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Allopolyploidy-Induced Rapid Genome Evolution in the Wheat (*Aegilops–Triticum*) Group

Hakan Ozkan,[1] Avraham A. Levy, and Moshe Feldman[2]

Department of Plant Sciences, The Weizmann Institute of Science, Rehovot 72100, Israel

To better understand genetic events that accompany allopolyploid formation, we studied the rate and time of elimination of eight DNA sequences in F1 hybrids and newly formed allopolyploids of *Aegilops* and *Triticum*. In total, 35 interspecific and intergeneric F1 hybrids and 22 derived allopolyploids were analyzed and compared with their direct parental plants. The studied sequences exist in all the diploid species of the Triticeae but occur in only one genome, either in one homologous pair (chromosome-specific sequences [CSSs]) or in several pairs of the same genome (genome-specific sequences [GSSs]), in the polyploid wheats. It was found that rapid elimination of CSSs and GSSs is a general phenomenon in newly synthesized allopolyploids. Elimination of GSSs was already initiated in F1 plants and was completed in the second or third allopolyploid generation, whereas elimination of CSSs started in the first allopolyploid generation and was completed in the second or third generation. Sequence elimination started earlier in allopolyploids whose genome constitution was analogous to natural polyploids compared with allopolyploids that do not occur in nature. Elimination is a nonrandom and reproducible event whose direction was determined by the genomic combination of the hybrid or the allopolyploid. It was not affected by the genotype of the parental plants, by their cytoplasm, or by the ploidy level, and it did not result from intergenomic recombination. Allopolyploidy-induced sequence elimination occurred in a sizable fraction of the genome and in sequences that were apparently noncoding. This finding suggests a role in augmenting the differentiation of homoeologous chromosomes at the polyploid level, thereby providing the physical basis for the diploid-like meiotic behavior of newly formed allopolyploids. In our view, this rapid genome adjustment may have contributed to the successful establishment of newly formed allopolyploids as new species.

INTRODUCTION

Polyploidy has played an important role in the evolution of higher plants. Between 50 and 70% of all angiosperm species are of polyploid origin (Stebbins, 1971; Soltis and Soltis, 1993; Masterson, 1994). Recent restriction fragment length polymorphism (RFLP) analyses and genome sequencing have shown that even classic diploid plant species such as maize and Arabidopsis are actually ancient polyploids (Helentjaris et al., 1988; The Arabidopsis Genome Initiative, 2000). Also, vertebrates may be ancient polyploid organisms (Sidow, 1996; Spring, 1997). The widespread occurrence of polyploidy has been attributed to the potential of polyploid species to adapt to a wider range of habitats and survive better in unstable climates than their diploid progenitors (Ehrendorfer, 1980; Levin, 1983; Novak et al., 1991). Polyploid evolution therefore has been a subject of intensive study for more than half a century. There are several reviews of polyploid evolution, including types or categories of polyploids, mode of formation of polyploids, ecological and evolutionary attributes, polyploidy as a speciation mechanism, cytogenetic behavior of polyploids, and genetic consequences of polyploidy (Stebbins, 1971; Dewet, 1980; Levin, 1983; Soltis and Soltis, 1999; Wendel, 2000).

During the last two decades, molecular data have provided new insights into polyploid evolution, leading to significant progress in understanding the mechanism and evolutionary aspects of polyploidy. Yet, few studies have been performed on the genomic changes that occurred upon the formation of polyploids, particularly allopolyploids, changes that might have led to a more harmonious behavior and activity of the different constituent genomes. It is assumed that these genomic changes facilitated the establishment of the newly formed polyploids as successful species (Sasakuma et al., 1995; Song et al., 1995; Wendel et al., 1995; Feldman et al., 1997; Liu et al., 1998a, 1998b; Comai et al., 2000).

One of the intriguing questions on the evolution of polyploids in plants regards the mechanism(s) of stabilizing the newly formed allopolyploids. Little is known about the prevalence of this process and the factors involved. Previous

[1] Current address: Department of Field Crops, Faculty of Agriculture, University of Cukurova, Adana 01330, Turkey.

[2] To whom correspondence should be addressed. E-mail moshe.feldman@weizmann.ac.il; fax 972-8-9344160.

studies (Feldman et al., 1997) have indicated that the stabilization of allopolyploids involves two major events: (1) cytological or chromosome diploidization, in which meiotic pairing of homoeologous chromosomes (genetically related chromosomes of different genomes) is suppressed, thus leading to a pairing pattern similar to that of diploids, that is, exclusive bivalent pairing of homologous chromosomes; and (2) genetic diploidization, in which duplicate genes are either silenced or expressed at reduced levels. A well-known example of diploidization at the chromosome level has been described for tetraploid (durum) and hexaploid (bread) wheat. In this case, two dominant genes, *Ph1* and, to a lesser extent, *Ph2*, were considered to be responsible for the cytological diploidization by suppressing homoeologous pairing (Riley and Chapman, 1958; Mello-Sampayo, 1971; Sears, 1976). However, *Ph1*-like genes were not found in polyploids of the related genus *Aegilops*, which exhibits diploid-like meiotic behavior. Moreover, plants of hexaploid wheat deficient for *Ph1* exhibit much lower levels of homoeologous pairing ($\sim$0.6 multivalents per cell; Sears, 1977) than was expected from the level of pairing in hybrids among the three diploid progenitors. This fact indicates that diploid-like meiotic behavior in polyploid wheat depends on factors other than *Ph1*.

In a recent study (Feldman et al., 1997), it was found that low copy, presumably noncoding DNA sequences, which are present in all diploid species of the Triticeae, were eliminated from one of the two genomes of tetraploid wheat and from two of the three genomes of hexaploid wheat. Newly formed allopolyploids of *Aegilops* and *Triticum* are an excellent system for studying the nature, frequency, and time of this sequence elimination. The genome relationships between the naturally occurring *Aegilops–Triticum* allopolyploids and their diploid progenitors have been well established (Kimber and Tsunewaki, 1988; Feldman et al., 1995). Recent comparisons in the *Aegilops–Triticum* group between the genomes of synthetic allopolyploids and those of their diploid progenitors showed that allopolyploidy-induced sequence elimination occurred very early in the history of the nascent allopolyploid species (Feldman et al., 1997; Liu et al., 1998a). In these studies, however, 3- to 6-year-old synthetic allopolyploids were compared with their parental accessions rather than with the exact parental plants. It was impossible, therefore, to distinguish between genomic changes, either those that occurred as a result of hybridity at the diploid or polyploid level (in F1) or those that occurred as a result of polyploidy (in the first generations of the allopolyploids), and segregation of parental polymorphisms within the diploid progenitor accessions. Also it was impossible to determine either the timing or the rate of change.

The main objective of this research was to study short-term changes induced by allopolyploidization in the wheat (*Aegilops–Triticum*) group. Specifically, this research had the following objectives: (1) to study the type of changes in several low-copy DNA sequences that exist in all of the diploid species of the Triticeae but occur in only one genome in natural polyploids, either in one homologous pair (chromosome specific) or in several pairs of the same genome (genome specific); (2) to determine the prevalence and time (in terms of generations) of genomic changes; and (3) to compare the pattern, rate, and time of sequence elimination in newly synthesized allopolyploids whose genomic constitution is analogous to that of natural polyploids with those of allopolyploids not analogous to natural polyploids. We crossed several species of *Aegilops* and *Triticum* and produced a number of F1 hybrids and first generations (S1, S2, and S3) of newly synthesized allopolyploids at different ploidy levels. By analyzing this material, it was found that rapid genomic changes are a general phenomenon in newly formed allopolyploids of the wheat group. Elimination of genome-specific sequences (GSSs) started in the F1 generation and was completed in the first and second generations of the allopolyploids, whereas elimination of chromosome-specific sequences (CSSs) started in the first generation of the allopolyploids and was completed in the second or third generations. Sequence elimination of either GSSs or CSSs started earlier and was more prominent in allopolyploids whose genomic constitution was analogous to that of natural polyploids than in allopolyploids not analogous to natural polyploids.

RESULTS

Each of the five CSSs and the three GSSs was labeled and hybridized to genomic DNA, digested with four or five different restriction enzymes, from F1 hybrids, S1, S2, and, in five cases, S3 generations of newly synthesized allopolyploids and their parental plants. Two types of deviations from the expected hybridization pattern were observed: "loss" of a sequence from the genome(s) donated by one of the two parents, and, rarely, "gain" of a novel fragment not detected in the parental lines. The loss of a sequence was predominant. In most cases, loss was complete, with all bands from one parent being absent in the allopolyploid (Figures 1 to 4). Partial loss (disappearance of one or several but not all bands from one parent) also was observed in several cases, especially for GSS probes (Figure 1). The loss pattern was observed with all enzymes tested that produced recognizable polymorphism between the parents (Figure 1). In most cases, the loss pattern was unidirectional, namely, elimination of fragments from one parental genome and preservation of those of the other genome. However, partial loss of fragments from both parents was observed in several cases in "nonnatural" allopolyploids, but complete loss from both parents was never found. The gain of a novel fragment was observed only in F1 plants between TL02 and TU02 when the DNA was digested with EcoRI but not with the other enzymes (data not shown). Moreover, this change was not observed in any of the newly synthesized allopolyploids and was not inherited in the allopolyploid TL02–TU02. It was as-

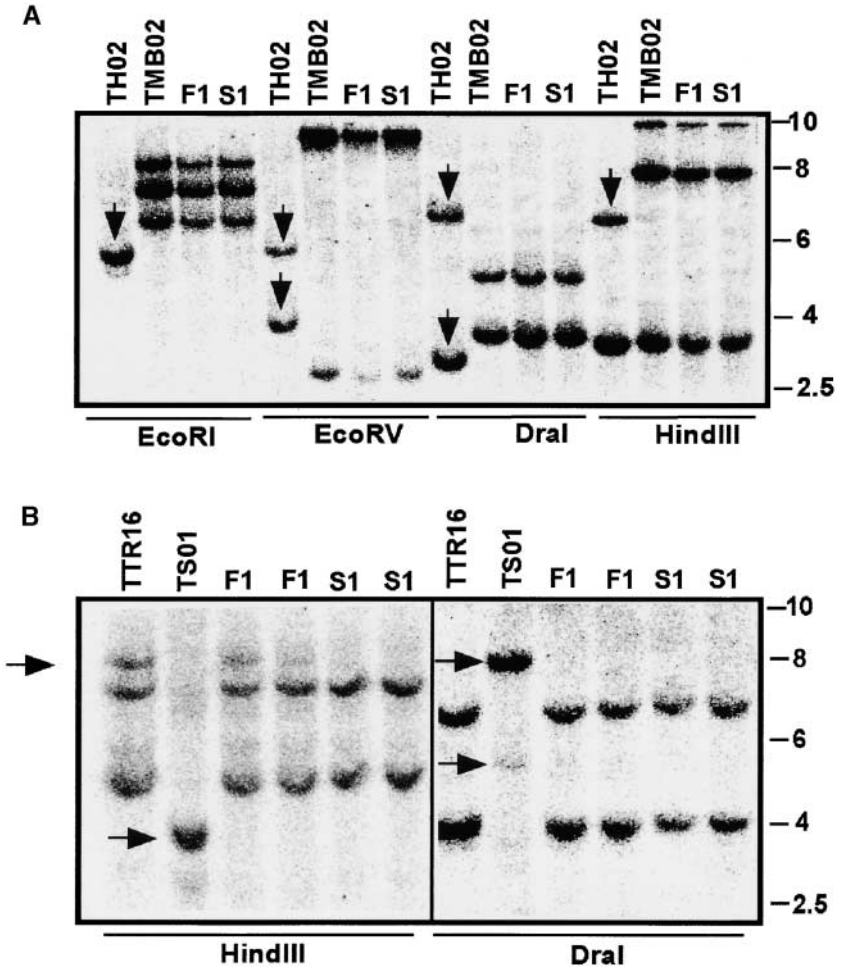

Figure 1. DNA Gel Blot Hybridization of the Genome-Specific Sequence PSR593 to Genomic DNA from F1 Hybrids and Newly Formed Allopolyploids.

(A) Hybridization to genomic DNA from the F1 hybrid between *Ae*. *sharonensis* (TH02) and *T*. *monococcum* ssp *aegilopoides* (TMB02), from the S1 generation of the allotetraploid that derived from this hybrid, and from the two parental plants. DNA was digested using EcoRI, EcoRV, DraI, and HindIII. Arrows indicate the bands from the genome of TH02 that disappeared in F1 and in the S1 generation of the allopolyploid. Fragment size is indicated at right in kilobases.

(B) Hybridization to genomic DNA from the F1 hybrid between *T. turgidum* ssp *durum* (TTR16) and *Ae*. *speltoides* (TS01), from the S1 generation of the allohexaploid derived from this hybrid, and from the two parental plants. DNA was digested using HindIII and DraI. Arrows indicate the bands that disappeared in F1 and/or in the S1 generation of the allohexaploid. The upper band of TTR16 is present in F1 of the HindIII digest but is absent in S1. No such difference between F1 and S1 was noted with DraI. This difference between HindIII and DraI probably results from methylation. Fragment size is indicated at right in kilobases.

sumed therefore that the band gain resulted from epigenetic modifications (e.g., methylation), and it was not investigated further.

To exclude the possibility that the loss of a fragment(s) reflects a change in size attributable to methylation of one of the restriction sites rather than fragment elimination, genomic DNA of the parental plants, F1 plants, and newly synthesized allopolyploids was digested with a series of four to five enzymes, including DraI, which is not sensitive to cytosine methylation. Each digest was probed with the five CSSs and three GSSs. Because fragment loss was found in all enzymes, including DraI (Figure 1), it was concluded that band disappearance was the result of DNA elimination rather than of modifications.

Altogether, 35 F1 hybrid combinations (13 diploids, 12 triploids, and 10 tetraploids) and 22 allopolyploids (eight tetraploids, eight hexaploids, and six octoploids) were produced and analyzed (Table 1). Eleven of the allopolyploids

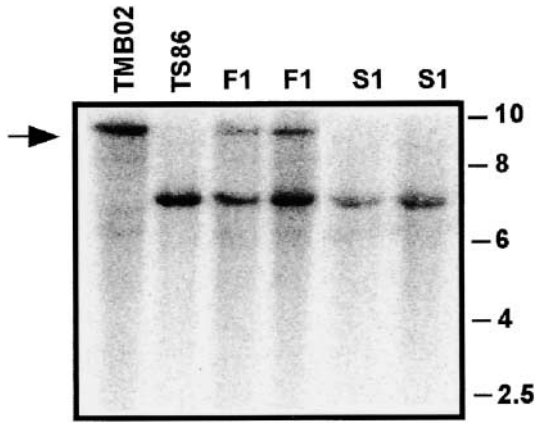

Figure 2. DNA Gel Blot Hybridization of the CSS WPG90 to Genomic DNA from the F1 Hybrid between *T. monococcum* ssp *aegilopoides* (TMB02) and *Ae. speltoides* (TS86), from the S1 Generation of the Allotetraploid That Derived from This Hybrid, and from the Two Parental Plants.

DNA was digested with EcoRI. The arrow indicates the band from the genome of TMB02 that disappeared in the S1 generation of the allopolyploid. Fragment size is indicated at right in kilobases.

(five tetraploids and six hexaploids) had a genomic constitution analogous to that of "natural" allopolyploids, and the remaining 11 (three tetraploids, two hexaploids, and six octoploids) had a "nonnatural" genomic constitution that was not analogous to that of natural allopolyploids (Table 1). Mean sequence elimination in these F1 hybrids and in the S1, S2, and, in some cases, S3 allopolyploid generations at the different ploidy levels is presented in Table 2; the elimination frequency of the individual GSSs and CSSs is given in Table 3. These data show that sequence elimination is a rapid process that occurs as early as in the F1 hybrid and continues for several generations after allopolyploidization. A significant difference between GSSs and CSSs in the timing of elimination was noticed: elimination of GSSs often started in F1 and was almost completed in S2 or S3, whereas that of CSSs usually started in S1 (except for PSR618, which was eliminated at low frequency [6%] in F1 of TH01 × TU04) and was almost completed in S2 and S3.

The various GSSs and CSSs differed with regard to both the timing and frequency of elimination (Table 3). The elimination rate of the GSSs WPG176 and PSR593 was much higher than that of PSR551. Similarly, the elimination rate of the CSSs WPG15, PSR301, and PSR618 was very high, whereas that of WPG90 and PSR743 was low (Table 3). In nonnatural amphiploids, WPG90 and PSR618 were eliminated at a higher frequency than PSR301, PSR743, and WPG15 (Table 3).

Comparing the rate and pattern of sequence elimination in natural and nonnatural allopolyploids showed that elimination of both GSSs and CSSs started significantly earlier and was more rapid in natural allopolyploids (Tables 2 and 3). Also, in allooctoploids, all of which were nonnatural, the GSSs and CSSs were eliminated at a very slow rate.

In all of the natural allohexaploids (i.e., *T. turgidum–Ae. tauschii*), the GSSs and CSSs that, in common wheat, are located on either the A or the B genome were eliminated from the D genome. Interestingly, in all of these synthetic allopolyploids, the direction of elimination was similar to that of the natural hexaploid wheat.

Most allopolyploids used in this study were produced by colchicine treatment of F1 plants. However, three allohexaploids, TTR04–TQ27, TTR16–TQ27, and TTR19–TQ27, were obtained spontaneously by unreduced gametes of F1 plants that were not treated with colchicine (Table 1). Rapid elimination of GSSs and CSSs was observed in the spontaneously produced allopolyploids as well as in those obtained by colchicine treatment.

Genetic Effect on Sequence Elimination

Study of F1 hybrids and allopolyploids that were produced from crosses of one parental line of one species to two or three different parental lines of another species, for example, line TTH01 of *T. turgidum* ssp *carthlicum* with two different lines (TQ17 and TQ27) of *Ae. tauschii*, line TQ27 of *Ae. tauschii* with three different lines (TTR04, TTR16, and TTR19) of *T. turgidum* ssp *durum*, and line TAA01 of *T. aestivum* ssp *aestivum* with two different lines (TL01 and TL02) of *Ae. longissima* (Table 1), enabled us to assess the effect of different genetic backgrounds on sequence elimination. The pattern and rate of GSS and CSS elimination were the same in each group of these allopolyploids, indicating that the different backgrounds of the diploid parental lines did not affect sequence elimination in the F1 hybrids or in the S1 and S2 allopolyploid generations.

The *Ph1* gene, which is located on chromosome arm 5BL, is considered to be the genetic system that determines exclusive homologous pairing in polyploid wheat; plants deficient in this gene exhibit homoeologous pairing, facilitating some degree of intergenomic recombination (Riley and Chapman, 1958). To determine if intergenomic recombination plays a role in allopolyploidy-induced sequence elimination, we studied the effect of the absence of this gene on the pattern and frequency of changes in the various GSSs and CSSs. Allooctoploids with and without *Ph1* were produced by crossing the wild-type (*Ph1Ph1*) and the mutant (*ph1ph1*; produced by E.R. Sears) lines of the hexaploid wheat TAA01 to line TL01 of *Ae. longissima* (Table 1). DNA gel blot hybridization did not reveal differences with regard to the pattern or frequency of elimination of the various GSSs and CSSs between allooctoploids carrying and allooctoploids lacking *Ph1* (data not shown). No sequence elimination was noted in the F1 hybrids and S1 allopolyploid generation of these two crosses, whereas in the S2 generation, the GSSs PSR593 and WPG176 and the CSSs PSR618 and WPG90

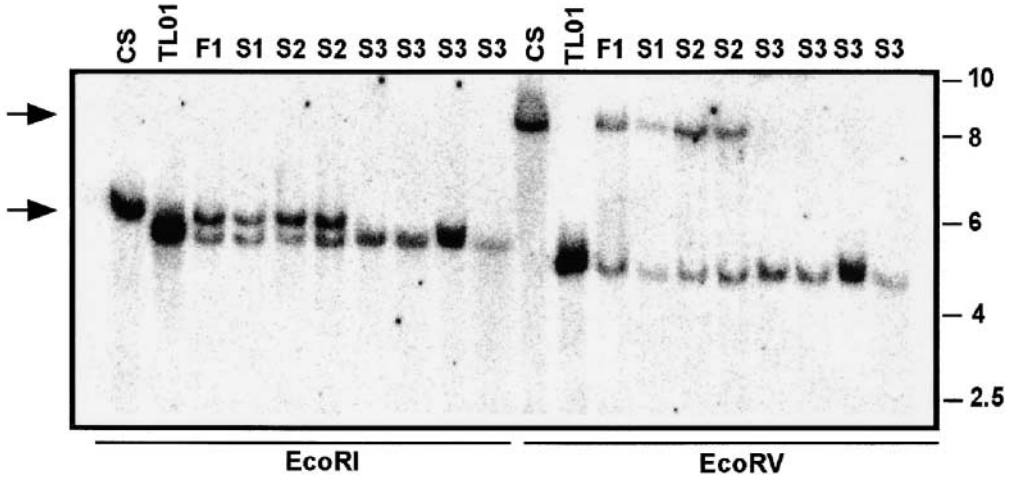

Figure 3. DNA Gel Blot Hybridization of the GSS PSR551 to Genomic DNA from the F1 Hybrid between *T. aestivum* ssp *aestivum* cv Chinese Spring (CS) and *Ae. longissima* (TL01), from the S1, S2, and S3 Generations of the Allooctoploid That Derived from This Hybrid, and from the Two Parental Plants.

DNA was digested using EcoRI and EcoRV. Arrows indicate the band from the genome of TL01 that disappeared only in the S3 generation of the allopolyploid. Fragment size is indicated at right in kilobases.

were eliminated completely from both allopolyploids (data not shown). Also, allohexaploids carrying or lacking the *Ph1* gene were produced by crossing line TTR16 (cv Langdon) of tetraploid wheat and the substitution line disomic 5D nullisomic 5B of line TTR16 (produced by L.R. Joppa) to *Ae. speltoides* line TS01 (Table 1). Only the F1 hybrids of these two crosses were analyzed, and in both of them only GSS PSR593 was eliminated (Figure 4). These data indicate that the absence of *Ph1* does not create conditions that increase the frequency or change the pattern of sequence elimination in the hybrids or allopolyploids.

To study the effect of the cytoplasm on the pattern and rate of sequence elimination, TAA01 was crossed as female to *Ae. speltoides* line TS01 and as male to *Ae. speltoides* line TS42, and F1 hybrids and allooctoploids were obtained (Table 1). Elimination pattern and frequency of GSSs and CSSs were the same in the two F1 hybrids and in the S1 and S2 generations of these two allopolyploids (data not shown). Apparently, the cytoplasm did not affect sequence elimination in the F1 hybrid in the early generations of these two allopolyploids.

DISCUSSION

Rapid Elimination of GSSs and CSSs in Newly Formed Allopolyploids

Previous studies have shown that allopolyploidization is a revolutionary event through which a new species is formed in one step. It generates two genomic "shocks" on the newly formed allopolyploid species: hybridity, in which two divergent genomes are joined together to form one nucleus; and polyploidy, resulting in duplicated genomes. In response to these two unanticipated shocks, the genomes of the newly formed allopolyploids react in a burst of irreversible genomic reorganizations and modifications. These changes include, among others, structural rearrangements on the chromosome level (Leitch and Bennett, 1997) and sequence level (Song et al., 1995; Wendel et al., 1995), regulation of gene expression (Scheid et al., 1996; Galitski et al., 1999; Matzke et al., 1999; Comai et al., 2000), activation of transposons (Matzke and Matzke, 1998; Voytas and Naylor, 1998; Zhao et al., 1998; Soltis and Soltis, 1999; Hanson et al., 2000), and amplification, reassortment, or elimination of highly repetitive sequences (Hanson et al., 1998; Salina et al., 2000) and low-copy sequences (Feldman et al., 1997; Liu et al., 1998a).

An obvious shortcoming of many of the studies cited above was the absence of the parental plants; therefore, the allopolyploids were compared with the progeny of the parental accessions, which, because of intra-accession polymorphism, might differ from the parental plants. To overcome this problem and, at the same time, to determine the timing and the rules of sequence elimination, 35 different F1 hybrids and 22 different combinations of allopolyploids at different ploidy levels were produced and analyzed. Half of the newly synthesized allopolyploids had genomic combinations analogous to those of natural allopolyploids (natural), and the remaining half had genomic combinations that do not exist in natural allopolyploids (nonnatural). In each combination, the DNA gel blot hybridization patterns of the F1

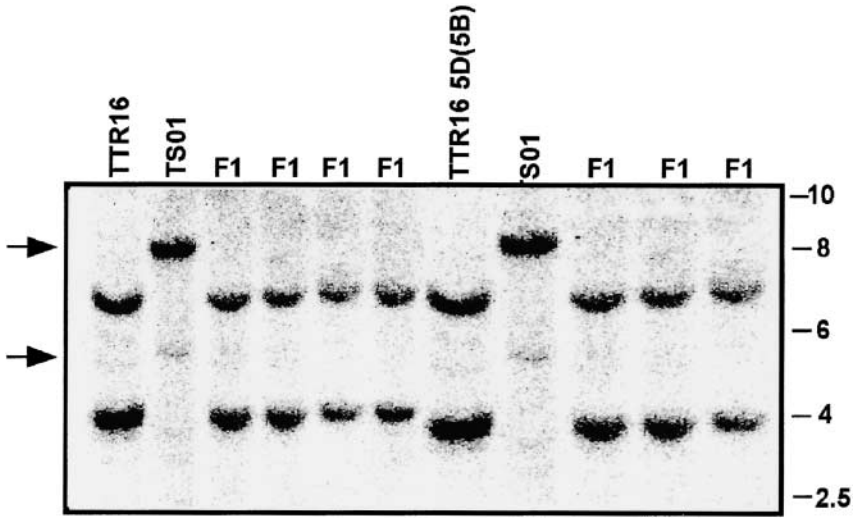

Figure 4. DNA Gel Blot Hybridization of the GSS PSR593 to Genomic DNA from the F1 Hybrid between *T. turgidum* ssp *durum* (TTR16) having *Ph1* and *Ae. speltoides* (TS01), from the F1 Hybrid between *T. turgidum* ssp *durum* (TTR16) Disomic 5D Nullisomic 5B Lacking *Ph1* and *Ae. speltoides* (TS01), and from Their Parental Plants.

DNA was digested with DraI. Arrows indicate the bands from the genome of TS01 that disappeared in the F1 hybrid. Fragment size is indicated at right in kilobases.

hybrid and of the S1, S2, and, in five combinations, S3 generations of the allopolyploids were compared with those of the parental plants.

The integrity of the chromosome complement of all of the hybrids and the allopolyploids was checked cytologically, and aneuploid plants were discarded and not analyzed. Moreover, DNA gel blot hybridization with RFLP markers located on the proximal and distal regions of the short and long arms of the seven homoeologous groups (H. Ozkan, A.A. Levy, and M. Feldman, unpublished data) further supported the conclusion that the observed sequence elimination did not result from a loss of chromosome or chromosome arm.

The strict comparison of parents, hybrids, and allopolyploids, and the large number of interspecific and intergeneric crosses used, enabled us to draw a number of conclusions. The results showed clearly that the studied GSSs and CSSs underwent rapid elimination in the newly formed allopolyploids, with different patterns according to the sequence type and the genomes. Some sequences began the process of elimination in F1 (especially PSR593 and WPG176), others in the first and second allopolyploid generations, and still others during subsequent generations. In any case, as found by Feldman et al. (1997) and Liu et al. (1998a), complete elimination of these sequences was achieved by the S3 to S6 generations of the synthetic allopolyploids. In the natural allopolyploids, 50 to 80% of these sequences were eliminated in only one generation and 75 to 100% were eliminated in the second generation. The elimination rate of these sequences in nonnatural amphiploids was significantly slower. The rapid elimination of these sequences from the natural genomic combinations correlates with their ability to be established in nature as successful species.

There is a basic difference in the elimination pattern of GSSs and CSSs. Elimination of GSSs starts in F1 and continues into the first and second generations of the allopolyploids; thus, it may result from hybridity. Elimination of CSSs starts in S1 and may result from allopolyploidy. This significant difference between the two types of sequences indicates that the causes and biological consequences of elimination may be different for GSSs and CSSs.

DNA gel blot hybridization showed that band disappearance in F1 and allopolyploids was an all-or-none event, that is, there was no weak hybridization signal that could suggest the chimeric nature of the tissues from which the genomic DNA was extracted. Consequently, it was assumed that elimination of GSSs in F1 happened during or soon after F1 zygote formation. Similarly, elimination of CSSs in the S1, S2, or S3 generations of the newly formed allopolyploids occurred during meiosis, during the mitotic cycles of the male and female gametophytes, or, more likely, during or soon after the formation of the allopolyploid zygote.

Results obtained in this study extend and support previous observations (Feldman et al., 1997; Liu et al., 1997, 1998a) showing that changes in CSSs and GSSs occur soon after allopolyploidization and are nonrandom, directional, and highly reproducible. First, the new hybridization pattern exhibited by three or four randomly chosen individual plants of F1, S1, or S2 of a given combination was identical. In contrast to these data from the wheat group, Song et al. (1995) observed differences between individual plants

of the same generation of synthetic allopolyploids of *Brassica*. However, they did not study changes in CSSs and GSSs but in randomly selected nuclear DNA clones (19 anonymous clones, 63 cDNA clones, and seven nuclear genes of known function). Recent RFLP studies, however, using 120 anonymous genomic DNA clones found no evidence for genome change in the polyploid *Brassica juncea* compared with its two diploid progenitors (Axelsson et al., 2000). This phenomenon requires further clarification in *Brassica*. Second, in the wheat group, no further change was observed in individual plants of succeeding allopolyploid generations probed with sequences that detected changes in earlier generations. Third, we show that the elimination of a particular sequence is nonrandom, consistently targeting the same genome(s) in the polyploids. Thus, an identical pattern of changes was detected in natural and newly synthesized allopolyploids with the same genomic constitution. For example, in the newly synthesized allopolyploids of

Table 1. List of the F1 and Synthesized Allopolyploids of *Triticum*, *Aegilops*, and *Secale* That Were Produced in this Work[a]

Cross Combination	Genomic Constitution of F1	Synthesized Allopolyploids
Natural allopolyploid combinations		
Tetraploids ($2n = 4x = 28$)		
Ae. sharonensis (TH01) × *Ae. umbellulata* (TU04)	S^lU	+
Ae. longissima (TL02) × *Ae. umbellulata* (TU02)	S^lU	+
Ae. longissima (TL05) × *T. urartu* (TMU06)	S^lA	+
Ae. sharonensis (TH02) × *T. monococcum* ssp *aegilopoides* (TMB02)	S^lA^m	+
T. monococcum ssp *aegilopoides* (TMB02) × *Ae. speltoides* (TS86)	A^mS	+
Ae. longissima (TL01) × *T. monococcum* ssp *aegilopoides* (TMB02)	S^lA^m	−
Ae. longissima (TL02) × *T. monococcum* ssp *aegilopoides* (TMB02)	S^lA^m	−
Ae. bicornis (TB01) × *T. urartu* (TMU06)	S^bA	−
Hexaploids ($2n = 6x = 42$)		
T. turgidum ssp *cartlicum* (TTH01) × *Ae. tauschii* (TQ17)	BAD	+
T. turgidum ssp *cartlicum* (TTH01) × *Ae. tauschii* (TQ17)	BAD	+
T. turgidum ssp *durum* (TTR04) × *Ae. tauschii* (TQ27)	BAD	+
T. turgidum ssp *durum* (TTR16) × *Ae. tauschii* (TQ27)	BAD	+
T. turgidum ssp *durum* (Lang 5D[5B]) × *Ae. tauschii* (TQ17)	BAD	−
T. turgidum ssp *durum* (TTR19) × *Ae. tauschii* (TQ27)	BAD	+
T. turgidum ssp *dicoccoides* (TTD20) × *Ae. tauschii* (TQ27)	BAD	+
Nonnatural allopolyploid combinations		
Tetraploids ($2n = 4x = 28$)		
Ae. speltoides (TS86) × *Ae. caudata* (TD01)	SC	+
T. urartu (TMU38) × *Ae. tauschii* (TQ27)	AD	+
Ae. bicornis (TB01) × *Ae. tauschii* (TQ27)	S^bD	+
Ae. longissima (TL01) × *Ae. tauschii* (TQ27)	S^lD	−
Ae. longissima (TL02) × *Ae. tauschii* (TQ27)	S^lD	−
Hexaploids ($2n = 6x = 42$)		
T. turgidum ssp *durum* (TTR16) × *Ae. speltoides* (TS01)	BAS	+
T. turgidum ssp *durum* (TTR19) × *Ae. sharonensis* (TH01)	BAS^l	+
T. turgidum ssp *durum* (Lang 5D[5B]) × *Ae. speltoides* (TS01)	BAS	−
T. turgidum ssp *durum* (TTR16) × *Ae. longissima* (TL01)	BAS^l	−
T. turgidum ssp *durum* (TTR16) × *Ae. longissima* (TL02)	BAS^l	−
Octoploids ($2n = 8x = 56$)		
T. aestivum ssp *aestivum* (TAA01) × *Ae. speltoides* (TS01)	BADS	+
Ae. speltoides (TS42) × *T. aestivum* ssp *aestivum* (TAA01)	SBAD	+
T. aestivum ssp *aestivum* (TAA01) × *Ae. longissima* (TL01)	$BADS^l$	+
T. aestivum ssp *aestivum* (TAA01) × *Ae. longissima* (TL02)	$BADS^l$	+
T. aestivum ssp *aestivum* (TAA01) × *Ae. sharonensis* (TH01)	$BADS^l$	−
T. aestivum ssp *aestivum* (TAA01) × *Ae. bicornis* (TB01)	$BADS^b$	−
T. aestivum ssp *aestivum* (TAA01) × *S. cereale* (SC01)	BADR	+
T. aestivum ssp *aestivum* (TAA01 *ph1 ph1*) × *Ae. longissima* (TL01)	$BADS^l$	+
T. aestivum ssp *aestivum* (TAA01 *ph1 ph1*) × *Ae. speltoides* (TS01)	BADS	−
T. aestivum ssp *aestivum* (TAA01 *ph1 ph1*) × *Ae. sharonensis* (TH01)	$BADS^l$	−

[a] All allopolyploids were produced by colchicine treatment of F1 seedlings except for TTR04–TQ27, TTR16–TQ27, and TTR19–TQ27, which were obtained by spontaneous formation of unreduced gametes on F1 plants.

Table 2. Mean Sequence Elimination (%) in F1 Hybrids and S1, S2, and S3 Allopolyploid Generations of *Triticum*, *Aegilops*, and *Secale*[a]

		CSS						GSS					
		4×		6×		8×		4×		6×		8×	
Type of Allopolyploid	Generation	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Natural	F1	8	3	7	0	–	–	8	31	7	67	–	–
	S1	5	67	6	50	–	–	5	80	6	67	–	–
	S2	3	92	5	75	–	–	4	92	5	100	–	–
Nonnatural	F1	5	0	5	0	10	0	5	10	5	13	10	13
	S1	3	36	2	29	6	10	3	50	2	34	6	28
	S2	–	–	2	58	5	53	–	–	2	50	5	53
	S3	–	–	1	100	3	65	–	–	2	84	3	100

[a] *n*, number of combinations; –, data not available; %, mean sequence elimination in every generation was calculated from the percentage of disappearing DNA gel blot bands in every genomic combination.

different lines of *T. turgidum* and *Ae. tauschii*, a genomic combination similar to that of common wheat (BBAADD), the direction of elimination was the same as in the natural hexaploid. Sequences that are located on chromosomes of genomes B or A in polyploid wheat were eliminated from the D genome in the synthetic allopolyploids. Similar directional changes also were reported for processes of diploid hybrid speciation in *Helianthus* (Rieseberg et al., 1995). Those authors found that the genomic structure and composition of natural hybrid species were fixed within a few generations after the initial hybridization event and remained relatively static thereafter.

In contrast to previous work (Liu et al., 1998a), no correlation was found between ploidy level and direction of elimination. Also, we did not find that the type and origin of the sequences determined the direction of elimination. Similar results were obtained by Salina et al. (2000). They found that the subtelomeric repeat Spelt1, which constitutes ~2% of the *Ae. speltoides* genome, was deleted by the first generation of several newly synthesized allopolyploids having *Ae. speltoides* as one parent. Neither the level of polyploidy nor the direction of the cross affected the pattern of elimination of this highly repetitive sequence.

This well-orchestrated, nonrandom, and reproducible response of the wheat genomes to allopolyploidy is in contrast to previous reports on the disorganized response of plants to genomic shocks (Walbot and Cullis, 1985). One could argue that a particular set of sequences (CSSs and GSSs) was analyzed in this work, thus explaining the differences. In a related study, Shaked et al. (2001) analyzed a random set of loci in the same allopolyploids that are described in this work. They found that sequence elimination is the major response of the wheat genome to allopolyploidization, and their results confirm the nonrandom nature of sequence elimination. Together, our results imply that rapid and nonrandom sequence elimination is an essential adjustment required for the harmonious coexistence of the two or more different genomes in the nucleus of wheat allopolyploids. This rapid response of the genome is probably essential because there is no grace period for the new allopolyploid, and it must survive the traumatic situation of genomic disharmony characteristic of the first generation(s) to establish itself as a new species. The directional elimination indicates that, at the diploid level, there is a preadaptive condition to genomic changes and predetermination of the direction of the elimination. According to McClintock (1984), such predetermination of genomic modifications might originate from repeated encounters during the evolutionary history of the group with particular genetic shocks. This would suggest that interspecific or intergeneric hybridization and/or duplication or allopolyploidy occur relatively frequently in nature.

Mechanism of Allopolyploidy-Induced Sequence Elimination

Although the molecular mechanism of sequence elimination is not known (for discussion, see Shaked et al., 2001), a number of rules and characteristics of this process can be derived from our results. An obvious and critical question arising from the results of Feldman et al. (1997) and Liu et al. (1997, 1998a, 1998b) is whether findings from synthetic allopolyploids, which were produced by colchicine treatment of F1 plants, can be extended to natural allopolyploids. In this study, three allopolyploids analogous to natural polyploids (i.e., three different combinations of *T. turgidum* ssp *durum*–*Ae. tauschii*) were obtained spontaneously as a result of the formation of unreduced gametes. These allopolyploids exhibited the same pattern of CSS and GSS elimination as the colchicine-treated allopolyploids. Also, the allopolyploids *Ae. peregrina*–*Secale cereale*, *Ae. kotschyi*–*S. cereale*, and *Ae. ovata*–*S. cereale*, obtained through tissue culture by Barbara Wojciechowska (Institute of Plant Genetics, Polish Academy of Sciences, Posnan), exhibited elimination of CSSs and GSSs (data not shown). These results clearly show that elimination is induced by allopolyploidization and not by the method of chromosome doubling

(e.g., colchicine treatment); thus, it is likely to be a general phenomenon in stabilization of new wheat allopolyploids.

No connection was noted in this work between the origin of the sequences (maternal or paternal) or their chromosomal location and the rate and pattern of their elimination. The degree of divergence between the parental genomes was not associated with the frequency of genomic changes, unlike for *Brassica*, in which a positive correlation was reported (Song et al., 1995).

It seems that the pattern, rate, and time of elimination of the various CSSs and GSSs are affected by the genomic combination of the allopolyploid, with rapid elimination occurring in combinations that exist in nature. Different lines of the same parental species had no effect on these parameters of elimination.

Altered patterns of DNA methylation have been shown to be a frequent outcome in new allopolyploids (Song et al., 1995; Liu et al., 1998b; Shaked et al., 2001). However, the loss of the hybridization fragment(s) found here and in other works (Feldman et al., 1997; Liu et al., 1998a; Shaked et al., 2001) was observed in all enzymes that produced diagnosable polymorphism. Moreover, we checked hybrids and allopolyploids with methylation-sensitive and -insensitive isoschizomeric restriction enzymes and found the same RFLP profiles (data not shown). This shows that loss of hybridization fragments in the newly formed allopolyploids results from elimination rather than from methylation.

The cytoplasm may provide selection pressure on portions of the alien nuclear genome, stabilizing the newly produced allopolyploid by establishing a "harmonious relationship between the cytoplasmic and the nuclear genome" (Soltis and Soltis, 1993). In *Brassica*, Song et al. (1995) found directional genomic changes that were probably caused by nuclear-cytoplasmic interactions. However, Liu et al. (1998a) found no differences in a pair of allooctoploids derived from reciprocal crosses and suggested that the cytoplasm did not play a role in determining the direction of elimination. To address this question further, TAA01 was crossed as female with TS01 and as male with TS42, two lines of *Ae. speltoides*. Similar elimination patterns were observed in the F1 hybrids and the derived allopolyploids. Therefore, although cytoplasmic factors may influence the modification of DNA sequences, they are not a major factor affecting elimination of the CSSs and GSSs surveyed in the present study.

Intergenomic recombination has been proposed by Song et al. (1995) as a major factor contributing to genomic changes in newly synthesized allopolyploids. In addition, other possible examples of genome and chromosomal repatterning have been shown by comparative genome mapping (Helentjaris et al., 1988; Whitkus et al., 1992). Genomic in situ hybridization also has provided evidence for intergenomic transfer of DNA in the alloploid *Milium montianum* (Bennett et al., 1992). On the other hand, Feldman et al. (1997) and Liu et al. (1998a) claimed that elimination of GSSs and CSSs in wheat was not caused by intergenomic recombination because one of the parental lines included in their study had *Ph1*, a gene that suppresses homoeologous pairing at first meiotic metaphase. To address this question, four different hybrids and two allopolyploids differing in their *Ph1* genotype were produced and analyzed in this work. Similar elimination patterns were observed in these amphiploids, suggesting that intergenomic recombinations might not be a major factor affecting sequence elimination. This conclusion is in accord with that of Axelsson et al. (2000), who found no evidence for intergenomic recombination in synthetic allopolyploids of *Brassica.*

Table 3. Elimination Frequency (%) of the Different CSSs and GSSs in F1 Hybrids and S1 and S2 Allopolyploid Generations of *Triticum*, *Aegilops*, and *Secale*

Type of Allopolyploid	Number of Combinations	Percent Elimination of CSSs						Percent Elimination of GSSs				All Sequences (Mean)
		PSR743	PSR618	PSR301	WPG15	WPG90	Mean	PSR551	PSR593	WPG176	Mean	
Natural												
F1	15	0	6	0	0	0	1.2 b	7	46	67	40.0 a[a]	15.8 b
S1	11	11	64	100	100	27	60.4 a	45	82	91	72.7 a	65.0 a
S2	8	11	88	100	100	38	67.4 a	45	88	100	77.7 a	71.3 a
Mean	–	–	–	–	–	–	43.0	–	–	–	63.4	50.7[b]
Nonnatural												
F1	20	0	0	0	0	0	0.0 b	0	17	13	10.0 a[a]	3.8 b
S1	11	18	30	9	6	20	16.6 ab	0	36	50	28.7 a	21.1 b
S2	7	18	71	29	6	88	42.4 a	10	86	88	61.3 a	49.5 a
Mean	–	–	–	–	–	–	19.7	–	–	–	33.3	24.8[b]
Mean, all combinations	–	–	–	–	–	–	31.3	–	–	–	48.4	37.7

Arc cosine transformation of the percentage values was compared by analysis of variance. Within each group of allopolyploids (natural and nonnatural), values followed by common letters are not significantly different ($P \leq 0.05$).

[a] Significantly higher ($P \leq 0.05$) than in the F1 of the CSSs in the natural and nonnatural allopolyploids.

[b] Significantly higher ($P \leq 0.009$) than the nonnatural all sequences mean.

Recently, Voytas and Naylor (1998) suggested that retrotransposons might play a role in genomic change. Evidence for the activation of several kinds of retrotransposons in natural allopolyploids (Zhao et al., 1998; Hanson et al., 2000) and in newly formed allopolyploids (K. Kashkush, M. Feldman, and A.A. Levy, unpublished data) has been obtained. However, it is difficult to ascribe the nonrandom, directional, and highly reproducible elimination reported here to the activity of retrotransposons.

Possible Significance of Sequence Elimination for Genomic Differentiation and Diploid-Like Meiotic Behavior of the Newly Formed Allopolyploids

The revolutionary rather than evolutionary formation of a new lineage of plant species through allopolyploidy requires a variety of rapid genomic changes to allow for the two diverged genomes to act in harmony within the nucleus of the newly formed allopolyploid. Such changes are prerequisite for the successful establishment of the newly formed allopolyploid species. Among such changes, elimination of low-copy, presumably noncoding CSSs and GSSs, which are ubiquitous among the diploid species of the tribe Triticeae and are highly conserved, occurs at the onset of the polyploid speciation event. Such nonrandom elimination of DNA sequences augments the divergence of homoeologous chromosomes, that is, it accelerates the evolution of genomic allopolyploids from segmental allopolyploids; thus, it can provide the physical basis for the diploid-like meiotic behavior (i.e., the exclusive pairing of homologous chromosomes) of the newly formed allopolyploids. The work of Shaked et al. (2001) provides a quantitative assessment of the process of allopolyploidy-induced sequence elimination, showing that it can affect up to 15% of the sequences of a genome. Such an extensive change may shift the pairing pattern in the raw allopolyploid from multivalent pairing, with resulting polysomic inheritance, to bivalent pairing, with dis-

Table 4. Species and Lines of *Triticum*, *Aegilops*, and *Secale* Used in This Study

Species, Subspecies, and Cultivars	Line Designation	Genome
Diploids ($2n = 2x = 14$)		
T. urartu	TMU06	AA
	TMU38	AA
T. monococcum ssp *aegilopoides*	TMB02	A^mA^m
Ae. speltoides	TS01	SS
	TS42	SS
	TS86	SS
Ae. bicornis	TB01	S^bS^b
Ae. sharonensis	TH01	S^lS^l
	TH02	S^lS^l
Ae. longissima	TL01	S^lS^l
	TL02	S^lS^l
	TL05	S^lS^l
Ae. tauschii	TQ17	DD
	TQ27	DD
Ae. umbellulata	TU02	UU
	TU04	UU
Ae. caudata	TD01	CC
S. cereale	SC01	RR
Tetraploids ($2n = 4x = 28$)		
T. turgidum ssp *dicoccoides*	TTD20	BBAA
ssp *durum* cv Inbar	TTR04	BBAA
cv Langdon	TTR16	BBAA
cv Cappelli	TTR19	BBAA
ssp *carthlicum*	TTH01	BBAA
Hexaploids ($2n = 6x = 42$)		
T. aestivum ssp *aestivum* cv Chinese Spring	TAA01	BBAADD
Substitution line		
T. turgidum ssp *durum* cv Langdon 5D(5B)	Produced by L.R. Joppa	BBAA
Mutant line		
T. aestivum ssp *aestivum* cv Chinese Spring, *ph1 ph1*	Produced by E.R. Sears	BBAADD

omic inheritance, thus preventing intergenomic recombinations and yielding viable gametes. Such a shift therefore enables the allopolyploid to benefit from a permanent heterozygosity between homoeoalleles and might have been an essential prerequisite for the establishment of these new allopolyploids as successful species.

It has been proposed by Feldman et al. (1997) that the diploid-like meiotic behavior of polyploid wheat has been brought about by two independent systems that complement each other. The first system is based on nonrandom elimination of DNA sequences, first from one of the two pairs of homoeologous chromosomes at the tetraploid level, and later, from the third pair of homoeologous chromosomes at the hexaploid level. These elimination events occur soon after the formation of the allopolyploids, resulting in increased differentiation between homoeologous chromosomes. The second system is based on the *Ph1* gene located on the long arm of chromosome 5B (Riley and Chapman, 1958; Sears, 1976), the main gene that suppresses homoeologous pairing in polyploid wheat. This gene presumably evolved at later evolutionary stages, and its effect is additive to the elimination system. Thus far, genes with action like *Ph1* have not been found in *Aegilops* polyploids that exhibit characteristic diploid-like meiotic behavior. Therefore, rapid and extensive molecular changes that instantly convert several low-copy, noncoding-specific sequences into CSSs or GSSs in a newly formed allopolyploid represent an efficient way to augment the divergence between homoeologous chromosomes and, thus, prevent intergenomic recombinations.

Indeed, a positive linear relationship was found in S1 to S3 generations of the newly synthesized allopolyploids between the percentage of seed fertility and the percentage of elimination of CSSs and GSSs, and a negative relationship was found between multivalents per cell and the percentage of CSS and GSS elimination (Ozkan, 2000). These relationships, which were more obvious in natural than in nonnatural combinations, indicate that elimination of CSSs and GSSs in early generations serves to reduce homoeologous pairing, improve homologous pairing, and enhance the fertility of newly synthesized amphiploids.

Recently, Liu et al. (1997) reported that on chromosome arm 5BL, CSSs and GSSs are clustered in two chromosomal regions. It was assumed that on other chromosome arms as well, the CSSs and GSSs are clustered in specific chromosomal regions. In contrast to other regions that contain group (homoeologous)-specific and nonspecific DNA sequences, these regions are rich in homology-specific sequences and, thus, may act as homology-determining regions. As such, they may play a critical role in homology search and initiation of pairing at early meiotic stages.

In conclusion, allopolyploidization in the wheat group induces rapid, nonrandom, and directional elimination of CSSs and GSSs. This elimination, which augments the differentiation of the homoeologous chromosomes, provides the physical basis for the diploid-like meiotic behavior of the nascent allopolyploids. The resultant strict bivalent pairing prevents intergenomic recombination and brings about higher fertility and permanent heterosis between homoeoalleles, thus fostering the successful establishment of the newly formed allopolyploid species in nature. It is assumed that CSSs, being the only homology-specific sequences in polyploid wheat, play an important role in the initiation of meiotic pairing and that their mode of distribution determines the pairing pattern that characterizes tetraploid and hexaploid wheat.

METHODS

Plant Material

The various lines of *Aegilops* and *Triticum* used for the production of F1 hybrids and synthetic allopolyploids are listed in Table 4. All of these lines are maintained in our collection. Single plants used as parents were bagged and selfed so that the genotype of F1 hybrids and synthetic allopolyploids could be traced to specific parental plants.

Interspecific and intergeneric crosses were performed between species of the same ploidy level as well as between species of different ploidy levels. Altogether, 35 different F1 hybrids were obtained (Table 1). At the three to four tiller stage, the hybrids were treated with 0.2% (w/v) colchicine (Sigma) for 5 hr at room temperature and then washed in tap water for 1 hr. After treatment, plants were grown in the greenhouse until maturity, and all spikes were bagged. Three allopolyploids were derived from spontaneous formation of unreduced gametes on F1 plants that were not treated with colchicine. In all, 22 different allopolyploids were obtained (Table 1), of which 11 are natural (i.e., they have a genomic combination that exists in nature) and the remaining 11 are nonnatural (i.e., they have a genomic combination that does not exists in nature) (Table 1). All 22 allopolyploids were analyzed in S1 and S2 generations, but only five allopolyploids were analyzed from the S3 generation because of slow growth.

Because three of these allopolyploids were produced by fertilization of unreduced gametes, the designation of the different allopolyploid generations is S (selfed) and not C (colchiploidy). Accordingly, polyploid tissues on F1 plants are S0, the seed produced on S0 tissues (i.e., after meiosis) and plants developed from them are S1, etc. Chromosome number was determined in all F1 hybrids and newly synthesized allopolyploids. Only those having the expected euploid chromosome number were analyzed.

DNA Probes

Two types of low-copy genomic sequences were used: genome-specific sequences (GSSs), which in polyploid wheat occur in several or in all chromosomes of a given genome, and chromosome-specific sequences (CSSs), which in polyploid wheat occur in only one pair of homologous chromosomes. All of these sequences are present in all of the diploid species of the Triticeae. Detailed information about these GSSs and CSSs is given in Table 5. Two CSSs and one GSS were isolated from a chromosome arm DNA library developed in our laboratory (Vega et al., 1994), and they have been characterized and mapped to specific chromosomal regions (Liu et al., 1997). The other sequences were isolated from a genomic library of bread wheat (Gale et al., 1995) and kindly provided by M.D. Gale (John Innes Centre,

Table 5. CSSs and GSSs Used in this Study

Designation[a]	Size (kb)	Chromosome Arm Location in Common Wheat
CSSs		
PSR743	2.200	7AX[b]
WPG15	0.279	5BL
WPG90	0.279	5BL
PSR618	2.800	5BS
PSR301	2.120	6BX[b]
GSSs		
PSR551	1.200	2BS, 6BS
PSR593	2.500	2BS, 4BS, 7BL
WPG176	0.260	3BL, 4BX, 5BL

[a] PSR (Plant Science Research) sequences were kindly provided by M.D. Gale (John Innes Centre, Norwich, UK); WPG (Weizmann Plant Genetics) sequences were produced in our laboratory. All sequences showed polymorphism between the parental lines.
[b] Arm location is unknown.

Norwich, UK). All sequences showed polymorphism between the parental plants; thus, it was possible to ascertain the parental origin of these sequences.

DNA Gel Blot Analysis

Young leaves of F1 plants, allopolyploids, and their parents were collected and stored in liquid nitrogen until extraction. DNA was extracted using the cetyl-trimethyl-ammonium bromide procedure (Kidwell and Osborn, 1992). The extracted DNA (10 μg) was digested with the five restriction enzymes EcoRI, EcoRV, HindIII, DraI, and BamHI (Boehringer Mannheim; 1 unit/μg DNA) unless specified otherwise. Digested DNA was separated by agarose electrophoresis (1.35 V/cm for 30 hr; Sambrook et al., 1989). The agarose gel was transferred to Hybond N^+ filters (Amersham) using the manufacturer's recommended protocol. Membranes were washed with 2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate), wrapped in seal wrap, and kept in plastic boxes at −20°C until hybridization.

The probes were radioactively labeled by the random hexamer method with α-^{32}P-dCTP according to Feinberg and Vogelstein (1983). Prehybridization, hybridization, and posthybridization washing conditions were as described by Liu et al. (1997). Filters were exposed to x-ray film for 5 to 8 days at −80°C or to a phosphorimager screen overnight. Visualization was accomplished using MACBAS program, version 1 (Fuji, Tokyo, Japan).

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