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RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye

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Summary. Genetic maps of chromosomes 3A, 3B and 3D of wheat and 3R of rye were developed using 22 DNA probes and two isozyme marker systems. Analysis of the 49 loci mapped showed extreme clustering around the centromere in all four maps, with large 'gaps' in the distal chromosome regions, which is interpreted as being due to strong localisation of recombination towards the ends of the wheat and rye chromosomes. In the centromeric regions gene orders are highly conserved between the three wheat genomes and the rye genome. However, the unpredictable behaviour of the DNA clones that map in distal chromosome locations may indicate that the genomes are diverging most rapidly in the regions of higher recombination. A comparison of cDNA and genomic probes showed the latter to be much more efficient for revealing RFLP. Some classes of gDNA clones, i.e. chromosome-specific sequences and those hybridizing in a non-homoeologous manner, were seen to be most polymorphic. Correlations between map locations and RFLP levels showed no clear relationship. In addition to anonymous DNA clones, the locations of known function clones, sedoheptulose-1,7-bisphosphatase (*XSbp*), carboxypeptidase I (*XCxp1*) and a bZIP protein (*XEmbp*), were ascertained along with those for two isozyme loci, *Mal-1* and *Est-5*.

Key words: Wheat-Rye-RFLP – Genetic maps – Isozymes

Introduction

Several morphological characters of bread wheat, *Triticum aestivum* (2n = 6x = 42), have been shown to be controlled by genes located on the homoeologous group 3 chromosomes. One of the most important traits, red grain colour, is controlled by genes on the long arms of chromosomes 3A, 3B and 3D (Sears 1944, Metzger and Silbaugh 1970). These *R* genes are particularly important because of their association with dormancy, and thus pre-harvest sprouting, which is the most serious constraint to consistent grain quality in northern Europe and other temperate wheat-growing areas. Other important genes located on 3D include *s1*, which defines the *sphaerococcum* spike type characterising 'shot' wheat, on the long arm (Sears 1947), and *ph2*, the second most potent chromosome pairing control gene, on the short arm (Sears 1982). Other significant genes listed in the Catalogue of Gene Symbols (McIntosh 1988 and subsequent supplements) include a range of genes controlling stem rust, *Puccinia graminis* Pers., leaf rust, *Puccinia recondita* Rob. ex Desm., and powdery mildew, *Erysiphe graminis* DC. In particular, *Sr24* from *Agropyron elongatum* and transferred to 3D in the cv 'Agent,' has been used effectively in Australian wheats.

Several isozyme loci are also located on the homoeologous group 3 chromosomes. *Est-5* (Ainsworth et al. 1984) and *Per-3* (Liu et al. 1990) are highly polymorphic and are hence of potential value in intervarietal comparisons, while the *Est-1, Est-2, Got-3, Hk-2, Pde-1, Tpi-1, Mal-1, Ndh-3,* and *Ndh-4* isozyme loci have potential for use in inter-genomic chromosome manipulation.

The development of extensive genetic maps in wheat has been hampered by the large genome size, the large number of linkage groups, and the relatively low levels of variation shown in restriction fragment length polymorphism (RFLP) analysis (Chao et al. 1989). Previous genetic mapping of the homoeologous group 3 has been limited to chromosome 3D, incorporating only three markers (from the short arm to the long arm): *Lr32*

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(Kerber 1988) – centromere – *Got-D3* (Hart et al. 1976) – *Ch2* (Koba and Tsunewaki 1978).

The red grain colour genes, *R*, and the *ph2* locus are among our primary targets for manipulation by marker 'tags,' and thus mapping was undertaken to develop a RFLP skeleton for further analysis of these and other traits. In this paper we describe the mapping of 22 DNA probes and two isozyme markers on group 3 chromosomes. Homoeologous loci were, where possible, also mapped in rye (*Secale cereale*, $2n = 2x = 14$) chromosome 3R to aid the interpretation of the wheat data and also to investigate the degree of map correspondence between the wheat and rye homoeologues.

Materials and methods

Genetic stocks

The nullisomic-tetrasomic (NT) and ditelosomic (DT) lines of the wheat cv 'Chinese Spring' (CS) (Sears 1954) were used to assign chromosome and arm locations to loci identified by DNA probes, as previously described by Sharp et al. (1989). For some probes, CS/*S. cereale* cv 'Imperial' (Driscoll and Sears 1971) and CS/*Hordeum vulgare* cv 'Betzes' (Islam et al. 1981) single chromosome addition lines were used to confirm chromosome locations in rye and barley.

All DNA probes were tested on a range of wheat genotypes to assess their efficiency for revealing RFLP. This panel included 'Chinese Spring,' 'Synthetic' (IPSR1190903, McFadden and Sears 1946; Sears 1976), 'Hope,' 'Cappelle-Desprez', 'Sportsman', 'Highbury', 'Sicco', 'Timgalen,' 'RL4137,' 'VPM1,' 'Condor,' 'Pavon,' and 'Frontana.' For some probes (pλc.3, pGC19, PSR78, PSR598, PSR902, PSR903, PSR904, PSR907) the latter three genotypes were replaced with 'Bezostaya I,' 'Mara,' 'Hobbit'S',' 'Inia 66,' and the line SQ1, a breeding line selected from the cross 'TW269/9' × 'Highbury,' and provided by S.A. Quarrie, Cambridge Laboratory.

For the cross-hybridization experiments of the wheat DNA probes with barley genomic DNA, two *H. vulgare* cultivars, 'Betzes' and 'Golden Promise', and one *H. spontaneum* line (IPSR #2370) were employed. Cross-hybridization with rye was tested using two inbred lines, Ds2 and RxL10 (Masojc and Gale 1991).

The clones were mapped in F_2 populations, comprising 120 individuals, using leaves from either F_2 plants, or from 12 F_3 progeny derived from each F_2 plant bulked together. The wheat populations were derived from the crosses CS × 'Synthetic' and 'Timgalen' × RL4137', and the rye population was from a cross between Ds2 × RxL10.

Markers

Anonymous DNA probes. Seven cDNAs, from the library described by Chao et al. (1989) with PSR numbers in the range 51–200, and six gDNA clones, from a leaf DNA *Pst*I genomic library with PSR numbers in the range 300–460, constructed by R.L. Harcourt, Cambridge Laboratory, which had previously been shown to have homologous sequences on homoeologous group 3 chromosomes, were further investigated.

These probes were supplemented with five gDNA clones from a newly constructed wheat-germ PstI genomic library. For this, approximately 40 µg of wheat germ DNA from a mixture of varieties was digested until completion with 160 U *Pst*I (BRL). The fragments were separated on a 1% low-melting-point agarose gel, and DNA sequences in the size range 500–3500 bp were isolated. The purified fragments were ligated into the *Pst*I site of PUC8 and used to transform the *E. coli* bacterial strain ED8800. Individual colonies were grown and checked for the presence of inserts. Highly repeated sequences were discarded after hybridization with total wheat genomic DNA. The low-copy-number clones investigated have PSR numbers in the range 540–699. Further libraries similarly constructed provided clones with PSR numbers in the range 900–999.

Known function probes. Three DNA probes with known function, listed with their sources in Table 1, were also mapped.

Isozyme loci

The grain esterase loci, *Est-5*, were analysed using the method described by Ainsworth et al. (1984). Malic enzyme, *Mal-1*, was analysed using the method described by Liu and Gale (1988).

RFLP procedures

All techniques of DNA extraction, restriction enzyme digestion, gel electrophoresis, Southern transfer, probe labelling, and filter hybridization were as described by Sharp et al. (1988) with the following modifications. After gel electrophoresis the DNA was transferred to Hybond N^+ nylon membranes (Amersham). The stringency of the post-hybridization washes was also increased. Two low stringency washes in 2 × SSC/1%SDS for 15 min were followed by two 15-min high stringency washes in 0.2 × SSC/1% SDS. After exposure, the size of the hybridizing bands was calculated on a digitising tablet by reference to λ*Pst*I and λ*Hin*dIII marker lanes.

Linkage analysis

Linkage analysis was performed using MAPMAKER 2.0, supplied by E. S. Lander, Whitehead Institute for Biomedical Research, Cambridge, USA. The method used was to establish linkage groups using LOD > 2, followed by establishment of the map order from the three-point data and accepting only orders preferred over all others with LOD > 2.5. Other points were placed in the preferred locations using the multipoint analysis. Where the preferred location was established with a LOD < 2.5, this is indicated on the map. The Kosambi function was used for conversion of recombination values to map distances.

Results

Classes of group 3 probes

The anonymous gDNA and cDNA clones and three known function clones, all previously shown to hybridize with sequences located on the group 3 chromosomes, were further analysed by nullisomic-tetrasomic and ditelosomic analyses and tested for cross-hybridization to barley and rye genomic DNA. Several classes of hybridization patterns were observed: Probes that hybridize strongly to wheat, barley, and rye and

a) hybridize within a wheat homoeologous group: pλc.3, PSR56, PSR74, PSR78, PSR116, PSR125, PSR156, PSR170 (cDNAs) and PSR305, PSR347, PSR354, PSR394, PSR543, PSR570, PSR578, PSR598, PSR902 (gDNAs) (Fig. 1 A)
b) hybridize within a homoeologous group but have additional odd copies on chromosome(s) of other groups: pGC19, pSBP (cDNAs) (Fig. 1 B);

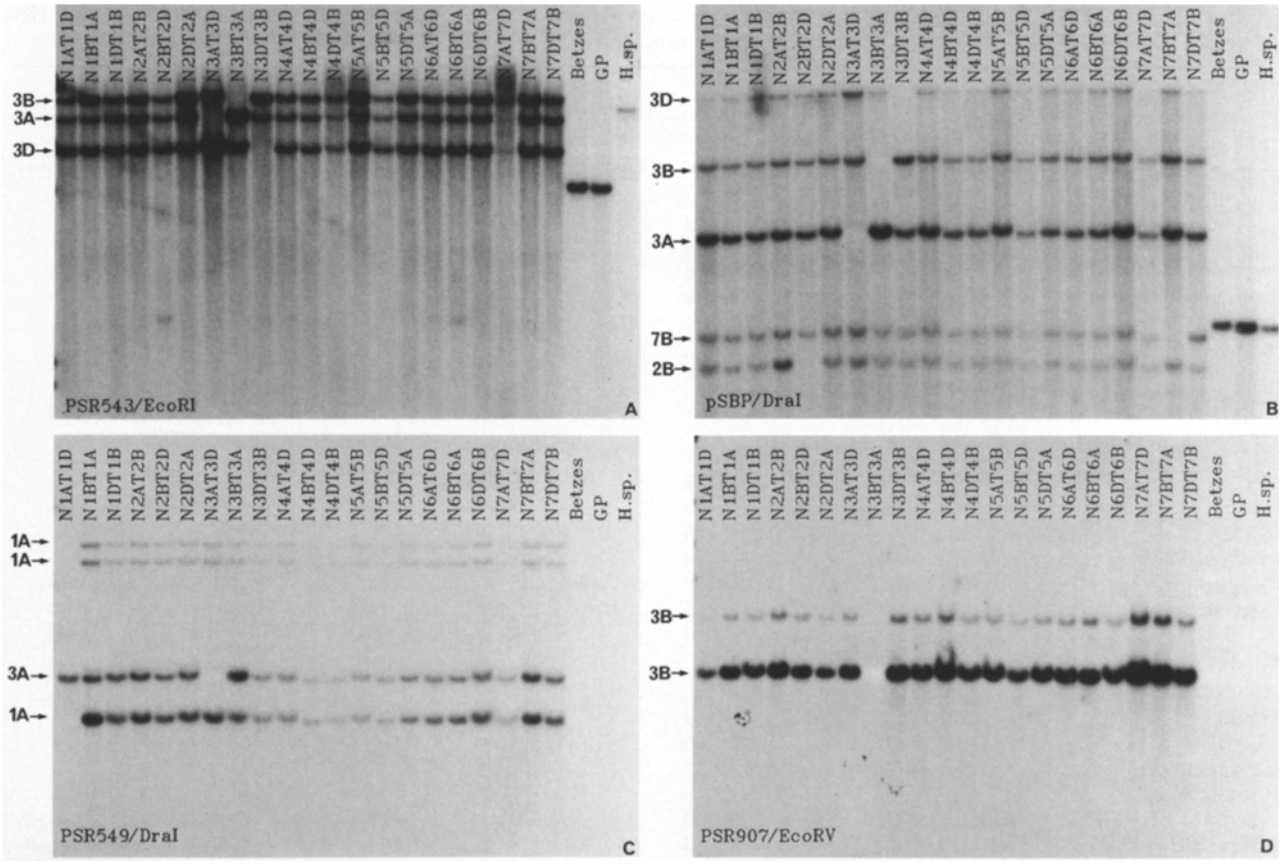

Fig. 1 A–D. Hybridization patterns obtained with different classes of probes with 'Chinese Spring' nullisomic-tetrasomic lines and three barley genotypes. 'Betzes', 'Golden Promise' (*GP*) and an accession of *H. spontaneum* (*H. sp.*). **A** Class *a* probe, located on 3A, 3B, and 3D; **B** class *b* probe, located on 3A, 3B, 3D, 2B, and 7B; **C** class *e* probe with copies on 1A and 3A; **D** class *f*, or chromosome-specific probe, with many copies located on 3B

Table 1. Known function clones

Locus	Clone	Function	Source	Reference
XCxp1	pλc.3	Carboxypeptidase-1	G. Fincher	Doan and Fincher (1988)
XEmbp	pGC19	bZIP protein	R. Quatrano	Guiltinan et al. (1990)
XSbp	pSBP	Sedoheptulose-1,7-bisphosphatase	T. Dyer	Personal communication

Probes that hybridize weakly or not at all to barley and rye, and

c) hybridize within a homoeologous group: PSR383 (gDNA)
d) hybridize within a homoeologous group with additional odd copies on chromosome(s) of other groups: PSR903 (gDNA)
e) hybridize only to apparently non-homoeologous loci: PSR904, PSR549 (gDNAs) (Fig. 1 C)
f) hybridize to only one wheat chromosome: PSR454, PSR907 (gDNAs) (Fig. 1 D).

An overview of the chromosomal locations of the group 3 probes, and their copy numbers in the A, B, D, H, and R genomes is given in Table 2.

Variability at the RFLP loci

Polymorphism in wheat. The 25 probes were hybridized against panels of wheat varieties, including the parents of the two mapping populations, digested with *Eco*RI, *Eco*RV, *Dra*I, and *Hin*dIII. The degree of polymorphism, expressed as percentage potential heterozygosity (H), is

Table 2. Chromosomal location and potential heterozygosity values for wheat, copy numbers in wheat (W), barley (B), and rye (R), and relative hybridization in barley and rye for 25 group 3 probes

Probe	Chromosomal location	H (%)[b]			Copy number[c]			Signal strength[d]	
		A	B	D	W	B	R	B	R
cDNA									
pλc.3[a]	3AL 3BL 3DL	29	4	4	1	1	1	+++	+++
pSBP[a]	3AL 3BL 3DL	0	0	11	1				
	2BS	–	19	–	1	1	1	+++	+++
	7BL	–	21	–	1				
pGC19[a]	3BL	–	22	–	1				
	5AL 5BL 5DL	0	46	0	1	1	4	+++	+++
	6AL 6BS	0	70	–	1				
	7DL	–	–	25	1				
PSR56	3AL 3BL 3DL	24	0	0	1	4	4	+++	++
	7AL 7BL 7DL	0	32	18	1				
PSR74	3AL 3BL 3DL	0	9	14	1	1	1	+++	+++
PSR78	3AL 3BL 3DL	7	25	0	1	1	1	+++	+++
PSR116	3AL 3BL 3DL	0	0	4	1	1	1	+++	+++
PSR125	3AL 3BL 3DL	0	14	0	1	1	1	+++	+++
PSR156	3AL 3BL 3DL	28	9	7	1	1	1	+++	+++
PSR170	3AL 3BL 3DL	25	32	14	2	2	2	+++	+++
	5AS 5B	46	63	–	1				
gDNA									
PSR305	3AS 3BS 3DS	26	27	32	1	~10	1	++	+++
PSR347	3AL 3BL 3DL	7	0	0	1	1	1	+++	+++
PSR354	3AL 3BL 3DL	4	11	11	1	1	1	++	++
PSR383	3AS 3BS 3DS	0	0	0	1	–	–	–	–
PSR394	3AL 3BL 3DL	0	0	28	1	1	1	+++	+++
PSR543	3AL 3BL 3DL	37	4	7	1	1	1	+++	+++
PSR570	3AL 3BL 3DL	31	54	14	1	1	1	++	++
PSR578	3AL 3BL 3DL	27	22	27	1	1	1	+++	++
PSR598	3AS 3BS 3DS	37	5	17	1	1	1	++	++
PSR902	3AS 3BS 3DS	0	17	0	2	1	1	+++	+++
PSR903	3AS 3BS 3DS	0	42	13	1				
	2DS	–	–	18	1	–	–	+	+
	5DS	–	–	4	1				
PSR904	3AL 3DL	26	–	13	1	–	–	+	+
	6AS	10	–	1					
PSR549	3AL	35	–	–	1	–	4	–	++
	1AL	28	–	–	4				
PSR454	3BL	–	72	–	~30	–	–	–	+
PSR907	3BS	–	58	–	~30	–	–	–	–

[a] See Table 1
[b] H, Potential heterozygosity
[c] The copy number is estimated as the minimum number of hybridizing bands, over four restriction digests, observed per genome
[d] The relative strength of the hybridization signal in comparison to wheat: +++, strong signal; ++, relative strong signal; +, weak signal; –, no hybridization

calculated as $H = 1 - \sum p_i^2$, where p_i is the frequency of each allele i in the population (Nei 1973). Mean values of H over the four restriction enzymes at each wheat locus in the A, B, and D genomes are shown in Table 2. A comparison of the RFLP levels in the two mapping populations, CS × 'Synthetic' and 'Timgalen' × 'RL4137,' reveals that the variability is less in the latter cross. Of the ten cDNA probes, nine show at least one RFLP in CS × 'Synthetic,' while only three detect polymorphism in 'Timgalen' × 'RL4137'. A similar result was obtained with the gDNA clones, where, of 15 probes, 13 could be mapped on at least one genome in the CS × 'Synthetic' cross, while only seven could be mapped in 'Timgalen' × 'RL4137'. RFLP, where present, was usually detected with more than one restriction enzyme. Analysis of the RFLP levels among the variety panels showed *Eco*RI to be the most (H = 22%) and *Eco*RV the least effective (H = 15%) in revealing polymorphism. Overall the D genome loci were less polymorphic (12%) than those in the A (18%) and B (22%) genomes.

Polymorphism in rye. The probes were also tested for RFLP between the rye inbred lines Ds2 and RxL10 with three enzymes, *Eco*RI, *Eco*RV and *Hin*dIII. All clones except a single cDNA clone (PSR125) and four gDNA clones (PSR347, PSR354, PSR394 and PSR578) showed RFLP.

Intrachromosomal mapping

Wheat, CS × Synthetic. The best fit maps for 3A, 3B, and 3D are shown in Fig. 2. Nine single copy DNA sequences were mapped on chromosome 3A in a single linkage block spanning the centromere. Twelve loci were placed on chromosome 3B in three linkage blocks. These included: a centromeric block of nine loci, including one of the two-sequence copies detected by PSR902, the multicopy chromosome-specific locus *Xpsr907,* and the most distal 3S locus, *Xpsr305,* detected by a class *a* probe, single copy in wheat and rye, but moderately repeated in barley (data not shown); the *XEmbp* locus on 3BL (Devos et al. 1991), which was not significantly linked with other 3BL markers; and a linkage block comprising the *Est-B5* locus and *Xpsr454*, detected by the chromosome-specific clone PSR454. Fourteen loci were mapped on 3D: 13 of these comprised a large group spanning the centromere, including one of two copies detected by PSR170. *Est-D5* was unlinked to the centromeric block. All but the loci identified with '*' in Fig. 2 represent fixed map locations, preferred over all other possible maps by a LOD difference > 2.5. For the marked loci, the most likely position on the map is shown. No unequivocal order could be determined for *Xpsr156, Xpsr116*, and *Xpsr354* on 3DL. The orientation of the loci independent of the centromeric clusters on 3B and 3D could not be established conclusively. The two-point linkage data suggest, however, that the order of loci on 3B is, from the centromeric cluster, *XEmbp – Xpsr454 – Est-5*, as shown in Fig. 2. For all the wheat loci, the map locations are consistent with the arm locations obtained by ditelosomic analysis.

Wheat, Timgalen × RL4137. Four loci were mapped on chromosome 3A in two linkage blocks. The centromeric group comprised three loci (ordered from the short arm to the long arm), *Xpsr598* – 21.3 cM ± 3.0 – *Xpsr78* – 3.1 cM ± 1.1 – *Xpsr170*, which were unlinked with the 3AS locus, *Xpsr305*. The four loci mapped on chromosome 3B formed one linkage block entirely located on the long arm; the locus order is *Xpsr354* – 13.9 cM ± 2.4 – *Xpsr570 – 6.2 cM ± 1.6 – Xpsr578* – 4.0 cM ± 1.3 – *Xpsr74.*

Rye, Ds2 × RxL10. Thirteen RFLP markers were placed on the rye 3R map; *XCxp1, XSbp,* five gDNAs, and six cDNA sequences, with PSR170 detecting two loci separated by 0.5 cM (Fig. 2). Thus, together with the location of the isozyme locus, *Mal-1,* 15 loci were mapped on 3R.

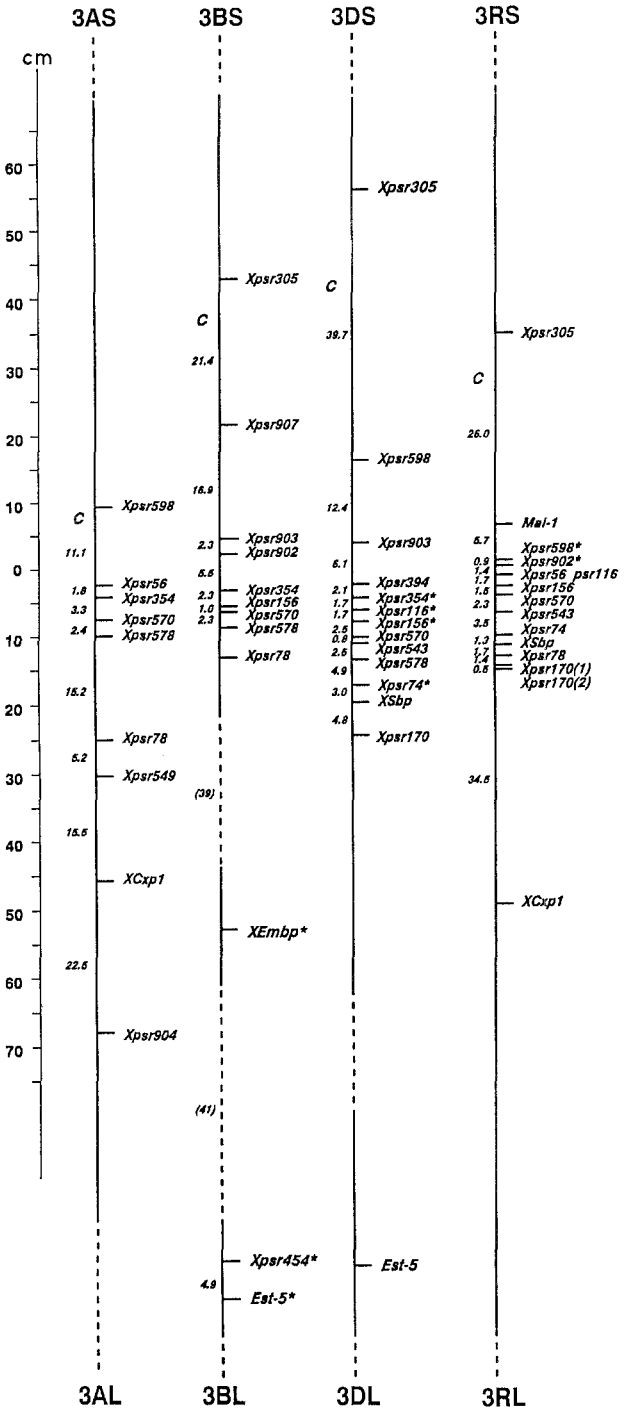

Fig. 2. Genetic maps of chromosomes 3A, 3B and 3D of wheat and 3R of rye. *Note: Broken lines* indicate a lack of evidence (LOD < 2.0) for linkage between adjacent loci. On 3BL, map distances suggested by the two-point data are shown in *parenthesis.* * Indicates map location preferred with LOD differences less than 2.5

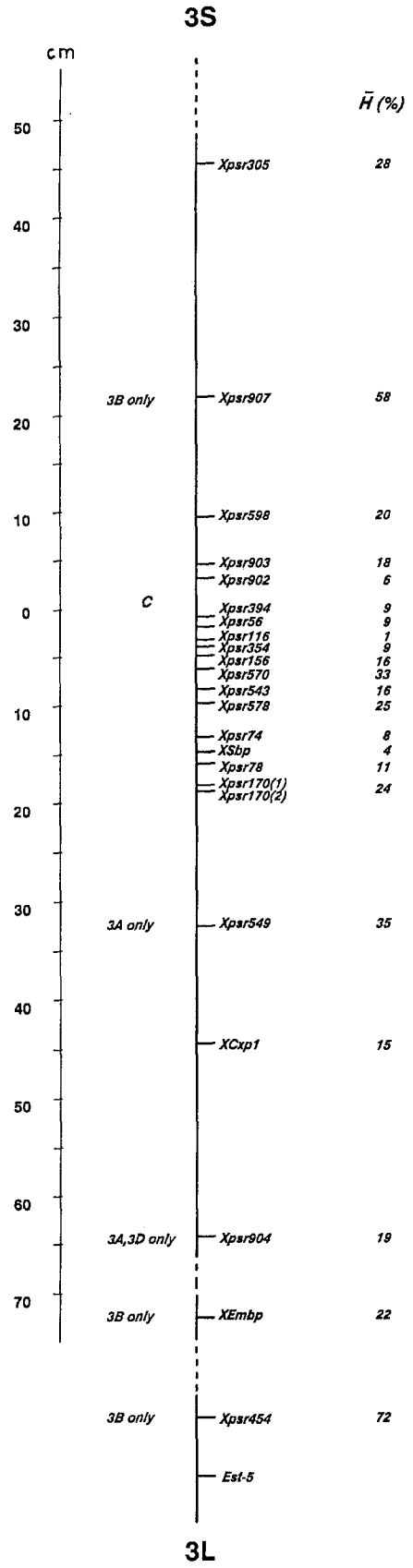

Fig. 3. Consensus homoeologous group 3 chromosome map. *Note:* H (%) values are means over the three wheat genomes

Definitive locations with LOD differences > 2.5 were obtained for most of the loci. The exceptions are *Xpsr598* and *Xpsr902*, where only a preferred (LOD differences < 1.5) map location is shown in Fig. 2. Analysis of the wheat ditelosomics and the CS/'Imperial'3RL and S ditelosomic addition lines had shown *Mal-R1* to be located on the long arm of the homoeologous group 3 chromosomes (Liu and Gale 1988). The short arm location obtained here was, however, preferred over any long arm location by a LOD difference > 5.

Homoeologous group 3 consensus map. The precise correspondence of the maps of 3A, 3B, 3D, and 3R justified the construction of the consensus map (Fig. 3). However, because of the unexplained inconsistency between the ditelosomic analyses and the map location, *Mal-1* has been omitted.

Potential heterozygosity and map location. Potential heterozygosity (H) levels are shown for individual loci in Table 2 and for sets of DNA loci in the consensus map shown in Fig. 3. Overall, the genomic clones ($\bar{H} = 19\%$) detected almost twice as much polymorphism as the cDNAs ($\bar{H} = 10\%$), while the highest levels of polymorphism were shown by the chromosome-specific clones PSR454 and PSR907. There was, however, a wide range of H values; for example while *Xpsr454-3B* showed an RFLP level of 72%, 26 loci (29% of the 89 loci investigated) displayed no RFLP at all among the varieties tested. In addition, no correlation between RFLP levels observed and map location is apparent (Fig. 3).

Discussion

The homoeologous group 3 genetic maps of wheat and rye

The conserved co-linearity over the A, B, and D genomes of wheat and the R genome of rye for the homoeologous group 3 chromosomes allowed the establishment of a consensus map of 24 points in three linkage groups. The application of the CS aneuploid lines, particularly the nullisomic-tetrasomic lines to assign chromosomal locations and the ditelosomic stocks to assign restriction fragments to chromosome arms, greatly aided the analysis. This information allows the location of the centromere to a single linkage interval and provides an arm location, but not orientation, of linkage blocks independent of the centromeric clusters. Most importantly, prior knowledge of the arm locations provides some degree of independent confirmation of the multipoint linkage analysis.

A most striking feature of all four maps is the extreme clustering of mapped loci in the region of the centromere. Overall some 16 loci have been located in a centromeric block spanning only about 25 cM, while the remaining

eight points cover a minimum of a further 150 cM. Similar centromeric clustering, albeit to a lesser extent, has been observed by Chao et al. (1989) in wheat group 7 chromosomes and Wang et al. (1991) in rye chromosome 1. It is extremely likely that this non-random distribution of points along the chromosome reflects a tendency for recombination events to occur most frequently in the distal chromosome regions. This was similarly observed in elegant experiments combining cytological and genetic recombination data for wheat chromosomes 6B (Dvořák and Chen 1984) and 1B (Snape et al. 1985; Curtis and Lukaszewski 1991). However, the long genetic maps obtained here, e.g. at least 100 cM for 3BL, would suggest that the cytologically observed chiasmata considerably underestimate the actual number of recombinational events. Indeed, the extension of the RFLP-based genetic map distances beyond that expected from classical meiotic chiasmata analysis (Sallee and Kimber 1979), appears to be a general feature of the emerging Triticeae chromosome maps.

Although there is no clear evidence of cDNA probes mapping to different regions of the chromosomes than gDNAs, examination of the consensus map does indicate that different classes of clones may be unequally distributed along the chromosome. Of the 17 single copy clones that hybridize in a homoeologous fashion across wheat, barley, and rye chromosomes, 15 are concentrated in apparently co-linear blocks of tightly linked genes spanning the centromeres of 3A, 3B, 3D, and 3R. Outside of this block, only two (*Xpsr305* and *XCxp1*) of the eight mapped loci are class *a* clones, although even PSR305 is unusual in that it detects only one copy in wheat and rye, but is moderately repeated in barley. The remaining six mapped loci include two chromosome-specific sequences (*Xpsr454* and *Xpsr907*), an odd copy on 3BL detected by pGC19 (*XEmbp*), two non-homoeologous loci (*Xpsr904* and *Xpsr549*), and the *Est-5* isozyme loci. The coincidence of the presence of non-homoeologous loci in the chromosome region of high recombination leads us to speculate that these regions are accumulating mutations most rapidly and, in consequence, that the initial divergence of the wheat genomes is being observed first at the ends of the chromosomes.

Where comparisons over genomes can be made, i.e. for homoeologous clones for which polymorphism is present in more than one genome, complete co-linearity can be observed over the A, B, D, and R genomes. This appears to extend to the most distal marker on 3S (*Xpsr305*) and, at least 40 cM out on 3L (to *XCxp1*), and probably further to the *Est-5* loci, which are present on all three wheat chromosomes. However, divergence between genomes, to the extent that inter-genomic cross-hybridization is restricted, has taken place in some single or low copy sequences, particularly away from the centromere. A further cause of non-co-linearity between 3R and the wheat homoeologues is likely to be a translocation, in rye relative to wheat, of the distal regions of 3RL to 6RL (see below).

The recombination frequencies between the same loci in the different genomes, where they can be compared, do vary, e.g. *Xpsr578-Xpsr78* is 15.2 cM $\pm$ 2.5 in 3A and 4.4 cM $\pm$ 1.4 in 3B, and *Xpsr305* is 55.1 cM $\pm$ 4.6 from the centromere in 3D and only 33.4 cM $\pm$ 3.7 in 3R. Little evidence of a pattern to this variation can be observed in the limited data available. However, a study of Fig. 2 does suggest that chromosome 3D may recombine more frequently than its homoeologues.

Since the main wheat mapping population employed here, CS × 'Synthetic,' represents an extremely wide cross, effectively involving crosses between a cultivated AB × *T. dicoccum* genome and a cultivated D × *Ae.squarrosa* genome, the question of whether the recombination frequencies observed are typical of those expected in a conventional intervarietal wheat cross is relevant. The limited data available from another narrower intervarietal cross, 'Timgalen' × 'RL4137,' are actually remarkably consistent with the loci order and, to a lesser extent, the map distances observed in the CS × 'Synthetic' cross.

RFLP levels

The trend for gDNAs to detect more RFLP than cDNAs is at odds with similar comparisons reported by Landry et al. (1987) in lettuce, Havey and Muehlbauer (1989) in lentil, and Miller and Tanksley (1990) in tomato. However, the result is not unexpected in wheat. Sharp et al. (1988) showed in a study of RFLP and isozyme polymorphism at β-amylase loci that conservation of sequence around transcribed genes extends well beyond the coding region. The cDNAs and a proportion of the *Pst*I gDNA probes will target these conserved regions because *Pst*I will preferentially excise fragments in 'CpG' or 'methylation-free' islands associated with transcribed genes (Cheung et al. 1991, 1992). A proportion of the genomic clones are, however, likely to be derived from other unique or low copy sequences that will not have been subjected to the same degree of pressure for conservation.

The chromosome and genome specific low copy repeats, PSR454 and PSR907, are both genomic clones and indicate clearly that some genomic clones are qualitatively different from cDNAs. Both identify about 30 copies that are located in small regions of 3BL and 3BS respectively. Recombination analyses have detected no intra compound locus recombination for PSR454 (Harcourt and Gale 1991) and PSR907 (data not shown) among 120 individual F_2 progenies. H values for *Xpsr454* and *Xpsr907*, calculated for hybridizing band patterns (Table 2) rather than for single restriction fragments as above, are among the highest we have observed in wheat.

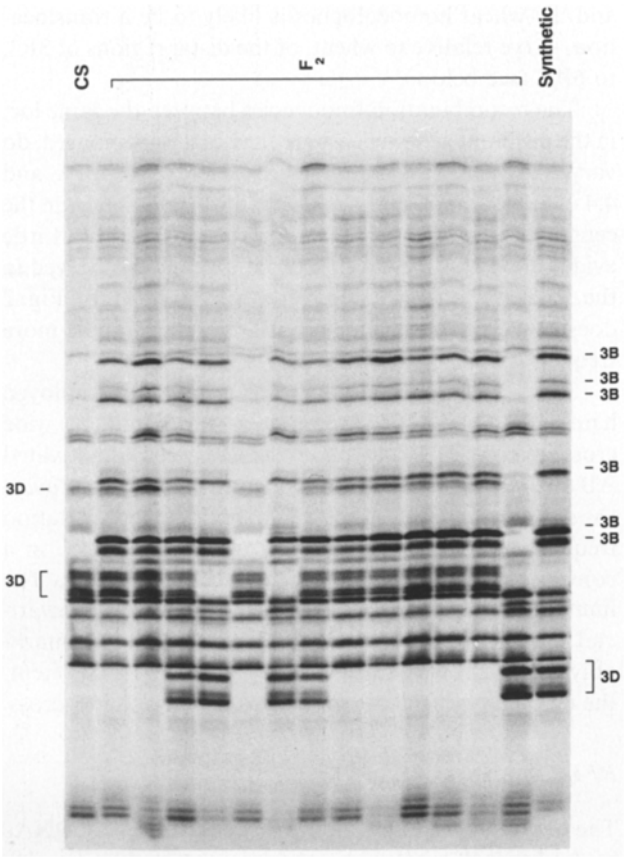

Fig. 4. F_2 segregation of grain esterase, *Est-5*, isozyme

The levels of RFLP detected here with cDNA clones compare closely with these obtained by Chao et al. (1989) in a study of 54 homoeologous group 7 chromosome cDNA loci. However, the threefold higher levels in 7B relative to 7A and 7D are not repeated here, although the levels observed in 3D were lower, by about a third, than in 3A and 3B.

The probability of detecting RFLP in any situation is clearly restriction enzyme dependent. McCouch et al. (1988), Chao et al. (1989), Miller and Tanksley (1990), and Graner et al. (1990) have all shown large effects in different species. The commonly observed positive relationship between fragment size generated by restriction enzyme and RFLP detected was, however, not apparent in this study. Indeed, the enzyme giving the largest fragments, *Eco*RV, showed the lowest level of polymorphism.

Duplicated loci

Most of the clones used in this study detected single copy sequences, i.e. one fragment per haploid genome as judged by the minimum number of fragments observed over several enzymes. A few clones identify multiple copies on a single chromosome arm, the smallest chromosome unit resolved with the standard aneuploid stocks. It is clearly important to ascertain that the individual fragments comprise a tight linkage block. In the present study, this has been ascertained for: the compound loci *Xpsr454-3B* and *Xpsr907-3B* detected by chromosome-specific probes; the duplicated loci *Xpsr170(1)* and (2), where the two subloci are separated by only 0.5 cM on 3D; and the *Est-5* isozyme loci (Fig. 4), which have multi-banded phenotypes. It cannot be assumed that duplicated loci will lie close together as has recently been shown with *Xpsr120* on the long arm of chromosome 5R, where 4 loci were mapped spanning a distance of 60 cM (Liu et al. 1992).

A3RL/6RL translocation

The existence of a 3RL/6RL translocation, relative to wheat, has been postulated on the basis of 6RL locations of grain esterase, *Est-5* (Ainsworth et al. 1986), NADH-dehydrogenase, *Ndh-3* (Liu and Gale 1991), and a number of morphological characters including grain colour and *sphaerococcum* grain shape (Miller 1984), all of which are located on group 3 long arms in wheat. Of the 18 3L DNA probes which hybridized to rye, only one, PSR454, provided a 6R location from analysis of the CS/*S. cereale* cv 'Imperial' single chromosome addition lines. Although the hybridization pattern for *Xpsr454-6R* was very weak, RFLP could be scored and a two-point linkage between *Est-R5* and *Xpsr454-6R* of 2.5 cM $\pm$1.0 was established. However, this block was unlinked with other available 3RL or 6RL markers. This result adds to the circumstantial evidence for the translocation, but provides no further information regarding its extent or reciprocality.

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