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A 2500-Locus Bin Map of Wheat Homoeologous Group 5 Provides Insights on Gene Distribution and Colinearity With Rice

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ABSTRACT

We constructed high-density deletion bin maps of wheat chromosomes 5A, 5B, and 5D, including 2338 loci mapped with 1052 EST probes and 217 previously mapped loci (total 2555 loci). This information was combined to construct a consensus chromosome bin map of group 5 including 24 bins. A relatively higher number of loci were mapped on chromosome 5B (38%) compared to 5A (34%) and 5D (28%). Differences in the levels of polymorphism among the three chromosomes were partially responsible for these differences. A higher number of duplicated loci was found on chromosome 5B (42%). Three times more loci were mapped on the long arms than on the short arms, and a significantly higher number of probes, loci, and duplicated loci were mapped on the distal halves than on the proximal halves of the chromosome arms. Good overall colinearity was observed among the three homoeologous group 5 chromosomes, except for the previously known 5AL/4AL translocation and a putative small pericentric inversion in chromosome 5A. Statistically significant colinearity was observed between low-copy-number ESTs from wheat homoeologous group 5 and rice chromosomes 12 (88 ESTs), 9 (72 ESTs), and 3 (84 ESTs).

BREAD wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is an allohexaploid species composed of three related genomes A, B, and D, each containing seven pairs of chromosomes. In spite of the polyploid nature of wheat and its large genome size (16,000 Mb; Arumuganathan and Earle 1991), the size of the gene-containing portion of the wheat genome is only a small percentage of the total genome and is probably similar to other grasses, such as rice (*Oryza sativa* L.), with much smaller genomes (430 Mb; Arumuganathan and Earle 1991). Therefore, genomic techniques focused on the gene portion of the genome are easier to implement in the large genome of hexaploid wheat.

Expressed sequence tags (ESTs) provide a rapid and efficient method for sampling a genome for transcribed gene sequences. Most of the EST markers represent functional genes, although a small portion corresponds to active retroelements (Echenique *et al.* 2002). The sequence information associated with each EST facilitates an immediate comparison with reference model

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species and can be used to integrate the physical and genetic chromosome maps of wheat with the genome sequence of rice.

As a part of a National Science Foundation-funded wheat EST project, an effort was initiated to map thousands of ESTs in the wheat genome. A deletion mapping system (Endo and Gill 1996; Qi *et al.* 2003) was selected for this task due to the speed and simplicity of the process. Most of the RFLP fragments can be mapped in chromosome bins using a single hybridization and a single restriction enzyme without a preliminary screen for polymorphism. This is possible because the rapid divergence of the intergenic regions in the wheat genomes generates high levels of RFLP polymorphism among genomes (Cenci *et al.* 2003; Wicker *et al.* 2003).

To facilitate a detailed analysis of the thousands of loci mapped in the chromosome bins, the work was divided by homoeologous chromosome groups that were assigned for coordination to seven laboratories. This study focused on the distribution of EST markers on homoeologous group 5 chromosomes and on their relationships with the rice genome.

Chromosomes from homoeologous group 5 carry important genes associated with domestication (*Q*), grain quality (*Ha*), plant responses to seasonal changes (*Vrn1* and *Vrn2*), frost tolerance (*Fr1* and *Fr2*), regulation of homoeologous chromosome pairing (*Ph1*), salt and dehydration tolerance (*Esi2, Esi4, Esi14, Esi28, Esi32, Esi47, Dhn2*, and *Dhn1*), heat tolerance (*Hsp16.9*), and numerous resistance genes to pathogens (*e.g.*, *Tsn1*, *Lr18*, *Snb3*, and *Sr30*) (McIntosh *et al.* 2003).

MATERIALS AND METHODS

ESTs were sequenced at the USDA-ARS Western Regional Research Center in Albany, California from 42 libraries representing a wide range of tissues, developmental stages, and environmental stresses and distributed to 10 mapping laboratories. Information on the cDNA libraries is available in Zhang *et al.* (2004) and the development of EST singletons was described by Lazo *et al.* (2004). The deletion lines used in the NSF-EST project were described by Qi *et al.* (2003). For this study, we focused on the Chinese Spring nulli-tetrasomic, ditelosomic, and 23 deletion lines involving homoeologous group 5. The physical maps, including the positions of the breakpoints for homoeologous group 5 chromosomes, can be found in the supplemental online materials (http://wheat.pw.usda.gov/pubs/2004/Genetics/).

Additional plant materials were used to characterize the structural changes observed in chromosome 5A. Three translocation lines (T5RS·5AL, T5RS·5BL, and T5RS·5DL) with chromosome arms 5RS from different rye sources and 5AL (from Chinese Spring), 5BL (from line E12162), and 5DL (from Anza/Wheaton) were introduced by backcrossing into the wheat variety Pavon 76 by A. J. Lukaszewski (University of California, Riverside, CA). In these translocations the most proximal regions of the 5L arms were from the original wheat donors, but the distal regions were likely replaced by Pavon 76 chromosome segments. We also generated a monotelosomic Mt5AS from the cross between Dt5AS-Mt5AL × N5AT5D (this plant is sterile and cannot be propagated by seed). A dDt5A line (two copies of Dt5AS and Dt5AL) was also included in the study. The arm location of the markers involved in a putative pericentromeric inversion were also determined in *Lophophyrum elongatum* Host ditelosomic addition lines Dt5ES and Dt5EL (Dvořák 1980; Tuleen and Hart 1988) and *Hordeum vulgare* L. line Dt5HL (Islam *et al.* 1981). The *T. monococcum* L. DV92 × G3116 mapping population was used to map some of the conflictive ESTs into the genetic maps (Dubcovsky *et al.* 1996).

DNAs from the deletion lines were digested only with restriction enzyme *Eco*RI for the EST mapping, but five additional enzymes (*Eco*RV, *Bam*HI, *Dra*I, *Hin*dIII, and *Sac*I) were used to digest the DNAs from the different cytogenetic stocks used in characterization of the putative inversion. Procedures for deletion-bin mapping were described by Akhunov *et al.* (2003a).

Consensus map construction: ESTs that detected sequences mapped to more than one homoeologous group 5 chromosome (database searched March 17, 2003) were obtained by querying the wEST-SQL database (http://wheat.pw.usda.gov/cgi-bin/westsql/sql.cgi), reorganized in a Microsoft Access database, and used to construct the consensus map. Combination of the bins from the three chromosomes resulted in 11 bins in the short arm designated C-1S and 1S to 10S and 13 bins in the long arm designated C-1L and 1L to 12L, with numbers increasing from the centromere to both telomeres (Figure 1). The diagnostic deletion line delimiting each consensus bin is indicated in parentheses before the consensus bin name in Figure 1. ESTs were placed within the consensus bins using the following criteria. If all the ESTs from bin 1 in chromosome X were present in bin 2 of chromosome Y, then bin 1 was included in bin 2. If only part of the ESTs mapped to bin 1 on chromosome X were included in bin 2 but the rest were included in adjacent bin 3 on chromosome Y, then the breakpoint between bin 2 and bin 3 was considered to be within bin 1 in chromosome X. ESTs mapped only on a single chromosome were incorporated into the consensus bins as follows. If a chromosome bin was within a consensus bin, all these ESTs were included into the corresponding consensus bin. Otherwise, ESTs were assigned to larger combined consensus bins, indicated to the right of the consensus bins in Figure 1.

Previously mapped RFLP probes and genes on the maps of homoeologous group 5 chromosomes (Gill *et al.* 1996; Faris *et al.* 2000; Sarma *et al.* 2000; Zhang *et al.* 2000; Qi and Gill 2001; Faris and Gill 2002) were included in the consensus bins presented in the supplemental online materials (http://wheat.pw.usda.gov/pubs/2004/Genetics/).

Wheat-rice comparison: ESTs that hybridized with a single locus in each genome or those where all the RFLP bands were mapped were obtained from the wEST-SQL database and used as core markers for wheat-rice synteny comparisons. We also included in this comparison ESTs with six or fewer bands in their hybridization profiles, for which at least two bands mapped in homoeologous group 5. This criterion was used to balance the advantage of increasing the number of ESTs in the analysis and the disadvantage of including ESTs that hybridize with multiple loci, which can therefore complicate the wheat-rice comparisons. Autoradiogram images for each of the selected ESTs were individually verified before inclusion in this analysis.

The EST nucleotide sequences (GenBank, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide) were compared against the public rice genome sequence database of ordered BAC and P1 artificial chromosome (PAC) clones (TIGR, http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1). At the time of this analysis (March 17, 2003) most rice chromosomes were completely sequenced with the exception of chromosomes 12 (94%), 9 (93%), and 11 (79%).

Sequences showing similarities ≥70%, over at least 100-bp segments, were considered as significant matches and were used in the analysis of the wheat-rice colinearity. To test the significance of the colinearity between a particular wheat bin and a rice chromosome, we first identified the rice chromosome having the largest number of significant matches to that particular wheat bin and then used a binominal distribution to calculate the probability of finding the observed number under the null hypothesis of no colinearity (random distribution). Probabilities <0.01 were considered as evidence of significant colinearity.

Since wheat chromosome bins could be similar to more than one rice chromosome, or noncolinear segments could be included in otherwise colinear regions, we also tested the significance of the colinearity with the rice chromosome having the second-highest number of ESTs. We first eliminated the rice chromosome with the highest score from the total number of ESTs analyzed in that particular consensus bin. Then we repeated the calculation of the binomial probability using the remaining ESTs.

Gene distribution among genomes and within the chromosome arms: We tested the hypothesis of equal number of ESTs per chromosome using chi-square statistics. We used a similar statistical analysis to test the homogeneity of EST content distribution along chromosome arms. Each arm was divided into proximal and distal halves, and then the distal half was further divided into proximal and distal quarters. The observed frequencies of ESTs in these intervals were tested against the null hypothesis of homogeneous EST distribution along the chromosome arms. Under the null hypothesis, the expected number of ESTs is proportional to the length of the bin.

RESULTS

Consensus map: Twenty-two deletion breakpoints divide chromosome 5A into 9, 5B into 11, and 5D into 8 bins. A total of 1052 EST probes were used to map 2338 loci into these bins. The complete list of ESTs has been deposited in the supplemental online materials (http://wheat.pw.usda.gov/pubs/2004/Genetics/). The online material also includes 51 and 166 loci previously mapped on the short arms and long arms of chromosomes of group 5, respectively (Gill *et al.* 1996; Faris *et al.* 2000; Sarma *et al.* 2000; Qi and Gill 2001; Faris and Gill 2002). The total number of mapped loci is currently 2555, resulting in a mean density of 1.1 loci/Mb.

The physical order of EST markers along the chromosomes was almost identical for 5A, 5B, and 5D, except for a distal 5AL/4AL translocation and a 5A putative pericentromeric inversion described below. On the basis of this extensive colinearity, we established a consensus physical map for wheat homoeologous group 5 chromosomes. The consensus map includes 11 bins in the short arm and 13 bins in the long arm, with bin numbers increasing from centromere to telomere (Figure 1). In

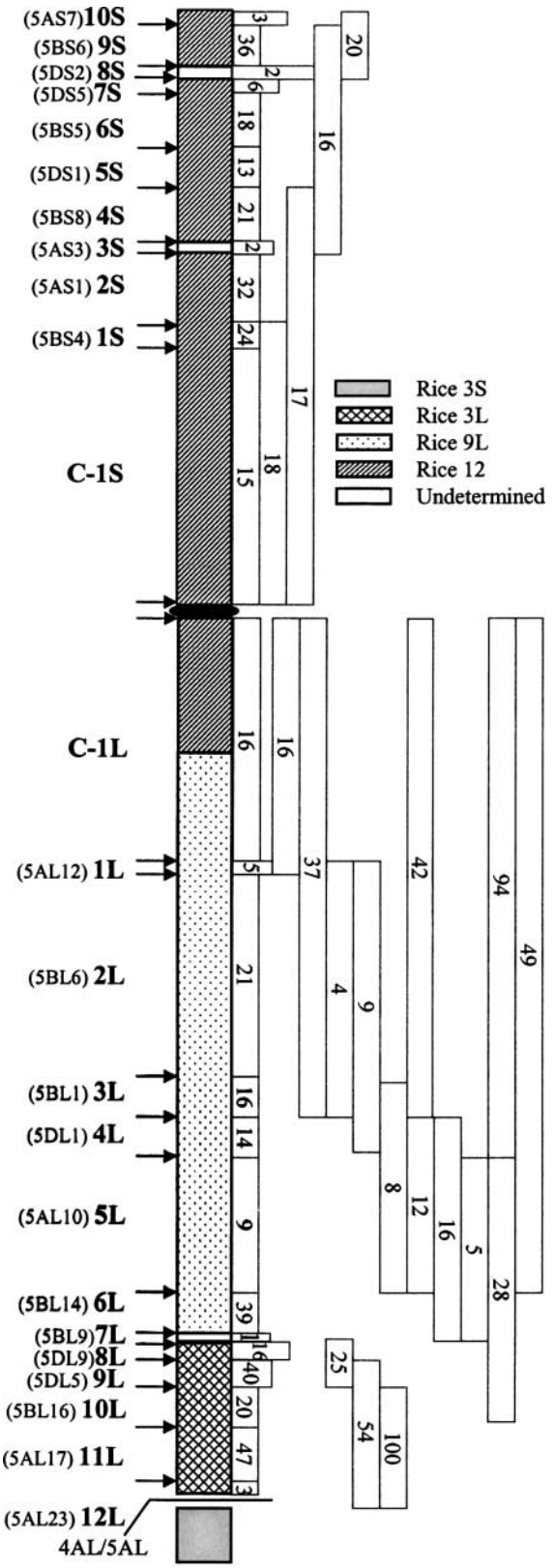

Figure 1.—Consensus physical map of wheat homoeologous group 5 chromosomes. Consensus bin names are shown on the left (the corresponding deletion bin names are in parentheses). Numbers inside bars show the number of EST probes mapped into each consensus interval. Larger bars to the right represent combined consensus bins. Different shadowing patterns within the consensus chromosome indicate colinearity with different rice chromosomes. The 4AL/5AL translocation breakpoint is indicated at the bottom.

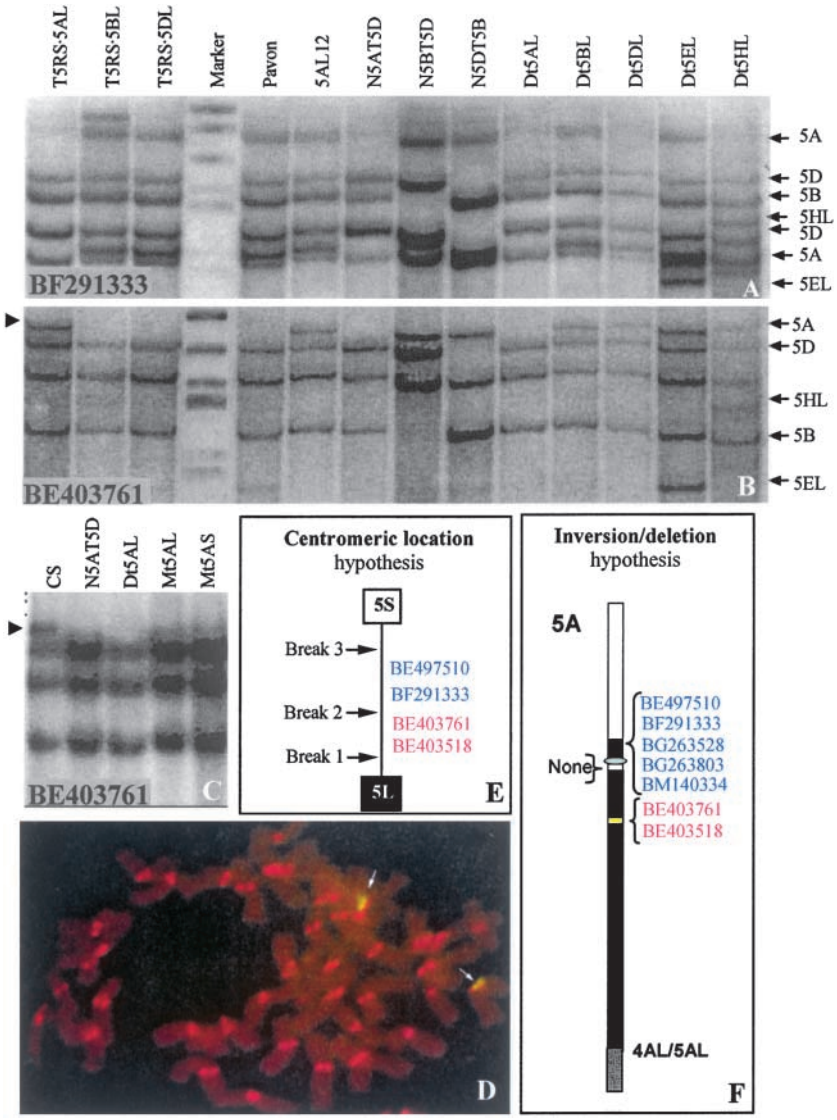

FIGURE 2.—Southern blot hybridization of probes included in the putative pericentric inversion on chromosome 5A. Hybridization of *Eco*RI-digested DNAs of wheat-rye translocation lines, nulli-tetrasomic and telosomic lines for homoeologous group 5, and *Lophopyrum elongatum* and barley telosomic addition lines is shown. (A) Probe BF291333. (B and C) Probe BE403761. (D) Multicolor FISH with pAW-RC.1, a rye-specific centromeric probe (labeled with fluorescein-12-dUTP and visualized by yellow-green fluorescence), and pAet6-J9, a grass common centromeric probe (labeled with rhodamine-6-dUTP and visualized by red fluorescence), showing the presence of a complete rye centromere in the T5RS·5AL translocation (indicated with arrows; provided by Dr. Peng Zhang; FRANCKI 2001; ZHANG 2002). (E) Schematic of the centromeric location hypothesis. According to this hypothesis these ESTs are located within the functional centromeric region and the arrows indicate different centromeric breakpoints. (F) Schematic of the inversion/deletion hypothesis. ESTs indicated in blue are located within the inversion and ESTs indicated in red are located within a region that is deleted in Dt5AL.

both arms the distal halves have three times more consensus bins than the proximal halves, resulting in smaller consensus bins in the distal part of the chromosomes. A total of 419 probes were assigned to these 24 consensus bins, whereas the other 570 probes were mapped into combined consensus bins because they were mapped into only one or two chromosomes or into combined bins in the original chromosomes. A total of 63 probes were assigned to chromosomes or chromosome arms, but not to bins. A few consensus bins have a small number of ESTs because of an unequal partition of the original chromosome bins into small consensus bins (8S, 3S, and 8L) or because of the presence of small distal deletions (*e.g.*, 10S).

Structural rearrangements: Two exceptions were found to the colinearity observed among chromosomes 5A, 5B, and 5D. The first one was the known 4AL/5AL-translocation on the distal part of chromosome 5AL corresponding to bin 5AL23 (NARANJO *et al.* 1987). From a total of 73 ESTs mapped on bin 5AL23, 68 were mapped on 5AL, 4BL, and 4DL bins, whereas 5 were mapped on 5AL, 5BL, and 5DL bins.

Nine EST probes (BE403518, BE403761, BE425161, BE497510, BF291333, BF474334, BG263528, BG263803, and BM140334) detected homoeoloci in centromeric bins on 5AS, 5BL, and 5DL (Figure 2, A and B), suggesting the presence of a small pericentric inversion (Figure 2F). The same probes detected RFLP fragments on chromosome arms 5EL and 5HL in ditelosomic-addition lines of *L. elongatum* and *H. vulgare* (Figure 2, A and B). Three of these ESTs—BE497510, BF474334, and BE425161—were found to be different parts of the same gene contig and their results are all reported as part of the BE497510 results hereafter (Figure 2F).

Assignment of these loci to the short arm of chromosome 5A was initially determined only by the absence of the RFLP fragments in the Dt5AL line, because the sterile Dt5AS stock was not included in the general

TABLE 1

Distribution of probes and loci among group 5 chromosomes in wheat

	All ESTs			ESTs with all bands mapped		
Chr[a]	Loci	Probes	Duplications	Loci	Probes	Duplications
$5A_c$	785 (34%)	636 (33%)	149 (35%)	167 (30%)	145 (30%)	22 (27%)
5B	883 (38%)	702 (37%)	181 (42%)	208 (37%)	172 (36%)	36 (44%)
5D	668 (28%)	570 (30%)	98 (23%)	185 (33%)	161 (34%)	24 (29%)
χ^2 *P*	<0.0001	0.001	<0.0001	0.10	0.31	0.12

Duplications are numbers of loci minus number of EST probes. χ^2 test indicates the probability of a departure from a hypothetical 1:1:1 proportion among genomes.

[a] $5A_c$ = corrected $5A = 5A - 5AL23_{4A} + 4AL_{5A} + 7BS_{5A}$. Chr, chromosome.

mapping project. Therefore, we used different cytogenetic stocks to characterize further some of these probes. The 5A RFLP fragments detected with EST probes BE497510, BF291333, and BM140334 were also absent in the wheat-rye translocation line T5RS·5AL (Figure 2A). The short-arm location of the fragment identified by the BE497510 probe on chromosome 5A was further confirmed by its presence in Mt5AS.

EST probes BE403518 and BE403761 showed unexpected patterns. The 5A RFLP fragments detected with these two probes (absent in N5AT5D) were simultaneously absent from Dt5AL and Mt5AS (Figure 2C, arrowhead), but present in the T5RS·5AL line (Figure 2B, arrowhead) and in a double ditelosomic line dDt5A carrying a pair of 5AS and a pair of 5AL telocentrics. We confirmed by *in situ* hybridization that the T5RS·5AL chromosome had a rye centromere, indicating that a complete 5RS arm was present in this stock (Figure 2D). Linkage mapping of these two probes in *T. monococcum* showed that BE403761 was completely linked to the centromere of chromosome $5A^m$. BE403518 hybridized with multiple fragments and the only polymorphic fragment was mapped on chromosome $7AS^m$ linked to *Xabc152*. The observed results of the probes with altered colinearity in the centromeric region of homoeologous group 5 were explained by two alternative hypotheses presented in Figure 2, E and F, and described in detail in the DISCUSSION.

Ten EST probes from homoeologous group 5, with all bands mapped, hybridized with restriction fragments from only one genome (2 from 5A, 5 from 5B, and 3 from 5D). Seven of them showed no sequence similarity with rice. The other 3 (BF200575, BE424034, and BG313878) showed significant similarities with ESTs from the colinear regions in rice chromosomes 9 and 12, suggesting that they were deleted from two of the three wheat genomes.

Distribution of ESTs among chromosomes: To compare the number of ESTs hybridizing with at least one locus on chromosomes 5A, 5B, and 5D, chromosome 5A was reconstructed to its ancestral state before the structural changes took place. The symbol $5A_c$ was used to indicate that the number of ESTs hybridizing with at least one locus in chromosome 5A was corrected by eliminating those present in the 5AL23 bin and originated from the translocated 4AL arm (68) and adding those from the 5AL arm translocated to 4AL (50) and 7BS (8) (Tables 1 and 2).

A significantly higher number of EST probes hybridizing with at least one locus were mapped on 5B (702) compared to $5A_c$ (636) and 5D (570) (χ^2, $P < 0.001$, Table 1). This paralleled a significantly higher number of duplicated loci on the 5B chromosome ($P < 0.001$).

To eliminate any potential effect of different levels of polymorphism among genomes we repeated the previous analysis using only the ESTs for which all the RFLP fragments were mapped. For this reduced subset of 559 ESTs, the 5B chromosome continued to show the largest proportion of probes (172), loci (208), and duplicated loci (44%, Table 1). However, the reduced numbers of ESTs used in the second analysis resulted in no significant differences (Table 1).

Distribution of mapped loci along the chromosome arms: Using the corrected numbers for $5AL_c$, the proportions of EST probes between the long and the short arms of all three chromosomes were almost identical ($5AL_c/5AS = 3.0$, $5BL/5BS = 3.1$, and $5DL/5DS = 3.1$). The total numbers of loci assigned to the short and long arms were not equal to the total number assigned to chromosomes because 54 loci were not allocated to defined bins, and 173 were assigned only to complete chromosome arms or chromosomes.

In spite of the similar ratios between the number of probes located in the long and short arms, some differences were detected in the distribution of the probes within the chromosome arms. For all six arms we found a significantly higher number of EST loci and probes in the distal half of the arm relative to that in the proximal half (χ^2, $P < 0.01$, Table 2). However, when we divided the most distal half from each arm into two approximately equal parts, we found significant differences only in three of the six arms. Chromosome

TABLE 2

Distribution of group 5 wheat EST loci and duplicated loci along the centromere-telomere axis

		Proximal ½ *vs.* distal ½					Proximal ¼ *vs.* distal ¼ (within distal ½)				
Chr[a]	Arm location[b]	Proportion of the arm	Observed loci	*P*[c]	Observed duplications	*P*[c]	Proportion of the arm	Observed loci	*P*[c]	Observed duplications	*P*[c]
5AS	Prox.	0.40	35	<0.005	5	<0.005	0.35	46	<0.005	14	NS
	Dist.	0.60	135		28		0.25	89		14	
$5AL_c$	Prox.	0.57	258	<0.005	48	<0.005	0.21	117	NS	18	<0.005
	Dist.	0.43	269		60		0.22	152		42	
5BS	Prox.	0.56	76	<0.005	15	<0.025	0.25	68	NS	18	NS
	Dist.	0.44	117		27		0.19	49		9	
5BL	Prox.	0.55	66	<0.005	15	<0.005	0.21	102	<0.005	23	<0.01
	Dist.	0.45	364		76		0.24	262		53	
5DS	Prox.	0.63	63	<0.005	8	<0.01	0.15	29	NS	2	NS
	Dist.	0.37	85		14		0.22	56		12	
5DL	Prox.	0.60	145	<0.005	16	<0.005	0.16	103	<0.005	19	NS
	Dist.	0.40	322		56		0.24	219		37	

The "proximal ½ *vs.* distal ½" column includes the comparison between the proximal and distal halves of each arm, whereas the "proximal ¼ *vs.* distal ¼ (within distal ½)" column indicates the comparison between the proximal and distal regions of the distal half.

[a] Chr, chromosome; $5AL_c$ = corrected 5AL = 5AL − $5AL23_{4A}$ + $4AL_{5A}$ + $7BS_{5A}$.

[b] Prox., proximal half of the arm; Dist., distal half of the arm.

[c] *P* indicates the probability of a significant χ^2 test against the null hypothesis of number of ESTs proportional to the physical length of the combined bin. NS, not significant.

arms 5AS, 5BL, and 5DL showed a significantly higher ($P < 0.01$) number of EST probes and loci in the most distal quarter relative to that in the proximal quarter whereas the three other arms did not follow this pattern (Table 2).

The difference between the number of loci and the number of probes mapped in the bins of a particular chromosome was used to estimate the level of intrachromosomal duplications. This includes duplications within the same bin and among different bins of the region being compared. The number of intrachromosomal duplications in the distal halves of the six chromosome arms from homoeologous group 5 was significantly higher (χ^2, $P < 0.01$) than the number in the proximal halves. To test the homogeneity of the duplication distribution within the distal halves, these regions were divided further into two similar quarters. Statistically significant higher numbers of duplications in the distal quarter were confirmed only for $5AL_c$ and 5BL arms (Table 2). It should be pointed out that the absolute number of duplications indicated in Table 2 is an overestimate, because internal *Eco*RI sites within the region of hybridization also can create duplicated fragments within a bin.

The increased number of ESTs mapped in the distal halves of the chromosomes can be one of the causes of the increased number of duplications observed in these chromosome regions. To investigate this possibility, the proportion of duplications was calculated by dividing the number of duplications by the number of loci mapped in a particular region. Five of the six arms showed a higher proportion of duplicated loci in the distal half of the chromosomes than in the proximal half. The exception to this pattern was 5BL, which showed 23% of duplications in the proximal region and 21% in the distal region, which suggests that the higher level of duplications was not entirely caused by a larger number of loci present in the distal regions.

Comparative wheat-rice genome analysis: A total of 430 EST probes that hybridized with six or less bands, and for which at least two bands were mapped to homoeologous group 5 (of the 980 included in the consensus map), were selected for the wheat-rice comparisons. The other ESTs were excluded because they hybridized with multiple bands, and the mapping of paralogous loci could result in an overestimate of the noncolinear loci. From the 430 selected ESTs, 357 were significantly similar (see MATERIALS AND METHODS) to sequences present in the public rice genome database of ordered BAC/PAC clones (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) and were used for the statistical tests of colinearity (Table 3). Hereafter, wheat chromosomes are preceded by a W and rice chromosomes by an R to simplify the descriptions.

In the short arm, most of the ESTs (64% = 67 ESTs, Table 3) were significantly similar to rice genes located within a 66-cM segment covering the complete long arm of R12 (Figure 1). The statistical analysis showed that 9 of the 11 consensus bins from W5S had significantly more common probes with R12 than expected from a random distribution ($P < 0.01$, Table 3). The four

TABLE 3

Comparison between wheat group 5 consensus bin map and the rice genome

Wheat consensus bin	Rice chromosome 1	2	3	4	5	6	7	8	9	10	11	12	No match	Total matches	*P* first	*P* second
8S-10S	1	2					1					2		6	0.083	0.044
3S-10S	1	1					1		1			2	1	6	0.083	0.317
10S												2	1	2	0.007	—
9S	4	2								1		13	16	20	<0.001	0.002
8S													2	0	—	—
7S			1					1				3	1	5	0.0051	0.174
6S					2					1		6	1	9	<0.001	0.023
C-1S-5S												4	1	4	<0.001	—
5S												3		3	0.001	—
4S	1		1	1				1				7		11	<0.001	0.317
3S												1	1	1	0.083	—
C-1S-2S	1											2		3	0.020	0.091
2S		1							2		2	10	1	15	<0.001	0.069
1S		1										9	1	10	<0.001	0.091
C-1S							1	2		1	2	3	6	9	0.033	0.097
C-1L	1				2		1	2	3			3	4	12	0.072	0.041
C-1L-2L									1				1	1	0.083	—
C-1L-4L	2		3			2	2		10	1	1	6	7	27	<0.001	0.003
C-1L-5L	1	1	1		2		1	2	14		1	9	2	32	<0.001	<0.001
C-1L-6L									3			3	1	6	0.009	0.001
1L									2			1		3	0.020	0.091
1L-4L		1									1	2		4	0.037	0.174
1L-5L			1	1		1		1				1		5	0.353	0.317
2L		1			1			1	6			1	1	10	<0.001	0.317
3L									7		1	1	1	9	<0.001	0.174
3L-6L									3				1	3	0.001	—
4L									9			1		10	<0.001	0.091
4L-8L		2							5				2	7	<0.001	0.008
5L							2		4					6	0.001	0.008
6L		1					1		5		2	1	4	10	0.001	0.069
7L													1	0	—	—
7L-10L			4					1					1	5	<0.001	0.091
8L			3					1				1		5	0.005	0.174
9L			18			1	1	1		2				23	<0.001	0.069
9L-12L			7										3	7	<0.001	—
10L			14				1		1				1	16	<0.001	0.174
10L-12L	1	1	19	1					2				8	24	<0.001	0.069
11L	2		17		1		2					2	3	24	<0.001	0.128
12L			1											1	0.0833	—
5L-11L			1								2			3	0.0197	—

combined consensus bins from the short arm showed also higher similarity to rice genes from R12 (Table 3).

The exceptions were the small consensus bins 3S and 8S. No common ESTs between wheat and rice were found in consensus bin 8S. Only one of the two ESTs assigned to the 3S consensus bin had a significant match with a rice gene (Table 3) located within the expected region on R12. However, because of the low number of ESTs mapped on this bin, the statistical comparison was not significant ($P = 0.08$). Wheat bin 9S included 13 ESTs significantly similar to rice chromosome R12, but 4 additional ESTs were assigned to R1 (Table 3, $P < 0.01$). If these 4 ESTs were the result of a single translocation event, they would probably map on a contiguous region on R1. However, we found that these 4 ESTs corresponded to two regions located ~73 and 136 cM on R1.

In the long arm of W5, 253 of the 294 analyzed ESTs showed similarities with sequences from R12 (32 ESTs), R9 (75 ESTs), and R3 (89 ESTs) (Table 3). The additional 57 wheat ESTs with significant matches corresponded to the other nine rice chromosomes (Table 3).

The colinearity between the order of the bins on W5S and R12L described above was extended to the

centromeric bins on W5L and R12S. These wheat ESTs were significantly similar to rice genes located within the region between 6 and 49 cM (centromeric region) on the linkage map of R12S. The ESTs assigned to the long arm centromeric bins C-1L (12) and 1L (3) showed significant or close to significant similarity with sequences on both R12 and R9 (Table 3), suggesting that this breakpoint in colinearity was located somewhere within these two bins. Combined bins C-1L-4L, C-1L-5L, and C-1L-6L also showed significant similarity with rice genes located on these two rice chromosomes (Table 3). Analysis of the combined bins was necessary because a large number of ESTs were mapped into combined bins C-5AL12-0.57* and C-5BL14-0.75* due to the presence of incorrect proximal deletion lines for 5AL and 5BL available to most of the mapping laboratories.

The region of the long arm of wheat chromosome 5 corresponding to consensus bins 2L to 6L showed a significant similarity with rice chromosome R9L. Two exceptions to this general trend were detected in bin 5L and in combined bin 4L-8L. Bin 5L contained four ESTs that were similar to sequences on R9L. Two additional ones had similarity to R7; however, these two ESTs did not match adjacent sequences in R7. In the combined interval 4L-8L, five ESTs were similar to R9L, but two ESTs were assigned to R2. As for the ESTs from bin 5L, these ESTs were mapped into a separate region in R2, suggesting that they do not belong to a single translocation event.

ESTs mapped to the most distal bins, 8L to 11L, were significantly similar ($P < 0.01$) to genes from two separate regions of R3L. The first region extended from the centromere to 100 cM, whereas the second one extended from 136 cM to the end of R3L. Rice BAC/PAC sequences located between these two regions were used to screen the database of mapped wheat ESTs. Approximately 20% of the significant matches corresponded to W4S and 60% to W4L (W4A matches were located on opposite arms to 4B and 4D due to a known pericentric inversion). The other 20% was distributed among five different wheat chromosomes.

We could not confirm the syntenic relation of consensus bins 7L and 12L due to the insufficient number of probes assigned to these bins. Finally, 14 ESTs mapped in the distal region of W5AL (most ESTs in bin 5AL23 originated from a translocation with homoeologous group 4) were colinear to the regions between 1 and 6 cM on R3S. This segment is in an inverted orientation relative to the colinearity between bins 8L to 11L and rice R3L (Dubcovsky *et al.* 1998).

The conservation of large chromosome blocks from wheat homoeologous group 5 with rice chromosomes R12, R9, and R3 can be used to infer the order of the loci within the physical bins. Although these predictions should be taken with caution, they provide a valuable first approximation to the order of loci within a particular bin. We have included in the supplemental online materials (http://wheat.pw.usda.gov/pubs/2004/Genetics/) a table with the predicted order of the wheat ESTs based on the order of the corresponding rice genes within the physical map of the rice BAC/PAC clones.

DISCUSSION

Physical maps and consensus maps: Deletion maps are a simple and rapid way to build cytogenetically based RFLP maps of wheat (Werner *et al.* 1992; Gill *et al.* 1993; Kota *et al.* 1993), providing a first insight into the structure and evolution of the wheat genome (Gill *et al.* 1996; Qi and Gill 2001; Faris and Gill 2002; Akhunov *et al.* 2003a).

Consensus maps combine the information of the individual deletion maps, adding information about the order of markers within a bin in one genome, on the basis of the distribution of the same markers in bins of the other wheat genomes (Werner *et al.* 1992; Gill *et al.* 1996). Many of the previous consensus maps have been constructed by ordering the consensus breakpoints according to the fraction length (FL) values derived from the bins located to the different homoeologous chromosomes (Gill *et al.* 1996; Sarma *et al.* 2000; Qi and Gill 2001). The large number of ESTs included in this study facilitated a more precise approach for defining the consensus bins. In this procedure (see materials and methods) the information derived from the numerous ESTs that mapped to more than one homoeologous chromosome was used to define precisely the consensus bins. Even using this improved approach, the three wheat genomes are not perfectly conserved and, given the resolution of the deletion bins, it is likely that we cannot detect many deviations in gene content and order among them.

Colinearity among chromosomes 5A, 5B, and 5D and its exceptions: Results from this and previous studies have shown good colinearity among chromosomes 5A, 5B, and 5D (Gill *et al.* 1996; Faris *et al.* 2000; Sarma *et al.* 2000; Qi and Gill 2001; Sorrells *et al.* 2003). However, this colinearity has been disrupted by two structural changes on chromosome 5A. The first one is a large reciprocal translocation between the long arms of chromosome 5AL and 4AL that occurred in the diploid ancestor of the A genome (Naranjo 1992; Devos *et al.* 1995). We found 5 of 73 ESTs from the 5AL23 bin outside the translocation (5AL, 5BL, 5DL location), suggesting that the 4AL/5AL translocation point was located distally, but close to the 5AL23 deletion line breakpoint. A similar result was reported before (Faris and Gill 2002).

In addition, a previously unknown alteration in the colinearity in the centromeric region of chromosomes from homoeologous group 5 was discovered in this study. Two alternative hypotheses are presented to ex-

plain those results: the inversion/deletion hypothesis (Figure 2F) and the centromeric location hypothesis (Figure 2E).

The inversion/deletion hypothesis proposes that a pericentric inversion in the centromeric region of chromosome 5A was the cause of the opposite-arm location of the five ESTs indicated in blue in Figure 2F (5AS, 5BL, 5DL). These ESTs were also mapped in the long arms of barley 5HL and *L. elongatum* 5EL chromosomes, confirming their ancestral long-arm location. This was also supported by their colinearity with a segment of rice chromosome arm R12S between 27.1 and 39.4 cM, corresponding to the long arms of wheat homoeologous group 5.

However, no EST mapped to the long arm of 5AL while mapping to the short arms of 5B and 5D, suggesting that the breakpoint on the 5AL arm in this putative inversion was more distal than the breakpoint on the 5AS arm. One probe, BE403618, was mapped on bins C-5AL12 and 5BS4, but the inclusion of this probe within the putative inversion was ruled out because 5BS4 is not the most proximal bin in 5BS, and the ESTs mapped in the more proximal C-5BS4 bin were mapped in colinear locations in 5AS and 5DS outside the putative inversion.

A second assumption of the inversion/deletion hypothesis is that the Dt5AL stock carries a secondary interstitial deletion in the proximal region of 5AL including loci BE403518 and BE403761 (Figure 2F). This deletion explains the simultaneous absence of the 5A RFLP fragments from the short (Mt5AS) and the long arms (Dt5AL) of chromosome 5A in the hybridization profiles of BE403518 and BE403761. The presence of these fragments in the T5RS·5AL translocation line is explained by the absence of the secondary 5AL interstitial deletion in T5RS·5AL.

The centromeric location hypothesis (Figure 2E) is based on the assumption that this group of noncolinear loci is located within the functional centromeric region, and that different breakpoints explain the different results observed in this study. The first proposed breakpoint, the closest to the long arm, is used to explain the results observed in the Dt5AL arm, in which all probes show missing 5A RFLP fragments. The second proposed breakpoint on the centromeric region of chromosome 5A (Figure 2E, breakpoint 2), located proximal to BE403761 and BE403518, is used to explain the presence of these loci in the T5RS·5AL line and their absence in the Mt5AS line. The last breakpoint (Figure 2E, breakpoint 3) is the closest to the short arm and is used to explain the presence of all the analyzed RFLP fragments in Dt5BL, Dt5DL, Dt5EL, Dt5HL, T5RS·5BL, and T5RS·5DL.

The location of expressed genes within a functional centromere has been demonstrated in yeast (Kuhn *et al.* 1991) and in Arabidopsis (Copenhaver *et al.* 1999) and was also suggested for the centromeric region of wheat chromosome 1B (Sandhu *et al.* 2001; Francki *et al.* 2002). Cytogenetic studies have shown that the centric breakage fusions in Robertsonian wheat-rye translocations can occur at different positions within the primary constriction (Zhang *et al.* 2001), providing an explanation for the different results observed in this study in the wheat-rye translocation lines.

Since none of the observed results can be used to rule out one of the two proposed hypotheses, additional research will be necessary to determine which of them is correct.

Distribution of ESTs among chromosomes: The large sample of ESTs used in this study, representing a random sample of approximately one-third of the wheat genes, provided a powerful tool to analyze the distribution of genes among genomes and within chromosome arms. The 5B chromosome has a larger number of mapped loci than 5A and 5D, which is the pattern when total loci mapped per genome is calculated, and the B genome has more than the A and D (Qi *et al.* 2004). The 5BL arm appears to have the highest number of mapped ESTs among all wheat long-arm chromosomes. A previous report indicated that the B genome has twice as many unique loci as the A and D genomes (Akhunov *et al.* 2003b), providing a possible explanation to the higher number of mapped loci observed in this study.

However, the higher level of RFLP polymorphism usually observed in the B genome compared to that in the other two genomes could provide an alternative explanation for the higher number of ESTs mapped to the B genome. The lower level of intergenomic polymorphism in the A and D genomes could result in a larger proportion of comigrating bands that cannot be mapped by deletion mapping. To test this possibility we repeated the comparison using only those probes for which all RFLP fragments were mapped. This excludes the putative effect of different levels of RFLP polymorphism. In this second analysis, the B genome also showed the largest proportion of mapped probes and loci, although the reduced number of ESTs resulted in nonsignificant statistics (Table 1). These results suggested that part, but not all, of the increase in the number of ESTs mapped on chromosome 5B was due to its higher level of polymorphism.

Distribution of ESTs within chromosome arms: The distal regions of the wheat genomes are known to show higher levels of RFLP than the proximal regions (Dvořák *et al.* 1998). As a result of this gradient, many proximal probes were excluded from the wheat linkage maps because of a lack of polymorphism between the parental lines of the mapping populations. Because most of the RFLP probes used in the first characterizations of the wheat deletion lines were selected from clones previously mapped in linkage maps, this resulted in an underestimation of the number of proximal loci. This study used a random sample of ESTs originating from multiple cDNA libraries, resulting in a better estimate

of the number of loci in the proximal regions. We mapped 395 and 146 EST probes in the proximal half regions of the long and short arms of homoeologous group 5 chromosomes, respectively.

The results obtained here were used to reexamine the conclusions from previous studies suggesting differences in gene density along the wheat chromosomes. Previous studies consistently showed that the proximal bins from homoeologous group 5 had a lower density of markers than the distal bins (Gill *et al.* 1996; Faris *et al.* 2000; Sarma *et al.* 2000; Qi and Gill 2001; Faris and Gill 2002). Some of these studies calculated the gene density along the chromosome by dividing the number of genes by the size of the bin in megabases. Although this is a relatively good approach for large bins, it results in imprecise estimates for small bins due to the limitations in the cytological determination of metaphase chromosomes lengths. To avoid this problem, we used a conservative approach and pooled bins covering relatively large fractions of the chromosomes. All six arms of homoeologous group 5 chromosomes showed a significantly higher number of genes in the distal halves compared with a null hypothesis of uniform distribution along the chromosome arm, confirming previous reports. However, division of the distal halves of the six arms into two quartiles failed to show significant differences in gene number in three of the six chromosome arms (Table 2). Among the three nonsignificant arms, 5BS had 4% fewer loci than expected in the most distal quartile, whereas 5DS and $5AL_c$ had 9–10% more loci than expected in this quartile. This result contradicts a simple model of increased gene density with distance from the centromere and suggests a more uniform distribution of gene densities between the two distal quartiles of some of the arms of homoeologous group 5 chromosomes.

To test if the higher level of polymorphism observed in loci from the distal regions of the wheat chromosomes (Dvořák *et al.* 1998) was affecting our estimates of the distribution of loci along the chromosome arms we repeated the statistical analyses from Table 2 using only those probes with all the restriction fragments mapped. For this limited set of loci, we observed results similar to those with the complete set of ESTs, suggesting that the observed differences were not determined by difference in polymorphism. The only exception was the comparison between the two distal quartiles of 5DL that was significant with the complete set of loci but not with the subset with all bands mapped.

Gene duplication: The same partition of the homoeologous group 5 chromosome arms was used to investigate the intrachromosomal distribution of gene duplications. For the six chromosome arms analyzed in this study, we found a significantly higher number ($P < 0.01$) of intrachromosomal duplicated loci in the distal halves relative to the null hypothesis of uniform distribution. This result is in agreement with previous observations indicating a predominance of multigene loci in the distal regions of wheat chromosomes (Akhunov *et al.* 2003a). However, as in the gene density study, the division of the distal halves into two quarters resulted in nonsignificant differences for four of the six arms analyzed, suggesting that not all the arms had a larger number of duplications toward the telomeres within the distal halves of the chromosomes.

Comparative analysis of wheat homoeologous group 5 genes with the rice genome: The wheat and rice genomes diverged >50 million years ago and differ by nearly 40-fold in genome size. However, large chromosome blocks showed good colinearity between the two genomes (Kurata *et al.* 1994; Moore *et al.* 1995; Van Deynze *et al.* 1995; Feuillet and Keller 2002; Sorrells *et al.* 2003).

Wheat homoeologous group 5 was previously shown to have one of the most complex syntenic relationships with rice among all wheat groups (Moore *et al.* 1995; Sorrells *et al.* 2003), resulting in an incomplete picture of its colinearity with rice. The large numbers of ESTs used in this study and the completion of the first draft of the rice genome sequence (Goff *et al.* 2002) were useful to refine the comparison between wheat chromosome 5 and the rice genome.

Previous comparative studies reported colinearity between W5S and rice chromosomes R12, R11, R5, and R1 (Sarma *et al.* 2000; Qi and Gill 2001; Lamoureux *et al.* 2002). We confirmed here that the colinearity between W5S and R12 extends from almost the complete length of this wheat chromosome arm to the proximal region of W5L, suggesting the presence of similar centromere locations. We could not confirm a significant similarity with other rice chromosomes. The similarities between W5S and rice chromosome R11 most likely originated from the duplication of a large segment of chromosome R12 in R11 (Wu *et al.* 1998).

Sarma *et al.* (2000) reported that the colinearity between W5L and R9 was disrupted by the insertion of parts of several linkage groups of rice. We showed here that the R9 region between 100 and 136 cM shows similarity with W4. The two R9 regions flanking this 36-cM interval showed 69 ESTs colinear with W5L, suggesting the presence of a large translocation into R9 or out of W5. The few ESTs similar to other rice chromosomes detected in bins 5L (R7) and 4L-8L (R2) were not adjacent in the rice genome, suggesting that they do not represent single translocation or transposition events.

Although the large-scale colinearity between wheat and rice chromosomes can be explained by few breakpoints in the rice genome (Moore *et al.* 1995), more complex relationships have been described in studies that compared the same chromosomes at the subcentimorgan or sequence level (Bennetzen and Ramakrishna 2002; Feuillet and Keller 2002). A comparison of the available Triticeae-rice microcolinearity studies showed a better conservation in the proximal

regions of the wheat chromosomes (Roberts *et al.* 1999; Sanmiguel *et al.* 2002; Yan *et al.* 2003) than in the distal parts of the same chromosomes (Kilian *et al.* 1997; Feuillet and Keller 1999; Li and Gill 2002; Yan *et al.* 2004). The *Ha* locus in the distal region of W5S represents an exception to this trend (Chantret *et al.* 2004).

The better wheat-rice microcolinearity observed in the proximal regions of the chromosomes is in agreement with the general evolutionary trends previously described for the large Triticeae chromosomes. These studies have shown that new loci, originating by duplication and transposition, as well as fixed deletions are more frequent in high-recombination regions at the distal ends of the wheat chromosomes (Akhunov *et al.* 2003a,b). As a result of these trends the distal regions of wheat chromosome arms have been evolving faster than the proximal regions, resulting in more frequent exceptions to the colinearity and microcolinearity between wheat and rice in the distal chromosome regions.

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