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An intervarietal molecular marker map in *Triticum aestivum* L. Em. Thell. and comparison with a map from a wide cross

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Abstract An intervarietal molecular marker map covering most of the nuclear genome was developed in *Triticum aestivum*. One hundred and six androgenetic-derived doubled haploid lines obtained from the F_1 between monosomics of 'Chinese Spring' and 'Courtot' were analysed for genetic mapping. The map covered 18 of the 21 chromosomes with an identical distribution of markers in the A and B genome, and only small segments of the D genome. Distorted markers were mapped using Bailey's 2-point method and revealed skewed regions on 1A, 1DS, 2A, 2B, 4AS and 6B. Comparison with a wide cross ['Opata' × Synthetic hexaploid (*T. tauschii*/'Altar 84')] showed colinearity for markers on homologous chromosomes, but revealed a large proportion (25%) of markers mapped on non-homoeologous chromosomes, i. e. heterologous markers. The origin of the material and distortion segregation are discussed with particular emphasis on investigations of D-genome markers.

Key words *Triticum aestivum* · Doubled haploids · Intervarietal map · Distortion segregation · Genetic map comparison

Introduction

Restriction fragment length polymorphism (RFLP) maps have already been constructed in many higher plants (for a review, see O'Brien 1990). In bread wheat, (*Triticum aestivum* L. em. Thell. 2n = 6x = 42), an allohexaploid species organized into seven homoeologous groups and three genomes (A, B and D), linkage maps of homoeologous groups 1 (Van Deynze et al. 1995), 2 (Devos et al. 1993; Nelson et al. 1995b), 3 (Devos et al. 1992; Nelson et al. 1995c), 4 , 5 (Devos et al. 1995b; Nelson et al. 1995a; Xie et al. 1993), 6 (Jia et al. 1996; Marino et al. 1996) and 7 (Chao et al. 1989; Nelson et al. 1995a) have been published separately as well as in the form of complete maps (Liu and Tsunewaki 1991; Gale et al. 1995).

Due to the low level of RFLP polymorphism in bread wheat (Chao et al. 1989), the strategy of genome mapping used populations derived from wide crosses, such as 'Chinese Spring' or 'Opata' with a synthetic wheat (reconstructed by hybridizing an AABB *T. dicoccum* with a *T. tauschii* DD genome donor) (Gale et al. 1995; Van Deynze et al. 1995; Nelson et al. 1995a, b, c), or 'Chinese Spring' with *T. spelta* (Liu and Tsunewaki 1991). However, the dissection of complex traits, which is an important task in plant breeding, requires informative markers in an intraspecific context, particularly for marker-assisted selection.

In wheat, the transfer of information from mapping reference populations to agronomic crosses should take into account homoeology relationships. Map comparisons have been made in maize (Beavis and Grant 1991; Bentolila et al. 1992; Murigneux et al. 1993), barley (Laurie et al. 1993; Sherman et al. 1995), *T. durum* 6A and 6B chromosomes (Chen et al. 1994) and wheat (Van Deynze et al. 1995) in order to analyse colinearity of markers and to study recombination.

The objective of the study presented here was to establish a molecular map for wheat using an intervarietal doubled haploid (DH) population obtained from a cross between 'Chinese Spring' and the French cultivar 'Courtot' and to compare it to a previously published map derived from the wide cross ('Opata' × synthetic wheat).

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Materials and methods

Wheat genotypes

'Chinese Spring' and the French semi-dwarf cultivar 'Courtot' display the same chromosomal structure (Denis et al. 1982). By using the complete sets of monosomic lines available in both of these varieties and reciprocal crosses, we were able to obtain plants with 42 and 41 chromosomes.

From 42-chromosome F_1 hybrids, a population of 275 double haploid (DH) lines was produced by anther culture (Félix et al. 1996). The RFLP mapping population consisted of a random sample of 106 DH lines. The 41-chromosome plants corresponded to reciprocal monosomics. These lines, together with the available nullisomic-tetrasomic (NT) set of lines in 'Chinese Spring' (kindly provided by the Cereal Research Department, Norwich, UK), were used for the chromosomal assignment of linkage groups. The NT lines were used for chromosomal identification of codominant markers which encompassed the vast majority of the RFLP markers; the reciprocal monosomics were more specifically used for assignment of hemizygous markers if the case arose in which the 'Chinese Spring' band was not detectable.

DNA probes

The cDNA and gDNA clones used as probes were kindly provided by various laboratories and are described in Table 1. An important source of clones, originating from 2 *Pst*I libraries, was obtained from etiolated seedlings of 'Courtot' (FBA clones) and 'Chinese Spring' (FBB clones) by the laboratory of F. Quétier, Université Paris Sud, France. Two microsatellites were included in the map: one, denoted *Xpsp3000(Gli-1-1)*, corresponded to a γ gliadin pseudogene (Devos et al. 1995a), and the second, 1DS specific and denoted *Xwms106* (Plaschke et al. 1995), was kindly provided by M. Röder, Gatersleben, Germany.

Biochemical markers

The SDS-PAGE method was used to study the segregation of storage protein alleles in the DH population (Félix et al. 1996). Loci *Gli-A1*, *Gli-A5*(1AS), *Glu-A1*(1AL), *Glu-B3*(1BS), *Glu-D3*(1DS) and *Gli-A2*(6AS) were mapped.

RFLP analysis

DNA was extracted from young leaves (2 weeks of growth) using the CTAB method (Saghai-Maroof et al. 1984). Restriction digestion was done at 37 °C for a minimum of 4 h with 10 μg of DNA using 30 units of enzyme in the buffer provided by the manufacturer (Boehringer Mannheim). Seven restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Bgl*II, *Ssp*I and *Sac*I) were used to evaluate polymorphism in the parents. Procedures for electrophoresis, alkali transfer by Southern blotting, probe labelling with a non radioactive technique such as digoxigenin-11-dUTP (Boehringer Mannheim) and filter hybridization were those described previously (Lu et al. 1994). However, an alkali-labile digoxigenin-11-dUTP was used to avoid the residual traces of the previous signal.

Linkage analysis

Goodness-of-fit for all loci to a 1 : 1 segregation ratio was tested by means of chi-square analysis. MAPMAKER/EXP version 3.0 (Lander et al. 1987) was used to construct the map for loci showing no segregation distortion. Linkage groups were established by calculating recombination frequencies with identical conditions (maximum recombination fraction = 0.35 and minimum LOD score = 3.0). A contingency G test was applied (Garcia-Dorado and Gallego 1992) to establish linkage groups involving loci showing segregation distortion. The estimation of the recombination fraction between 2 loci linked to independent viability factors (u, v) was carried out using the Bailey's "r_b" estimator (Bailey 1949) which is

Table 1 Description of DNA probes used in this study

Probe	Plasmid	Cloning site	DNA type	Origin	References	Number of clones tested	Number polymorphic clones
BCD	pBS-SK	*Eco*RI	cDNA	*Hordeum vulgare*	(Heun et al. 1991; Kleinhofs et al. 1993)	16	7
CDO	pBS-SK	*Eco*RI/*Xho*I	cDNA	*Avena sativa*	(Heun et al. 1991)	23	8
MTA	pUC118	*Eco*RI	cDNA	*Triticum aestivum*	(M. F. Gautier, P. Joudrier, INRA Montpellier)	3	3
MTD	pUC118	*Kpn*I/*Bam*HI	cDNA	*Triticum durum*	(M. F. Gautier, P. Joudrier, INRA Montpellier)	1	1
	pUC118	*Pst*I	cDNA	*Triticum durum*	(M. F. Gautier, P. Joudrier, INRA Montpellier)	4	4
	pSPORT1	*Not*I/*Sat*I	cDNA	*Triticum durum*	(Labhilili et al. 1995)	3	1
PSR	pUC18	*Sma*I	cDNA	*Triticum aestivum*	(Gale et al. 1995)	19	13
PSR	pUC18	*Pst*I	gDNA	*Triticum aestivum*	(Gale et al. 1995)	14	5
	pUC18	*Acc*I	gDNA	*Triticum aestivum*	(Gale et al. 1995)	3	0
WG	pGEM4-Z	*Pst*I	gDNA	*Triticum aestivum*	(Heun et al. 1991)	38	16
KSU	pUC18	*Pst*I	gDNA	*Triticum tauschii*	(Gill et al. 1991)	65	43
	pBSK	*Pst*I	gDNA	*Triticum tauschii*	(Gill et al. 1991)	2	1
	pBR322	*Pst*I	gDNA	*Triticum tauschii*	(Gill et al. 1991)	3	2
GLK	pUC119	*Pst*I	gDNA	*Triticum aestivum*	(Liu and Tsunewaki 1991)	46	27
	pUC119	*Eco*RI	gDNA	*Triticum aestivum*	(Liu and Tsunewaki 1991)	6	4
	pUC119	*Hin*dIII	gDNA	*Triticum aestivum*	(Liu and Tsunewaki 1991)	5	5
FBA	pBSK	*Pst*I	gDNA	*Triticum aestivum*	(Nelson et al. 1995a,b,c; Marino et al. 1996)	82	53
FBB	pBSK	*Pst*I	gDNA	*Triticum aestivum*	(Nelson et al. 1995a,b,c; Marino et al. 1996)	50	30
TAM	pUC8	*Pst*I/*Bam*HI	gDNA	*Triticum aestivum*	(Devey and Hart 1993)	8	5

$r_b = \sqrt{bc}/(\sqrt{ad} + \sqrt{bc})$ with $u = \sqrt{(ab/cd)}$ and $v = \sqrt{(ac/bd)}$ (where b, c are recombinant gametes and a, d are parental gametes).

When a satisfactory order was found, distorted markers were included in the whole map. Where possible, the same general procedure was applied to detect linkage between groups composed of distorted loci and normally segregating loci. The genetic distances were calculated with the Kosambi function (Kosambi 1944).

Comparison between maps

In order to evaluate synteny relationships and common coverage, a comparison was made between this map (hereafter referred to as CTCS) and an interspecific-type map for 'Opata' × Synthetic hexaploid ('Altar 84'/*T. tauschii*), (referred to as SICOP for laboratory convenience), as many probes were common to both maps. Information concerning the SICOP map was drawn from published data in the Graingenes database (USDA National Agricultural Library Plant Genome Program) and from GENOBLE phase I program data (P. Leroy, personal communication). When a given marker was located on both maps, with the same position, the corresponding loci were noted as homologous (if they were on the same chromosome) or homoeologous (if they were on homoeologous chromosomes). They were noted as heterologous when found on different homoeologous groups. As both maps originate from crosses involving different population types, a regression analysis (Snedecor and Cochran 1989) was applied for all common intervals to study recombination.

Results

RFLP between parental varieties

Considering the whole set of clones tested (391), 58% (228) exhibited polymorphism in both parents (Table 1). These data showed that 'Courtot' and 'Chinese Spring' were good candidates for the construction of an intervarietal population map. However, the polymorphism was evaluated on a pattern basis and not on a band basis. In a polyploid species, such as wheat, an informative probe is generally polymorphic for only one genome or, more rarely, for two genomes out of three. A polymorphic probe/enzyme combination for all three genomes was never detected in our set of data. In several cases, the combination of 1 probe with different enzymes revealed different loci (e.g. for the clone PSR135 corresponding to 1 locus on 2AS with *Dra*I and to another on 2BS with *Eco*RI). In the case of probes giving several polymorphic patterns, only 1 probe/enzyme combination was chosen. Therefore, not all the possible polymorphic probe/enzyme combinations were assigned.

Segregation distortion

The polymorphic probes were hybridized on the DH progeny, revealing 293 loci. A general chi-square segregation test was done for all the loci recorded. On the whole, the CTCS population was not skewed, with 51.1% of the alleles coming from 'Courtot' and 48.9% of the alleles from 'Chinese Spring' ($\chi^2 = 0.047$, $P = 0.85$). Chi-square segregation tests were also systematically calculated for each locus. Eighty-one molecular markers (27%) deviated significantly from the 1:1 ratio with χ^2 ranging from 3.92 ($P = 0.05$) to 89.60 ($P < 0.001$) (Table 2). Markers with segregation distortion in favour of 'Courtot' were more numerous (54) than those in favour of 'Chinese Spring' (17). Among the latter, loci on 6B exhibited the maximum bias (Table 2).

Table 2 Chi-square values and bias percentages of distorted markers classified by chromosome. Excess of allele in favour of one parent is noted by percentages in bold characters

Chromosome[a]	Locus	Chi-square value	Courtot	Chinese Spring
1A	*Xfbb196b*	4*	40	**60**
	XksuE18b	4*	40	**60**
	Xfba298	6.37*	37.74	**62.26**
	Xfba26b	29.36***	23.31	**76.69**
	Xfba299	39.38***	19.24	**80.76**
	Xbcd22	10.24**	34	**66**
	Xfba266a[b]	12.46***	**67.3**	32.7
1DS	*Xwms106*	4.56*	**60.38**	39.62
	XksuE18a	7.21**	**63.37**	36.63
	XksuE19a	8.16**	**64.08**	35.92
	Xfba250	9.51**	**65.35**	34.65
	Xglk558	8.65**	**64.42**	35.58
	Xfba8	8.00**	**63.8**	36.2
	Xfba383a	7.07**	**63.1**	36.9
2A	*Xpsr107*	3.92*	**59.8**	40.2
	Xpsr135b	6.06*	**62.13**	37.87
	Xcdo770	3.92*	**59.8**	40.2
	Xfba71b	5.03*	**60.95**	39.05
	Xpsr388	5.03*	**60.95**	39.05
	Xglk554	5.13*	**61.16**	38.84
	Xglk594a	16.00***	**69.52**	30.48
	XksuD23b	14.48***	**68.57**	31.43
	Xwg645b	13.03***	**67.61**	32.39
	XksuF1a	12.22***	**66.98**	33.02
	Xfba209b	11.33***	**66.66**	33.34
2B	*Xpsr135a*	7.53**	**63.46**	36.54
	Xfbb185	8.49**	**64.15**	35.85
	Xbcd1434a	5.03*	**60.95**	39.05
	Xfbb75b	6.62*	**62.74**	37.26
	XksuF11b	5.95*	**61.9**	38.1
	Xfbb353	18.30***	**71.28**	28.72
	Xfba61b	19.66***	**71.84**	28.16
	XksuD22	16.96***	**70.2**	29.8
	Xfba62b	16.32***	**69.9**	30.1
	Xfba64b	16.32***	**69.9**	30.1
	XksuE3c	16.00***	**69.52**	30.48
	Xfba345a	14.76***	**68.93**	31.07
	Xglk594b	12.70***	**67.64**	32.36
	Xfbb324a	11.66***	**66.66**	33.34
	Xfba209a	13.55***	**68.31**	31.69
	XksuF1b	9.66**	**66.1**	34.9
	XksuD23a	6.5*	**62.5**	37.5
	Xwg645a	8.16**	**64.07**	35.93
	Xfbb284b	8.00**	**63.8**	36.2
	Xfba209c	9.33**	**65.04**	34.96
	Xfba314a	8.49**	**64.15**	35.85

Table 2 Continued

Chromosome[a]	Locus	Chi-square value	Courtot	Chinese Spring
	XksuF11a	6.94**	**62.85**	37.15
	Xfbb75a	7.07**	**63.1**	36.89
	Xfbb226	8.65**	**64.42**	35.58
	Xfbb278a	6.62*	**62.74**	37.26
3DS	*Xglk538b*	5.95*	**61.9**	38.1
4AS	*Xfba78*	13.03***	**67.61**	32.39
	Xfba137	14.48***	**68.57**	31.43
	Xfba40	16***	**70**	30
	Xfbb248	13.88***	**68.26**	31.74
	Xfbb120	13.03***	**67.61**	32.39
4BL	*Xcdo1312a*	28.80***	**76.2**	23.8
	Xwg282	8.65**	**64.42**	35.58
	Xfbb178	4.56*	**60.37**	39.63
6B	*XksuG8a*	10.37**	34.28	**65.72**
	Xpsr167	10.37**	34.28	**65.72**
	Xpsr8(Cxp3)	22.15***	26.92	**73.08**
	XksuD17	89.60***	3.8	**96.2**
	Xfbb156b	77.88***	7.7	**92.3**
	Xfbb61	87.62***	3.89	**96.11**
	Xfba328	83.35***	5.66	**94.34**
	Xpsr2	48.00***	16.2	**83.8**
	XksuG12a	29.58***	23.58	**76.42**
	Xcdo836c	12.22***	33.01	**66.99**
	Xcdo836a	9.84**	34.61	**65.39**
7A	*Xpsr165a*	4.2*	**60**	40

* = 0.05, ** = 0.01, *** > 0.001

[a] The unlinked markers have not been localized and they do not figure in this table

[b] This marker is mapped on a distal position on 1AL, whereas other 1A-specific skewed markers are mapped on the short arm

Construction of the map

Out of the 293 loci noted, 264 belonged to 26 linkage groups (Fig. 1). All the groups were assigned to individual chromosomes by nulli-tetrasomic or monosomic reciprocal families analysis. Of the 72 dominant markers, 43 displayed a band present in 'Courtot' and absent in 'Chinese Spring'. Monosomic reciprocal families were particularly suitable for assignment of this type of marker. An example is illustrated in Fig. 2 for probe FBA209 (*Xfba209c* being a 'Courtot'-specific dominant marker). Rearrangements of chromosome arms between 4AL, 5AL and 7BS were decribed for the first time by Naranjo et al. (1987) on the basis of a lack of homoeologous pairing of *Ph1*- and *Ph2*-deficient wheat x rye hybrids. Devos et al. (1995b) and Nelson et al. (1995a) gave a more precise picture of the rearranged chromosomes by means of RFLP markers, the former presenting a general scheme for the evolutionary sequence of the rerrangements. In the present work, assignment information from probes KSUC2, CDO1312 (5A, 4B, 4D) and PSR103, PSR160 (4A, 7A, 7D) allowed the detection of 2 linkage groups involved in this rearrangement (Fig. 1). Additional markers would be necessary to complete this information. Eighteen out of 21 chromosomes were defined. No markers could be assigned to chromosome 2D. Chromosomes 4D and 5D were only represented by 1 marker each and are omitted from Fig. 1.

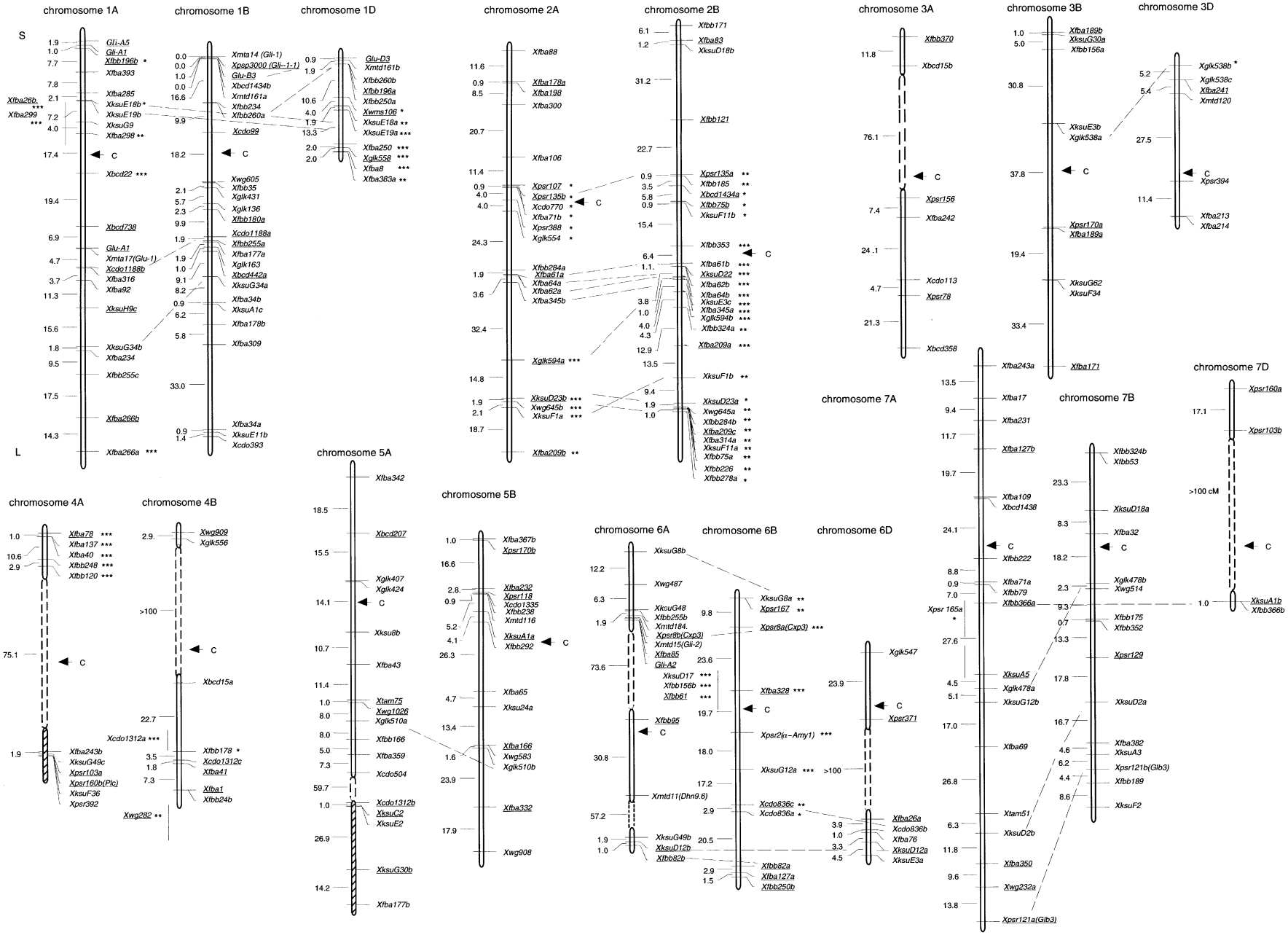
Distribution of markers

The present map covered 1772 cM with 266 markers (Table 3), which represented two-thirds and one-half of the lengths of the 'Chinese Spring × Synthetic' (Gale et al. 1995, 2575 cM) and the SICOP (Nelson et al. 1995 a, b, c; Van Deynze et al. 1995; Marino et al. 1996, 3551 cM) maps, respectively. There was a considerable imbalance between the A and B genomes, and the D genome. Similar percentages of markers (42.8% and 45.1%) on the A and B genomes covered a similar length of the map (46% for both genomes). The D genome was underrepresented with only 32 markers (12%) covering 8% (140.8 cM) of the map (Table 3). The longest map was obtained for chromosome 7A (217.6 cM) with 20 markers regularly spaced on the chromosome, whereas homoeologous group 4 chromosomes were constituted by only a few markers densely distributed.

Mapping of distorted loci

Homoeologous groups 1 and 2 and chromosome 6B were those principally affected by bias; 71 markers (26%) covering about 278 cM (15.7% of the map) showed deviation (Table 3). The maximum likelihood function is irrelevant for distorted loci mapping because of the risk of error in declaring two independent markers as linked, and vice versa (Lorieux 1993). As an alternative, contingency G tests were calculated for each pair of distorted loci. Nine linkage groups were established, whereas 10 markers remained unlinked. The mapping of chromosomes 1A, 1DS, 2A, 2B, 3DS, 4AS, 4BL, 6B was completed by the contribution of

Fig. 1 Molecular marker map constructed for 18 chromosomes from 106 DH lines from the CTCS cross. Recombination fractions are provided on the left of the chromosomes. *Dotted* chromosomal segments represent a recombination fraction >0.35. *Underlined* markers were assigned to specific chromosomes by nulli-tetrasomic and reciprocal monosomic lines. *Cross-hatched* regions represent chromosomal fragments involved in ancestral rearrangement between the 4AL, 5AL and the 7BS. The symbols *, **, ***, denote the loci which deviate significantly from the expected ratio 1:1 at the 5%, 1% and 0.1% levels, respectively. *Vertical bars* on the *left* of chromosomes 1A, 4B, 6B and 7A indicate skewed markers which could not be arranged on the chromosome with a sufficient degree of certainty. *Dashed lines* between chromosomes link homoeologous markers. The position of the centromere (*c*) is denoted by an *arrow*


chromosome 1A
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chromosome 3A
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chromosome 5A
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chromosome 6B
chromosome 6D
chromosome 7A
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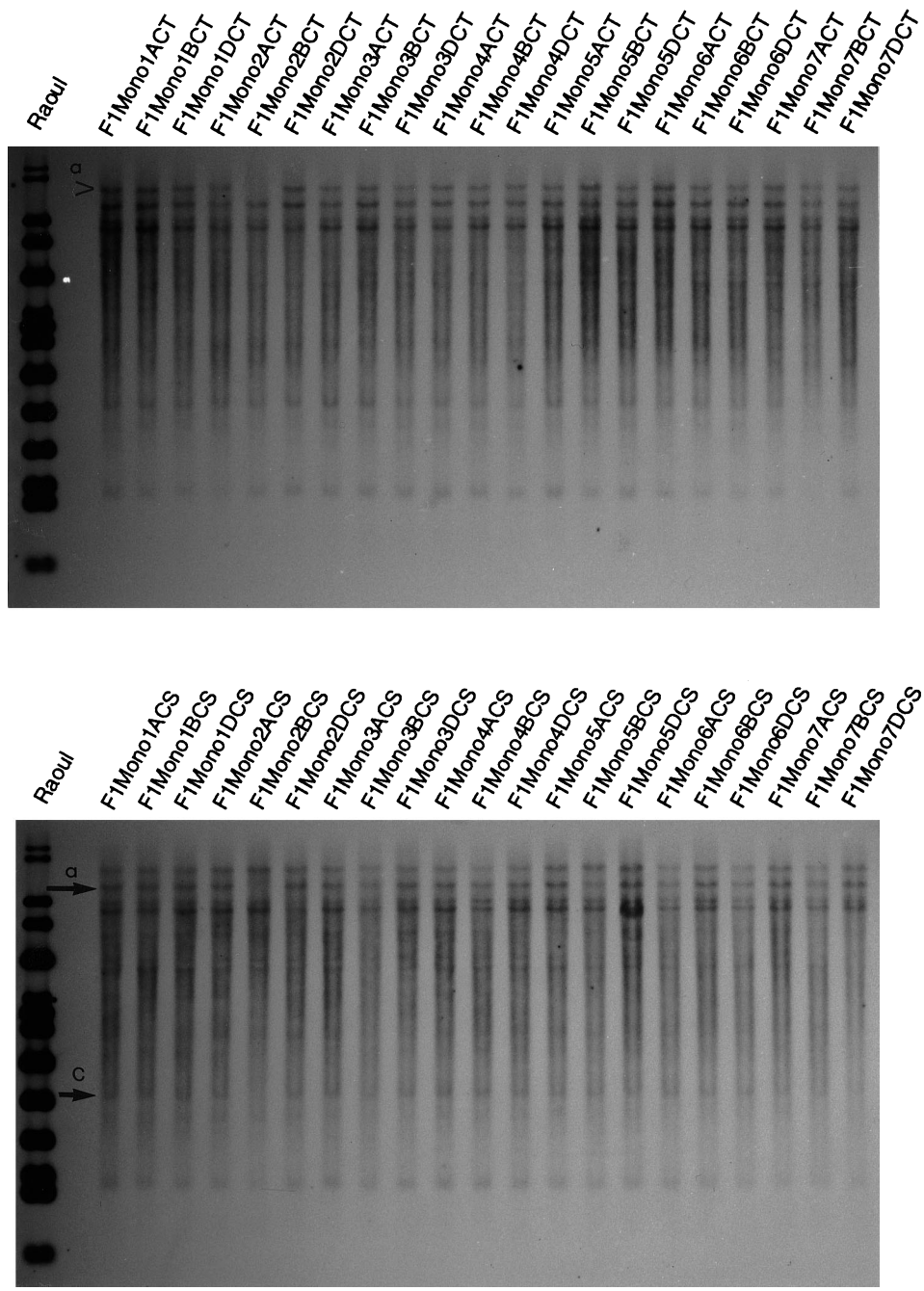


Fig. 2 Hybridization of probe FBA209 to *Bgl*II-restricted genomic DNA from F_1 reciprocal monosomic lines. Each reciprocal monosomic line is noted "F_1mono" followed by the name of the parental chromosome present in the heterozygous structure. *Lane 1* Raoul marker (Appligene), *lanes 3–23* monosomic reciprocal hybrids. → denotes 'Courtot'-specific allelic bands for *Xfba209a* (codominant) and for *Xfba209c* (dominant). > denotes a 'Chinese Spring'-specific allelic band for *Xfba209a* (codominant). Consequently, all these bands are assigned to chromosome 2B

distorted markers. The product formula was irrelevant for *Xfba26b*, *Xfba299* on chromosome 1A, *Xcdo1312a*, *Xwg282* on chromosome 4BL, *XksuD17*, *Xfbb156b*, *Xfbb61* on chromosome 6B and *Xpsr165a* on chromosome 7A (Fig. 1), and the estimation of r_b did not allow a unique order to be found. The position of these loci on the map was defined by a probability region (Fig. 1).

Except for *Xfbb196b* and *XksuE18b* on chromosome 1A, *Xglk538b* on 3D, and *Xpsr165a* on 7A, distorted markers were not randomly distributed on the genome. Tables 2 and 3 show 5 regions with a majority of 'Courtot' alleles, ranging from 3 markers on 4BL to 25 markers on 2B covering 85.8 cM. Two regions deviated significantly in favour of 'Chinese Spring' alleles: one on 1AS, and a second, covering at least 91.2 cM and spanning 11 markers, on 6B.

Comparison with an "interspecific-type cross"

An analysis was made for 155 markers in the CTCS map that showed counterparts in the SICOP map. The

Table 3 Distribution of mapped markers among the seven homoeologous groups and the three genomes for all markers (A) and distorted markers (B). The numbers in brackets represent the sum of distances between markers constituting a chromosome, expressed in centiMorgans

Genome	Homoeologous group							
	1	2	3	4	5	6	7	Total
A. A	24 (153.8)	21 (161.7)	7 (69.3)	11 (16.4)	17 (141.9)	14 (54.1)	20 (217.6)	114 (814.8)
B	26 (136)	29 (147)	10 (127.4)	10 (38.2)	16 (118.4)	14 (116.1)	15 (133.7)	120 (816.8)
D	12 (36.6)	0	7 (49.5)	1 (0)	1 (0)	7 (36.6)	4 (18.1)	32 (140.8)
Total	62 (326.4)	50 (308.7)	24 (246.2)	22 (54.6)	34 (260.8)	35 (206.8)	39 (369.4)	266 (1772.4)
B. A	7 (17.4)	11 (46.1)	0	5 (14.5)	0	0	1 (0)	24 (78)
B	0	25 (85.8)	0	3 (0)	0	11 (91.2)	0	39 (177)
D	7 (23.2)	0	1 (0)	0	0	0	0	8 (23.2)
Total	14 (40.6)	36 (131.9)	1 (0)	8 (14.5)	0	11 (91.2)	1 (0)	71 (278.2)

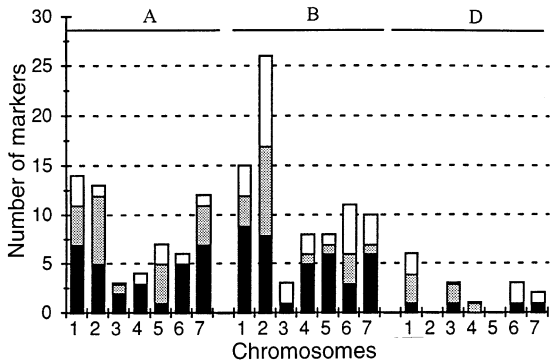


Fig. 3 Comparison of CTCS markers with the corresponding SICOP markers over the whole genome. The *x-axis* represents the chromosomes partitioned into the three genomes A, B and D, and the *y-axis* represents the number of markers compared between the two populations. *Black*, *grey* and *white* bars indicate homologous, homoeologous and heterologous markers, respectively, common to the two maps

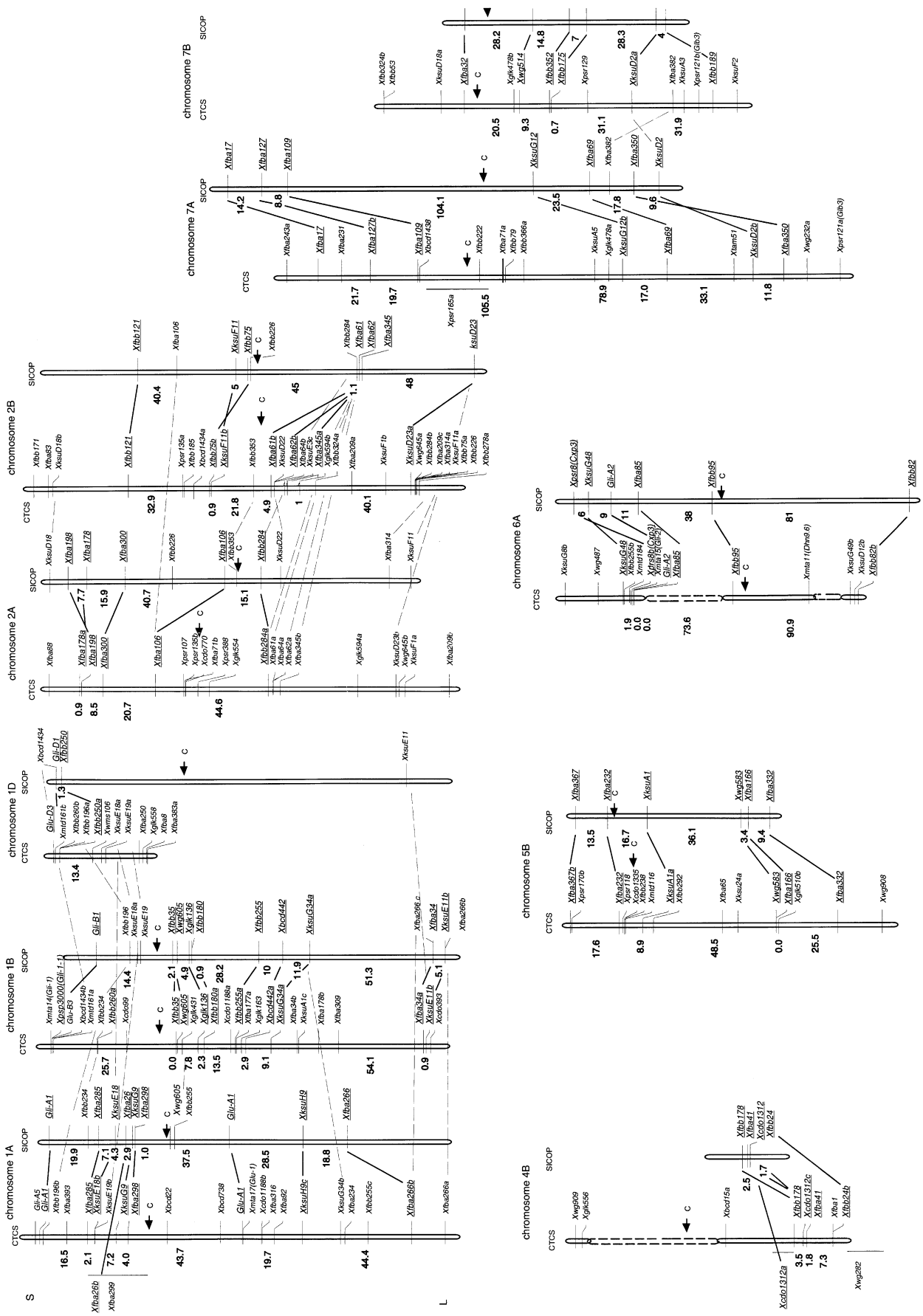
results are illustrated in Fig. 3. Seventy-two loci (46.4%) were homologous, 43 loci (27.8%) were homoeologous and 40 loci (25.8%) were heterologous. Conservation of homologous and homoeologous regions (representing 75% of the compared markers) was preponderant for almost all chromosomes, but 25% of the markers revealed loci scattered on chromosomes of different homoeologous groups. Markers could be defined from probes according to their origin (cDNA/gDNA) or their patterns (single copy/multicopy). cDNA clones represented 10% of the homologous markers, 16% of the homoeologous markers and 5% of the heterologous markers. In the same way, single-copy clones produced 37%, 25% and 15% of the homologous, homoeologous and heterologous markers, respectively. These data showed that heterologous markers were mainly derived from genomic clones possessing a complex spectrum of bands.

The comparison of homoeologous group 1 and chromosomes 2A, 2B, 4B, 5B, 6A, 7A, 7B is reported in Fig. 4. The most important feature was the colinearity of markers. For all the above-mentioned chromosomes, a regression model describing the relationship between SICOP and CTCS intervals indicated that the model explained 65% of the variability. Therefore, the correlation coefficient ($r = 0.80$) indicated a moderately strong relationship between the intervals of the two maps. There were discrepancies in some chromosome regions: on chromosome 6A, the segment delimited by *XksuG48-Xfba85* represented only 1.9 cM in the CTCS map and 26 cM in the SICOP map. On the other hand, on chromosome 7B, the segment encompassing *XksuD2a-Xfbb189* represented 31.9 cM in the CTCS map and 4 cM in the SICOP map (Fig. 4).

A special case was observed for probe FBB255. With the same probe/enzyme combination, different patterns were observed in both populations allowing 2 loci to be mapped at different sites on the same chromosome arm, 1AL. On chromosome 2B in the CTCS map, probes KSUF11 and FBB75 identified 2 loci: *XksuF11a*, *Xfbb75a* on 2BL and *XksuF11b*, *Xfbb75b* on 2BS. On the SICOP map, probe KSUF11 was mapped on 2BS but also on 2AL (Fig. 4). As pointed out by Nelson et al. (1995b), a duplication of loci seems evident. The same hypothesis could be postulated for distal markers *Xfbb226* and *Xfbb284b* on 2BL in the CTCS map, considering the position of their counterparts in the SICOP map and their multiband RFLP patterns. On chromosome arm 7AL, there is an inversion of *XksuD2* and *Xfba350* markers between SICOP and CTCS maps, which is confirmed by the comparison with the 7B homoeologues. Therefore, the occurrence of a limited inversion of these particular markers on the SICOP map seems conceivable (Fig. 4).

Discussion

Even with incomplete coverage for some chromosomes, the CTCS molecular map is the first intervarietal map


chromosome 1A
chromosome 1B
chromosome 1D
chromosome 2A
chromosome 2B
chromosome 4B
chromosome 5B
chromosome 6A
chromosome 7A
chromosome 7B
SICOP
CTCS
S
L
C

published for bread wheat that covers most of the genome. To date, owing to the low level of RFLP polymorphism in wheat, mapping strategies have concentrated on particular chromosomes by using recombinant inbred substitution lines (Chao et al. 1989; Chen et al. 1994) or wide crosses (Liu and Tsunewaki 1991; Gale et al. 1995; Nelson et al. 1995a, b, c, Van Deynze et al. 1995; Marino et al. 1996), which significantly increased the level of polymorphism. Intervarietal populations, such as those derived from 'Timgalen' $\times$'RL4137', led to the present mapping data concerning homoeologous groups 7 (Chao et al. 1989), 2 and 3 (Devos et al. 1992, 1993), and chromosome 4A (Liu et al. 1992). Our results (map covering 1772 cM with 266 loci) showed the difficulty in constructing a map with an intervarietal cross.

An advantage of using cvs 'Courtot' and 'Chinese Spring' was the simultaneous availability of DH lines and monosomic reciprocal F_1 hybrids. As illustrated in Fig. 2, these hybrids were used for assigning dominant-type markers where only a 'Courtot' allelic band was detectable. A high percentage (27%) of dominant markers was revealed in the CTCS population. This is in contrast with rice mapping results (McCouch et al. 1988) but conforms to *T. tauschii* mapping data, where 16% of the markers displayed null alleles (Gill et al. 1991). As suggested by these authors, a significant proportion of null alleles could be a consequence of insertion-deletion events. This type of DNA rearrangement could be seen from analysing several polymorphic probe/enzyme combinations. Polymorphism detected by a unique enzyme, which is indicative of a point mutation, was negligible in the CTCS population (data not shown).

The lack of polymorphism in the D-genome mapping is a striking feature. This has already been observed in wheat (Chao et al. 1989; Kam-Morgan et al. 1989; Liu and Tsunewaki 1991), but it became very clear when our D-genome map was compared to the results from interspecific-derived maps: this is most probably related to the intraspecific situation and is in agreement with the hypothesis of a recent and monophyletic introduction of the D genome in bread wheat (Lagudah et al. 1991b). Genomes A and B displayed a similar degree of coverage in the CTCS map as observed by Liu and Tsunewaki (1991) and by Nelson et al. (1995c). Chao et al. (1989) revealed twice as much variation in the B genomes than in the A and D genomes, suggesting that the B genome would be more mutable, or more variable. In any case, the lack of markers in the D genome seems to be a persistent problem. A higher degree of polymorphism in the D genome of wheat had been expected when using *T. tauschii* specific DNA libraries developed for *T. tauschii* mapping studies (Kam-Morgan et al. 1989; Lagudah et al. 1991a; Gill et al. 1991). In the present work, among 43 polymorphic clones originating from the *T. tauschii* library (KSU), only 5 defined markers mapped in the D genome (*XksuE18, XksuE19 on* 1DS, *XksuD12, XksuE3 on* 6DL, *XksuA1* on 7DL) (Fig. 1). Other approaches to isolate a specific DNA genomic library involve microdissection and microcloning of a chromosome via polymerase chain reaction (PCR) amplification, as reported for barley (Schondelmaier et al. 1993) or for wheat (Albani et al. 1993). However, a DNA fragment isolated from a single chromosome may not necessarily mark a locus on this particular chromosome, underlining the inefficiency of these approaches in producing genome-specific probes. The screening of recently developed markers (microsatellites, AFLP) should be performed to reveal polymorphism within the D genome. Microsatellites appear to be a valuable tool, as they are mostly genome specific and they show a higher intrinsic polymorphism than RFLP markers (Röder et al. 1995): for example, a microsatellite assigned to 1D chromosome by Plaschke et al. (1995) was incorporated in the CTCS map.

In the CTCS population, 81 markers deviated significantly from the 1 : 1 ratio. Most of them were located in specific chromosomal regions. When true linkage between 2 loci was detected by the G-test and the estimation of one out of two viability factors was close to one, Bailey's formula and the maximun likelihood method gave similar results (data not shown). However, a few remaining markers could not be precisely mapped using these methods. The development of new theoretical models which take into account particular bias situations appears necessary.

The clustering of skewed markers has been verified in the genetic mapping of androgenetic populations of barley (Graner et al. 1991; Heun et al. 1991) and maize (Bentolila et al. 1992; Murigneux et al. 1993), with the underlying hypothesis of the linkage of such markers to specific genes controlling in vitro androgenesis (embryogenesis, plantlet regeneration, albinism in cereals). 'Courtot' and 'Chinese Spring' were both characterized by a low responsiveness to androgenesis (unpublished results). It was thus not surprising to find deviations favouring one or other of the parent. Eighty genotyped DH lines were evaluated for androgenetic response, and quantitative trait loci (QTL) detection is being performed.

Fig. 4 Comparison between the CTCS and SICOP maps for common markers on homoeologous group 1 chromosomes and the 2A, 2B, 4B, 5B, 6A, 7A and 7B chromosomes. Markers *underlined* and linked by *bold-type* lines indicate homologous markers in common to the two maps. *Dashed lines* between chromosomes link homoeologous markers common to the two maps. The numbers on the *left* of the chromosomes indicate distances between homologous markers. *Dashed* chromosomal segments represent a recombination fraction > 0.35. *Vertical bars* to the *left* of chromosomes 1A, 4B and 7A in the CTCS map indicate skewed markers which could not be placed on the chromosome with a sufficient degree of certainty. The position of the centromere (*c*) is denoted by an *arrow*

It may be noted that segregation distortion has also appeared in F_2 progenies, as shown in mapping studies in wheat (Liu and Tsunewaki 1991), rice (McCouch et al. 1988), lettuce (Landry et al. 1987) and tomato (Helentjaris et al. 1986). Further, comparisons between DH lines and F_2 progenies in barley (Thompson et al. 1991, Graner et al. 1991), rice (Guiderdoni 1991), and between DH lines and RI progenies in maize (Murigneux et al. 1993) showed deviations either common to both reproductive systems or specific to one of them. So, in DH populations, a distortion segregation is not necessarily attributable to in vitro gametic selection.

In our map , the excess of 'Chinese Spring' alleles on chromosome 6B represents the most important bias. In mapping F_1-derived populations developed either by androgenesis or by the *Hordeum bulbosum* method, Wang et al. (1995) found a gametic selection in androgenetic lines for the 6B centromeric region. However, this chromosome has never been found to be significantly involved in androgenetic ability (Martinez 1994). A hypothesis of a considerable abortion of microspores due to the *Ki* pollen killer gene (Loegering and Sears 1963) was postulated by Liu and Tsunewaki (1991) for the distorted locus *Xglk705* on chromosome 6B. The same hypothesis can be put forward concerning our population, assuming that 'Courtot' carries the *ki* allele.

Comparison between the CTCS and SICOP maps has allowed the global colinearity of both maps to be confirmed. No significant chromosomal rearrangement was detected on the CTCS map, which is in agreement with the absence of trivalent or tetravalent associations in the F_1 of 'Courtot' × 'Chinese Spring' (Denis et al. 1982). This observation is important as events such as inversions or translocations would complicate the mapping of regions involved by inhibiting recombination. Distance discrepancies were shared in approximately equal numbers between the two maps. As the populations compared in our study did not originate from the same parents, several hypotheses could explain localized discrepancy in recombination (difference in sex, number of meioses, environmental factors when developing the populations).

The comparison based upon enzyme/probe combination and localization of loci (Fig. 3) showed that the two maps had only 50% of the homologous markers in common. The use of information obtained from a reference map for another population is hampered by two major points: the polyploid condition of wheat, which yields at least 3 loci for each pattern, and the lack of polymorphism. Thus, the probability of finding the same marker with the same probe/enzyme combination in two crosses may be small. If a different enzyme is used, only minimal information may be obtained from the reference map because the newly revealed locus will most likely be different from the previous one and can be found on a different chromosome, either homoeologous or heterologous. Knowledge of allelic bands through genotype collection analysis would make the establishment of useful relationships easier.

Additional efforts will be necessary to obtain a better coverage of homoeologous groups 3 and 4 as well as of the D-genome chromosomes using RFLP or other types of markers (RAPD, microsatellites, AFLP . . .). However, this work should allow the detection of quantitative trait loci, as the CTCS segregation displays polymorphism for many biological and economically important characters.

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