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Genome evolution in polyploids

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Abstract

Polyploidy is a prominent process in plants and has been significant in the evolutionary history of vertebrates and other eukaryotes. In plants, interdisciplinary approaches combining phylogenetic and molecular genetic perspectives have enhanced our awareness of the myriad genetic interactions made possible by polyploidy. Here, processes and mechanisms of gene and genome evolution in polyploids are reviewed. Genes duplicated by polyploidy may retain their original or similar function, undergo diversification in protein function or regulation, or one copy may become silenced through mutational or epigenetic means. Duplicated genes also may interact through inter-locus recombination, gene conversion, or concerted evolution. Recent experiments have illuminated important processes in polyploids that operate above the organizational level of duplicated genes. These include inter-genomic chromosomal exchanges, saltational, non-Mendelian genomic evolution in nascent polyploids, inter-genomic invasion, and cytonuclear stabilization. Notwithstanding many recent insights, much remains to be learned about many aspects of polyploid evolution, including: the role of transposable elements in structural and regulatory gene evolution; processes and significance of epigenetic silencing; underlying controls of chromosome pairing; mechanisms and functional significance of rapid genome changes; cytonuclear accommodation; and coordination of regulatory factors contributed by two, sometimes divergent progenitor genomes. Continued application of molecular genetic approaches to questions of polyploid genome evolution holds promise for producing lasting insight into processes by which novel genotypes are generated and ultimately into how polyploidy facilitates evolution and adaptation.

Introduction

Recent years have witnessed an explosion in our understanding of the organization and structure of eukaryotic genomes. One generalization that has been confirmed and extended by the data emerging from the global thrust in genome sequencing and mapping is that most 'single-copy' genes belong to larger gene families, even in putatively diploid organisms. Accompanying this enhanced appreciation of genomic redundancy has been an effort to understand its functional significance and its genesis. This latter motivation has invigorated an interest in evolutionary aspects of the problem, which in turn has led to a renewed awareness of the importance of polyploidy as the primary mechanism for generating genomic redundancy. Although polyploidy has long been recognized as a prominent speciation process in plants [70, 112, 130, 188, 191, 200, 201], recent investigations have demonstrated that genome doubling has been significant in the evolution of all vertebrates and in many other eukaryotes [125, 153, 157, 180, 197, 233]. Given this preeminence, it is worth asking what we know about the process from a molecular evolutionary genetic point of view. Because polyploid evolution appears to be more of an ongoing, dynamic process in plants than in most other eukaryotes, it is not surprising that most empirical studies on polyploidy focus on various model angiosperm systems, as will this review.

It is difficult to overstate the importance of genome doubling in the evolutionary history of flowering 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 certainly 50% and might be more than 70% [70, 117, 130, 200, 201]. As

a consequence, there has been a long history of interest in various aspects of polyploidy in plants, including classification of the various types of polyploids (e.g., autopolyploid, genomic allopolyploid, segmental allopolyploid), mode and frequency of formation, potential evolutionary significance, and correlations with life-history attributes and ecological parameters. These subjects have been thoroughly reviewed [70, 78, 96, 97, 112, 114, 117, 130, 162, 188, 190, 191, 199–201, 206] so they need not be discussed here. Instead, the present focus will be on gene and genome evolution in polyploids. In particular, recent studies have led to novel insights and perspectives into the molecular evolutionary genetics of polyploid formation, stabilization and subsequent evolution; it is these subjects that will be highlighted in the present review.

Before proceeding it is necessary to first consider what it meant by the term polyploidy. Because genome doubling via autopolyploidy or allopolyploidy has been continuing since angiosperms first appeared definitively in the Cretaceous and because this remains an active, ongoing process, many angiosperm genomes have experienced several cycles of polyploidization at various times in the past. The more ancient of these past genome doubling events may be difficult to discern, due to potentially rapid evolutionary restoration of diploid-like chromosomal behavior and/or other evolutionary changes following polyploidization. Most angiosperms thus are appropriately considered to have 'paleopolyploid' genomes, which may be revealed as such through comparative mapping or other approaches [20, 60, 63, 108, 109, 141, 164, 179, 195]. Throughout the angiosperms, more recent polyploidization events have been superimposed on these more ancient genome doubling events, followed often by additional rounds of 'diploidization' and evolutionary divergence among previously doubled genomic sequences. This cyclical process of duplication and divergence leads to a concept of the modern angiosperm genome as one characterized by a series of nested duplications of varying antiquity, only some of which descend to the present relatively unscathed by evolutionary disruptions such as loss of duplicated sequences, chromosomal repatterning, or other aspects of 'diploidization'. Only the most recent genome duplications are likely to be classically recognized as constituting 'polyploid' speciation events. Examples of such obvious polyploids abound, and include many of the world's most important agricultural commodities [86], such as wheat, soybean, potatoes, sugarcane and cotton. Most of these same species, as well as countless others, experienced more ancient cycles of genome duplication, as is evident in the genomes of *Brassica* [108, 109], cotton [164], soybean [179], and many important cereals [12, 60, 63, 102, 140].

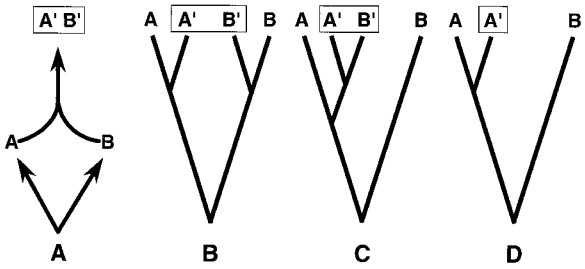

Figure 1. Expected consequences of polyploidy on gene and genome evolution. Divergence between two diploids (A and B) followed by hybridization and chromosome doubling yields an allopolyploid with a genome (A′B′) that is expected to be additive with respect to its progenitors (panel A). If the resulting duplicated genes or other genomic sequences evolve independently, the two homoeologous copies in the allopolyploid (A′ and B′, boxed) will not be phylogenetically sister to each other. Instead, each copy will be sister to the orthologous copy in one of the two parental diploids (panel B). Violations of independence may take many forms, such as inter-locus gene conversion (panel C) or loss of one of the two homoeologues (panel D).

Some of what is known about polyploid genome evolution stems from the analytical perspective provided by genome doubling itself. Irrespective of whether a polyploidization event involved the merger of two fully differentiated genomes (allopolyploidy), a simple doubling of a single genome (autopolyploidy), or something in between, all or nearly all genes and other genomic sequences will have become duplicated in the process. In allopolyploids, these duplicated (homoeologous) copies of a particular chromosome, chromosome segment, or gene were contributed by different donor taxa at the time of polyploid formation (Figure 1, panel A). Thus, their descendant sequences in the polyploid are expected to be phylogenetically sister to their counterparts (orthologues) from the respective diploids rather than to each other (Figure 1, panel B). In addition, the new polyploid genome is expected to be additive with respect to its diploid progenitors. Rejection of this 'null hypothesis' (Figure 1, panel C) may reflect a number of causes [223], including some that are germane to polyploid genome evolution, such as concerted evolution of genes in different genomes or other interactions among homoeologous sequences.

While the scenario modeled in Figure 1 fails to accommodate some important aspects of gene and genome evolution in polyploids, such as changes in

duplicate gene expression, divergence in function, or chromosomal repatterning, it underscores the importance of a sound organismal phylogenetic framework for making inferences regarding molecular evolutionary events. Moreover, it highlights the necessity of having comparisons be appropriate, that is, among strictly orthologous genes and their two homoeologous descendants [34], rather than among some mixture of paralogous and orthologous sequences. Because these dual requirements of firm phylogenetic underpinnings and sound inferences of orthology are difficult to meet in many polyploid groups of plants, much of the experimental work on gene and genome evolution in polyploids involves model systems in just a few genera, i.e. *Brassica*, *Gossypium*, *Nicotiana*, *Tragopogon*, and *Triticum-Aegilops*. Each of these groups is characterized by relatively recent polyploidy, the existence of extant diploid progenitors or reasonable models thereof, and usually a well-developed set of genetic and cytogenetic tools that are important for inferring orthology or making inferences about genome evolution. Notwithstanding the importance of these model systems, insights into one or more aspects of polyploid genome evolution have emerged from studies of many different taxonomic groups, including older polyploids such as *Zea* [60, 84, 161] and many pteridophytes [59, 82, 155].

Because polyploidization is both an ancient and an ongoing process, it may be useful to consider the molecular evolutionary events that characterize the earliest stages of polyploid formation separately from those that are responsible for longer-term genomic changes. An allopolyploid speciation event, for example, entails the merger of two, often differentiated genomes into a common nucleus in only one of the two parental cytoplasms. Though we still know relatively little about many if not most details of the process, the initial stages in this biological reunion evidently are molded by an array of molecular genetic mechanisms and processes that collectively lead to polyploid stabilization. This suite of mechanisms and processes may be rather different, at least in part, from those responsible for longer-term gene and genome evolution in polyploids. The latter classically are thought of as the mechanisms leading to chromosomal and genic diploidization, as well as to functional diversification among duplicated genes. Recent insights into both of these aspects, i.e. the initial stages of polyploid formation and longer-term genic and genomic evolution, will be discussed here.

Evolution of duplicated genes

Given the prevalence of polyploidy in plants it is not surprising that many have addressed the potential evolutionary significance of its most obvious genetic consequence, namely, gene duplication [114, 117, 144, 200, 202, 203]. That genome-wide genetic redundancy may lead to new evolutionary opportunity was underscored by S.G. Stephens nearly half a century ago [203, p. 249], when he commented that: '…One might expect…that a mechanism in which new functions could be added and the old ones retained would have considerable selective advantage…the only likely manner of achieving this "improvement" would be by increasing the number of genetic loci….' In this remark Stephens highlighted a key aspect of the evolution of polyploids, namely, that there may be divergence in function among duplicated genes following polyploidization. This single and important concept has become widely embraced as an explanation for the evolutionary success of polyploids. Duplication leads to relaxation of selection on one gene copy, allowing divergence between the duplicated genes and the acquisition of new function [51, 92, 107, 119, 144, 147, 148, 156]. This divergence in function may be manifested in myriad evolutionarily significant ways, viz., greater biochemical and physiological flexibility, enhanced environmental adaptability, or the evolution of novel physiologies or morphologies [5, 7, 33, 70, 114, 117, 170, 200].

We might ask how much we actually know about this process that is so central to polyploid speciation theory. Polyploidy certainly results in genome-wide gene duplication, and it is also clear that plants contain numerous multigene families, the members of which often show functional diversification. Nonetheless, there are relatively few examples where 'divergence after duplication' has convincingly been demonstrated. In large part this undoubtedly reflects the stringent requirements for experimental verification of functional diversification associated with polyploidization: that two duplicated genes are truly homoeologous (as opposed to paralogous); that functional diversification has occurred; and that differentiation in function occurred subsequent to polyploid formation and not earlier at the diploid level. From an experimental standpoint, orthologous and homoeologous relationships among candidate genes are most likely to be verifiable in recent polyploids, such as in the model genera discussed above. In these relatively young polyploids, however, sufficient time may

not have elapsed for measurable functional diversification to have taken place between genes duplicated via polyploidy, at least for some unknown portion of the genome. The study of older polyploids may be more promising in this respect, but in these cases one is likely to have reduced confidence in inferences of homology and of diploid parentage. When viewed in this context, the relatively limited number of empirical demonstrations of divergence after duplication is not really surprising. Moreover, many additional examples are likely to emerge in the near future, as the tools of genome mapping and functional analysis become more widely applied to evolutionary questions.

Although divergence in function is widely thought of as a common and perhaps the most provocative consequence of gene duplication, it is not the only possible outcome. This has been recognized for at least 50 years. Stephens [203, p. 250], for example, commented that '...In newly formed amphidiploids numerous loci must be duplicated and any subsequent disappearance of duplicate functions must be attributed to (a) loss or inactivation of one of the loci or (b) divergence in function.' Gene silencing is thus highlighted as a second evolutionary consequence of genome doubling, although in Stephens' time the processes responsible for 'loss or inactivation' (e.g. pseudogenization and epigenetic modification, discussed below) were even less clear than they are today. In some respects the fate of duplicated genes may be viewed as a race between survival and decay, as mutation in coding and regulatory regions leads both to differences in protein expression or function (diversification) as well as pseudogene formation (silencing).

As might be expected from both the prevalence of polyploidy and its perceived significance, the subject of gene duplication has long attracted the interest of population geneticists [28, 106, 129, 142, 146, 148, 216, 217, 220]. This work, which traces back at least as far as Haldane and Fisher in the 1930s [53, 73], models one or more aspects of gene diversification or silencing using as input parameters factors such as mutation rates, fitness differences among alleles at duplicated loci, and effective population sizes. Of particular relevance here is the relative likelihood of functional diversification versus gene silencing. Most modeling studies suggest that few duplicated genes escape the accumulation of deleterious mutations, so that pseudogene formation occurs at a rate often estimated to be an order of magnitude or more higher than that of functional divergence. Walsh [217], for example, estimated that under a variety of reasonable population sizes and mutation rates, the likelihood of evolving a new gene function was low, unless fitness differentials and/or population sizes were large. In this light, empirical studies on rates of gene silencing are noteworthy, in that these rates often are much lower than that predicted by population genetic models [50, 142, 156].

The realization that rates of gene silencing are much lower than those predicted by population genetic models is potentially illuminating, in that it necessitates a biological explanation. This in turn evokes the potential significance of a third possible fate of genes duplicated by polyploidy, namely, long-term maintenance of similar if not identical function [93, 142, 156, 216]. A common observation is that both copies of a duplicated gene often persist in a functional state for long periods of evolutionary time [33, 45, 50, 93, 186]. Several possible explanations for this observation recently have been forwarded [32, 62, 67, 93, 142, 156, 216], involving the interplay among natural selection, the nature of genetic redundancy, and myriad aspects of protein interactions and pathways.

The foregoing suggests that there are three primary possibilities for the evolutionary fate of duplicated genes: (1) functional diversification; (2) decay, through mutation, leading to 'silencing' of one of the two duplicated copies; and (3) retention of original or similar function. Empirical evidence that bears on each of these possibilities will be discussed below, as will the responsible mechanisms, where known. To these three primary fates will be added a fourth prospect, namely, interaction among duplicated genes. Several phenomena are included in this final category, conceptually intertwined by the loss of independence among the duplicated copies that results from interaction between homoeologues (cf. Figure 1).

Acquisition of new function

As mentioned above, as yet there are few compelling examples of functional diversification that can convincingly be traced to differentiation of specific pairs of genes that originated via polyploidy. To some extent, however, this issue is time-scale-dependent. Because most genes exist as members of multigene families and because most if not all angiosperms lineages have an ancient if not a recent polyploid ancestry, it is clear that, ultimately, genome doubling events must have generated a significant portion of the existing genetic redundancy in a given genome. In conjunction

with the widespread observation of functional diversification among members of plant nuclear gene families [29], it becomes axiomatic, therefore, that there has been 'divergence after duplication'. In these cases of long-term diversification, however, specific homology relationships among the genes involved generally are obscure, thereby limiting understanding of both the nature of selection and of the specific molecular events responsible for the acquisition of novel function.

Notwithstanding these limitations, comparative analyses of amino acid and nucleotide sequences have provided clues into the process of functional diversification. For example, purifying selection apparently has operated on both members of 17 duplicate gene pairs in tetraploid frogs [93]. This suggests that the traditional view of divergence after duplication [144, 145], whereby one copy is released from functional constraint and through mutation either randomly decays or acquires new function, ultimately to '... emerge triumphant as a new gene locus' [145, p. 261], does not universally apply. In some cases, functional differentiation among duplicated genes has been shown to be accompanied by accelerated rates of amino acid substitution [119, 147, 148]. This excess of replacement substitutions has been interpreted to implicate positive selection as an important force in shaping functional diversification.

Similar ideas have been brought forward for multigene families in plants. Accelerated nonsynonymous rates of substitution have been observed, for example, in members of the chalcone synthase (CHS) gene family in *Petunia* and *Ipomoea*, and these have been interpreted as possibly reflecting functional divergence [41, 94]. In a few cases, sequence data have been combined with studies of enzyme substrate specificity or other aspects of function. Particularly notable in this respect is the analysis of differentiation among chalcone synthase gene family members in the Asteraceae [83]. The novel proteins involved, which differ from other *Chs* gene family members ontogenetically and catalytically and are only 70% similar to other *Chs* genes at the amino acid level, are inferred to have arisen following gene duplication prior to diversification of the Asteraceae.

Some of the more compelling examples of functional diversification following polyploidy involve maize. The maize genome has long been known to harbor numerous duplicate factors [165], which, using molecular genetic approaches, have more recently been mapped into parallel or duplicated linkage groups [84, 231]. Sequence analysis of 14 pairs of duplicated genes located on these parallel linkage groups confirms the paleotetraploid nature of the maize genome [60]. Based on the amount of sequence divergence, the tetraploidization event is suggested to have occurred about 11.4 million years ago [60].

Included among the loci implicated to have been duplicated via polyploidy are both regulatory and structural genes. A comparison of these highlights an important aspect of polyploidization, namely, its impact not only on structural genes, but on regulatory genes that control developmental pathways and networks. A case in point involves the pair of duplicated genes *R* and *B*, which map to duplicated linkage groups on chromosomes 10 and 2, respectively, and which encode helix-loop-helix transcriptional activators that regulate anthocyanin biosynthesis. The two genes differentially regulate tissue-specific patterns of purple pigmentation in various maize tissues. Although relative rate tests [60] suggest that the two genes are evolving at equivalent rates (only exons were included in the analysis), the rate of replacement substitution between *R* and *B* is highest among all locus pairs studied. This suggests either that positive selection has been operating to diversify function, or that there has been limited functional constraint on one or both gene copies. Additional molecular genetic analyses verify that some portions of these genes, and their homologues in other grasses, are evolving rapidly [90, 161]. This pattern of rapid evolution is not restricted to *R* and *B*. Indeed, among the 14 locus pairs studied, the mean ratio of replacement to synonymous substitutions is 50% higher in 'regulatory' than in 'structural' genes [159]. Perhaps even more noteworthy is the fact that in maize, *R* and *B* differ primarily in their pattern of expression rather than in protein function. This indicates that not only is divergence in the coding region of regulatory genes potentially important, but that the most significant evolutionary events have been those that regulate *R* and *B*, including molecular changes in *cis*- and possibly *trans*-acting factors.

These examples from maize are instructive in that they provide insights into the nature of molecular evolutionary diversification that might be expected following polyploid-induced gene duplication. In addition, they underscore the possibility that the most profound changes are likely to be novel patterns of gene regulation rather than diversification in protein function per se. Divergence among duplicated genes has long been suggested to occur at the regulatory level [2, 51, 202]; the examples from maize and elsewhere are providing glimpses into the responsi-

ble molecular evolutionary events. From a broader perspective, analyses such as those described in this section highlight the evolutionary significance of regulatory gene diversification, as emphasized in several recent reviews [6, 38, 39, 159, 160]. It is tempting to speculate that much of the evolutionary success of polyploidy in angiosperms, which entails a saltatory doubling of regulatory factors, is tied directly to regulatory gene evolution, both at the protein level and through differential regulation of the regulatory genes themselves.

Gene silencing

As an alternative to maintenance of duplicated genes and divergence in function, 'gene silencing' might occur, leading to loss of duplicate gene expression. This process has long been recognized as an evolutionary possibility [144, 145, 203], but it was not until isozymes became widely employed as genetic markers that the phenomenon could readily be monitored. Because enzymes encoded by duplicated genes were often easily separated electrophoretically, their *in vitro* expression patterns provided direct evaluations of the extent of gene silencing [1, 59, 65, 66, 82, 170, 187, 232]. In some cases, inferences of gene silencing were bolstered by comparisons to diploid progenitors and/or genetic analyses, to rule out, for example, co-migration of duplicated enzymes [e.g. 232]. These analyses also demonstrated that loss of expression might occur for either member of a duplicate gene pair, as is the case for the well-known example of gene silencing represented by the doubled leucine aminopeptidase loci in tetraploid *Chenopodium* [232].

Among the several generalizations to emerge from several decades of enzyme electrophoretic studies is that in young polyploids both copies of a duplicated gene usually retain expression [reviewed in 33, 66, 186]. This is true, for example, in polyploid *Tragopogon* from the western United States [170], which first appeared in the early 1900s after the introduction of the progenitor diploids from the Old World [143, 150], and in hexaploid wheat, which first formed perhaps 8000 years ago [47] and which still expresses the majority of its initially triplicated isozyme genes [79–81]. In older polyploids, however, loss of duplicate gene expression is common in both plants and animals. A widely cited example involves tetraploid catostomid fish, where it is suggested that half of all duplicated allozyme loci have been silenced in the 50 million years since polyploidization [49, 118]. About 25% of the duplicated single-copy fraction has been eliminated since polyploidy doubled the ancestral genome of modern soybeans [240]. In ferns, allozyme expression patterns indicative of the diploid condition [66] often are observed, even in species with chromosome numbers suggestive of polyploidy [reviewed in 82, 187]. Most evidence suggests that this 'genetic diploidy' reflects extensive and perhaps repeated episodes of gene silencing after a polyploidization event or events. A glimpse into the diploidization process is offered by the report of progressive loss of duplicated phosphoglucose isomerase activity in tetraploid (relative to its congeners) *Pellaea rufa*; different individuals express varying levels of one of the duplicated *Pgi* genes, culminating in plants with a completely diploidized PGI phenotype [59].

As reflected in the foregoing discussion, most empirical evidence bearing on the phenomenon of loss of duplicate gene expression derives from isozyme loci, because historically these were the simplest readily scored genetic markers available. It has been noted, however, that enzyme-encoding loci might respond differently to polyploidization than other classes of genes [45]. This is apparent in both hexaploid and tetraploid wheats, where the previously noted retention of enzyme multiplicity contrasts with a 'massive and nonrandom' diploidization of endosperm proteins [45, 56]. Hexaploid wheat lines, which have three genomes (A, B, and D), variably express high-molecular-weight glutenin bands encoded by only two of the three genomes (B and D), indicating silencing of the A-genome homoeologues. In tetraploid wheat (AB genome), many wild accessions display an additive profile of high-molecular-weight glutenin bands, but diploidization, through loss of one or more A-genome bands, is common in cultivated lines. Feldman *et al*. [45] highlighted the contrast between diploidization of seed-storage proteins and a lack thereof for enzyme-encoding loci, and proposed that this difference reflects selection for efficient production of appropriate levels of seed storage proteins. The genomic effects of selection are processes like those that lead to gliadin and glutenin gene silencing as well as dosage compensation.

This classic work on wheat storage proteins illustrates the truism that the evolutionary fate of duplicated sequences will vary widely among taxa and among the various components of the genome. In some situations and species, gene silencing may be an incremental process, as exemplified by PGI in *Pellaea*, whereas in other cases genomic change might be more

saltational. Ribosomal genes duplicated as a result of polyploidy provide many examples of the latter. In the Triticeae [40], *Nicotiana* [215], *Festuca* [208], *Brassica* [185], *Glycine* [36, 178], and *Scilla* [212], entire 18S-26S and/or 5S rDNA arrays not only have been 'silenced' after polyploidization, they have been completely lost. In some if not all of these cases, array loss might have occurred suddenly, via a single unequal inter-chromosomal exchange or deletion event. In the light of the above, 'gene silencing' may best be seen as reflecting a variety of different phenomena, including the classical process of slow decay leading to pseudogene formation, genomic deletion of entire arrays and possibly individual genes, and epigenetic modification (discussed below). Accordingly, global measures of gene silencing rates, estimated mostly from allozyme data, are unlikely to have uniform or even broad applicability, notwithstanding the body of theory concerned with rates of gene silencing [118, 142, 217, 220].

The diversity of silencing phenomena alludes to a spectrum of underlying mechanisms, not all of which are well understood. Conventional gene silencing through deleterious mutations has been described many times for plant pseudogenes. There remain few cases where this mutational process has been detailed for gene-pairs shown to have acquired their duplicate status via polyploidy, but the process is conceived of as being similar to that described for duplicate PGI-encoding genes in *Clarkia* [67] and chlorophyll *a/b*-binding protein genes in *Polystichum munitum* [155]. In both cases numerous lesions were evident in the silenced genes, reflecting point mutations, insertions and deletions. To date, the most detailed description of pseudogene formation for a duplicated gene is for *PgiC2* in *Clarkia mildrediae* [67]. Of the 23 exons 18 were sequenced, of which nine exhibited indels, causing frameshifts and a stop codon. Deletions ranged from 3 to 52 nucleotides, some causing loss of exon-intron splice junctions, while there were three insertions totaling 8 bp. Introns were also degenerate, with a total of 21 insertions, one of which was 857 bp in length with a stem-loop structure and direct terminal repeats suggestive of a transposable element.

Transposable elements themselves might be the causative agent of gene silencing [69, 104, 121, 127, 135, 227–230]. In hexaploid wheat, for example, loss of glutenin expression at the *Glu-1* locus is due to an 8 kb insertion of a retrotransposon in the coding region [77]. Non-functional nitrate reductase genes in tobacco were shown to be caused by insertion of a *copia*-like retrotransposon called *Tnt1* [69]. Insertions into regulatory regions might also alter expression, as documented for pea *rbcS* [230], the maize regulatory gene *R-s* [135], the *nivea* chalcone synthase gene in *Antirrhinum* [121] and many other examples [104, 127, 227–230]. As discussed below, polyploidy may be associated with increased transposable element activity; perhaps element-induced gene silencing is tolerated in polyploids to a greater extent than in diploids because of the buffering effect of gene duplication.

Not all cases of gene silencing will have progressed to the extent that *Clarkia mildrediae PgiC2* has. During the initial stages of gene silencing, and with apologies to Mark Twain (in his famous cable from Europe to the Associated Press), the reports of a gene's death may be an exaggeration. In an intriguing paper, Marshall *et al.* [128] raise the possibility that genes might evolutionarily 'flicker' on and off, whereby silencing happens as a consequence of point mutations in coding or regulatory regions, and function is restored by back mutation or gene conversion with a functional homologue. Their statistical analysis suggests that this type of resurrection may be evolutionarily realistic over time spans of several million years. Clearly, the likelihood of gene rescue will be higher when the number of lesions that caused silencing is small and when they involve easily reversed steps, such as back mutations for point substitutions. Other factors also are likely to be important in gene resuscitation, such as the number of intact homologues elsewhere in the genome, and the frequency of gene conversion or inter-genic recombination. Although no examples have yet been described of genic 'death and resurrection' in plants, the process may have functional significance, particularly if novel genic combinations are formed by the rescue process.

Regain of function might be more common and potentially have even greater significance for genes silenced by epigenetic means. In this regard, the example of intergenomic suppression of endosperm proteins in allopolyploid wheat is especially relevant. Galili and Feldman [57] extracted tetraploid (AABB) wheat lines from common wheat (AABBDD) and observed a loss of several gliadin and glutenin bands in the tetraploid derivatives, which logically were attributed to protein genes located in the missing D genome. More surprising was the observation of several novel protein bands in the extracted tetraploids, which were either missing or weakly expressed in the hexaploid parent. These data suggest that the D genome suppresses expression of some of the protein

genes resident in either the A or B genomes, or perhaps both. When experimental allohexaploids were synthesized between the extracted tetraploid and a D-genome diploid, the suppressive effect was reestablished. These data thus comprise evidence for intergenomic suppression of expression after polyploidization.

As pointed out by Galili and Feldman [57, p. 655], gene silencing through mutational or deletional processes are long-term evolutionary consequences of polyploidy, whereas 'intergenomic suppression or repression of redundant genes operates immediately after polyploidization and is therefore of particular importance for the establishment and successful adaptation of newly formed polyploids as new taxa'. This speculation may yet turn out to be prophetic, especially in the light of the recent plethora of examples of epigenetic silencing that have emanated from work with plant transgenes. Specifically, in recent years it has become clear that transgenes introduced into recipient genomes often become inactivated, due to a variety of different *cis*- and *trans*-acting mechanisms operating pre- or post-transcriptionally, including cytosine methylation and enhanced RNA turnover [89, 95, 101, 131-134, 137, 138, 151, 174, 198]. In addition, introduced genes might cause *trans*-inactivation of homologous endogenous genes, through homology-dependent gene silencing mechanisms [55, 101, 138]. When considered in the present context, it is especially noteworthy that changes in ploidy level alone can result in modified patterns of gene expression [151, 174]. As noted by others [54, 133, 174], these epigenetic silencing phenomena may have significance with respect to the evolution of polyploids.

A final type of epigenetic silencing concerns the well-known phenomenon of nucleolar dominance [163], whereby nucleoli are formed in association with ribosomal genes from only one of the two progenitors of a polyploid or diploid hybrid. Recently it was shown that in both natural and synthetic *Brassica* allopolyploids, rRNA genes from only one progenitor are active in vegetative tissues, meaning that the rDNA arrays from the alternative parent were epigenetically silenced [26]. This repression is developmentally regulated, though, as silenced rDNA genes are expressed in floral tissues. The evolutionary significance of this form of gene silencing, involving developmental transitions in epigenetic silencing patterns, remains a matter of speculation, but it is conceivable that in some cases similar forms of tissue-specific expression may be visible to selection.

Long-term retention of duplicated genes

A frequent evolutionary outcome of gene duplication is retention of function for both gene copies. This fate appears to be a common one, as evidenced in a large number of studies involving a diverse array of organisms and genes [33, 45, 50, 93, 186]. For example, and notwithstanding the many reports of gene silencing at enzyme-encoding loci (as discussed above), a common observation in polyploids is duplicate gene expression. As mentioned previously, rates of gene silencing often are lower than those predicted by population genetic models [93, 142, 156, 216], raising the question as to why so many genes escape pseudogenization.

Selection in one form or another is often invoked as an explanation for redundancy [32, 67, 92, 93, 111, 119, 147, 148, 216]. For example, Larhammar and Risinger [111] note the persistence of duplicate gene expression following polyploidization in fish, and raise the question of whether selection acts to preserve duplicate genes only in those cases where increased transcript levels are favored. Others have suggested that redundancy may create subtle fitness advantages that might, for example, only be evident in particular stages of the life cycle or under particular environmental conditions [32]. Selection may also operate by molding partial and perhaps subtle differences in expression between the two gene copies [2, 31, 32, 51, 93, 118]. The latter underscores the point that persistence of duplicate genes does not necessarily imply retention of identical protein function or expression; rather, functional diversification may simply be cryptic.

Persistence of duplicate genes might also be favored in cases where mutations in one gene copy lead to negative interactions with the products of other essential genes [67, 92]. In discussing the persistence of a duplicated *Pgi* locus in *Clarkia*, for example (albeit in a diploid), Gottlieb and Ford [67] propose that mutants in either the original gene or its duplicate derivative are selected against because of negative catalytic effects on intergenic heterodimers formed between monomers encoded by the two loci. They also point out that mutations which reduce protein interactions or cause complete inactivation might be those most likely ultimately to lead to gene silencing, as well as the corollary that diversifying selection may act to

change tissue- or age-specific gene expression patterns in cases where deleterious intergenic proteins may be formed. Finally, Gottlieb and Ford make the interesting point that persistence of shared function itself might result in both gene copies becoming 'essential', in that transcript or protein levels will have become optimized and therefore mutations in either gene copy will be deleterious.

This notion that duplicated genes exist not in isolation but in the context of myriad other genes, gene products and cellular constituents has important implications for the possibilities of retention of function and functional diversification. This idea has been stressed in a number of recent papers [62, 92, 142, 156, 216]. Nadeau and Sankoff, for example [142, p. 1265], invoke 'coevolution of genes in pathways' in attempting to account for the unexpectedly low rate of gene silencing in multigene families from humans and mice. Several authors have posited that long-term persistence of duplicated genes may result from pleiotropy or selection against point mutations in multi-function proteins or proteins that comprise components of multi-protein complexes [62, 92, 216]. In the latter case, mutations in one constituent protein may result in lowered functionality of an entire complex and a selectively inferior phenotype, in a fashion analogous to the example of duplicated *Pgi* in *Clarkia* [67]. Because of the complexities of gene networking and regulation, there might be selection against any mutation that generates a molecular 'poison' [156, p. 1353] through altering the function of either duplicate copy. As pointed out by Wagner [216, p. 787], we are far from an understanding of 'the rich substructure of genes and their embedding into superstructures – genetic networks – that the theory may need to capture to explain the abundance of functional diversification'. With respect to polyploidy, the perspective of genetic superstructures and networking may prove to be essential for achieving a fuller understanding of the consequences of merging two genomes into a single nucleus.

Interactions among duplicated genes

Among the possibilities generated by polyploidization are novel interactions among duplicated sequences or their protein products. The former represent interactions at the DNA sequence level, mediated by various mechanisms that alter the actual sequence of one or both duplicated copies. The latter comprise higher-level interactions potentially involving complex networks of factors that alter expression levels or patterns. Examples include the well-known phenomena of dosage effect and dosage compensation [13, 14, 58, 72, 115, 171]. It is often observed that in polyploids expression level is positively correlated with gene copy number [e.g. 171], whereas in some cases 'dosage compensation' is observed, in that overall expression levels are maintained despite copy number increases [15, 72]. A recent study in maize [72] is illustrative of the diversity of effects polyploidy may have on genic expression levels. In this study expression levels were monitored for 18 maize genes in a genomic dosage series ranging from haploid through tetraploid. Most genes exhibited a dosage effect, and several displayed dosage compensation. Non-linear relationships were also observed, however, as was a remarkable example (involving a thiol protease gene) of down-regulation as gene dosage increased. Transcript levels have also been shown to be influenced by many different chromosomal regions [71], underscoring the complexity of the regulatory networks. Because the effects of natural polyploidy on gene expression have not been systematically investigated, the relative frequencies of dosage compensation and down-regulation are not known. Available data, however, emphasize the possibility of radical regulatory changes issuing from the union of two diploid genomes in a common polyploid nucleus.

In addition to changes operating at the level of gene expression, the genes themselves might interact via recombination, gene conversion, or other mechanisms. As discussed above, in many cases gene duplication leads to peaceful coexistence, in that both copies continue to evolve along what appear to be independent evolutionary trajectories. This coexistence classically has been manifested as retention of duplicate expression for morphological markers or allozymes. More recently, and more commonly, persistence of duplicated genes has been evidenced as additive restriction site profiles or sequence data. Evidence that the duplication reflects polyploidy usually requires comparison with putative progenitor diploids or their near-relatives, in addition to other information (e.g. Southern blot analysis, genetic mapping data) that helps verify that truly orthologous and homoeologous sequences are being compared [34]. It is the phylogenetic partitioning of the sequences into two classes, each containing a sequence from one of the two diploid ancestors and one of the two polyploid homoeologues, which constitutes the best evidence of genic origins and relationships. These inferences

are phylogenetically grounded and hence they assume independence among the sequences involved. On occasion, however, duplicated sequences interact and violate the assumption of independence, in the process generating conflict [223] with expected phylogenetic relationships (cf. Figure 1). Several incompletely understood mechanisms are responsible for the phenomenon of 'non-independence' which, singly or in combination, may lead to reciprocal recombination, gene conversion, or sequence homogenization resulting from concerted evolution [3, 4, 43, 85, 241]. Even sequences on chromosomes for which there is no cytologically detectable evidence of affinity may be subject to these interactions, indicating that at least transient meiotic or mitotic associations may occur among non-homologous chromosomes [91, 172, 224, 237]. Polyploidy increases the number of duplicated sequences resident in the genome, and hence homology-dependent recombination or gene conversion mechanisms [91, 120, 154, 158, 175] may lead to novel intergenic interactions.

Most of the evidence for interaction among polyploidy-induced duplicated sequences involves ribosomal DNA [18, 168, 172, 210, 224, 237]. In higher plants, as in most eukaryotes, ribosomal genes encoding the large and small subunit RNAs are tandemly repeated in arrays at one or more chromosomal loci, each containing hundreds to thousands of repeats. A characteristic feature of plant rDNA is that repeats within arrays often exhibit minimal sequence heterogeneity [but see 21, 22], the repeats having been homogenized via repeated cycles of unequal crossing over or gene conversion [43, 85]. When arrays exist at more than a single locus, the possibility arises for inter-chromosomal interactions, as has been shown for some polyploid as well as diploid plants [18, 168, 172, 210, 215, 224, 237]. In *Gossypium*, for example, *in situ* hybridization work [75] has shown that rDNA loci exist at more than one chromosomal location in diploid cotton, including the progenitor genomes (A and D) of allopolyploid (AD genome) species. Although there appears to have been evolutionary change in the number of repeats per locus following polyploidization, copy number and chromosomal distribution are approximately additive in allopolyploid *Gossypium* species [75, 224]. Sequence data from the internal transcribed spacer (ITS) region reveal that little heterogeneity exists among repeats within either progenitor diploid genome or within the allopolyploid genomes, showing that concerted evolution has operated to homogenize repeats not only within arrays but between them [224]. Because ITS sequences from the two diploid progenitors differ at many sites, it was possible to evaluate the fate of the two repeat types in the allopolyploid derivatives by restriction site analysis. This showed that different allopolyploid species contain only one of the two parental rDNA types (A or D), demonstrating that there has been inter-genomic homogenization of repeats subsequent to polyploidization. In addition, phylogenetic analysis showed that four of the five allopolyploid species contain rDNA repeats much like those contributed by the D-genome diploid progenitor, while the fifth species possesses A-genome repeat types. These results demonstrate that concerted evolution among rDNA repeats can occur in either direction, toward either parental type, after allopolyploid formation.

Inter-locus homogenization of alternative rDNA repeat types has been reported in other polyploid plants as well, including *Microseris* [168, 210], *Paeonia* [172, 237], and *Saxifraga* [18], suggesting that the process may be common. In one respect, inter-locus replacement mimics gene silencing, in that only one of the two parental sequence types remains, but it differs in that there has not necessarily been a change in functional copy number (disregarding the absence of relevant rDNA expression data). These studies also demonstrate that sequence 'conversion' from one progenitor diploid type to the other is not the only possible outcome. In both *Paeonia* and *Microseris*, as well as in synthetic *Medicago* hybrids [30], novel rDNA types have been recovered that appear to have arisen through gene conversion events between alternative repeat types. Whether there is functional significance to either inter-locus replacement of rDNA repeat types or recombination among repeats is an open question, but the demonstration of inter-genomic interactions and novel allelic recombinants following polyploidization suggests that this is as a possibility, if not for ribosomal genes then perhaps for other functionally relevant factors.

The question arises as to the mechanism that underlies inter-locus rDNA interactions. Unequal crossing-over and gene conversion are likely possibilities. Perhaps inter-chromosomal exchanges are facilitated in taxa such as *Gossypium* and *Paeonia* by the near-telomeric location of the rDNA arrays, which may permit unequal crossing without deleterious recombination among non-homologous chromosomes [4, 224]. In this respect, it is notable that some polyploid plants exhibit multiple rDNA repeat types that persist for long periods of time following poly-

ploidization [24, 40, 105, 205, 218], and that in at least some of these cases one or more rDNA arrays occupy chromosomal locations distant from the telomere [40, 100, 126]. It might be that the correlation between the fate of duplicated sequences in polyploids and chromosomal location [224, 237] applies to other repetitive DNAs and perhaps to non-repetitive sequences as well. As an example, 5S repeats in *Gossypium*, which occur in arrays localized by fluorescent *in situ* hybridization (FISH) to centromeric regions [75], appear to retain their subgenomic origin following allopolyploidization [35], as they do in polyploid wheats [42, 110]. The converse situation, involving the fate of duplicated 5S repeats that occur near the telomere in polyploid plants, has not yet been explored.

There are as yet few examples where the possibility of inter-locus interactions has been formally evaluated for single-copy genes. As mentioned previously, in part this reflects experimental difficulties associated with isolating proven orthologues and homoeologues from phylogenetically well understood diploids and their polyploid derivatives. In allotetraploid cotton, these interactions may be relatively infrequent. No evidence of intergenic exchanges have been observed for orthologues at 16 loci isolated and sequenced from diploid and allopolyploid *Gossypium*, encompassing a diversity of genes, including alcohol dehydrogenases [182, 183], subtilisin-like proteases, α-mannosidases, and cellulose synthases (Cronn and Wendel, unpublished). On the other hand, the literature contains numerous reports of recombination and gene conversion among single-copy genes, although in only a few cases do the data permit even a tentative conclusion that these events involve homoeologous gene copies duplicated as a result of polyploidy. Perhaps the best example is the study of glucan endo-1,3-β-D-glucosidase genes in *Nicotiana tabacum* [196]. Tobacco is an allotetraploid (genomic constitution ST) derived from ancestral diploids much like modern *N. sylvestris* (S-genome) and *N. tomentosiformis* (T-genome). Genes derived from both progenitor genomes exist in tobacco, as revealed by restriction site digestion and Southern hybridization analysis. In addition to these 'normal' genes, two apparently recombined cDNA clones have been recovered from tobacco, which display alternating blocks of sequence similarity to the two parental forms. This is interpreted as reflecting reciprocal exchanges or gene conversion between genes from the diploid donors after formation of the ancestor of modern allotetraploid tobacco. Because the glucan endo-1,3-β-D-glucosidase gene family has been incompletely characterized in *Nicotiana* and because sequences from the diploids were not reported, orthology relationships remain uncertain, and so the conclusion reached should be regarded as tentative.

At present we do not know how common intergenomic gene conversion or recombination is in polyploids, nor is it clear what the genomic and genetic factors are that promote or inhibit the responsible mechanisms. It also remains to be demonstrated that the novel alleles that may result from these interactions are functionally distinct from their antecedents, and hence potentially evolutionarily significant.

Genetic diversity in homoeologous genes in allopolyploids

When genes become duplicated as a consequence of polyploidization they may continue to evolve at the same rate as in their diploid ancestors and as each other, or they may be subject to pressures that lead to differential rates of sequence evolution. This leads to a useful null hypothesis for the evolution of duplicated homoeologous genes following allopolyploidization, namely, that evolutionary rates will be equal. A corollary expectation is that both gene copies will accumulate infraspecific diversity at an equivalent rate. This need not be true, of course, as is evident from the possibilities of functional diversification and gene silencing, as discussed above, and because of many other genomic processes that might differentially affect homoeologues. Nonetheless, the model may be useful in informing a search for the underlying explanation for differential diversity when it is observed. For example, if one homoeologue becomes pseudogenized while the other remains under purifying selection, then nucleotide diversity is expected to increase in the former locus at a faster rate than in the latter. Although there are still few cases where infraspecific diversity has been measured for homoeologous locus pairs, the fact that the duplicated genes reside in the same nucleus provides a powerful tool for isolating potentially important genomic forces from population-level factors such as breeding system or effective population size. Because population-level factors are expected to affect both homoeologues equivalently, observed differences in diversity are more easily attributed to genetic or genomic processes.

One noteworthy example involves tetraploid and hexaploid members of the wheat group (*Aegilops-Triticum*), whose genomes have long been known

to differ in their variability [242]. In several studies using a diversity of markers, the B genome of polyploid wheat has been shown to harbor more diversity than the other genomes [37, 48, 116, 181]. Recently, Feldman and colleagues surveyed genetic diversity in wild and cultivated tetraploid emmer wheat (*Triticum turgidum*), and showed (unpublished data) that this generality extends to 14 pairs of mapped, homoeologous RFLP loci. The explanation for the differential accumulation of diversity in the A and B genomes of tetraploid wheat is not known, but it may reflect external forces such as differential selection and interspecific introgression, and/or internal mechanisms involving such processes as differential methylation, mutation or recombination.

At present, the only published study of nucleotide diversity levels for homoeologous locus pairs is from *Gossypium.* About 1 kb of *AdhA* sequence was generated from both the A- and D-genomic homoeologues for 22 accessions (44 alleles per genome) of *G. hirsutum* and for five accessions (10 alleles per genome) of *G. barbadense* [183]. In both allotetraploid species, estimates of nucleotide diversity were higher for *AdhA* from the D genome than from the A genome, by a factor of two or more. Although absolute nucleotide diversity estimates were sufficiently low that the inference of differential diversity among homoeologues was not supported by statistical tests, the results are directionally consistent with data from a second pair of homoeologues (*AdhC*) that encode a different member of the *Adh* gene family [182, and unpublished results]. Whether this genome-specific bias will be found to apply to other homoeologous locus pairs is an open question. Similarly, at present the underlying mechanistic basis for differential diversity and its potential evolutionary significance are matters of speculation.

Evolution of duplicated genomes

Many of the potentially important processes in polyploid genome evolution operate above the organizational level of duplicated genes. Notwithstanding the possibility of autopolyploid speciation [reviewed in 162, 188, 191], many if not most genome duplications entail the merger of two, often highly differentiated genomes into a common nucleus in only one of the two parental cytoplasms. These allopolyploidization events have long been thought to be associated with evolutionary innovation mediated by genome recombination and perhaps other higher-order genomic interactions. Nearly four decades ago, for example, Zohary and Feldman emphasized the importance of genomic interactions in polyploid evolution in the wheat group [242]. Although our present understanding of genome interactions in polyploids is still relatively rudimentary, recent applications of molecular genetic techniques have confirmed that an array of phenomena and processes collectively lead to polyploid stabilization and evolution. Some of these are disclosed by observations of older polyploids, while other potentially significant phenomena have been revealed through analyses of young and even synthetic allopolyploids. For purposes of discussion, these various aspects of genome evolution in polyploids will be introduced in four non-mutually exclusive categories: (1) chromosomal structural evolution; (2) rapid, non-Mendelian genomic changes; (3) inter-genomic invasions; and (4) cytonuclear stabilizations.

Chromosomal diploidization and structural evolution

One of the important realizations to emerge from the widespread use of genetic mapping technologies is that many plants previously considered to be diploid, based on either comparative chromosome number data, meiotic chromosome behavior, or both, are actually stabilized or 'chromosomally diploidized' polyploids. RFLP studies have shown that in many chromosomally diploidized taxa, such as *Brassica* [27, 108, 109, 209], *Glycine* [179], *Gossypium* [20, 179], and *Zea* [84], probes that reveal duplicated loci often map to parallel, duplicated linkage groups. These data are suggestive of ancient polyploidy, and in some cases, further series of 'nested duplications' are evident, indicating even more ancient cycles of genome doubling and chromosomal diploidization. In *Brassica*, for example, the diploid species *B. nigra* ($n = 8$), *B. oleracea* ($n = 9$) and *B. rapa* ($n = 10$) have genomes containing triplicated copies of an ancestral genome [109]. Each of these triplicated ancestral genomes is structurally similar to the genome of *Arabidopsis* [108], lending additional support to the interpretation that diploid *Brassica* species are descended from a hexaploid ancestor.

Inferences of paleopolyploidy based on mapping data often are supplemented by other sources of evidence. DNA sequence data may be useful, for example, as shown by Gaut and Doebley [60], who studied 14 pairs of duplicated loci in maize. They inferred two different groups of coalescence times, and interpreted these to reflect ancestral tetraploidiza-

tion between diploids whose genomes were incompletely differentiated from each other. Based on these data and the comparative mapping analyses, it appears that the modern maize genome ($n = 10$) is descended from a segmental allopolyploidization speciation event. *In situ* hybridization and special chromosome visualization techniques also are powerful tools that are likely to find increasing utility in exploring ancient polyploidization events. Gómez *et al.* [63], for example, report a 45 kb BAC (bacterial artificial chromosome) from *Sorghum bicolor* ($n = 10$) that preferentially hybridizes to centromeric regions of 5 of the 10 sorghum chromosomes, providing strong supporting evidence [cf. 231, 235] for a tetraploid genomic ancestry for sorghum while simultaneously identifying the two genomic complements of five chromosomes each. In *Gossypium*, comparative RFLP mapping studies suggest that diploid cotton species ($n = 13$) are in fact paleotetraploid, and that this ancient genome doubling occurred at least 20–40 million years ago [20, 164]. This history remains visible cytogenetically: Muravenko *et al.* [141] used BrdU-Hoechst-Giemsa banding analysis to demonstrate that modern diploid cotton genomes contain two sets of either six or seven chromosomes, presumably reflecting the more ancient diploid condition.

In addition to extensive collinearity and retention of synteny, chromosomal rearrangements are commonly observed in comparative mapping studies, even in diploid plants [e.g. 17, 140]. Thus it is not surprising that various types of chromosomal rearrangements, including inversions and translocations, have been detected in all of the examples cited above involving ancient polyploids. In some cases rearrangements may be confirmed or novel insights may be obtained using genomic *in situ* hybridization (GISH) techniques. In particular, the possibility of inter-genomic chromosomal exchanges may be evaluated. These have been observed, for example, by using GISH techniques in tetraploid tobacco [103], *Milium* [9], and polyploid oats [25, 98]. This powerful molecular cytogenetic tool permits quantitative analysis of the size and number of inter-genomic interchanges that have occurred following polyploidization. A cautionary note arises, however, from the realization that inter-genomic sequence homogenization might occur, as discussed above for rDNA in *Gossypium* [224]. This raises the possibility that in any particular case, GISH signal in the 'wrong' genome may reflect inter-genomic concerted evolution rather than reciprocal or non-reciprocal translocations.

The frequent observation of inter-genomic translocations in polyploids suggests that recombination between homoeologous chromosomes may be common. A compelling illustration of this phenomenon is provided by a recent GISH analysis of synthetic tetraploid ($2n = 4x = 28$) progeny between *Lolium multiflorum* and *Festuca pratensis* [243]. Despite the fact that this was an inter-generic cross, extensive inter-genomic recombination was observed in all 72 mitotic cells examined, which represented 25 different F_8 plants. From 22 to 38 translocations were observed per cell, involving a minimum of 20 of the 28 chromosomes, with a range of 0 to 7 translocations per chromosome. These data demonstrate extensive recombination of chromatin from the two genera. Extrapolation of these results to natural situations involving polyploids suggests that an important component of the evolutionary success of polyploids may be the large number of different gametic combinations that are generated by independent assortment combined with inter-genomic exchanges. This is merely a new twist on an old proposal [e.g. 200]. In attempting to account for the evolutionary success, wide variability, and ecological and geographical amplitude of polyploids in *Triticum-Aegilops*, for example, Zohary and Feldman [242] underscored the importance of recombination between differential genomes.

Inter-genomic mixing may have significance not only with respect to polyploid evolution, but to diploids as well. In colchicine-doubled hybrids between diploid *Lolium multiflorum* ($2n = 14$) and hexaploid *Festuca arundinacea* ($2n = 6x = 42$), some of the fertile progeny unexpectedly had diploid chromosome numbers [152]. In all cases examined, these diploids showed regular meiotic pairing. GISH analysis revealed that the diploids are recombinant with respect to *Festuca arundinacea* and *Lolium multiflorum*, with various proportions of chromosomes and chromosome segments contributed by the two parents. Once again, extension of these results to natural situations raises the intriguing prospect that many natural 'diploids' harbor in their genomes cryptic evidence of past hybridization events with polyploids. A case in point involves the species *Gossypium gossypioides*, which is a diploid D-genome diploid cotton from Oaxaca, Mexico. Analyses of ribosomal genes [35, 225] and other repetitive sequences [238] have shown that the *G. gossypioides* genome is extensively introgressed with sequences from the A-genome. Because A-genome diploids presently are restricted geographically to the Old World whereas allopolyploid

(AD-genome) cottons are endemic to the New World, as is *G. gossypioides*, it appears likely that *G. gossypioides* has experienced introgression from polyploid cotton followed by restoration of the diploid condition. The observation of restoration of euploids following inter-ploidal crosses in other genera [19, 124, 204] is consistent with this interpretation. Because so few plant groups have been studied using a combination of GISH and phylogenetic techniques, the frequency and significance of interspecific, inter-ploidy introgression simply is not known. Nonetheless, it remains a tantalizing evolutionary possibility.

At present, it seems safe to state that genome mixing occurs in both diploid and polyploid lineages, although our understanding of both frequency and mechanism is limited. It also is clear that inter-genomic chromosomal recombination and introgression happen on the long term in many polyploid lineages. Some of this recombination may reflect interspecific hybridization, while other inter-genomic exchanges will have arisen from contact between genomes within a single nucleus. It seems improbable, however, that structural changes *per se* (e.g. translocations, inversions) play a significant role in 'chromosomal diploidization' or 'evolutionary stabilization' of polyploids [20]. This inference is based on the observation that there is no real difference in the kind and magnitude of chromosomal structural changes that distinguish diploid genomes versus those in polyploids, as well as repeated observations in many plant groups that structural changes do little to inhibit pairing (e.g. the common observation of paired chromosomes in translocation heterozygotes). It may be, therefore, that following polyploidization the more significant mechanisms leading to rapid restoration of regular, diploidized meiotic pairing (i.e. suppression of homoeologous pairing) involve factors such as the well-known *Ph* gene in wheat [see 44, 176, 213, 214]. Perhaps other non-Mendelian, saltational phenomena are also important, as described in the following section.

Rapid genome evolution

An expected consequence of the merger of two nuclear genomes during polyploidization is that the resulting nascent polyploid will contain the full genomic complement of both of its parents. This expectation of additivity serves as a convenient null hypothesis of predicted genomic contributions to the 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 study of synthetic experimental allopolyploids. Recent studies in *Brassica* and in wheat are especially revealing in this regard, as they demonstrate that nascent allopolyploids often do not show genomic additivity with respect to their parents. Instead, as described below, their genomes display remarkable patterns of non-Mendelian genomic change accompanying polyploidization. These studies, more than any other, are responsible for a growing awareness of the 'dynamic nature of polyploid genomes' [189].

The experiments in *Brassica* [192] involved reciprocal synthetic allopolyploids between the diploids *B. rapa* and *B. nigra* and between *B. rapa* and *B. oleracea*. Thus, two different hybrids were generated in each of two cytoplasms. After 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 genes, and anonymous genomic clones revealed a high frequency of unexpected fragment profiles in each generation. 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 the 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*.

Experiments in wheat yielded similar striking examples of polyploidy-induced genomic change, albeit with some novel twists. Bread wheat is an allohexaploid (AABBDD) derived from hybridization between *Triticum turgidum* (AABB) and a taxon similar to modern *Aegilops tauschii* (DD). Feldman *et al*. [46] studied RFLP patterns in diploid and allopolyploid wheats using 16 low-copy, non-coding probes that were either chromosome-specific or were confined to several chromosomes within a single polyploid genome. Of these, 9 yielded a strong hybridization signal in all diploid genomes, suggesting that these sequences are relatively conserved and indicating that they were present in each of the progenitor genomes at the onset of polyploidization. Thus, the expectation

was that each of these 9 sequences would be detected in both tetraploid and in all three hexaploid wheat genomes. Using aneuploid and nullisomic-tetrasomic stocks, however, Feldman *et al.* showed that each sequence was retained in only one of the allopolyploid genomes, having been eliminated from one of the two tetraploid genomes, with a second round of sequence elimination accompanying the formation of hexaploid wheats. Remarkably, insofar as it has been studied, similar patterns were observed in synthetic allohexaploids, suggesting that polyploidy-induced sequence elimination is a directed, non-random process. In a follow-up study, Liu *et al.* [123] monitored RFLP fragment profiles in synthetic tetraploids, hexaploids, octoploids, and decaploids in *Triticum* and *Aegilops* using a similar set of probes as employed in the initial study. Consistent with the earlier results, rapid, 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 latter echoes results from *Brassica* [192] and an earlier study in wheat where novel RFLP patterns were observed in synthetic allopolyploids with an rDNA spacer as a probe [173].

Most of the probes used in the *Brassica* work [192] were coding sequences whereas those employed in the wheat analyses [46, 123] represented non-coding portions of the genome. To address the question of whether the phenomenon of polyploidy-induced sequence elimination extends to coding sequences, Liu *et al.* [122] studied RFLP fragments in the same set of synthetic allopolyploids as in their first analysis, using as probes coding sequences that mapped to each of the 42 chromosome arms in hexaploid wheat. Although fragment loss and gain were observed, as in *Brassica*, there was no evidence for sequence elimination, as for all probes examined parental fragments were detected for at least some of the restriction enzymes employed. Methylation was implicated as the mechanism responsible for the fragment changes observed.

Whether the phenomenon of polyploidy-induced sequence elimination has functional significance remains an open question, but Feldman *et al.* [46] noted that it has the effect of converting sequences that initially exist on homoeologous chromosomes into chromosome-specific sequences, thereby increasing divergence between homoeologous chromosomes. Accordingly, homoeologous chromosome pairing may be hindered while strictly homologous pairing is favored, leading Feldman *et al.* to offer the intriguing speculation that the phenomenon provides a physical basis for rapid restoration of diploid-like chromosome pairing following polyploidization.

These experiments in *Brassica* and wheat demonstrate that rapid, non-Mendelian change may occur during polyploid formation or in the earliest stages of polyploid stabilization. As a consequence, nascent polyploid genomes cannot be assumed to be completely additive with respect to their progenitors. At present, relatively little is known regarding the nature of the sequences that are subject to polyploidy-induced elimination or modification, nor is it understood what the various factors are that might influence the prevalence and patterns of non-additivity. Liu *et al.* [123] point out the additional complication that in wheat, elimination of a sequence from one genome may be effected by the presence or absence of related sequences in the alternative genome(s). Moreover, sequence elimination appears to be related to the relative ploidy level of the two parents, with elimination being more common from the parent that has the lower chromosome number. It seems almost certain that several different mechanisms underlie the phenomenon of rapid genome change; there is no *a priori* reason, for example, to attribute sequence elimination in wheat to the same mechanism as RFLP fragment loss and gain in *Brassica*. Clearly more research is needed using these and other experimental systems before we can achieve a fuller understanding of the prevalence and scope of the phenomena incorporated under the umbrella heading ‘rapid genome change’. Nonetheless, it is still worth speculating about the responsible mechanisms.

One of the potential explanations for rapid genome change in nascent polyploids is increased or altered patterns of DNA methylation. This was brought forward as a partial explanation for unexpected fragment patterns in both wheat [122] and *Brassica* [192], based on different RFLP profiles observed using isoschizomers that vary in methylation sensitivity. Cytosine methylation, especially in CpG dinucleotides and CpNpG trinucleotides, is common in plants and is thought to play a role in the regulation of gene expression, timing of DNA replication, and other aspects of plant development [52, 166]. DNA methylation may also be significant as a host defense response to either viral infection or transposable element activity [54, 131–133, 149, 236]. In this respect, it has been proposed that the primary function of DNA methylation actually may be to repress the activity of transposable elements [236]. This proposition is made attractive by the prominence of transposable elements in plant

genomes [10, 11, 23, 68, 229]. As noted by several authors [89, 95, 101, 131–134, 137, 138, 151, 174, 198], the high frequency of silencing of introduced transgenes in plants may reflect this epigenetic response.

Epigenetic silencing may be especially relevant to genome evolution in polyploids. At the minimum, the union of two genomes into a single nucleus may be perceived as introduction of 'foreign DNA' by the responsible signal transduction pathways. In addition, it may be that transposable elements are released from suppression during polyploidization. As Barbara McClintock suggested in her Nobel Laureate speech [136], one genomic response to 'challenge' may be release of suppression of transposable element activity. Indeed, enhanced transposable element activity has been shown to accompany stress in several systems [87, 88, 226, 229]. Perhaps polyploidy itself represents a form of genomic 'shock', causing increased transposable element activity and an accompanying cellular response of elevated DNA methylation or other forms of epigenetic silencing. Indeed, McClintock stated that 'Species crosses are another potent source of genomic modification' [136, p. 799] and that 'All evidence suggests that genomic modifications of some type would accompany formation of such new species' [136, p. 800]. More recently, it was shown in *Arabidopsis* that ploidy level itself alters epigenetic silencing patterns [174], as does aneuploidy in tobacco [151]. The mutagenic effects of transposable elements are well known and their potential role in gene evolution has previously been discussed. Several authors have noted the connection between the potential proliferation of transposable elements and polyploidy, not only in the sense that genome doubling may enhance element activity, but that the effects of insertional mutagenesis will be ameliorated by gene duplication and its implied redundancy [54, 133]. Thus, an important dimension of polyploidy may be bursts of genic and regulatory evolution mediated by transposable element insertion during polyploid formation or shortly thereafter. It is noteworthy in this respect that retrotransposable elements have colonized alternative genomes following allopolyploidization in cotton [74, 76, 238], as will be discussed below. As noted by others [e.g. 174], the epigenetic response itself may lead to accelerated rates of mutation, thereby augmenting the process of rapid genomic change in polyploids.

Not all responses to genomic stress involve transposable elements or epigenetic silencing. For example, in heat shock-treated *Brassica nigra*, about one-third of the ribosomal DNA repeats were lost [219]. Although the responsible mechanism is unknown, it is unlikely to have involved transposable elements or epigenetic silencing. Similarly, the rapid and non-random loss of specific sequences from all but one genome in wheat allopolyploids [46, 123] cannot be attributed to either of these processes. Hence, other mechanisms clearly are involved in rapid non-Mendelian change in polyploids. These may include various processes of sequence amplification [169], unequal crossing over, gene conversion, and chromosome rearrangements caused by translocations or other cytogenetic events. The latter evidently played a role in rapid genome changes in *Brassica* [192] but are unlikely to be involved in the case of wheat [46, 122, 123]. At present, little evidence bears directly on the importance of any of these mechanisms in the stabilization of nascent polyploids, but it seems likely that these and related mechanisms will be involved to various degrees.

Intergenomic invasion

As noted in this review several times, one of the principal genomic consequences of allopolyploidy is evolutionary interdependence among genomes that formerly were isolated in separate taxa. This is exemplified by a number of phenomena already discussed, including homoeologous recombination or other mechanisms that lead to inter-genomic exchange of chromosome segments, inter-genomic concerted evolution of divergent sequences, and inter-genic, inter-genomic recombination. A final illustration of this interdependence emerges from recent work on repetitive sequences in allopolyploids, especially allopolyploid *Gossypium*. Phylogenetic and sequence divergence data [177, 182, 222] suggest that the two ancestral diploid genomes (African-Asian A genome and American D genome; $n = 13$) of allopolyploid 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 [184, 221], about 1–2 million years ago. During the long history of diploid divergence, significant genome-size evolution transpired, so that modern descendants have DNA contents that differ by nearly a factor of two [8, 64, 139]. Not surprisingly, the single-copy fraction is similar [61], indicating that the genome size differences reflect differential accumulation and elimination of repetitive sequences during the millions of years of independent evolution of the diploids in different hemispheres. Al-

lopolyploid species have nearly additive genome sizes with respect to the diploid progenitors (2C values for A-, D-, and AD-genome cottons are ca. 3.8, 2, and 5.8 pg, respectively).

At first appearance, the near-additivity of allopolyploid genome sizes suggests a relative stasis of the repetitive fraction since polyploidization occurred in the mid-Pleistocene. Recent work, however, has clearly shown that this is not the case. A total of 83 non-cross-hybridizing repetitive DNAs were isolated from tetraploid *G. hirsutum*, collectively representing about one quarter of the cultivated cotton genome [239]. Slot-blot and Southern hybridization analyses [238] demonstrated that about three-fourths of these are largely restricted to the A genome, and that these repetitive DNAs collectively account for about half of the genome size differences between the two diploid progenitors of allopolyploid cotton. In contrast, only 4 of the repetitive DNAs were found to be D-genome-specific. The existence of genome-specific repetitive sequences permits an evaluation of their subgenomic integrity following polyploidization. When 20 of the 83 repetitive families were used in *in situ* hybridization experiments in allopolyploid cotton, most families that are restricted to the A genome at the diploid level exhibited hybridization signal not only on A-subgenome chromosomes but on D-subgenome chromosomes as well [76, 238]. 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 suggest that since polyploids formed, colonization of alternative genomes by genome-specific repetitive sequences has been common.

This phenomenon of inter-genomic 'horizontal transfer' was studied further by Hanson *et al.* [76], who examined eight repetitive families in detail. Of these, two were A-genome-specific while the other six hybridized strongly to both *G. hirsutum* subgenomes. 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. These data, as well as the data of Zhao *et al.* [238], show 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. From a mechanistic standpoint, one or more processes of inter-genomic interaction are implicated. These include DNA 'overwriting' through gene conversion, inter-genomic recombination and exchange, and the activity of transposable elements. In this respect it is noteworthy that database searches of the 83 cotton repetitive DNAs yielded matches to known transposons [238]. Moreover, specific *copia*-like retrotransposable elements restricted to the A genome [211] were similarly shown to have spread among all 52 chromosomes of allotetraploid cotton [74]. Replicative transposition is thus implicated as one process of inter-genomic interaction.

Because there are few comparable studies of 'genome colonization' after polyploidization it is difficult to generalize based on this work from *Gossypium.* Several considerations suggest, though, that the process may be general and have significance with respect to polyploid genome evolution. These include the ubiquity and abundance of retroelements in plant genomes [10, 11, 68, 229], the potential for polyploidy to cause a release from mechanisms that constrain transposition at the diploid level [87, 88, 133, 226], and the fact that genic duplication may relax functional constraints to the extent that element insertions are occasionally permitted in regulatory or coding regions [104, 121, 127, 135, 227–230]. Further insights into this issue are anticipated in the coming years, as the tools of molecular cytogenetics and phylogenetics converge on the problem.

Nuclear-cytoplasmic interactions

Plant growth and development entails a coordinated regulation of expression not only of nuclear genes but also of those in the chloroplast and mitochondrial genomes [16, 113, 207]. Presumably evolution operates to fine-tune the myriad regulatory interactions and metabolic networks involved in cytonuclear coordination. When polyploidization occurs, such that the nuclear genome becomes doubled but the organellar genomes do not, the stoichiometry between organellar genes and those in the nucleus is changed, potentially leading to regulatory disruptions or other sub-optimal physiological effects. These problems may be exacerbated by allopolyploidization, where potential differences between two formerly isolated but now merged nuclear genomes must become reconciled with each other and with only one (usually) of the two sets of cytoplasmic genes. It seems, therefore, that an important dimension of polyploid genome evolution may be cytonuclear in scope, involving either dramatic or subtle evolutionary optimizations or transformations in genic function and regulation.

At present, virtually nothing is known about the process of cytonuclear stabilization following polyploidization. In several cases the 'footprint' of whatever mechanisms are involved has been sought by monitoring nuclear genome-specific changes in synthetic and natural allopolyploids. In the *Brassica* RFLP study described above [192], no significant cytoplasmic effect was observed in reciprocal synthetic *B. rapa* × *B. oleracea* allopolyploids (A and C genomes), but an effect was detected in reciprocal synthetic *B. rapa* × *B. nigra* allopolyploids (A and B genomes). Specifically, a biased loss of B-genome RFLP fragments was observed among nine F_5 individuals derived from an initial AB allopolyploid generated in the A cytoplasm. The direction of fragment loss bias is consistent with earlier suggestions based on RFLP mapping data [194] that the AB nuclear genome of the natural allopolyploid *B. juncea* was more similar to the A than to the B genome diploid progenitor. In other cases, this cytoplasmic effect on RFLPs has not clearly been observed, for example, in *Gossypium* [20], wheat [122, 123], and other *Brassica* allopolyploids [192, 193]. Song *et al.* [192] suggest that there may be a relationship between genetic distance and biased cytonuclear effects in reciprocal allopolyploids. They point out that since *Brassica* A and C genomes are more similar to each other than are the A and B genomes, the absence of a cytonuclear effect in AC allopolyploids and its presence in AB allopolyploids may be related to the level of cytonuclear compatibility.

These investigations of RFLP changes in allopolyploids may suggest in a very general way processes involved in cytoplasmic-nuclear interactions, but the studies to date have not been designed to sharply focus on the 'real action' of cytonuclear evolutionary stabilization. Clues into the complexities of the regulatory interactions and evolution implied by polyploidization are emerging from model systems like rubisco [167]. This all-important multiprotein complex is a model for investigating cytonuclear integration in that its small subunits (SS) are encoded by a small family of nuclear *rbcS* genes whereas the gene (*rbcL*) encoding the large subunit (LS) is located on the plastid genome. Expression of rubisco is coordinately regulated by a complex adjustment of both SS and LS stoichiometries. At least two principal governing mechanisms are implicated, one involving LS mRNA translation initiation and the other SS protein turnover rates [167]. Application of insights from model molecular genetic/physiological models like rubisco to natural allopolyploid systems will be a fertile arena for future investigations, and will require an integrated approach using tools from several different disciplines.

Future prospects

In this review I have attempted to encapsulate our understanding of the molecular evolutionary genetics of a prominent mode of plant speciation. Fundamental processes affecting the evolutionary fate of duplicated genes and genomes were highlighted, as were many of the novel insights and perspectives that recently have been gained into polyploid stabilization and long-term evolution. Many of these recent insights have emerged from interdisciplinary efforts, often involving tools from molecular genetics and cytogenetics as well as their application to phylogenetically well understood groups, such as model systems in *Brassica*, *Gossypium*, *Triticum* and *Zea*. These studies have profoundly altered our views on genome evolution in polyploids, in particular by emphasizing the myriad interactions that are made possible by gene and genome duplication. Notwithstanding these many recent contributions, a common thread in this review is that our knowledge of mechanism, function and evolutionary significance is still in its infancy for the aspects of polyploid genome evolution that might matter most. For example, we still have only a rudimentary understanding of the mechanisms and significance of epigenetic silencing, the underlying controls of chromosome pairing, and the frequency, mechanisms and evolutionary significance of rapid genome changes. Similarly, we know little about the evolutionary accommodation that must occur with respect to cytonuclear integration and the coordination of disparate sets of regulatory factors contributed by two nuclear genomes at the onset of polyploidization. Continued application of molecular genetic approaches to questions of polyploid genome evolution holds great promise for producing lasting insight.

It would seem that enhanced understanding of phenomena and mechanisms at these molecular genetic levels are prerequisite to developing a fuller appreciation of the contribution of polyploidy to morphological evolution and ecological adaptation. Polyploidy has long been recognized to be associated with novel morphologies and adaptations, but how genome duplication ultimately translates into novel evolutionary opportunity has remained obscure. Interdisciplinary approaches are likely to be the most revealing in this

respect. It may be, for example, that polyploidization events are associated with bursts of transposable element activity, which lead to intra- and inter-genomic insertions into coding and regulatory regions of both structural and regulatory genes, in the process generating arrays of novel genotypes and morphological or physiological phenotypes upon which selection might act. One wonders whether this type of history underlies the recent observation, for example, that many fiber-related genes in cotton are located in the allopolyploid subgenome that corresponds to the non-lint-bearing diploid ancestor [99]. This and similar possibilities [e.g. 234] are now open to experimental study, suggesting that in certain instances the molecular genetic basis of polyploidy-induced adaptations may soon be revealed. This is an exciting prospect, and it is one whose promise undoubtedly will increasingly be realized in the future.

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