Genome Evolution Due to Allopolyploidization in Wheat

Moshe Feldman¹ and Avraham A. Levy

Department of Plant Sciences, The Weizmann Institute of Science, Rehovot 76100, Israel

ABSTRACT The wheat group has evolved through allopolyploidization, namely, through hybridization among species from the plant genera *Aegilops* and *Triticum* followed by genome doubling. This speciation process has been associated with ecogeographical expansion and with domestication. In the past few decades, we have searched for explanations for this impressive success. Our studies attempted to probe the bases for the wide genetic variation characterizing these species, which accounts for their great adaptability and colonizing ability. Central to our work was the investigation of how allopolyploidization alters genome structure and expression. We found in wheat that allopolyploidy accelerated genome evolution in two ways: (1) it triggered rapid genome alterations through the instantaneous generation of a variety of cardinal genetic and epigenetic changes (which we termed “revolutionary” changes), and (2) it facilitated sporadic genomic changes throughout the species’ evolution (i.e., evolutionary changes), which are not attainable at the diploid level. Our major findings in natural and synthetic allopolyploid wheat indicate that these alterations have led to the cytological and genetic diploidization of the allopolyploids. These genetic and epigenetic changes reflect the dynamic structural and functional plasticity of the allopolyploid wheat genome. The significance of this plasticity for the successful establishment of wheat allopolyploids, in nature and under domestication, is discussed.

HYBRIDIZATION and polyploidization are ubiquitous modes of evolution in plants and in other eukaryotes (reviewed by Van de Peer *et al.* 2009). The wheat group (genera *Aegilops* and *Triticum*) emphasizes the impact of hybridization and polyploidization on species evolution in nature and under domestication. For example, bread wheat has a complex genome consisting of three related genomes that derived from three different diploid species; it is called an allohexaploid (*allo*, from Greek, meaning “different”). Pasta wheat is an allotetraploid. Overall (Table 1), the wheat group contains 13 diploid species and 18 allopolyploid species (Kihara 1924, 1954; Sax 1927; Sears 1948, 1969; Kihara *et al.* 1959; Morris and Sears 1967; Van Slageren 1994; and reference therein). Attempts to determine the timing of wheat speciation, using DNA data, suggest that the diploid progenitors of allopolyploid wheat have diverged from a common progenitor some 2.5–4.5 MYA (Huang *et al.* 2002). Because of this fairly recent divergence, most of the diploid wheat species have relatively limited morphological and molecular variation, occupy only a few well-defined

ecological habitats, and are distributed throughout relatively small geographical areas (Eig 1929; Zohary and Feldman 1962; Kimber and Feldman 1987; Van Slageren 1994). In historical terms, allotetraploid wheat developed about 300,000 to 500,000 years ago (Huang *et al.* 2002), while allohexaploid wheat was formed only about 10,000 years ago (Feldman *et al.* 1995; Feldman 2001). According to Stebbins (1950), newly formed allopolyploids are often characterized by limited genetic variation, a phenomenon he referred to as the “polyploidy diversity bottleneck.” This bottleneck arises because only a few diploid genotypes were involved in the allopolyploid speciation events, because the newly formed allopolyploid is immediately isolated reproductively from its two parental species, and because time was not sufficient for the accumulation of mutations.

Despite the diversity bottleneck in newly formed allopolyploids and despite the fact that all *Aegilops* and *Triticum* allopolyploids formed much later than their ancestral diploids, they differ radically from their diploid progenitors. The allopolyploids show wider morphological variation, occupy a greater diversity of ecological habitats, and are distributed over larger geographical area than their diploid progenitors (Eig 1929; Zohary and Feldman 1962; Kimber and Feldman 1987; Van Slageren 1994). They are also dynamic colonizers

Table 1 The species of the wheat group (the genera *Aegilops*, *Amblyopyrum*, and *Triticum*)

Ploidy level	Species	Genome ^a
Diploids (2n = 2x = 14)	<i>Amblyopyrum muticum</i> (=Ae. mutica)	TT
	<i>Aegilops speltoides</i>	SS
	<i>Ae. bicornis</i>	S ^b S ^b
	<i>Ag. Longissima</i>	S ^l S ^l
	<i>Ae. sharonensis</i>	S ^l S ^l
	<i>Ae. searsii</i>	S ^s S ^s
	<i>Ae. tauschii</i> (=Ae. squarrosa)	DD
	<i>Ae. caudata</i>	CC
	<i>Ae. umbellulata</i>	UU
	<i>Ae. comosa</i>	MM
	<i>Ae. uniaristata</i>	NN
	<i>Triticum monococcum</i>	A ^m A ^m
	<i>T. urartu</i>	AA
Tetraploids (2n = 4x = 28)	<i>Ae. biuncialis</i>	UUMM
	<i>Ae. geniculata</i> (=Ae. ovata)	<u>MMUU</u>
	<i>Ae. neglecta</i> (=Ae. triaristata 4x)	UUMM
	<i>Ae. columnaris</i>	UUMM
	<i>Ae. triuncialis</i>	UUCC; CCUU
	<i>Ae. kotschy</i>	<u>SSUU</u>
	<i>Ae. peregrina</i> (=Ae. variabilis)	<u>SSUU</u>
	<i>Ae. cylindrica</i>	DDCC
	<i>Ae. crassa</i> 4x	<u>DDMM</u>
	<i>Ae. ventricosa</i>	<u>DDNN</u>
	<i>T. turgidum</i>	BBAA
	<i>T. timopheevii</i>	GGAA
Hexaploids (2n = 6x = 42)	<i>Ae. recta</i> (=Ae. triaristata 6x)	UUMMNN
	<i>Ae. vavilovii</i>	DDMMSS
	<i>Ae. crassa</i> 6x	DDDDMM
	<i>Ae. juvenalis</i>	DD <u>MMUU</u>
	<i>T. aestivum</i>	BBAAADD
	<i>T. zhukovskyi</i>	GGAAA ^m A ^m

^a Genome designations according to Kimber and Tsunewaki (1988); underlined designation indicates a modified genome.

compared to their diploid progenitors (Zohary and Feldman 1962). Therefore, students of wheat evolution have been fascinated by this paradox and have dedicated themselves to unraveling the processes and mechanisms that contributed to the build up of genetic and morphological diversity of allopolyploids and to their great evolutionary success in term of proliferation and adaptation to new habitats, including under domestication. During the past several decades, methods and materials have been developed that facilitate studies of genomic alterations triggered by allopolyploidization. Studies of natural and synthetic wheat and related allopolyploids, as well as genome sequencing data, indicate that a broad range of DNA rearrangements occur during, immediately, or within a few generations following allopolyploidization. These rapid changes contribute to increased diversity at the intra-specific level.

What triggers these changes is a fascinating and still unanswered question, but it is clear that these developments

are rapid and extensive, including the loss of coding and noncoding DNA sequences, transposon activations, and gene silencing or duplication and pseudogenization (Levy and Feldman 2004; Feldman and Levy 2005, 2009; and references therein). We therefore have distinguished between revolutionary changes—which occur rapidly, within a few generations—and evolutionary changes, which take place gradually in the polyploid lineage, on an evolutionary time scale of hundreds or thousands of generations (Feldman and Levy 2005, 2009). Note that these evolutionary changes may also be relatively faster than run-of-the-mill evolution due to the buffering of mutations in the polyploid genome (Mac Key 1954, 1958; Sears 1972; Thompson *et al.* 2006), which speeds neo- or subfunctionalization of genes and brings on diploidization and divergence from the diploid progenitor genomes. In this manuscript, we focus on the contribution of wheat and related species to our understanding of evolutionary processes that shape allopolyploid genomes.

Early Studies on Interspecific Hybridization

A newly formed allopolyploid wheat species results from an interspecific or intergeneric hybridization, followed by spontaneous somatic or, more likely, meiotic chromosome doubling in the sterile F1 or, alternatively, derived from crosses between parental species with unreduced gametes (Ramsey and Schemske 1998). Indeed, early studies of synthetic intergeneric hybrids of wheat showed that the frequency of unreduced gametes in the F1 hybrids could be as high as 50% (Kihara and Lilienfeld 1949) and identified the occurrence of spontaneous chromosome doubling, thus demonstrating the possibility of species formation via allopolyploidy in the wheat group (Von Tschermak and Bleier 1926). Thus, a newly formed allopolyploid is a hybrid that contains two or more different genomes enveloped within a single nucleus. Consequently, the evolutionary process of allopolyploidization exerts considerable stress on the young species whose genomes are not always compatible, as seen with the hybrid necrosis syndrome (Caldwell and Compton 1943; Hermesen 1963; Tsunewaki 1970). This stress was referred to by Barbara McClintock as a genomic shock that could activate transposons and further reduces the fitness of the hybrid (McClintock 1984). This raised the question, discussed below, on the extent and modes of hybridization that actually occur in nature.

Zohary and Feldman (1962) studied the extent and pattern of hybridization and allopolyploidization that occur in nature in *Aegilops* and *Triticum* species. Evidence that many wheat allopolyploids were genetically interconnected through hybridizations between various allopolyploid species and therefore did not evolve independently was presented. These wheats were divided into three groups, each containing several allopolyploids that share a single, common genome but differ in their other genome(s) (Zohary and Feldman 1962). The allopolyploids within each group grow in mixed populations in many sites, where they can easily hybridize (Feldman 1965a). In laboratory hybridization studies of allopolyploids,

it was found that the common genome acts as a buffer ensuring some seed fertility following pollination by either one of the parents, while the chromosomes of the dissimilar genomes, brought together from different parents, may pair to some extent and exchange genetic material (Feldman 1965b, c). Consequently, the dissimilar genomes of these allopolyploids are recombined, or modified genomes (Zohary and Feldman 1962), which contain chromosomal segments that originated from two or more diploid genomes. These species overlap in their genetic variation ranges and are interconnected by a series of morphological intermediate forms. Thus, despite the genetic barriers and the genomic shock involved, interspecific hybridization, which is rare at the diploid level, has played a decisive role in the establishment of a wide range of genetic variation in the allopolyploid species (Zohary and Feldman 1962). This has probably significantly contributed to their evolutionary success.

In addition, the newly formed allopolyploids—especially annual, predominantly self-pollinated species, like those of the wheat group—must overcome several immediate challenges to survive at the cytological, genetic, and epigenetic levels (Levy and Feldman 2002, 2004; Feldman and Levy 2005, 2009, 2011). We argue, in the following sections, that overcoming these challenges, and increasing the nascent allopolyploid fitness, is achieved through genomic plasticity, namely through the immediate triggering of a variety of cardinal genetic and epigenetic changes that affect genome structure and gene expression.

Cytological Diploidization

Bread wheat is an allohexaploid species ($2n = 6x = 42$, genomes BBAADD) that originated from hybridization events involving three different diploid progenitors classified in the genera *Triticum* and *Aegilops*: (i) *T. urartu*, the donor of the A genome (Dvorak 1976; Chapman *et al.* 1976), (ii) a yet-undiscovered extant or extinct *Aegilops* species closely related to *Ae. speltoides*, the donor of the B genome, and (iii) *Ae. tauschii*, the donor of D genome (McFadden and Sears 1944, 1946; Kihara 1944). On the basis of genetic similarities, the 21 pairs of homologous chromosomes of bread wheat (seven pairs in each genome) fall into seven homeologous groups, each containing one pair of chromosomes from the A, B, and D genomes, respectively (Sears 1954; Figure 1). Hence, homeologous group 1, for example, contains the pair 1A, 1B, and 1D. In each group, homeologous chromosomes, which are derived from a relatively recent, common ancestral chromosome, *i.e.*, only 2.5–4.5 MYA (Huang *et al.* 2002), still share a high degree of gene synteny and DNA sequence homology. However, they differ from one another by a number of noncoding and highly repetitious DNA sequences (Flavell 1982), and many functional gene complexes (Wicker *et al.* 2011 and references therein). In spite of this genetic relatedness, the homeologues do not pair with each other at meiosis, a phenomenon that still requires clarification.

In allopolyploid wheat, the restriction of pairing to homologous chromosomes, *i.e.*, cytological diploidization, has developed through two independent but complementary systems. The first system to be studied is based on the genetic control of pairing. The second depends on the physical divergence of the homeologous chromosomes.

In 1958, a gene was discovered on the long arm of chromosome 5B of bread wheat that restricts meiotic pairing to completely homologous chromosomes (Riley and Chapman 1958; Sears and Okamoto 1958). Gene(s) with similar effect have not been found in the homeologous chromosomes 5A and 5D of common wheat (Sears 1976). Discovery of this gene has had a great impact on wheat cytogenetics: it became a subject of intensive cytogenetic studies that attempted to understand its mode of action, evolution, and breeding significance. The gene, called *Ph1* (pairing homeologues; Wall *et al.* 1971), was further positioned some 1.0 cM from the centromere of the long arm of chromosome 5B (5BL) (Okamoto 1957; Sears 1984). It is a dominant gene that suppresses pairing of the homeologues (intergenomic pairing) while allowing that of homologs (intragenomic pairing) (Riley 1960; Sears 1976; and reference therein). Consequently, only bivalents are formed during allopolyploid wheat meiosis.

The mechanism controlling the *Ph1* mode of action is still unclear. Six extra doses of the *Ph1*-containing arm of chromosome 5B caused partial asynapsis of homologs at meiosis, concomitantly allowing some pairing of homeologous chromosomes and inducing a high frequency of interlocking bivalents (Feldman 1966). Similar effects were observed by premeiotic treatment with colchicine (Driscoll *et al.* 1967; Feldman and Avivi 1988). It was therefore assumed that the hexaploid nucleus still maintains some organizational aspects of the individual ancestral genomes; *i.e.*, each genome occupies a separate region in the nucleus, which in turn, is recognized by *Ph1* (Feldman 1993 and references therein).

A model was thus proposed whereby *Ph1* exerts its effect at premeiotic stages, before the commencement of synapsis, and affects the premeiotic alignment of homologous and homeologous chromosomes. It therefore controls the regularity and pattern of pairing (Feldman 1993 and references therein). In euploid bread wheat, two doses of *Ph1*, while scarcely affecting homologous chromosomes, keep the homeologues apart, thereby leading to exclusive homologous pairing in meiosis. In the absence of *Ph1*, the three genomes are mingled together in the nucleus and consequently, the homeologues can also pair with each other. Six doses of *Ph1* or premeiotic treatment with colchicine induces separation of chromosomal sets and the random distribution of chromosomes in the premeiotic nucleus, leading to an increased distance between homologs. This results in partial asynapsis of homologs that are relatively distant and some pairing of homeologues that happen to lie close to one another. Thus, an interlocking of bivalents can occur with their chromosomal constituents coming to pair from a relative distance and catching other chromosomes between them (Feldman 1993 and references therein).

Figure 1 Schematic of the wheat karyotype. The wheat karyotype is arranged into genomes A, B, and D and into seven homeologous groups (e.g., group 1 consists of chromosomes 1A, 1B, and 1D). This arrangement is after Sears (1954), who classified homeologous chromosomes based on their ability to compensate for each other's absence. Examples of the different types of sequences are drawn on top of the chromosomes, namely: group-specific sequences that are present in only one homeologous group, chromosome-specific sequences (CSSs) that are present in only one chromosome pair, genome-specific sequences (GSSs) that can be on more than one chromosome pair but only in one of the genomes, and nonspecific sequences that are present on both homeologous and nonhomeologous chromosomes. The nonspecific sequences are mainly dispersed repeats (adapted from Levy and Feldman 2004).

Studying the effects of *Ph1* on centromere behavior in meiotic cells, Vega and Feldman (1998) concluded that the *Ph1* gene affects the interaction between the centromeres and microtubules, possibly through a microtubule-associated protein (MAP) whose activity is located near the centromere. G. Moore and co-workers have localized *Ph1* to a 2.5-Mb interstitial region of wheat chromosome 5B (Griffiths *et al.* 2006; Yousafzai *et al.* 2010). This region contains a structure consisting of a segment of subtelomeric heterochromatin that inserted into a cluster of *cdc2*-related genes after polyploidization (Griffiths *et al.* 2006). The correlation of the presence of this structure with *Ph1* activity in related species, and the involvement of heterochromatin with *Ph1* and *cdc2* genes with meiosis, makes the structure a good candidate for the *Ph1* locus. These mapping data suggest that the mode of action of this complex locus is determined by the effect of cyclin-dependent kinases on cell-cycle progression (Griffiths *et al.* 2006; Al-Kaff *et al.* 2008; Yousafzai *et al.* 2010). However, direct evidence as to the identity of the coding gene and its mode of action is still missing. Recently, K. Gill and associates (personal communication) identified a candidate gene in the *Ph1* region that is different from the one that was proposed by the lab of G. Moore. Silencing of the newly identified gene disrupts the alignment of chromosomes on the metaphase plate in early stages of meiosis, suggesting a role of microtubules in its function (K. Gill, personal communication).

It was suggested by several authors that *Ph*-like genes exist in diploid wheat species (Okamoto and Inomata 1974; Avivi 1976; Waines 1976; Maan 1977) and that they became more effective at the polyploid level as a result of duplication. This dosage-dependent effect might have been selected to yield an allopolyploid with improved fertility.

The suppressive effect of *Ph1* on homeologous pairing in interspecific and intergeneric wheat hybrids is absolute. However, its effect on homeologous pairing in bread wheat itself might not be indispensable as plants deficient for this gene exhibit relatively little homeologous pairing. This is evidenced from the small number of multivalents (less than one per cell), resulting from intergenomic pairing in these plants (Sears 1976). Interestingly, and in accord with the above, *Ph*-like gene(s) have not been found in any of the allopolyploid species of the closely related *Aegilops* genus (Sears 1976). Nevertheless, these species also exhibit exclusive bivalent pairing of fully homologous chromosomes. Hence, additional mechanisms that ensure homologous pairing must be called for and are discussed below.

Novel molecular tools for investigating wheat genomics has advanced our understanding of the exclusive intra-genomic pairing in bread wheat. Using micromanipulation, Vega *et al.* (1994, 1997) isolated more than 30 isochromosomes of the long arm of chromosome 5B from first meiotic metaphase spreads of a monoisosomic 5BL line of bread wheat. The dissected isochromosomes were amplified by degenerate oligonucleotide-primed PCR and a large number of DNA sequences were produced from this arm (Vega *et al.* 1994, 1997). These sequences were classified by us (Feldman *et al.* 1997) into the following four classes (Figure 1): (i) non-specific sequences (mainly repetitious sequences) that are found in all or many of the wheat chromosomes; (ii) group-specific sequences (mainly coding sequences) that occur in the chromosomes of one homeologous group, e.g., 1A, 1B, and 1D; (iii) genome-specific sequences that occur in several chromosomes of one genome, e.g., 1A, 2A, 3A, etc.; and (iv) chromosome-specific sequences (CSSs) that occur in only one homologous chromosome pair, e.g., 1A and 1A. Most of the CSSs are noncoding sequences (Feldman *et al.* 1997) and are present in all the diploid species of *Aegilops* and *Triticum*, but occur in only one pair of chromosomes of allopolyploid wheat, suggesting that they were lost during or after allopolyploidization (Feldman *et al.* 1997).

A most surprising discovery is that allopolyploidization in the wheat group causes immediate nonrandom elimination of specific noncoding, low-copy, and high-copy DNA sequences (Feldman *et al.* 1997; Liu *et al.* 1998a,b; Ozkan *et al.* 2001; Shaked *et al.* 2001; Han *et al.* 2003, 2005; Salina *et al.* 2004). The extent of DNA elimination was estimated by determining the amounts of nuclear DNA in natural allopolyploids and in their diploid progenitors, as well as in newly synthesized allopolyploids and their parental plants (Ozkan *et al.* 2003; Eilam *et al.* 2008, 2010). Natural wheat and related allopolyploids contain 2–10% less DNA than the sum of their diploid parents, and synthetic allopolyploids

exhibit a similar loss, indicating that DNA elimination occurs soon after allopolyploidization (Nishikawa and Furuta 1969; Furuta *et al.* 1974; Eilam *et al.* 2008, 2010). Also, the narrow intraspecific variation in DNA content of the allopolyploids supports that the loss of DNA occurred immediately after the allopolyploid formation and that there was almost no subsequent change in DNA content during the allopolyploid species evolution (Eilam *et al.* 2008). In triticale (a synthetic allopolyploid between wheat and rye, *Secale cereale*), Boyko *et al.* (1984, 1988) and Ma and Gustafson (2005) found that there was a major reduction in DNA content in the course of triticale formation, amounting to ~9% for the octoploid and 28–30% for the hexaploid triticale. In this synthetic allopolyploid, the various genomes were not affected equally: the wheat genomic sequences were relatively conserved, whereas the rye genomic sequences underwent a high level of variation and elimination (Ma *et al.* 2004; Ma and Gustafson 2005, 2006). Similarly, in hexaploid wheat, genome D underwent a considerable reduction in DNA, while the A and B genomes were not reduced in size (Eilam *et al.* 2008, 2010).

Bento *et al.* (2011) reanalyzed data concerning genomic analysis of octoploid and hexaploid triticale and different synthetic wheat hybrids, in comparison with other polyploid species. This analysis revealed high levels of genomic restructuring events in triticale and wheat hybrids, namely major parental band disappearance and the appearance of novel bands. Furthermore, the data showed that restructuring depends on parental genomes, ploidy level, and sequence type (repetitive, low copy, and/or coding); is markedly different after wide hybridization or genome doubling; and affects preferentially the larger parental genome (Bento *et al.* 2011).

Screening the parental wheat diploid species for a number of CSSs, each located on different chromosome arms in common wheat, it was found that many of them occur in all the diploids (Feldman *et al.* 1997). However, in the allopolyploids these sequences occur in only one chromosome pair and are absent from the homeologous chromosomes (Feldman *et al.* 1997). By analyzing newly formed *Triticum* and *Aegilops* allopolyploids with genomic combinations similar to those of the natural allopolyploids, it was shown that the CSSs were eliminated from one genome immediately or a few generations after the formation of the allopolyploid (Feldman *et al.* 1997; Ozkan *et al.* 2001). Thus, chromosome-specific sequences are found in each homologous pair, but different homeologous pairs have their own unique signatures.

Since CSSs are the only sequences that determine chromosomal homology, it is assumed that they are implicated in recognition and initiation of homologous pairing at meiosis. Therefore, if upon allopolyploid formation these sequences are eliminated from one pair of homeologous chromosomes in tetraploids or from two pairs in hexaploids, a cytological diploidization process that strongly augments the physical divergence of the homeologous chromosomes takes place. It is then extremely difficult or even impossible for them to pair at meiosis. Thus, cytological diploidization leads

to exclusive intragenomic pairing, *i.e.*, diploid-like meiotic behavior.

DNA elimination seems not to be random at the intrachromosomal level as well (Liu *et al.* 1997). For example, these authors found that the CSSs on chromosome arm 5BL in allohexaploid wheat are not distributed randomly but cluster in terminal (subtelomeric), subterminal, and interstitial regions of this arm. Such structures make these regions extremely chromosome specific—or homologous. Hence, it was tempting to suggest that these chromosome-specific regions are equivalents to the classical “pairing-initiation sites” that play a critical role in homology search and initiation of pairing at meiosis (Feldman *et al.* 1997). Computer modeling also shows that homeolog divergence in association with pairing stringency drives disomic inheritance (Le Comber *et al.* 2010),

To sum up, cytological diploidization in allopolyploid wheat was engendered by two independent, complementary systems. One is based on the physical divergence of chromosomes and the second, on the genetic control of pairing. The *Ph*-gene system superimposes itself on and takes advantage of—and thereby reinforces—the above-described system of the physical differentiation of homeologous chromosomes. In addition, stringent selection for fertility might well favor the development of two systems to effect the suppression of multivalent formation and the promotion of bivalent pairing in nature and, more so, in domesticated material.

The process of cytological diploidization in wheat group allopolyploids has been critical for their establishment in nature. The restriction of pairing to completely homologous chromosomes ensures regular segregation of genetic material, high fertility, genetic stability, and disomic inheritance that prevents the independent segregation of chromosomes of the different genomes. Homologous pairing also allows for the maintenance of favorable intergenomic genetic interactions. On the other hand, disomic inheritance sustains the asymmetry in the control of many traits by the different genomes (Feldman *et al.* 2012). In addition, since cytological diploidization facilitates genetic diploidization, existing genes in double and triple doses can be diverted to new functions through mutations, thereby favoring the creation of favorable, new intergenomic combinations.

Structural Changes That Occur in the Polyploid Lineage through Time

Several structural changes are known to have sporadically occurred during the history of allopolyploid wheat genomes. These generate an additional source of variation that, some of which, could not have taken place in the diploid parental genomes and occur almost exclusively in an allopolyploid background. Such intergenomic changes include the horizontal transfer of chromosomal segments, repetitive sequences, transposons, or genes via intergenomic translocations resulting from interchange between nonhomologous or homeologous chromosomes. Intergenomic translocations that characterize

specific populations or biotypes are widespread in allohexaploid wheat (Maestra and Naranjo 1999 and references therein). Invasion of the A genome by sequences from B—most probably transposons—was detected in wild allotetraploid wheat using genomic *in situ* hybridization (GISH) (Belyayev *et al.* 2000). The possibility of intergenomic transfer adds to allopolyploid genomic plasticity and enables the creation of new genetic combinations beyond those possible through the intragenomic mechanisms of the individual genomes.

Moreover, in contrast to the diploids, which are genetically isolated from each other and have undergone divergent evolution, wheat group allopolyploids exhibit convergent evolution because they contain genetic material from two or more unique diploid genomes that can be exchanged via hybridization and introgression, resulting in new genomic combinations. Examples for introgression between allotetraploid *Aegilops* species were provided by Zohary and Feldman (1962) and Feldman (1965a,b,c). Additional evidence for the existence of introgressed genomes in allopolyploid *Aegilops* was obtained by C-banding analysis (Badaeva *et al.* 2004). Introgression of a DNA sequence from allopolyploid wheat to lines of the allotetraploid *Aegilops* species, *Ae. peregrina*, was also described (Weissmann *et al.* 2005).

In addition to evolutionary changes that are almost unique among allopolyploids, other types of mutations (e.g., point mutations, microsatellite instability, transposition, etc.) may contribute, perhaps in an accelerated manner, to evolutionarily relevant structural or functional changes in allopolyploids. For example, presence of duplicate or triplicate genetic material in wheat allopolyploids might have relaxed constraints on gene structure and function. Thus, the accumulation of genetic variation through mutation or hybridization might be more readily tolerated in an allopolyploid than in a diploid species. While there is no direct evidence for this assumption, there is indirect support from experimental data showing a higher resistance of allohexaploid wheat to irradiation compared to their diploid progenitors (Mac Key 1954, 1958; Sears 1972). Such an increase in mutation resistance with increased ploidy was shown in yeast to be correlated with higher evolutionary ability and fitness (Thompson *et al.* 2006).

Genetic or Functional Diploidization

In some cases, an extra genetic dose can be beneficial, while in others it may be deleterious. Most duplicated genes that code for enzymes are active in allopolyploid wheats (Hart 1987). The extra gene itself might provide a favorable effect or it could lead to the buildup of positive intergenomic interactions if genes or regulation factors on homeologous chromosomes are divergent. In some duplicated genes, however, increased dosage has led to redundancy or to a deleterious effect. In these cases, functional diploidization processes bring the redundant or unbalanced gene systems into a diploid-like mode of expression. Functional or genetic diploidization can

be achieved through elimination, inactivation, or diversion of the redundant, duplicated genes to new functions.

In studies on the genetic control of high-molecular-weight (HMW) glutenin subunits, an important component of wheat storage proteins, allopolyploidy was found to affect gene function through a variety of genetic and epigenetic mechanisms (Galili and Feldman 1984; Forde *et al.* 1985; Galili *et al.* 1986; Waines and Payne 1987). The levels of HMW glutenin subunits in the endosperm was determined in a series of wheats containing different doses of chromosomes 1A, 1B, or 1D, which carry the genes controlling the production of these proteins. It was found that HMW glutenin subunit production was nonlinear in response to gene dosage, indicating the operation of a mechanism of dosage compensation (Galili *et al.* 1986). Such dosage compensation is commonly observed as a way to instantaneously reduce the negative effect of overproduction and inefficiency of genes that exist in super optimal doses (Birchler *et al.* 2001).

Another aspect regulating gene action in newly formed allopolyploids is intergenomic suppression (Galili and Feldman 1984; Galili *et al.* 1986). By crossing hexaploid with tetraploid wheat, backcrossing the pentaploid offspring of each generation back to the hexaploid parent, and finally selfing the pentaploid to obtain tetraploid plants, Kerber (1964) extracted the A and B genomes of allohexaploid wheat (genome BBAADD) to produce an extracted allotetraploid wheat line lacking the D genome. This line facilitated the study of intergenomic relationships between the D genome genes and those of A and B. When compared with the mother allohexaploid, polyacrylamide gels of storage proteins of the extracted tetraploid line exhibited several bands with increased staining intensity as well as new bands (Galili and Feldman 1984). These novel bands are assumed to have resulted from a novel activity of genes located on the A or B genomes, which are no longer repressed by the removed D genome. Re-addition of the D genome to the extracted allotetraploid instantaneously resuppressed these genes. Galili and Feldman (1984) suggested that these endosperm-protein genes were repressed immediately following the formation of allohexaploid wheat, about 10,000 years ago, but they still have retained their potential for being activated.

Likewise, using microarray analysis, a group of genes located on chromosomes of genomes A and B were found to be strictly regulated by the D genome. They are not expressed in allohexaploid wheat; they are expressed in an extracted allotetraploid (genome BBAA) and they are silenced again upon re-adding the D genome (B. Liu, personal communication). Similarly, Kerber and Green (1980) described an intergenomic suppression of a rust-resistance gene in genome D by gene(s) in genome A or B. Intergenomic suppression of disease-resistance genes is a common phenomenon in wheat and related allopolyploids, as was noted in several natural and newly formed allopolyploids (Y. Anikster, J. Manisterski, and M. Feldman, unpublished data). Comparable results were obtained by Dhaliwal and co-workers (Aghaee-Sarbarzeh *et al.* 2001) in *Triticum durum*–*Aegilops*

amphiploids. They found that dominant leaf-rust- and stripe-rust-resistance genes from the *Aegilops* parents were suppressed by genes in the AB genomes of the wheat parent. Another well-studied example of intergenomic suppression is the silencing in triticale of the ribosomal RNA genes of rye in the presence of the wheat genome (Appels *et al.* 1986 and references therein). Cytosine methylation is involved in this silencing as suggested by reactivation of the rye ribosomal RNA genes by the demethylation agent 5-azacytidine or methylation sensitive/insensitive isoschizomers (Houchins *et al.* 1997). Intergenomic suppression is a common control mechanism for instantaneously reducing the negative effect of overproduction and the inefficiency of genes that exist in super-optimal doses.

In bread wheat, HMW glutenin genes are located on chromosomes of homeologous group 1, *i.e.*, 1A, 1B, and 1D. These chromosomes carry two genes: Glu1-1, coding for the slow-migrating subunit, and Glu1-2, coding for the fast migrating subunit (Payne *et al.* 1981). Galili and Feldman (1983b) analyzed 109 different lines of allohexaploid wheat representing a wide spectrum of genetic backgrounds and found that 22 lines (20.2%) had no HMW glutenin subunits controlled by chromosome 1A, 44 lines (40.4%) had only one subunit controlled by 1A, and 43 lines (39.4%) had two such subunits. Moreover, in all lines having one subunit controlled by 1A, this subunit was always the slow-migrating variety; *i.e.*, only *Glu-1A-1* was active. Hence, in 60% of the hexaploid lines studied, *Glu-A1-2* was inactive despite the fact that this gene is regularly active in diploid wheat (Waines and Payne 1987).

The HMW glutenin subunits in 456 accessions of the wild allotetraploid wheat *T. turgidum* subsp. *dicoccoides*, originating from 21 different populations in Israel, were studied (Levy and Feldman 1988; Levy *et al.* 1988). In 82% of the accessions, the fast-migrating subunit of genome A was absent, and in 17% of the accessions the slow-migrating subunit of this genome was also absent. In all 11 studied lines of the primitive domesticated allotetraploid wheat, *T. turgidum* subsp. *dicoccum*, the fast-migrating subunit of genome A, was absent, *i.e.*, *Glu-A1-2* was inactive, and in all 19 lines of modern allotetraploid wheat, subsp. *durum*, *Glu-A1-1* was also inactive, while only Glu-B1-1 and Glu-B1-2 were active (Feldman *et al.* 1986). Moreover, the HMW glutenin loci of genome B were much more polymorphic than those of genome A (Felsenburg *et al.* 1991). The reduced polymorphism of the A genome loci apparently reflects the nonrandom inactivation of the HMW glutenin genes, as well.

Thus, in both allotetraploid and allohexaploid wheat, inactivation of HMW glutenin genes was massive and nonrandom and occurred in the glutenin genes of genome A (Galili and Feldman 1983a,b; Feldman *et al.* 1986; Levy *et al.* 1988; and reference therein). In hexaploid wheat, this tendency has been found for HMW gliadin genes, as well (Galili and Feldman 1983a,b). The nonrandom nature of the process is shown by the fact that not only were the HMW glutenin genes of the A genome specifically affected but their

order of inactivation was also nonrandom, starting with the rapidly migrating subunits and continuing with the slowly migrating ones. The fast-migrating glutenin gene of genome A was not inactivated by elimination, but from the positioning of a terminating sequence inside the transcribed portion of the gene (Forde *et al.* 1985).

The development of molecular genetic techniques for the study of plants in general and wheat in particular has provided tools for further investigating genome changes due to allopolyploidization that are involved in the development of functional diploidization. Examining newly formed allopolyploid wheat by cDNA-AFLP, Shaked *et al.* (2001) found that genes were either eliminated or silenced via cytosine methylation of DNA sequences. They found that alterations in cytosine methylation (demethylation or new methylation) affected about 13% of a random set of genomic loci. Several cDNAs were expressed in the allopolyploids but not in the diploid progenitors (Shaked *et al.* 2001; Kashkush *et al.* 2002). Kashkush *et al.* (2002) studied the response of the transcriptome to allopolyploidization in the first generation of newly formed allopolyploid wheat and in its two diploid progenitors. They found that transcript disappearance was the result of gene loss or silencing, the latter being associated with cytosine methylation. The silenced/lost genes included rRNA genes as well as genes involved in metabolism, disease resistance, and cell-cycle regulation. The activated genes with known functions were all retroelements. Similarly, He *et al.* (2003) found that the expression of a significant fraction of the genome (7.7%) is altered in a synthetic hexaploid wheat.

Following allopolyploidization events in wheat, the steady-state level of expression of long terminal repeats (LTR) in retrotransposons was massively elevated (Kashkush *et al.* 2002, 2003). In addition, the transcriptional activity of the *Wis2-1A* LTR element was shown to be associated with the production of read-out transcripts flanking toward host DNA sequences, a process that occurred in a genome-wide manner (Kashkush *et al.* 2003). In many cases, these read-out transcripts were associated with the expression of adjacent genes, depending on their orientation. They knocked down or knocked out the gene product if the read-out transcript was in the antisense orientation relative to the orientation of the gene transcript (such as the *iojap-like* gene), or they overexpressed the gene if the read-out transcript was in the sense orientation (such as the *puroindoline-b* gene) (Kashkush *et al.* 2003). The mechanisms by which transcriptional activation of TEs influences the expression of neighboring genes are poorly understood. Recent studies (Kraitshtein *et al.* 2010; Yaakov and Kashkush 2011) on tracking methylation changes around transposons in the first generations of a newly formed wheat allopolyploid showed massive changes in methylation and that read-out activity was restricted to the first generations of the nascent polyploid species.

Transcriptional activation of transposons in allopolyploids is part of the “genomic shock” syndrome that results from interspecific hybridization and polyploidization as proposed

by McClintock (1984). The mechanism that leads to this activation is not well understood.

Recently, Kenan-Eichler *et al.* (2011) found that small RNAs (siRNAs) that are thought to repress transposons under normal conditions can be disregulated in allopolyploid. The repression of these repressors is correlated with hypomethylation of the transposons, which in turn may enable their transcriptional activation (Kenan-Eichler *et al.* 2011). An additional pathway of small RNAs-mediated epigenetic control of genome stability and expression might be achieved through the activity of microRNAs. Changes in microRNAs expression in newly synthesized wheat were observed, such as that of miR168, which targets the *Argonaute1* gene (Kenan-Eichler *et al.* 2011). Changes in microRNAs expression were also found in *Arabidopsis* allopolyploids that presumably have led to changes in gene expression, growth vigor, and adaptation (Ha *et al.* 2009).

Inactivation of homeoalleles may be a nonrandom effect. The data of Koeber and co-workers (Bottley *et al.* 2006) suggested that for leaf transcripts, there is a modest bias toward silencing of the D genome copies, but this pattern does not extend to root transcripts. Interestingly, He *et al.* (2003) found that *Ae. tauschii* genes (genome DD) were affected much more frequently than *T. turgidum* genes (genome BBAA) in a BBAADD synthetic allopolyploid, and D genome homeoalleles were silenced twice as frequently as those from the A or B genomes in a newly synthesized hexaploid wheat line. Silencing of the same genes was also found in the bread wheat cultivar Chinese Spring (He *et al.* 2003).

Thus, allopolyploidization triggers gene silencing, gene elimination, or gene activation and transposon activation via genetic and epigenetic alterations immediately upon allopolyploid formation.

Concluding Remarks

Formation of an allopolyploid species is accomplished in a small number of steps. However, its establishment and survival in nature probably depend on its ability to self, the number of allopolyploid individuals that are formed, and perhaps also exchange genes with its progenitors, as well as on a high level of genomic plasticity that enables it to overcome potential incompatibilities in its genome and to gain new traits. The studies reported here suggest that allopolyploid wheat can achieve genomic plasticity through the induction of a series of cardinal nonadditive genomic changes. Some of them, genetic and epigenetic, are rapid and non-Mendelian, occurring during or immediately after the formation of the allopolyploid (revolutionary changes; Table 2). Other changes occur sporadically over a long period of time during the evolution of the allopolyploid (evolutionary changes; Table 2). From a population point of view, the chances of several individuals of a nascent allopolyploid being established as a new species is almost null, unless they exhibit increased fitness compared to their parents or new traits that can enable them to colonize new niches. This must occur within a

few generations, otherwise the new species will rapidly become extinct.

The revolutionary changes described here may contribute to the establishment of new allopolyploid species. Instantaneous elimination of sequences from one genome in the new plant increases the divergence of the homeologous chromosomes, leading to exclusive intragenomic pairing and improving fertility. Mechanisms enabling the loss of deleterious genes (*e.g.*, the removal of genetic incompatibilities), positive gene dosage effects, and new intergenomic heterotic interactions may all rapidly increase fitness of the nascent species.

Evolutionary changes, however, contribute to the buildup of genetic variability and thereby increase adaptability, fitness, competitiveness, and colonizing ability. In nature, most hybridization events do not lead to the formation of a new species. However, the wheat group is remarkably equipped with a battery of molecular mechanisms that enable the appearance of phenotypic novelty and successful speciation through allopolyploidy. Future work should help to clarify (i) the role of specific genes and DNA sequences in allopolyploid speciation; (ii) the mechanisms that confer robustness to the genome under the shock of allopolyploidy; (iii) the activation of transposable elements; (iv) the mechanisms that enable the orchestration of chromosome division; and (v) the control of bivalent pairing during meiosis.

The rapid processes of cytological and genetic diploidization allow for the development of two contrasting and highly important genetic phenomena in allopolyploid wheat that presumably contribute to their evolutionary success: (i) the buildup and maintenance of enduring intergenomic favorable genetic combinations and (ii) genome asymmetry in the control of a variety of morphological, physiological, and molecular traits, namely, the total or predominant control of certain traits by a single constituent genome. While the first phenomenon was taken for granted by plant geneticists, genomic asymmetry in interspecific hybrids and allopolyploids was mainly recognized in ribosomal RNA genes (reviewed in Pikaard 2000). Only recently has this been documented in allopolyploid wheat, where genome A was found to control morphological traits while genome B in allotetraploid wheat and genomes B and D in allohexaploid wheat were shown to control reactions to biotic and abiotic factors (Feldman *et al.* 2012 and references therein). Intergenomic pairing might have led to disruption of the linkage of the homeoalleles, which contribute to positive intergenomic interactions and might have also led to the segregation of genes that participate in the control of certain traits by a single genome. Intergenomic recombination could therefore produce many intermediate phenotypes that may negatively affect the functionality, adaptability, and stability of the allopolyploids.

The revolutionary and evolutionary genomic changes reported for wheat allopolyploids emphasize the dynamic plasticity of their genomes with regard to structure and function alike. These changes might have improved and currently improve the adaptability of the newly formed allopolyploids

Table 2 Genomic changes in allopolyploid wheat thought to promote and facilitate speciation (adapted from Feldman *et al.* 2012)

Structural	Functional
1. Revolutionary changes occur during or soon after allopolyploidization, lead to diploidization and are often reproducible	
Genetic <ul style="list-style-type: none"> • Elimination of low-copy DNA sequences • Elimination, reduction or amplification of high-copy sequences • Intergenomic invasion of DNA sequences • Elimination of rRNA and 5S RNA genes 	Genetic <ul style="list-style-type: none"> • Gene loss/loss of function • Rewiring of gene expression through novel intergenomic interactions • Novel dosage responses (positive, negative, dosage compensation) • Gene suppression or activation • Transcriptional activation of transposons that may affect nearby genes • New transposon insertion/excision
Epigenetic <ul style="list-style-type: none"> • Chromatin remodeling • Chromatin modification • Heterochromatinization • DNA methylation • Small RNA activation or repression 	Epigenetic <ul style="list-style-type: none"> • Gene silencing or activation through changes in methylation or small RNAs or through chromatin modifications • Transposon silencing or activation through demethylation or changes in small RNAs or through chromatin modifications
2. Evolutionary changes facilitated by allopolyploidy in wheat occur during the evolution of the species by promoting species biodiversity (current knowledge is limited to the genetic rather than epigenetic changes) <ul style="list-style-type: none"> • Chromosomal re-patterning (intra- and intergenomic translocations) • Introgression of chromosomal segments from alien genomes and production of recombinant genomes • Intergenomic transposons invasion 	<ul style="list-style-type: none"> • Subfunctionalizations • Neofunctionalizations • New dosage effects through copy number variation • New allelic variations

and facilitated their rapid colonization of new ecological niches. No wonder, therefore, that domesticated allohexaploid wheat exhibits a wider range of genetic flexibility than diploid species and has been able to adapt itself to an impressive variety of environments over a very short evolutionary time scale.

Ohno's (1970) proposal that evolution moves forward through polyploidy (whole-genome duplication) is presently generally accepted. The current use of accurate and sensitive molecular methods, *e.g.*, sequence analysis, shows that polyploidy (recent and ancient) is a widespread phenomenon occurring in up to 80% of angiosperms (Soltis and Soltis 1993; Masterson 1994; Otto and Whitton 2000), in 95% of pteridophytes (Grant 1971), and in the lineage of all vertebrates (Van de Peer *et al.* 2009 and references therein). Species that were considered typical diploids (*e.g.*, maize, rice, *Arabidopsis*, yeast, and humans) are in fact ancient polyploids, *i.e.*, paleopolyploids, that underwent one or more rounds of chromosome doubling during their evolution (Van de Peer *et al.* 2009 and references therein). The progenitors of paleopolyploids cannot be identified due to both cytological and genetic diploidization. The fact that all polyploids, including paleopolyploids, recent allopolyploids, and diloidized autopolyploids, underwent cytological and genetic diploidization supports the concept that polyploidy without diploidization is an evolutionary dead end. The works reported here, showing rapid diploidization, suggest that polyploids that did not diploidize could not survive rather than an alternative model of gradual decay of redun-

dant loci. Hence, the processes described above and similar processes described in other plant polyploids (*e.g.*, Chen and Ni 2006; Chaudhary *et al.* 2009; Tate *et al.* 2009; Buggs *et al.* 2011; and references therein) suggest that diploidization was essential for the successful establishment of polyploid plant and animal species.

Acknowledgments

The constructive comments of four anonymous reviewers and of the editor are acknowledged with appreciation.

Literature Cited

- Aghaee-Sarbarzeh, M., H. S. Dhaliwal, and H. Singh, 2001 Suppression of rust resistance genes from distantly related species in *Triticum durum*-*Aegilops* amphiploids, pp. 195–201 in Proceedings of the First Regional Conference on Yellow Rust in the Central and West Asia and North Africa Region, Karaj, Iran, edited by R. Johnson, A. Yahyaoui, C. Wellings, A. Saidi, and H. Ketata, May 8–14.
- Al-Kaff, N., E. Knight, I. Bertin, T. Foote, N. Hart *et al.*, 2008 Detailed dissection of the chromosomal region containing the *Ph1* locus in wheat *Triticum aestivum*: with deletion mutants and expression profiling. *Ann. Bot. (Lond.)* 101: 863–872.
- Appels, R., L. B. Moran, and J. P. Gustafson, 1986 The structure of DNA from the rye (*Secale cereale*) NOR R1 locus and its behaviour in wheat backgrounds. *Can. J. Genet. Cytol.* 28: 673–685.
- Avivi, L., 1976 The effect of genes controlling different degrees of homoeologous pairing on quadrivalent frequency in induced autotetraploid lines of *Triticum longissimum*. *Can. J. Genet. Cytol.* 18: 357–364.

- Badaeva, E. D., A. V. Amosova, T. E. Samatadze, S. A. Zoshchuk, N. G. Shostak *et al.*, 2004 Genome differentiation in *Aegilops*. 4. Evolution of the U-genome cluster. *Plant Syst. Evol.* 246: 45–76.
- Belyayev, A., O. Raskina, A. Korol, and E. Nevo, 2000 Coevolution of A and B genomes in allotetraploid *Triticum dicoccoides*. *Genome* 43: 1021–1026.
- Bento, M., J. P. Gustafson, W. Viegas, and M. Silva, 2011 Size matters in Triticeae polyploids: larger genomes have higher re-modeling. *Genome* 54: 175–183.
- Birchler, J. A., U. Bhadra, M. P. Bhadra, and D. L. Auger, 2001 Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. *Dev. Biol.* 234: 275–288.
- Bottley, A., G. M. Xia, and R. M. D. Koebner, 2006 Homoeologous gene silencing in hexaploid wheat. *Plant J.* 47: 897–906.
- Boyko, E. V., N. S. Badaev, N. G. Maximov, and A. V. Zelenin, 1984 Does DNA content change in the course of Triticale breeding? *Cereal Res. Commun.* 12: 99–100.
- Boyko, E. V., N. S. Badaev, N. G. Maximov, and A. V. Zelenin, 1988 Regularities of genome formation and organization in cereals. I. DNA quantitative changes in the process of allopolyploidization. *Genetika* 24: 89–97.
- Buggs, R. J. A., L. Zhang, N. Miles, J. A. Tate, L. Gao *et al.*, 2011 Transcriptomic shock generates evolutionary novelty in a newly formed, natural allopolyploid plant. *Curr. Biol.* 21: 551–556.
- Caldwell, R. M., and L. E. Compton, 1943 Complementary lethal genes in wheat causing a progressive lethal necrosis of seedlings. *J. Hered.* 34: 67–70.
- Chapman, V., T. E. Miller, and R. Riley, 1976 Equivalence of the A genome of bread wheat and that of *Triticum urattu*. *Genet. Res.* 27: 69–76.
- Chaudhary, B., L. Flagel, R. M. Stupar, J. A. Udall, N. Verma *et al.*, 2009 Reciprocal silencing, transcriptional bias and functional divergence of homeologs in polyploid cotton (*Gossypium*). *Genetics* 182: 1–15.
- Chen, Z. J., and Z. Ni, 2006 Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. *Bioessays* 28: 240–252.
- Driscoll, C. J., N. L. Darvey, and H. N. Barber, 1967 Effect of colchicine on meiosis of hexaploid wheat. *Nature* 216: 687–688.
- Dvorak, J., 1976 The relationship between the genome of *Triticum urattu* and the A and B genomes of *Triticum aestivum*. *Can. J. Genet. Cytol.* 18: 371–377.
- Eig, A., 1929 Monographisch-kritische Übersicht der Gattung *Aegilops*. *Feddes Repert.* 55(Beih.): 1–228.
- Eilam, T., Y. Anikster, E. Millet, J. Manisterski, and M. Feldman, 2008 Nuclear DNA amount and genome downsizing in natural and synthetic allopolyploids of the genera *Aegilops* and *Triticum*. *Genome* 51: 616–627.
- Eilam, T., Y. Anikster, E. Millet, J. Manisterski, and M. Feldman, 2010 Genome size in diploids, allopolyploids, and autopolyploids of mediterranean triticeae. *J. Bot.* 210: 10.1155/2010/341380.
- Feldman, M., 1965a Further evidence for natural hybridization between tetraploid species of *Aegilops* Section *Pleionathera*. *Evolution* 19: 162–174.
- Feldman, M., 1965b Fertility of interspecific F_1 hybrids and hybrid derivatives involving tetraploid species of *Aegilops* Section *Pleionathera*. *Evolution* 19: 556–562.
- Feldman, M., 1965c Chromosome pairing between differential genomes in hybrids of tetraploid *Aegilops* species. *Evolution* 19: 563–568.
- Feldman, M., 1966 The effect of chromosomes 5B, 5D and 5A on chromosomal pairing in *Triticum aestivum*. *Proc. Natl. Acad. Sci. USA* 55: 1447–1453.
- Feldman, M., 1993 Cytogenetic activity and mode of action of the pairing homoeologous (*Ph1*) gene of wheat. *Crop Sci.* 33: 894–897.
- Feldman, M., 2001 The origin of cultivated wheat, pp. 1–56. *In The Wheat Book*, edited by A. Bonjean, and W. Angus. Lavoisier Tech & Doc, Paris.
- Feldman, M., and L. Avivi, 1988 Genetic control of bivalent pairing in common wheat: the mode of *Ph1* action, pp. 269–279 in *The Third Kew Chromosome Conference*, edited by P. E. Brandham. Kew Publishing, Richmond, Surrey, UK.
- Feldman, M., and A. A. Levy, 2005 Allopolyploidy—a shaping force in the evolution of wheat genomes. *Cytogenet. Genome Res.* 109: 250–258.
- Feldman, M., and A. A. Levy, 2009 Genome evolution in allopolyploid wheat—a revolutionary reprogramming followed by gradual changes. *J. Genet. Genomics* 36: 511–518.
- Feldman, M., and A. A. Levy, 2011 Instantaneous genetic and epigenetic alterations in the wheat genome caused by allopolyploidization, pp. 261–270 in *Transformations of Lamarckism, From Subtle Fluids to Molecular Biology*, edited by S. B. Gissis, and E. Jablonka. The MIT press, Cambridge, MA.
- Feldman, M., G. Galili, and A. A. Levy, 1986 Genetic and evolutionary aspects of allopolyploidy in wheat, pp. 83–100 in *The Origin and Domestication of Cultivated Plants*, edited by C. Barigozzi Elsevier, Amsterdam.
- Feldman, M., F. G. H. Lupton, and T. E. Miller, 1995 Wheats, pp. 184–192 in *Evolution of Crop Plants*, Ed. 2nd, edited by J. Smartt and N. W. Simmonds. Longman Scientific, London.
- Feldman, M., B. Liu, G. Segal, S. Abbo, A. A. Levy *et al.*, 1997 Rapid elimination of low copy DNA sequences in polyploid wheat: A possible mechanism for differentiation of homoeologous chromosomes. *Genetics* 147: 1381–1387.
- Feldman, M., A. A. Levy, T. Fahima, and A. Korol, 2012 Genome asymmetry in allopolyploid plants - wheat as a model. *J. Exp. Bot.* 63: 10.1093/jxb/errs311932.
- Feldman, M., A. A. Levy, B. Chalhoub, and K. Kashkush, 2012 Genome Plasticity in Polyploid Wheat, *Polyploidy and Genome Evolution*, edited by Soltis, P. S., and D. Soltis. Springer-Verlag, Berlin/Heidelberg (in press).
- Felsenburg, T., A. A. Levy, G. Galili, and M. Feldman, 1991 Polymorphism of high molecular weight glutenins in wild tetraploid wheat: Spatial and temporal variation in a native site. *Isr. J. Bot.* 40: 501–508.
- Flavell, R. B., 1982 Sequence amplification, deletion and rearrangement: major sources of variation during species divergence, pp. 301–323. *In Genome Evolution*, edited by G. A. Dover and R. B. Flavell, Academic Press, New York.
- Forde, J., J. M. Malpica, N. G. Halford, P. R. Shewry, O. D. Anderson *et al.*, 1985 The nucleotide sequence of an HMW glutenin subunit gene located on chromosome 1A of wheat (*Triticum aestivum* L.). *Nucleic Acids Res.* 13: 6817–6832.
- Furuta, Y., K. Nishikawa, and T. Tanino, 1974 Stability in DNA content of AB genome component of common wheat during the past seven thousand years. *Jpn. J. Genet.* 49: 179–187. doi:10.1266/jgg.49.179.
- Galili, G., and M. Feldman, 1983a Genetic control of endosperm proteins in wheat. 2. Variation in high molecular weight glutenin and gliadin subunits of *Triticum aestivum*. *Theor. Appl. Genet.* 66: 77–86.
- Galili, G., and M. Feldman, 1983b Diploidization of endosperm protein genes in polyploid wheats, pp. 1119–1123 in *Proc. 6th Int. Wheat Genet. Symp.*, Kyoto, Japan, edited by S. Sakamoto, Faculty of Agriculture, Kyoto University, Kyoto, Japan.
- Galili, G., and M. Feldman, 1984 Inter-genomic suppression of endosperm protein genes in common wheat. *Can. J. Genet. Cytol.* 26: 651–656.
- Galili, G., A. A. Levy, and M. Feldman, 1986 Gene-dosage compensation of endosperm proteins in hexaploid wheat *Triticum aestivum*. *Proc. Natl. Acad. Sci. USA* 83: 6524–6528.
- Grant, V., 1971 *Plant Speciation*, Columbia University Press, New York.

- Griffiths, S., R. Sharp, T. N. Foote, I. Bertin, and M. Wanous *et al.*, 2006 Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. *Nature* 439: 749–752.
- Ha, M., J. Lu, L. Tian, V. Ramachandran, K. D. Kasschau *et al.*, 2009 Small RNAs serve as a genetic buffer against genomic shock in *Arabidopsis* interspecific hybrids and allopolyploids. *Proc. Natl. Acad. Sci. USA* 106: 17835–17840.
- Han, F. P., G. Fedak, T. Quillet, and B. Liu, 2003 Rapid genomic exchanges in interspecific and intergeneric hybrids and allopolyploids of Triticeae. *Genome* 46: 716–723.
- Han, F. P., G. Fedak, W. L. Guo, and B. Liu, 2005 Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. *Genetics* 170: 1239–1245.
- Hart, G. H., 1987 Genetic and biochemical studies of enzymes, pp. *Wheat and wheat improvement*, Ed. 2nd, edited by E. G. Heyne American Society of Agronomy, Madison, Wisconsin.
- He, P., B. R. Friebe, B. S. Gill, and J. M. Zhou, 2003 Allopolyploidy alters gene expression in the highly stable hexaploid wheat. *Plant Mol. Biol.* 52: 401–414.
- Hermesen, J. G. T., 1963 Hybrid necrosis as a problem for the wheat breeder. *Euphytica* 12: 1–16.
- Houchins, K., M. O. Dell, R. B. Flavell, and J. P. Gustafson, 1997 Cytosine methylation and nucleolar dominance in cereal hybrids. *Mol. Gen. Genet.* 255: 294–301.
- Huang, S., A. Sirikhachornkit, X. Su, J. Faris, B. Gill *et al.*, 2002 Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. *Proc. Natl. Acad. Sci. USA* 99: 8133–8138.
- Kashkush, K., M. Feldman, and A. A. Levy, 2002 Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. *Genetics* 160: 1651–1659.
- Kashkush, K., M. Feldman, and A. A. Levy, 2003 Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. *Nat. Genet.* 33: 102–106.
- Kenan-Eichler, M., D. Leshkowitz, L. Tal, E. Noor, C. Melamed-Besudo *et al.*, 2011 Wheat head polyploidization results in de-regulation of small RNAs. *Genetics* 188: 263–272.
- Kerber, E. R., 1964 Wheat: reconstitution of the tetraploid component (AABB) of hexaploids. *Science* 143: 253–255.
- Kerber, E. R., and G. J. Green, 1980 Suppression of stem rust resistance in hexaploid wheat cv Canthach by chromosome 7DL. *Can. J. Bot.* 58: 1347–1350.
- Kihara, H., 1924 Cytologische und genetische studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden, *Mem. Cell. Sci., Kyoto Imp. Univ., Ser. B1*: 1–200.
- Kihara, H., 1944 Die Entdeckung der DD-Analysatoren beim Weizen. *Agric and Hort.* (Tokyo) 19: 889–890.
- Kihara, H., 1954 Considerations on the evolution and distribution of *Aegilops* species based on the analyzer-method. *Cytologia* (Tokyo) 19: 336–357.
- Kihara, H., and F. A. Lilienfeld, 1949 New synthesized 6x-wheat. *Proceedings of Eighth International Congress of Genetics*, edited by G. B. A. R. Larsson, Stockholm, Sweden, *Hereditas* (Suppl.), pp. 307–319.
- Kihara, H., H. Yamashita, and M. Tanaka, 1959 Genomes of 6x species of *Aegilops*. *Wheat Inf. Ser.* 8: 3–5.
- Kimber, G., and M. Feldman, 1987 Wild wheats: an introduction. pp. 1–142, Special Report 353. College of Agriculture, Columbia, Missouri.
- Kimber, G., and K. Tsunewaki, 1988 Genome symbols and plasma types in the wheat group, in pp. 1209–1211, *Proceedings of the 7th International Wheat Genetics Symposium*, edited by T. E. Miller and R. M. D. Koebner, Cambridge, UK.
- Kraitshtein, Z., B. Yaakov, V. Khasdan, and K. Kashkush, 2010 Genetic and epigenetic dynamics of a retrotransposon after allopolyploidization of wheat. *Genetics* 186: 801–889.
- Le Comber, S. C., M. L. Ainouche, A. Kovarik, and A. R. Leitch, 2010 Making a functional diploid: from polysomic to disomic inheritance. *New Phytol.* 186: 113–122.
- Levy, A. A., and M. Feldman, 1988 Ecogeographical distribution of HMW glutenin alleles in populations of the wild tetraploid wheat *Triticum turgidum* var. *dicoccoides*. *Theor. Appl. Genet.* 75: 651–658.
- Levy, A. A., and M. Feldman, 2002 The impact of polyploidy on grass genome evolution. *Plant Physiol.* 130: 1587–1593.
- Levy, A. A., and M. Feldman, 2004 Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. *Biol. J. Linn. Soc. Lond.* 82: 607–613.
- Levy, A. A., G. Galili, and M. Feldman, 1988 Polymorphism and genetic control of high molecular weight glutenin subunits in wild tetraploid wheat *Triticum turgidum* var. *dicoccoides*. *Heredity* 61: 63–72.
- Liu, B., G. Segal, J. M. Vega, M. Feldman, and S. Abbo, 1997 Isolation and characterization of chromosome-specific sequences from a chromosome arm genomic library of common wheat. *Plant J.* 11: 959–965.
- Liu, B., J. M. Vega, G. Segal, S. Abbo, M. Rodova *et al.*, 1998a Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy non-coding DNA sequences. *Genome* 41: 272–277.
- Liu, B., J. M. Vega, and M. Feldman, 1998b Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. II. Changes in low-copy coding DNA sequences. *Genome* 41: 535–542.
- Ma, X.-F., and J. P. Gustafson, 2005 Genome evolution of allopolyploids: a process of cytological and genetic diploidization. *Cytogenet. Genome Res.* 109: 236–249.
- Ma, X.-F., and J. P. Gustafson, 2006 Timing and rate of genome variation in triticales following allopolyploidization. *Genome* 49: 950–958.
- Ma, X.-F., P. Fang, and J. P. Gustafson, 2004 Polyploidization-induced genome variation in triticales. *Genome* 47: 839–848.
- Maan, S. S., 1977 Fertility of amphiploids in *Triticinae*. *J. Hered.* 68: 87–94.
- Mac Key, J., 1954 Mutation breeding in polyploid cereals. *Acta Agriculturae Scandinavica* 4: 549–557.
- Mac Key, J., 1958 Mutagenic response in *Triticum* at different levels of ploidy, pp. 88–111, in *Proc. 1st Inter Wheat Genetics Symp.*, edited by C. B. Jenkins, Winnipeg, Manitoba, Canada.
- Maestra, B., and T. Naranjo, 1999 Structural chromosome differentiation between *Triticum timopheevii* and *T. turgidum* and *T. aestivum*. *Theor. Appl. Genet.* 98: 744–750.
- Masterson, J., 1994 Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. *Science* 264: 421–424.
- McClintock, B., 1984 The significance of responses of the genome to challenge. *Science* 226: 792–801.
- McFadden, E. S., and E. R. Sears, 1944 The artificial synthesis of *Triticum spelta*. (Abstr.). *Rec. Genet. Soc. Amer.* 13: 26–27.
- McFadden, E. S., and E. R. Sears, 1946 The origin of *Triticum spelta* and its free-threshing hexaploid relatives. *J. Hered.* 37: 81–89, 107–116.
- Morris, R., and E. R. Sears, 1967 The cytogenetics of wheat and its relatives, pp. 19–87 in *Wheat and Wheat Improvement*, edited by K. S. Quisenberry, and L. P. Reitz. American Society of Agronomy, Madison, WI.
- Nishikawa, K., and Y. Furuta, 1969 DNA content per nucleus in relation to phylogeny of wheat and its relatives. *Jpn. J. Genet.* 44: 23–29 10.1266/jjg.44.23.
- Ohno, S., 1970 *Evolution by Gene Duplication*, Springer, New York.

- Okamoto, M., 1957 Asynaptic effect of chromosome V. *Wheat Inf Serv.* 5: 6.
- Okamoto, M., and N. Inomata, 1974 Possibility of 5B-like effect in diploid species. *Wheat Inf Serv* 38: 15–16.
- Otto, S. P., and J. Whitton, 2000 Polyploid incidence and evolution. *Annu. Rev. Genet.* 34: 401–437.
- Ozkan, H., A. A. Levy, and M. Feldman, 2001 Allopolyploidy-induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. *Plant Cell* 13: 1735–1747.
- Ozkan, H., M. Tuna, and K. Arumuganathan, 2003 Nonadditive changes in genome size during allopolyploidization in the wheat (*Aegilops-Triticum*) group. *J. Hered.* 94: 260–264.
- Payne, P. I., L. M. Holt, and C. N. Law, 1981 Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. *Theor. Appl. Genet.* 60: 229–236.
- Pikaard, C. S., 2000 The epigenetics of nucleolar dominance. *Trends Genet.* 16: 495–500.
- Ramsey, J., and D. Schemske, 1998 Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Syst.* 29: 467–501.
- Riley, R., 1960 The diploidization of polyploid wheat. *Heredity* 15: 407–429.
- Riley, R., and V. Chapman, 1958 Genetic control of the cytologically diploid behavior of hexaploid wheat. *Nature* 182: 713–715.
- Salina, E. A., O. M. Numerova, H. Ozkan, and M. Feldman, 2004 Alterations in subtelomeric tandem repeats during early stages of allopolyploidy in wheat. *Genome* 47: 860–867.
- Sax, K., 1927 Chromosome behavior in *Triticum* hybrids. pp. 1267–1284 in *Verhandlungen des V. Intern. Kongresses für Vererbungswissenschaft*, Berlin, Germany.
- Sears, E. R., 1948 The Cytology and Genetics of the Wheats and their Relatives. *Adv. Genet.* 2: 239–270.
- Sears, E. R., 1954 The aneuploids of common wheat. *Missouri Agric. Exp. Stn. Res. Bull.* 572: 1–58.
- Sears, E. R., 1969 Wheat cytogenetics. *Annu. Rev. Genet.* 3: 451–468.
- Sears, E. R., 1972 The nature of mutation in hexaploid wheat. *Symp. Biol. Hung.* 12: 73–82.
- Sears, E. R., 1976 Genetic control of chromosome pairing in wheat. *Annu. Rev. Genet.* 10: 31–51.
- Sears, E. R., 1984 Mutations in wheat that raise the level of meiotic chromosome pairing, pp. 295–300 in *Gene Manipulation in Plant Improvement*, edited by J. P. Gustavson Stadler Symposium, Columbia, MO.
- Sears, E. R., and M. Okamoto, 1958 Intergenomic chromosome relationships in hexaploid wheat. *Proc. X Int. Congr. Genet.* 2: 258–59.
- Shaked, H., K. Kashkush, H. Ozkan, M. Feldman, and A. A. Levy, 2001 Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell* 13: 1749–1759.
- Soltis, D. E., and P. S. Soltis, 1993 Molecular data and the dynamic nature of polyploidy. *Crit. Rev. Plant Sci.* 12: 243–273.
- Stebbins, G., L., 1950 *Variation and Evolution in Plants*. Columbia University Press, New York.
- Tate, J. A., P. Joshi, K. A. Soltis, P. S. Soltis, and D. E. Soltis, 2009 On the road to diploidization? Homoeolog loss in independently formed populations of the allopolyploid *Tragopogon miscellus* (Asteraceae). *BMC Plant Biol.* 9: 80 10.1186/1471-2229-9-80.
- Thompson, D. A., M. M. Desai, and A. W. Murray, 2006 Ploidy controls the success of mutators and nature of mutations during budding yeast evolution. *Curr. Biol.* 16: 1581–1590.
- Tsunewaki, K., 1970 Necrosis and chlorosis genes in common wheat and its ancestral species. *Seiken Zihō* 22: 67–75.
- Van de Peer, Y., S. Maere, and A. Meyer, 2009 The evolutionary significance of ancient genome duplications. *Nat. Rev. Genet.* 10: 725–732.
- Van Slageren, M. W., 1994 *Wild Wheats: A Monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae)*, Agricultural University, Wageningen, The Netherlands.
- Vega, J. M., and M. Feldman, 1998 Effect of the pairing gene *Ph1* on centromere misdivision in common wheat. *Genetics* 148: 1285–1294.
- Vega, J. M., S. Abbo, M. Feldman, and A. A. Levy, 1994 Chromosome painting in plants: *in situ* hybridization with a DNA probe from a specific microdissected chromosome arm of common wheat. *Proc. Natl. Acad. Sci. USA* 91: 12041–12045.
- Vega, J. M., S. Abbo, M. Feldman, and A. A. Levy, 1997 Chromosome painting in wheat, pp. 319–332 in *Chromosomes Today*, Vol. 12, edited by N. Henriques-Gil, J. S. Parker, and M. J. Puertas. Chapman and Hall, London.
- Von Tschermak, E., and H. Bleier, 1926 Über fruchtbare *Aegilops-Weizenbastarde*, *Der Deutsch. Bot. Ges.* 44: 110–132.
- Waines, J. G., 1976 A model for the origin of diploidizing mechanisms in polyploid species. *Am. Nat.* 110: 415–430.
- Waines, J. G., and P. I. Payne, 1987 Electrophoretic analysis of the high-molecular-weight glutenin subunits of *Triticum monococcum*, *T. urartu* and the A genome of bread wheat (*T. aestivum*). *Theor. Appl. Genet.* 74: 71–76.
- Wall, A. M., R. Riley, and M. D. Gale, 1971 The position of a locus on chromosome 5B of *Triticum aestivum* affecting homoeologous meiotic pairing. *Genet. Res.* 18: 329–339.
- Weissman, S., M. Feldman, and J. Gressel, 2005 Sequence evidence for sporadic intergeneric DNA introgression from wheat into wild *Aegilops* species. *Mol. Biol. Evol.* 22: 2055–2062.
- Wicker, T., K. F. X. Mayer, H. Gundlach, M. Martis, B. Steuermagel *et al.*, 2011 Frequent gene movement and pseudogene evolution is common to the large and complex genomes of wheat, barley, and their relatives. *Plant Cell* 23: 1706–1719.
- Yaakov, B., and K. Kashkush, 2011 Massive alterations of the methylation patterns around DNA transposons in the first four generations of a newly formed wheat allohexaploid. *Genome* 54: 42–49.
- Yousafzai, F. K., N. Al-Kaff, and G. Moore, 2010 Structural and functional relationship between the *Ph1* locus protein 5B2 in wheat and CDK2 in mammals. *Funct. Integr. Genomics* 10: 157–166.
- Zohary, D., and M. Feldman, 1962 Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. *Evolution* 16: 44–61.

Communicating editor: A. S. Wilkins