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Polyploidy and novelty: Gottlieb's legacy

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Nearly four decades ago, Roose & Gottlieb (Roose & Gottlieb 1976 *Evolution* **30**, 818–830. (doi:10.2307/2407821)) showed that the recently derived allotetraploids *Tragopogon mirus* and *T. miscellus* combined the allozyme profiles of their diploid parents (*T. dubius* and *T. porrifolius*, and *T. dubius* and *T. pratensis*, respectively). This classic paper addressed the link between genotype and biochemical phenotype and documented enzyme additivity in allopolyploids. Perhaps more important than their model of additivity, however, was their demonstration of novelty at the biochemical level. Enzyme multiplicity—the production of novel enzyme forms in the allopolyploids—can provide an extensive array of polymorphism for a polyploid individual and may explain, for example, the expanded ranges of polyploids relative to their diploid progenitors. In this paper, we extend the concept of evolutionary novelty in allopolyploids to a range of genetic and ecological features. We observe that the dynamic nature of polyploid genomes—with alterations in gene content, gene number, gene arrangement, gene expression and transposon activity—may generate sufficient novelty that every individual in a polyploid population or species may be unique. Whereas certain combinations of these features will undoubtedly be maladaptive, some unique combinations of newly generated variation may provide tremendous evolutionary potential and adaptive capabilities.

1. Introduction

The concept of polyploidy has long evoked the origin of novelty—or has it? Although widely recognized as a process that generates new species, and thus novel biodiversity, polyploidy has not always been considered a source of 'new' characteristics. Allopolyploids were historically viewed as intermediate to their parents, with autopolyploids simply higher ploidy versions of their progenitors [1–3]. In fact, allopolyploids are typically discovered *because* of their morphological intermediacy, and autopolyploids are typically *not* discovered because of their morphological similarity to their parents. Long-standing views of allopolyploids as 'fill-in' taxa ([4] and earlier treatments by, e.g. [3,5]) extended this concept of intermediacy to habitats and ecological roles. Indeed, as required for establishment and survival of homoploid hybrids, 'hybridization of the habitat' [6] was considered by some to be essential for the success of allopolyploids. Perhaps counter to this view was the perception that allopolyploids might have broader ecological amplitudes than their parents, allowing them to move into harsher habitats, such as previously glaciated arctic and alpine areas (although Ehrendorfer [7] noted such distributions, he cautioned against generalizations and causal explanations linking polyploidy and ecology or distribution). Nonetheless, emphasis on gross morphology—with associated inferences of intermediacy and parental similarity for allo- and autopolyploids, respectively—has perhaps continued to mask remarkable forms of novelty, from the genomic to biochemical to ecological levels.

Studies of diploid hybrids clearly reveal that an expectation of morphological intermediacy is overly simplistic (e.g. [8–12]): hybrids are mosaics of characters that range from intermediate to parental to transgressive of the parental features. Thus, our expectations for allopolyploids should likewise transcend strict intermediacy of traits. Here, we revisit Roose & Gottlieb's [13] classic paper on

biochemical novelty in allopolyploid species of *Tragopogon* as a paradigm for viewing polyploidy and novelty at a range of biological scales.

2. Biochemical novelty in *Tragopogon*: a paradigm for understanding the 'success' of polyploids

Nearly four decades ago, genetic and biochemical additivity in polyploid genomes was demonstrated in recent allotetraploids of *Tragopogon* (Asteraceae). Using allozyme data, Roose & Gottlieb [13] showed that the recently derived (post-1920s; [14]) allotetraploids *T. mirus* and *T. miscellus* (both $2n = 24$) combined the allozyme profiles of their diploid ($2n = 12$) parents (*T. dubius* and *T. porrifolius*, and *T. dubius* and *T. pratensis*, respectively) at nine and seven loci, respectively. This classic paper addressed the link between genotype and biochemical phenotype and provided a mechanism—enzyme multiplicity—that might explain the expanded ranges of polyploids relative to their diploid progenitors (see below). This documented additivity was at the core of the allopolyploid paradigm, in which genes from the parental species are represented and expressed in the new polyploid. Roose & Gottlieb [13] provided evidence for the null model of additivity of gene expression in allopolyploids.

Perhaps the greatest value of Roose and Gottlieb's paper, however, was not in documenting additivity, but in demonstrating novelty at the biochemical level. Enzyme multiplicity—the production of novel enzyme forms in the allopolyploids—can provide an extensive array of polymorphism for a polyploid individual [13] (figure 1). Note that a fully heterozygous allotetraploid individual can harbour as many as 10 different enzyme forms of a dimeric enzyme, instead of three, as would be found in a heterozygous diploid parent. This tremendous polymorphism in the allotetraploid results from a single pair of homeologous loci (i.e. genes duplicated via polyploidy). Consider the enzymatic diversity that must exist within a single allopolyploid individual when the vast number of multimeric enzymes (dimeric, tetrameric and beyond) is considered.

The novelty of Roose & Gottlieb's [13] findings is that an allopolyploid is far more than the sum of its genomic parts: through production of interlocus heteromeric enzymes, novel enzyme forms, presumably with novel activities and perhaps (ultimately) novel function, are generated. A population of genetically variable allotetraploid individuals can exhibit even greater biochemical diversity—and presumably flexibility. Moreover, novel alleles may be generated from the parental copies through recombination, such that part of an allele may be contributed by one parent and the other part by the second parent [15]. Although rare, such novel alleles may lead to further phenotypic novelty. In addition to possible selection for genetic variants, such diversity among individuals provides the opportunity for selection on interlocus interactions and the development of novel networks as well [16,17]. The foundational studies by Gottlieb and collaborators on enzyme additivity and conservation of isozyme number in plants [13,18] provide both the baseline expectations against which observations of molecular and chromosomal additivity can be evaluated and the concept of novelty through interacting parental (homeologous) genomes.

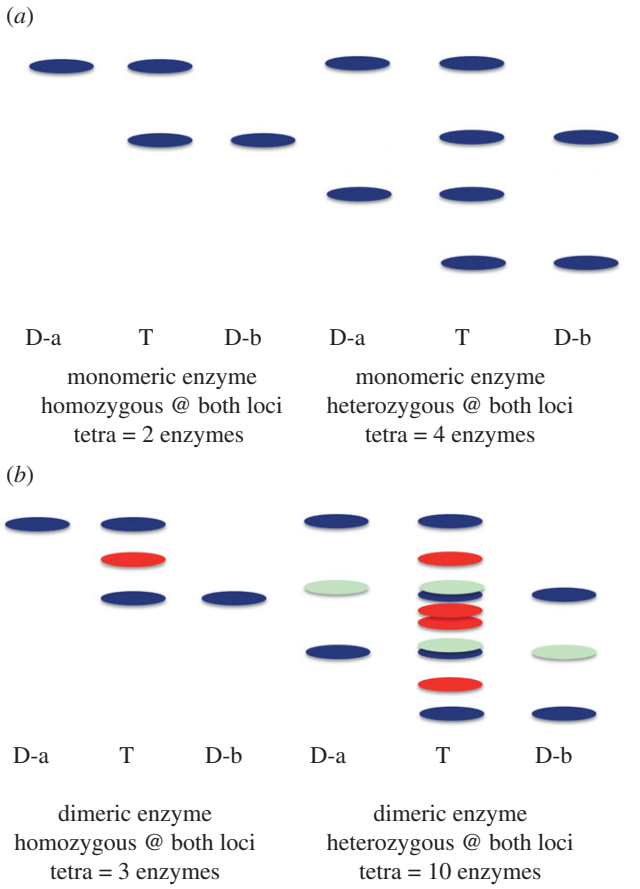

Figure 1. Enzyme multiplicity in a diploid and allotetraploid individual, showing increased numbers of enzyme forms in both monomeric (*a*) and dimeric (*b*) enzymes, drawn to illustrate enzyme bands on a gel. D-a and D-b refer to two diploid individuals; T refers to an allotetraploid individual. In (*b*), red bands are interlocus heterodimeric enzymes, and green bands are heterodimeric enzymes with subunits encoded by different alleles at the same locus. Note that an allotetraploid individual can produce up to 10 different forms of dimeric enzyme, if the individual is heterozygous at both homeologous loci, in contrast to three different forms in a heterozygous diploid.

Gottlieb's studies of polyploidy (in *Tragopogon*, as well as *Stephanomeria* [19] and *Clarkia* [20]) focused on allopolyploids, but it is clear that some of the same sorts of novelty may arise in autopolyploids, as Levin [21, p. 1] beautifully described:

> . . . that autopolyploidy may greatly alter the cytological, biochemical, genetic, physiological, and developmental character of organisms, and may provide them with unique or transgressive tolerances and developmental patterns which could suit them to conditions which are beyond the limits of their diploid progenitors. In a sense, chromosome doubling produces macromutants which may offer a population novel avenues of response to the exigencies of the environment. The products of chromosome doubling provide a basis for punctuated evolution within a microevolutionary time scale.

Levin noted a range of possible novel effects in autopolyploids, ranging from nucleotypic effects [22], such as cell size, to novel gene expression, physiological response, growth rate, developmental features, reproductive output, mating system and ecological tolerances to diverse environmental pressures. Perhaps most importantly, chromosome doubling *per se* may generate effects that lead to novelty, apart from features that arise via the union of previously separated genomes. In fact, Levin [21] suggests that nucleotypic effects may '"propel" a population into a new adaptive sphere', perhaps accounting for the distribution of polyploids, both auto- and

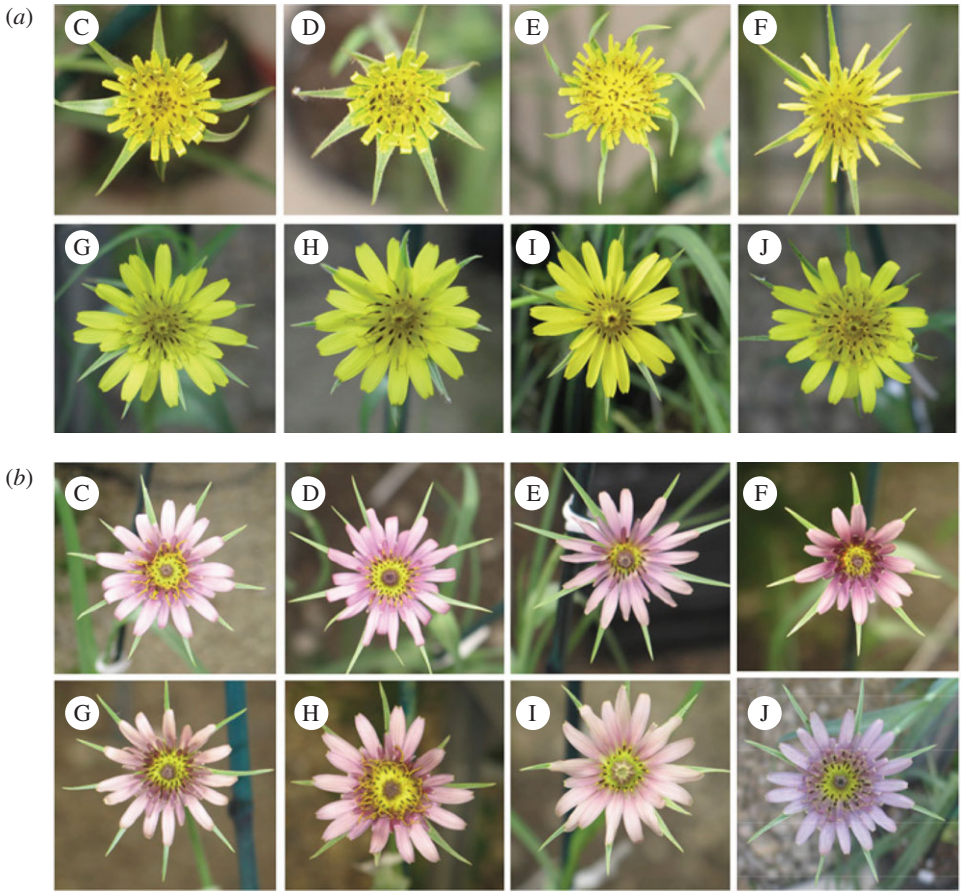

Figure 2. Variation in inflorescence colour and morphology in synthetic hybrids and allopolyploids in *Tragopogon*. (*a*) *Tragopogon miscellus* and *T. pratensis-T. dubius* hybrids. C–F are the 'short-liguled' form of *T. miscellus*, with *T. pratensis* as the maternal parent and *T. dubius* as the paternal parent; G–J are the 'long-liguled' form and are the reciprocal crosses of C–F. C, D, F, H, J are 4*x*; E, G, I are 2*x*. (*b*) C–F are derived from crosses with *T. porrifolius* as the maternal parent and *T. dubius* as the paternal parent; G–J are reciprocal crosses. C, D, E, H are 4*x*; F, G, I, J are 2*x*. Reproduced with permission from [24].

allopolyploids, in areas beyond those of the diploid parents. Now, 30 years beyond Levin's [21] paper, we still have little understanding of the relative effects of polyploidy due to hybridity versus genome duplication *per se* (although some studies have sought to tease apart these influences on patterns of gene expression; see review by Yoo *et al*. [23]).

3. Novel features in polyploids: from DNA sequences to geographical distributions

Rieseberg and colleagues, among others, over the span of a decade or so effectively made the case for plurality in our view of morphological features in diploid hybrids: they range from intermediate to parental to transgressive [9–12]. A comprehensive analysis of morphological variation in allopolyploids has, to our knowledge, not been conducted, but anecdotal evidence supports similar findings. As examples, *Tragopogon mirus* and *T. miscellus* present spectacular arrays of variation in inflorescence structure, petal colour and receptacle colour (figure 2), but extensive analyses of morphology in *Tragopogon* and other polyploids are needed. Here, we review a range of other features for which novel genotypes or phenotypes are reported for polyploids. These examples were selected to demonstrate the range of biological levels of organization over which novelty has arisen and are not intended to be comprehensive. The emphasis is on allopolyploids—in keeping with the scenario proposed by Roose & Gottlieb [13]—but similar analyses of autopolyploids are warranted and would be welcome additions to our knowledge of the genetic and phenotypic effects of polyploidy.

(a) Chromosomal novelty

Powerful, modern cytogenetic techniques—fluorescent *in situ* hybridization (FISH) and genomic *in situ* hybridization (GISH)—have facilitated detailed analysis of genome restructuring following allopolyploidization (reviewed in [25,26]). For example, a combination of FISH and GISH revealed that the recently and repeatedly formed allotetraploids *T. miscellus* and *T. mirus* possess extensive chromosomal variability, both within and among populations [27–30]. In their extensive survey of natural populations of *T. miscellus* of independent origin, Chester *et al.* [28] significantly found that none of the populations examined was fixed for a particular karyotype; 76% of the individuals studied possessed intergenomic translocations, and 69% exhibited aneuploidy for one or more chromosomes. The aneuploidy detected was noteworthy in that it was nearly always reciprocal. For example, three copies of a given chromosome might be present from one parent, and one chromosome of the other diploid parent; or four copies of a chromosome from one parent and none from the other diploid parent (figure 3). Very similar results have also been obtained for *T. mirus*, although reciprocal aneuploidy was not as frequent in this species [30]. Interestingly, the same chromosomal processes of translocation and reciprocal aneuploidy are also present in natural hybrids between the two allotetraploids, *T. mirus* and *T. miscellus* [31].

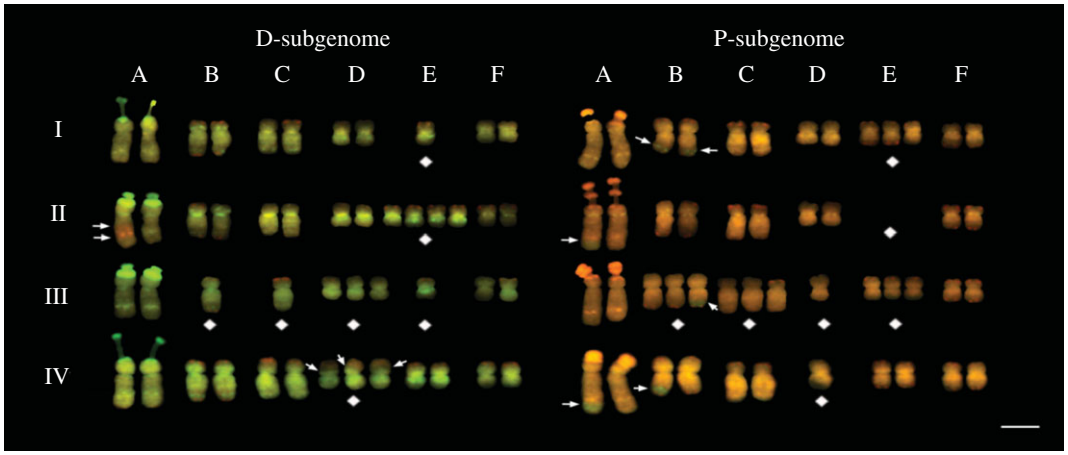

Figure 3. Compensated aneuploidy in *Tragopogon miscellus*, showing examples of novel karyotypes in individuals with 3 : 1 and 4 : 0 ratios of parental chromosomes, in four individuals (I–IV). D-subgenome refers to the contribution of *T. dubius* and P-subgenome to that of *T. pratensis*. A–F represent chromosome pairs. Note that none of the individuals has an additive karyotype with 12 chromosomes from each parent. White diamonds represent deviations from disomy. Arrows indicate intergenomic translocations. Reproduced with permission from [29].

Significantly, similar results have been obtained using FISH and GISH in a study of synthetic allotetraploid lines of *Brassica napus* [32]. These authors detected extensive chromosomal variation, including intergenomic translocations as well as reciprocal aneuploidy in multiple synthetic lines of *B. napus* polyploids [32]. Taken together, the extensive chromosomal variation present after only 40 generations in the recent allotetraploids *T. mirus* and *T. miscellus*, coupled with similar observations for synthetic lines of *B. napus*, suggests that substantial and prolonged chromosomal instability might be common in natural populations following whole-genome duplication. Moreover, this chromosomal variation represents novelty at the karyotypic level, which may have further consequences for genetic novelty in allopolyploids.

(b) Genetic novelty: presence–absence variation

Despite complete additivity of parental genomes upon polyploid formation, accumulating data indicate that additivity is not retained at all loci and that loss of one parental gene copy (homeologue) is typical of many (most?) allopolyploids. This process of homeologue loss renders an allotetraploid essentially diploid at an affected locus, and an allotetraploid is therefore a mosaic of loci that retain both parental copies coupled with others that have either one parental copy or the other. Furthermore, homeologue loss can happen very quickly following polyploid formation, as in *Tragopogon mirus* and *T. miscellus* [33–38].

Analyses of homeologue loss in *Tragopogon* tetraploids was initiated on a small scale using CAPS (cleaved amplified polymorphic sequence) analysis [33–35,38] and later extended to over 100 loci using Sequenom methods [36,37]. In all analyses, close to 10% of duplicate loci showed loss of one parental homeologue, a stunning result, given the young age (approx. 80 years or 40 generations) of these polyploids. Typically, the same loci underwent loss across populations of independent formation, suggesting differential selection for singleton versus duplicate copies across loci. Furthermore, loss of a given parental homeologue was rarely fixed in a population, leading to polymorphism within populations, which, when amplified across all loci in the genome, may result in each individual of a population (and maybe species) being genetically unique, although this hypothesis requires further testing. Recent [37] and ongoing (IE Jordon-Thaden, LF Viccini, B Jordon-Thaden, RJA Buggs, PS Soltis, DE Soltis 2014, unpublished data) analyses demonstrate that, at some duplicate loci, only one parental allele and not both may be lost, yielding a 2 : 1 imbalance of parental copies. Chromosomal mechanisms, such as homeologous recombination and non-reciprocal translocations, may account for loss of parental copies (as in *Brassica napus* [39]), and ongoing studies are aimed at integrating genetic and chromosomal data to improve our understanding of the mechanisms involved in homeologue loss in *Tragopogon*.

Spectacular levels of homeologue loss in maize (*Zea mays*) are reported as copy number variation (CNV) and presence/absence variation (PAV) [40]. This structural variation was long suspected among inbred lines of maize but could not be investigated in detail until the assembly of the maize genome. Relative to other crown eukaryotes, two prominent inbred lines of maize, B73 and Mo17, revealed unprecedented levels of CNV and PAV. Further analyses incorporating data for additional inbred lines indicate extensive structural variation in all pair-wise comparisons and demonstrate that homeologue loss is ongoing. Haplotype-specific variants contain hundreds of single-copy, rather than duplicate, loci that may contribute to phenotypic diversity in maize and to heterosis when these lines are crossed [40]. In all, approximately 8000–9000 genes are single-copy in maize, an ancient polyploid that is perhaps 5–12 Myr old, and only approximately 4000–5000 are retained in duplicate (reviewed in [41]). Schnable *et al.* [42] suggest that homeologue loss (fractionation) has been biased, such that the maize genome is more similar to one parental genome, which is dominant over the other subgenome.

(c) Novelty in gene expression

Global gene expression patterns in polyploids may show additivity of parental expression or novelty, the latter resulting from a combination of additive, parental and truly novel expression at individual loci. Non-additive expression in allopolyploids has been variously described as transcriptome dominance [43], bias [44], nucleolar dominance (in reference to rRNA genes; [45]), genome dominance [42,46] and homeologue expression bias [47]. The factors contributing to these patterns—and what the patterns themselves represent—are complex, as is the terminology [23,47]. However, it is clear that biased expression in allopolyploids, when summed over

the entire genome, represents a form of novelty, yielding combinations of gene expression patterns not present in diploid parents and deviation from an expectation of additivity. Experiments on polyploid gene expression have used a variety of techniques, including microarrays, quantitative polymerase chain reaction (qPCR), CAPS analysis of cDNA relative to genomic DNA, Sequenom MassARRAY and RNA-Seq; thus, the data and their interpretations vary among studies from overall patterns of gene expression, as in microarrays, to homeologue-specific analyses of additivity, bias or novelty at specific duplicate gene pairs. Recent studies of allopolyploid cotton [48,49] and coffee [50] focused not only on global gene expression patterns in allopolyploids relative to their parental species in a genome-wide manner, but also on how homeologue expression bias is linked to expression-level dominance. Such studies will be particularly effective at determining the extent of novel gene expression in polyploids.

To illustrate the complexity of gene expression patterns, we present here a summary of gene expression studies, spanning a range of techniques, for allopolyploid cotton. *Gossypium hirsutum* ('cotton'; $2n = 4x = 52$) formed approximately 1–2 Ma via allopolyploidzation between A-genome (similar to modern *G. arboreum* and *G. herbaceum*) and D-genome (similar to modern *G. raimondii*) progenitors. Non-additive expression has been addressed through studies of homeologue silencing, biased expression and organ-specific expression differences, with results demonstrating differential homeologue expression in different plant organs [51–54], at different developmental time points [55,56] and at different evolutionary stages [56,57]. Global transcriptome profiling using microarrays [44,58] and RNA-Seq [48,49] has shown biased expression-level dominance towards one or the other of the diploid parents. Moreover, Yoo *et al.* [48] showed that a staggering 40% of the genes investigated exhibited non-additive expression patterns in cotton leaf tissue, and the degree of non-additive expression increased over time and includes transgressive and novel gene expression. In sum, gene expression in cotton deviates substantially from a null model of additivity, with bias and novelty at the level of both individual duplicate gene pairs and the genome as a whole.

In contrast to his views on the potential of allopolyploids to generate biochemical and evolutionary novelty, Gottlieb championed the concept of 'parental legacy' in gene expression, that is, the extent to which gene expression patterns of homeologues in an allopolyploid are a legacy of expression patterns that were already present in the progenitor species [59], as opposed to novelty. He cautioned against assuming equal expression of homeologues in a new polyploid and then interpreting differences in parental contributions as evidence of divergence following polyploidization. Instead, he noted that diploid parental species may differ in expression patterns and levels, and these differences may be maintained in an allopolyploid, such that differences in homeologue expression may in fact represent additivity of the parental profiles. Roose & Gottlieb's [60] analysis of *Adh3* expression in *Tragopogon miscellus* and its diploid parents is an example of such parental legacy. Buggs *et al.* [59] further relate patterns due to parental legacy to current concepts of *cis*- and *trans*-regulation. The extent to which differences in homeologue expression in allopolyploids are due to parental legacy versus divergence following allopolyploidization is unclear because most studies do not thoroughly examine broad ranges of expression in the parental species; Buggs *et al.* found that a substantial fraction (18–55%) of expression differences in *Tragopogon* allotetraploids could be due to parental legacies but that these maximum estimates cannot be further evaluated due to insufficient data for the diploid parents (due in part to extinction of populations of these species). Future studies should ensure adequate sampling of diploid parental species to evaluate the role of parental legacy in allopolyploid gene expression.

(d) Alternative splicing, polyploidy and novelty

RNA alternative splicing (AS) is a series of processes that remove introns from a pre-mRNA transcript and reconnect exons in multiple ways [61–64]. AS may have many effects on gene expression, but most relevant to our paper is that AS creates multiple forms of mRNA from a single gene, leading to multiple protein isoforms. Although the level of AS appears to vary among species, current estimates indicate that as many as 61% of intron-containing genes in *Arabidopsis thaliana* undergo AS [65]. Thus, even within a diploid species, AS can yield an array of protein products not predicted by the genome sequence alone (figure 4).

But what happens in polyploids? One prediction is that an even larger pool of mRNA forms could be produced in an allopolyploid with divergent parental pre-mRNA transcripts, yielding further opportunities for novel protein formation beyond those produced in the diploid parents. However, few studies have analysed the impact of gene or genome duplication on AS. Some studies suggest that duplicate genes have little effect on AS [66,67], whereas others suggest that AS decreases with gene duplication (whether single-gene or whole-genome duplication), and that alternatively spliced isoforms between duplicates may differ dramatically in their effects [68,69]. The negative correlation between AS and duplication may relate to the 'expense' of operating the AS machinery: if the cost of generating multiple isoforms through AS is high, then selection would favour reducing it in those cases where multiple isoforms may be present through other mechanisms, such as gene duplication or polyploidy. Initial investigations of AS in relation to gene and genome duplication in plants have focused on *Arabidopsis* [70], *Brassica napus* [71] and wheat [72]. Additional studies of the role of AS in generating novel isoforms in polyploids are needed; for further information on AS in polyploids, see Yoo *et al.* [23].

(e) Transposons and novelty

Since Barbara McClintock's first discovery of transposition in maize in the early 1950s, transposable elements (TEs) have been widely studied in bacteria, plants and animals. Because TEs can change their position within a genome, they are considered important factors associated with genome reorganization and epigenetic changes [73,74]. Furthermore, transposon activity can be influenced by either environmental stress [75,76] or genomic changes, including polyploidy [77]. In polyploids, changes in transposon activity are twofold, with effects on both transcriptional activity and transpositional activity. Changes in transcriptional activity are usually detected by comparing the steady-state level of expression of a transposon in a polyploid and its parents [77]. Alterations in transpositional activity occur if the number of TE insertions differs between a polyploid and its parents [77]. Changes of TE activity correlated with allopolyploidy are also accompanied by epigenetic changes at those TE sites, such as DNA methylation [78].

Novel transcriptional activity of transposons has been detected in several allopolyploid plants. Microarray and reverse

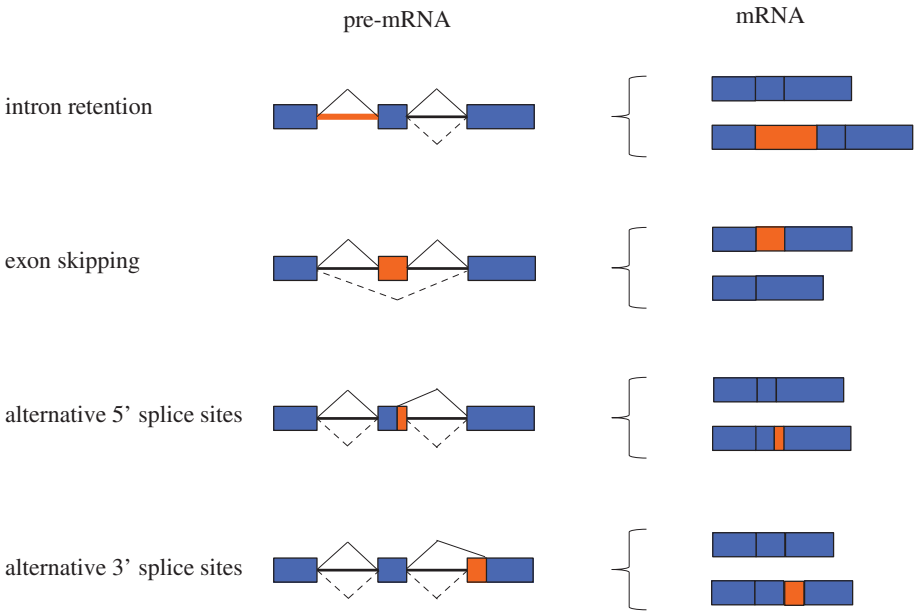

Figure 4. Common types of alternative splicing events. Blue bar, constitutive region; orange bar and line, alternative region.

transcriptase-PCR analysis in synthetic *Arabidopsis* polyploids showed strong activity of *Sunfish* (*Suf*), an *En-spm-like* transposon, in synthetic allotetraploid lines and hybrids, compared with no expression in the parental autotetraploid *A. thaliana* and *A. arenosa* [79]. Besides the *Suf* family, the microarray results suggested that allopolyploidization might also increase the activation of two *En-spm-like* elements and a *Ty-1* copia-like retrotransposon. Similar novel transcriptional activity of TEs was also found in newly synthesized allotetraploid wheat. By applying cDNA–amplified fragment length polymorphism (-AFLP) and RT-PCR approaches, Kashkush *et al.* [80] found that the *Wis* transposon showed higher transcriptional activity in synthetic allotetraploid wheat; furthermore, the high activation of this TE resulted in silencing of the adjacent downstream gene [81].

Novel transpositional activation of transposons has also been detected after allopolyploidization in some species. Such a burst in transpositional activity has been proposed in allopolyploids, based on the fact that all genes are duplicated and the genome can therefore tolerate higher levels of TE activity [82]. An increase in the copy number of a *Tnt1* retrotransposon was detected in allotetraploid tobacco [83], but no similar transposition burst was detected after genome doubling in the allopolyploid *Spartina anglica* [84]. In synthesized allohexaploid wheat (*T. aestivum*), both AFLP-based analysis and small RNA high-throughput sequencing data showed that the abundance of *Veju* long terminal repeats decreased in allopolyploid wheat and is associated with a decrease in CG methylation [85,86]. By applying 454 pyrosequencing and a site-specific PCR approach, a significant increase in copy numbers of *Au SINE* was found in natural wheat polyploids (*T. turgidum* ssp. *dicoccoides*, *T. turgidum* ssp. *Durum* and *T. aestivum*), compared with no significant increase in the copy number of this transposon in the first four generations for newly formed allopolyploid lines [87]. In *Nicotiana* allopolyploids, TE transpositional dynamics vary among TEs [88].

In some cases, transcriptional activity of a transposon correlates with transpositional activity. In the study by Madlung *et al.* [79] noted above, despite an increase in transcriptional activity after allopolyploidy, limited transpositional activation of the *Sunfish* transposon in allopolyploid *Arabidopsis* was detected through a methyl-insensitive Southern blot analysis [79]. In polyploid coffee (*Coffea arabica*), microarray analysis showed that the expression level of *Tip100* in allopolyploid *C. arabica* is higher than the sum of the expression levels of *Tip100* in its parents, *C. eugenioides* and *C. canephora* [89]. FISH results showed an increase in *Tip100* copy number and a more prevalent interstitial chromosomal location in allotetraploid *Coffea* compared with the parental species, indicating an increase in transpositional activity in this species compared with the parental species [89]. Thus, although novel TE activity is noted in allopolyploids in both *Arabidopsis* and *Coffea* relative to their parents, in *Arabidopsis*, transcriptional and transpositional activities of TEs are negatively correlated, while in *Coffea* they are positively correlated. Clearly, additional studies of TE activity—both transcriptional and transpositional—are needed in polyploids.

(f) Novel ecophysiological variation

One of the associations traditionally made with polyploidy is an increase in cell size, or *gigas* effect, resulting from nucleotypic effects of an enlarged genome. The implications of increased cell size can be many, from larger size of stomata and vessel elements, to an overall larger plant and changes in various processes [21,90,91]. While exceptions exist, this association has held up over time, with many, if not most, polyploids harbouring cells that are larger than those of their progenitor(s). Several investigations have uncovered a pattern of increased stomatal guard cell length coinciding with a decrease in stomata per unit leaf area [92,93]. Despite this apparent balancing act, these polyploids often demonstrate transgressive responses to drought stress [94–97]. For example, Li *et al.* [98] investigated the response to water deficiency in *Betula papyrifera* and found that polyploids delayed stomatal closure until reaching a much lower leaf water potential than that of the diploid, allowing the gas-exchange necessary for photosynthesis to continue longer. Maherali *et al.* [96] found similar increases of stomatal size, as well as xylem architecture, in both natural and synthetic tetraploids relative to diploid *Chamerion angustifolium*, with the synthetic tetraploid displaying a drought tolerance intermediate to that of the natural tetraploid and diploid. These

results suggest that in *Chamerion* the physiological changes associated with whole-genome duplication *per se* represent a large first step towards the ecological divergence observed between the natural cytotypes. However, the problem is complex, as the issue of parental legacy was not addressed, and the possibility exists for evolution post-polyploidization, as demonstrated in *Achillea* [99]. The physiological effects associated with cell size and water use may not be entirely beneficial, involving trade-offs (e.g. increased risk of xylem cavitation), and the way these are balanced over time likely influences the establishment of polyploids in novel habitats [97,100]. Yet to be empirically linked with polyploidy (to the best of our knowledge) is the role of cell size increase in cuticle thickening, which has been demonstrated to be inversely correlated with water loss, and could serve as another immediate change following polyploidy that works to restrict water loss during drought [101].

In one of the earliest discussions of polyploid dosage effects, Roose & Gottlieb [13] postulated that genome duplication should increase the concentration of gene products as a result of having extra gene copies. In the past decade, several studies have upheld the conclusions of Roose and Gottlieb, taking this idea an additional step further by linking the increased expression with specific transgressive traits following polyploidization. Commonly studied traits include a suite of abiotic tolerances, comprising heat, cold, salt and light stress. For example, Liu *et al.* [102] found that peroxidase and superoxide dismutase, among other cellular mechanisms for combating harmful reactive oxygen species (peroxides, free radicals, etc., that increase in response to environmental stressors), were more active in a synthetic autotetraploid *Dendranthema nankingense* (Asteraceae) than in the diploid progenitor. Tolerance to either heat or cold stress, like cell size and water use efficiency, may also involve some degree of trade-offs; in *Dendranthema*, tetraploids were more resistant to extreme cold, but had a reduced tolerance towards heat stress, which in nature could be a mechanism to drive niche divergence between cytotypes [102]. Vyas *et al.* [95] discovered that increased levels of RuBisCO in first-generation synthetic tetraploid *Phlox drummondii* created an unbalanced complement of photosynthetic components, but still yielded a net photosynthetic gain that increased over subsequent generations as balance between gene products was optimized, pointing again towards the importance of both whole-genome duplication *per se* in generating novelty immediately, also the role of subsequent evolution in shaping it. The allopolyploid *Glycine dolichocarpa* exhibits enhanced photoprotection under conditions of excess light through overexpression of genes associated with both xanthophyll production and electron flow [103].

Ten years after Roose & Gottlieb's findings in *Tragopogon*, Stebbins [104] suggested that it is increased heterozygosity and not increased gene dosage due to duplicate gene copies that accounts for transgressive polyploid phenotypes. This topic continues to receive attention in discussions of heterosis [105]. Compared to the examples above pertaining to the *gigas* effect and increased dosage, fewer studies have been able to demonstrate a clear link between the role of heterozygosity in polyploids and phenotypic novelty. While possible, it is unlikely that autopolyploids would display transgressive physiological effects due solely to heterozygosity (likely cell size, dosage effects and subsequent evolution play larger roles in divergence via autopolyploidy, although heterozygosity is also higher in autopolyploids [106,107]), and no studies to the best of our knowledge have yet demonstrated otherwise. In allopolyploids, however, homeologous (representing fixed heterozygosity) contributions within the same biochemical pathway can generate new and unique interactions. The clearest example of this was provided by Wang *et al.* [108] in the allopolyploid *Arabidopsis suecica*, in which one homeologous copy of the *FRI* locus influenced the *FLC* expression of the other genome, resulting in a flowering time that was much later than either diploid parent. The importance of homeologous interactions in generating unique phenotypes cannot be understated, but due to the difficulty in generating clear links between heterozygosity and function, comprehensive analyses linking to physiological change are slow to emerge.

In addition to the above studies demonstrating links between specific mechanisms and polyploid novelty, a large body of literature reports various forms of phenotypic divergence that have led to the isolation of polyploids from their progenitor(s). Ramsey [99] explored the role that autopolyploidy in *Achillea* played directly in the shift from a mesic to a xeric habitat, finding that whole-genome duplication *per se* conferred an immediate increase in survivorship in a xeric environment and therefore likely contributed to the ecological isolation of cytotypes observed today. Other prominent examples include changes in phenology [109], salt accumulation [110], pollinator assemblages [111] and mineral-related stress (e.g. serpentine soil) [112]. Taken together, these studies illustrate some of the many ways in which a nascent polyploid might escape minority cytotype exclusion and become established though a shift in niche space, temporal isolation or otherwise [113].

(g) Novel niches and geographical distributions

Numerous contending theories and results regarding polyploid distributions have filled botanical, ecological and evolutionary journals for decades [2,3,7,114–116]. However, the classic, pioneering work of Clausen *et al*. [117] epitomized the variety and array of ecogeographic diversity that can arise from whole-genome duplication. Broadly, four outcomes emerged from their evaluation of 21 polyploid complexes and the relative ecological and geographical distributions of each polyploid and its progenitors: sympatry, limited intermediacy, broad intermediacy and transgressive. While these results were largely descriptive, modern ecogeographic investigations into polyploids and their progenitors using a variety of techniques (e.g. niche modelling, genetic analyses, transplant experiments, etc.) have produced the same assortment of outcomes [118–120]. Here, rather than review previously proposed hypotheses of the distributions of polyploids relative to their parents in specific regions (e.g. the arctic; [7,114,121]), we provide discussion in the context of Clausen *et al.* [117], with an emphasis on novel distributions of polyploids.

Although often difficult to detect morphologically, sympatric distributions of multiple cytotypes [117] are becoming more evident as the ease of assessing chromosome counts or genome size advances and cryptic cytotypes become more apparent [122–124]. Ecological studies of these sympatric cytotypes have found minute niche differentiation such as in phenology or pollinator syndrome to allow these polyploids to cohabitate with their progenitors [111]. The second general ecogeographic distribution of polyploids, limited intermediate

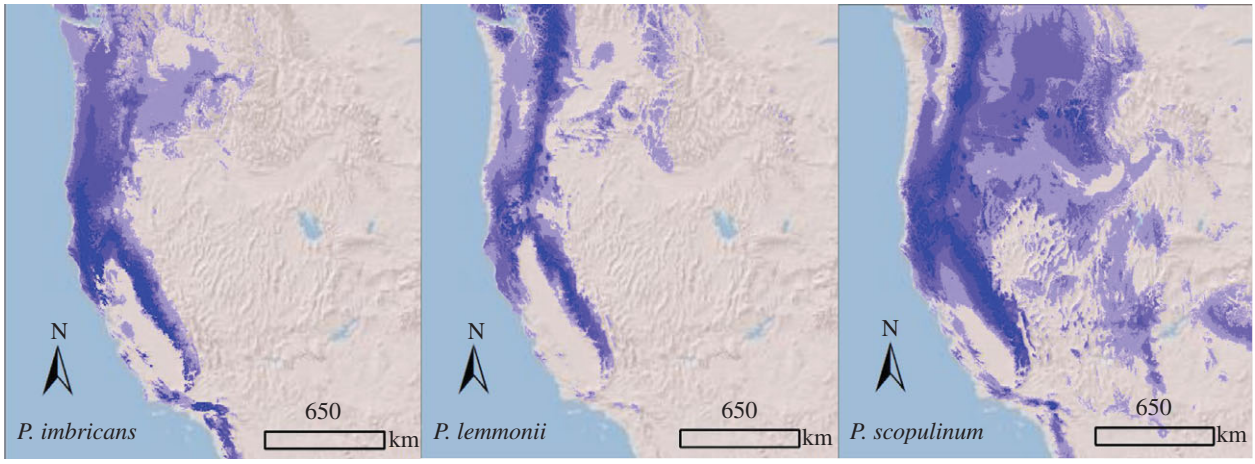

Figure 5. Ecological niche models for the allotetraploid *Polystichum scopulinum* and its diploid parents, *P. imbricans* and *P. lemmonii*, in western North America. Intensity of colour indicates probability of occurrence, with lavender designating lower probability than dark blue. Note that the predicted niche of *P. scopulinum* is greater than the sum of the niches of its parents.

distributions, was found in the California endemics *Madia citrigracilis* and *Penstemon neotericus* [117]. Described as intermediates in ecological preference between their parent species yet geographically confined, these polyploids are hypothesized to be recently formed or lacking the adaptive novelty of other polyploid species to extend beyond their current ranges. Theodoridis *et al.* [116] found similar ecogeographic patterns in four European *Primula* species, ranging from 2*x* to 8*x*, with the polyploids inhabiting significantly smaller ranges and niche breadth than the diploid. Given the distributions of these polyploid *Primula* species in northern Europe or the Alps, it is likely that they also represent recent formations that expanded into these areas following Pleistocene glaciation. In the third polyploid distribution outcome, the polyploid inhabits an intermediate range and ecology relative to its progenitors [117]. This pattern was described for the allopolyploid *Iris versicolor*, which inhabits wet lowlands and dry talus in a range geographically transitional between its parents *I. setosa*, which is found in prairies, and *I. virginica*, which is swamp-specific. Similarly, McIntyre [118] discovered ecogeographic intermediacy in three cytotypes (2*x*, 4*x*, 6*x*) of three *Claytonia* species in western North America.

The most widely discussed polyploid ecogeographic pattern, transgressive distribution, describes a range and ecological habitation of a polyploid beyond those of its parent species. Recently, transgressive polyploids have gained notoriety because of their pervasiveness among invasive species [125]. In addition, these cases best demonstrate the genetic impact of a 'macromutation' such as whole-genome duplication by providing the genetic fodder for morphological, physiological and/or ecological novelty [21]. The allopolyploid fern *Polystichum scopulinum* provides a simple case study of transgressive ecogeography, ranging beyond the extent of that of its parent species, *P. imbricans* and *P. lemmonii* [126]; *P. scopulinum* occurs commonly at high elevations throughout much of western North America from Arizona to British Columbia, whereas *P. imbricans* is found at low to mid-altitudes from California to British Columbia, and *P. lemmonii* is restricted to serpentine soils from northern California to British Columbia. Our recent analyses further indicate that *P. scopulinum* inhabits dissimilar niches from both *P. imbricans* and *P. lemmonii* (figure 5; Schoener's $D = 0.47$, 0.52, respectively; DB Marchant, DE Soltis, PS Soltis 2014, unpublished data). While the distribution of *P. imbricans* was most closely linked to precipitation seasonality (20.8%) and temperature seasonality (37.9%), that of the polyploid was more related to overall annual precipitation (29.5%) and the mean temperature of the wettest quarter (20.3%).

Recently formed polyploids in *Spartina* have become extremely invasive and now serve as excellent case studies of transgressive ecogeography. For example, *Spartina anglica*, a neoallopolyploid formed from the hexaploid *S. alterniflora* (introduced from North America) and tetraploid *S. maritima* (a European native) and subsequent genome duplication of the F_1 hybrid *S.* × *townsendii*, has been expanding its range and overtaking its parents since its formation in southern England over a century ago (reviewed in [127]). Studies indicate that a considerable portion of its transgressive ecogeography is a result of physiological novelty, in the form of increased oxygen transport efficiency and greater hydrogen sulfide removal, both of which permit ecological expansion into low-marsh zones, previously inaccessible by its predecessors [127–129]. Additional examples of transgressive polyploids and their novel adaptations are reviewed in te Beest *et al.* [125].

With the optimization and ease of accessing digitized data from museum specimens for niche modelling analyses [130], studies investigating ecogeographic distributions of species are becoming increasingly more holistic in their breadth and variables analysed. Recent publications have assessed polyploid ecogeography using these techniques in a variety of systems [116,118–120,131]; however, few conclusive patterns have emerged. Future studies must take into account phylogeny, type of polyploid and a wide variety of ecological variables at a scope so far unattained, yet achievable with current digitized resources.

4. Implications of genetic and phenotypic novelty for the evolution of polyploid plants

Polyploidy generates not only new species but also novel genomes with novel phenotypes in traits that span cellular to ecological levels of organization. Such novelty was described and addressed by Les Gottlieb and his students and collaborators for *Tragopogon*, *Stephanomeria* and *Clarkia*, in particular,

but with consideration for polyploid plants in general. Furthermore, Levin [21] built on Gottlieb's and others' observations to disentangle the relative roles of genome duplication and hybridity in generating novelty. The compelling result from this collection of work is that polyploids cannot be viewed strictly as additive or intermediate to their progenitors; instead, the effects of duplicated genomes may be novel in both predictable and unpredictable ways, perhaps catapulting polyploids into new adaptive landscapes. Moreover, recent work at the genetic and chromosomal levels indicates that, contrary to traditional models, polyploid species (and populations) are not genetically uniform but highly variable. The dynamic nature of polyploid genomes—with alterations in gene content, gene number, gene arrangement, gene expression and transposon activity, to name a few—may generate sufficient novelty that every individual in a polyploid population or species may be unique. Whereas certain combinations of these features will undoubtedly be maladaptive, some unique combinations of newly generated variation may provide tremendous evolutionary potential and adaptive capabilities.

Polyploidy has been linked to increased speciation rates via the role of 'reciprocal silencing' of homeologous loci [132]; in fact, divergent resolution of even a single duplicated gene can lead to reproductive isolation, and genetic incompatibilities between populations can be compounded when such reciprocal silencing or loss occurs at a genome scale (reviewed in [133]). Thus, not only does polyploidization yield a single new species, but also gene and genome dynamics following polyploid formation are predicted, in many cases, to trigger a species radiation [133], such as those observed at deep phylogenetic levels in yeast [17,134], teleost fishes [135,136] and angiosperms (e.g. [137–139], but see [140]). The impacts of genetic and phenotypic novelty in shaping variable polyploid populations are thus amplified through accelerated formation of reproductive barriers between genetically divergent individuals, leading to greater diversity than generated through the original polyploidization event. This emerging view of dynamic polyploid evolution has its roots in the work of Les Gottlieb and others who demonstrated that even recently formed polyploids harbour unexpected genetic and biochemical novelty.

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