Non-Mendelian Phenomena in Allopolyploid Genome Evolution

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Abstract: Perhaps all flowering plants have experienced one or more episodes of polyploidization at some time in their evolutionary history. Recent evidence indicates that this genome doubling may be accompanied by a variety of non-Mendelian phenomena, some of which operate during hybridization and polyploid formation while others manifest more gradually on an evolutionary timescale. Here we review these phenomena, drawing attention to recent paradigm shifts necessitated by new insights from model plant systems. Allopolyploid formation in some plant groups is associated with an unexplained and in some cases directed process of genomic alteration leading to non-additivity with respect to parental genomes. Novel intergenomic interactions become possible as a consequence of the merger of two previously isolated diploid genomes, variously leading to intergenomic colonization and/or homogenization of formerly diverged sequences. Several epigenetic processes may accompany nascent allopolyploidy, such as nucleolar dominance, gene silencing and mobile element activation, the latter also resulting in genetic change. These myriad phenomena do not characterize all polyploid systems, and some nascent allopolyploids appear to be genomically quiescent. Although a direct connection to adaptation remains to be established, the diversity of genetic responses to allopolyploid formation and their apparent high frequency suggest that non-Mendelian phenomena contribute directly to polyploid stabilization and diversification.

I. INTRODUCTION

Polyploidy, resulting from either duplication of a single but complete genome (autopolyploidy) or from combination of two or more differentiated genomes (allopolyploidy), is a prominent mode of speciation in plants [1-8], and has also been significant in the evolution of vertebrates and possibly many other eukaryotes, including humans [9-16]. It is difficult to overstate the importance of polyploidy in the evolutionary history of plants. While estimates vary regarding the proportion of angiosperms that have experienced one or more episodes of chromosome doubling at some point in their evolutionary history, it is at least 50% and may be higher than 70% [2,6]; perhaps 95% of pteridophytes have experienced at least one episode of polyploidization in their past [1,2]. Many important crop plants, including wheat, oat, coffee, potato, canola, soybean, sugarcane, tobacco and cotton are typical polyploids.

Because most ancient polyploids have undergone an evolutionary process of chromosomal and perhaps genic "diploidization", their polyploid history may be obscured at the cytological and classic genetics levels. Consequently, the polyploid nature of many plant genomes was not evident until the advent of comparative genomics and whole-genome sequencing. Recent and prominent examples include maize [17,18] and *Arabidopsis* [19-21] — both species were traditionally recognized as diploids, but in fact their genomes harbor compelling evidence of historical cycles of genome doubling. Given these and other recent examples from plants [e.g. 22,23-26], it is probably safe to state that there are no *bona fide* diploid species in the plant kingdom.

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Given the prominence of polyploidy in plants, it is not surprising that there has been a great deal of interest in its biological significance. Various aspects of polyploidy have attracted attention, including classification of the various types of polyploids, mode and frequency of formation, significance vis-à-vis adaptation and diversification, and correlations with life-history attributes and ecological parameters. These subjects have been thoroughly reviewed elsewhere [6-8,27-32] and will not be discussed here. More recently, attention has been focused on genetic and genomic attributes of polyploidy, including the immediate and long-term consequences of genome doubling [reviewed in 33].

Because of rapid progress in the field, there has been growing awareness of the diversity of phenomena associated with polyploidy, particularly its surprising non-Mendelian attributes (i.e., those not characterized by conventional transmission genetics). The present review is aimed at these phenomena, with the goal of providing a convenient entry into a rapidly expanding literature. We will revisit some of the central principles of polyploid genome evolution [cf. 33] as well as draw attention to recent paradigm shifts necessitated by new insights from model plant systems. Particular emphasis will be placed on the mysterious process of rapid and in some cases directed structural changes that occur in polyploid genomes upon their formation; novel intergenomic interactions that become possible as a consequence of the merger of two formerly isolated genomes; and epigenetic mechanisms that may accompany nascent allopolyploidy, such as nucleolar dominance, gene silencing and mobile element activation. These myriad phenomena do not characterize all polyploid systems, and in fact some nascent allopolyploids appear to be genomically quiescent in this respect. We will summarize experimental data from model polyploid systems, explore the possible mechanisms and biological significance of the various

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Table 1. Model plant Allopolyploids and Non-Mendelian Phenomena Reported

System	Plant material	Phenomena and references
Brassica	5-generation-old synthetic allotetraploids produced by reciprocal crosses between *B. rapa* and *B. nigra*, and between *B. rapa* and *B. oleracea*	· Rapid and random structural genomic changes, including loss and/or gain of parental RFLP fragments [35] · DNA methylation changes [35] · Novel phenotypes, including variation in flowering time [135]
Wheat (*Aegilops-Triticum*)	F_1 hybrids, 1-5 generation-old synthetic allopolyploids between various species combinations and at various ploidal levels; natural tetra- and hexaploid wheats	· Rapid random/non-random structural genomic changes, including loss and/or gain of parental RFLP fragments [40,41,45] · Rapid and 'programmed' sequence elimination of low-copy sequences [44,46,47] · DNA methylation changes [40,45,83] · Rapid epigenetic gene silencing [83] · Retrotransposon activation [83] · Rapid gene loss [83] · Gene silencing and dosage compensation [81,82,147] · Reciprocal intergenomic invasion by repeats [122] · Elimination of repeats [148]
Arabidopsis	Synthetic allotetraploids between *Arabidopsis thaliana* (2n = 20) and *Cardaminopsis arenosa* (2n = 32); a natural tetraploid species, *Arabidopsis suecica*; synthetic allotetraploids between *A. thaliana* and *A. lyrata*	· Rapid epigenetic gene silencing [61,80] · DNA methylation changes [61,80] · Reversibility of silenced genes via epigenetic means [80] · Multiple phenotypic variation and instability [61] · Nuclear dominance [68,69,73]
Cotton (*Gossypium*)	Natural allotetraploids (*ca.* 1-2 million-year-old)	· Inter-locus concerted evolution [58] · Intergenomic colonization by repeats and transposons [55-57]
Tobacco (*Nicotiana tabacum*)	Natural allotetraploid	· Elimination and rearrangement of rDNA [111]
Rice (*Oryza sativa*)	Intergeneric F_1 hybrid and introgressed lines between rice and wild rice (*Zizania latifolia*) (9 generation- old)	· DNA methylation changes [79,124] · Transposon activation [79]

phenomena, and discuss possible reasons for the observed differences among polyploid taxa.

II. RAPID GENOMIC CHANGES IN NEWLY SYNTHESIZED ALLOPOLYPLOIDS

According to the classical view of allopolyploidy, the merger of two distinct but related genomes should result in genomic additivity with respect to the parental species. This expectation serves as a convenient null hypothesis of the predicted genomic contributions to a polyploid nucleus. Naturally occurring polyploids may not, however, provide robust tests of the hypothesis, because their genomes, as well as those of their diploid progenitors, will have continued to evolve since polyploid formation, thereby obscuring initial conditions. Because of this, insights into the earliest stages of polyploid genome evolution are likely to require the study of synthetic experimental allopolyploids. Recent studies in *Brassica* and in *Aegilops-Triticum* are especially revealing in this regard, as they demonstrate that nascent allopolyploids often do not show genomic additivity with respect to their parents. Instead, their genomes display remarkable patterns of non-Mendelian genomic changes accompanying hybridization and polyploidization. These studies, more than any others, are responsible for a growing recognition of the dynamic [34] and unpredictable nature of polyploid genomes.

The first study demonstrating extensive and rapid genomic changes accompanying polyploid formation was by Song *et al.* [35], who used newly synthesized reciprocal synthetic allopolyploids in *Brassica*. Two different hybrids were generated in each of the two parental cytoplasms, one between the diploids *B. rapa* and *B. nigra* and the other from *B. rapa* and *B. oleracea*. Following colchicine-doubling, F_2 individuals were recovered from which progenies up to the F_5 generation were synthesized by self-pollination. Southern

hybridization analysis using 89 nuclear probes corresponding to cDNAs, known-function genes, and anonymous genomic clones revealed a high frequency of unexpected fragment profiles in each generation [see, however, 36]. These genomic changes included loss of parental fragments, recovery of parental fragments in the F_5 that were not detected in the F_2, and the frequent appearance of novel fragments, especially in allopolyploids involving *B. rapa* and *B. nigra*. This latter observation reflects the quantitative conclusion that nearly twice as much change was detected in crosses involving the distant relatives *B. rapa* and *B. nigra* as in the more closely related *B. rapa* and *B. oleracea*. The changes were apparently random, as individuals from the same and different generations exhibited a great degree of variation.

A similar phenomenon of unexplained gain and loss of DNA fragments was soon thereafter described from synthetic allopolyploids of the *Aegilops-Triticum* group. The wheat group provides an ideal system to study polyploid genome evolution because several allopolyploid species are young, their diploid progenitors are extant, and the phylogenetic relationships among the diploid species and between the diploids and polyploids are reasonably well-understood [37-39]. Moreover, allopolyploids can be readily synthesized in the laboratory by colchicine treatment. Perhaps of more significance, the young (~ 8,500 year-old) natural hexaploid species *Triticum aestivum* (common or bread wheat), vital to the development and present sustenance of human civilization, is a classic example of speciation via allopolyploidy. This species is an allohexaploid, with a genomic constitution BBAADD, formed from a natural hybridization event between the allotetraploid *Triticum turgidum* (BBAA) and a taxon similar to modern *Aegilops tauschii* (DD), with genome doubling most probably resulting from fertilization between unreduced gametes [37,38].

Liu *et al.* [40] studied RFLP patterns in nine sets of parental lines and 3- to 6-generation-old synthetic allopolyploids of various *Aegilops-Triticum* species at several different ploidy levels. Several restriction enzymes were used and the probes included 41 anonymous genomic or cDNAs and two protein-coding genes whose functions were known. To maximize genomic coverage, probes were selected that map to proximal and distal regions of the short and long arms of each of the seven homoeologous chromosome groups. Contrary to the expected genomic additivity, and in accord with the findings in *Brassica*, extensive changes in RFLP patterns were observed in all nine allopolyploids, including loss of parental fragments and/or appearance of novel fragments. As was the case with *Brassica*, some of the changes were apparently random, as evidenced by differences in restriction fragment profiles among individuals for a given synthetic amphiploid and between synthetics and their natural counterparts. Some changes, however, were uniform among all individuals tested and showed concordance with the RFLP patterns observed in natural polyploids. Liu et al [40] presented evidence to show that the observed changes accompanying allopolyploid formation were due to methylation, as opposed to actual sequence elimination.

In addition to establishing that hybridization and polyploid formation may be accompanied by an unexpectedly high level of genomic non-additivity, the foregoing studies raised the possibility that some of the genomic changes may be "directed", as opposed to random, as suggested by earlier work on ribosomal RNA genes in synthetic allopolyploid wheat [41]. Classic cytogenetic data and recent comparative genome mapping studies have shown that the three constituent genomes (A, B and D) of common wheat are highly syntenic to each other and to other grasses [23,42]. Hence it was surprising when Liu *et al.* [43] and Feldman *et al.* [44] discovered that there exists a class of low-copy, apparently non-coding sequences in wheat that are exclusively localized to one pair of homologues (chromosome-specific sequences, or CSSs) or to several pairs of homologues of the same genome (genome-specific sequences, or GSSs). More surprising was the observation that some of these sequences are conserved, as deduced from Southern hybridization analysis, among the diploid progenitors. The presence of these sequences in all diploid progenitors of bread wheat contradicted their apparent absence from one or more of its constituent genomes.

Feldman *et al.* [44] studied nine of these sequences in both tetraploid and hexaploid natural wheat by using a variety of aneuploid lines. Particularly useful were the nullisomic-tetrasomic lines, in which one pair of homologues is replaced by another pair of homoeologous chromosomes (e.g., nullisomic for chromosome 5 from the A-genome, tetrasomic for chromosome 5 from the B-genome), thereby permitting diagnosis of the genomic distribution of particular sequences. All nine sequences studied, which are conserved in the parental diploid genomes, were invariably either chromosome- or genome-specific in the tetraploids and hexaploids. This observation indicated that the process responsible for the "conversion" from ubiquity to specificity operated twice, once upon formation of the BBAA tetraploids and a second time coincident with the allopolyploidization event that gave rise to hexaploid wheat. The striking occurrence of this phenomenon at two different ploidy levels suggested that the responsible mechanism operated in a directed or non-random fashion. To find out whether sequence divergence or sequence elimination (deletion) was the underlying mechanism, a 5-generation-old synthetic hexaploid wheat was studied, which was analogous in genome constitution to common wheat. In this experiment all sequences that showed diagnostic polymorphism already became either chromosome- or genome-specific by the fifth generation following polyploid formation, thus strongly implicating sequence elimination as the operative process.

In a follow-up study, Liu *et al.* [45] monitored RFLP fragment profiles in three to six generation-old synthetic tetraploids, hexaploids, octoploids, and decaploids in the *Aegilops-Triticum* complex, using a similar set of probes as employed in the study of Feldman *et al.* [44]. Consistent with the earlier results, rapid and non-random sequence elimination was observed from one or more genomes in every allopolyploid studied, in addition to a less common appearance of novel fragments. The non-random nature of the phenomenon was shown by the observation that in cases where a natural counterpart of the synthetic amphiploid existed, sequence elimination was found to have invariably

occurred as expected from the hybridization profile of the extant natural allopolyploid. For example, if the probe sequence was B-genome-specific in natural bread wheat, then in the synthetic amphiploids it was always eliminated from the A-genome (or A^m) parent in tetraploids formed between A (or A^m) and S (B)-genome diploids, and additionally from the D-genome in AABBDD hexaploids. Thus, only the B genome retained the sequence subsequent to tetraploid and hexaploid formation.

The foregoing experiments established that in at least some polyploid systems, rapid sequence elimination may not only characterize nascent allopolyploids, but that this process is highly "directed" and is repeatable under both natural and experimental conditions. Albeit intriguing, we note that because the studied sequences have a pre-selected and anomalous property, namely, not being present in all genomes of natural polyploid wheat, it is unclear how representative they are of the genome as a whole, and by extension, what the extent is of the phenomenon of polyploidy-induced sequence elimination. In addition, the above studies involved comparisons of synthetic amphiploids with their parental *lines* (descendents of parental plants) instead of the exact parental *plants*, leaving open the possibility that segregation of parental heterozygosity was responsible for the observed DNA losses. One might also wonder about the consequences of mode of synthesis of the amphiploids, which in the present case were obtained by chromosome doubling with colchicine; although unlikely, it is possible that unexpected genomic non-additivity resulted from this chemical treatment. Finally, because two events are involved in allopolyploidization, i.e., hybridization and genome doubling, and because the initial F_1 hybrid was not studied, it is not possible to distinguish whether genomic merger *per se* or genome doubling was the instigator of genomic change.

To address these experimental concerns and extend our understanding of the phenomenon, Feldman and coworkers synthesized and studied new amphiploids [46,47]. In one study [46], 35 interspecific or intergeneric F_1 hybrids, their exact parental plants, and 22 derived allopolyploids of *Triticum* and *Aegilops* were analyzed by Southern blotting, using eight different CSSs or GSSs as hybridization probes. This study not only confirmed the earlier result that allopolyploidy is accompanied by rapid and nonrandom sequence elimination [44,45], but also provided several novel insights into the phenomenon. *First*, rapid elimination of CSSs and GSSs appears to be a general, nonrandom, directional and highly reproducible phenomenon in newly synthesized allopolyploids in *Aegilops-Triticum*. *Second*, sequence elimination is not caused by colchicine treatment, as amphiploids arising spontaneously from the union of unreduced gametes or obtained by tissue culture showed the same pattern of elimination. *Third*, initiation of the elimination process for GSSs was already apparent in F_1 individuals and was completed by the second or third generation of selfing following allopolyploid formation, whereas elimination of CSSs started in the first allopolyploid generation and was completed by the second or third allopolyploid generation. This finding suggests that there may be a basic difference in the elimination pattern for GSSs and CSSs, with elimination of GSSs triggered by the union of divergent genomes (hybridization), and elimination of CSSs by genome doubling (polyploidy). *Fourth*, the data suggested that sequence elimination started earlier and was completed sooner in allopolyploids whose genomic compositions have natural counterparts than in allopolyploids that do not exist in nature.

This last observation is important, in that it suggests that specific attributes of particular genomic combinations influence the timing and rate of sequence elimination. This reinforces the earlier speculation [44,45] that sequence elimination plays a role in the initial stabilization and establishment of newly formed allopolyploids as new species in nature. Perhaps only those genomic combinations that could effectively "deal" with the merger of two genomes, in part by a process of directed sequence elimination, could survive and form new lineages; those that were less effective in this respect may have suffered a higher frequency of unbalanced or inviable gametes and been less fit.

In the second study [47], two different F_1 hybrids between diploid species of the *Tricitum-Aegilops* group were surveyed at a large number of genomic loci using amplified fragment length polymorphism (AFLP) analysis, with follow-up confirmation using Southern blotting. AFLP analysis permitted the discovery of a large number of bands (373 and 246, respectively, in the two progenies) that could be monitored for transmission to the hybrid and derived allopolyploid generations. This unbiased (vs. using pre-selected CSSs and GSSs) and large-scale survey showed that sequence elimination is a major and immediate response of the wheat genome to wide hybridization and genome doubling. Shaked *et al.* [47] also showed that it affects a large fraction (see below) of the genome and that identical elimination patterns occurred in independently synthesized polyploid plants. Perhaps most astonishing is the result that in one of the two combinations of diploids, up to 14% of the genomic loci of one parent's genome were eliminated in a single generation, with most of the changes resulting from hybridity (F_1) as opposed to genome doubling.

A profound realization emerges from these studies on *Brassica* and *Aegilops-Triticum*, namely, that allopolyploidy can not only lead to the establishment of new species in a single generation, as has long been recognized [6,8,28], but that in the process the constituent genomes may be dramatically and virtually instantaneously altered. At present, one can only speculate about the immediate morphological, physiological, and ecological consequences of rapid and directed hybridization-induced and polyploidy-induced genomic reorganization, but the potential relevance to adaptation and diversification is evident (see **section VIII**, below). This issue takes on added importance when one considers the prevalence of wide hybridization and polyploidy in plants.

One question that naturally arises is whether the results for *Brassica* and *Aegilops-Triticum* will turn out to be typical or aberrant. It already is evident that *rapid genomic change* is not a hallmark of all nascent plant allopolyploids. For example, a recent study in cotton (*Gossypium*) assayed approximately 22,000 genomic loci in nine sets of synthetic allopolyploids using AFLP fingerprinting, yet nearly perfect

additivity was found with respect to parental AFLP banding profiles [48]. This result extends that inferred from phylogenetic analysis of 20 low-copy genomic loci in natural allotetraploid cotton (*Gossypium hirsutum*) and its extant two diploid progenitors [49,50]. Recovery of the expected phylogenetic topology for each of these gene trees demonstrates that since allopolyploid formation about 1-2 million years ago [51-53], most duplicated genes have not interacted, i.e., they have evolved independently. Similar results have also been obtained for repeated sequences, such as 5S ribosomal genes and spacers [54]. Contrary to this pattern of stasis and independence, some repetitive families, including transposable elements, have spread from one genome to the other following allopolyploid formation [55-57]. Moreover, homoeologous 18S-26S rDNA arrays in *G. hirsutum* have interacted to such an extent that only a single sequence type remains, the other having been 'overwritten' by some concerted evolutionary process [58].

These data collectively demonstrated that in natural allopolyploid cotton, there is a fundamental difference in the mode of evolution between low-copy loci and repetitive families. Yet even the latter do not appear to be subject to the kinds of rapid genomic changes as described above for *Aegilops-Triticum* and *Brassica*. A similar degree of genomic quiescence accompanying allopolyploid formation appears to be the case for the grass species *Spartina anglica*. This allopolyploid taxon originated in southern England approximately 110 years ago, from hybridization between the native *S. maritima* and the introduced North American *S. alterniflora*. Since its recent formation, *S. anglica* has rapidly expanded its geographic range, and now is a widespread species in salt marshes and estuaries in Western Europe and has been introduced and is rapidly expanding its range in Australia, China and New Zealand. One might suggest that this ecological success is attributable, at least in part, to its polyploidy, and that perhaps its rapid colonization history was facilitated by "rapid genome change" of the type reported in *Brassica* and wheat. Yet molecular analysis using RAPDs and ISSRs revealed genomic additivity with respect to parental genotypes [59]. Recent analyses using the more powerful REMAP (retrotransposon-microsatellite amplified polymorphism) and IRAP (inter-retrotransposon amplified polymorphism) techniques corroborate the relative genomic stasis of *S. anglica* [60]. Thus it is apparent that different groups of plant species are subject to dramatically variable genomic responses to allopolyploidization, ranging from apparent quiescence to widespread reorganization. At present, the mechanistic underpinnings of this heterogeneity remain obscure.

III. EPIGENETIC CHANGES IN SYNTHETIC AND NATURAL ALLOPOLYPLOIDS

It has long been noted that recently originated and synthesized allopolyploids may be associated with variability and instability in phenotypic characteristics, including lethality, sterility, homeotic transformation, flower variegation, and dominance of the hybrid phenotype by that of one parent [see 61,62,63]. Although the genesis of these instabilities is not understood, one plausible explanation is that they reflect altered gene expression resulting from intergenomic interactions or incompatibilities. It is envisioned that modification or disruption of intrinsic epigenetic control systems could alter expression patterns and developmental trajectories [62,64]. Until recently, however, there was virtually no empirical data on epigenetic changes associated with polyploidization, either natural or artificial.

The term epigenetic refers to changes in gene expression that do not entail a change in DNA sequence. Among the several mechanisms responsible for epigenetic phenomena, the most important appear to be DNA methylation and histone deacetylation [65-67]. An excellent example of the relationships between allopolyploidy, epigenetic modification and change in gene expression is provided by recent research into the well-known phenomenon of nucleolar dominance [68,69]. Nucleolar dominance refers to the process in hybrids or allopolyploids whereby nucleoli form, in association with ribosomal RNA genes, on chromosomes inherited from only one of the two parents (or in non-hybrids from only one of two or more rDNA arrays). The phenomenon was discovered nearly 70 years ago in plant hybrids, when unexpected changes were observed in the morphology of chromosomes from the genome of one parent [70]. It turned out that the changes reflected nucleoli formation that manifested at the cytogenetic level as secondary constrictions or satellites.

Nucleolar dominance has since been described and studied in numerous plant and animal hybrids and allopolyploids [68], but the molecular basis for the phenomenon has remained elusive. Recently, nucleolar dominance was studied at the molecular level in both natural and synthetic allopolyploids in two plant genera, *Brassica* [71,72] and *Arabidopsis* [73]. In contrast to earlier data on animals suggesting that nucleolar dominance was due to selective activation of dominant rRNA genes, it was found that in plants the phenomenon results from selective silencing of rRNA genes from the "recessive" (sub-dominant) genome through covalent chromatin modifications [71,72]. Notably, rRNA transcripts from only one of the parental genomes were detected in vegetative tissues in *both natural and synthetic* polyploids, indicating rapid occurrence of the phenomenon with the onset of polyploid formation as well as its long-term evolutionary conservation.

Further study demonstrated that cytosine methylation and histone deacetylation act as partners in the enforcement of rRNA gene silencing [72]. Direct evidence that cytosine methylation plays a role in nucleolar dominance stemmed from chemical treatment with 5-aza-2'-deoxycytosine, an inhibitor of cytosine methyltransferase, which induced a reactivation of under-dominant rRNA genes [72,73]. The histone deacetylase inhibitors, sodium butyrate and trichostatin A, also de-repressed silent rRNA genes. Interestingly, treatment with both aza-dC and trichostatin A was no more effective than either compound alone in de-repressing under-dominant rRNA genes, suggesting that cytosine methylation and histone deacetylation act as partners in the same repression pathway at the transcriptional level [68,69,71-73].

One of the more intriguing aspects of these studies is the observation that whereas nucleolar dominance is complete or nearly so in vegetative tissues, homoeologous rRNA genes were co-expressed in all floral organs. This not only proves both reversibility of the phenomenon and epigenetic control that acts at the transcriptional level, it suggests the possibility of partitioning of duplicate gene function following polyploidization, due to epigenetic phenomena alone. This, in turn, has been proposed as an important attribute facilitating adaptation and diversification of polyploids [74,75].

These studies on nucleolar dominance demonstrate that allopolyploidy can induce rapid epigenetic silencing of rRNA genes from one of the parental genomes. Since rRNA genes are exclusively transcribed by RNA polymerase I, it is of interest to ask if protein-coding genes, which are transcribed by RNA polymerase II, are subject to similar epigenetic regulation that is responsive to hybridization and polyploidy. Although in both the work on *Brassica* [35] and wheat [40] synthetic allopolyploids, DNA methylation changes in anonymous genomic or cDNAs were noted, the relevance of these observations to gene expression were not addressed. Several recent studies, however, have demonstrated that wide hybridization and genome doubling could induce rapid epigenetic modifications in both coding and regulatory sequences, as well as in or near mobile elements, resulting in gene silencing, novel expression and mobile element de-repression [61,62,76-79,80, Levy, pers. comm.].

The first extensive study of gene silencing and novel expression on a global level was that of Comai *et al.* [61] on polyploid *Arabidopsis X Cardaminopsis* hybrids. These authors found that lines derived from a synthetic allotetraploid (2n = 26) between a tetraploid *Arabidopsis thaliana* (2n = 20) and *Cardaminopsis arenosa* (2n = 32) exhibited a great deal of variation in morphology, fertility and flowering time. Although some of this variation could be attributed to segregation of the parental heterozygosity of *C. arenosa*, owing to the out-crossing nature of this species, the high level of phenotypic instability observed in the F_2 generation cannot be explained by Mendelian genetic processes. These anomalous phenotypic instabilities in newly synthesized allopolyploids implicate one or more genetic or epigenetic phenomena. To evaluate the effects on gene expression, Comai *et al.* [61] conducted a comparative analysis of the allotetraploid and its parents, using amplified fragment length polymorphism analysis on cDNAs (cDNA-AFLP). To identify changes due to allopolyploidization, but not to differences in ploidy level, lines of the same ploidy level were compared. Potential gene silencing was revealed by the observation that 20 of approximately 700 transcripts evaluated disappeared from the allotetraploid although they existed in the parents. Moreover, in two cases transcripts that did not exist in the parents appeared in the allotetraploid, suggesting novel expression. Gene silencing was confirmed by RT-PCR analysis on independently prepared mRNAs for three of the 10 cDNAs isolated; in all cases the presence of the corresponding genomic DNA was verified, thus strongly suggesting epigenetic silencing. Because the silenced genes were identified from a sample size of approximately 700, Comai *et al.* [61] estimated that at least 0.4% of the protein-coding genes in the F_2 generation of the synthetic allotetraploid were silenced. This is apparently a conservative estimate, because candidate genes not silenced from *all* F_2 plants were not included in the calculation and because partial silencing was not considered.

Further molecular characterization of the three genes and their flanking genomic regions by similarity searches of databases and by Southern blotting indicated that two genes are associated with repetitive sequences, one being part of a solo long terminal repeat (LTR) of a *copia*-like retrotransposon, and the other having a repeat in its 5' upstream region. This suggested a possible correlation between gene silencing and the presence of repeated sequences. No mutation was observed in the putative promoter region of one of the silenced genes, providing additional evidence that the silencing was epigenetic. Comparison of the cytosine methylation status in the LTRs by a pair of isoschizomers, *Hpa*II and *Msp*I, which have the same recognition site CCGG but differ in sensitivity to methylation at either cytosine, revealed epigenetic changes associated with allopolyploidy.

Additional evidence on epigenetic gene silencing in *Arabidopsis* came from a recent study on *Arabidopsis suecica*, a natural allotetraploid between *A. thaliana* and *Cardaminopsis arenosa.* Lee and Chen [80] conducted a global comparative gene expression analysis, also using the cDNA-AFLP method, in an accession of *A. suecica* and its two extant parental species. Co-expression was examined for over 4,400 fragments, of which about 11% potentially were candidates for gene silencing. Follow-up cloning, sequencing, and RT-PCR experiments verified differential expression of parental genes in the allopolyploid for 25 of the 110 fragments isolated, indicated a silencing rate of about 2.5%. These genes encode a variety of predicted proteins, including a number of transcription factors and a transposase. The epigenetic nature of the gene silencing events was confirmed by verifying the presence of DNA sequences, and by reactivating the silenced genes through blocking genomic cytosine methylation. A remarkable and important observation emerged from analysis of expression patterns on a local genomic scale: Lee and Chen analyzed five adjacent genes localized to a single *Arabidopsis thaliana* BAC, and found co-expression, silencing of a homoeologue from one parent, and silencing of a homoeologue from the other parent among the five genes studied. As stated by Lee and Chen, this indicates that "expression patterns of parental genes in polyploid genomes are complicated even in a small chromosomal domain."

The two studies discussed above were the first to demonstrate that allopolyploidy often is accompanied by epigenetic gene silencing and that this process can affect a variety of genes with different biological functions. Although the silencing events accompanied the onset of allopolyploid formation, reversibility for at least some of the expression changes is maintained in natural allopolyploids with the same genomic composition, suggesting evolutionarily stable epigenetic transformations. This in turn suggests selection to stabilize the epigenetic regulatory response. As pointed out by the foregoing authors [61,62,80], gene silencing by epigenetic instead of

mutational means in a natural allopolyploid provides the potential for developmental or tissue-specific reversibility, which may provide an evolutionarily adaptive regulatory flexibility.

The generality of the *Arabidopsis* results with respect to other natural polyploid systems is largely unknown, as few comparable studies exist. Classic work by Feldman and coworkers elegantly demonstrated over 15 years ago that in natural hexaploid (BBAADD genome) bread wheat (*circa* 8,500 year-old), genes encoding endosperm storage proteins have undergone a "massive and non-random" genetic diploidization via either gene silencing or dosage compensation [81,82]. The silencing was found also to occur in synthetic hexaploid wheat, and sometimes regain of expression of the silenced alleles was observed when tetraploid wheat was "extracted" from hexaploid populations. These observations showed that silencing was stimulated by the addition of the third (DD) genome to tetraploid (BBAA) wheat, and as with the *Arabidopsis* experiments described above [80], silenced genes could be reactivated, demonstrating that the expression changes did not arise from permanent structural changes at the DNA sequence level.

Ongoing research on synthetic allotetraploid wheat using the cDNA-AFLP approach indicates that rapid epigenetic gene silencing also occurs in this system [83]. In first generation (S_1) amphiploids between *Aegilops sharonensis* and *Triticum monococcum*, analogous in genomic constitution to natural tetraploid wheat, 60 of 3,072 transcripts were reproducibly altered in the allotetraploid, entailing 48 apparent silencings and 12 novel activations. It was further demonstrated that some of the silencing events were due to epigenetic alterations while others resulted from actual gene *loss* — a phenomenon not observed in either natural nor synthetic *Arabidopsis* allotetraploids [61,80]. Similar to the findings in *Arabidopsis*, epigenetic silencing in synthetic wheat is also associated with cytosine methylation modification, and a diverse set of genes are affected, including some involved in metabolism, disease resistance and cell cycle regulation. All novel transcripts that could be assigned a putative function are from retrotransposons, although transposition of the elements was not detected.

As discussed in section III, above, nine sets of synthetic cotton allopolyploids recently were examined using AFLP fingerprinting of genomic DNAs, yet there was nearly perfect additivity with respect to parental AFLP banding profiles [48]. This study included a modification of the standard AFLP procedure designed to assess possible changes in methylation status; by comparing *Eco*RI + *Hpa*II digests to those from *Eco*RI + *Msp*I, differences for the two isoschizomers should reflect the presence of cytosine methylation at internal Cs of CCGG sites. Nearly 10,000 AFLP fragments were evaluated by this method, and comparison of the parental fingerprints with those of the corresponding allopolyploid revealed nearly complete additivity. These results showed that *de novo* cytosine methylation changes at CCGG sites did not occur to an appreciable degree in the synthetic *Gossypium* allopolyploids. Relative to *Brassica* and *Triticum*, therefore, nascent *Gossypium* allopolyploids appear to be genomically quiescent.

It seems important to distinguish between epigenetic phenomena associated with polyploid formation from longer-term epigenetic modifications. As noted above, these are not wholly separable, as some of the epigenetic responses to allopolyploidization in *Arabidopsis* appear evolutionarily stable. In contrast, allopolyploid *formation* in *Gossypium* does not appear to be associated with radical methylation changes, yet our current work on expression analysis of a set of fiber development-associated genes in *natural* tetraploid cotton (*Gossypium hirsutum*), which probably is 1 – 2 million years old [53], reveals a number of cases of differential expression that may be caused by epigenetic modification (unpublished data). At present, it is safe to say that epigenetic gene silencing in both rRNA genes and protein-coding genes is an intriguing and frequent phenomenon that accompanies allopolyploid speciation in plants. The genomic extent and timing of onset of epigenetic alterations are likely to vary widely among various plant groups, as are its immediate and long-term evolutionary consequences.

IV. ACTIVITY OF TRANSPOSABLE ELEMENTS IN ALLOPOLYPLOID PLANTS

Transposable elements (TEs) are mobile DNA sequences that are widespread in the genomes of eukaryotes. Traditional explanations for the ubiquity and abundance of TEs include the "selfish DNA hypothesis", which states that the abundance of TEs in a host genome results from the ability of TE sequences to replicate faster than the host, and the "junk DNA hypothesis", which holds that the bulk of TE populations are inactive and neutral to the host and hence may accumulate without fitness consequences. Neither of these hypotheses, however, address whether TEs have significance with respect to the evolution of the host. In the last decade in particular, many studies have provided evidence that TEs can be a major source of genetic diversity in plants, and that they can respond to environmental stimuli by modulating potentially adaptive genetic change [reviewed by 84,85]. Transposable elements are now implicated in a diverse array of evolutionary significant phenomena, including genomic restructuring, insertion mutagenesis conferring tissue-specific or developmental regulatory changes, and epigenetic effects.

That TEs are responsive to biotic and abiotic stresses has been well documented, particularly for several characterized "active" retrotransposons in plants. Retrotransposons, also called class I elements, transpose by a "copy and paste" model; that is, the transposition intermediate is an element-encoded transcript (mRNA) rather than the element itself. Retrotransposons encompass two classes: non-LTR retrotransposons (including long-interspersed nuclear elements, or LINEs, and short interspersed nuclear elements, or SINEs) and LTR retrotransposons. LTR retrotransposons are flanked by long terminal repeats and usually encode all of the proteins required for their transposition, i.e., they are autonomous. Nevertheless, even LTR retrotransposons are largely quiescent during normal development, indicating that their activity is tightly regulated by the host.

Recent studies in diploid plants have demonstrated that repressed LTR retrotransposons can be activated by stress. For instance, the expression of both *Tnt1* and *Tto1* from tobacco is induced by different biotic and abiotic factors that can elicit plant defenses [reviewed by 86]. These two elements were also activated by tissue culture and their expression was further enhanced by protoplast isolation. Interestingly, expression of *Tos17* of rice, which is also activated by tissue culture, is not further elevated by protoplast isolation, suggesting that the transcriptional control of *Tos17* is different from that of *Tnt1* and *Tto1* [87]. Activated retrotransposons may transpose into various genomic loci, with some preferentially inserting into low-copy genic regions, resulting in heritable genomic and phenotypic changes [88-90]. It has been further shown that different *cis*-acting regulatory sequences exist in the LTRs of retrotransposons, raising the possibility that these may be differentially regulated or responsive to different stimuli. That this is the case was recently demonstrated in tobacco, where three different *Tnt1* families were shown to be induced by different stresses [91]. This result is significant not only in that it demonstrates differential coevolution of families of retroelements with their host genomes, and a concomitant responsiveness to physiological stresses, but also provides an important perspective on the great diversity of retroelements that exist in plant genomes.

These cases of retrotransposon activation were stimulated by experimental manipulations, such as tissue culture and various chemical treatments, and hence their relevance to natural processes may be questioned. An important demonstration of retrotransposon activation in natural populations was recently described for wild barley (*Hordeum spontaneum*) from Israel [92]. The element family in question is the LTR retrotransposon *BARE-1*, which comprises up to 3% of the large (1C 5pg) barley genome [93]. *BARE-1* levels and accumulation are correlated with edaphic and microclimatic conditions over broad ecological scales in barley, but differences may be remarkable even within a single canyon, where thousands of insertions appear to distinguish wild barley populations from adjacent sites [92]. A nearly three-fold range in element copy number exists among populations on the moister, north-facing and dryer, south-facing slopes of the canyon, suggesting lability of the element to environmental differences. Indeed, statistical analysis shows a significant correlation between *BARE*-1 copy number and one or more ecological variables, such as water availability.

Remarkably, earlier studies by these authors identified ABA (abscissic acid)-response elements within the *BARE*-1 LTR promoters [94], thus reinforcing the suggestion that the proliferation of *BARE*-1 is stress related. In addition, *BARE*-1 copy number does not change unidirectionally, as intra-element recombination between the LTRs is an active process, resulting in loss of the internal domains of the element and generation of solo LTRs. This indicates that recombinational loss of the element is an important factor limiting *BARE*-1 accumulation in natural barley populations. Significantly, the geographical sites with the highest *BARE*-1 copy number, i.e., those from the most water-stressed environments, have the highest ratio of full-length to solo LTRs, suggesting again a connection between environmental stress and dynamics of the element [92,95]. These relationships between *BARE*-1 activity, water-stress, and adaptation may be more than coincidental. It may be, for example, that selection is operating on one or more aspects of genome size that we presently do not perceive of as adaptively relevant [96]. In contrast to whole-genome selection, perhaps retroelement activation has led to adaptively relevant insertions that affect drought-tolerant pathways or other ecologically relevant physiologies [95].

The wild barley example underscores the notion that TEs are not only abundant but are also a highly dynamic component of plant genomes, capable of "sensing and responding" to various stresses. Noteworthy in this respect is Barbara McClintock's prediction, made nearly twenty years ago, that interspecific hybridization may constitute a sufficiently traumatic shock to the genome that dormant TEs would become activated and could thereby restructure the genome [97]. Because uncontrolled TE activity may lead to deleterious insertions, one might expect that these mutations would be tolerated in polyploids to a greater extent than in diploids due to the buffering effects of gene duplication [64,98,99]. Although direct evidence for a cause and effect relationship between wide hybridization (including allopolyploidy) and TE activity remains elusive, several lines of evidence are consistent with McClintock's hypothesis. Most of this evidence is indirect, such as the realization that most of the inter-genic space in the maize genome [which is an ancient tetraploid 18] is occupied by accumulated retrotransposons [100-103], or the observation that in allotetraploid cotton (*Gossypium*), species-specific repeats, including apparent TEs, have colonized the alternative genome since allopolyploid formation [55-57]. Similarly indirect but supportive evidence for hybridization-induced retroelement activation stems from classic work in *Nicotiana*. As shown by Gerstel and Burns over 30 years ago [104], interspecific hybridization between *N. otophora* and *N. tabacum* induced rapid and dramatic heterochromatin expansion in one of the *N. otophora* chromosomes, whose length could be magnified by a factor of 20- to 30-fold. Although this work predated molecular analysis, it could well be an example of TE activation by allopolyploidy, as suggested by Comai [62], because heterochromatin is predominantly composed of TEs.

Perhaps a more direct form of evidence emerges from recent studies of interspecific hybrids in both animals and plants, where particular classes of retroelements were specifically monitored. For example, wide crosses in *Drosophila* may lead to P-element mobilization and hybrid dysgenesis [105]. Similarly, interspecific hybridization between the two wallaby species *Macropus eugenii* and *Wallabia bicolor* leads to a massive increase in copy number for the *KERV-1* retrotransposon [76]. Element activity in this case is accompanied by genome-wide demethylation, suggesting epigenetic repression of the element in parental species and derepression in the hybrid. Moreover, as a consequence of the runaway replication of this retrotransposon, centromeric regions of one set of parental chromosomes in the hybrid are dramatically extended, as evident by cytological analysis. Comai [62] has drawn attention to the similarity between this last observation and those from tobacco, mentioned above. From a mechanistic

standpoint, activation of quiescent TEs in nascent allopolyploids may reflect the compromise in methylation-based epigenetic control systems that occur when divergent genomes are united prior to or followed by genome doubling. As a result, dormant TEs can be released from suppression and become transcriptionally and even transpositionally activated.

In plants, recent work has shown that the copy numbers of several classes of LTR retrotransposons are significantly elevated in rice lines into which genomic DNA from wild rice (*Zizania latifolia*) has been introgressed [79]. The elements apparently are only active briefly (completely inactive by the 9th generation), presumably due to rapid silencing. Copy number increase was associated with cytosine methylation changes (both hyper- and hypomethylation), suggesting epigenetic repression of the elements in the wild-type rice genome and/or methylation-mediated repression in the introgression lines. Characterization of the flanking regions of one of the transposed elements, *Tos17*, indicates that eight of the nine isolated insertions are known-function genes (Liu *et al.*, unpublished data), showing that this element has a propensity to target genic regions and increasing the likelihood that hybridization-induced retroelement activation has functional significance. Perhaps not coincidentally, the introgression line with the highest copy number of *Tos17* was found to have a *Tos17* insertion in the coding region of an RNA-dependent RNA polymerase (*RdRP*) gene; this polymerase has been shown in diverse organisms, including plants, to be involved in posttranscriptional gene silencing (PTGS) [106], thus suggesting a possible role of PTGS in TE repression. As mentioned earlier, *Tos17* is also activated by tissue culture [107], thus implicating a common mechanism of activation resulting from either wide hybridization or tissue culture. Perhaps these stresses are "perceived" by the rice genome as a sort of genomic shock (*sensu* McClintock), leading to TE mobilization through epigenetic changes or other means.

Although few natural plant hybrids and polyploids have been experimentally evaluated for TE activation, it already is apparent that rice is not unique. Recent cDNA-AFLP analyses in first generation synthetic *Aegilops-Triticum* allotetraploids has led to the discovery of transcripts not detected in either parental diploid [83]. The novel transcripts that could be identified were from retrotransposons, indicating transcriptional activation of TEs, although no immediate transposition was detected. It seems certain that additional examples of dormant TE activation will emerge in the near future, as more model polyploid systems are studied. Thus, as suggested earlier [33], an important dimension of polyploidy may be bursts of genic and regulatory evolution mediated by transposable element insertion during polyploid formation or shortly thereafter.

V. INTERGENOMIC INTERACTION

Allopolyploid formation entails a biological reunion between two genomes that have evolved independently for thousands to millions of years. Merged together into a single nucleus in only one of the two parental cytoplasms, the newly cohabiting genomes no longer evolve independent of one another but instead are interdependent and interact through a variety of molecular genetic mechanisms. Examples include homoeologous recombination or other interactions that lead to inter-genomic exchange of chromosome segments [e.g., in tobacco 108,109], inter-genomic concerted evolution of divergent sequences [58,110,111], and inter-genic, inter-genomic recombination or gene conversion [112]. These and other mechanisms potentially lead to novel function and have adaptive significance, as suggested not only by their prevalence but by quantitative genetic studies that demonstrate significant intergenomic epistasis [113].

There now are numerous cases described of inter-genomic interactions following allopolyploid formation, exemplified by recent studies of allotetraploid cotton (*Gossypium*). Phylogenetic and sequence divergence data indicate that the two ancestral diploid genomes (African-Asian A-genome and American D-genome; n = 13) of allotetraploid cotton (American AD-genome; n = 26) last shared a common ancestor 5-10 million years ago, and that they became reunited in a common nucleus, in the A-genome cytoplasm, approximately 1-2 mya [reviewed in 53]. During the millions of years that the diploids were evolving on different continents, significant genome-size differences arose, so that modern representatives of the two progenitors of allopolyploid cotton have DNA contents that differ by nearly a factor of two (2C = 3.8 pg and 2 pg, respectively, for A- and D-genomes). Not surprisingly, the single-copy fraction is similar, indicating that the genome size differences reflect differential accumulation and/or elimination of repetitive sequences during the millions of years of independent evolution of the diploids in different hemispheres [114]. Allopolyploid species have nearly additive genome sizes with respect to the diploid progenitors (2C = 5.8 pg).

This near-additivity of allopolyploid genome sizes may be construed as suggesting that there have been few genomic interactions following merger of the two diploid genomes in the mid-Pleistocene. Recent work, however, shows that this is not the case. Zhao *et al.* [115], for example, isolated 83 non-cross hybridizing sequences corresponding to the most abundant repetitive DNAs in the *G. hirsutum* genome. Slot-blot and Southern hybridization analyses performed on both diploid and allopolyploid *Gossypium* species demonstrated that approximately three-fourths of these repetitive sequences are largely restricted to the A-genome, and that these repetitive DNAs collectively account for approximately half of the genome size differences between the two diploid progenitors of allopolyploid cotton. In contrast, only four of the repetitive DNAs were found to originate from the smaller, D-genome. The existence of genome-specific repetitive sequences permitted an evaluation of their genomic integrity following polyploidization. When 20 of the 83 repetitive families were used in fluorescent *in situ* hybridization (FISH) experiments in *allopolyploid* cotton, most families that are restricted to the A-genome at the diploid level exhibited hybridization signal not only to the A-genome chromosomes but to the D-genome chromosomes as well. Although there was considerable variation among the different repetitive

sequences in the degree of hybridization, most families exhibited an even distribution of signal among the allopolyploid chromosomes. These data show that since allopolyploid formation, colonization of alternative genomes by formerly genome-specific repetitive sequences has been common in cotton.

This phenomenon of inter-genomic "horizontal transfer" was studied further by Hanson *et al.* [55,57], who examined eight repetitive families of unknown-function and a characterized LTR-retrotransposon. Of the eight repetitive families, two were A-genome specific while the other six hybridized strongly to both genomes of the allopolyploid *G. hirsutum*. No signal was detected for any of these six repetitive sequences, however, when they were used as hybridization probes against chromosomes from the diploid D-genome. In the case of the retrotransposon, although it is exclusively A-genome specific at the FISH level, clear signals were detected on all 56 somatic chromosomes of tetraploid cotton. These data, as well as the data of Zhao *et al*.[56], established that since polyploidization there has been substantial colonization of the D-genome by A-genome repetitive elements, although not to the extent that this is reflected in DNA content estimates. Alternatively, the constant DNA content or C-value suggests that horizontal transfer of repeats has been compensated by DNA loss on a comparable scale. From a mechanistic standpoint, several processes of inter-genomic interaction are implicated, including DNA "overwriting" through gene conversion, intergenomic recombination and exchange, and particularly, the activity of TEs. In this respect it is noteworthy that, apart from the characterized retrotransposon, database searches of the 83 cotton repetitive DNAs yielded matches only to known transposons [56].

The intergenomic interactions described for *Gossypium* allopolyploids likely are common in other plants. Thus, the phenomenon of interlocus concerted evolution among the constituent genomes of allopolyploids, as originally described for *Gossypium* [58], has been reported in a number of other plant systems [111,116-121]. Similarly, inter-genomic colonization by repetitive sequences from alternative parental diploid genomes has been reported in wild allotetraploid wheat (*Triticum dicoccoides*) [122]. In this case genomic *in situ* hybridization (GISH) analyses demonstrated that repetitive sequences from the ancestor of the B genome have spread throughout the A and B chromosome sets in tetraploid wheat, and to a lesser extent the phenomenon has been reciprocal; i.e., there has been some movement of sequences or gene conversion in the alternative direction. Not all allopolyploids, however, are equally susceptible to processes of intergenomic interaction; in *N. tabacum*, for example, GISH investigations [123] reveal little evidence for inter-genomic colonization of the type observed in cotton and wheat, despite the fact that rDNA arrays are subject to concerted evolution. Because there still are relatively few studies on "intergenomic horizontal transfer" following polyploidization it is difficult at this time to assess the prevalence and significance of the phenomenon, as well as the factors that are responsible for the variation among plants in the degree to which each responsible mechanism operates. Insights into these questions are anticipated in the coming years, as the tools of molecular cytogenetics and phylogenetics continue to converge on the problem.

VI. MECHANISMS RESPONSIBLE FOR NON-MENDELIAN GENETIC CHANGE

From the foregoing account it is evident that hybridization and polyploidy can trigger a number of non-Mendelian genetic responses. Given the diversity of sequences involved and their differential response dynamics, it seems probable that these non-Mendelian changes are mediated by at least several different molecular genetic mechanisms. Although in nearly all cases relatively few details are understood at the molecular genetic level, the responsible processes may include intergenomic recombination [108,109], gene conversion or other homologous genetic interactions [40,44,46,47,55-58,110-112], transposable element activity [55-57,79,83,91,92], epigenetic changes [40,44,46,47,61,62,80], and a suite of more mysterious mechanisms. These mechanisms were each introduced and described briefly in the relevant sections, above. Here we note that heritable DNA methylation modifications have been detected in all cases where rapid genomic changes are observed [35,40,44,46,47,124] and appear to be minimized in systems like *Gossypium* where rapid genome change does not appear to accompany polyploid formation [48]. The importance of epigenetic mechanisms is also underscored by the studies on *Arabidopsis* allopolyploids [61,80], where cytosine methylation was found to be the underlying cause of the observed gene silencing. Thus, in nascent plant allopolyploids both rapid genomic changes and gene silencing often are caused by an epigenetic and hence potentially reversible mechanism; as noted above, the evolutionary significance of this realization is not clear, but may be related to the adaptive success of polyploidy in plants.

Among the more remarkable and mysterious phenomena associated with polyploidy is irreversible DNA loss, as described in *Brassica* and wheat, which apparently is not associated with DNA methylation or other epigenetic phenomena. Thus far, virtually nothing is known about the molecular mechanisms that mediate this response. Ozkan *et al.* [46] ruled out several possibilities as the cause of sequence elimination, including colchicine treatment, intergenomic recombination, DNA methylation, cytoplasmic effects and TE mobilization. Shaked *et al.* [47] suggested several possibilities, including gene conversion between the homoeologous alleles, site-specific recombination, and crossing over between direct repeats that flank the eliminated sequences, followed by loss of the excised circle. As suggested by Shaked *et al.* [47], insight into the relevance of these speculations may emerge from characterizing the deleted regions, their flanking sequences, and the boundaries of the eliminated DNA segments.

As noted in section **II**, not all newly formed allopolyploid plants appear equally susceptible to the kinds of genomic instabilities that characterize wheat and *Brassica*. Examples of relative genomic quiescence include *Gossypium* (Malvaceae) and *Spartina* (Poaceae), which have rather

different life-histories and phylogenetic relationships. This observation suggests that as more plant groups are studied a high level of variance in genomic responses to allopolyploid formation will continue to be revealed, both with respect to magnitude and mechanisms. The reasons for this difference among plant groups are even more puzzling and poorly understood than are the mechanisms responsible for polyploidy-induced sequence elimination. In *Gossypium* there does not appear to be a relationship between genetic or cytogenetic distance and non-Mendelian genomic aberrations, and as discussed by Liu *et al.* [48] this same disconnect exists in other plant polyploids between multivalent formation or other manifestations of structural differentiation and rapid genomic change as revealed through Southern hybridization or AFLP analysis.

Given that the degree of genetic or chromosomal divergence between the parents has relatively little predictive value with respect to genomic aberrations in the nascent allopolyploid, what might be the most important determinants? It may be that specific DNA sequences and/or proteins involved in non-homologous chromosome interactions are responding differently in the various allopolyploid systems. Comai [62], for example, recently speculated that mismatch-repair systems in plants might be compromised when divergent genomes are united. He suggested that high levels of genomic mismatch might titrate available pools of mismatch repair enzymes, and thus non-homologous interactions (such as ectopic recombination between TEs) would go uncorrected and be revealed as genomic instability. Perhaps these and other proteins involved mediate the level of non-homologous chromosome interaction, and do so variably among different plant allopolyploids. An additional clue may have been offered by the recent demonstration of species-specific differences in DNA double-strand break repair pathways in plants [125]. It may be that differential activities (or different inducibility thereof) in one or more enzymatic systems responsible for DNA repair are responsible for the different responses to allopolyploidy. Finally, epigenetic systems such as DNA methylation/demethylation [65] may mediate genomic interactions through effects on ectopic recombination [126,127], and as discussed in section **V** also may be related to activation of quiescient mobile elements [64,76,99,126]; to the extent that these mechanisms differ among allopolyploids, we might therefore expect variation in levels of genomic instability.

VII. IMPLICATIONS FOR POLYPLOID EVOLUTION

Because it entails the sudden merger of two differentiated genomes, allopolyploidy is a remarkable and dramatic form of speciation. As shown in this review, myriad genetic and genomic adjustments may accompany this "shock" as the progenitor genomes, long accustomed to diploidy, respond to the requirements of the new cellular environment. These many adjustments need to accommodate the constraints of essential cellular processes [128] and must do so in an ecological context that may or may not be novel with respect to those of the parental diploids. Thus, allopolyploid speciation entails numerous and varied molecular evolutionary alterations that are required to coordinately regulate the newly formed allopolyploid genome in guiding growth and development and reproduction. Our glimpse into non-Mendelian phenomena, as discussed in the present review, provides a small portal through which we might begin to appreciate the nature and scope of molecular evolutionary change involved in allopolyploid speciation. Although our view at present is mostly obscured by ignorance, it is of interest to speculate about the adaptive relevance of non-Mendelian responses to polyploidization.

A. Polyploidy-Induced Sequence Elimination May Promote Chromosomal Diploidization

Because allopolyploidy usually entails the merger of genomes that are sufficiently homologous that homoeologous chromosomes may pair during meiosis, the earliest generations in allopolyploid formation must experience strong selection for exclusive bivalent pairing. This is evidenced in modern allopolyploids by the common observation that homoeologous pairing is much lower than one might expect from chromosome associations formed in hybrids between the extant parental diploids. One of the best examples of this phenomenon is wheat, where both tetraploids and hexaploids exhibit exclusive bivalent formation. Both historical cytogenetic analyses and modern comparative genomic data show that the homoeologous chromosomes of the different diploid species of *Triticum* and *Aegilops* are structurally similar and colinear [e.g., 23,42]. Classic genetic studies demonstrated decades ago that two dominant genes, *Ph1*, and to a lesser extent *Ph2*, are responsible for the exclusive diploid-like meiotic pairing behavior in polyploid wheat [129-131]. Recent molecular cytogenetic data suggest that the function of *Ph1* is to act as a 'local editor' to ensure specific centromere association between homologous chromosomes [132]. However, based on the observation that *Ph1*-like genes are not found in allopolyploid species of *Aegilops*, which also exhibit diploid-like meiotic behavior and, the fact that hexaploid wheat plants deficient for *Ph1* exhibit low levels of homoeologous pairing, Feldman and colleagues have argued that factors other than the *Ph* genic system are involved in meiotic pairing in polyploid wheat [44,46].

Feldman *et al.* [44] proposed that rapid and non-random sequence elimination, as described in this review, may provide an alternative mechanism for enforcing exclusive homologous chromosome pairing. It is envisioned that elimination of low-copy, non-coding sequences from one of the two genomes of tetraploid wheat and from two of the three genomes of hexaploid wheat would instantly convert the targeted sequences from conserved to genome-specific, in the process perhaps forming a major class of homologue-specific sequences. Sequence elimination thus would augment differentiation between homoeologous chromosomes in a single step, and this polyploidy-generated *de novo* difference could presumably serve as a physical basis for exclusive homologous pairing.

The suggestion that non-random sequence elimination is essential to the initial establishment and stabilization of nascent allopolyploids [44] has gained indirect support from the recent report [46] that sequence elimination is both non-

random and highly reproducible and that sequence elimination occurs early, during both hybridization and allopolyploidization. Particularly interesting is the observation that sequence elimination was more prominent and occurred earlier in synthetic allopolyploids that have a natural counterpart than in those that do not exist in nature. This result, as well as the positive correlation between sequence-elimination frequency and fertility [46], raise the intriguing possibility that allopolyploid speciation will be evolutionarily promoted in species groups that evolved a predisposition for the (at present, unknown) mechanisms of molecular interactions that underlie sequence elimination. Presumably, these mechanisms will have originally evolved for other purposes.

Despite the attractiveness of this speculation, no data demonstrate a direct connection between sequence elimination and homologous chromosome pairing. Indeed, the possibility exists that polyploidy-induced sequence elimination has *no adaptive significance*, and that it constitutes a dramatic but ultimately unimportant side-effect of allopolyploid formation, resulting from unavoidable molecular genetic interactions that arise from the union of two differentiated genomes. Presumably additional clues as to the function, if any, of polyploidy-induced sequence elimination will emerge as more is learned about the responsible mechanism(s), and as it is investigated in additional systems.

B. Do Non-Mendelian Changes Contribute to Diversification and Adaptation?

A widely accepted explanation for the success of polyploidy in plants is that polyploid formation is accompanied by an instantaneous doubling of the raw material necessary for adaptation to changing environments or new ecological niches [6,8,29,31,32,133]. Although this proposition is reasonable and has been discussed for over 50 years, to date a direct connection between genome doubling and adaptation has not been shown, but instead has been inferred from circumstantial or correlative evidence. Given the technological advances of the last decade, whereby it is now possible to isolate and study the function of homoeologues in polyploids, the exciting opportunity is presented to discover functional diversification among duplicated genes, perhaps establish an actual cause and effect relationship between functional diversification and either physiological or morphological innovation, and thereby possibly demonstrate polyploidy-facilitated adaptation.

The existence of a suite of non-Mendelian mechanisms in polyploid evolution adds a new wrinkle to the long-standing speculations about polyploidy and adaptation. Perhaps rapid structural or epigenetic change in nascent allopolyploids generates functional divergence or novel metabolic interaction, which conceivably could translate into phenotypic novelty visible to natural selection. Also, epigenetic modifications may lead to permanent genetic changes via the creation of mutational "hot-spots"[134]. Various hints of these connections have emerged from recent studies using model plant polyploids. In the synthetic *Arabidopsis* allopolyploids discussed earlier in this review, phenotypic mutants affecting a large number of traits were recovered [61]. Although these variants were not subjected to analyses of reproductive success in experimental or natural settings, the mere fact that such variation was generated, at least in part, by non-Mendelian genetic phenomena associated with polyploidization raises the possibility of adaptive significance. In this regard the recent demonstration of *de novo* phenotypic variation for flowering time in newly synthesized *Brassica* allopolyploids [135] is noteworthy, as phenological aspects of the reproductive cycle are undoubtedly subject to intense selection in many natural settings.

Additional clues into the potential adaptive significance of polyploidy have emerged from recent work involving allopolyploid cotton. As discussed earlier, there is no evidence for either rapid genomic changes [48] or long-term intergenomic interactions between low-copy, genic sequences [49,50] in this plant system. However, indirect evidence for novel genetic interactions following polyploidy has emerged from studies of QTL affecting the quality and quantity of fibers [136], and by comparative morphological analysis of fiber development in diploid and allopolyploid *Gossypium* species [137]. These studies and others suggest that expression novelty, perhaps mediated by either epigenetic or genetic alterations, has been rendered possible by the union of divergent genomes into one nucleus and cytoplasm.

In addition to expression novelty *per se*, one aspect of polyploids that may have contributed to evolutionary success is genetic diploidization, either through mutational or epigenetic means. Genetic diploidization in the present context refers to the phenomenon whereby expression levels in a polyploid are reduced to those of one of the diploid progenitors, by either gene silencing or dosage compensation [29,33]. Heretofore it has been assumed that this process is achieved on an evolutionary timescale by slow mutational processes. As discussed in the present review, however, both structural changes and epigenetic gene silencing may actually accompany polyploid formation *from the outset* (see sections **II** and **III**). This increases the likelihood that some of the silencing events are physiologically and adaptively significant, in ways we have not as yet perceived.

If gene silencing events associated with nascent allopolyploids are relevant to evolutionary "stabilization" or actual "adaptation", it is unclear which of several possible epigenetic mechanisms may be involved. Homology-dependent gene silencing (HDGS) could induce gene silencing, but as was pointed out by Matzke *et al.* [64], there is no evidence that HDGS has contributed to genetic diploidization in polyploids. Instead, a novel type of epigenetic silencing that occurs solely as a consequence of change in ploidy level has been demonstrated in *Arabidopsis* [138]. Comai [62] proposed that several types of epigenetic processes may have played a role in allopolyploidy-induced rapid epigenetic silencing and phenotypic instability, including mechanisms such as paramutation [139] that are based on homologous (allelic or ectopic) interaction.

In some polyploids mobilization of quiescent TEs and an elevated tolerance to their activity may bear directly on adaptation [95]. As discussed in section **V**, compelling data

have accumulated to indicate that TEs are a significant source of genetic diversity [84,85]. The finding that TEs may be associated with genic regulatory regions further suggests an important role for TEs in gene evolution [140-144]. A case in point is the rice disease-resistance multigene family *Xa21*, into which 15 transposon-like elements have been inserted [145]. While 13 of the elements transposed into non-coding regions (including the 5' upstream region and introns), two elements inserted into coding regions and created ORFs that encode truncated proteins with potentially altered biochemical functions due to changes in hydrophobicity. It was suggested by the authors that TE insertions may have generated disease-resistance genes with novel properties [145]. In addition to this example, many TEs have strong promoter sequences [e.g., in LTRs of retrotransposons 146], suggesting that element insertion may lead to altered expression patterns. In addition, the possibility exists for TE-mediated ectopic recombination to produce swapping of promoter regions and hence novel expression patterns. Thus, we are probably just beginning to appreciate the numerous avenues by which TEs may generate genetic novelty, and given the potential activation caused by genome merger, this may especially be the case for polyploids [33,99].

A final and potentially important role for TEs may be element-mediated, rapid, *de novo* karyotypic evolution, which may facilitate reproductive isolation and genome stabilization. As demonstrated in hybrid wallabies [76], retrotransposon-mediated massive heterochromatin expansion caused dramatic genomic remodeling in the hybrid genome and hence rapid differentiation from the parental genomes. Although this phenomenon remains relatively unexplored in other organisms [78], and is mechanistically mysterious at present, TE activity and the associated genomic alterations may be central to the process. Hence, explorations of the myriad effects of hybridization-induced TE mobilization are likely to continue to yield new insights in the coming years.

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