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RFLP-based genetic maps of wheat homoeologous group 7 chromosomes

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Summary. Restriction fragment length polymorphism (RFLP) mapping was attempted using 18 cDNA clones, 14 anonymous and 4 of known function, which had been shown to have homologous DNA sequences on the group 7 chromosomes of wheat. The loci identified by these probes have been mapped on one or more chromosomes in this homoeologous group using linkage data derived from various F_2, random inbred, doubled haploid and single chromosome recombinant populations. The maps also include three isozyme loci, five disease resistance loci, two anthocyanin pigment loci and a vernalisation response locus. The mapping data have been used to determine the extent of map co-linearity over the A, B and D genomes, the degree of RFLP variability in the three genomes and the relative efficiency of various restriction enzymes in detecting RFLPs in wheat. The strategy for future mapping in wheat, particularly the use of "alien" genomes or segments, such as that from *Aegilops ventricosa* used here, is discussed.

Key words: Wheat – Genetic mapping – RFLPs – Isozymes

Introduction

The genetic map of hexaploid wheat (*Triticum aestivum*, 2n = 6x = 42) is poorly developed compared with diploid cereals such as maize and barley. Nevertheless, the availability of a comprehensive set of aneuploid stocks has allowed a number of genes, including those controlling morphological traits, disease resistances and isozyme markers, to be located to chromosomes. However, the relatively large number of wheat chromosomes and the hexaploid genome constitution, which results in the phenotype of recessive mutations being masked by effective homoeoloci of the other genomes, have hampered rapid progress in intrachromosomal mapping.

Restriction fragment length polymorphisms (RFLPs), which have already been extensively used to construct genetic maps both in man and in other plants, provide a potential means of rapidly extending the wheat map. RFLPs are effectively unlimited in number, usually act as co-dominant genetic markers, and because individual loci are recognized, their use is not generally confounded by polyploidy. Indeed, since most fragments hybridizing to cDNA probes are triplicated over the A, B and D genomes, RFLP analysis in wheat is particularly efficient, since it allows simultaneous genotype classification to be made at at least three independent loci with a single Southern hybridisation.

We have located 18 RFLP loci on the homoeologous group 7 chromosomes of wheat by hybridisation of cDNA probes to restriction enzyme-digested genomic DNA. In this paper we describe the construction of genetic maps of chromosome *7A*, *7B* and *7D* using these probes. The maps also include three isozyme loci: aminopeptidase (*Amp-3*), endopeptidase (*Ep-1*) and α-amylase (*α-Amy-2*); five loci carrying genes conferring disease resistance: to leaf rust *Puccinia recondita* (*LrVPM*, *Lrm* and *Lr14a*), eyespot *Pseudocercosporella herpotrichoides* (*Pch1*) and powdery mildew *Erysiphe graminis* (*Pm5*); a locus controlling vernalisation requirement (*Vrn5*), and two loci controlling anthocyanin pigmentation (*Pc* and *Rc3*).

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Materials and methods

Genetic stocks

Aneuploid genetic stocks were employed to ascertain the homoeologous group and chromosomal arm locations of the fragments identified by hybridisation with the cDNA probes. These were the nullisomic-tetrasomic (NT) and ditelosomic (DT) stocks of cv Chinese Spring (CS) (Sears 1966; Sears and Sears 1978), the single chromosome addition lines of *Hordeum vulgare* cv Betzes in CS (Islam et al. 1981), of *Secale cereale* cv Imperial in CS (Driscoll and Sears 1971) and of *Aegilops umbellulata* in CS (Kimber 1967), along with Betzes and the CS/Imperial and CS/*Ae. umbellulata* amphiploids from which the respective addition series were derived.

The mapping was based on data collected from 51 F_2 plants from the cross Timgalen × RL4137, 50 F_2 plants from Holdfast × RL4137, 58 single-seed descent lines from the cross Sportsman × Highbury developed by J. W. Snape, Cambridge Laboratory, IPSR and 48 doubled haploid derivatives of the F_1 hybrid from a cross of the intervarietal chromosome substitution lines, Sicco (CS*5B*) × Highbury (CS*5B*) (Snape et al. 1986). Further mapping of particular chromosomes was achieved using sets of 91 single chromosome recombinant lines between chromosomes *7B* of CS and Hope derived from the cross CS × CS (Hope *7B*) (Law 1966), and 68 between chromosomes *7D* of Hobbit 'S' and VPM1 from Hobbit 'S' × Hobbit 'S' (VPM1 *7D*) (Worland et al. 1988).

The chromosome nomenclature used here is that agreed to by the 7th International Wheat Genetics Symposium, where the previous designations of *4A* and *4B* were reversed, and the *L* (long) and *S* (short) arm nomenclature was adopted. Thus, homoeologous arms within group 4 are now (previous designations in parenthesis): *4AS*(*4BS*)≡*4BL*(*4Aβ*)≡*4DL*, and *4AL*(*4BL*) ≡*4BS* (*4Aα*)≡*4DS*.

RFLP probes

Known function probes. DNA probes with known function and their sources are listed in Table 1.

Anonymous cDNA probes. A wheat cDNA library was constructed using mRNA isolated from leaves of CS. Ten grams of frozen leaves were ground in liquid nitrogen and added to homogenizing buffer (0.1 *M* NaCl, 50 m*M* TRIS-HCl pH 9.0, 10 m*M* EDTA, 2% SDS, 0.1 mg/ml proteinase K). After standing at room temperature for 15 min, an equal volume of phenol/chloroform was added and the mixture was shaken for 10 min. The aqueous phase was re-extracted twice with phenol/chloroform and once with chloroform, and then adjusted to 0.5 *M* Na^+ by addition of 5 *M* NaCl before mixing with oligo dT cellulose. The poly A^+ mRNA was eluted and fractions containing mRNA were pooled and ethanol precipitated.

cDNA was synthesized using the Amersham cDNA synthesis kit. cDNA fragments larger than 500 bp were purified from low melting point agarose and blunt end ligated into the SmaI site of pUC18 plasmid vector. The clones were picked and grown in 96-well microtiter plates. Bacterial stock was stored under − 70 °C after addition of DMSO to 14%. Clones containing genes for Rubisco small subunit and CAB binding proteins were identified by screening the clones by colony hybridisation using cloned wheat sequences of these genes as probes (Maniatis et al. 1982), and were excluded from further screening.

Isolation of genomic DNA and Southern hybridization

The techniques for plant genomic DNA isolation, restriction enzyme reactions, gel electrophoresis, Southern transfer, filter hybridization and probe labelling using the "oligolabelling" method were as described previously (Sharp et al. 1988a). However, the transfer buffer used was 0.4 *M* NaOH, 0.6 *M* NaCl, and both Zetaprobe (BioRad) and Gene Screen Plus (Du Pont, NEN) were used as transfer membranes.

Isozyme analysis

The electrophoretic method used for endopeptidase isozymes was that of Koebner et al. (1988). Aminopeptidase-3 (*Amp-3*) isozymes (E.C. 3.4.11.2.) were separated by isoelectric focusing, and visualised by the linking of the cleavage product of methionyl-β-naphthylamide with Fast Black K (R. M. D. Koebner, unpublished results).

Linkage analysis

Segregation analysis, tests of the significance of linkage and the estimation of the recombination frequencies between linked loci in the F_2, doubled haploid and single chromosome recombinant mapping families were performed using the LINKAGE-1 computer program of Suiter et al. (1983). Recombination frequencies based on data from the doubled haploid and single-seed descent lines were estimated using methods described by Snape (1988). The map distances in centiMorgans (cM) were calculated using the Kosambi mapping function (Kosambi 1944).

Results

Chromosomal location of RFLP loci

The initial assignment to homoeologous chromosome group of the DNA fragments defined by each of the cDNA clones was achieved by analysing the hybridisation patterns of Betzes, the CS/Imperial and CS/*Ae. umbellulata* amphiploids and the three sets of wheat-alien individual chromosome addition lines. In general, all but

Table 1. cDNA clones of known function used as probes

Probe	Clone	Function	Source	Reference
SSU	pW9	Rubisco small subunit	N-H. Chua	Broglie et al. 1983
CAB	cabIIA	Chlorophyll a/b binding protein	N-H. Chua	Lamppa et al. 1985
Pepc	Sorco	Phosphoenolpyruvate carboxylase	P. Westhoff	Personal communication
Nra	bNRp10	Nitrate reductase	A. Kleinhofs	Cheng et al. 1986
Wx	pcwx27	Waxy	W. Rhode	Rhode et al. 1988
α-Amy2	4868	α-amylase	D.C. Baulcombe	Lazarus et al. 1985

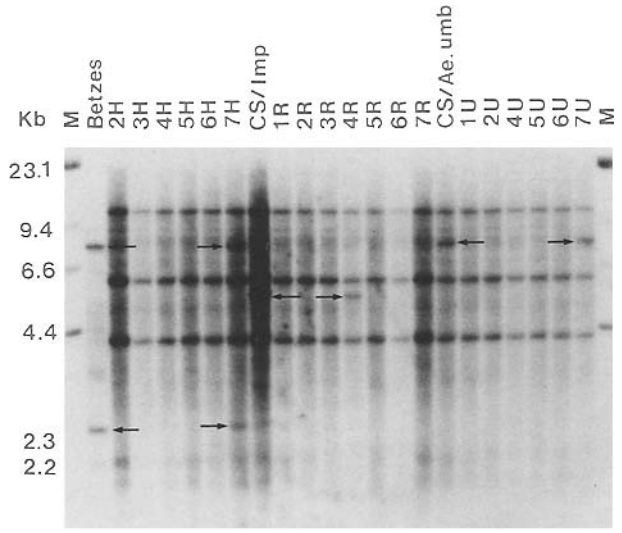

Fig. 1. Hybridisation of probe PSR152 to HindIII-restricted genomic DNA from the wheat-alien amphiploids and wheat-alien chromosome addition lines. *Arrows* indicate the non-wheat fragment(s) present in the barley and amphiploid tracks and in the *7H, 4R* and *7U* addition lines. M = lambda HindIII digest marker

one of the addition lines within each set gave a pattern of hybridisation identical to that of CS, while the remaining addition line and the amphiploid (where available) showed a combined pattern of CS and the alien species. An example of this initial screen is shown in Fig. 1, where PSR152 detects a single fragment in rye and *Ae. umbellulata,* three in CS and two in barley, presumably resulting from an internal cut in the DNA sequence in barley corresponding to the wheat cDNA probe. The same alien fragments can be seen to occur in the addition lines *7H, 4R* (*4RL·7RS*), and *7U*. One restriction enzyme, HindIII, was usually sufficient to distinguish the alien from the wheat fragments. Where this was not so, other enzymes were also used. In this way, 18 cDNA clones, including 14 anonymous and 4 known function clones, were located to alien homoelogous group 7 chromosomes.

The gene synteny among homoeologous chromosomes within the Triticeae has proved to be adequate, so far, to predict wheat chromosome location from those in the three alien species. Furthermore, for homoeologous group 7 loci, the 4/7 translocation in *S. cereale* (Koller and Zeller 1976) allows chromosome arm locations in wheat to be predicted, such that a probe identifying fragments located on *7H, 7R* and *7U* indicates a long-arm location in wheat, while *7H, 4R* and *7U* locations indicate a short-arm location of the detected wheat loci, as is the case with PSR152 shown in Fig. 1.

The assignment of the individual wheat fragments to their respective group 7 chromosome and determination

Table 2. Chromosomal arm location, copy number, and polymorphism levels at the loci detected

Probe	Arm location	Copy no.	% Polymorphism detected			
			7A	*7B*[b]	*7D*	*7D*[a]
103	*7AS 7BS 7DS*	1	0	42.2	1.4	16.7
105	*7AL 7BL 7DL*	2	0	0	0	16.7
108	*7AS 7BS 7DS*	1	0	0	0	33.3
	2AS 2BS 2DS	1				
117	*7AL 7BL 7DL*	1	0	11.6	0	30.0
	3AL 3BL 3DL	2				
119	*7AS 4AL 7DS*	1	40.2	0	29.2	0
121	*7AL 7BL 7DL*	2	0	36.9	0	60.0
	1AL 1BL 1DL	1				
129	*7AL 7BL 7DL*	1	0	4.1	0	10.0
150	*7AS 7BS 7DS*	1	0	29.3	0.7	50.0
	2AS 2BS 2DS	2				
152	*7AS 7BS 7DS*	1	0	2.8	0	70.0
160	*7AS 4AL 7DS*	1	20.6	64.0	49.3	20.0
165	*7AL 7BL 7DL*	2	0	6.1	0	50.0
169	*7AL 7BL 7DL*	1	0	0	0	10.0
65	*7AS 7BS 7DS*	1	0	11.7	9.6	20.0
72	*7AL 7BL 7DL*	2	3.2	52.1	0	10.0
Pepc	*7AL 7BL 7DL*	1	12.2	9.5	0	0
Nra	*7AS 4AL 7DS*	1	2.0	10.9	0	0
	6AS 6BS 6DS	1				
Wx	*7AS 4AL 7DS*	1	6.4	6.4	0	0
α-Amy2	*7AL 7BL 7DL*	3	0	8.5	0	–
Average			4.7	16.5	5.0	23.3

[a] Comparison between VPM1 and Hobbit'S'7D
[b] Also includes that part of *7B* translocated to *4A*

of the arm location for each probe was performed by analyses of the fragment patterns detected in the CS homoeologous group 7 NT and DT aneuploid lines. An example is shown in Fig. 2a, where PSR152 is confirmed to hybridise with single sequences on the short arms of the three group 7 chromosomes. Of the 18 cDNA clones, 9 detected DNA fragments on the long arms and 9 on the short arms of the group 7 chromosomes. The analyses were extended to include DNA from CS N4A-T4B and DT4AS and DT4AL stocks when evidence confirming the proposed translocation of part of the short arm of *7B* to *4A* (Naranjo et al. 1987) was obtained. An example of such a case is shown in Fig. 2b, where PSR160 is shown to hybridise with sequences on *7AS, 7DS* and *4AL*. Four of the loci on *7AS* and *7DS* (defined by probes PSR119, PSR160, Nra and Wx), were found to have homoeoloci on *4AL* and not on *7BS* (Fig. 2b and Table 2).

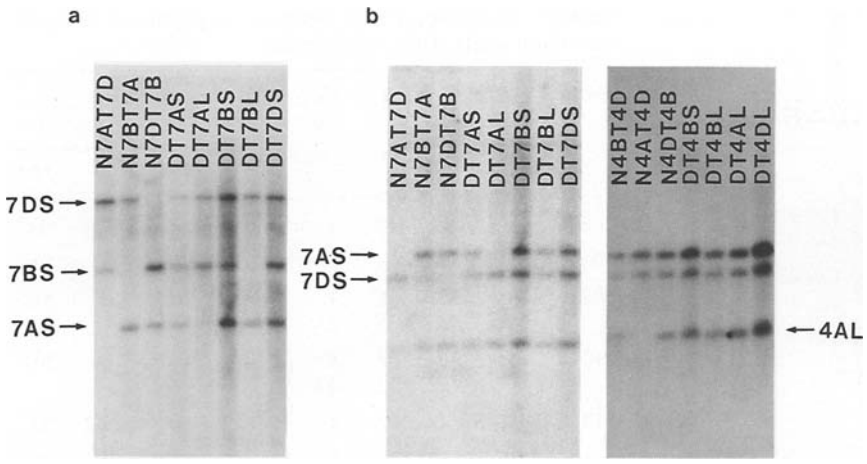

Fig. 2a and b. Hybridisation of HindIII- restricted genomic DNA of **a** the homoeologous group 7 NT and DT lines with probe PSR152, **b** the group 7 and group 4 NT and DT lines with probe PSR160

Copy numbers of the sequences detected

Of the 18 clones, 9 detected "single copy" sequences, as defined by their detection of a minimum of three fragments in a range of restriction digests, with one fragment located in each of the A, B and D genomes. More fragments were observed with some enzymes and this presumably occurred when the genomic DNA was cut at sites within the span of DNA hybridizing to the probe. Genomic reconstruction experiments to confirm the copy numbers were not carried out.

Of the 9 probes detecting either "duplicated" (a minimum of six fragments) or "triplicated" (nine fragments) sequences, 4 (PSR105, PSR165, PSR72 and α-Amy2) detected fragments located on the same homoeologous group 7 chromosome arm, while 5 (PSR108, PSR117, PSR121, PSR150 and Nra) detected fragments located on other homoeologous groups in addition to those located on group 7 chromosomes. CS NT and DT lines of other homoeologous groups were analysed to assign these fragments to a chromosome arm. This information is summarised in Table 2. The duplication of *XNra* loci on the chromosomes of group 6 and 7 has been reported previously (Kleinhofs et al. 1988), as has the presence of multiple copies of *α-Amy-2* genes on the group 7 chromosomes (Lazarus et al. 1985; Huttley et al. 1988).

Variability at RFLP loci

The 18 clones were hybridized to restricted genomic DNA from parental lines of crosses used for mapping (Timgalen, RL4137, Holdfast, Sportsman, Highbury and Sicco) to identify informative probe/restriction enzyme combinations. The CS homoeologous group 7 NT lines were also analysed for each probe/enzyme combination so that RFLPs detected could be assigned to particular chromosomes. For each probe, 13 restriction enzymes, all hexanucleotide cutters, were assayed for the detection of RFLPs. Polymorphisms were found for all three group 7 chromosomes with only one probe (PSR160), for two chromosomes with eight probes and for a single chromosome with six of the probes. Three of the probes detected no RFLPs among the parental varieties with the restriction enzymes assayed.

The degree of polymorphism detected varied greatly between the clones, as can be seen in Table 2. The levels of polymorphism were calculated by recording the presence or absence of RFLPs for the 15 possible pairwise comparisons among the 6 varieties for each probe, using each enzyme. The data were recorded separately for the *7A, 7B* and *7D* chromosomes, and any hybridisation patterns which were unclear were treated as missing data. The results shown in Table 2 are the total number of polymorphic pairwise comparisons revealed, with the restriction enzymes used expressed as a percentage of the total number of pairwise comparisons for each probe on each chromosome (maximum 15 comparisons × 13 restrictions enzymes = 195). It is clear that *7B,* including the portion translocated to *4A,* is the most polymorphic of the three chromosomes. Table 2 does not include data obtained from the CS × CS (Hope *7B*) family because RFLPs on *7A* and *7D* between CS and Hope were not assayed, and there is evidence that the *7B* chromosome of Hope contains chromatin derived from a tetraploid (AABB) emmer wheat (Law 1967). Similarly, the Hobbit 'S' × Hobbit 'S' (VPM1 *7D*) data have not been pooled with those from the other families, as here the comparison does not include the *7A* and *7B* chromosomes, and there is evidence for an alien (*Aegilops ventricosa*) origin of much of chromosome *7D* in VPM1 (Worland et al. 1988).

The ability of the various restriction enzymes to detect polymorphism at individual loci is shown in Table 3. These data were determined by dividing the total number of polymorphic pairwise comparisons with all the probes used by the total number of the pairwise comparisons (maximum 18 probes × 15 comparisons = 270) for each of

Table 3. The level of polymorphism detected by 13 restriction enzymes

Enzyme	% Polymorphism detected				*7D*[a]
	7A	*7B*[b]	*7D*	Within group 7	
ApaI GGGCC C	2.0	10.0	0	4.0	7.1
BamHI G GATCC	5.2	15.6	5.2	8.7	21.4
BglII A GATCT	0	16.8	6.0	7.6	23.5
DraI TTT AAA	6.8	21.2	9.2	12.4	35.3
EcoRI G AATTC	6.4	18.8	5.2	10.1	35.3
EcoRV GAT ATC	11.6	24.4	5.6	13.9	29.4
HindIII A AGCTT	7.6	13.2	9.6	10.1	23.5
KpnI GGTAC C	0	0	0	0	14.3
PvuII CAG CTG	0	11.1	0	3.7	21.4
SspI AAT ATT	0	28.6	14.3	14.3	ND
SstI GAGCT C	5.9	15.3	1.6	7.7	21.4
XbaI T CTAGA	0	25.2	2.8	9.3	ND
XhoI C TCGAG	0	9.1	1.4	3.5	ND
Average	3.5	16.1	4.7	8.1	23.3

[a] Comparison between VPM1 and Hobbit'S'7D
[b] Also includes that part of *7B* translocated to *4A*

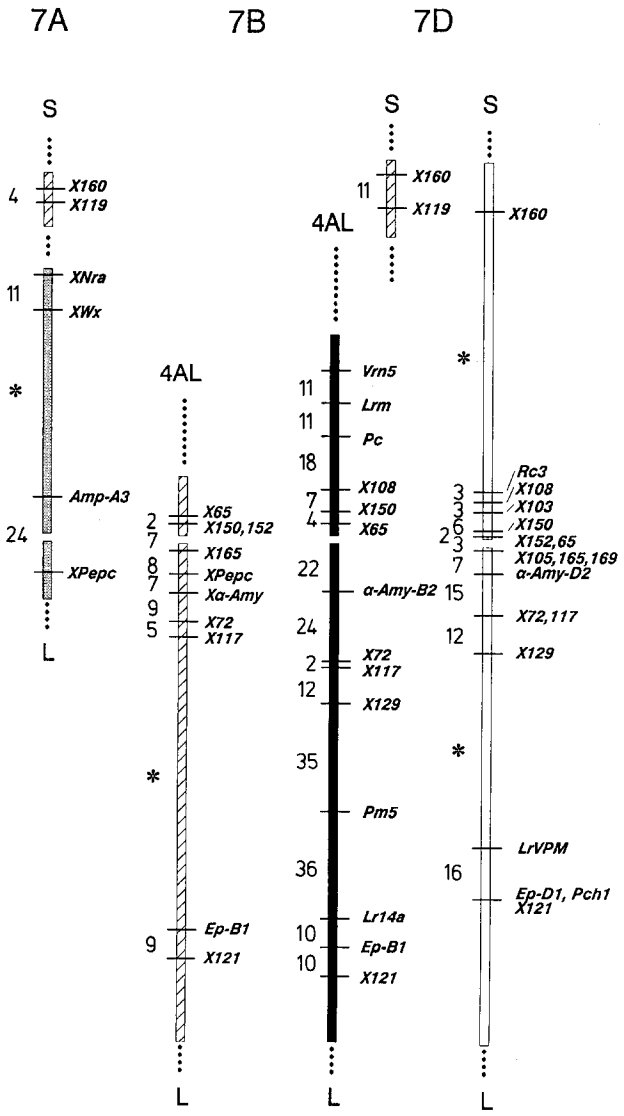

Fig. 3. Genetic maps of chromosomes *7A*, *7B* and *7D*. The map constructed using data from the Timgalen × RL4137 F_2 population is indicated by the *cross-hatched lines*, that from the Sicco (CS *5B*) × Highbury (CS *5B*) doubled haploid lines by the *stippled line*, that from the CS/Hope *7B* recombinant lines by the *solid line*, while that from the Hobbit'S'/VPM1 *7D* recombinant lines is indicated by the *open line*. * Indicates synteny based on the analysis of aneuploid stocks, but where the contingency Chi-square test reveals no linkage at the 5% significance level. The *dotted lines* indicate unmapped regions between the most distal loci and the telomeres, or indicate junctions between maps constructed in different populations. The positions of the centromeres are indicated by *breaks* in the maps. The order of some loci has been determined by reference to a map of barley chromosome 1 (*7H*) using the same probes (A. Kilian, A. Kleinhofs, P.J. Sharp, S. Chao and M.D. Gale, unpublished results)

the 13 restriction enzymes. All the enzymes which detected the highest levels of polymorphism (over 9% averaged over *7A*, *7B* and the wheat *7D*) have at least four of the six bases of their recognition sequences as A or T.

Many of the RFLPs detected represent deletion/insertion mutations. They were observed with several restriction enzymes, as has been found previously in wheat (Sharp et al. 1988b). For the subsequent analysis of the mapping families, the most convenient enzyme was chosen, considering its cost, the availability of previously prepared filters and the ease of observation of the RFLP.

Intrachromosomal mapping

The segregation of all loci in each of the populations studied fitted the expected Mendelian ratios (data not shown). The map distances obtained within individual families and the derived genetic maps are shown in Fig. 3. The information has not been pooled, although clearly a single consensus map of the three group 7 wheat chromosomes can be constructed.

The gene order obtained for *7D* in the Hobbit 'S' × Hobbit 'S' (VPM1 *7D*) cross is similar to that

derived for *7B*. A direct comparison to a map of *7D* using only variation within wheat itself cannot be made because the level of polymorphism in the wheat families studied here did not allow such an extensive map to be constructed. Interestingly, the recombination frequencies found with this cross are generally lower than those obtained for *7B* (Fig. 3). This may be related to the alien source of the VPM1 *7D* chromosome, resulting in reduced chromosome pairing due to reduced homology of the alien and wheat chromosome *7D*.

Some of the loci included on the map produced from Hobbit 'S' × Hobbit 'S' (VPM1 *7D*), endopeptidase (*Ep-D1*), eyespot resistance (*Pch1*), leaf rust resistance (*LrVPM*), α-amylase (*α-Amy-D2*) and red coleoptile (*Rc3*), were scored previously (Worland et al. 1988). Many of the VPM1 alleles at the RFLP loci scored appear to derive from the *Ae. ventricosa* accession used in the original interspecific cross (Maia 1967). This was determined by hybridizing each probe to digests of Hobbit 'S', VPM1 and *Ae. ventricosa* to test whether the alien species carried the allele found on VPM1 *7D*. Of the probes detecting *7D* RFLPs between Hobbit 'S' and VPM1, all but one probe (PSR129) showed the VPM1 allele that was also present in *Ae. ventricosa*. An example is shown in Fig. 4. With four probes, PSR119, Nra, Wx and Pepc, the *7D* fragments of Hobbit 'S', VPM1 and *Ae. ventricosa* were identical.

Two isozyme loci, *Ep-B1* and *Amp-A3*, were able to be included on the maps of *7B* and *7A*, respectively.

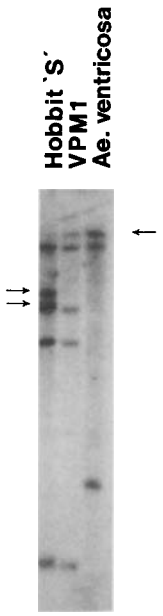

Fig. 4. Hybridisation of probe PSR103 to EcoRI-digested DNA from Hobbit'S', VPM1 and *Ae. ventricosa*. The *arrows* on the left indicate the Hobbit'S' *7D* fragments and that in VPM1 and *Ae. ventricosa* is indicated on the right

Timgalen possesses the allele *Ep-B1d*, while RL4137 has *Ep-B1b*, and this difference could be unequivocally scored. Similarly Sicco (*Amp-A3b*) and Highbury (*Amp-A3a*) show an allelic difference which allows ready assignation of genotype with respect to this locus.

The analysis of *7B* using the Sportsman × Highbury lines (data not shown) provided an additional interesting feature. Only in this cross did polymorphisms occur in both the duplicated sequences hybridizing to probe PSR72. This allowed a test of allelism for the two copies and indicated that the probe detects two loci, *Xpsr72-7B(1)* and *Xpsr72-7B(2)*, separated by a map distance of 9 cM. In the other families where probe PSR72 was used in mapping, only one of the two copies carried by all group 7 chromosomes segregated. In these families, therefore, the precise identity of the *Xpsr72* locus mapped cannot be determined.

The gene order on *7A*, *7B* and *7D* is conserved, or is consistent with conservation, in all families with one exception. There is an apparent discrepancy in the relative positions of the closely linked loci *Xpsr65*, *Xpsr150* and *Xpsr152* on chromosome *7B*, based on data from the Timgalen × RL4137 F_2 family, relative to the CS *7B*/Hope *7B* and Hobbit 'S' *7D*/VPM1 *7D* families. Close examination of the Timgalen × RL4137 F_2 data showed that the gene order here is based on only eight recombinant gametes, including one recombinant in the *Xpsr65-Xpsr150, 152* region, six recombinants in the *Xpsr150, 152-Xpsr165* region (spanning the centromere) and one double recombinant. Thus, although the order is that which fits the data most closely, a further linked marker in this population distal to these loci on *7BS* is required before this order can be confirmed.

Discussion

In this paper we have demonstrated an approach to mapping the wheat genome using RFLPs, isozymes and genes controlling other traits, some of agronomic significance. This has resulted in the production of more extensive genetic maps of chromosomes *7B* and *7D* than has previously been possible.

RFLP variation in wheat

It is clear from the data presented here that RFLPs are relatively infrequent in wheat. The 18 cDNA clones used detected polymorphism at the group 7 loci between pairs of the six varieties examined with 13 restriction enzymes in an average of only 8.7% of the comparisons. In lettuce, similar comparisons have detected polymorphism at levels of 27.4% with cDNAs and 11.1% with genomic clones (Landry et al. 1987), while in maize (Burr et al. 1983; Helentjaris et al. 1985) and rice (McCouch et al.

1988), the values are even higher. However, the low average figure hides a number of features of wheat RFLPs which are evident in Tables 2 and 3.

The three wheat chromosomes differ, with the *7B* chromosome RFLP loci being approximately three times as variable as their homoeoloci on *7A* and *7D*. This is unlikely to be a chance result obtained with this particular sample of probes, because the same trend is evident in work on other wheat homoeologous groups using probes from the same library (S. Chao, P. J. Sharp, F. J. Nicoll and M. D. Gale, unpublished results). It also concurs with variability detected at isozyme and storage protein loci. Examination of the "Catalogue of gene symbols for wheat" (McIntosh 1988), to determine the number of different alleles recorded at homoeoloci across the A, B and D genomes where at least one homoeolocus has been shown to be polymorphic, also reveals approximately twice as much variation in the B genome than in the A and D genomes.

The reasons for this difference between the genomes are as yet unclear. Two non-exclusive possibilities, however, present themselves – one presupposing that the B genome is more mutable, the other that the B genome progenitor species was more variable a priori and that this variability has been maintained through the allopolyploidization events which have led to the evolution of hexaploid wheat.

In the first case, it is assumed that hexaploid wheat arose following spontaneous chromosome doubling of a single AABB × DD hybrid. Thus, at this initial stage, no variability was present. The variation in modern hexaploid wheat subsequently arose by mutation. The suggestion has been made that some highly repeated DNA sequences and, therefore, some heterochromatin have evolved from the activity of transposable elements. Since the B genome chromosomes are characterised by far more heterochromatin and repeated sequences than those from the A and D genomes (reviewed by Flavell et al. 1987; May and Appels 1987), the B genome may be inherently more mutable via transposition, which appears to be favoured within rather than between chromosomes.

In favour of the second hypothesis is the fact that the donor of the B genome was an outbreeding species, or mixture of species in the Sitopsis section of the *Aegilops*, while the A genome donor was the inbreeding species, *T. urartu* (reviewed by Miller 1987). Thus, if *T. dicoccoides*, the original tetraploid amphiploid, arose more than once, then the species would be likely to be more variable at B genome loci than at A genome loci. Much of this variation is likely to have been cryptic to early farmers during the domestication of *T. dicoccum* and, hence, if the formation of the hexaploid was also a multiple occurrence, then the same variability may have been transmitted to the present-day crop. Whatever its origin, however, the difference in the levels of polymorphism indicates that it will be easier to construct detailed genetic maps of the wheat B genome chromosomes, as indeed is evident in Fig. 3.

Another important point arising from the data in Table 2 relates to the large differences between probes with respect to the variability they detect and the differences in variability between individual sets of homoeoloci. Of the 18 probes, 3 did not reveal any polymorphism in the pairwise comparisons of the wheat varieties studied, while some of the other probes revealed high levels of polymorphism. One, PSR160, detects a high level of polymorphism at all three homoeoloci. When this probe was hybridised to HindIII digests of 25 varieties, it revealed three *7A* alleles, six *4A* alleles, and six *7D* alleles (Gale et al. 1989). It will be important in wheat RFLP studies, therefore, to identify probes which detect highly polymorphic loci, so that the maps generated will be of greatest value to geneticists and breeders. Other types of library, for example, genomic clones, may have a higher proportion of such useful probes, although this is not the case in lettuce (Landry et al. 1987). This possibility is currently being investigated.

The restriction enzymes studied produced different frequencies of polymorphism (Table 3), with those having A-T rich recognition sequences detecting more polymorphisms. This is the opposite of the trend noted in human RFLP work, where sites containing CpG dimers are more polymorphic (Barker et al. 1984), but agrees with observations in other plant species – maize and tomato (Helentjaris et al. 1985), rice (McCouch et al. 1988), and lettuce (Landry et al. 1987). However, the comparison here is more precise, as the enzymes analysed all have 6-base recognition sequences (Table 3), whereas in the other plant studies cited above A-T rich 6-base sequences were usually compared with G-C rich 4-base sequences.

The linkage maps

As an experimental organism, wheat has the advantage of the availability of ditelosomic lines, which allow loci to be located to chromosome arms. This permits the centromeres to be located on the maps, often very precisely when they lie between two closely linked markers, as in the case of chromosome *7D* map. The chromosome maps indicate that the gene orders on each of the homoeologous chromosomes studied are almost identical. The evidence is most comprehensive for chromosomes *7B* and *7D* where, apart from the inconsistency in the *Xpsr65, Xpsr150, Xpsr152* region noted above, nine homoeoloci and the centromere are observed to lie in the same order and to be separated by similar map distances.

Chromosome *7B* does, of course, differ from *7A* and *7D*, in that part of the short arm of the "original" *7B* has

been translocated to the long arm of *4A*, as has been inferred from cytological evidence (Naranjo et al. 1987). The breakpoint on *7BS* must lie between *Pc* and the most proximal of *Xpsr160*, *Xpsr119*, *XNra* and *XWx*. Comparisons with a barley *7H* map (A. Kilian, A. Kleinhofs, P. J. Sharp, S. Chao and M. D. Gale, unpublished results) suggests that this is *XWx*. The translocated segment is relatively large, covering these four loci. As yet, no marker gene confirms the postulated translocation of part of *5AL* to *7BS* (Naranjo et al. 1987), although genetic material, in addition to that mapped here, probably does exist on *7BS*, as the present map extends for only approximately 60 cM.

The maps also allow some conclusions to be made with regard to the homoeologous relationships of particular loci. The similar map positions of the two anthocyanin pigment genes, *Pc* (Purple culm) on *7BS* and *Rc3* (Red coleoptile) on *7DS*, suggest they are homoeoloci. Similarly, the two leaf rust resistance genes, *Lr14a* (*7BL*) and *LrVPM* (*7DL*), may also be homoeoloci, even though the former is recognised as a race-specific allele in seedlings and the latter is recognised as an adult plant resistance.

Furthermore, the framework created by the RFLP map allows the ready inclusion of newly discovered loci, provided that allelic differences are present between the appropriate parents of a cross. The mapping of *Ep-B1* and *Amp-A3* provides an apposite application of this feature.

Five group 7 DNA sequences are duplicated elsewhere in the genome, but these do not show evidence for the duplication of any large chromosomal region. Only two sequences, detected by PSR108 and PSR150, are duplicated to the same chromosome group. Although *Xpsr108* and *Xpsr150* are closely linked on group 7, the single copy locus *Xpsr103* lies between them, so that the duplication of both *Xpsr108* and *Xpsr150* to, or from, the group 2 chromosomes is likely to have been an independent event.

Previous evidence in wheat, based on a few cytological and genetic markers on chromosome *6B* (Dvorak and Chen 1984; Kleinhofs et al. 1988) and *1B* (Flavell et al. 1987) has indicated that recombination occurs more frequently in the more distal regions of the chromosomes. The present maps of both chromosomes *7B* and *7D* have RFLP loci clustered to some extent around the centromere map positions on both arms (Fig. 3). This would indicate that recombination is indeed more frequent in distal chromosome regions, if the sequences analysed here are not preferentially located physically close to the centromere. Snape et al. (1985) suggested, for chromosome *1B*, that the large block of centromeric heterochromatin was responsible for this distribution. However, this may not be a factor reducing crossing-over in such regions, as chromosome *7D* is devoid of this material (Flavell et al. 1987). Distal clustering of recombination events does not appear to be an obvious feature of other maps constructed with cDNA probes in tomato (Helentjaris et al. 1986) or with genomic probes in rice (McCouch et al. 1988), although in these species the precise locations of the centromeres are more difficult to determine.

The 7D chromosome of VPM1

The high level of polymorphism between VPM1 and Hobbit 'S' for chromosome *7D* loci is clearly related to the alien origin from *Ae. ventricosa* of parts of the *7D* chromosome of VPM1 (Maia 1967). The VPM1 alleles at the *Pch1*, *Rc3* and *Ep-D1* loci have all been shown previously to derive from *Ae. ventricosa* (Worland et al. 1988). The RFLP evidence presented here adds another 12 marker loci, indicating the presence of substantial *Ae. ventricosa 7D* chromatin on the *7D* of VPM1. Since these RFLP markers include the most distal, *Xpsr160* and *Xpsr121*, and the most proximal, *Xpsr152*, *Xpsr65*, *Xpsr105*, *Xpsr165* and *Xpsr169* (Fig. 3), it must be considered that most of this chromosome in VPM1 has originated from *Ae. ventricosa*. The origin of a large internal segment of the short arm cannot be determined, since no RFLPs were found between Hobbit 'S', VPM1 and *Ae. ventricosa* at the *Xpsr119*, *XNra* and *XWx* loci. Only at the *Xpsr129-7D* locus do VPM1 and *Ae. ventricosa* have different alleles. It is possible, however, that this represents a difference between the *Ae. ventricosa* accession examined and that used to produce VPM1, and not that the *Xpsr129-7D* allele of VPM1 is of wheat origin, particularly since a similar inconsistency has been noted previously at the *α-Amy-D2* locus (Gale et al. 1984).

The *7D* map produced here using VPM1 demonstrates the utility of alien segments as a method of more rapid map construction than relying on the variation within *T. aestivum* itself. The map of *7B* produced from the CS × CS (Hope *7B*) cross may be another example, as the Hope *7B* chromosome contains genetic material (marked by *Pm5*, *Pc* and *Lr14a*) derived from a tetraploid wheat (Law 1967). The map distances on the *7D* map tend to be lower than those found for the same intervals on the *7B* maps (Fig. 3). Particularly noteworthy is the difference between the *7B Ep-B1-Xpsr121* recombination of 9.9 ± 3.32 cM and the complete linkage (maximum of 3 cM, $P = 0.05$) of the *7D* homoeoloci. *Pch1*, the eyespot resistance gene from *Ae. ventricosa* which is being used in many breeding programmes, also lies in this region and is completely linked to both markers. Reduced recombination in this case results in both *Ep-D1* and *Xpsr121* being particularly efficient markers for the resistance. This mapping result could be due either to a lower frequency of recombination between partially homologous, relative to fully homologous seg-

ments, or to an inversion in this region of the VPM1 chromosome relative to conventional wheats. In the latter case, any marker in the inverted segment would appear completely linked. Such paracentric inversions have been found to differentiate the linkage maps of the closely related species tomato and potato (Bonierdale et al. 1988).

The map of *7D* presented here enables the use of RFLP and other marker loci (e.g. *Ep-D1*) in the identification and isolation of plants with recombinant *7D* chromosomes possessing useful characteristics. Thus, lines can be identified with specific alleles at the disease resistance loci *Pch1* and *LrVPM*, but without other genetic material derived from *Ae. ventricosa*, which may have detrimental effects on agronomic performance.

Conclusions

The results described here demonstrate that RFLPs can provide the many markers necessary for the production of a detailed genetic map of wheat for application in research and breeding. This is possible even though wheat is a polyploid inbreeding species of comparatively recent origin. The results indicate that maps can be obtained by analysis of segregating populations from intervarietal crosses by screening many probe/enzyme combinations. However the process is more efficient when chromosomal segments derived from alien sources are allowed to recombine with the homoeologous wheat segments. Similarly, the general co-linearity of the maps means that mapping in one genome, for instance the D genome in segregating populations of *Ae. squarrosa* hybrids, should closely predict the maps in the other two wheat genomes. Maps constructed in rye or barley are likely to be of similar use as a basis for those of wheat.

However, it is also clear that probes which show high levels of intervarietal polymorphism must be identified, as these will be of more general value in breeding and research. Thus eventually, the mapping, including the location of genes controlling agronomically important traits and the evaluation of the usefulness of individual probes, must be carried out in wheat itself.

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