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Structural and Genetical Studies on the High-molecular-weight Subunits of Wheat Glutenin

Part 3. Telocentric Mapping of the Subunit Genes on the Long Arms of the Homoeologous Group 1 Chromosomes

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Summary. The genes controlling the synthesis of the high-molecular-weight subunits of glutenin on the long arms of chromosomes 1A and 1B were mapped to the ω-gliadin genes on the short arms by analysing the progeny of three test crosses by sodium dodecyl sulphate, polyacrylamide-gel electrophoresis. Only very weak linkages were detected: the percentage recombination ranged from 39% to 47% and as the values did not significantly differ from each other, the data was pooled. A mean recombination of 43% was obtained and the map distance between glutenin and gliadin genes was calculated to be 66 cM. The analysis of three crosses involving telocentric lines revealed that the glutenin subunit genes on chromosomes 1A, 1B and 1D are tightly linked to the centromere, the mean map distance being 9.0 cM.

Key words: Glutenin – *Triticum* – Gene mapping – Polyacrylamide-gel-electrophoresis

1 Introduction

The genes controlling the synthesis of the high-molecular-weight (HMW) subunits of glutenin are located at three complex loci, one on each of the long arms of chromosomes 1A, 1B and 1D (Bietz et al. 1975; Payne et al. 1980; Lawrence and Shepherd 1981a). In contrast, the genes controlling the gliadin proteins are located on the short arms of both the group 1 and group 6 chromosomes; all of the ω-gliadins, most of the γ-gliadins, and a few of the β-gliadins are controlled by genes on chromosomes 1A, 1B and 1D whereas all the α-gliadins, most of the β-gliadins and a few of the γ-gliadins are controlled by chromosomes 6A, 6B and 6D (Shepherd 1968; Wrigley and Shepherd 1973; Payne et al. 1982 for a review).

In the present study, crosses were made, some involving the homoeologous group 1 ditelocentric lines of 'Chinese Spring' to enable the following genetic distances to be estimated: (1) HMW subunit genes on the long arms of chromosomes 1A and 1B to the gliadin genes on their short arms and (2) the HMW subunit genes to the centromeres of all three group 1 chromosomes.

2 Materials and Methods

The hexaploid wheat varieties, the aneuploid lines of 'Chinese Spring' and various intervarietal chromosome substitution lines were obtained from collections maintained at the Plant Breeding Institute (PBI), Cambridge. Other substitution lines were both developed and maintained at the PBI.

2.1 Sodium Dodecyl Sulphate, Polyacrylamide-gel Electrophoresis (SDS-PAGE)

The protein constituents of the grains obtained from genetic crosses were analysed by electrophoresis as described previously (Payne et al. 1980; Payne et al. 1981a), except that 32-slot gels were used.

2.2 Acid, Polyacrylamide-gel Electrophoresis (APAGE)

To extract the gliadin proteins, a crushed half grain was incubated with 70% (v/v) ethanol (3.3 µl per mg grain) for 2–3 h at room temperature with occasional vortex mixing. The suspension was centrifuged at 8,000 g for 5 min in a Beckman Microfuge, the supernatant was mixed with 0.84 its volume of 60% (v/v) glycerol, 0.05% (w/v) pyronin G and the sample centrifuged again to re-pellet the sediment. Approximately 25 µl of supernatant was used for electrophoresis. The polyacrylamide gel slabs were made and subjected to electrophoresis according to Bushuk and Zillman (1977) but modified for the Pharmacia vertical electrophoresis system. The slabs measured 16.8 cm × 12.8 cm × 0.27 cm thick and consisted of 8% (w/v) acrylamide and 0.3% (w/v) methylenebisacrylamide buffered at pH 3.1. Electrophoresis was at 500 V and was continued for double the time taken for the tracking dye, pyronin G, to reach the base of the gel.

2.3 Cytological Estimation of Non-pairing in Ditelocentric Crosses

Anthers of F_1 plants estimated to be at the first metaphase of meiosis were fixed in one part of glacial acetic acid and three

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Table 1. Assignment of symbols to genes which code for gliadins and glutenin subunits

Protein	Gene location	Gene description
HMW subunits of glutenin	IAL	*Glu-A1*
	IBL	*Glu-B1*
	IDL	*Glu-D1*
ω-gliadins	IAS	*Gli-A1*
	IBS	*Gli-B1*
	IDS	*Gli-D1*

parts absolute ethanol and stained using a Feulgen procedure. Pollen mother cells were studied to estimate pairing of the telocentric chromosome with its homologous, whole chromosome.

3 Results

3.1 Assignment of Symbols to Genes Coding for the HMW Subunits of Glutenin and the ω-gliadins

The detailed assignment of symbols to genes on the homoeologous group 1 chromosomes which code for gliadins and glutenin subunits is listed in Table 1. The symbols conform with the standardised nomenclature for wheat chromosomes (McIntosh 1973) and they have been accepted by Dr. R.A. McIntosh, the primary authority for the recording of gene symbols in wheat (Anon 1968).

3.2 Recombination Frequencies Between Glutenin Subunit Genes and Gliadin Genes on the Group 1 Chromosomes

3.2.1 Genetic Theory

Two complex crosses were made and were of the type: (F_1 variety A × variety B) ♀ × variety C (♂). Parents of the primary crosses were chosen so that they had different allelic HMW subunits of glutenin for the chromosome in question and different allelic ω-gliadins. This enabled the genetic distances between *Glu-A1* and *Gli-A1*, and between *Glu-B1* and *Gli-B1* (two determinations) to be estimated. A similar crossing scheme was set up for the second mapping experiment involving *Glu-A1* and *Gli-A1* except the parents were euploid 'Chinese Spring', an intervarietal chromosome substitution line of 'Chinese Spring' and a ditelocentric line of the same variety (Table 2). This enabled the chromosome 1A ω-gliadins to be scored particularly easily by SDS-PAGE because no other ω-gliadins, coded by genes on other chromosomes, would be segregating.

In all the crosses analysed, only two parental-type progeny (P) and two types of recombinant (R) are produced and each can be recognised unambiguously. For example, in the cross ('Lancota' × 'Flanders') × 'Sonora 64', the parental-type progeny contain (1) subunit 13 and ω_2-gliadin (both from 'Lancota') or (2) subunit 6 and ω_1-gliadin (both from 'Flanders') whereas the recombinants contain (1) subunit 13 and ω_1-gliadin or (2) subunit 6 and ω_2-gliadin. This testcross procedure has distinct advantages over F_1 selfing and back cross methods as discussed by Payne et al. (1981 b).

The map distances in centiMorgans (cM) were calculated from the recombination frequency using the Kosambi function (Kosambi 1944); $cM = 25 \times \log_e [(100+2R) \div (100-2R)]$, where R is the recombination percentage. The standard deviation of the map distance was calculated from $2{,}500 \times S_R \div (2{,}500-R^2)$, where S_R is the standard deviation of R.

3.2.2 Evidence for the Chromosomal Location of the Genes Coding for the HMW Subunits of Glutenin and the ω-gliadins

The gene locations of the HMW subunits have been studied extensively using various aneuploid lines of

Table 2. Recombination frequencies between HMW subunit genes and gliadin genes on the group 1 chromosomes

The cross (A × B) × C	Chromosome	Progeny No.	P		R		χ^2	Conclusion	R (%)	Map distance (cM)
			glu A / gli A	B / B	A / B	B / A				
[CS × CS (Hope 1A)] × CS DT 1AL	1A	220	59	57	56	48	0.68	No linkage	47 ± 3.4[a]	
(Hope × Cappelle-Desprez) × Highbury	1A	348	101	102	80	65	9.25	Linkage	42 ± 2.6[a]	
(Hope × Cappelle-Desprez) × Highbury	1B	361	106	113	73	69	15.6	Linkage	39 ± 2.6[a]	
(Lancota × Flanders) × Sonora 64	1B	279	66	81	66	66	0.54	No linkage	47 ± 3.0[a]	
Pooled data	1A + 1B	1208	332	353	275	248	21.3	Linkage	43 ± 1.4[a]	66 ± 5.7[a]

[a] Standard deviation

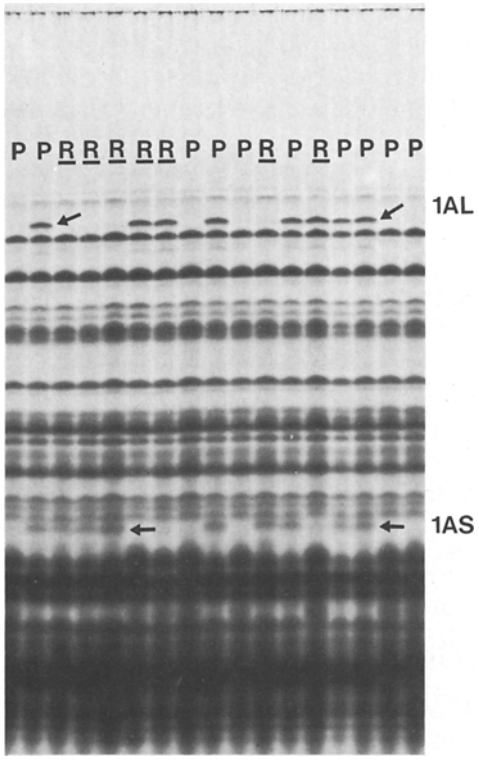

Fig. 1. Analysis of 17 progeny from the cross ['Chinese Spring' × 'Chinese Spring' ('Hope' 1A)] × 'Chinese Spring' DT 1AL by SDS-PAGE. The HMW subunit of glutenin controlled by the 'Hope' 1A chromosome is indicated by a diagonal arrow and by 1AL. The ω-gliadin controlled by genes on the 1A chromosome of 'Hope' is arrowed horizontally and marked 1AS. Parental-type progeny are marked P and recombinants R

several varieties and many intervarietal chromosome substitution lines (Payne et al. 1980; Lawrence and Shepherd 1981a). In the varieties and genetical lines used in this study, subunits having identical mobilities during SDS-PAGE to those whose gene location is known from the above studies, were assumed to be controlled by genes on the long arms of the same chromosome.

The chromosome 1B ω-gliadins were recognised from their distinctive mobilities in SDS-PAGE (Fig. 2). They have a slower mobility than any of the other ω-gliadins but have a slightly faster mobility than the HMW subunits of glutenin. Two-dimensional electrophoresis, using SDS-PAGE in the first dimension and APAGE in the second, confirms their identification as ω-gliadins (Payne et al. 1982). That their genes are located on chromosome 1B comes from an analysis of the aneuploid wheat lines described above (unpublished data).

The identification of chromosome 1A ω-gliadins in progeny, marked 1AS in Fig. 1, is simple and unambiguous. This is because a chromosome 1A substitution line was crossed with the corresponding recipient variety so that any changes in protein pattern must be due to genes located on chromosome 1A. The protein band, marked 1AS, has the correct mobility for an ω-gliadin.

The strongly-stained protein band marked as 1AS in Fig. 2 is inherited from 'Cappelle-Desprez' and has the electrophoretic mobility in SDS-PAGE which is expected for chromosome 1A and 1D ω-gliadins. This protein is absent in euploid 'Chinese Spring' and 'Chinese Spring' ('Cappelle-Desprez' 1D) but is present in 'Chinese Spring' ('Cappelle-Desprez' 1A). The protein (1AS, Fig. 2) must therefore be coded by chromosome 1A.

3.2.3 Results

A selection of the progeny analysed by SDS-PAGE from the cross involving 'Chinese Spring' and a chromosome substitution line of this variety which contains the 1A chromosome of 'Hope' is shown in Fig. 1. The HMW subunit from 'Hope' is indicated by diagonal arrows and the 'Hope' ω-gliadin by horizontal arrows. Almost as many recombinants were scored as were parentals (Fig. 1, P and R) and this was borne out in

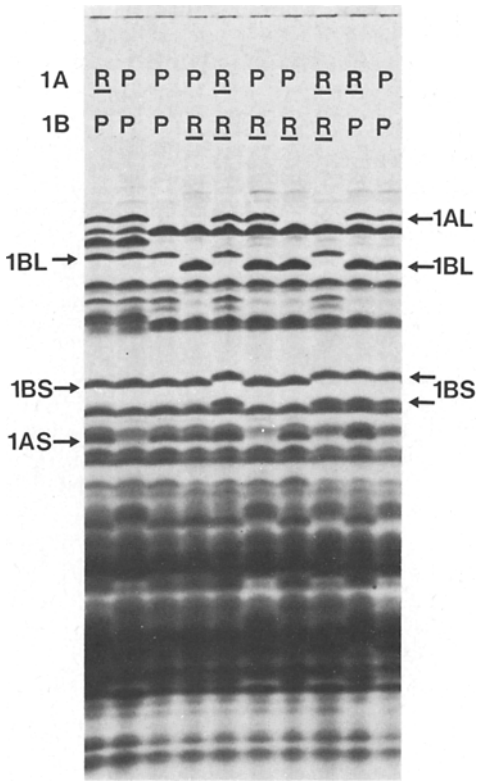

Fig. 2. Analysis of 10 progeny from the cross ('Hope' × 'Cappelle-Desprez') × 'Highbury' by SDS-PAGE. The HMW glutenin subunit variants (1AL, 1BL) and those of the ω-gliadins (1AS, 1BS) for mapping on chromosome 1A and 1B respectively are marked

the full analysis of 220 progeny (Table 2), the percentage recombination being 47%. Thus no significant linkage was demonstrated between the genes for the HMW glutenin subunit and the ω-gliadin showing they are widely separated on the two arms of chromosome 1A. Similar results and conclusions were reached from the cross ('Lancota' × 'Flanders') × 'Sonora 64' for the proximity of HMW subunit genes and ω-gliadin genes on chromosome 1B (Table 2; for SDS-PAGE separation, see Fig. 5 e of Payne et al. 1981 b).

In the case of the cross ('Hope' × 'Cappelle-Desprez') × 'Highbury', recombination was scored between *Glu-1* and *Gli-1* for both chromosome 1A (Fig. 2; 1AL versus 1AS) and chromosome 1B (Fig. 2; 1BL versus 1BS). In contrast to the two previous crosses, linkage between the storage-protein genes was significant although the recombination percentages, 42 for chromosome 1A and 39 for chromosome 1B, were only slightly lower (Table 2) than the previous results. When the four recombination percentages, which ranged from 39 to 47, were compared by a heterogeneity χ^2, they were shown not to be significantly different from each other ($\chi^2_{[3]}=4.77$, $P=0.2-0.1$). It was thus possible to pool the data and obtain a combined estimate of recombination between *Glu-1* and *Gli-1* loci on chromosomes 1A and 1B. Recombination was calculated to be 43% and the linkage between the genes was significant (Table 2). Using the Kosambi function (Kosambi 1944) the map distance between *Glu-1* and *Gli-1* was calculated to be 66 ± 5.7 cM.

A further comparison can also be made amongst the classified progeny of the cross ('Hope' × 'Cappelle-Desprez') × 'Highbury'. As mentioned, this cross permits the classification of allelic variation at *Glu-A1, Gli-A1, Glu-B1, Gli-B1* and also at *Glu-D1*. It is possible to determine the degree of association that occurs not only between loci on the same chromosome, but also between loci on different chromosomes. Such between-chromosome studies may reveal evidence of a non-random segregation of quasi-linkage which has been reported in other organisms and is often referred to as affinity (Wallace 1953). The segregational patterns between the glutenin and gliadin genes are given in Table 3. Apart from those storage protein genes which occur on the same chromosome, no significant associations were found.

3.3 Recombination Frequency Between Glu-A1 on the Long Arm of Chromosome 1A and the Centromere

3.3.1 Genetic Theory

The following crosses were made:

CS ditelosomic 1AL × CS (*T. sp.* 1A)

↓

F_1 × CS ditelosomic 1AL

♀ ♂

CS ditelosomic 1AL is a line of 'Chinese Spring' (CS) which lacks the short arm of chromosome 1A. CS (*T. sp.* 1A) is an intervarietal chromosome substitution line which is genetically identical to CS except the 1A chromosome of *Triticum spelta* has replaced its homologue in CS. This line was selected because the HMW glutenin subunit and the ω-gliadins of the *T. spelta* 1A chromosome can be distinguished easily when progeny

Table 3. Joint segregation of HMW subunits of glutenin coded by chromosomes 1A, 1B and 1D and ω-gliadins coded by chromosomes 1A and 1B in progeny of the cross ('Hope' × 'Cappelle-Desprez') × 'Highbury'

Protein type		Glutenin Subunits					
		Chromosome 1A Hope	Cappelle	Chromosome 1B Hope	Capelle	Chromosome 1D Hope	Cappelle
Glutenin subunits							
Chromosome 1B	Hope	80	95	–	–	–	–
	Cappelle	93	93	–	–	–	–
Chromosome 1D	Hope	74	96	83	87	–	–
	Cappelle	99	92	92	99	–	–
ω-gliadins							
Chromosome 1A	Hope	101[a]	80[a]	86	95	85	96
	Cappelle	65[a]	102[a]	83	84	81	86
Chromosome 1B	Hope	84	95	106[a]	73[a]	84	95
	Cappelle	89	93	69[a]	113[a]	86	96

The recombinant numbers in each group are underlined

[a] Significant at $P<0.05$ by χ^2 analysis

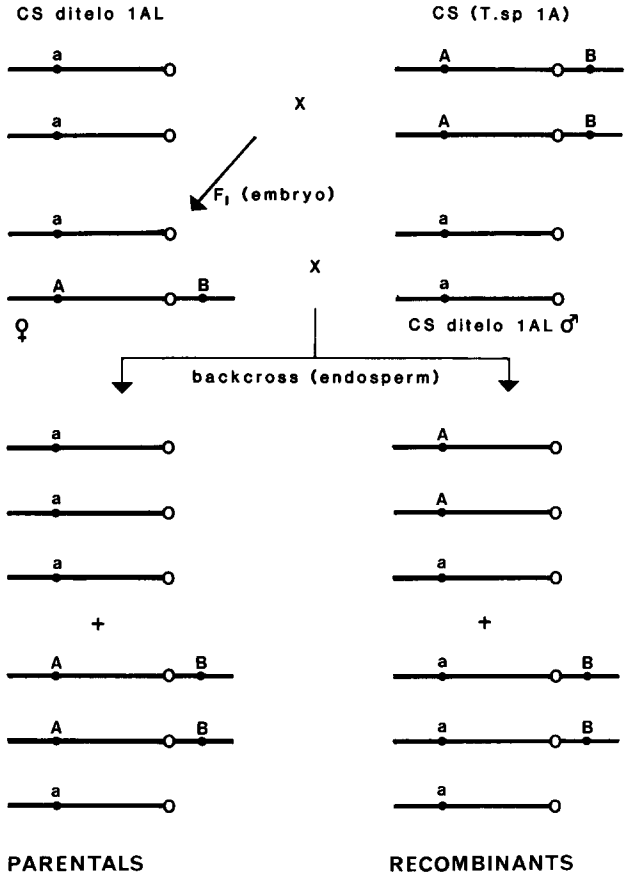

Fig. 3. Progeny types expected from the cross [CS DT 1AL×CS (*T. sp.* 1A)]×CS DT 1AL. If no cross over occurs between *Glu-A1* and the centromere, two parental type progeny are formed (left). The recombinants arise from crossing over between these two points on the chromosome (right). *Glu-A1* locus: gene a codes for the HMW subunit of glutenin in CS and gene A codes for the equivalent protein in *T. spelta*. *Gli-A1* locus: gene B, which codes for the *T. spelta* ω-gliadin, is the marker for the short arm of chromosome 1A from *T. spelta*

are screened by SDS-PAGE. Apart from chromosome 1A, the chromosome constitution of the two parents is identical so that allelic variation for other storage proteins is eliminated, making the interpretation on polyacrylamide gels both simple and unambiguous.

The possibe types of progeny expected from this cross are derived in Fig. 3. Chromosomes were not examined at mitosis to determine whether the short arms of chromosome 1A of *T. spelta* were present or not in the progeny of the second cross because this information was given in SDS-PAGE by the presence or absence of *T. spelta* ω-gliadin. As in previous crosses, there were two parental types only (1) *T. spelta* HMW subunit and ω-gliadin both present and (2) *T. spelta* proteins both absent, and two recombinants, (1) *T. spelta* HMW subunit present and *T. spelta* ω-gliadin absent and (2) *vice versa*.

Telocentric chromosomes can be used to determine gene-centromere distances provided the telocentric chromosome pairs with the complete chromosome at the same frequency as occurs for complete homologues. Otherwise the estimates of the map distances between the gene and the centromere will be underestimated. To establish whether this was a major source of error in any of the telocentric crosses, F_1 plants were examined cytologically and the extent of non-pairing determined at metaphase I of meiosis as described in the methods.

3.3.2 Results

A selection of SDS-PAGE analyses is shown in Fig. 4. In contrast to the gliadin-glutenin mapping experiments, many parental-type progeny were obtained. In the complete analysis of 210 progeny, only 16 recombinants were detected (Table 4) showing that the HMW glutenin genes on chromosome 1A are tightly linked to the centromere, the map distance being 7.7 ± 1.8 cM. Non-pairing was low (Table 5) so the map distance was only very slightly underestimated.

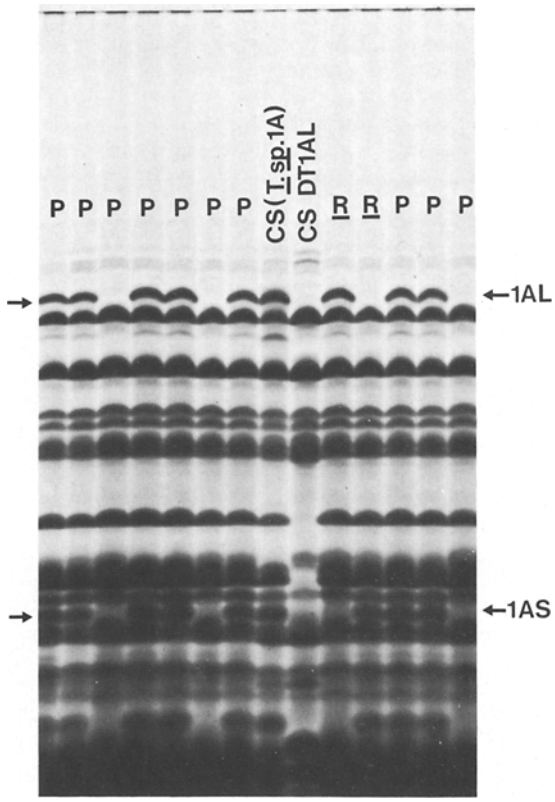

Fig. 4. Ditelocentric mapping of *Glu-A1*. The parents ['Chinese Spring' DT 1AL and 'Chinese Spring' (*T. sp.* 1A)] and 12 progeny are analysed by SDS-PAGE. The presence or absence of the short arm of a *T. spelta* 1A chromosome is detected by the presence or absence of a *T. spelta* ω-gliadin marked 1AS. The presence or absence of the *Glu-A1* allele from *T. spelta* which produces subunit 1 is shown by the presence or absence of the band marked 1AL

Table 4. Recombination frequencies between HMW glutenin subunit genes and the centromere

Chromosome	Progeny No.		P		R		Linkage to centromere (%R)	cM
		T. sp 1A short arms	//	×	//	×		
		T. sp 1A HMW subunits	//	×	×	//		
1A	210		90	104	10	6	7.6±1.8[a]	7.7±1.8[a]
			P		R			
		Koga 1B short arms	//	×	//	×		
		Koga 1B HMW subunits	//	×	×	//		
1B	195		93	84	8	10	9.2±2.1[a]	9.3±2.2[a]
			P		R			
		Koga 1D short arms	//	×	//	×		
		Koga 1D HMW subunits	//	×	×	//		
1D	178		80	80	7	11	10.1±2.3[a]	10.2±2.4[a]
1A, 1B+1D	583		263	268	25	27	8.9±1.2[a]	9.0±1.2[a]

[a] Standard deviation

Table 5. Estimation of non-pairing between long-arm telocentric chromosomes and whole chromosomes during meiosis

Chromosome	Nuclei No.	Paired	Non-paired	% Non-pairing
1A	104	94	10	9.6
1B	102	97	5	4.9
1D	103	98	5	4.9
1A+1B+1D	309	289	20	6.5

3.4 Recombination Frequency Between Glu-B1 and the Centromere

3.4.1 Genetic Theory

The crosses made were:

CS ditelosomic 1BL × 'Koga II'

F_1 × CS ditelosomic 1BL

♀ ♂

Although the types of parentals and recombinants are comparable to the equivalent cross using the 1A ditelosomic stocks of CS, they are distinguished differently by SDS-PAGE. This is because CS produces HMW subunits of glutenin coded by chromosome 1B but not subunits coded by chromosome 1A. The two parental types were identified by having either (1) CS chromosome 1B HMW subunits present at 3 doses with the absence of both the chromosome 1B HMW subunits and the chromosome 1B ω-gliadins of 'Koga II' or (2) 1 dose of CS chromosome 1B HMW subunits and from 'Koga II', 2 doses each of the chromosome 1B HMW subunits and ω-gliadins. In contrast, the recombinants have either (1) 1 dose of the CS HMW subunits, 2 doses of the 'Koga II' HMW subunits and the 'Koga II' chromosome 1B ω-gliadins lacking, or (2) 3 doses of the CS HMW subunits, the absence of the 'Koga II' HMW subunits but the presence at two doses of the 'Koga II' ω-gliadin. As shown in Fig. 5, these 4 types of progeny can be readily distinguished by SDS-PAGE.

3.4.2 Results

As with the equivalent cross using CS ditelosomic 1AL, few recombinant types were present amongst the progeny (Table 4), indicating that the HMW subunit genes are located on the long arm close to the centromere. Chromosome non-pairing was also low (Table 5). The map distance was calculated as 9.3±2.2 cM.

3.5 Recombination Frequency Between Glu-D1 and the Centromere

3.5.1 Genetic Theory

The crosses made were equivalent to those for the mapping on the long arm of chromosome 1B:

CS ditelosomic 1DL × 'Koga II'

F_1 × CS ditelosomic 1DL

♀ ♂

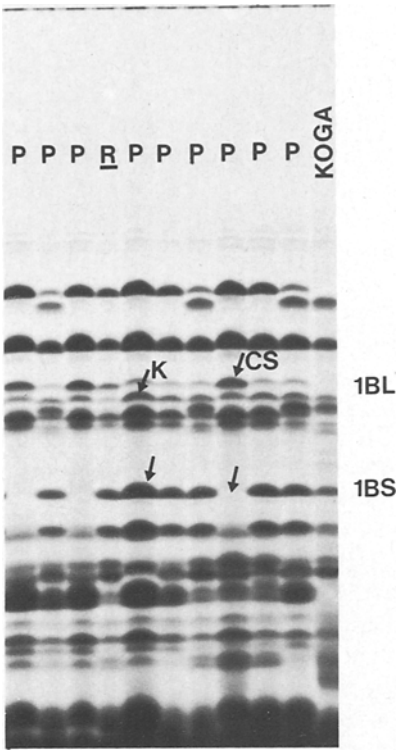

Fig. 5. Ditelocentric mapping of *Glu-B1*. The analysis by SDS-PAGE of one of the parents ('Koga II') and 10 progeny are illustrated. The presence or absence of the short arm of the 'Koga II' 1B chromosome is detected by the presence or absence of the 'Koga II' ω-gliadin marked 1BS. In the 1BL area of the electrophoretogram, the presence of three doses of the 'Chinese Spring' chromosome 1B HMW subunit is marked "CS" and the presence of one dose of the same subunit plus two doses of the 'Koga II' chromosome 1B HMW subunit is marked "K"

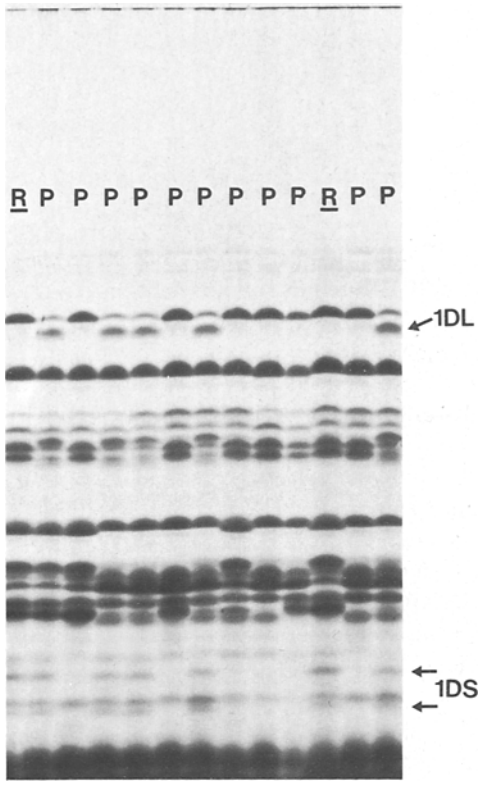

Fig. 6. Ditelocentric mapping of *Glu-D1*. Subunit 5, the HMW subunit derived from 'Koga II' is marked 1DL. The presence of the short arm of chromosome 1D from 'Koga II' is determined by the presence or absence of a pair of uncharacterised bands marked 1DS

The types of progeny expected are equivalent to those from the chromosome 1B ditelocentric cross. Because the ω-gliadins of 'Koga II' could not be identified readily by SDS-PAGE, individual grains were cut into sections: one was used to characterise the chromosome 1D HMW subunits by SDS-PAGE (Fig. 6) and another by APAGE to identify the relevant ω-gliadins (arrowed, Fig. 7). It was shown that in the 75 progeny analysed, two protein bands detected by SDS-PAGE (marked 1DS, Fig. 6) were always present in grains which possessed the slow-moving chromosome 1D ω-gliadins in acid gels (arrowed, Fig. 7) and they were absent in grains lacking chromosome 1D ω-gliadins. In the remaining grains, therefore, only one gel system, SDS-PAGE, was used.

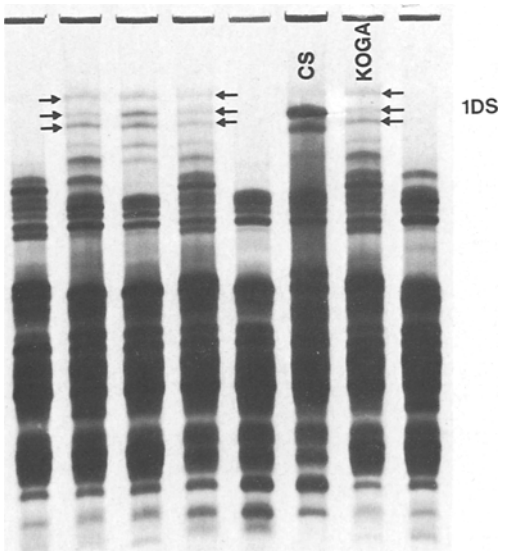

Fig. 7. APAGE of ethanol extracts from progeny derived for the ditelocentric mapping of *Glu-D1*. The presence of the 'Koga II' 1DS chromosome halves is shown by three horizontal arrows and marked 1DS. The chromosome 1D ω-gliadins of 'Koga II' and 'Chinese Spring' are also shown

3.5.2 Results

A similar percentage of recombinants was detected in this ditelocentric cross compared to that of the previous

two, the map distance from the HMW subunit genes to the centromere being 10.2 ± 2.4 cM (Table 4). Only 4.9% of those meiotic nuclei examined appeared not to have paired (Table 5).

4 Discussion

The telocentric mapping experiments described here show that the HMW glutenin subunit genes are tightly linked to the centromere on the long arms of chromosomes 1A, 1B and 1D. When the linkage data for each chromosomes were compared they were shown not to be significantly different from each other (heterogeneity $\chi^2_{[2]}=0.77$, P = 0.7–0.5) and so were pooled (Table 2). The combined estimate of percentage recombination was 8.9 ± 1.2 and the map distance, 9.0 ± 1.2 cM. Similarly, the three sets of telocentric non-pairing data were indistinguishable (heterogeneity $\chi^2_{[2]}=2.56$, P = 0.3–0.2) and the pooled estimate was shown to be 6.5 ± 2.8%. It is possible to use this information to correct the estimate of recombination using the formula

$$\frac{R}{P+R} = p \cdot x,$$

where the observed number of parental-types and recombinants are P and R respectively; x is the frequency of pairing and p is the corrected frequency of recombination. This gives an adjusted estimate of recombination of 9.5%, which is hardly indistinguishable from the original estimate of 8.9%. Pairing failures were therefore not a major distortion in estimating the map distances between the *Glu-1* genes and their respective centromeres.

The finding that the HMW glutenin genes *Glu-A1* and *Glu-B1* are located at similar positions close to the centromere on the long arms of their respective chromosomes supports the concept that they are homoeoallelic. The same genes are, however, only weakly linked to the gliadin genes *Gli-A1, Gli-B1* and *Gli-D1* indicating that the latter are located distally on the short arms of the group 1 chromosomes. Direct, but preliminary, evidence from the ditelocentric mapping of *Gli-A1* and *Gli-B1* to the respective centromeres supports this view (Payne, Holt, Worland and Law, unpublished data).

In similar studies to ours, Lawrence and Shepherd (1981b) showed an independent segregation of glutenin and gliadin genes on chromosome 1B in progeny of a cross between an Australian variety and an Indian breeding line. Their value for recombination between the two genes (48.8%) is significantly different from our pooled value of 43.3%. However, this small difference in recombination is not entirely unexpected since it is known that the relatedness of parents of the crosses and the temperature at which the plants are grown at the time of meiosis can affect the rate of crossing over (Law 1961; Wilson 1959). To obtain much more reliable map distances, other marker genes are required which lie in between the two storage-protein genes.

The gene symbols for gliadin and glutenin were chosen where possible so that they are comparable with the symbols used to describe the genes coding for the hordeins, the storage proteins of barley. Thus, the ω-gliadin genes are described as *Gli-1* because in barley the C-hordeins are homologous to the ω-gliadins (Shewry et al. 1980a) and their genes are located at the *Hor-1* locus (Doll and Brown 1979). Unfortunately, difficulties arise in gene descriptions of the HMW subunits of glutenin and D-hordein, for, although they are homologous proteins as judged by biochemical analysis (Field et al. 1982) the gene mapping nomenclature is different. In barley, all the storage proteins are called hordein (Shewry et al. 1980b) whereas in wheat those proteins which spontaneously aggregate have been traditionally called glutenin rather than gliadin (Wall 1979). Hence the two loci bearing genes for these two related groups of proteins are called *Hor-3* for barley (Shewry and Miflin 1982) and *Glu-1* for wheat. Another major locus for storage proteins in barley is *Hor-2* which codes for B-hordein and shows between a 6–13% linkage to *Hor-1* on chromosome 5 (Shewry et al. 1980c; Sozinov et al. 1979). Unfortunately, the N-terminal amino-acid sequence of B-hordein cannot be determined because the N-terminal amino acid is chemically blocked (Shewry et al. 1980a). Less stringent biochemical studies indicate a possible homology with the α- and β-gliadins (Shewry et al. 1980b). If this homology is subsequently shown to be authentic, then the wheat locus would be appropriately described as *Gli-2*. Currently, very little is known about the complexity of the genes coding for the α- and β-gliadins except that they are located on the short arms of the group 6 chromosomes (Payne et al. 1982) and are probably located at one, complex locus (Mecham et al. 1978; Sozinov and Poperelya 1980). The position on the short arms of *"Gli-2"* in relation to the centromere is not known. If *Hor-2* and *Gli-2* are indeed homologous, then a translocation must have occurred to account for *Hor-1* and *Hor-2* being on the same chromosome and closely linked in barley and *Gli-1* and *Gli-2* being on different chromosomes in wheat, as was suggested by Kasarda (1981).

The complex, locus, *Gli-1,* probably contains genes for the γ-gliadins in addition to those for ω-gliadins because Damidaux et al. (1980) did not detect any recombination between ω- and γ-gliadins coded by chromosome 1B in 209 progeny of a tetraploid wheat cross. These results are supported by the extensive work of Sozinov and his colleagues (for review see Sozinov and Poperelya 1980) who showed that the storage proteins coded by genes on each of the short

arms of the group 1 chromosomes are inherited as "blocks". Mecham et al. (1978) also demonstrated a close linkage between γ- and ω-gliadin genes in two hexaploid wheat crosses but they did detect a very low level of recombination, indicating two short-arm loci rather than one, but very tightly linked, about 0.7–4.0 cM apart. As pointed out by these authors, interpretation of some progeny in one dimensional-gels, where many different proteins coded by several different chromosomes are segregating, can be difficult. To try and resolve the issue of whether the γ- and ω-gliadin genes occur at one locus or at two tightly-linked loci, we have set up three crosses between genetic lines, one for each group 1 chromosome. The type of cross, exemplified for chromosome 1B is: [CS×CS ('Timstein' 1B)]×CS ditelosomic 1BL. The F_1 progeny from the primary cross will only be heterozygous for the chromosome 1B genes and so the subsequent segregation of the gliadin genes in progeny of the secondary cross can be followed unambiguously because the third parent, CS ditelosomic 1BL, lacks completely the same gliadin genes on the short arms of chromosome 1B. As proteins coded by all other chromosomes will not be segregating, the risk of misinterpretation of the APAGE separations will be reduced considerably.

In conclusion, the wheat genome may be envisaged as containing a limited number of unlinked but complex loci which contain many genes coding for a large number of different storage proteins. The genes at each locus display allelic variation and to different extents. Many different combinations of alleles are therefore possible and this explains the great variation in protein electrophoretic pattern between varieties (Bushuk and Zillman 1978, Zillman and Bushuk 1979). The particular combination of storage proteins in a variety principally governs its protein quality (Payne et al. 1981a) and is partially responsible for determining whether the variety is suitable for conversion into bread, biscuits or animal feeds.

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Note Added in Proof

After acceptance of this manuscript, we became aware of a paper in Russian by A. I. Rybalka and A. A. Sozinov (1979) entitled "Mapping the locus of Gld 1B, which controls the biosynthesis of reserve proteins in soft wheat" (Tsitologiyai Genetika 13, 276–282). Using telocentric mapping procedures, they obtained a recombination of $41.6 \pm 2.5\%$ between the gliadin locus on chromosome 1BS and the centromere. This is similar to our value of 34%, calculated by subtracting the recombination between *Glu-1* and the centromere from the recombination between *Glu-1* and *Gli-1*.