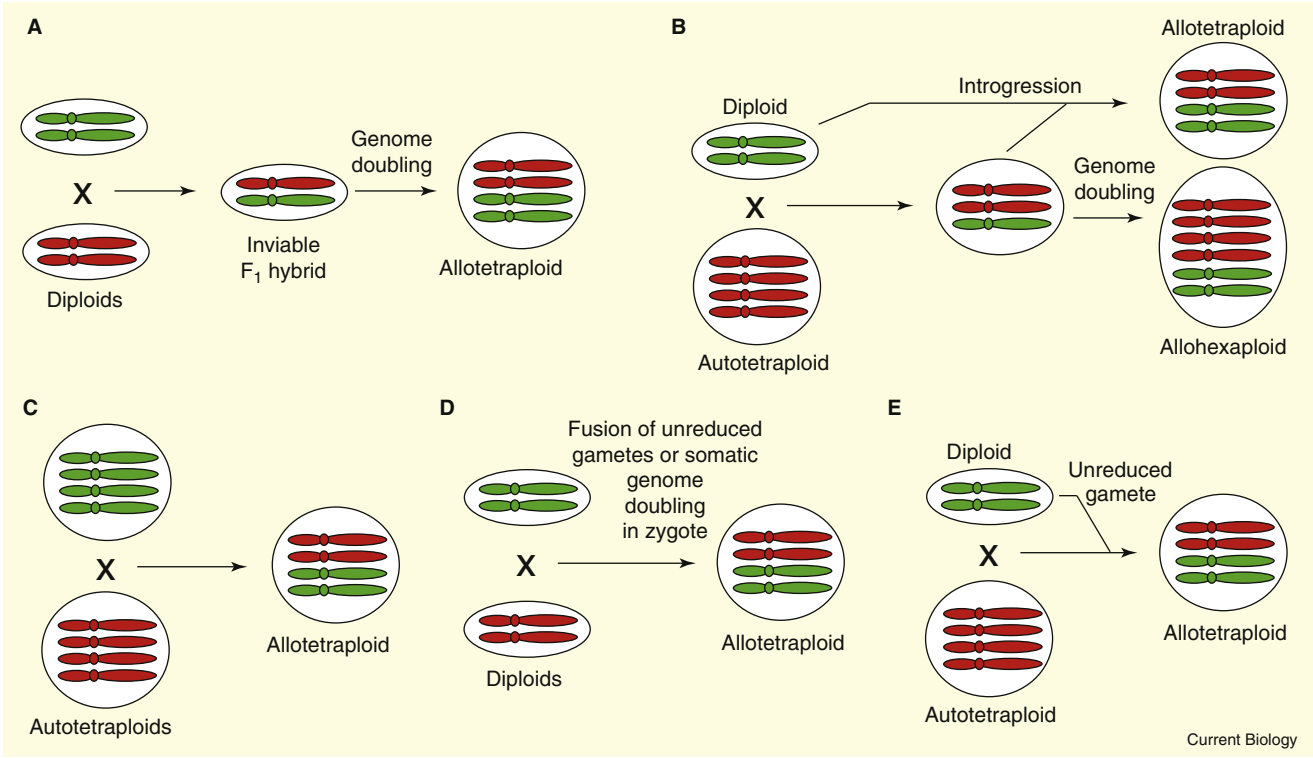

Figure 2. Allopolyploidy.

(A) Interspecific hybridisation yielding an inviable F_1 hybrid (due to problems with meiotic pairing), followed by genome doubling to yield a fertile allotetraploid. (B) Interspecific hybridisation between two species of differing ploidy to yield a sterile F_1 triploid, followed by genome duplication to produce a viable allohexaploid. The triploid may also backcross to the diploid parent, yielding allotetraploid progeny. This can also occur when the initial triploid is the result of fusion between one normal and one unreduced gamete from the same species and results in an autotetraploid. (C) Interspecific hybridisation between two tetraploid individuals resulting in an allotetraploid. Higher ploidies yield the same result as long as the resulting chromosome complement is balanced. (D) Fusion of two unreduced gametes from different species to produce an allotetraploid. An allotetraploid may also result from somatic genome duplication in the hybrid zygote. (E) Interspecific hybridisation involving fusion of an unreduced gamete from a diploid with a normally reduced gamete from a tetraploid.

resynthesised *Brassica napus* allotetraploids [55] have been implicated in phenotypic changes such as seed yield [66], flowering time [67] and disease resistance [68]. It is clear then that the combination of diverged genomic material in a hybrid nucleus provides a substantial source of genetic novelty that can facilitate the evolutionary divergence and success of a new hybrid species. Conclusive evidence for true transgressive segregation in allopolyploids has yet to be forthcoming, but its effects could be reflected in the altered patterns of gene expression commonly described in polyploids that may play an important role in the genetic flexibility of allopolyploids.

Changes to Transcriptome As a Consequence of Hybridisation and Polyploidy

Non-Additive Gene Expression in Allopolyploids

With the advent of techniques for medium- to large-scale analysis of gene expression, there has been a huge interest in the impact of hybridisation and polyploidisation on transcription. These studies have provided some intriguing insights into how altered patterns of gene expression in hybrids — especially allopolyploids — might contribute to the ability of these hybrids to meet a broader range of environmental challenges than their progenitors. Microarray analyses of changes to gene expression in both homoploid and allopolyploid hybrid systems have shown large-scale alterations to gene expression on a genome-wide level. Strikingly, a large proportion of these changes have been shown to be non-additive, that is to say, the expression level of the hybrid is not simply a mix of the two parental expression levels. Such non-additive changes represent a means by which hybrids can possess phenotypes that are not intermediate to those of the parental taxa. Studies of synthetic lines of allotetraploid *Arabidopsis suecica* (which likely formed naturally from the union of an unreduced pollen grain from diploid *A. thaliana* and a normal egg cell from tetraploid *A. arenosa*; Figure 3A) using oligonucleotide arrays showed non-additive changes to the expression of approximately 5% of genes represented on the array [69]. Importantly, around 56% of these genes were affected similarly in two independently generated lines of the synthetic allopolyploids. Wang *et al.* [69] showed that the affected genes did not appear to be limited to any particular chromosomal region and belonged to a broad range of functional categories, with cell defence, hormonal regulation and aging being particularly prevalent. In both synthetic lines, the majority of non-additively expressed genes (65% and 76%, respectively) displayed downregulation compared with the parental midpoint. An overwhelming proportion of these genes was found to display higher expression in the *A. thaliana* autotetraploid parent compared with the *A. arenosa* parent, suggesting coordinated repression of the *A. thaliana* transcriptome. This

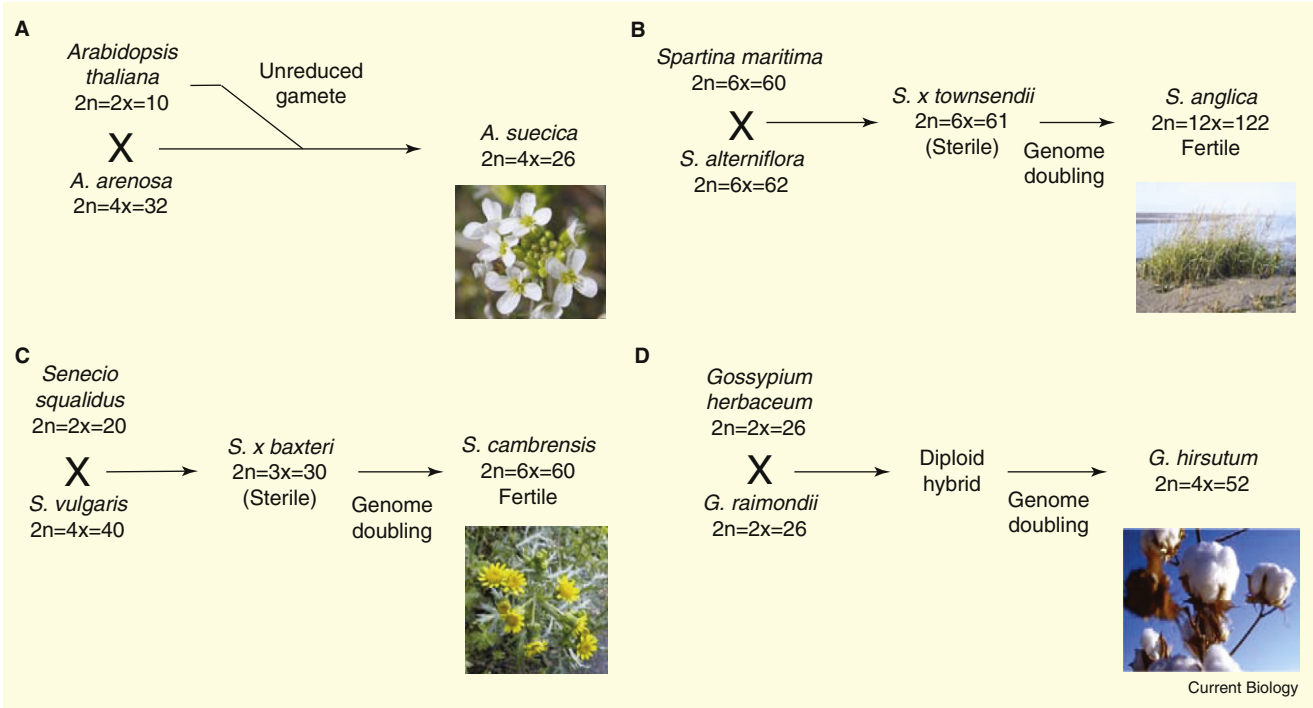

Figure 3. Allopolyploid origins of *Arabidopsis suecica*, *Spartina anglica, Senecio cambrensis* and *Gossypium hirsutum*, four model species for analysis of genetic and epigenetic changes resulting from allopolyploidisation.

(A) Fusion of an unreduced (2n) pollen grain from diploid *Arabidopsis thaliana* with a normally reduced egg cell from tetraploid *A. arenosa* gives rise to the allotetraploid *A. suecica*. (B) Hybridisation between the hexaploids *Spartina maritima* and *S. alterniflora* produces *S. x townsendii*, which is infertile due to unbalanced chromosome number. Autopolyploidisation of this initial hybrid produces the viable allododecaploid *S. anglica*. (C) Hybridisation of diploid *Senecio squalidus* with tetraploid *S. vulgaris* forms a sterile triploid, *S. x baxteri*. Subsequent genome duplication yields the fertile allohexaploid *S. cambrensis.* (D) Ancient hybridisation of the diploid species *Gossypium herbaceum* and *G. raimondii* resulted in an inviable diploid F_1. Subsequent genome duplication yielded a fertile allotetraploid, which has since diverged into five cotton species, including *G. hirsutum*.

idea was in accordance with previous experiments by the authors, who had noted silencing of the ribosomal RNA (rRNA) genes from the *A. thaliana* genome in *A. suecica* allopolyploids as a consequence of nucleolar dominance [70,71]. This phenomenon results largely in masking of *A. thaliana* phenotypes in both synthetic and natural *A. suecica* hybrids, because a large proportion of the *A. thaliana* gene copies are not expressed due to inactivity of the *A. thaliana* translational machinery.

Because large-scale changes to gene expression were not observed in synthetic autotetraploid *A. thaliana*, when compared with the allotetraploid *A. suecica*, Wang *et al.* [69] concluded that polyploidisation in itself has little impact on changes to gene expression, with interspecific hybridisation instead being the primary cause of altered levels of gene expression observed in *A. suecica*. However, in the *Arabidopsis* system, polyploidy arises prior to hybridisation, so this system cannot provide any information regarding the effects of genome duplication on an already hybrid nucleus. By contrast, interspecific hybridisation more frequently results in unbalanced, sterile F_1 hybrids, and polyploidisation provides a means by which hybrid fertility can be restored (autoallopolyploidy).

So what happens to gene expression in cases where hybridisation precedes genome duplication? Study of the allohexaploid origin of *Senecio cambrensis* (Welsh ragwort) has provided an opportunity to 'decouple' the events of hybridisation and polyploidy. *S. cambrensis* has formed on at least two occasions in the British Isles within the last century [72] as a result of hybridisation between the endemic groundsel *S. vulgaris* (tetraploid) and the invasive species *S. squalidus* (diploid), itself the product of homoploid hybridisation between species native to Mount Etna, Sicily [73]. Hybridisation between the two taxa results in the formation of a sterile triploid, *S. x baxteri* that can then give rise to fertile allohexaploid offspring via the union of unreduced gametes (Figure 3C). Initial microarray experiments that analysed changes to gene expression in the two hybrid taxa, relative to the parents, suggested that expression levels were more greatly perturbed in the triploid *S. x baxteri* than in the allohexaploid *S. cambrensis* [74]. The immediate alteration to gene expression resulting from hybridisation suggested a transcriptional form of 'genome shock' [50], as first proposed by Comai *et al.* [75]. The *Senecio* data also suggested that genome duplication, leading to the formation of the allohexaploid, had an 'ameliorating' effect on this hybridisation-induced 'transcriptome shock' [76]. Further analyses using synthetic *S. cambrensis* allohexaploids produced via colchicine treatment of *S. x baxteri* shoots revealed that this ameliorating effect was an immediate consequence of genome duplication and that many of the genes involved were affected similarly in both the synthetic allohexaploids and also in wild *S. cambrensis* [76]. These studies support the findings in *Arabidopsis* that interspecific hybridisation results in widespread changes to gene expression and also demonstrate that subsequent genome duplication can have a distinct secondary

effect on gene expression. This may be a general phenomenon in cases where hybridisation precedes genome duplication during allopolyploid formation as similar observations were made in synthetic wheat allohexaploids [77].

Non-additive changes to gene expression in hybrids could result in phenotypic novelty and consequently lead to speciation and a new evolutionary direction. In the *Arabidopsis* system, for example, the formation of the allotetraploid *A. suecica* results in altered regulation of the flowering genes *FRI* and *FLC* [78]. In this case, the *FRI* locus of the *A. arenosa* genome enhances expression of the *A. thaliana FLC* locus, leading to inhibition of early flowering. Indeed, natural allopolyploid *A. suecica* displays even later flowering than the synthetic lines, suggesting that this phenotypic change may have arisen due to allopolyploidy and then was fixed by natural selection. A change in flowering time would immediately enhance the reproductive isolation of the new allopolyploid from its parents. Reducing the possibility of introgression back to the tetraploid *A. arenosa* parent in this manner would be an important factor in the establishment of early *A. suecica* as a true species.

Changes to gene expression could also influence how polyploid species interact with other organisms, such as pollinators, pathogens or herbivores. Autopolyploidy has been shown to result in non-uniform effects on plant–herbivore interactions, with autopolyploids of *Heuchera grossulariifolia* displaying improved resistance to some herbivores, but increased susceptibility to others [79]. Similar effects have been observed in allopolyploid hybrids of *Nicotiana*. In this study, three independent lines of synthetic allotetraploids were assessed for inheritance of anti-herbivore defence mechanisms [80]. The results showed that parental defence systems were flexibly inherited in the allopolyploids: the initial jasmonate signalling mechanism was present in all three synthetic lines, but assorted downstream responses either were absent in some of the hybrids or showed changes in magnitude. The authors concluded that this would allow for rapid divergence within allopolyploids over a few generations, consistent with models predicting that initial allopolyploids function as generalists but are able to exploit novel ecological niches as a result of increased genetic flexibility [15,81]. It is crucial, therefore, that future research combines the results of molecular studies with field experiments to test these models by performing common garden fitness assays of resynthesised polyploids alongside their diploid progenitors.

The transgressive phenotypes observed in interspecific hybrids are a key factor in the ecological divergence of these taxa, and changes to gene expression may provide another route by which these phenotypes can arise in addition to transgressive segregation of parental QTLs. Analyses of non-additive gene expression in wild and synthetic allohexaploid *S. cambrensis* and the intermediate triploid hybrid *S. x baxteri* showed that a proportion of non-additively expressed genes also displayed expression levels that were substantially (>1.5-fold) different from those of progenitor species [82]. Similar results were recorded in intervarietal diploid hybrids of maize [83], confirming that such transgressive patterns of gene expression can arise immediately as a consequence of hybridisation. However, further experimentation will be required to link these expression changes to transgressive phenotypes and thus to ecological divergence in natural populations, as has been done for studies of homoploid hybrid sunflowers [84].

Alterations to Location of Gene Expression

While microarray analyses have provided insights into the large-scale changes to gene expression in both established and newly formed hybrids, other techniques have provided startling insights into the ways in which allopolyploidy may lead to altered gene expression. Studies of wild allotetraploid cotton (Figure 3D) by Adams *et al.* [85] using cDNA single-strand conformation polymorphism (cDNA-SSCP) to distinguish parental gene copies showed that 11 out of 16 analysed gene pairs displayed evidence of organ-specific gene silencing in at least one tissue out of the 10 surveyed. In other words, different parental gene copies were switched on or off in a tissue-specific manner. Some of the gene pairs showed reciprocal silencing of parental gene copies in different organs compared with the diploid progenitors (Figure 4). This process, known as subfunctionalisation, had been proposed as a potential mechanism by which duplicated genes could be maintained in polyploid genomes (reviewed in [86,87]), in contrast to the classical model of Ohno [88] which states that duplicated genes are subject to silencing and then lost as a consequence of accumulated mutations. In the genetic model of subfunctionalisation [89], the two gene copies suffer deleterious but complementary mutations, such that both copies are necessary for a normal phenotype. The form of subfunctionalisation shown by Adams *et al.* [90] arises through divergent expression of the two gene copies.

While wild allotetraploid cotton arose ~1.5 million years ago and has since diverged into several lineages, recent evidence from resynthesised allotetraploids [91] has shown that altered gene expression patterns can arise rapidly and thus may be a factor in the ecological divergence of neopolyploids from their progenitors, since selection may act upon favourable expression patterns. This possibility is even more interesting in light of the observation in wild allotetraploid cotton that differential expression of the parental gene copies (though not involving total gene silencing) could be induced by abiotic stress [92], potentially allowing the hybrid to express whichever parental gene copy allows the optimum response to a stressor. This suggests a mechanism by which allopolyploids could effectively 'put the best foot forward' when confronted with a new environmental challenge [59]. Again, there is the need to perform direct fitness comparisons of allopolyploids and their diploid progenitors using field experiments to determine whether this does indeed lead to a fitness advantage.

Mechanisms of Gene Expression Change in Polyploids

What mechanisms serve to bring about the dramatic changes in gene expression observed in allopolyploid hybrids? Novel assortments of gene-regulatory systems (e.g., the flowering-time genes of *Arabidopsis*) and transgressive segregation of parental QTLs have already been discussed as drivers of speciation in *A. suecica* and *Helianthus*, respectively. However, a number of other processes are also believed to be involved in alterations to the hybrid transcriptome (reviewed in [92]). The phenomenon of nucleolar dominance, whereby the rRNA genes of one parent are silenced in the hybrid, had previously been observed in *A. suecica* [70] and its downstream effects on expression of other parental genes confirmed by microarray analysis [69]. In this form of nucleolar dominance, the repression of *A. thaliana* rRNA genes is not connected to competition for limiting transcription factors, as some models have predicted [93]. Instead,

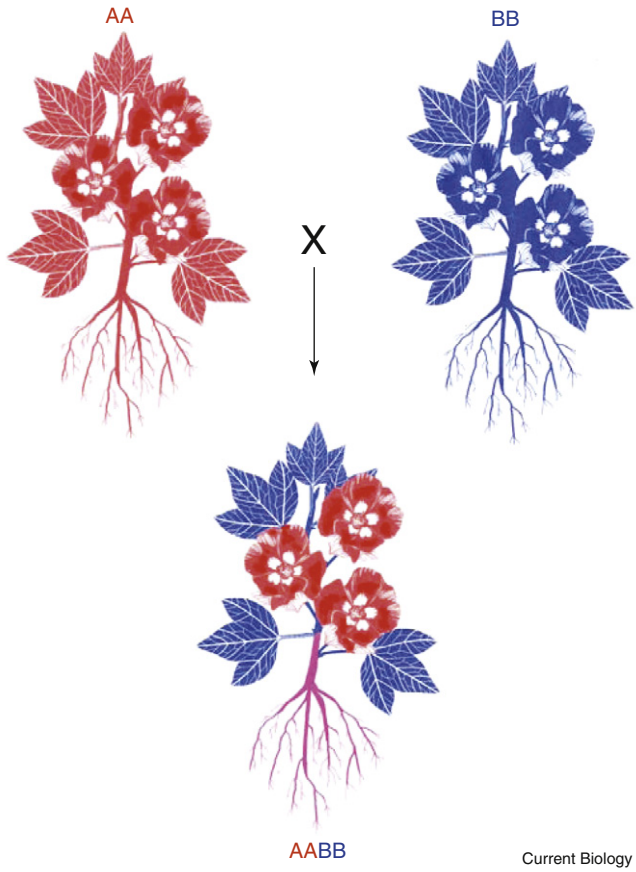

Figure 4. Subfunctionalisation as a consequence of altered expression patterns in allopolyploids.

Parental gene copies (red and blue) are differentially expressed in the allopolyploid hybrid. Only the gene copy from the A genome contributor is expressed in flowers, while only the copy from the B genome parent is expressed in stem and leaf. Both parental copies are equally expressed (purple) in root tissue. This form of subfunctionalisation has been demonstrated most clearly in allotetraploid cotton (Figure 3D and see [85]).

the available evidence suggests that epigenetic changes, such as DNA methylation and histone modifications, serve to remodel the chromatin structure of the *A. thaliana* rRNA genes into an inactive state, blocking RNA polymerase I transcription [94].

Epigenetic modifications are hypothesised to play a role in silencing duplicated genes in circumstances where the activity of both copies might be counterproductive [95]. For example, there are many classes of protein that are dimeric or polymeric in nature. Studies in *Drosophila* have shown that different gene homeologues can encode different monomeric subunits of these complexes and, when these subunits are assembled, protein complexes formed from subunits of different origins (heterodimeric proteins) may be functionally compromised [96]. If this is the case in allopolyploids, increased fitness could result from silencing of one genomic copy of the subunit-encoding genes, thus preventing heterodimer formation. The timing and relationship of the mechanisms involved in silencing as a consequence of allopolyploidy are still being examined, though some preliminary information from *Arabidopsis* suggests that DNA methylation acts upon a different subset of heterochromatic genes during hybridisation than those affected by histone acetylation [95]. The authors suggest that the chromatin structure of these loci is remodeled by histone acetylation (immediately preventing transcription) in early polyploid generations and, thereafter, histone and DNA methylation serve to reinforce gene silencing in a stable manner.

Epigenetic regulation of gene expression in polyploids through cytosine methylation has received a great deal of attention in recent years. Much of the initial research has been carried out in synthetic lines of *A. suecica*, which were found to display considerable phenotypic variation in the F_2 generation and several cases of unstable phenotypes compared with the F_1 [97]. An analysis of gene expression using cDNA-amplification fragment length polymorphism (AFLP) showed that a number of genes were potentially silenced in the allotetraploid lineages. Southern blot assays using methylation-sensitive restriction enzymes indicated that these genes were methylated in the hybrids. Subsequent analysis [98] suggested that there were no gross changes to the overall level of cytosine methylation in the allotetraploid genome; therefore, the researchers tested the possibility that the hybrids were undergoing reorganisation of their methylation state. Using methylation-sensitive AFLP (MSAP), they discovered that 8.3% of loci scored showed differential methylation between the parents and the F_3 synthetic allotetraploids. The majority of these (76.9%) were consistent across the four allotetraploid individuals studied, with 62.5% of this group showing demethylation and the remainder displaying an increase in methylation [98]. These results suggested that the methylation pattern of allopolyploids is subject to widespread modification in a concerted manner.

Similar results were reported in studies of the allododecaploid *Spartina anglica* [41], which forms via hybridisation of the hexaploids *S. alterniflora* and *S. maritima*. The initial hybridisation results in the sterile hybrid *S. x townsendii*, subsequent to which genome duplication produces the fertile *S. anglica* (Figure 3B). Using MSAP analysis, Salmon *et al.* [41] observed even higher levels of methylation change than those observed in *A. suecica*, with nearly 30% of parental fragments displaying altered behaviour in the initial hybrid *S. x townsendii* and/or the allopolyploid *S. anglica*. The authors speculated that this might be due to the possible allopolyploid nature of the parental species themselves, along with the higher ploidy of the final hybrid. Interestingly, most of the methylation changes (71.4%) were conserved between the initial hybrid and *S. anglica*, adding further weight to the argument that it is hybridisation, rather than polyploidy, that is the primary factor in driving epigenetic changes to gene expression in allopolyploids. However, some fragments displayed differential methylation between *S. x townsendii* and *S. anglica*, including the reappearance of parental bands absent in *S. x townsendii*. This fluctuation in methylation state may provide an answer to why genome duplication has a secondary effect on gene expression [76], a possibility that we are currently testing by subjecting synthetic lines of allohexaploid *S. cambrensis* to MSAP analysis (Hegarty, M.J., Edwards, K.J., Abbott, R.J., and Hiscock, S.J., unpublished data).

While chromatin remodeling can account for some of the transcriptional changes arising as a consequence of allopolyploidy, some silenced genes are not reactivated in plants with impaired methylation and acetylation pathways [99]. Instead, each gene may be regulated via interactions between homeologous loci. This phenomenon, known as

paramutation, produces heritable changes in expression due to *trans*-regulation of one allele by its counterpart and was observed in tetraploid *A. thaliana* containing both active and inactive alleles of a transgene encoding hygromycin phosphotransferase [100]. The tetraploids showed *trans*-inactivation of the active allele by the silenced copy in cases where a plant heterozygous for the two forms had been selfed. This effect was not observed in diploid *A. thaliana* and indicated that both active and inactive alleles go through meiosis together. Such pairing-based *trans*-inactivation is consistent with findings in tetraploid tomatoes, where the ratio of paramutagenic alleles to paramutable alleles causes variation in the frequency of the silencing effect [101]. Paramutation effects can therefore vary depending upon the ploidy level and parental genome dosages in an allopolyploid individual.

It has been suggested [102] that many paramutation phenomena could be the result of different repeat sequences in homeologous gene copies triggering abnormal production of small interfering RNAs (siRNAs), leading to alterations in RNA-mediated gene silencing within allopolyploids. Given the inter-relationship between siRNAs, microRNAs (miRNAs) and other epigenetic mechanisms, such as histone acetylation and DNA methylation, it has been speculated that RNA-mediated regulation of gene expression may be similarly affected in allopolyploid systems. Indeed, a study of synthetic wheat allotetraploids provides evidence for the involvement of this mechanism, because reactivation of transposons in the synthetic lines was correlated with upregulation or downregulation of neighbouring genes, depending on whether the readout transcripts were in the sense or antisense orientation [103]. In addition to the theory that differences in repeat regions between parental homeologues might lead to aberrant siRNA production, it has also been proposed that incompatibilities between divergent parental genomes could result in three specific effects in polyploids. The interaction of the two genomes could result in either changes to the accumulation of siRNAs and miRNAs, or changes to the efficiency of the siRNA/miRNA biosynthetic machinery, or alterations to the specificity of the targets [92]. In the first such scenario, transcription will be affected differently depending upon the fidelity of the small RNAs for their targets. Low fidelity, where small RNA accumulation is increased (due to ploidy change), will result in silencing/downregulation of both parental transcripts, but high fidelity will affect only targets from one parent. These proposals remain predictions for the moment for, as Chen and Ni [92] rightly point out, there is still much that remains to be understood about the expression of small RNAs in allopolyploid systems.

Conclusions

The advent of new molecular tools to investigate both genomic and transcriptional effects of hybridisation and polyploidy has provided a great deal of fresh insight into the complex genetic alterations that occur during these important evolutionary processes. These insights have, as always with more rigorous scientific probing, raised as many questions as they have answered. It is now possible to say that we understand some of the genetic changes that can occur as a consequence of hybridisation and polyploidy, but only with the caveat that we are still in the process of elucidating *how* and *why* they occur. It is also unclear why some effects are observed in particular plant systems and not others; in cotton, for example, there is no evidence for the widespread chromosomal rearrangements or changes to DNA methylation that have been observed in other allopolyploids [44]. Another important question relates to whether the transcriptional changes (affecting hundreds of genes) observed in microarray experiments manifest themselves at the protein level. Recent evidence from proteomic studies suggests that changes in protein levels are indeed observed: in a study of synthetic *Brassica* allopolyploids, Albertin *et al.* [104] identified a large number of proteins (305 in stem, 200 in root) displaying non-additive changes to translation when compared with the parental taxa. *In silico* analysis showed that these proteins belonged to no single particular functional class and that different subunits of the same functional pathway could be affected in opposite directions, mirroring data from gene expression analyses.

The impact of polyploidy at the phenotypic level has the potential to be vast. Recent genomic and transcriptional studies of polyploid plants have generated genetic models to explain why polyploidy could be such a potent adaptive force in plant evolution. The time has now come to test these models using field-based transplant experiments similar to those used so elegantly to demonstrate the adaptive significance of hybridisation in the evolution of homoploid hybrid sunflowers [63–65]. Future studies of polyploidy must therefore strive to correlate genetic changes, occurring at the level of the genome and transcriptome, with changes occurring at the phenotypic level and at the level of individual fitness to determine whether the genetic and epigenetic changes observed in polyploids are indeed adaptive.

Acknowledgments

We thank three anonymous reviewers for their constructive comments on improving this manuscript and Christopher Thorogood for producing Figure 4. Research in S.J.H.'s lab is supported by the Natural Environment Research Council (NERC), the Leverhulme Trust, and the Lady Emily Smyth Research Station, University of Bristol.

References

1. Lutz, A.M. (1907). A preliminary note on the chromosomes of *Oenothera lamarkiana* and one of its mutants, *O. gigas*. Science *26*, 151–152.
2. Gates, R.R. (1909). The stature and chromosomes of *Oenothera gigas* De Vries. Arch. Zellforsch *3*, 525–552.
3. Winge, O. (1917). The chromosomes: their number and general importance. C.R. Trav. Lab. Carlsberg. *13*, 131–275.
4. Clausen, R.E., and Goodspeed, T.H. (1925). Interspecific hybridization in *Nicotiana*. II. A tetraploid *glutinosa-tabacum* hybrid, an experimental verification of Winge's hypothesis. Genetics *10*, 279–284.
5. Müntzing, A. (1930). Über chromosomenvermehrung in *Galeopsis*-Kreuzungen und ihre phylogenetische bedeutung. Hereditas *14*, 153–172.
6. Darlington, C.D. (1937). Recent Advances in Cytology, second edition (Philadelphia: P. Blakiston's Son & Co.).
7. Stebbins, G.L. (1950). Variation and Evolution in Plants (New York: Columbia University Press).
8. Grant, V. (1981). Plant Speciation, second edition (New York: Columbia University Press).
9. Coyne, J.A., and Orr, H.A. (2004). Speciation (Sunderland: Sinauer Associates).
10. Masterson, J. (1994). Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science *264*, 421–423.
11. Stebbins, G.L. (1971). Chromosomal Evolution in Higher Plants (London: Addison-Wesley Press).
12. Simillion, C., Vandepoele, K., Van Montagu, M.C.E., Zabeau, M., and Van de Peer, Y. (2002). The hidden duplication past of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA *99*, 13627–13632.
13. Blanc, G., and Wolfe, K.H. (2004). Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. Plant Cell *16*, 1667–1678.
14. Levin, D.A. (2002). The Role of Chromosomal Change in Plant Evolution (New York: Oxford University Press).

15. Otto, S.P., and Whitton, J. (2000). Polyploid incidence and evolution. Annu. Rev. Genet. *34*, 401–437.
16. Soltis, D.E., and Soltis, P.S. (1993). Molecular data and the dynamic nature of polyploidy. Crit. Rev. Plant Sci. *12*, 243–273.
17. Soltis, D.E., and Soltis, P.S. (1999). Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. *14*, 348–352.
18. Tate, J.A., Soltis, D.E., and Soltis, P.S. (2005). Polyploidy in plants. In The Evolution of the Genome, T.R. Gregory, ed. (Elsevier Science & Technology, Academic Press), pp. 371–426.
19. Levin, D.A. (1975). Minority cytotype exclusion in local plant populations. Taxon *24*, 35–43.
20. Husband, B.C. (2000). Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. Proc. R. Soc. Lond. B. *267*, 217–223.
21. Li, B.H., Xu, X.M., and Ridout, M.S. (2004). Modelling the establishment and spread of autotetraploid plants in a spatially heterogeneous environment. J. Evol. Biol. *17*, 562–573.
22. Baack, E.J. (2005). To succeed globally, disperse locally: effects of local pollen and seed dispersal on tetraploid establishment. Heredity *94*, 538–546.
23. Ehrendorfer, F. (1980). Polyploidy and distribution. In Polyploidy: Biological Relevance, W.H. Lewis, ed. (New York: Plenum Press), pp. 45–60.
24. Brochmann, C., and Elven, R. (1992). Ecological and genetic consequences of polyploidy in Arctic *Draba* (Brassicaceae). Evol. Trends Plants *6*, 111–124.
25. Stebbins, G.L. (1985). Polyploidy, hybridization, and the invasion of new habitats. Ann. Missouri Bot. Gard. *72*, 824–832.
26. Barrier, M., Baldwin, B.G., Robichaux, R.H., and Purugganan, M.D. (1999). Interspecific hybrid ancestry of a plant adaptive radiation: allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotic genome duplications. Mol. Biol. Evol. *16*, 1105–1113.
27. Baack, E.J., and Stanton, M.L. (2005). Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus Adoneus*): niche differentiation and tetraploid establishment. Evolution *59*, 1936–1944.
28. Buggs, R.J., and Pannell, J.R. (2007). Ecological differentiation and diploid superiority across a moving ploidy contact zone. Evolution *61*, 125–140.
29. Brown, A.H.D., and Marshall, D.R. (1981). Evolutionary changes accompanying colonization in plants. In Evolution Today. Proceedings of the Second International Congress of Systematic and Evolutionary Biology, G.G.E. Schudder and J. Reveal, eds., pp. 351–363.
30. Ramsey, J., and Schemske, D.W. (1998). Pathways, mechanisms and rates of polyploid formation in flowering plants. Annu. Rev. Ecol. Syst. *29*, 467–501.
31. Soltis, D.E., Soltis, P.S., Schemske, D.W., Hancock, J.F., Thompson, J.N., Husband, B.C., and Judd, W.S. (2007). Autopolyploidy in angiosperms: have we grossly underestimated the number of species? Taxon *56*, 13–30.
32. Baack, E.J. (2005). Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): minority cytotype exclusion and barriers to triploid formation. Am. J. Bot. *92*, 1827–1835.
33. Rausch, J.H., Morgan, M.T., and Husband, B. (2005). The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. Evolution *59*, 1867–1875.
34. Soltis, P.S., and Soltis, D.E. (2000). The role of genetic and genomic attributes in the success of polyploids. Proc. Natl. Acad. Sci. USA *97*, 7051–7057.
35. Rieseberg, L.H. (1997). Hybrid origins of plant species. Annu. Rev. Ecol. Syst. *28*, 359–389.
36. Chapman, M.A., and Burke, J.M. (2007). Genetic divergence and hybrid speciation. Evolution *61*, 1773–1780.
37. Rieseberg, L.H., Sinervo, B., Linder, C.R., Ungerer, M.C., and Arias, D.M. (1996). Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. Science *272*, 741–745.
38. Feldman, M., Liu, B., Segal, G., Abbo, S., Levy, A.A., and Vega, J.M. (1997). Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homeologous chromosomes. Genetics *147*, 1381–1387.
39. Ungerer, M.C., Baird, S.J.E., Pan, J., and Rieseberg, L.H. (1998). Rapid hybrid speciation in wild sunflowers. Proc. Natl. Acad. Sci. USA *95*, 11757–11762.
40. Shaked, H., Kashkusk, K., Ozkan, H., Feldman, M., and Levy, A. (2001). Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridisation and allopolyploidy in wheat. Plant Cell *13*, 1749–1759.
41. Salmon, A., Ainouche, M.L., and Wendel, J.F. (2005). Genetic and epigenetic consequences of recent hybridisation and polyploidy in *Spartina* (Poaceae). Mol. Ecol. *14*, 1163–1175.
42. Tate, J.A., Ni, Z., Scheen, A.C., Koh, J., Gilbert, C.A., Lefkowitz, D., Chen, Z.J., Soltis, P.S., and Soltis, D.E. (2006). Evolution and expression of homeologous loci in *Tragopogon miscellus* (Asteraceae), a recent and reciprocally formed allopolyploid. Genetics *173*, 1599–1611.
43. Rieseberg, L.H., and Willis, J.H. (2007). Plant speciation. Science *317*, 910–914.
44. Liu, B., Brubaker, C.L., Cronn, R.C., and Wendel, J.F. (2001). Polyploid formation in cotton is not accompanied by rapid genomic changes. Genome *44*, 321–330.
45. Gale, M.D., and Devos, K.M. (1998). Comparative genetics in the grasses. Proc. Natl. Acad. Sci. USA *95*, 1971–1974.
46. Moore, G. (2002). Meiosis in allopolyploids: the importance of "Teflon" chromosomes. Trends Genet. *18*, 456–463.
47. Leitch, I.J., and Bennett, M.D. (1997). Polyploidy in angiosperms. Trends Plant Sci. *2*, 470–476.
48. Wendel, J.F. (2000). Genome evolution in polyploids. Plant Mol. Biol. *42*, 225–249.
49. Song, K., Lu, P., Tang, K., and Osborn, T.C. (1995). Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc. Natl. Acad. Sci. USA *92*, 7719–7723.
50. McClintock, B. (1984). The significance of responses of the genome to challenge. Science *226*, 792–801.
51. Weil, C.F., and Wessler, S.R. (1993). Molecular evidence that chromosome breakage by *Ds* elements is caused by aberrant transposition. Plant Cell *5*, 512–522.
52. Jin, Y.-K., and Bennetzen, J.L. (1994). Integration and nonrandom mutation of a plasma membrane proton ATPase gene fragment within the Bs1 retroelement of maize. Plant Cell *6*, 1177–1186.
53. Matzke, M.A., and Matzke, A.J.M. (1998). Polyploidy and transposons. Trends Ecol. Evol. *13*, 241.
54. Ungerer, M.C., Strakosh, S.C., and Zhen, Y. (2007). Genome expansion in three hybrid sunflower species is associated with retrotransposon proliferation. Curr. Biol. *16*, R872–R873.
55. Gaeta, R.T., Pires, J.C., Iniguez-Luy, F., Leon, E., and Osborn, T.C. (2007). Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell *19*, 3403–3417.
56. Riddle, N.C., and Birchler, J.A. (2003). Effects of reunited diverged regulatory hierarchies in allopolyploids and species hybrids. Trends Genet. *19*, 597–600.
57. Hammerle, B., and Ferrus, A. (2003). Expression of enhancers is altered in *Drosophila melanogaster* hybrids. Ecol. Dev. *5*, 221–230.
58. Kermicle, J., and Alleman, M. (1990). Gametic imprinting in maize in relation to the angiosperm life cycle. Development (suppl.), 9–14.
59. Hegarty, M.J., and Hiscock, S.J. (2007). Polyploidy: Doubling up for evolutionary success. Curr. Biol. *17*, 927–929.
60. Barkan, A., and Martiennssen, R.A. (1991). Inactivation of maize transposon *Mu* suppresses a mutant phenotype by activating an outward-reading promoter near the end of *Mu1*. Proc. Natl. Acad. Sci. USA *88*, 3502–3506.
61. Raizada, M.N., Benito, M.I., and Walbot, V. (2001). The *MuDR* transposon terminal inverted repeat contains a complex plant promoter directing distinct somatic and germinal programs. Plant J. *25*, 79–91.
62. Rieseberg, L.H., Archer, M.A., and Wayne, R.K. (1999). Transgressive segregation, adaptation and speciation. Heredity *83*, 363–372.
63. Rieseberg, L.H., Raymond, O., Rosenthal, D.M., Lai, Z., Livingstone, K., Nakazato, T., Durphy, J.L., Schwarzbach, A.E., Donovan, L.A., and Lexer, C. (2003). Major ecological transitions in wild sunflowers facilitated by hybridization. Science *301*, 1211–1216.
64. Lexer, C., Welch, M.E., Durphy, J.L., and Rieseberg, L.H. (2003). Natural selection for salt tolerance quantitative trait loci (QTLs) in wild sunflower hybrids: Implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. Mol. Ecol. *12*, 1225–1235.
65. Lexer, C., Welch, M.E., Raymond, O., and Rieseberg, L.H. (2003). The origin of ecological divergence in *Helianthus paradoxus* (Asteraceae): selection on transgressive characters in a novel hybrid habitat. Evolution *57*, 1989–2000.
66. Osborn, T.C., Butrulle, D.V., Sharpe, A.G., Pickering, K.J., Parkin, I.A.P., Parker, J.S., and Lydiate, D.J. (2003). Detection and effects of a homoeologous reciprocal transposition in *Brassica napus*. Genetics *165*, 1569–1577.
67. Pires, J.C., Zhao, J.W., Schranz, M.E., Leon, E.J., Quijada, P.A., Lukens, L.N., and Osborn, T.C. (2004). Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). Biol. J. Linn. Soc. Lond. *82*, 675–688.
68. Zhao, J.W., Udall, J.A., Quijada, P.A., Grau, C.R., Meng, J.L., and Osborn, T.C. (2006). Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homoeologous non-reciprocal transposition in *Brassica napus* L. Theor. Appl. Genet. *112*, 509–516.
69. Wang, J., Tian, L., Lee, H.-S., Wei, N.E., Jiang, H., Watson, B., Madlung, A., Osborn, T.C., Doerge, R.W., Comai, L., *et al*. (2006). Genomewide nonadditive gene regulation in *Arabidopsis* allotetraploids. Genetics *172*, 507–517.
70. Chen, Z.J., Comai, L., and Pikaard, C.S. (1998). Gene dosage and stochastic effects determine the severity and direction of uniparental ribosomal RNA gene silencing (nucleolar dominance) in *Arabidopsis* allopolyploids. Proc. Natl. Acad. Sci. USA *95*, 14891–14896.
71. Pikaard, C.S. (1999). Nucleolar dominance and silencing of transcription. Trends Plant Sci. *4*, 478–483.

72. Ashton, P.A., and Abbott, R.J. (1992). Multiple origins and genetic diversity in the newly arisen allopolyploid species, *Senecio cambrensis* Rosser (Compositae). Heredity *68*, 25–32.
73. James, J.K., and Abbott, R.J. (2005). Recent, allopatric, homoploid hybrid speciation: the origin of *Senecio squalidus* (Asteraceae) in the British Isles from a hybrid zone on Mount Etna, Sicily. Evolution *59*, 2533–2547.
74. Hegarty, M.J., Jones, J.M., Wilson, I.D., Barker, G.L., Coghill, J.A., Sanchez-Baracaldo, P., Liu, G., Buggs, R.J.A., Abbott, R.J., Edwards, K.J., *et al*. (2005). Development of anonymous cDNA microarrays to study changes to the *Senecio* floral transcriptome during hybrid speciation. Mol. Ecol. *14*, 2493–2510.
75. Comai, L., Madlung, A., Josefsson, C., and Tyagi, A. (2003). Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? Phil. Trans. Royal Soc. Lond. Ser. B *358*, 1149–1155.
76. Hegarty, M.J., Barker, G.L., Wilson, I.D., Abbott, R.J., Edwards, K.J., and Hiscock, S.J. (2006). Transcriptome shock after interspecific hybridisation in *Senecio* is ameliorated by genome duplication. Curr. Biol. *16*, 1652–1659.
77. Feldman, M., and Levy, A.A. (2005). Allopolyploidy - a shaping force in the evolution of wheat genomes. Cytogenet. Genome Res. *109*, 250–258.
78. Wang, J., Tian, L., Lee, H.-Y., and Chen, Z.J. (2006b). Nonadditive regulation of *FRI* and *FLC* loci mediates flowering-time variation in *Arabidopsis* allopolyploids. Genetics *173*, 965–974.
79. Nuismer, S.L., and Thompson, J.N. (2001). Plant polyploidy and non-uniform effects on insect herbivores. Proc. R. Soc. Lond. B *268*, 1937–1940.
80. Pearse, I.S., Krügel, T., and Baldwin, I.T. (2006). Innovation in anti-herbivore defense systems during neopolyploidy - the functional consequences of instantaneous speciation. Plant J. *47*, 196–210.
81. Levin, D.A. (2003). The ecological transition in speciation. New Phytol. *161*, 91–96.
82. Hegarty, M.J., Barker, G.L., Brennan, A.C., Edwards, K.J., Abbott, R.J., and Hiscock, S.J. (2008). Changes to gene expression associated with hybrid speciation in plants: further insights from transcriptomic studies in Senecio. Phil. Trans. Royal Soc. Lond. Ser. B, in press.
83. Stupar, R.M., Hermanson, P.J., and Springer, N.M. (2007). Nonadditive expression and parent-of-origin effects identified by microarray and allele-specific expression profiling of maize endosperm. Plant Physiol. *145*, 411–425.
84. Lai, Z., Gross, B.L., Zou, Y., Andrews, J., and Rieseberg, L.H. (2006). Microarray analysis reveals differential gene expression in hybrid sunflower species. Mol. Ecol. *15*, 1213–1227.
85. Adams, K.L., Cronn, R., Percifield, R., and Wendel, J.F. (2003). Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. Proc. Natl. Acad. Sci. USA *100*, 4649–4654.
86. Kellogg, E.A. (2003). What happens to genes in duplicated genomes. Proc. Natl. Acad. Sci. USA *100*, 4369–4371.
87. Otto, S.P. (2003). In polyploids, one plus one does not equal two. Trends Ecol. Evol. *18*, 431–433.
88. Ohno, S. (1970). Evolution by Gene Duplication (Berlin: Springer-Verlag Press).
89. Lynch, M., and Force, A. (2000). The probability of duplicate gene preservation by subfunctionalization. Genetics *154*, 459–473.
90. Adams, K.L., Percifield, R., and Wendel, J.F. (2004). Organ-specific silencing of genes in a newly synthesized cotton allotetraploid. Genetics *168*, 2217–2226.
91. Liu, Z., and Adams, K.L. (2007). Expression partitioning between genes duplicated by polyploidy under abiotic stress and during organ development. Curr. Biol. *17*, 1669–1674.
92. Chen, Z.J., and Ni, Z. (2006). Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays *28*, 240–252.
93. Reeder, R.H. (1985). Mechanisms of nucleolar dominance in animals and plants. J. Cell. Biol. *101*, 2013–2016.
94. Chen, Z.J., and Pikaard, C.S. (1997). Epigenetic silencing of RNA polymerase I transcription: a role for DNA methylation and histone modification in nucleolar dominance. Genes Dev. *11*, 2124–2136.
95. Chen, Z.J., and Tian, L. (2007). Roles of dynamic and reversible histone acetylation in plant development and polyploidy. Biochim. Biophys. Acta. *1769*, 295–307.
96. Phillips, J.P., Tainer, J.A., Getzoff, E.D., Boulianne, G.L., Kirby, K., and Hilliker, A.J. (1995). Subunit-destabilizing mutations in *Drosophila* copper/zinc superoxide dismutase: neuropathology and a model of dimer dysequilibrium. Proc. Natl. Acad. Sci. USA *92*, 8574–8578.
97. Comai, L., Tyagi, A.P., Winter, K., Holmes-Davis, R., Reynolds, S.H., Stevens, Y., and Byers, B. (2000). Phenotypic instability and rapid gene silencing in newly formed *Arabidopsis* allotetraploids. Plant Cell *12*, 1551–1567.
98. Madlung, A., Masuelli, R.W., Watson, B., Reynolds, S.H., Davison, J., and Comai, L. (2002). Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic *Arabidopsis* allotetraploids. Plant Physiol. *129*, 733–746.
99. Wang, J., Tian, L., Madlung, A., *et al*. (2004). Stochastic and epigenetic changes of gene expression in *Arabidopsis* polyploids. Genetics *4*, 1961–1973.
100. Mittelsten Scheid, O., Afsar, K., and Paszkowski, J. (2003). Formation of stable epialleles and their paramutation-like interaction in tetraploid *Arabidopsis thaliana*. Nat. Genet. *34*, 450–454.
101. Hagemann, R., and Berg, W. (1978). Paramutation at the *sulfurea* locus of *Lycopersicon esculentum* Mill. VII. Determination of the time of occurrence of paramutation by the quantitative evaluation of the veriegation. Theor. Appl. Genet. *53*, 709–719.
102. Chen, Z.J. (2007). Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu. Rev. Plant Biol. *58*, 377–406.
103. Kashkush, K., Feldman, M., and Levy, A.A. (2003). Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nat. Genet. *33*, 102–106.
104. Albertin, W., Alix, K., Balliau, T., Brabant, P., Davanture, M., Malosse, C., Valot, B., and Theillement, H. (2007). Differential regulation of gene products in newly synthesized *Brassica* allotetraploids is not related to protein function nor subcellular localization. BMC Genomics *8*, 56–70.