The Plant Genome
Review & Interpretation

Polyploidy and Crop Improvement

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Abstract

All crop plants are polyploid and some genomes have been duplicated more recently than others. Advancements in cytogenetic and molecular tools, including high-density genetic mapping, florescent in situ hybridization, and genome and EST sequencing, have enabled new insights into genome composition and the history of genome duplications in crop plants. We review this evidence and discuss the relevance of genome duplication to crop improvement. Polyploidy provides genome buffering, increased allelic diversity and heterozygosity, and permits novel phenotypic variation to be generated. Polyploid formation is often accompanied with loss of duplicated chromatin, changes in gene expression, novel epistatic interactions, and endosperm effects. All of these factors need be considered in a genome-wide context for optimizing marker assisted selection and crop plant improvement.

Abbreviations: Ks, synonymous sites; FLC, FLOWERING LOCUS C.

Published in Crop Sci 46(S1) (2006). Published 7 Sept. 2006.
doi:10.2135/cropsci2006.07.0489tpg

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One of the most spectacular advances of the genomics era has been a renewed appreciation of the pervasiveness and importance of genome doubling in plant evolution. Although the prevalence of polyploidy in plants has classically been recognized from comparative analyses of chromosome numbers (Stebbins, 1950; Grant, 1981) and other biosystematic approaches (e.g., Masterson, 1994), it turns out that this mostly cytogenetically-based perspective greatly underestimated the role polyploidy has played in shaping modern plant genomes. With the advent of genome sequencing and the availability of extensive EST data sets and high-density, molecular marker-based maps, it became clear that *all* plant genomes harbor evidence of cyclical, recurrent episodes of genome doubling (Wendel, 2000; Bowers et al., 2003; Blanc and Wolfe, 2004; Paterson et al., 2004; Seoighe and Gehring, 2004; Cui et al., 2006). These events have occurred at temporal scales ranging from ancient to contemporary, and are suspected to have fundamental significance to plant adaptation and function.

Given the importance of polyploidy in plants, it is not surprising that the subject has received considerable attention and has provided the focus for a number of reviews (Ramsey and Schemske, 1998; Osborn et al., 2003a; Soltis et al., 2004a; Wendel and Doyle, 2005; Adams and Wendel, 2005a; Adams and Wendel, 2005b; Durand and Hoberman, 2006; Chen and Ni, 2006). These reviews provide excellent entries into the literature on modes, mechanisms and frequency of polyploid

formation, possible ecological and functional consequences of gene and genome doubling, and the diverse array of molecular genetic mechanisms that characterize the evolution of duplicated genomes. In this mini-review, we focus on crop plants, drawing attention to some of the advances in our understanding of polyploidy that are relevant to crop improvement.

Polyploid Terminology and Modes of Formation

Traditionally, polyploidy refers to either duplication of a single genome (autopolyploidy) or from the combination of two or more differentiated genomes (allopolyploidy) (Kihara and Ono, 1926; Stebbins, 1947; Stebbins, 1971; Grant, 1981). Wendel and Doyle (2005) noted that polyploids form in many ways, from individual diploids doubling their chromosome complements (strict autopolyploid) to hybridization between individuals from highly divergent species (strict allopolyploid). Thus, there are both taxonomic (the same or different species) and cytogenetic (ability of chromosomes to pair) dimensions to these terms. Clearly there is broad overlap between the taxonomic and genetic definitions of polyploids, and in actuality these two modes of formation represent endpoints in a taxonomic-genetic continuum.

The many new tools of gene and genome sequencing and high-density genetic maps, among others, have provided novel perspectives on genome history and their composition in crop plants.

Evidence indicates that both allopolyploidy and autopolyploidy are common in nature, and that allopolyploidy probably predominates (Ramsey and Schemske, 1998; Soltis et al., 2004a; Wendel and Doyle, 2005). Both forms are common among plants important to human nutrition (Hilu, 1993), as are the "intermediate" types of polyploids such as segmental allopolyploids. Traditionally, the most useful evidence bearing on the genomic status (diploid or polyploid) and mode of formation (auto- or allopolyploid) of polyploids has derived from comparative analysis of chromosome numbers, supplemented by an analysis of karyotypic features (e.g., size, location of centromeres), and often pairing behavior in interspecific hybrids (Stebbins, 1950; Grant, 1981). Thus, within taxonomic groups, an allopolyploid was often identified because it displayed a numerical summation of the chromosome complements and karyotypes of two or more genomes of the taxonomic group to which it belongs.

Inferring the Genomic Composition of Polyploids

Using the classical sources of evidence listed above, many domesticated crops have long been recognized as having polyploid genomes. Wheat, canola, tobacco, peanut, and cotton, for example, possess allopolyploid genomes. Other domesticated crops have a history of autopolyploidy, including watermelon, strawberries, potato and alfalfa. Each of these (and related) cases was inferred following a long and rich history of botanical, genetic, and often archeological sleuthing, such that the genomic donors, or their closest living descendants, could be hypothesized and/or verified. An excellent example is offered by the cotton genus (*Gossypium*), for which several classic and modern reviews document this lengthy process of discovery, from original detection of chromosome number variation to genomic designations and inferences of progenitors (Hutchinson et al., 1947; Endrizzi et al., 1985; Wendel and Cronn, 2003).

This wealth of classical literature has provided a foundation for understanding the genomes of many of our most important crop species, but a quantum leap in our appreciation of crop genomes was ushered in by the genomic era. The many new tools of gene and genome sequencing and high-density genetic maps, among others, have provided novel perspectives on genome history and their composition in crop plants. For example, high density genetic maps uncovered a polyploid history for maize, which classically was considered to be diploid. With the advent of molecular marker-based genetic maps, for which maize was among the earliest, genome duplications were immediately recognized (Helentjaris et al., 1988; Wendel et al., 1989). Using additional molecular evidence, including DNA sequence data and population genetic considerations of coalescence times for alleles at different loci and high density genetic maps, Gaut and Doebley (1997) and Lynch and Conery (2000) suggested that this apparently diploid genome actually has a tetraploid origin. None of these insights were possible from the classical tools available before the genomics era.

An additional illustrative application of the diverse suite of modern tools available for unraveling the origin of crop plant genomes is exemplified by soybean. The soybean genome has been described as having both allo- and autopolyploid origin. An allopolyploid soybean genome was first hypothesized based on cytogenetic (Singh and Hymowitz,

1985) and molecular studies (Lee and Verma, 1984; Shoemaker et al., 1996), a proposal that gained support from detailed genetic mapping studies that revealed many duplicated genomic regions (Grant et al., 2000; Walling et al., 2006). An autopolyploid origin has also been recently hypothesized based on phylogenetic analysis of nuclear genes (Doyle et al., 2003; Straub et al., 2006); however, in this sort of analysis the diagnosis of allopolyploidy in soybean was limited by absence of the diploid progenitors or their close relatives. Recently, a novel cytogenetic approach was used to provide nearly incontrovertible evidence for an allopolyploid origin for soybean (Jackson, 2006, unpublished data); searching soybean genomic sequence data, several classes of tandem repeats were discovered, two of which, Soybean-91 (SB-91) and Soybean-92 (SB-92), were identified as putative centromeric repeats based on repeat length and frequency. Using florescence in situ hybridization (FISH), SB-92 distinguished 10 chromosome pairs suggesting that the soybean nucleus contains two distinct, co-resident genomes having two types of centromeres, presumably reflecting divergence in its two diploid progenitors.

Polyploidy is Cyclical and is Followed by Gene Loss and Diversification

An interesting twist on the soybean story is that the genetic map data revealed multiple nested duplications that appeared to reflect an even more ancient round of polyploidy at some point in the ancestry of the genus (Shoemaker et al., 2006). The implication is that even the ancestral "diploid" genome donors of modern "allopolyploid" soybean were themselves stabilized paleopolyploids from an earlier round of genome duplication. This nested history of cyclical or episodic polyploidy is the rule rather than the exception for all plant genomes that have been investigated in detail. Examples include *Arabidopsis* (Vision et al., 2000; Bowers et al., 2003), even with its quintessentially streamlined genome, as well as an ancient duplication at the base of the grasses (Paterson et al., 2004) and a more recent superimposition of an additional polyploidization in the maize lineage (Gaut and Doebley, 1997; Gaut, 2001), legumes (Shoemaker et al., 2006), and cotton (Rong et al., 2004).

Ancient duplication events of crop plant genomes can also be detected in EST sequences. At present there exist tens of thousands to hundreds of thousands of EST sequences for most major crop species. By conducting similarity searches among ESTs within species, it is possible to identify genes duplicated by various evolutionary processes, including those retained since a whole-genome duplication event. Because nucleotide substitutions at synonymous sites (Ks) evolve in a quasi-neutral manner, the amount of divergence between any locus-pair will be a proxy for the age of the duplication (in the absence of gene conversion). In a distribution of Ks values among pairs of putatively duplicated loci, ancient genome duplications appear as "peaks" or "bumps" because many genes were duplicated at the same time. Searching for such peaks within plant EST assemblies, Blanc and Wolfe (2004a), Schlueter et al. (2004), and Cui et al. (2006) identified ancient duplication events within many different 'diploid' plant species covering a broad spectrum of angiosperm diversity, including many of our most important crop species. Some of these duplication events may be shared (Bowers et al., 2003), but a recent analysis of cotton, chocolate, and *Arabidopsis* ESTs suggests that separate genome duplication events occurred in the cotton and *Arabidopsis* lineages after speciation (Rapp, Udall, Wendel, 2006, unpublished). Using predicted proteins rather than EST sequences and a more complete model, Maere et al. (2005) verified this approach of dating with similar results. Thus, the concept of an iconic "diploid" plant genome is an antedated one that oversimplifies genomic evolutionary history.

The foregoing sequence-based approaches have provided powerful tools for diagnosing and defining the history of genome duplications, and also have demonstrated that each duplication event has been followed by a subsequent loss of much of the duplicated material. This process of genomic diploidization appears to be accompanied by significant amounts of gene loss after genome doubling, and is likely responsible for much of the deviation in colinearity among relatively closely related plants, such as the cereals (Paterson et al., 2003). One of the more intriguing aspects of differential retention of duplicated genes concerns the patterns of gene loss versus survivorship. In *Arabidopsis*, some classes of genes have been preferentially retained whereas other classes have been preferentially lost (Seoighe and Gehring, 2004; Blanc and Wolfe, 2004a; Blanc and Wolfe, 2004b; Chapman et al., 2006). The chromosomal location of retained versus lost genes also is of interest, including the degree to which retained genes are clustered. Recently, Thomas et al. (2006) used the remnant clustering of retained duplicates in *Arabidopsis* to characterize a bias in the process of gene loss (fractionation) with respect to homeolog. Looking at possible functions of retained duplicates, they suggested that the likelihood of duplicate retention was correlated with the number of functional interactions among the gene products.

The concept of duplicate gene retention and loss is inseparable from a consideration of duplicate gene function. In *A. thaliana*, Blanc and Wolfe (2004b) showed

that, about 2/3 of recently duplicated gene pairs have undergone functional diversification, consistent with theory that indicates that duplicated genes are likely to survive mutational pseudogenization only when they acquire something new and useful to do (Lynch and Conery, 2000; Lynch and Force, 2000). Some gene pairs have completely subfunctionalized, others appear to have retained their ancestral function, and some display a mix between these two extremes, with overlapping novel and retained functions (Thomas et al., 2006; Chapman et al., 2006). Scrutiny of individual genes duplicated by an ancient polyploid event has uncovered interesting results. For example, Causier et al. (2005) found two orthologs of *A. thaliana* and *Antirrhinum majus* that have reciprocally evolved to opposite functions. Without careful consideration, extrapolation of model plant functional genomics may be limited by paralog subfunctionalization because gene function in model species may not correctly predict gene function in particular crop plants.

The Role of Polyploidy in Crop Improvement

For more than 60 yr, polyploidy has been considered to be important largely because of concepts of genome "buffering," increased allelic diversity, increased or "fixed" heterozygosity, and the opportunity for novel phenotypic variation to arise from duplicated genes acquiring new function (Stebbins, 1950). Recognition that modern plant genomes harbor a complex history of polyploidization followed by fractionation and duplicate gene diversification provides the opportunity to reevaluate the importance of polyploidy for crop improvement. In this section, we explore the possible connections between gene and genome doubling and plant improvement.

Genome Buffering and Allele Dosage

Echoing the consensus of many contemporary scientists, Stebbins (1971) proposed that the presence of multiple genomes in polyploid plants would retard the response to natural (or artificial) selection due to mutation and recombination. This view was based on the assumption of equal mutation rates for polyploids and diploids and often tetrasomic segregation ratios. Both of these assumptions may represent oversimplifications, however. Clearly, some *traits* may be 'buffered' from mutation by genome redundancy, even when the underlying genes are mutated. For example, consider the *waxy* locus that encodes granule bound starch synthase I (GBSS). GBSS largely determines the amount of amylose. Genome buffering for the *waxy* locus was first characterized in potato where a gene dosage population was obtained by crossing two genotypes that were duplex for the null GBSS allele (Flipse et al., 1996). Although GBSS activity was linearly correlated with dosage, amylose content in tubers was not; hence, there was "buffering" against the mutant alleles. Similarly, in allohexaploid wheat, each of the three *waxy* loci on three homeologous chromosomes (7AS, 7BS, and 7DS) encodes a different GBSS isoform (Denyer et al., 1995; Yamamori et al., 2000). Natural mutations for each of the three loci exist, but not the extreme phenotype containing all null alleles in a single genotype. Because of 'buffering', synthesis of a full *waxy* wheat required molecular markers to combine null alleles using appropriate crosses (Yamamori et al., 2000). In both potato and wheat, simple phenotypic segregation ratios of amylose content determined by one or two GBSS null alleles, as expected in the absence of buffering, appear instead as a quantitative trait due to multiple copies and its nonlinear accumulation of amylose.

In addition to buffering phenotype against single-locus mutants, polyploidy affects allelic dosage. When a polyploid nucleus is formed, every gene in the genome becomes instantly duplicated, leading to novel dosage effects that may be important to phenotype (Guo et al., 1996). Several studies have suggested that purifying selection of key regulator genes has preserved function after duplication (Chapman et al., 2006). One such gene is FLOWERING LOCUS C (FLC), a master control gene of flowering time that functions in an additive or rheostatic manner (Michaels and Amasino, 1999) in *Arabidopsis* and *Brassica* (Schranz et al., 2002). A *Brassica* homolog of *A. thaliana* FLC was duplicated three times after the divergence of *Arabidopsis* and *Brassica* (Yang et al., 2006) and all four copies operate in the same manner in *B. rapa* (Kole et al., 2001; Schranz et al., 2002; Pires et al., 2004). In two recent QTL studies of allotetraploid *B. napus*, Quijada et al. (2006) and Udall et al. (2006) identified flowering time QTL that span the genomic position of each of the eight copies of *BnFLC*. Collectively, these QTL for flowering time explained a large portion of genetic variance in field trials, suggesting that alleles at all *FLC* loci can have incremental effects on flowering time. An agronomic possibility suggested by this observation is that it might be possible to manipulate alleles at the eight (or possibly fewer) FLC loci to convert an annual spring *B. napus* cultivar to one with a biennial growth habit.

Increased Allelic Diversity and Heterozygosity

It is a truism that allelic copy *number* increases with ploidy level, potentially leading to novel phenotypes via dosage effects, as described above. Allelic *diversity* also increases during allopolyploidy, when two (or more) divergent genomes become joined in a common nucleus. This intergenomic heterozygos-

ity will apply not only to single loci but to the entire genome, and hence to specific chromosome blocks of possible interest. For example, intergenomic heterozygosity has been shown to have positive effects on oilseed production in *B. napus* (Osborn et al., 2003b). Osborn et al. (2003b) found lower oilseed seed yields associated with a loss of intergenomic heterozygosity when recombinants of homeologous recombination were evaluated alongside lines containing the parental chromosomal configurations. Effects of intergenomic heterozygosity have similarly been suggested to impact QTL for seed yield and other traits in additional populations of *B. napus* (Udall et al., 2006; Quijada et al., 2006). In cotton, diploid *G. arboreum* and *G. herbaceum* (A genome species), and tetraploid *G. barbadense* and *G. hirsutum* (AD genome species) have been domesticated for their epidermal seed trichomes (cotton fiber), while the D genome diploids of Central and South America produce short, tightly adherent and useless fiber on their seed (Applequist et al., 2001; Wendel and Cronn, 2003). In terms of fiber production, the tetraploid cottons dominate the global market because they produce longer, finer, and stronger fiber than do their diploid relatives. In a QTL mapping study, Jiang et al. (1998) found several QTL located on the D genome, suggesting that D genome loci had been recruited for the synthesis of fiber subsequent to polyploid formation. In bread wheat, rye translocations have been used to introgress novel phenotypic variation, including abiotic stress resistance (Singh et al., 1998), greenbug resistance (Sebesta and Wood, 1978), Hessian fly resistance (Friebe et al., 1999), and potentially increase seed yield (Villareal et al., 1996). While genes introgressed from various rye segments may exist in a hemizygous condition within the wheat nucleus, such phenotypic effects and chromosome segment substitutions are possible because of hexaploid gene redundancy—minus a few tolerable, but sometimes important, exceptions (Lukaszewski, 2000).

Having only a single genome type and exhibiting multisomic inheritance, autotetraploids do not have intergenome heterozygosity; however, it may be possible to combine or pyramid blocks of genes containing diverse alleles into a single polyploid line, with the goal being to maximize allelic diversity. For example, Chase (1963) proposed an 'analytic breeding' method for autotetraploid potato, where improvements could be made at the diploid level and then transferred to the tetraploid level (Chase, 1964). Ironically, because autopolyploidy was thought to be maladaptive (Stebbins, 1950), the most dramatic example of increased heterozygosity is in autotetraploid alfalfa, where a single locus can potentially have up to four different alleles. Bingham et al. (1994) demonstrated that maximum heterozygosity was obtained after intermating double-cross progeny for one or more additional generations beyond the final F_1 line, resulting in a phenomenon they termed 'progressive heterosis'. Similar strategies to maximize allele diversity first at the diploid level have also been developed to improve banana (Ortiz, 1997) and sweet potato (Orjeda et al., 1991).

One important aspect of allelic diversity is that the concept applies not only to coding regions but also to regulatory sequences, which, like the coding regions they control, experience independent evolutionary pressures while separated in diploid progenitors. Because much if not most evolutionary change is thought to arise from altered gene expression rather than from protein evolution per se (Wittkopp et al., 2004; Haberer et al., 2004; Stupar and Springer, 2006; Clark et al., 2006), an important dimension to polyploidy may be the increased variance of expression that accompanies increased allelic diversity in regulatory regions. Regulatory divergence at the diploid level leads to novel allele combinations in derivative polyploids when both loci are reunited into a single nucleus. An example of this is provided by recent work in *Arabidopsis*, where cis-regulatory changes were demonstrated between *A. thaliana* and *A. arenosa* FLC alleles (Wang et al., 2006a). In artificial (synthetic) allotetraploid *Arabidopsis*, these two alleles behaved as "strong" and "weak," notwithstanding the conserved coding region.

The foregoing example highlights several aspects of allelic diversity and dosage that may need to be considered in crop improvement efforts. This includes the several challenges inherent in identifying and distinguishing coding sequence effects on phenotype from those arising from regulatory diversity. In addition, even when the desired "allele" (regulatory or coding) has been chosen, there may be several strategies for optimizing the plant breeding effort. These include: (i) increasing allelic dosage and if desired transgressive segregation by homologous or homeologous recombination, (ii) selecting a single "dominant" allele for its phenotypic effects but relaxing dosage selection, thereby reducing the number of selected markers and their accompanying linkage drag. and (iii) combinations of the above.

Novel Phenotypic Variation

Novel phenotypic variation is known to accompany polyploidization (Soltis et al., 2004a). In synthetic allotetraploid *Brassica*, for example, significant *de novo* variation was found for flowering time (Schranz and Osborn, 2000) and for several life history traits (Schranz and Osborn, 2004). In tobacco, three independent synthetic allopolyploid *Nicotiana* × *mierata* were examined for traits induced by herbivore feeding (Pearse et al., 2006). Inheritance of

metabolite compounds was initially complex but appeared to stabilize over a few generations. There are many different mechanisms that could give rise to these types of novel phenotypic variation (see below) and some of this phenotypic variation may provide a selectional basis for crop improvement.

Polyploidization and Genetic Bottlenecks

While the potential allelic diversity within a polyploid genome is greater than that of diploid genomes, often there exists higher levels of natural variation in related diploid species because the process of polyploid formation entailed a genetic bottleneck. The relatively recent realization that many polyploids originate repeatedly from identical or similar progenitors (Soltis et al., 2004b) has required that we reconsider the severity of polyploidization-associated genetic bottlenecks, but in general, a bottleneck is expected. Accordingly, much effort has been expended at gene pool enrichment through trait introgression and interploidal hybridization in nearly every polyploid crop, including wheat, canola, cotton, and potato, to name a few. Indeed, much of the 'untapped' genetic variation needed to continue to feed the world lies within non-crop, often diploid relatives of our major crop species (Tanksley and McCouch, 1997). Examples abound of interploidal introgression from wild relatives; in *Brassica*, these include seed color (Chen et al., 1988), photoperiod insensitivity (Akbar, 1989), clubroot resistance (Bradshaw et al., 1997; Manzanares-Dauleux et al., 2000), and silique shattering resistance (Prakash and Chopra, 1990; Morgan et al., 1998). In cotton, recent studies of Australian (Ahoton et al., 2003) and Hawaiian (Waghmare et al., 2005) species have focused on introgression of genetic variation from diploid and tetraploid taxa, respectively. Additional efforts introgressing specific traits into cultivated tetraploid cotton are numerous and have been reviewed (Percival et al., 1999; Mergeai, 2006). In autopolyploids, transfer of genetic material between diploid and tetraploid levels is somewhat simplified by a single genome, a common cytoplasm, diploid gametes, and a recognition of endosperm balance (Bushell et al., 2003; Carputo et al., 2003). As examples, efforts have been made to improve alfalfa and potato at the diploid level and then transfer the improvements to the tetraploid level of commercial cultivars (Kimbeng and Bingham, 1997; Carputo et al., 2003).

Genomic Consequences of Polyploidy

The successful merger of two divergent genomes or the doubling of one genome requires a series of genetic and genomic adjustments that govern proper centromere recognition, chromosome pairing, and balanced assortment of chromosomes during meiosis. Genome doubling may further be complicated by other factors that may collectively be referred to as 'genomic shock' (McClintock, 1984), even though many of these were not known when McClintock coined the phrase. These include a diverse suite of genetic and epigenetic mechanisms that influence gene expression and function as well as genomic organization.

Loss of Duplicated Chromatin

As discussed above, the history of plant genomes is replete with duplications followed by fractionation of the duplicated genome, including frequent loss of duplicated genes (Adams and Wendel, 2005; Lockton and Gaut, 2005; Yang et al., 2006; Clark et al., 2006). In recently formed polyploids, some duplicated genes or fragments have been lost shortly after polyploid formation (Song et al., 1995; Pontes et al., 2004; Skalicka et al., 2005). In resynthesized *Brassica*, for example, Song et al. (1995) observed that rapid genomic changes accompanied neopolyploidization of interspecific F_1 hybrids, including the loss of parental RFLP fragments. In a related study of 49 independently resynthesized *Brassica* lines, Lukens et al. (2006) found that changes in the S_0 generation were uncommon, and that nearly all changes were explained by indels in the *B. rapa* (A) genome. In subsequent generations, several fragment losses were found (along with a concomitant duplication) due to homeologous recombination resulting in non-reciprocal translocations. That these types of genomic changes in polyploids may be phenotypically relevant was shown by Pires et al. (2004), who illustrated that homeologous recombination altered the number of "early" and "late" FLC alleles, thereby generating increased flowering time variation among the resynthesized lines.

Gene loss after polyploidization also has contributed to phenotypic variation in wheat. Loss of parental genes and fragments was demonstrated in synthetic wheat allopolyploids (Liu et al., 1998) and their close relatives (Feldman et al., 1997), both immediately after polyploid formation (synthetic allotetraploids; Shaked et al., 2001; Kashkush et al., 2002), and in later generations (synthetic allohexaploids; Ma et al., 2004). Insights into some of the possible phenotypically relevant consequences of gene loss have emerged from a recent study at the hardness locus in wheat (*Ha*; Chantret et al., 2005). This study is particularly intriguing in that the authors provide evidence for independent loss and rearrangements of a region containing multiple duplicated genes surrounding the hardness locus, during domestication at both the tetraploid and hexaploid levels. The mechanism of gene-region loss appears to be intrastrand recombination between long terminal repeats of retrotransposable elements.

Gene Expression Changes are Widespread

Genome changes that accompany polyploid formation also impact gene expression levels (see Wendel, 2000; Osborn et al., 2003a; Adams and Wendel, 2005; Chen and Ni, 2006 for reviews) and changes in gene expression that accompany polyploidization has been the subject of numerous, recent investigations (Comai, 2000; Kashkush et al., 2002; Wu et al., 2003; He et al., 2003; Adams et al., 2003; Adams et al., 2004; Wang et al., 2004; Madlung et al., 2005; Hegarty et al., 2005; Lai et al., 2006; Tate et al., 2006; Wang et al., 2006b). Little is known regarding the precise intergenomic interactions that occur after polyploidization, but a general picture is emerging regarding the scope and scale of the phenomenon. From a mechanistic standpoint, up- or downregulation of gene expression levels is thought to depend on *cis*- and *trans*-acting effects on a gene-by-gene basis. For example, the promoter of gene 1 in genome A may be receptive to transcription factors encoded in both genomes A and B while the promoter of gene 2 (genome A) may have been modified such that it only responds to transcription factors of genome A. Considering the number of eukaryotic genes in any polyploid genotype grown in multiple environments, there are likely an infinite number of first and higher-order interactions. The outcomes of such interactions will depend on many factors, including the amount of regulatory divergence between genomes A and B and the epigenetic state of both loci at and following hybridization. While it is not yet possible to predict the outcomes of these complex regulatory interactions for gene expression in polyploids, the process has been discussed and modeled in several important papers (Riddle and Birchler, 2003; Comai, 2005; Veitia, 2005).

One generalization that has emerged from the empirical literature is that gene expression in polyploids often is non-additive. In particular, repression or silencing of gene expression has frequently been found in synthetic and natural allopolyploids, and this is observed far more frequently that up-regulation or novel gene expression. Using cDNA-AFLP techniques, approximately 5% of loci were repressed in *Tragopogon* (Soltis et al., 2004b) and cotton (Adams et al., 2004) polyploids, whereas about 10% of genes were reported as repressed in *Arabidopsis* polyploids (Wang et al., 2004). Measurements of polyploid gene expression on a larger scale have also suggested repression of gene expression (Hegarty et al., 2005; Wang et al., 2006b). For example, Wang et al. (2006b) used microarrays to identify hundreds of genes that are differentially expressed between two tetraploid *Arabidopsis* entities (synthetic autotetraploid *A. thaliana* lines and *A. arenosa*, a natural allotetraploid). In two synthetic allotetraploid derivatives of these parents, there was global alteration of gene expression, with a predominant downregulation of *A. thaliana* genes.

In principle, absolute levels of gene expression (defined here as transcript concentration at a particular stage and cell or organ type, under identical growing conditions) may be maintained between diploid and polyploids, yet the contributions to the transcript pool from each homeolog may be unequal. In the *Arabidopsis* study by Wang et al. (2006b), comparisons of the average expression level of the genes that were differentially expressed in the tetraploid parents to expression levels found in two synthetic allotetraploids suggested that about 95% of the repressed genes were those where the *A. thaliana* transcript was up-regulated relative to *A. arenosa*. The authors infer a genome-wide bias against *A. thaliana* gene expression, consistent with overall plant phenotype (the allotetraploids look more like *A. arenosa* than *A. thaliana*) and the direction of previously reported nucleolar dominance (Chen and Pikaard, 1997).

Homeologous expression biases have been measured directly in wheat using pyrosequencing (Mochida et al., 2003) and in cotton using single-stranded conformational polymorphism (SSCP) analysis (Adams et al., 2003; Adams et al., 2004) and bioinformatics combined with SSCP (Yang and Chen, 2006) or custom microarrays (Tate et al., 2006). First described in recently synthesized and natural cotton (Adams et al., 2003; Adams et al., 2004), biases in homeologous expression in eight genes were found to be tissue-dependent, random in terms of function, and, on average, slightly biased with respect to the two co-resident genomes. The results suggested an almost immediate initiation of subfunctionalization with the onset of polyploid formation, where gene expression has somehow been compartmentalized to different tissue types. In each of the above studies, biases in homeolog expression have been inferred in the polyploid nucleus. At present, little is known regarding the functional consequences of such homeolog bias, but it is tempting to speculate that as our understanding improves, this knowledge may be harnessed for purposes of plant breeding. Perhaps developmentally regulated, intergenomic expression diversity will be found to endow allopolyploids with greater plasticity of response to stress, as found in F_1 hybrids of maize (Guo et al., 2004).

A cautionary note relevant to most studies to date is that gene expression has usually been evaluated by examining mRNA rather than protein levels; variation in mRNA levels may not translate directly to protein levels for a variety of reasons. Looking at gliaden and glutenin proteins from wheat endosperm, Galili and Feldman (1984) observed suppression of the D-genome isoform in a synthetically derived allohexaploid line.

Similar observations have been made in *Brassica*, where a *B. rapa* isoform of glucosephosphate isomerase was silenced in seedlings of resynthesized allotetraploids (Chen et al., 1989). More recently, and using a more global approach, Albertin et al. (2006) assessed changes in the entire proteome in allopolyploid roots and stems relative to diploid progenitors in *Brassica*. They found proteomic patterns slightly closer to *B. rapa* than to *B. oleracea*, supporting claims that expression level changes are reflected in the proteome and that an at least partly stochastic mechanism of expression may exist for transcriptional regulation at the genome level.

This process of genomic diploidization appears to be accompanied by significant amounts of gene loss after genome doubling, and is likely responsible for much of the deviation in colinearity among relatively closely related plants, such as the cereals.

Developing a mechanistic understanding of gene expression in polyploids requires several complementary perspectives. One might be construed as quantitative, involving modeling of interactions among variably present and variably acting, *trans*-activating proteins (Riddle and Birchler, 2003; Comai, 2005; Veitia, 2005). A second requirement is developing an enhanced understanding of the various molecular mechanisms that determine the *cis*- and *trans*-effects, such as methylation, RNAi, and transposon activation. At present, there exist few relevant studies, but illustrative examples may be provided for each of these and other mechanisms. In wheat, for example, Nomura et al. (2005) described *cis*-effects controlling benzoxazinone levels that originated in diploid relatives of wheat and which have been retained through polyploidization. In a survey of 49 independently resynthesized *B. napus* lines, Lukens et al. (2006) found few genomic changes, but substantial changes in DNA methylation, whereas in the same population, Pires et al. (2004) reported few changes in methylation in later generations. Kashkush et al. (2003) in an interesting and potentially important discovery, showed that readout transcription of a transposable element could affect transcript levels of neighboring genes. Though unlikely to cause tissue-dependent or genome-wide gene suppression, retrotransposons have been activated in recent polyploids, and their reinsertion may cause suppression of expression of nearby genes (Kashkush et al., 2003; Madlung et al., 2005). The epigenetic state of the genome may be controlled by the RNAi pathway (Pontes et al., 2006). Chen and Ni (2006) recently discussed the possible role of RNAi in gene regulation in polyploids, and developed a simple conceptual model for perspective.

The foregoing examples are intended to illustrate at least part of the spectrum of molecular mechanisms that may influence gene expression in polyploids. From the standpoint of crop improvement, we hasten to add that to date, connections of nonadditive gene expression to phenotypic variation in important traits remain elusive. Perhaps the alterations described until now have only incremental effects, too small to be measured or, perhaps, they have not affected 'important' genes. Because the phenomenon of gene expression alteration in polyploids is so prominent, however, it is likely that this vacuum will soon be filled.

Novel Epistatic Interactions

No gene acts alone, so of course novel epistatic interactions also are possible in allopolyploid plants. The gene *FLC* is epistatically activated by *FRI* to suppress flowering in *Arabidopsis* (Johanson et al., 2000). With *FLC*, Wang et al., (2006a) recently provided an elegant example of nonadditive expression levels in synthetic *Arabidopsis* polyploids. The gene *FLC* is epistatically activated by *FRI* to suppress flowering in *Arabidopsis* (Johanson et al., 2000). When the *A. thaliana* and *A. arenosa* genomes were combined in an allopolyploid, *AaFRI* activated *AtFLC*, not *AaFLC* due to *cis*-modifications at the *AaFLC* locus, creating a very late flowering plant. Thus, the most efficient allelic combination was not restricted by intergenomic interactions; in fact, the *trans*-acting *A. arenosa* transcription factor (*AaFLC*) followed the epigenetic activation queues of the *A. thaliana* genome (*AtFLC*) via methylation and acetylation. Such genetic combinations and transgressive phenotypes are not possible at the diploid level and highlight the added flexibility of the polyploid genome and may partially explain the response to selection during polyploid crop improvement.

Endosperm Effects

During hybridization, plants undergo double fertilization, where one sperm fertilizes the egg to form the diploid zygote and the other combines with two haploid polar nuclei to form the triploid endosperm. Thus up until now, our discussion has considered only one half of the genome mergers that occur during hybridization! Perhaps because the genetic mate-

rial of the endosperm is not included in the germline, the effects of polyploidization on endosperm biology have not been frequently considered. Proper genome dosage has long been known to affect endosperm development in maize (Birchler, 1993) and potato (Ehlenfeldt and Ortiz, 1995), where a 2:1 endosperm balance of maternal-to-paternal ratio haploid genomes is necessary if postzygotic barriers that terminate seed development are to be obviated.

While making crosses to develop allotetraploid *Arabidopsis*, it was noticed that tetraploid *A. thaliana* × diploid *A. suecica* hybridizations produced many more seeds than other pairwise combinations (Comai et al., 2000). In a recent and elegant study, Josefsson et al. (2006) discovered derepression of heterochromatic repeats, including retrotransposons, in incompatible crosses. They proposed a dosage-dependent induction (DDI) model of chromatin as a requirement of proper endosperm development in interspecific hybrids. The model hypothesizes a fine adjustment between regulator and target sites in male and female gametes; because these diverge at the diploid level following speciation, subsequent hybridization of now diverged systems could alter regulatory repression of heterochromatic repeats. In essence, "the female gamete must provide sufficient quantity of repressive factors to saturate available binding sites in the male gamete." Proper regulation of dosage-dependent chromatin could be the underlying mechanism for endosperm balance sensitivity found in interspecific hybridization of crop plants. Continued research is necessary, but understanding endosperm (or chromatin) requirements during interspecific hybridizations may unlock one of the largest genetic transmission barriers for the formation of allopolyploid plants.

Genome Space

Because nearly all modern breeding programs use molecular markers in combination with phenotypic evaluations for selection, additional understanding of polyploidy can aid crop improvement. It has often been said that the ideal molecular marker is the gene itself and, increasingly, the genes underlying phenotypic variation have been characterized. Eventually characterized genes, including selectable markers and transgenes, will saturate the genomic landscape of cultivated plants. However, even with this complete knowledge of the gene-to-phenotype equation, and perfect genetic maps, generating the individuals that have the desired genotype at all loci of interest would likely take a prohibitory large population. Thus, in practice, sections of the genome are incrementally recombined and selected to eventually create the desired haplotype (Servin et al., 2004), while controlling linkage drag (Hospital, 2001). With numerous targets, the amount of the genome allowed to recombine for a genetic response to selection of other traits is greatly reduced. By understanding the polyploid nature of the genome, the number of essential markers (or genes) might be optimized by selecting the most effective allele at paralogous or homeologous loci, particularly because many allelic effects appear to be nonadditive. Conversely, for genes characterized to act as rheostats, additional copies could be pyramided to increase the phenotypic variance beyond a two-copy genome.

Summary

Breeding of polyploid crops has been ongoing ever since crop plants were first domesticated. While genetic gains have been obtained via selection, evaluation, and recombination, successful selection for crop improvement may increasingly depend on understanding and unraveling the complexities of genetic variation that underlies the phenotype. The genomic revolution has vastly expanded our knowledge of plant genomes, leading to a clearer understanding of the dynamics of polyploid plant genomes, and the spectrum of phenomena that accompany polyploidy in both model and crop plants. Many of the examples discussed here feature allopolyploid crops, but new ways of understanding autopolyploids will certainly be brought to light once the genomes of *Medicago truncatula* and *Solanum tuberosum* have been fully sequenced and assembled. A deeper understanding of polyploidy holds great promise for crop improvement by improving the connection between genotype and phenotype and bridging gaps for the genetic transmission of agronomic traits between crop species and their unadapted relatives.

Acknowledgments

We thank the National Science Foundation and the U.S. Department of Agriculture—National Research Initiative for their financial support.

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