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Genetic linkage between endosperm storage protein genes on each of the short arms of chromosomes 1A and 1B in wheat

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Summary. The storage proteins of the endosperm of wheat grain which are known to be controlled by genes on the short arms of the homoeologous group 1 chromosomes are (1) the ω-gliadins, (2) most of the γ-gliadins, (3) a few β-gliadins and (4) the major low-molecular-weight subunits of glutenin. Several crosses were made between varieties or genetic lines which had contrasting allelic variants for some of these proteins and which were coded by genes on chromosomes 1A or 1B. The progeny were analysed by one or more of several electrophoretic procedures. The results of all the analyses are consistent with the hypothesis that chromosomes 1A and 1B each contain just one, complex locus, named *Gli-A 1* and *Gli-B 1* respectively, which contain the genes for the ω-, γ- and β-gliadins and the low-molecular-weight subunits of glutenin.

Key words: Gliadin – Glutenin – *Triticum* – Gene linkage – Electrophoresis

1 Introduction

The majority of the gliadin proteins of wheat endosperm are simple polypeptides without subunit structure under normal conditions of extraction. These, the "classical" gliadins, typically fractionate into α-, β-, γ- and ω-gliadins by electrophoresis in starch or polyacrylamide gels using aluminium lactate buffer (Bushuk and Zillman 1978). When fractionated by two dimensional electrophoretic systems many more components separate, approximately 40 (Wrigley 1970), and half of them (all the ω-gliadins, most of the γ-gliadins and a few of the β-gliadins) are now known to be coded by genes on the short arms of chromosomes 1A, 1B and 1D (Wrigley and Shepherd 1973; Payne et al. 1982 b).

The linkage between the gliadin genes on each of these chromosomes has been studied by several groups, particularly those in the USSR. Sozinov and colleagues were the first to convincingly demonstrate that gliadins, when coded by genes on the same chromosome, are inherited strictly as a block (Sozinov et al. 1979; Sozinov and Poperelya 1980). Similar conclusions were reached by Bebyakin and Balabolina (1977) and by Mitrofanova (1979). In the USA, Mecham et al. (1978) analysed the selfed, F_2 progeny of two crosses. Gliadin proteins were shown to be inherited as several blocks but in one cross a small amount of recombination occurred within a block (0.5%–4%) between ω- and γ-gliadins thought to be coded by chromosome 1B. Very recently, Branlard (1983) analysed by starch gel electrophoresis the inheritance of gliadin proteins in the F_2 generation of two crosses between French varieties. As well as concluding that some individual gliadin components are coded by two independent genes, Branlard (1983) showed that band 44 was heterogeneous and consisted of two gliadins whose genes he concluded occur on the same chromosome but 25 recombination units apart.

Thus, from all these results it is not clear whether each of the short arms of the homoeologous group 1 chromosomes have a single, complex locus coding for the classical gliadins or two or more loci. Recently, another major group of storage proteins in wheat, the low-molecular-weight (LMW) subunits of glutenin were also shown to be located on the short arms of the homoeologous group 1 chromosomes (Jackson et al. 1983).

In this communication, the linkage between genes for the ω-, γ- and β-gliadins on chromosomes 1A and 1B have been reappraised by analysing the progeny of crosses, some of which have been designed to give results which are much easier to interpret than those published previously. The linkage between these genes and those for the LMW subunits of glutenin was also determined.

2 Materials and methods

The intervarietal chromosome substitution lines of 'Chinese Spring' (CS), namely CS ('Timstein' 1B), CS (*Triticum spelta* 1A), CS ('Cappelle-Desprez' 1B), CS ('Hope' 1B) and 'Koga II' ('Bersée' 1B) and the ditelocentric (DT) lines, CS DT 1AL and CS DT 1BL, were taken from stocks held in the Cytogenetics Department of the Plant Breeding Institute, Cambridge. A substitution line, taking CS ('Timstein' 1B) as an example, has the same genetic constitution as euploid 'Chinese Spring' except that its 1B chromosome has been replaced by its homologue from the variety 'Timstein'. A ditelocentric line has lost a homologous pair of chromosome arms, as in CS DT 1BL where the short arms of chromosome 1B are missing. One nullisomic (N), tetrasomic (T) line of 'Chinese Spring', namely CS NIA TID, was also used in one of the crosses. This line lacks chromosome 1A but contains twice the normal dosage of 1D chromosomes, thereby having the same chromosome number, 42, as euploid 'Chinese Spring'. Varieties used in crosses, or as references for gel electrophoresis, were taken from the Institute's collection.

2.1 Sodium dodecyl sulphate, polyacrylamide-gel electrophoresis (SDS-PAGE)

The method used to fractionate the total proteins of the wheat endosperm after reduction with 2-mercaptoethanol was described previously (Payne et al. 1980; Payne et al. 1981). The method fractionates proteins mainly according to their molecular weight.

2.2 Acid, polyacrylamide-gel electrophoresis (APAGE)

This method was used to fractionate gliadin proteins according to a combination of molecular size and charge at low pH. The method, modified from that of Bushuk and Zillman (1978) has been described previously (Payne et al. 1982a).

2.3 Two-dimensional electrophoresis (APAGE followed by SDS-PAGE)

Samples were prepared and fractionated by APAGE using aluminium lactate buffer at pH 3.1, as described previously except that gels were cast in glass tubes of internal diameter 5 mm and 14 cm long and 64 µl of the gliadin preparation were applied. After electrophoresis, the gel rods were frozen horizontally in 5 ml of equilibration solution (10% (w/v) glycerol, 2.3% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 62.5 mM Tris-HCl, pH 6.8) as described by O'Farrell (1975) and stored at −10 °C until required. Prior to electrophoresis in the second dimension, the gel rods were taken from storage and gently rotated at room temperature in 20 ml of fresh equilibration solution for 45 min, with a change of solution after 15 min. For the second dimension, a 10% (w/v) polyacrylamide gel slab 16 cm × 14 cm × 4 mm thick with a 1 cm layer of stacking gel was made, as described by Laemmli (1970). An APAGE gel rod was placed horizontally onto the stacking gel of the SDS-PAGE slab. In some experiments, 20 µl of the gliadin preparation was mixed with 20 µl of 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 0.001% (w/v) pyronin Y, 10% (v/v) glycerol and 0.063 M Tris-HCl, pH 6.8. The mixture was set in a small volume of 1% v/v agarose made up in 0.13 M Tris-HCl, pH 6.8, and placed at the end of the pH 3.1 gel rod. Gel slabs were subjected to electrophoresis at a constant current of 20 mA (about 40 V) for 20 h. Gels were stained as described previously (Payne et al. 1980).

2.4 Classification of individual protein bands into storage protein groups and their genes to chromosomes

Varieties and genetic lines were selected for crossing which had contrasting allelic variation in two or more of the following groups: ω-gliadins, γ-gliadins, β-gliadins and LMW subunits of glutenin. In cross 1 (Table 1), variation was restricted to gliadin groups. Accordingly, proteins were extracted in aqueous ethanol and fractionated, without reduction of disulphide bonds, by APAGE. The protein group, to which each of the segregating proteins belonged, was deduced from their relative mobilities (Fig. 1., using the nomenclature of Woychick et al. 1961). In this electrophoretic system, the LMW glutenin subunits occur in their aggregated state as glutenin and so they remain at the origin of the gel or migrate in a polydispersed manner, imparting a background stain to the gel (Charbonnier 1973).

In the remaining crosses (2–4, Table 1), which all involved linkage studies with LMW glutenin subunits, the proteins of progeny were necessarily extracted with SDS and the reducing agent, 2-mercaptoethanol, and then fractionated by SDS-PAGE. Unfortunately this procedure does not directly distinguish LMW glutenin subunits from gliadins, or γ-gliadins from some ω-gliadins because these groups have overlapping electrophoretic mobilities in this system. To identify which electrophoretic band belonged to which protein group, protein extracts from each of the parents of the crosses were fractionated by Sephadex G-100 gel filtration chromatography as described by Payne and Corfield (1979). The proteins which eluted immediately after the void volume, because of their large size, were defined as high-molecular-weight (HMW) gliadin/LMW glutenin (for a discussion of terms see Jackson et al. 1983). After reduction the fraction was fractionated by SDS-PAGE so enabling the segregating LMW subunit of glutenin in the progeny to be identified. The polypeptides which were retarded by the column because of their smaller size, and which eluted as a second broad fraction were defined as classical gliadin, the ω-gliadins being the first to elute (Payne and Corfield 1979). To be absolutely sure of the designation of gliadins to the ω- γ- and β-classes, all parents were fractionated by a two-dimensional electrophoretic procedure. APAGE was used in the first dimension to split the gliadins into their sub-groups, and SDS-PAGE in the second dimension to enable the segregating gliadins screened by SDS-PAGE in progeny testing to be identified (results not shown).

In two of the four crosses analysed, namely [CS × CS (Timstein 1B)] × CS DT 1BL and [CS × CS (*T. spelta* 1A)] × CS DT 1AL, or × CS NIA TID, the chromosome location of the genes controlling the segregating proteins is unambiguous because only genes on one pair of chromosomes can be segregating. The ω-, γ-, and β-gliadins analysed for linkage in the cross ('Hope' × 'Cappelle-Desprez') × 'Highbury' were all shown to be controlled by genes on chromosome 1A from an analysis by APAGE of the intervarietal chromosomal substitution lines CS ('Hope' 1A) and CS ('Cappelle-Desprez' 1A). For the remaining cross (Table 1, cross 2) the 'Bersée' ω-gliadin and LMW glutenin subunit under study were shown to be controlled by genes on chromosome 1B by SDS-PAGE analysis of the intervarietal chromosome substitution line 'Koga II' ('Bersée' 1B). Such a line was not available for 'Sappo', so less direct methods were necessary to implicate chromosome 1B in the control of the ω-gliadin and the two LMW glutenin subunits derived from this parent. When analysed by the two-dimensional electrophoretic procedures (APAGE × SDS-PAGE as described in the methods, and by the methods of O'Farrell et al. 1977) the three proteins mapped to identical positions to complementary proteins found in 'Cappelle-

Table 1. Protein classes analysed for linkage amongst progeny of various crosses. Gli is short for gliadin and glu for glutenin. Apart from 'Sappo' × 'Bersée', all crosses were of the type (Parent 1 × Parent 2) ♀ × Parent 3♂

Cross			Chromosome bearing the segregating genes	Protein class (and number of components segregating)	
Parent 1	Parent 2	Parent 3		Parent 1	Parent 2
1. 'Chinese Spring'	CS (Timstein 1B)	CS DT 1BL	1B	ω-gli (3)	ω-gli (2)
			1B	γ-gli (1)	γ-gli (1)
			1B	β-gli (1)	β-gli (1)
2. 'Sappo'	'Bersée'	–	1B	ω-gli (1)	ω-gli (1)
			1B	LMW glu (2)	LMW glu (1)
3. 'Hope'	'Cappelle-Desprez'	'Highbury'	1A	ω-gli (2)	ω-gli (1)
			1A	γ-gli (1)	LMW glu (2)
4. 'Chinese Spring'	CS (*T. spelta* 1A)	CS DT 1AL	1A	γ-gli (1)	ω-gli (1)
'Chinese Spring'	CS (*T. spelta* 1A)	CS N1A T1D	1A	–	LMW glu (2)

Desprez' (results not shown). The ω-gliadin and the two LMW glutenin subunits in this variety were shown to be controlled by chromosome 1B genes by SDS-PAGE of the substitution line CS ('Cappelle-Desprez' 1B) thus strongly suggesting control by this chromosome of the equivalent proteins in 'Sappo'.

A summary of the crosses made and the identity of the proteins analysed genetically is given in Table 1.

3 Results

3.1 Linkage between endosperm protein genes on chromosome 1B

3.1.1 [CS × CS (Timstein 1B)] × CS DT 1BL. The first two parents in this complex cross were chosen because they are genetically similar and differ from each other only with respect to their 1B chromosomes. The third parent was chosen because it has the same genetic complement of 'Chinese Spring' except it lacks the short arms of chromosome 1B and therefore genes controlling the proteins under study. The segregation of the chromosome 1B proteins from the first two parents can therefore be followed unambiguously without interference from either homoeologous proteins from the third parent or from segregating proteins whose genes, derived from any parent, are located on other chromosomes.

The primary parents of this cross have contrasting ω- and γ-gliadins (Table 1) and so the progeny were analysed by APAGE. The fractionation shown in Fig. 1, slot 1 contains the pattern of chromosome 1B ω- and γ-gliadins for 'Chinese Spring' (arrows on left-hand side) whereas the fractionation obtained for slot 7 contained only 'Timstein' ω- and γ-gliadins coded by the same chromosome (arrows on right-hand side). The protein patterns of slots 2 to 6 are all either of the 'Chinese Spring' type or of the 'Timstein' type; no evidence of recombination between the protein groups was detected, either in the examples shown in Fig. 1 or in any of the 203 progeny examined in total.

Subsequent to this analysis, it was observed that two faint bands of protein at the base of the gel in Fig. 1 were also segregating and so their genes must also be

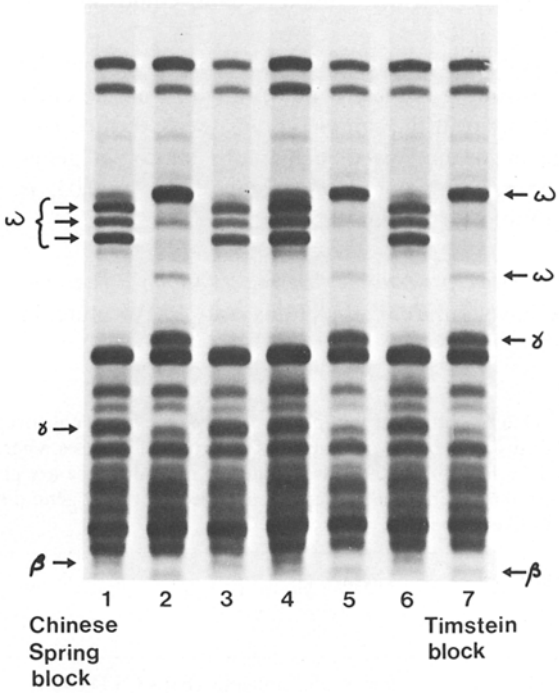

Fig. 1. APAGE separations of gliadins from seven progeny of the cross [CS × CS (Timstein 1B)] × CS DT 1BL. Samples applied to *slots 1, 3, 4* and *6* have chromosome 1B ω-, γ-, and β-gliadins inherited from 'Chinese Spring' (*arrowed on left-hand side)* and *slots 2, 5* and *7* have the equivalent proteins inherited from 'Timstein' (*arrowed on right-hand side*)

located on 1BS. The protein bands have the mobilities expected of β-gliadins. After all the APAGE fractionations of the 203 progeny were re-scrutinised it became clear that the β-band arrowed in Fig. 1, slot 1 was always inherited with ω and γ-gliadins of 'Chinese Spring' and the single band marked in Fig. 1, slot 7 was always inherited with the corresponding gliadins from 'Timstein'.

The identity of these faint bands was investigated further by analysing the two progeny types by two-dimensional analysis (APAGE×SDS-PAGE). The faint band of the 'Chinese Spring' type (Fig. 1, slot 1) corresponds to a β-gliadin spot (Fig. 2A, thin arrow), previously shown by the same two dimensional gel system to be controlled by genes on the short arm of chromosome 1B (Payne et al. 1982b). The faint band seen in the 'Timstein' derived progeny (Fig. 1, slot 7) corresponded to a faint spot (Fig. 2B, thin arrow pointing to β), which is presumably allelic to the complementary spot in 'Chinese Spring'. Another β-gliadin coded by chromosome 1B appears to be the same in both 'Chinese Spring' and 'Timstein' (Fig. 2A, B, thick arrows). Although limited allelic variation does occur for this protein (E.A. Jackson, unpublished), all the variants would be totally obscured in separations of the type shown in Fig. 1 because they co-migrate with blocks of strongly staining chromosome 6B and 6D β-gliadins. Consequently, linkage data could not be obtained.

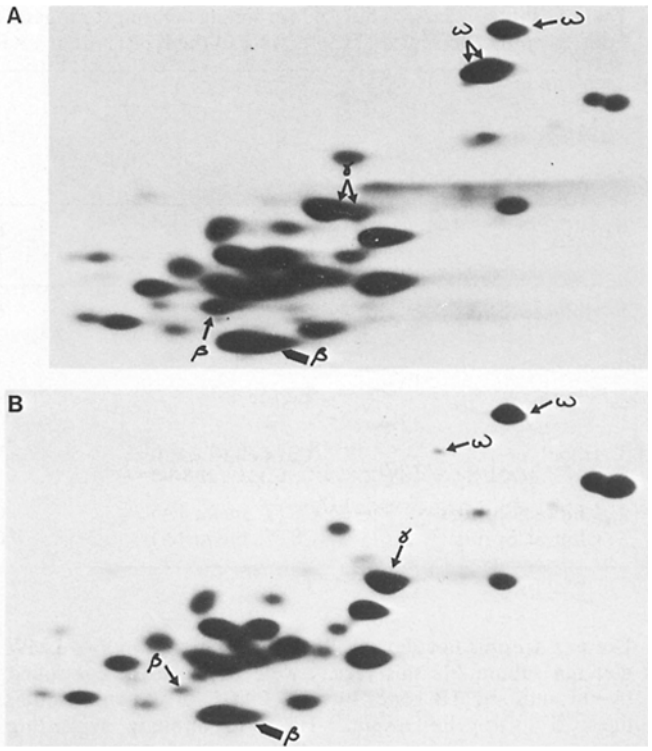

Fig. 2A, B. Two-dimensional fractionation of gliadin proteins from two progeny of the cross [CS×CS (Timstein 1B)]×CS DT 1BL. The *arrows* in (**A**) point to gliadins inherited from chromosome 1B genes of 'Chinese Spring' and in (**B**) to allelic gliadins inherited from 'Timstein'

The combined results from this cross show that the genes controlling the ω-, γ- and β-gliadins under study (Table 1) occur at the same complex locus, termed *Gli-B1*. The maximum distance that this cluster of genes could be apart, given a probability level of 0.05, is 1.5 cM (Table 2) (Hanson 1959).

3.1.2 F_2 selfed progeny of 'Bersée'×'Sappo'. The two parents have different ω-gliadins and LMW glutenin subunits coded by chromosome 1B and so the F_2 grains were analysed by SDS-PAGE. In Fig. 3, the two progeny analysed on the left-hand side have a LMw subunit and an ω-gliadin coded by chromosome WB from 'Bersée' and the two on the right have the relevant proteins from 'Sappo'. The F_1 progeny were selfed on this occasion rather than crossed to a genetic line of 'Chinese Spring' (such as CS DT 1BL) or a third variety because protein bands from either of the latter two

Table 2. Determination of the maximum distance between storage protein genes on the short arms of chromsomes 1A and 1B. The maximum distance between genes was determined in cases where no recombination was detected at the probability level of 0.05 as described by Hanson (1959). Two values of gene distance are given for the cross 'Sappo'×'Bersée' as discussed in the text. In the case where recombination had apparently occurred, the gene distance and its standard deviation was calculated by the method of maximum likelihoodness

Gene classes	Cross	Chromosome	Progeny no.	Recombinant no.	Maximum distance (cM)	Average distance (cM)
ω-, γ-, β-gli	[CS×CS (Timstein 1B)]×CS DT 1BL	1B	203	0	1.46	–
ω-gli and LMW glu	'Sappo'×'Bersée'	1B	160	0	0.93	–
			160	2	–	0.62±0.44
ω-, γ-gli and LMW glu	('Hope'×'Cappelle-Desprez')×'Highbury'	1A	348	0	0.86	–
ω-, γ-gli and LMW glu	[CS×CS (*T. spelta* 1A)]×CS DT 1AL	1A	98 } 291	0	1.02	–
	×CS N1A T1D	1A	193 }			

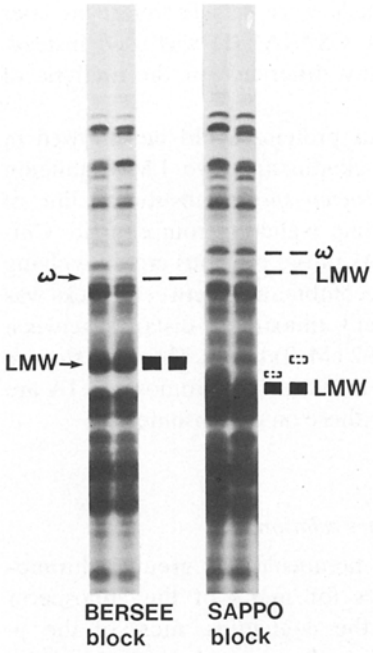

Fig. 3. SDS-PAGE separations of protein extracts from four, F_2 selfed progeny of the cross, 'Bersée' × 'Sappo'. Samples on the right have inherited the ω-gliadin and the LMW-glutenin subunits from 'Sappo' and those on the left have inherited complementary, allelic proteins from 'Bersée'. These proteins are also shown diagrammatically. The *open rectangles* indicate background proteins which have similar mobilities to the LMW subunits under study

would inevitably co-migrate with some of the LMW glutenin subunits under study and so obscure the results. Of the 311 progeny analysed, 78 had inherited the chromosome 1B ω-gliadins for 'Bersée' only and 91 had inherited the allelic ω-gliadins from 'Sappo' only, giving a total of 169 homozygotes for these genes. Of the 142 heterozygotes obtained, 71 had the 'Sappo' chromosome 1B ω-gliadin bands predominating and an equal number had the 'Bersée' ω-gliadins predominating. The results are hence close to the expected 1 : 1 : 1 : 1 ratio.

Two-dimensional electrophoresis of the proteins of the two parents showed that minor proteins, probably coded by genes on several different chromosomes, co-migrate with the LMW glutenin subunits under study (results not shown). Two such contaminating proteins are shown diagrammatically as open rectangles in Fig. 3. Because of this complication the two heterozygous types of chromosome 1B LMW glutenin subunits could not be classified by 1-dimensional gel electrophoresis. For linkage studies, therefore, only 160 progeny, which were classified as homozygous for chromosome 1B ω-gliadins, were classified into LMW glutenin subunit types. In all but two progeny, only two classes were detected: (1) chromosome 1B ω-gliadins and LMW glutenin subunits from 'Bersée' only and (2) the corresponding proteins from 'Sappo' only. The two exceptional progeny contained the 'Sappo' ω-gliadin only but the broad LMW glutenin band was intermediate in intensity between that found for progeny homozygous for this band (i.e. like 'Sappo') and that expected for heterozygous progeny. The two individuals were thought most likely to be homozygous for the 'Sappo' protein but which gave a disturbed gel pattern because they contained a particular assortment of 'background' proteins. If this is the case, then no recombinants were detected in progeny of this cross, indicating that the ω-gliadins and LMW glutenin subunits are coded by genes at the same complex locus, the maximum distance the genes are apart being 0.93 cM (Table 2). However, if the two progeny were heterozygous then recombination between ω-gliadin and LMW glutenin genes must have occurred. The map distance between the two gene groups in this case would be 0.62 ± .44 cM (Table 2).

3.2 Linkage between endosperm protein genes on chromosome 1A

3.2.1 ('Hope' × 'Cappelle') × 'Highbury'. Six proteins coded by genes on chromosome 1A were observed to be segregating by SDS-PAGE in this cross. Three of them were inherited from 'Hope' and consisted of two ω-gliadins and one γ-gliadin and the others, from 'Cappelle-Desprez', consisted of two LMW glutenin

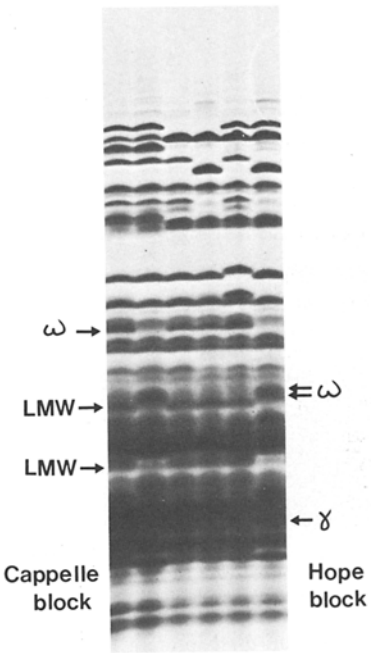

Fig. 4. SDS-PAGE separations of total protein extracts from six progeny of the cross ('Hope' × 'Cappelle-Desprez') × 'Highbury'. The block of chromosome 1A-mediated proteins from 'Hope' are *arrowed on the right* and those from 'Cappelle-Desprez' are marked *on the left*

subunits and one ω-gliadin (Fig. 4). Other proteins of the two primary parents which are also known to be coded by chromosome 1AS from two-dimensional analysis (results not shown) are obscured by proteins from one or more of the three parents used in this cross so their segregation could not be followed by SDS-PAGE.

Each set of three proteins was strictly inherited in blocks and no recombination was found between them in 348 progeny. However, two of the progeny were unusual in lacking both of the chromosome 1A blocks although they did contain the complementary proteins from the third parent, 'Highbury', at the expected dosage. Examination of the SDS-PAGE separations for the presence of those high-molecular-weight (HMW) subunits of glutenin coded by genes on the long arm of chromosome 1A indicated that one of the progeny was monosomic for this chromosome and the other was likely to be telosomic.

It was calculated that the maximum distance between the genes coding for the proteins studied, given a probability level of 0.05, is 0.86 cM (Table 2).

3.2.2 [CS×CS (T. spelta 1A)]×CS DT 1AL and *[CS×CS (T. spelta 1A)]×CS NIA TID.* In initial studies CS DT 1AL was used as the third parent. Unfortunately it produced little fertile pollen, so only a limited number of seeds were set. Therefore in later work, the more fertile CS NIA TID was used instead. This did not cause any difference in the analysis of linkage.

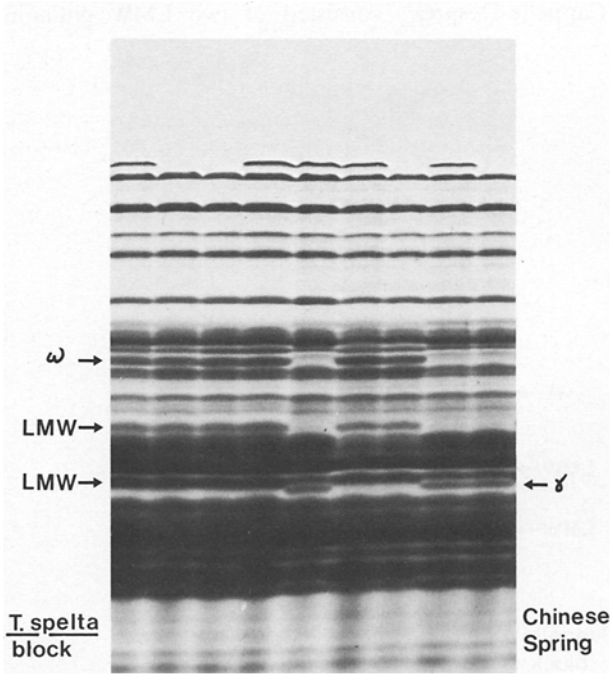

Fig. 5. SDS-PAGE separations of total protein extractions from nine progeny of the cross [CS×CS (*T. spelta* 1A)]×CS DT 1AL. The 'Chinese Spring'-inherited, chromosome 1A γ-gliadin is marked by an *arrow on the right* and the *Triticum spelta* chromosome 1A ω-gliadin and LMW subunits of glutenin are marked *on the left*

The linkage of four proteins could be followed in these crosses; one ω-gliadin and two LMW glutenin subunits from the *Triticum spelta* substitution line of 'Chinese Spring' and one γ-gliadin from euploid 'Chinese Spring' (Fig. 5). As in the previous cross involving chromosome 1A, no recombination between blocks was observed in 291 progeny (maximum distance between genes at $P > 0.05$ is 1.02 cM, Table 2). Thus, the storage protein genes on the short arm of chromosome 1A are clustered together, like those on chromsome 1BS.

4 Discussion

Genetics and evolutionary relationships

The short arms of the homoeologous group 1 chromosomes carry the genes for many of the endosperm storage proteins: all the ω-gliadins, most of the γ-gliadins, a few β-gliadins (Payne et al. 1982 b) and the LMW subunits of glutenin (Jackson et al. 1983). For this communication, several crosses were made to determine the linkage between the genes on chromosomes 1A and 1B coding for these groups of proteins. In none of the progeny was a recombinant positively identified. The results from the crosses are summarised in Table 2. Unfortunately, the two principal γ-gliadins coded by chromosome 1D do not show allelic variation that can be distinguished by our electrophoretic procedures, so linkage studies for this chromosome were not attempted. For any one cross involving either chromosome 1A or chromosome 1B, the segregation of all the polypeptides in all the relevant protein groups derived from both parents could not be followed (Table 1). Two-dimensional electrophoresis of numerous progenies would be required for this. Nevertheless, from the amalgamation of the information listed in Table 1, we believe it reasonable to infer that all the storage protein genes which occur on the short arms of each of the group one chromosomes occur together at a single, complex locus. The locus, from an extension of previous studies (Payne et al. 1982 a) is here defined as *Gli-A1* for chromosome 1A, *Gli-B1* for chromosome 1B and *Gli-D1* for chromosome 1D.

This hypothesis is consistent with the work of Sozinov and colleagues (reviewed by Sozinov and Poperelya 1980, 1982) who showed that gliadin polypeptides were strictly inherited in blocks in segregating progenies of many crosses and that no recombination was detected between ω- and γ-gliadins coded by chromosome 1A or those by chromosome 1B, or between ω-gliadins on chromosome 1D. In contrast, Mecham et al. (1978) whilst showing a close link-

age between ω and γ-gliadin genes on chromosome 1B, suggested that a low level of recombination had occurred in F_2 progeny of the cross between INIA 66R and Justin. In all, five recombinants were claimed in 136 progeny, one between γ_4- and ω_2-gliadins inherited from INIA 66R and four between γ_6- and ω_5-gliadins from Justin. In the type of cross studied, approximately 50% of the progeny will have been heterozygous for ω- and γ-gliadin genes and this, together with the segregation of many other proteins, would have made interpretation difficult, in spite of well-resolved, gel electrophoresis patterns. The authors now believe that at least some, and possibly all, of the five progeny they classified as recombinants were in fact heterozygous parentals (D. D. Kasarda, personal communication).

The only results which are contrary to our hypothesis are those recently published by Branlard (1983). Although Branlard's work was not specifically directed to measurement of linkage, several of his results indicate more than one locus containing gliadin genes on some chromosomes. For instance, band 44 in the variety 'Joss' was shown by two-dimensional analysis to consist of two proteins and genetical analysis of F_2 progeny derived from a cross between this variety and 'Mayo 54' indicated that their controlling genes occur on the same chromosome but 25 recombination units apart. Evidence of recombination occurring between ω-gliadins coded by chromosome 1D in progeny of the same cross was also presented (Branlard 1983; Table 3). 'Joss' has three such proteins, bands 22, 26 and 30 and these were inherited in 147, 186 and 187 of the progeny respectively, whereas the equivalent bands from 'Mayo 54', 21, 25 and 28 occurred in 166, 149 and 163 of the progeny. In contrast to these results, Sozinov and Poperelya (1980), who compared the same or very similar sets of chromosome 1D ω-gliadins in a similar type of cross between 'Bezostaya 1' and 'Odesskaya 26', could not detect any recombinants in 416 progeny. Thus, incorporating our results with those of Mecham *et al.* (1978) and Sozinov and Poperelya (1980), the balance of evidence is heavily against the results of Branlard (1983). It is possible that misclassification could have occurred in the latter study due to incomplete resolution of all six bands in heterozygous individuals.

As the genes for the LMW subunits of glutenin, the ω-gliadins, the γ-gliadins and the group 1 β-gliadins are adjacent to each other, they may have all arisen from a common ancestral gene. It will only be possible to assess this properly when the primary structures of the different proteins are known, either from amino-acid sequencing or by sequencing the DNA of the storage protein genes cloned in plasmids. Superficially, all three protein groups resemble each other but this is not surprising for they have all become adapted during evolution to act as nitrogen store for use by the germinating embryo before it becomes fully autotrophic. However, the ω-gliadins and γ-gliadins do appear to form distinctive groups. Thus, the ω-gliadins lack sulphur amino acids, unlike γ-gliadin, and they have much higher contents of glutamine and proline and much lower amounts of charged amino acids (Kasarda et al. 1976, for review). In addition, and more convincingly, their N-terminal amino acid sequences are unrelated (Bietz et al. 1977; Shewry et al. 1980). On current evidence therefore ω-gliadins and γ-gliadins can at best be only distantly related. Much less still is known about the biochemistry of the LMW subunits of glutenin. As discussed by Jackson et al. (1983) they appear to resemble the γ-gliadins more than the ω-gliadins on the basis of amino-acid composition and electrophoretic mobility. However, preliminary results with whole, unfractionated LMW glutenin subunits (Shewry et al. 1983) suggest that the principal N-terminal amino acid sequence is dissimilar to the N-terminal sequences of both ω- and γ-gliadins.

The number of major ω-, γ- and β-gliadins and major LMW glutenin subunits produced by each of the three *Gli-1* loci in the variety 'Chinese Spring' is shown in Table 3. They must be regarded as underestimates of the true number on two accounts. Firstly, when gel systems are highly loaded with proteins, then additional minor components appear which are controlled by genes at the same loci as the major components (Sozinov and Poperelya 1980; Fig. 6). Secondly the gel systems used only fractionate by differences in either molecular weight or charge so that any protein types which have arisen by the substitution of one amino acid for another with the same charge, would pass undetected. Since no recombination has been detected between protein products for any of the *Gli-1* loci, there is no direct evidence from classical genetical studies that they are actually coded by different structural genes. However, there is growing evidence from molecular genetics that the storage protein genes are present as multiple copies. In maize for instance, storage protein genes occur on 3 of the 10 chromosomes and there appears to be, by DNA hybridisation studies, about 100 structural genes (Viotti et al. 1979).

In wheat, therefore, the *Gli-1* locus may be envisaged as consisting of a set of three major families of genes coding for the three major protein groups, each family having arisen by gene duplication and mutation. The

Table 3. Numbers of storage proteins coded by genes at *Gli-A1*, *Gli-B1* and *Gli-D1* in 'Chinese Spring'

	Gli-A1	*Gli-B1*	*Gli-D1*
ω-gliadin[a]	1	3	2
γ-gliadin[a]	2	2	2
β-gliadin[a]	1	2	0
LMW glutenin[b]	1	5	8
	5	12	12

[a] Results of Payne et al. (1982)
[b] Results of Jackson et al. (1983)

number of genes at the *Gli-1* locus is not known. Kasarda (1980) has speculated that the gene family (called by us, *Gli-A2*) on chromosome 6A and coding for A-gliadin may contain as many as 100 genes. In their study, as in ours, it is not clear yet whether minor components are the products of different genes or the products of incomplete post-transcriptional or post-translational modifications.

Relationship to bread-making quality and to plant breeding

Storage protein has a major influence on bread-making quality, both with respect to protein type (Orth and Bushuk 1973) and protein amount (Finney and Barmore 1948). From the analysis of segregating populations from various varietal crosses, Payne et al. (1981) showed that certain high-molecular-weight (HMW) subunits of glutenin correlated with good bread-making quality as determined by the sodium dodecyl sulphate, sedimentation test (Axford et al. 1979) whereas other, allelic HMW subunits correlated negatively. In similar work, Burnouf and Bouriquet (1980) and Moonen et al. (1982) also found differential bread-quality relationships amongst allelic, HMW subunits of glutenin.

In addition to HMW glutenin subunits, different, allelic gliadins apparently also influence aspects of bread-making quality such as dough strength (Wrigley et al. 1981) and sedimentation volume (Sozinov and Poperelya 1980). These results are unexpected since it is generally agreed that glutenin rather than gliadin is associated with dough strength (Wall 1979) and that large sedimentation volumes (reflecting good quality) are due to the production of an extensive gel, formed exclusively from the larger, more insoluble glutenin molecules (Moonen et al. 1982). Because it has been shown in this communication that gliadins coded by genes on the group 1 chromosomes are tightly linked to LMW glutenin subunit genes, it is a distinct possibility that the quality associations with gliadins described above are due to linked genes and that the causal proteins are actually LMW subunits of glutenin. Thus, differences in the mixing properties and the strength of doughs, which can also have an important bearing on the texture and volume of baked loaves, may be envisaged as being caused primarily by different combinations of both HMW and LMW subunits of glutenin.

Two dimensional analysis of endosperm proteins in wheat has demonstrated their great complexity (Brown et al. 1981; Jackson et al. 1983). However, all these proteins appear to be coded by genes at just nine loci in the wheat genome; one on the long arm (Payne and Lawrence 1983) and one on the short arm of chromosomes 1A, 1B and 1D, and three on the short arms of the group 6 chromosomes (Sozinov and Poperelya 1980; Payne and Holt, unpublished findings). Thus, the prospect of plant breeders developing new varieties with specific technological properties by deliberately selecting for particular blocks of allelic storage proteins is less daunting than might otherwise have been imagined.

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