A Comparative Analysis of Chromosome Pairing at Metaphase I in Interspecific Hybrids between Durum Wheat *(Triticum turgidum* L.*)* and the Most Widespread *Aegilops* Species

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Key Words

Aegilops · Gene flow · Genome analysis · GISH · Homoeologous pairing · Wheat

Abstract

Homoeologous metaphase I (MI) associations in hybrids between durum wheat and its wild allotetraploid relatives *Aegilops neglecta*, *Ae. triuncialis* and *Ae. ventricosa* have been characterized by a genomic in situ hybridization procedure that allows simultaneous discrimination of A, B and wild species genomes. Earlier results in equivalent hybrids with the wild species *Ae. cylindrica* and *Ae. geniculata* have also been considered to comparatively assay the MI pairing pattern of the durum wheat × *Aegilops* interspecific combinations more likely to occur in nature. The general picture can be drawn as follows. A and B wheat genomes pair with each other less than the 2 wild constituent genomes do in any of the hybrid combinations examined. Interspecific wheat-wild associations account for 60–70% of total MI pairing in all hybrids, except in that derived from *Ae. triuncialis*, but the A genome is always the wheat partner most frequently involved in MI pairing with the wild homoeologues. Hybrids with *Ae. cylindrica*, *Ae. geniculata* and *Ae. ventricosa* showed similar reduced levels of MI association and virtually identical MI pairing patterns. However, certain recurrent differences were found when the pattern of homoeologous pairing of hybrids from either *Ae. triuncialis* or *Ae. neglecta* was contrasted to that observed in the other durum wheat hybrid combinations. In the former case, a remarkable preferential pairing between the wild species constituent genomes U^t and C^t seems to be the reason, whereas a general promotion of homoeologous pairing, qualitatively similar to that observed under the effect of the *ph1c* mutation, appears to occur in the hybrid with *Ae. neglecta*. It is further discussed whether the results reported here can be extrapolated to the corresponding bread wheat hybrid combinations.

Interspecific hybrids are the earliest intermediate forms for gene exchanges between related species, both in nature and for crop breeding objectives [Ellstrand et al., 1999; Zamir, 2001]. They merge parental species genomes, which thus can pair and originate recombinant chromosomes that stably incorporate genetic material from one species into the other species genome either in the meiosis of the F_1 hybrid itself and/or in derived selfing or backcrossing progenies. Many reports have examined chromosome pairing at metaphase I (MI) in interspecific combinations as a means to predict the chance and genome patterning of eventual intergenomic exchanges [see Benavente et al., 2008]. With information from crop × alien hybrids, such a MI-pairing-based knowledge can

serve both to design breeding strategies that ease the incorporation of wild traits into the crop genome [Qi et al., 2007] or to estimate the risk of unintended transference of crop genome regions to the wild relative genome [Stewart et al., 2003]. It is also very common that studies describing chromosome pairing in hybrids are aimed to infer the evolutionary history of their parental species, although the use of MI pairing data to deduce phylogenetic relationships between the interspecific hybrid constituent genomes has certain limitations [reviewed in Jauhar and Joppa, 1996].

Bread wheat (*Triticum aestivum* L.; 2n = 6x = 42, AABBDD) and, to a lesser extent, durum wheat (*T. turgidum* L.; 2n = 4x = 28, AABB) are the crop species in which human-directed interspecific hybridization has played the most relevant role for the development of new varieties, incorporating alien genes of agronomic interest, especially resistances to pathogens and tolerance to abiotic stress conditions [Islam and Shepherd, 1991; Ceoloni and Jauhar, 2006]. This is indeed the declared interest of many reports on the meiotic pairing behavior in wheat × alien combinations in the scientific literature [e.g. Jauhar and Peterson, 2006; Kang et al., 2008]. But it also underlies many other MI pairing studies that, since the pioneering work of Kihara and colleagues [Lilienfeld, 1951; Kihara, 1963], have investigated the genome composition and homoeologous chromosome relationships between diploid and polyploid species of the *Triticum-Aegilops* complex and related genera [e.g. Lucas and Jahier, 1988; Yen and Kimber, 1992; Cuñado, 1993].

Cultivated wheats can spontaneously hybridize with some of their wild *Triticum* and *Aegilops* relatives when they grow in sympatry and their flowering periods overlap [Jacot et al., 2004; Zaharieva and Monneveux, 2006]. Diploid relatives show a limited geographical distribution and cannot be found far from their origin center. By contrast, some tetraploid *Aegilops* species are quite ubiquitous and natural hybrids between wheat and some of them (i.e. *Ae. triuncialis, Ae. geniculata* and *Ae. neglecta*) are indeed documented as early as in the 19th century [see references in Van Slageren, 1994; Zaharieva and Monneveux, 2006]. Wheat × *Aegilops* hybrids are highly sterile, but descendents can be found after back-crossing to any of the parents or spontaneous amphiploidy. Thus, these hybrid-derived forms are acknowledged as the most likely bridges for wheat gene flow in nature [David et al., 2004].

In previous studies [Cifuentes et al., 2006; Cifuentes and Benavente, 2009a], we examined by means of genomic in situ hybridization (GISH) the MI pairing pattern of the individualized A and B wheat genomes in interspecific hybrids of durum wheat with the wild species *Ae. geniculata* (2n = 4x = 28; $U^gU^gM^gM^g$) and *Ae. cylindrica* (2n = 4x = 28; $C^cC^cD^cD^c$). Here we have extended the analysis to hybrids with the allotetraploids *Ae. triuncialis* ($U^tU^tC^tC^t$), *Ae. neglecta* ($U^nU^nX^nX^n$) and *Ae. ventricosa* ($D^vD^vN^vN^v$). Our main objective was to compare the pattern of wheat-wild pairing in the durum wheat hybrid combinations most relevant in a crop-to-wild gene flow frame. *Ae. triuncialis* L. is the most abundant wheat relative worldwide. *Ae. geniculata* Roth is the most common in the Mediterranean area while *Ae. neglecta* Req. ex Bertol. and *Ae. cylindrica* Host also show extensive geographic range in Central and West Asia, North Africa and Europe [Van Slageren, 1994; Maxted et al., 2008]. *Ae. ventricosa* Tausch is not as widely distributed as the remaining allotetraploid *Aegilops* species in the study, but it can be highlighted as the most successful donor of agronomic desirable traits for wheat breeding (mainly resistances) among them [Delibes et al., 1993; Friebe et al., 1996; Schneider et al., 2008]. On the other hand, these wild parents represent 5 distinct combinations of the diploid *Aegilops* genomes present in the allotetraploid species of the genus. It makes this set of interspecific hybrids suitable material to comparatively assess homoeologous pairing affinities between their constituent genomes, and between them and the A and B wheat genomes. We have also revised data from earlier reports on hybrids of *T. aestivum* with the same *Aegilops* species to examine to what extent the MI pairing pattern characterized in a durum wheat hybrid, much more suitable for cytological analyses, can be extrapolated to its bread wheat counterpart.

Materials and Methods

Interspecific hybrids were obtained by manual crosses between *T. turgidum* ssp *durum* and the allotetraploid wheat relatives *Ae. neglecta, Ae. triuncialis* and *Ae. ventricosa* (accessions PI 170209, PI 554364 and PI 277000, respectively, from the National Small Grains Collection, USDA-ARS). The durum wheat pollen donors were cultivars Langdon (for *Ae. neglecta* and *Ae. triuncialis*) and Cappelli (for *Ae. ventricosa*). Hybrid plantlets were grown in a green-house until flowering. Anthers of the emerging spikes containing pollen mother cells at metaphase I were fixed in 1:3 (v/v) acetic acid:ethanol and stored at −20°C for a minimum of 2 weeks. Anthers were squashed in 45% acetic acid and slides were stored at 4°C prior to in situ hybridization.

Total genomic DNAs were isolated from young leaves of the wild allotetraploid parental species and the diploids *T. monococcum* and *Ae. speltoides* (2n = 14; AA and SS, respectively) following standard protocols. Diploid species genomic DNAs were labeled with digoxigenin-11-dUTP (A genome) or biotin-16-dUTP

(S genome) by random priming and then mechanically sheared by autoclaving to 0.5–1.5 Kbp pieces. The pTa71 ribosomal DNA probe [Gerlach and Bedbrook, 1979] was labeled using nick translation. Labeling of probes was performed using standard kits from Roche following the manufacturer's instructions. The standard hybridization mixture contained differentially labeled A- and S-genome probes (4 and 8 ng/µl, respectively) and the ribosomal DNA probe (2.5 ng/µl), generally used as mixture of digoxigenin- and biotin-labeled pTa71 in a ratio of 2:3. Unlabeled genomic DNA from the corresponding wild parent, sheared to 0.3–0.7 Kbp by autoclaving, was always added in excess (400 ng/µl) to block shared DNA sequences. ISH protocol was essentially as described in Sanchez-Moran et al. [1999]. Digoxigenin-labeled probes were revealed with 5 ng/µl goat antidigoxigenin antibody conjugated with fluorescein isothiocyanate (FITC, Roche) whereas biotinylated probes were detected with 5 ng/µl avidin conjugated with Cy3 dye (Roche). Slides were screened using an Axiophot epifluorescent microscope (Zeiss) equipped with a double filter for fluorescein and avidin fluorescence. Images were captured with a CoolSnap digital camera. When required, further image processing for adjustment of brightness and contrast was performed with Adobe Photoshop v8.0.1. Statistical analyses were performed with Statistix v8.0.

Fig. 1. Micrographies from MI cells of interspecific hybrids between durum wheat and allotetraploid *Aegilops* species (2n = 4x = 28, generically ABYZ) after GISH combining differentially labeled A and S genomic DNA probes. Wheat constituent genomes are green (A chromatin) and red (B chromatin), while wild chromosomes (the generic Y and Z) are brown. Green or white signals on some B and wild chromosomes correspond to major nucleolus organizing region loci revealed by inclusion of the ribosomal pTa71 probe in the probe mix. **a** MI cell of durum wheat × *Ae. neglecta* with one intraspecific and 2 wheat-wild MI associations. **b** MI cell of durum wheat × *Ae. triuncialis* with 2 intraspecific and one wheat-wild MI associations. **c** MI cell of durum wheat × *Ae. ventricosa* showing the whole chromosome complement as univalents. The type of homoeologous MI pairing in the rod bivalents observed in **a** and **b** is indicated. Scale bar = 10 µm.

Results

The GISH hybridization mix used in this study allowed simultaneous identification of A and B wheat genome chromosomes and their discrimination from the wild genome homoeologues (generically, YZ chromosomes). Preliminary observation of MI cells served to confirm that the hybrids had 28 somatic chromosomes and the expected $A^7+B^7+YZ^{14}$ genome composition (fig. 1).

Meiotic configurations and frequency of MI associations in the hybrids under study are given in table 1. To facilitate further comparisons, the table includes formerly described data from hybrids of durum wheat cultivars Langdon and/or Cappelli with the wild species *Ae. geniculata* and *Ae. cylindrica*. The level of MI pairing was quite limited in all the ABYZ combinations. However, some remarkable differences were found. For example, hybrids derived from *Ae. cylindrica*, *Ae. geniculata* and *Ae. ventricosa* showed less or around 0.5 MI associations/cell whereas 2.75 MI associations/cell were recorded in durum wheat × *Ae. neglecta*.

Discrimination among A, B and wild chromatin by GISH allowed identification of the homoeologous genomes involved in each meiotic pairing configuration. The following types of MI associations could be distinguished: intraspecific associations involving both wheat genomes (A-B), int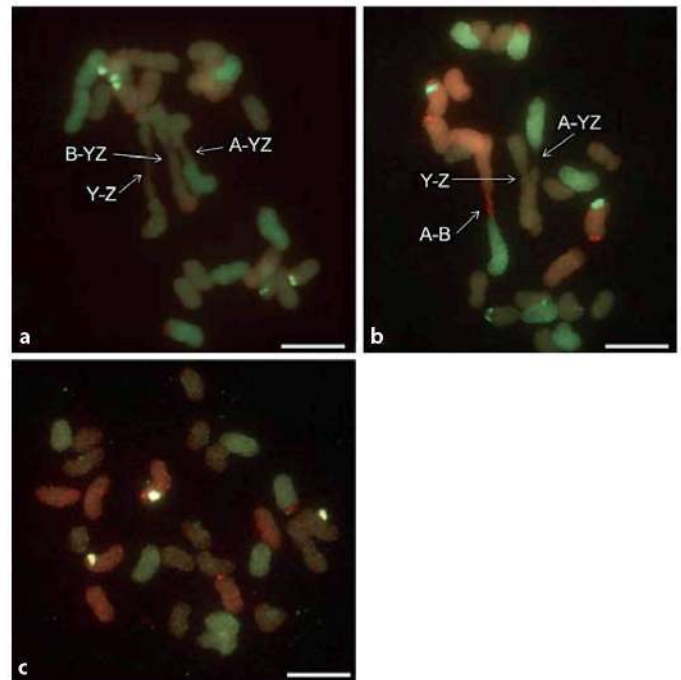raspecific associations involving both wild genomes (Y-Z), wheat-wild associations involving the A wheat genome (A-YZ) and wheat-wild associations involving the B wheat genome (B-YZ) (see fig. 1). The absolute frequencies of these types of MI association and their means per cell are given in table 2 while their relative proportions are represented in figure 2. A-B associations were less than 10% in all hybrids. The level of MI pairing between the wild homoeologues (Y-Z associations) ranged from 24%–30% in all wheat × *Aegilops* combinations except in those derived from *Ae. triuncialis* where it reached 46%. Correspondingly, wheat-wild MI associations represented about 2/3 of total associations in all hybrids but in ABU^{t}C^t, which showed less than 50% of interspecific MI pairing (fig. 2a). The wheat A genome

Table 1. Meiotic confignrations at metaphase I in dnrnm wheat × *Aegilops* hybrids

Hybrid	Cells	MI pairing confignration[a]					MI associations	
		I	rod II	ring II	III	IV	total	mean/cell
ABC^{c}D^c [1]	1,076	29,227	449	0	1	0	451	0.42
ABU^{g}M^g [2]	883	23,817	445	1	5	0	457	0.52
ABU^{n}X^n	109	2,475	258	1	17	2	300	2.75
ABU^{t}C^t	452	11,575	523	1	11	0	547	1.12
ABD^{v}N^v	507	13,914	141	0	0	0	141	0.28

[a] I = univalent; II = bivalent; III = trivalent; IV = quadrivalent.
[1] Data from genotypes c×L and c×Cp analyzed in Cifuentes and Benavente [2009a].
[2] Data from genotypes g003×L and g103×L analyzed in Cifuentes et al. [2006].

Table 2. Pattern of MI pairing in durum wheat × *Aegilops* interspecific hybrids

Hybrid	Intraspecific MI associations		Wheat-wild MI associations		Others[a]
	A-B	Y-Z	A-YZ	B-YZ	
ABC^{c}D^c [1]	31 (0.03)	117 (0.11)	268 (0.25)	32 (0.03)	3
ABU^{g}M^g [2]	44 (0.05)	131 (0.15)	246 (0.28)	34 (0.04)	2
ABU^{n}X^n	21 (0.19)	73 (0.67)	151 (1.39)	54 (0.50)	1
ABU^{t}C^t	37 (0.08)	250 (0.55)	220 (0.49)	37 (0.08)	3
ABD^{v}N^v	6 (0.01)	43 (0.08)	73 (0.14)	18 (0.04)	1
ABDU^{g}M^g	36	133	169	32	

The freqnency of associations per MI cell is given in parentheses. Nnmbers of equivalent MI associations observed in the bread wheat × *Ae. geniculata* hybrids examined in Cifuentes and Benavente [2009b] are also given (see text).
[a] Includes non-homologous associations and multiple (non two-by-two) chromosome arm associations.
[1] Data from genotypes c×L and c×Cp analyzed in Cifuentes and Benavente [2009a].
[2] Data from genotypes g003×L and g103×L analyzed in Cifuentes et al. [2006].

was always much more frequently associated with the wild homoeologues than B genome, A-YZ associations representing 85–90% of wheat-wild MI pairing in most cases (fig. 2b).

Contingency χ^2 tests were performed for two-by-two comparison of the MI pairing pattern in ABYZ hybrids derived from different wild species. Confirming the data illustrated in figure 2a, results in table 3 demonstrated that the frequency of wheat-wild MI association was significantly lower in ABU^{t}C^t than in the other ABYZ durum wheat × *Aegilops* combinations. The relative proportions of A-B and Y-Z MI associations were statistically identical in all ABYZ combinations except ABU^{t}C^t, which showed a higher frequency of intraspecific association between the 2 wild genomes (U^t-C^t MI associations). The contingency tests performed also demonstrated that all durum wheat × *Aegilops* hybrids showed the same ratio of A-YZ:B-YZ MI associations except those

derived from *Ae. neglecta*, which exhibited a very significant increase of wheat-wild associations involving the B wheat genome (see fig. 2b).

Discussion

Two former reports have described the homoeologous MI pairing pattern of hybrids between durum wheat and the wild species *Ae. geniculata* [Cifuentes et al., 2006] and *Ae. cylindrica* [Cifuentes and Benavente, 2009a]. A similar GISH approach has been used for the analysis of MI association in hybrids obtained from *Ae. neglecta*, *Ae. triuncialis* and *Ae. ventricosa*.

The existence of 4 distinct homoeologous genomes in all the wheat × *Aegilops* combinations examined and the employment of *Ph1* genotypes as durum wheat parents explain the reduced level of MI pairing in the hybrids.

Fig. 2. Graphic representation of data from durum wheat × *Aegilops* hybrids in table 2. **a** Contribution of the 2 types of intraspecific (A-B and Y-Z) and wheat-wild associations to total MI pairing. **b** Relative frequency of wheat-wild MI associations involving the A and B wheat genomes.
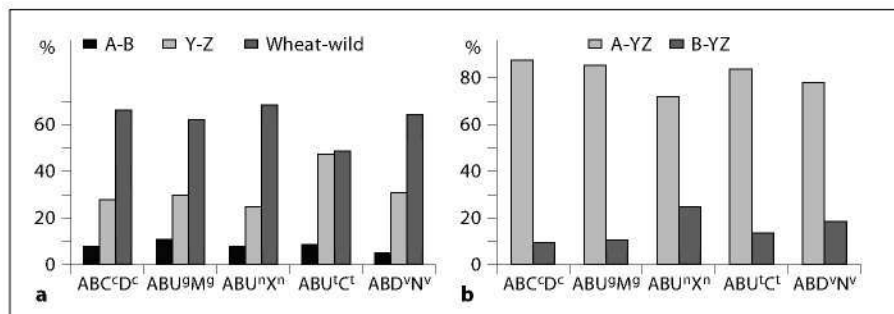
Table 3. Contingency χ^2 tests for comparison of MI pairing pattern between durum wheat × *Aegilops* hybrids

Hybrids compared	Intraspecific vs. wheat-wild	A-B vs. Y-Z	A-YZ vs. B-YZ
ABC^{c}D^c–ABU^{g}M^g	$\chi^2 = 2.89^{ns}$	$\chi^2 = 0.79^{ns}$	$\chi^2 = 0.31^{ns}$
ABC^{c}D^c–ABU^{n}X^n	$\chi^2 = 0.21^{ns}$	$\chi^2 = 0.07^{ns}$	$\chi^2 = 21.18^{***}$
ABC^{c}D^c–ABU^{t}C^t	$\chi^2 = 38.81^{***}$	$\chi^2 = 4.80^*$	$\chi^2 = 1.77^{ns}$
ABC^{c}D^c–ABD^{v}N^v	$\chi^2 = 0.18^{ns}$	$\chi^2 = 1.83^{ns}$	$\chi^2 = 5.20^*$
ABU^{g}M^g–ABU^{n}X^n	$\chi^2 = 3.88^*$	$\chi^2 = 0.26^{ns}$	$\chi^2 = 16.06^{***}$
ABU^{g}M^g–ABU^{t}C^t	$\chi^2 = 20.37^{***}$	$\chi^2 = 11.28^{***}$	$\chi^2 = 0.59^{ns}$
ABU^{g}M^g–ABD^{v}N^v	$\chi^2 = 0.55^{ns}$	$\chi^2 = 3.67^{ns}$	$\chi^2 = 3.32^{ns}$
ABU^{n}X^n–ABU^{t}C^t	$\chi^2 = 35.41^{***}$	$\chi^2 = 4.90^*$	$\chi^2 = 10.29^{**}$
ABU^{n}X^n–ABD^{v}N^v	$\chi^2 = 0.55^{ns}$	$\chi^2 = 2.14^{ns}$	$\chi^2 = 1.47^{ns}$
ABU^{t}C^t–ABD^{v}N^v	$\chi^2 = 14.05^{***}$	$\chi^2 = 0.02^{ns}$	$\chi^2 = 1.46^{ns}$

In all tests performed the number of degrees of freedom is equal to 1.
^{ns} Not significant ($p > 0.05$); * $p > 0.01$; ** $p > 0.001$; *** $p < 0.001$.

However, MI associations were noticeably more abundant in the hybrids derived from *Ae. neglecta* and *Ae. triuncialis* than in the remaining 3 ABYZ combinations, which showed less or about 0.5 associations per cell (table 1). It is unlikely that any biased influence of external non-genotypic conditions (i.e. temperature at flowering) affected pairing and/or chiasma formation because differences between individuals within each hybrid genotype were never significant even when cultivated in different years (results not shown). Giorgi et al. [1981] examined hybrids between durum wheat and *Ae. cylindrica* and *Ae. triuncialis*, which showed 1.86 and 4.53 associations per cell, respectively. The values are higher than those reported here, but maintain their relative ratio, which supports the existence of intrinsic factors, either of genotypic or genomic origin, resulting in differences in the extent of MI pairing among these interspecific combinations. It is worth noting that ABC^{c}D^c, ABU^{g}M^g and ABD^{v}N^v hybrids show not only similar MI pairing levels but also virtually identical MI pairing patterns (table 3) whereas certain recurrent differences are found when

wheat × wild hybrids from either *Ae. triuncialis* or *Ae. neglecta* are contrasted to the other ABYZ combinations.

The level of MI associations reached in a polyploidy interspecific hybrid depends upon the likelihood of MI association of all pairwise combinations (i.e. pairing affinities) among the genomes present in the hybrid, which determines the preferential MI pairing behavior in that hybrid [Jauhar and Joppa, 1996]. Therefore, the mere existence of different pairs of wild genomes in each durum wheat × *Aegilops* combination must represent a key source of variation in both the extent and the pattern of MI association among the ABYZ hybrids examined. It is further expected that which genomes are actually the generic Y and Z can affect not only the frequency of pairing between the 2 wild genomes (Y-Z associations) but also the level of wheat-wild MI pairing (A-YZ and B-YZ associations), while A-B associations should remain unaltered.

The homoeologous pairing pattern of the hybrid with *Ae. triuncialis* (ABU^{t}C^t) is characterized by a significantly higher frequency of intraspecific MI pairing due to a

remarkable increase of Y-Z (U^t-C^t) associations (tables 2 and 3). It can also be noted that Y-Z and A-YZ associations are those accounting for the increment of MI pairing in this hybrid compared to the lowest-pairing combinations ABC^cD^c, ABU^gM^g and ABD^vN^v (see mean per cell values in table 2). These results support the idea that U^t and C^t genomes show a higher pairing affinity for each other, and with the wheat A genome, than any of the other pairs of constituent genomes present in the allotetraploid wild species used in the study. The case of ABU^mX^n is different because all types of MI association, including A-B, are increased in this wheat $\times$ Aegilops combination (table 2), which suggests the presence of genetic factors promoting homoeologous pairing in the Ae. neglecta accession used as a parent. It is worth noting that the proportion of wheat-wild MI associations involving the B genome was significantly higher in ABU^mX^n (26.3%) than in any other of the ABYZ combinations examined (table 3; fig. 2b) because such MI pairing pattern modification has been demonstrated in hybrids from Ae. geniculata and Ae. cylindrica carrying the phlc mutation when compared with their counterparts with active Ph systems [Cifuentes et al., 2006; Cifuentes and Benavente, 2009a]. The finding of virtually identical homoeologous MI pairing pattern in hybrids derived from Ae. cylindrica, Ae. geniculata and Ae. ventricosa might then be a reflection of (1) comparable pairing affinities between their constituent genomes (C^c with D^c, U^g with M^g, and D^v with N^v) and between them and those of durum wheat, and (2) similar epistatic interactions between the wheat meiotic control system and the yet undisclosed diploidizing mechanism acting in these allotetraploid species [Cuñado and Santos, 1999].

In all the hybrid combinations, Y-Z associations were more frequent than A-B associations (table 2; fig. 2a). This is in agreement with the lower level of MI pairing reported in hybrids between T. urartu and Ae. speltoides, assumed to be the diploid donors of durum wheat genomes, compared to the corresponding values in hybrids between the diploid ancestors of the allotetraploid Aegilops used here [Lucas and Jahier, 1988]. Following a traditional assumption in the analyses of chromosome pairing in interspecific hybrids, the level of MI pairing between any 2 homoeologous genomes depends on their evolutionary relatedness [reviewed in Jauhar and Joppa, 1996]. If so, then A and B wheat genomes would be more distant from each other than any of the other 5 pairs of Aegilops genomes tested here. The constituent genomes of Ae. triuncialis (U^t and C^t) must be closely related since, as already noted, the proportion of Y-Z MI pairing was

significantly higher in ABU^tC^t than in any other ABYZ hybrid (table 3; fig. 2a). This broadly agrees with most phylogenetic trees based on molecular data analyses of diploid species of the genera Triticum and Aegilops [e.g. Dvorak and Zhang, 1992; Kellog et al., 1996]. Incongruities are, however, striking regarding the preferential MI pairing of A over B genome with any of their wild homoeologues (table 2; fig. 2b) since all molecular evidence supports the idea that the presumptive B genome donors are closer than, or as distant as, T. urartu to the remaining diploid Aegilops species [e.g. Sallares and Brown, 2004; Petersen et al., 2006]. It can be noted that A-D MI associations are also much more abundant than B-D MI associations in bread wheat haploids and interspecific hybrids [Jauhar et al., 1991; Naranjo and Fernandez-Rueda, 1996; Cifuentes and Benavente, 2009b], which is again far from reflecting the phylogenetic divergences among the 3 wheat ancestral genomes [Kellog et al., 1996; Petersen et al., 2006]. These observations reinforce the concept that the probability of 2 given genomes to pair with each other is not necessarily related to their genetic differentiation. This provides a solid argument to those that question the use of MI pairing analyses in interspecific hybrids to infer the evolutionary relationships among related species [Seberg and Petersen, 1998; see also Jauhar and Joppa, 1996].

Structural chromosome differentiation can be responsible for reduced genome pairing affinities by hampering synapsis and chiasma formation between structurally divergent homoeologues [Jauhar and Joppa, 1996; Naranjo et al., 1998]. Chromosomal rearrangements seem to be the rule in the evolution of allopolyploid Aegilops species [Badaeva et al., 2002; 2004]. Intergenomic reciprocal translocations have indeed been visualized by GISH in the parental accessions of Ae. triuncialis and Ae. ventricosa used in this study (results not shown) and the presence of additional rearrangements, undetectable by the technique, in these and the remaining wild parents cannot be excluded. It is, however, difficult to explain why this should result in the generalized A- over B-wild preferential MI pairing observed in the ABYZ hybrids. Alternatively, the lower pairing affinities detected here for the B genome might be ascribed to certain chromosomal changes, accumulated on this particular genome from a common ancestor, that do not reflect evolutionary timing within the Triticum-Aegilops complex.

The B genome is one of the most heterochromatic and shows the greatest average chromosome size among all genomes in wheat and their wild Aegilops relatives, with the exception of the X^n genome of Ae. neglecta [Gill and

Kimber, 1974a; Gill et al., 1991; Badaeva et al., 2002; 2004]. (For mean chromosome size comparisons of wild species, use D and U genomes in the ideograms reported in Badaeva's papers as reference). Genome size differences are also supported by DNA C-values estimated in the diploid donors and/or allotetraploid species used here [Bennett and Leitch, 2005]. A thorough revision of wheat-alien MI pairing studies in which individual wheat genomes have been discriminated reveals that, apart from the S genomes of species belonging to the Sitopsis section of *Aegilops*, the only *Triticeae* genomes that do not show A-over B- preferential pairing are the J and R genomes from *Thynopirum bessarabicum* and rye, respectively, both with great heterochromatin content [Gill and Kimber, 1974b; Endo and Gill 1984]. Several studies have demonstrated that most of wheat-rye MI associations in bread wheat × rye hybrids (2n = 4x = 28; ABDR) involve B genome chromosomes [e.g. Naranjo and Fernandez-Rueda, 1996; Cuadrado et al., 1997]. This has been assumed to be an indirect consequence of the higher pairing affinity between the A and D genomes, which would force R chromosomes to pair with their B homoeologues if with any at all. On the other hand, similar frequencies of A-wild and B-wild MI associations have been reported by Jauhar and Peterson [2006] in hybrids between durum wheat and *T. bessarabicum* (2n = 3x = 21; ABJ). It is worth noting that J chromosomes show an intermediate mean size between those of A and B genomes [Jauhar, 1992] and that the R genome has a greater mean chromosome size than even the wheat B genome [Mukai et al., 1992]. It is beyond the scope of this study to conclude whether such an apparent relationship between chromosome size divergences and pairing affinity for homoeologous associations involving the B genome is real or not.

The wild allotetraploid species used as parents are the most widespread among the closest wheat relatives. Therefore, the durum wheat hybrid combinations examined are surely the most frequent in nature. The possibility that stable genetic transference to these related species genomes can actually occur is substantiated by the finding of wheat-wild MI associations representing 60–70% of the total in most of the ABYZ hybrids (fig. 2a). However, the mean number of wheat-wild MI associations per pollen mother cell is close to 1.9 in the hybrid with *Ae. neglecta* ($ABU^{n}X^{n}$), while ranging from 0.2–0.5 in the remainder (table 2). This shows that the MI pairing level reached in each particular hybrid must also be taken into account to estimate the amount of wheat-wild recombinant chromosomes that can be potentially transmitted to its offspring. Our results further predict that, on average,

durum wheat genetic sequences located on the A genome have a much higher chance of being introgressed into wild genomes than those on the B genome. Some of the *Aegilops* parental species used here are employed as sources of agronomically desirable traits in wheat breeding programs [Delibes et al., 1993; Bai et al., 1995; Aghaee-Sabarzeh et al., 2002]. In this reverse genetic flow direction, our results allow one to expect that alien genes of interest will be more likely incorporated by homoeologous-recombination based strategies into A genome chromosomes.

Bread wheat is by far the most common wheat crop worldwide. Hence, there is interest in considering to what extent the homoeologous pairing pattern reported here for durum wheat hybrids can be extrapolated to bread wheat hybrids, which are both more likely as natural bridges for wheat gene flow to the wild and more commonly produced for introgression-based breeding objectives. On this point, a distinction needs to be made between hybrids from allotetraploid *Aegilops* parents having or lacking modified D genomes because homologous-like associations involving the wheat and wild D chromosomes can occur in the former case whereas only strictly homoeologous wheat-wild MI pairing is possible in the latter.

Two earlier GISH studies on hybrids of *Ae. geniculata* with durum and bread wheat [Cifuentes et al., 2006; Cifuentes and Benavente, 2009b] provide the most suitable information for comparison of homoeologous MI pairing patterns in ABYZ and ABDYZ hybrids where neither Y nor Z are D-derived genomes. Logically, only those types of MI associations occurring in either durum and bread wheat hybrids (A-B, Y-Z, A-wild and B-wild) can be contrasted, whereas those which are exclusive of bread wheat hybrids (i.e. A-D, B-D and D-wild MI associations) must be ignored. Statistical comparison of values reported in the mentioned studies (see table 2) reveals that both the A-B:Y-Z ratio and the relative proportion of wheat-wild MI associations involving A and B wheat genomes (A-YZ:B-YZ ratio) are similar in the durum and bread wheat hybrids with *Ae. geniculata* (A-B vs. U^{g}-M^{g} MI associations: $\chi^2 = 0.71$, d.f. = 1, p > 0.05; A-$U^{g}M^{g}$ vs. B-$U^{g}M^{g}$ MI associations: $\chi^2 = 1.41$, d.f. = 1, p > 0.05). There are no results on bread wheat hybrids with *Ae. neglecta* or *Ae. triuncialis* that can be contrasted with the reported here. However, some indirect evidence supports the possibility that the reasons noted above to explain the distinctive patterns of homoeologous association found in these 2 ABYZ combinations might be valid for the corresponding ABDYZ hybrids. For example, bread wheat × *Ae. ne-*

glecta hybrids (2n = 5x = 35; $ABDU^nX^n$) showed the highest MI pairing level among the bread wheat interspecific combinations examined by Abu Bakar and Kimber [1982], which included those derived from *Ae. geniculata* ($ABDU^gM^g$) and *Ae. triuncialis* ($ABDU^tC^t$). This agrees with the suggested existence of genetic factors promoting homoeologous association in *Ae. neglecta*, already described in other wheat relatives [Dvorak et al., 2006]. On the other hand, higher values of MI associations per cell are consistently found in hybrids between bread wheat and *Ae. triuncialis* than in hybrids from *Ae. geniculata* [for references see Zaharieva and Monneveux, 2006]. This fits the observed results in the present study and could also be explained by the greater pairing affinity between U^t and C^t genomes and between these and the A (and D?) wheat genome compared to the corresponding pairing affinities for *Ae. geniculata* constituent genomes.

Almost regular pairing occurs between the D genomes of wheat and *Aegilops* species derived from *Ae. squarrosa* [Mena et al., 1993; Zemetra et al., 1998]. This is the most obvious reason for the remarkably high level of MI association in bread wheat hybrids with *Ae. cylindrica* and *Ae. ventricosa* compared to other ABDYZ combinations [Abu Bakar and Kimber, 1982; see Zaharieva and Monneveux, 2006]. However, it can be questioned whether that may alter the distribution of wheat-wild MI pairing involving A and B wheat genomes in hybrids $ABDC^cD^c$ and $ABDD^vN^v$ when contrasted to that observed in durum wheat hybrids ABC^cD^c and ABD^vN^v (table 2; fig. 2b). On this matter, our previous results in interspecific combinations with *Ae. geniculata* demonstrate that the presence of the D bread wheat genome, even if involved in preferential pairing with the wild homoeologues, does not necessarily modify the homoeologous pairing affinities of the A and B genomes. Thus, $ABDU^gM^g$ hybrids show the same A-wild:B-wild ratio as ABU^gM^g hybrids (see above) despite the fact that D-wild associations represent 2/3 of wheat-wild MI pairing and more than 40% of total MI associations in bread wheat × *Ae. geniculata* hybrids [Cifuentes and Benavente, 2009b].

Cifuentes and Benavente [2009b] concluded that genetic sequences from the B genome are the least prone to be stably transferred from bread wheat to *Ae. geniculata*. The results and discussion presented here provide support to generalize this prediction to any of the most widespread wild wheat relatives whereas the MI pairing pattern of their hybrids with *T. aestivum* waits to be fully disclosed.

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References

Abu-Bakar M, Kimber G: Chromosome pairing regulators in the former genus *Aegilops*. Z Pflanzenzüchtg 89:130–138 (1982).

Aghaee-Sarbarzeh M, Ferrahi M, Singh S, Singh H, Friebe B, Gill BS, Dhaliwal HS: Ph-I-induced transfer of leaf and stripe rust-resistance genes from *Aegilops triuncialis* and *Ae. geniculata* to bread wheat. Euphytica 127:377–382 (2002).

Badaeva ED, Amosova AV, Muravenko OV, Samatadze TE, Chikida NN, et al: Genome differentiation in *Aegilops*. 3. Evolution of the D-genome cluster. Plant Syst Evol 231:163–190 (2002).

Badaeva ED, Amosova AV, Samatadze TE, Zoshchuk SA, Shostak NG, et al: Genome differentiation in *Aegilops*. 4. Evolution of the U-genome cluster. Plant Syst Evol 246:45–76 (2004).

Bai D, Scoles GJ, Knott DR: Rust resistance in *Triticum cylindricum* Ces. (4x, CCDD) and its transfer into durum and hexaploid wheats. Genome 38:8–16 (1995).

Benavente E, Cifuentes M, Dusautoir JC, David J: The use of cytogenetic tools for studies in the crop-to-wild gene transfer scenario. Cytogenet Genome Res 120:384–395 (2008).

Bennett MD, Leitch IJ: Plant DNA C-values Database (release 4.0). Available from http://data.kew.org/cvalues/homepage.html (2005).

Ceoloni C, Jauhar PP: Chromosome engineering of the durum wheat genome: Strategies and applications of potential breeding value, in Singh E, Jauhar JJ (eds): Genetic Resources, Chromosome Engineering, and Crop Improvement, vol 2: Cereals, pp 27–59 (CRC Press-Taylor & Francis Group, Boca Raton 2006).

Cifuentes M, Benavente E: Complete characterization of wheat-alien metaphase-I pairing in interspecific hybrids between durum wheat (*Triticum turgidum* L.) and jointed goatgrass (*Aegilops cylindrica* Host). Theor Appl Genet 118:1609–1616 (2009a).

Cifuentes M, Benavente E: Wheat-alien metaphase I pairing of individual wheat genomes and D genome chromosomes in interspecific hybrids between *Triticum aestivum* L. and *Aegilops geniculata* Roth. Theor Appl Genet 119:805–813 (2009b).

Cifuentes M, Blein M, Benavente E: A cytomolecular approach to assess the potential of gene transfer from a crop (*Triticum turgidum* L.) to a wild relative (*Aegilops geniculata* Roth.). Theor Appl Genet 112:657–664 (2006).

Cuadrado A, Vitellozzi F, Jouve N, Ceoloni C: Fluorescence in situ hybridization with multiple repeated DNA probes applied to the analysis of wheat-rye chromosome pairing. Theor Appl Genet 94:347–355 (1997).

Cuñado N: Genomic analysis in the genus *Aegilops*. 1. Interspecific hybrids between tetraploid species sharing a common genome. Heredity 70:9–15 (1993).

Cuñado N, Santos JL: On the diploidization mechanism of the genus *Aegilops*: meiotic behaviour of interspecific hybrids. Theor Appl Genet 99:1080–1086 (1999).

David JL, Benavente E, Bres-Patry C, Dusautoir JC, Echaide M: Are neopolyploids a likely route for a transgene walk to the wild? The *Aegilops ovata* × *Triticum turgidum* durum case. Biol J Linn Soc 82:503–510 (2004).

Delibes A, Romero D, Aguaded S, Duce A, Mena M, et al: Resistance to the cereal cyst-nematode (*Heterodera avenae* Woll.) transferred from the wild grass *Aegilops ventricosa* to hexaploid wheat by a 'stepping-stone' procedure. Theor Appl Genet 87:402–408 (1993).

Dvorak J, Deal KR, Luo MC: Discovery and mapping of wheat *Ph1* suppressors. Genetics 174: 17–27 (2006).

Dvorak J, Zhang HB: Reconstruction of the phylogeny of the genus *Triticum* from variation in repeated nucleotide sequences. Theor Appl Genet 84:419–429 (1992).

Ellstrand NC, Prentice HC, Hancock JF: Gene flow and introgression from domesticated plants into their wild relatives. Annu Rev Ecol Syst 30:539–563 (1999).

Endo TR, Gill BS: The heterochromatin distribution and genome evolution in diploid species of *Elymus* and *Agropyron*. Can J Genet Cytol 26:669–678 (1984).

Friebe B, Jiang J, Raupp WJ, Mcintosh RA, Gill BS: Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. Euphytica 91:59–87 (1996).

Gerlach WL, Bedbrook JR: Cloning and characterization of ribosomal-RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1885 (1979).

Gill BS, Kimber G: Giemsa C-banding and the evolution of wheat. Proc Nat Acad Sci USA 71:4086–4090 (1974a).

Gill BS, Kimber G: Giemsa C-banded karyotype of rye. Proc Nat Acad Sci USA 71:1247–1249 (1974b).

Gill BS, Friebe B, Endo TR: Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839 (1991).

Giorgi B, Barbera F, Bitti O: Intergeneric hybrids, meiotic behaviour and amphiploids involving a *Ph* mutant of *Triticum turgidum ssp. durum*, in Proceedings of the Symposium on Induced Variability in Plant Breeding, pp 96–100 (EUCARPIA, Wageningen, The Netherlands 1981).

Islam AK, Shepherd KW: Alien genetic variation in wheat improvement, in Gupta PK, Tsuchiya T (eds): Chromosome Engineering in Plants: Genetics, Breeding, Evolution, part A, pp 291–312 (Elsevier Science Publishers, Amsterdam 1991).

Jacot Y, Ammann K, Rufener Al, Mazyad P, Chueca C, et al: Hybridization between wheat and wild relatives, a European Union research programme, in den Nijs DB, Bartsch D, Sweet J (eds): Introgression from Genetically Modified Plants into Wild Relatives, pp 63–73 (CABI Publishing, UK 2004).

Jauhar PP: Synthesis and cytological characterization of trigeneric hybrids involving durum wheat, *Thinopyrum bessarabicum*, and *Lophopyrum elongatum*. Theor Appl Genet 84:511–519 (1992).

Jauhar PP, Joppa LR: Chromosome pairing as a tool in genome analysis: merits and limitations, in Jauhar JJ (ed): Methods of Genome Analysis in Plants, pp 9–37 (CRC Press, Boca Raton 1996).

Jauhar PP, Peterson TS: Cytological analyses of hybrids and derivatives of hybrids between durum wheat and *Thinopyrum bessarabicum*, using multicolour fluorescent GISH. Plant Breed 125:19–26 (2006).

Jauhar PP, Riera-Lizarazu O, Dewey WG, Gill BS, Crane CF, et al: Chromosome pairing relationships among the A, B, and D genomes of bread wheat. Theor Appl Genet 82:441–449 (1991).

Kang HY, Zhang HQ, Fan X, Zhou YH: Morphological and cytogenetic studies on the hybrid between bread wheat and *Psathyrostachys huashanica* Keng ex Kuo. Euphytica 162: 441–448 (2008).

Kellogg EA, Appels R, Mason-Gamer RJ: When genes tell different stories: The diploid genera of *Triticeae* (*Gramineae*). Syst Bot 21:321–347 (1996).

Kihara H: Interspecific relationships in *Triticum* and *Aegilops*. Seiken Ziho 15:1–12 (1963).

Lilienfeld FA: H. Kihara. Genome-analysis in *Triticum* and *Aegilops*. 10. Concluding Review. Cytologia 16:101–123 (1951).

Lucas H, Jahier J: Phylogenetic relationships in some diploid species of *Triticineae*: cytogenetic analysis of interspecific hybrids. Theor Appl Genet 75:498–502 (1988).

Maxted N, White K, Valkoun J, Konopka J, Hargreaves S: Towards a conservation strategy for *Aegilops* species. Plant Genet Resour 6: 126–141 (2008).

Mena M, Orellana J, Lopez-Braña I, Garcia-Olmedo F, Delibes A: Characterization of wheat/*Aegilops ventricosa* introgression and addition lines with respect to the M^v genome. Theor Appl Genet 86:197–204 (1993).

Mukai Y, Friebe B, Gill BS: Comparison of C-banding patterns and in situ hybridization sites using highly repetitive and total genomic rye DNA probes of 'Imperial' rye chromosomes added to 'Chinese Spring' wheat. Jpn J Genet 67:71–83 (1992).

Naranjo T, Fernandez-Rueda P: Pairing and recombination between individual chromosomes of wheat and rye in hybrids carrying the *ph1b* mutation. Theor Appl Genet 93: 242–248 (1996).

Naranjo T, Fernandez-Rueda P, Maestra B: Chromosome rearrangements and homoeologous pairing: implications for the introgression of alien genes into wheat, in Lelley T (ed): Proceedings of a Symposium on Current Topics in Plant Cytogenetics Related to Plant Improvement, pp 198–205 (WUV-Universitätsverlag, 1998).

Petersen G, Seberg O, Yde M, Berthelsen K: Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). Mol Phyl Evol 39:70–82 (2006).

Qi LL, Friebe B, Zhang P, Gill BS: Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res 15:3–19 (2007).

Sallares R, Brown TA: Phylogenetic analysis of complete 5' external transcribed spacers of the 18S ribosomal RNA genes of diploid *Aegilops* and related species (Triticeae, Poaceae). Genet Res Crop Evol 51:701–712 (2004).

Sanchez-Moran E, Benavente E, Orellana J: Simultaneous identification of A, B, D and R genomes by genomic in situ hybridization in wheat-rye derivatives. Heredity 83:249–252 (1999).

Schneider A, Molnar I, Molnar-Lang M: Utilisation of *Aegilops* (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica 163:1–19 (2008).

Seberg O, Petersen G: A critical review of concepts and methods used in classical genome analysis. Bot Rev 64:372–417 (1998).

Stewart CN Jr, Halfhill MD, Warwick SI: Transgene introgression from genetically modified crops to their wild relatives. Nat Rev Genet 4:806–817 (2003).

Van Slageren MW: Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub and Spach) Eig (Poaceae). Agricultural University of Wageningen-ICARDA (1994).

Yen Y, Kimber G: Genomic relationships of N-genome *Triticum* species. Genome 35:962–966 (1992).

Zaharieva M, Monneveux P: Spontaneous hybridization between bread wheat (*Triticum aestivum* L.) and its wild relatives in Europe. Crop Sci 46:512–527 (2006).

Zamir D: Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983–989 (2001).

Zemetra RS, Hansen J, Mallory-Smith CA: Potential transfer between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*). Weed Sci 46:313–317 (1998).