Heredity (1983), **50** (2), 179–189 0018-067X/83/06020179$02.00

© 1983. The Genetical Society of Great Britain

CHROMOSOMAL LOCATION OF *HOR 3*, A NEW LOCUS GOVERNING STORAGE PROTEINS IN BARLEY

P. R. SHEWRY*, R. A. FINCH†, SAROJ PARMAR*, J. FRANKLIN* AND B. J. MIFLIN*

** Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, U.K. † Plant Breeding Institute, Cambridge, U.K.*

Received 11.xi.82

SUMMARY

Hordein, the storage protein of barley grain, consists of three groups of polypeptides called B, C and D hordeins. Each group is coded for by a complex locus and these have been designated *Hor* 2, *Hor* 1 and *Hor* 3 respectively. Previous work has shown that *Hor* 1 and *Hor* 2 are on the short arm of chromosome 5. The results of crosses designed to evaluate the linkage relationships of *Hor* 3 show that it is located about 9 cM from the centromere on the long arm of chromosome 5. The loci *nec* 1 and *wst*5 are located 8·0 ± 1·5 and 37·4 ± 4·7 cM respectively distal to *Hor* 3. These results are discussed in relation to the chromosomal location of loci which code for homologous groups of storage proteins in wheat.

1. INTRODUCTION

Hordein, the prolamin storage protein of barley grain, is composed of a number of polypeptides which differ in their molecular weight, charge and isoelectric point (Shewry, Ellis, Pratt and Miflin, 1978*a*). The "B" and "C" groups of polypeptides account for about 95 per cent of the fraction and have been purified and characterized (Shewry *et al.*, 1980*c*). They differ in a number of their properties, notably their content of sulphur amino acids. We have recently purified a further group, D hordein, which consist of high molecular weight (HMW) polypeptides (apparent M_r 105,000) (Field, *et al.*, 1982). Furthermore we have shown that this group has properties in common with the HMW polypeptide components of wheat storage proteins (often called glutenins), notably a high content of glycine.

The numbers and electrophoretic properties of "B" and "C" hordein polypeptides differ between varieties (Shewry *et al.*, 1978*a*; Shewry, Pratt and Miflin 1978*c*; Shewry *et al.*, 1979). Genetic analysis of crosses has shown that the patterns of "C" and "B" hordein polypeptides are determined by separate loci, called *Hor* 1 and *Hor* 2 respectively, located on the short arm chromosome 5 (Solari and Favret, 1971; Oram, Doll and Køie, 1975; Shewry, Pratt, Finch and Miflin, 1978*b*; Netsvetaev, 1978; Sozinov *et al.*, 1978; Doll and Brown, 1979; Shewry *et al.*, 1980*b*; Jensen *et al.*, 1980). This location was confirmed by Lawrence and Shepherd (1981) using addition lines of barley into wheat. Molecular analysis of the *Hor* 2 locus using recombinant DNA techniques (Forde *et al.*, 1981) and the analysis of "B" hordein polypeptide fragmentation patterns (Faulks, Shewry and Miflin, 1981) both suggest that the locus consists of multiple

genes coding for two sub-families of related polypeptides, but the exact number and spatial organisation of these genes is not known.

Lawrence and Shepherd (1981) also showed that a quantitatively minor storage protein component of high molecular weight was coded for by genes on the long arm of barley chromosome 5, this location being similar to those of genes coding for the HMW glutenin polypeptides on the long arms of the homoeologous group 1 chromosomes of wheat. The properties of this component suggest that it is "D" hordein and we have therefore made crosses between lines with marker genes on chromosome 5 in order to determine the exact location of the "D" hordein locus, which we have called *Hor* 3 (Miflin and Shewry, 1981). The results of this study are reported here.

2. Materials and methods

(i) *Material*

Crosses were made among *Hordeum vulgare* L. cultivars Cambrinus, Nigrinudum and Sultan (all Plant Breeding Institute stocks) and between lines CI 2010 (Hungarian) from the World Barley Collection and S114 supplied by Dr J. Jensen of Risø National Laboratories, Roskilde, Denmark. Table 1 gives the alleles in each parent at the *Hor* 1, *Hor* 2 and *Hor* 3 and marker loci. Alleles at the first two loci have been named after the

Table 1

Genotypes of parents of the crosses

Varieties	Hordein alleles *Hor* 1	*Hor* 2	*Hor* 3	Marker loci		
Cambrinus	Pr	Ze	Cb	*Rps*4		
Nigrinudum	Ni	Ni	Ni		*B Mlnn*	
Sultan	Pr	Pr	Cb			
CI 2010 (Hungarian)	Hu	Hu	Ni		[*B*]	
S114	Pr	Ze	Cb			*nec*1 *wst*5

standard varieties containing them. Alleles at *Hor* 3 have not previously been named so we have designated the allele in Cambrinus, Sultan and S114 *Hor* 3 Cb after Cambrinus. This allele specifies a single major band of M_r 105,000 on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as shown in fig. 1 lanes a, c and e. The allele in Nigrinudum and CI 2010 (designated *Hor* 3 Ni) specifies a major band of slightly faster mobility (fig. 1 lanes b, d). The *Hor* 1 allele in CI 2010 (*Hor* 1 Hu) specifies a different polypeptide pattern from that of *Hor* 1 Pr (Sultan, Cambrinus and S114) but these could not be differentiated by the analytical procedures used for the single seeds (fig. 1, lanes d, e).

Markers in the parents (table 1) were the dominant genes *Rps*4 (*Yr*4), resistant reaction to barley yellow rust, *Puccinia striiformis* West race 23 but not 24 (Johnson and Finch, 1976); *Mlnn* (*JMlnn*), reaction to powdery

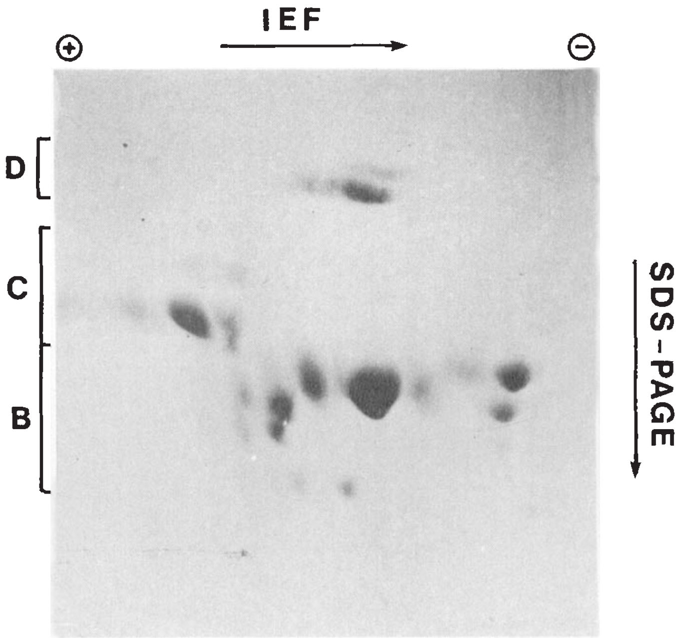

FIG. 3. Two-dimensional analysis of reduced and pyridylethylated hordein from a single F_2 seed from cross 2 (Nigrinudum × Sultan). The hordein alleles present are *Hor* 1 Ni Ni Ni, *Hor* 2 Ni Ni Ni and *Hor* 3 Ni Ni Cb. B, C and D are the groups of hordein polypeptides. The isoelectric focusing (IEF) separation was in the *p*H range 5–9.

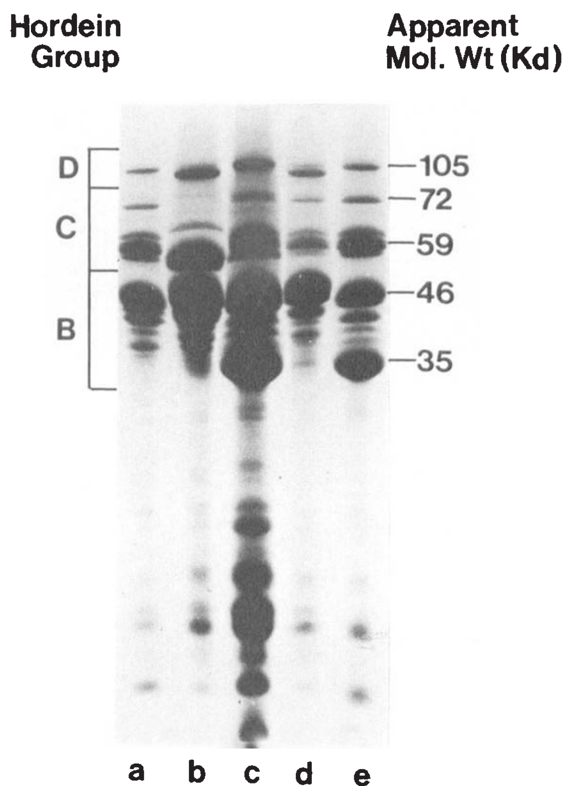

FIG. 1. SDS-PAGE of reduced and pyridylethylated hordein fractions from single seeds of the varieties used as parents in the crosses. (a) Sultan; (b) Nigrinudum; (c) Cambrinus, (d) CI 2010 (Hungarian); (e) S114. B, C and D are the groups of hordein polypeptides. Apparent molecular weights were determined in a previous study (Faulks *et al.*, 1981).

[facing p. 180]

mildew, *Erysiphe graminis* DC f. sp. *hordei* Marchal (Hiura, 1972) and B, black lemma and pericarp (Haus and Tsuchiya, 1971); and the recessive genes *nec* 1, necrotic leaf spotting (Jensen, 1973) and *wst*5, white streaks on young leaves (Jensen, 1973). The chromosomal locations of these loci and of *Hor* 1 and *Hor* 2 are shown in fig. 2(a) which is based on Jensen (1982).

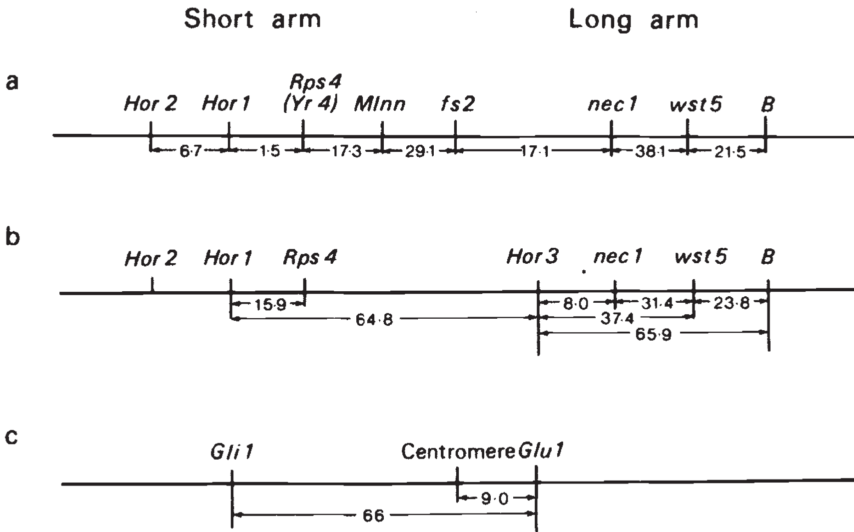

FIG. 2.(a) Part of the linkage map of chromosome 5 of barley, showing the map distances between marker loci in the parents of the crosses. The centromere is thought to be just to the left of *fs*2. Redrawn from Jensen (1982). (b) Map distances between *Hor* 3 and marker loci in the parents of the crosses, based on data reported here. (c) Map distances between the loci *Glu* 1, *Gli* 1 and the centromere on the homoeologous group 1 chromosomes of wheat, based on the data of Payne *et al.*, (1982). Map distances are in centimorgans (cM).

Five F_1 plants of Cambrinus × Nigrinudum (cross 1) and 7 of Nigrinudum × Sultan (cross 2) were grown together in a glasshouse at the Plant Breeding Institute. In cross 1, 110 bagged F_2 seeds (21–22 from each F_1) gave 109 F_2 plants and F_3 families. F_2 plants were scored as seedlings for reaction to yellow rust race 24 (cross 1 only) and at maturity for lemma and pericarp colour. From each F_2 plant, separate batches of F_3 seedlings were tested for reaction to yellow rust race 23 or mildew and five F_3 seeds were scored for hordein pattern.

In cross 2, 35 bagged F_2 seeds were sown from each F_1, 20 in sample X and 15 in sample Y. Failure of germination or technique prevented scores in some cases. In sample X, each seed was cut in two and the embryo-bearing part sown for a mildew reaction test and the other part scored for hordein patterns. Scores were obtained from 86 plants (6–18 from each F_1) for reaction to mildew, 92 seeds (6–19 from each F_1) for "C" hordeins and 97 seeds (6–20 from each F_1) for "D" hordeins. In sample Y, 50 F_2 plants (3–14 from each F_1) were scored for lemma and pericarp colour, and from each F_2 plant, F_3 seedlings were scored for reaction to mildew and five F_3 seeds for hordein patterns.

Two F_1 plants of CI 2010×S114 (cross 3) were grown together in a glasshouse at Rothamsted, 376 F_2 seeds gave 365 F_2 plants (226 and 139 from the two F_1 plants). F_2 plants were scored as seedlings for the *nec* 1 and *wst* 5 phenotypes. Five F_3 seeds from each F_2 plant were scored for hordein patterns.

(ii) *Tests for mildew and rust resistances*

In cross 1 reaction of intact first seedling leaf to Plant Breeding Institute stocks of yellow rust race 23 or 24 was scored two to three weeks after inoculation on a 0 to 4 scale. Resistant plants had only necrotic flecks or scored 0 to 1+; susceptible plants scored 3− to 4. The only intermediate was one F_3 plant which scored 2. Nigrinudum has a recessive gene on chromosome 4 giving resistance (usually a 0, 1−, 1 or 1+ score) to both races (Finch, Simpson and Johnson, 1978) whereas Cambrinus is resistant (usually necrotic flecks, only) to race 23 and susceptible to race 24 (Johnson and Finch, 1976). Therefore 10–12 F_3 seedlings from each F_2 plant were tested with race 23. An F_2 plant that had been susceptible to race 24 was deemed homozygous for *Rps* 4 if all its F_3 progeny had only necrotic flecks and heterozygous if 7–12 F_3 progeny were resistant, but not all merely with necrotic flecks. However, F_2 plants which had shown the Nigrinudum resistance to race 24 were deemed to have the *Rps* 4 allele only if 8 or more F_3 progeny showed only the necrotic reaction to race 23. Thus 3 F_2 plants each with all 12 F_3 progeny resistant but 11 in each family with 0 to 1+ scores, not merely necrotic flecks, were deemed homozygous for *rps* 4, as were 21 F_2 plants with 7–12 susceptible F_3 progeny each.

Cambrinus has 2 genes for mildew resistance on chromosome 4 (Wiberg, 1974) and Sultan has a gene on chromosome 5 (Torp, Jensen and Jørgensen, 1978). Resistance due to *Mlnn* in Nigrinudum was distinguished from that due to Cambrinus and Sultan genes with Plant Breeding Institute mildew isolate number 210 which is pathogenic on all 3 varieties, especially Sultan, but also evokes a necrotic reaction in Nigrinudum, only. If an F_2 plant did not show this necrotic reaction itself (cross 2, sample X), or in any of its 10–16 F_3 progeny tested (cross 1 and cross 2, sample Y), it was classed as homozygous for *mlnn*.

(iii) *Hordein pattern analysis*

Alleles at the *Hor* 1 and *Hor* 3 loci in seed from crosses 1 and 2 were determined by SDS-PAGE of reduced and pyridylethylated hordein fractions as described previously (Shewry *et al.*, 1978*b*). Alleles at *Hor* 2 and *Hor* 3 in cross 3 were determined using a modification of the single seed method of Doll and Andersen (1981). A crushed seed was placed in a 1·5 ml capped polypropylene centrifuge tube with 400 μl of 50 per cent (v/v) propan-2-ol containing 41 mM Tris and 40 mM boric acid, *p*H 8·6, and 1 per cent (v/v) 2-mercaptoethanol. The tube was suspended in a sonic cleaning bath for 30 min, centrifuged and 200 μl of the supernatant decanted to a fresh tube. 3·0 μl of redistilled 4-vinylpyridine was added and the tube shaken for 2 h at 20°C. 600 μl of distilled water was then added and the tube placed at 4°C overnight. The precipitated hordein was removed by centrifugation, redissolved in 8M urea containing 10 per cent (w/v)

SDS and separated by SDS-PAGE on a modified Laemmli gel (Forde *et al.*, 1981). Two-dimensional analyses of hordein fractions from single seeds were made as described by Shewry *et al.* (1980*b*).

(iv) *Statistical analysis*

Gene linkage was tested for by χ^2 tests and estimated by the method of maximum likelihood (Bailey, 1961). Map distance was calculated using the Kosambi function (Jensen and Jørgensen, 1975*a*). Genotype frequencies from different samples were pooled if homogeneous ($P > 0{\cdot}05$) in contingency χ^2 tests.

3. Results

The numbers of F_2 plants in the various genotypic classes, resulting from segregation at the loci studied, are given in tables 2–4 and the linkage χ^2 and probability values in table 5. Although the parents in crosses 1 and 2 (Nigrinudum and Cambrinus or Sultan) have different alleles at the *Hor* 2 locus they could not be distinguished easily from heterozygotes by one-dimensional SDS-PAGE and so segregation at this locus was not

TABLE 2

Frequency in the progeny of cross 1 of genotypes resulting from pairwise combinations of alleles at the loci Hor *1*, Hor *3*, Rps*4*, B *and* Mlnn

	Mlnn Mlnn, *Mlnn mlnn*	*mlnn mlnn*	*Hor* 1			*Hor* 3			Totals
			Ni Ni	Ni Pr	Pr Pr	Ni Ni	Ni Cb	Cb Cb	
*Rps*4 *Rps*4	16	11	1	9	17	6	16	5	27
*Rps*4 *rps*4	34	24	10	40	8	16	22	20	58
*rps*4 *rps*4	12	12	22	2	0	10	10	4	24
B B, *B b*	49	35	28	39	17	27	38	19	84
b b	13	12	5	12	8	5	10	10	25
Hor 3									
Ni Ni	16	16	14	12	6				32
Ni Cb	29	19	13	24	11				48
Cb Cb	17	12	6	15	8				29
Hor 3									
Ni Ni	16	17							33
Ni Pr	31	20							51
Pr Pr	15	10							25
Totals	62	47	33	51	25	32	48	29	109

studied. Similarly the *Hor* 1 alleles present in the parents of cross 3 (CI 2010 and S114) could not be distinguished and so segregation at this locus could not be scored. There was no evidence of recombination within the "B" hordein or "C" hordein patterns and so *Hor* 1 and *Hor* 2 were treated as single loci with multiple alleles. SDS-PAGE (fig. 1) showed that all the parents had single major "D" hordein bands. Two-dimensional separations by combined isoelectric focusing and SDS-PAGE showed that these "D"

TABLE 3

Frequency in the progeny of cross 2 of genotypes resulting from pairwise combinations of alleles at the loci Hor *1*, Hor *3*, Mlnn *and* B

	Hor 1			*Hor* 3		
	Ni Ni	Ni Pr	Pr Pr	Ni Ni	Ni Cb	Cb Cb
Cross 2(X)						
Mlnn Mlnn } *Mlnn mlnn*	18	30	7	15	33	11
mlnn mlnn	5	15	4	6	17	2
Hor 3						
Ni Ni	4	15	3			
Ni Cb	17	31	7			
Cb Cb	6	5	4			
Cross 2(*Y*)						
Mlnn Mlnn } *Mlnn mlnn*	10	22	11	9	23	11
mlnn mlnn	1	5	1	0	6	1
B B } *B b*	8	20	6	6	21	7
b b	3	7	6	3	8	5
Hor 3						
Ni Ni	4	3	2			
Ni Cb	6	18	5			
Cb Cb	1	6	5			

hordein bands each consisted of a number of components with different isoelectric points (fig. 3).

Segregation at the loci *Hor* 1, *Hor* 2, *Hor* 3 and *Rps*4 gave the expected 1:2:1 ratio of homozygotes:heterozygotes;homozygotes ($P > 0{\cdot}05$) in all sample populations. For *Mlnn*, *B*, *wst*5 and *nec*1 only two phenotypic classes at each locus were scored and the expected 3 dominant: 1 recessive ratio was found in all sample populations ($P > 0{\cdot}2$) except for *Mlnn* in cross 1 ($P < 0{\cdot}01$) where the ratio was apparently 9:7 ($P > 0{\cdot}9$). Interactions between *Mlnn* and the 2 mildew resistance genes in Cambrinus may account for this exception.

The χ^2 values indicate linkage ($P < 0{\cdot}05$) between *Hor* 3 and the loci *Hor* 1, *wst*5, *nec*1 and *B*, and the recombination values are given in table 5. These data clearly show that *Hor* 3 is located close to *nec*1 on the long arm of chromosome 5. Since the recombination percentage between *nec*1 and *wst*5 ($27{\cdot}8 \pm 2{\cdot}8$) is less than between *Hor* 3 and *wst*5 ($31{\cdot}7 \pm 2{\cdot}8$), *Hor* 3 is presumed to be proximal to *nec*1. The map distances calculated from these recombination percentages are given in table 5 while a map of the locations of these loci based on these results is presented in fig. 2(b). The locus *wst*5 also showed linkage with *B* ($22{\cdot}2 \pm 2{\cdot}6$ per cent recombination). The sum of estimates of the map distances between *wst*5 and *B* and *wst*5 and *Hor* 3 (61·2 cM) agreed well with the distance determined between *Hor* 3 and *B* (65·9 cM); surprisingly, *nec*1 was not linked with *B*. *Hor* 1 showed linkage with the yellow rust resistance locus *Rps*4, although the map distance ($15{\cdot}9 \pm 3{\cdot}0$ cM) was considerably greater than that calculated by Jensen (1982) (1·5 cM, see fig. 2(a)). Also, *Rsp*4 was not apparently linked with *Hor* 3. A further inconsistency was that *Hor* 1 showed linkage

TABLE 4

Frequency in the progeny of cross 3 of genotypes resulting from pairwise combinations of alleles at the loci Hor *2*, Hor *3*, wst*5*, B *and* nec*1*

	Hor 2			*Hor 3*							
	Hu Hu	Hu Ze	Ze Ze	Ni Ni	Ni Cb	Cb Cb	*Wst5 Wst5* / *Wst5 wst5*	*wst5 wst5*	*Nec1 Nec1* / *Nec1 nec1*	*nec1 nec1*	Totals
B B / *B b*	67	124	89	93	114	73	244	36	210	70	280
b b	17	49	19	14	47	24	34	51	61	24	85
Nec1 Nec1 / *Nec1 nec1*	62	133	76	102	156	13	231	40			271
nec1 nec1	22	40	32	5	5	84	47	47			94
Wst5 Wst5	60	130	88	95	131	52					278
Wst5 wst5 / *wst5 wst5*	24	43	20	12	30	45					87
Hor 3											
Ni Ni	28	42	37								107
Ni Cb	40	84	37								161
Cb Cb	16	47	34								97
Totals	84	173	108	107	161	97	278	87	271	94	365

TABLE 5

Linkage χ^2, probability values, recombination percentages and map distances for the loci segregating in crosses 1–3

Loci	Cross	Linkage χ^2 $d_f = 1$	Probability of independence	Recombination percentage (±S.E.)	Map distance in cM (±S.E.)
Hor 1 × *Hor* 3	1	3·26	<0·1	42·0±4·4	
	2(X)	0·07	>0·5	51·7±6·4	
	2(Y)	4·57	<0·05	33·1±6·6	
	1+2(X+Y)	4·24	<0·05	43·0±3·3	64·8±12·7
Hor 1 × *Rps*4	1	70·83	<0·001	15·4±2·7	15·9±3·0
Hor 1 × *Mlnn*	1	1·65	>0·1	56·2±4·8	
	2(X)	0·51	>0·5	45·2±6·7	
	2(Y)	0·02	>0·9	51·5±10·5	
	2(X+Y)	0·28	>0·5	47·0±5·6	
	1+2(X)	0·40	>0·5	52·4±3·9	
Hor 1 × *B*	1	2·42	>0·1	41·0±5·7	
	2(Y)	1·57	>0·2	39·5±8·1	
	1+2(Y)	3·96	<0·05	40·5±4·7	56·4±13·6
Hor 2 × *Hor* 3	3	0·82	>0·1	52·3±2·5	
Hor 2 × *wst*5	3	2·83	>0·05	55·3±3·1	
Hor 2 × *nec*1	3	0·45	>0·5	47·9±3·1	
Hor 2 × *B*	3	0·44	>0·5	52·1±3·1	
Hor 3 × *wst*5	3	36·2	<0·001	31·7±2·8	37·4±4·7
Hor 3 × *nec*1	3	238·1	<0·001	7·9±1·5	8·0±1·5
Hor 3 × *B*	1	3·12	<0·1	40·0±5·5	
	2(Y)	0·45	>0·5	44·9±8·7	
	3	4·0	<0·05	44·0±3·0	68·9±13·2
	1+2(Y)+3	7·01	<0·01	43·3±2·5	65·9±10·0
Hor 3 × *Rps*4	1	0·77	>0·3	46·2±4·3	
Hor 3 × *Mlnn*	1	0·68	>0·3	53·9±4·7	
	2(X)	0·50	>0·3	54·7±6·6	
	2(Y)	0·02	>0·8	48·4±10·9	
	2(X+Y)	0·29	>0·5	53·0±5·6	
*Rps*4 × *Mlnn*	1	0·21	>0·5	52·3±4·9	
B × *Mlnn*	1	0·12	>0·7	47·9±5·8	
*nec*1 × *wst*5	3	43·55	<0·001	27·8±2·8	31·4±4·1
*nec*1 × *B*	3	0·33	>0·5	47·7±3·8	
*wst*5 × *B*	3	71·87	<0·001	22·2±2·6	23·8±3·2

with *B* when the data from crosses 1 and 2(Y) were combined, although not with the data from either cross alone. There was no evidence for linkage between the Nigrinudum mildew resistance gene (*Mlnn*) and *Hor* 1, *Hor* 3 or *Rps*4, although it would have been expected from the chromosome map of Jensen (1982) (fig. 2(a)).

4. DISCUSSION

The linkage data for *Hor* 3 and the loci *Hor* 1, *nec*1, *wst*5 and *B* are consistent with the location of *Hor* 3 about 8 cm to the centromere side of *nec*1, on the long arm of chromosome 5. The map distances calculated for these loci are in good agreement with the chromosome map of Jensen (1982) (fig. 2a). However, some of the other recombination frequencies are not in agreement with this map; *Hor* 1 and *Rps*4 are 15·9 cM apart in cross 1 but only 1·5 cM apart in Jensen's map (fig. 2(a)), and the loose

linkage between *Hor* 1 and *B* in crosses 1+2(Y) combined, which was only significant at a low probability ($P > 0{\cdot}05$), is clearly inconsistent with the known locations of these loci.

We have previously experienced problems in determining *Rps*4 (*Yr*4) (Shewry *et al.*, 1980*b*) but we do not think these were responsible for the results in the present study, however; Jensen (1982) has noted that the proposed position (fig. 2(a)) of this locus is not certain. Why ***Mlnn*** shows no linkage with any of the other loci is not known. The location of this gene on chromosome 5 is based only on a loose linkage with *nec*1 ($36{\cdot}8 \pm 4{\cdot}2$) reported by Jensen and Jørgensen (1975*b*). It may be that *Mlnn* is not actually located within the map shown in fig. 2(a), but this requires confirmation.

It is of interest to compare the locations of the loci coding for hordein polypeptides with those coding for related proteins present in wheat and rye (Miflin, Field and Shewry, 1983). The HMW components of wheat, which are homologous with "D" hordein, are coded for by alleles at *Glu* 1 which have been mapped 9 cM from the centromeres on the long arms of the homoeologous group 1 chromosomes (Payne *et al.*, 1982) (fig. 2(c)). The centromere of barley chromosome 5 is thought to be located just on the short arm side of *fs*2 which would place *Hor* 3 about 9 cm from the centromere in a similar position to *Glu* 1. The ω-gliadins of wheat are homologus with "C" hordein (Shewry, Autran, Nimmo, Lew and Kasarda, 1980*a*) and these are coded for by alleles at *Gli* 1, which are located at 66 CM from *Glu* 1 on the short arms of the same chromosomes (Payne *et al.*, 1982) (fig. 2(c)). This distance is strikingly similar to that between *Hor* 1 and *Hor* 3 ($64{\cdot}8 \pm 3{\cdot}3$ cM). The γ-gliadins of wheat have similar amino acid compositions to "B" hordein but are also coded for by *Gli* 1 alleles (Sosinov and Poperelya, 1980) which may indicate that *Gli* 1 of wheat is equivalent to *Hor* 1 and *Hor* 2 of barley. Rye has genes coding for low and high molecular weight prolamins on the short and long arms respectively of chromosome 1R (Lawrence and Shepherd, 1981). Other wheat gliadins are coded for by alleles at loci (provisionally called *Gli* 2) on the short arms of the group 6 chromosomes (Payne *et al.*, 1982). These loci appear to be absent from barley and rye.

During the completion of this study we became aware of a similar study of the location of the genes for "D" hordein by Blake, Ullrich and Nilan (1982). However their results showed that the controlling locus was only about 35 cM from *Hor* 1, which would place it on the short arm or, in the centromeric region. This is not consistent with the results reported here.

Acknowledgements. We are grateful to Drs J. Jensen and H. Doll (Risø National Laboratory, Roskilde, Denmark) for helpful discussions and for critical comments on this manuscript. Dr J. Jensen also supplied seed of S114. We are also grateful to Dr P. I. Payne (PBI, Cambridge) for making available a reprint of his manuscript and Ms A. J. Faulks for running the two-dimensional gel showing in fig. 3.

5. References

BAILEY, N. T. J. 1961. *Introduction to the Mathematical Theory of Linkage*. Oxford University Press.

BLAKE, T. K., ULLRICH, S. E. AND NILAN, R. A. 1982. Mapping of the *Hor* 3 locus encoding "D" hordeins in barley. *Theor. Appl. Genet. 63*, 367–371.

DOLL, H. AND ANDERSEN, B. 1981. Preparation of barley storage protein, hordein, for analytical sodium dodecyl sulphate-polyacrylamide gel electrophoresis. *Anal. Biochem.*, *115*, 61–66.

DOLL, H. AND BROWN, A. D. H. 1979. Hordein variation in wild (*Hordeum spontaneum*) and cultivated (*H. vulgare*) barley. *Can. J. Genet. Cytol.*, *21*, 391–404.

FAULKS, A. J. SHEWRY, P. R. AND MIFLIN, B. J. 1981. The polymorphism and structural homology of storage polypeptides (hordein) coded by the *Hor* 2 locus in barley (*Hordeum vulgare* L.). *Biochem. Genet.*, *19*, 841–858.

FIELD, J. M., SHEWRY, P. R., MIFLIN, B. J. AND MARCH, J. F. 1982. The purification and characterization of homologous high molecular weight storage proteins from the grain of wheat rye and barley. *Theor. appl. Genet.*, *62*, 329–336.

FINCH, R. A., SIMPSON, E. AND JOHNSON, R. 1978. Ethiopian barleys with resistance to yellow rust (*Puccinia striiformis* West) and brown rust (*P. hordei* Otth.). *Barley Newsl.*, *21*, 56–57.

FORDE, B. G., KREIS, M., BAHRAMIAN, M. B., MATTHEWS, J. A., MIFLIN, B. J., THOMPSON, R. D., BARTELS, D. AND FLAVELL, R. B. 1981. Molecular cloning and analysis of cDNA sequences derived from poly A^+ RNA from barley endosperm: identification of B hordein related clones. *Nucleic Acids Research*, *9*, 6689–6707.

HAUS, T. E. AND TSUCHIYA, T. 1971. Black lemma and pericarp. *Barley Genet. Newsl.*, *1*, 148.

HIURA, U. 1972. Mildew resistance. *Barley Genet. Newsl.*, *2*, 194.

JENSEN, J. 1973. Locating some genes on barley chromosome 5. *Barley Genetics Newsl.*, *3*, 25–27.

JENSEN, J. 1982. Coordinators Report: Chromosome 5. *Barley Genetics Newsl.*, *12*, 87–90.

JENSEN, J. AND JØRGENSEN, J. H. 1975*a*. The barley chromosome 5 linkage map. I. Literature survey and map estimation procedure. *Hereditas*, *80*, 5–16.

JENSEN, J. AND JØRGENSEN, J. H. 1975*b*. The barley chromosome 5 linkage map. II. Extension of the map with four loci. *Hereditas*, *80*, 17–26.

JENSEN, J., JØRGENSEN, J. H., JENSEN, H. P., GIESE, H. AND DOLL, H. 1980. Linkage of the hordein loci *Hor* 1 and *Hor* 2 with the powdery mildew resistance loci *M*l-*k* and *M*l-*a* on barley chromosome 5. *Theor. appl. Genet.*, *58*, 27–31.

JOHNSON, R. AND FINCH, R. A. 1976. Resistant to *Puccinia striiformis* West (stripe rust). *Barley Genetics Newsl.*, *6*, 130.

LAWRENCE, G. J. AND SHEPHERD, K. W. 1981. Chromosomal location of genes controlling seed proteins in species related to wheat. *Theor. appl. Genet.*, *59*, 25–31.

MIFLIN, B. J., FIELD, J. M. AND SHEWRY, P. R. 1983. Cereal storage proteins and their effects on technological properties. In J. Daussant, J. Mosse & J. Vaughan (eds.) *Seed Proteins*, Academic Press, London. In press.

MIFLIN, B. J. AND SHEWRY, P. R. 1981. The genes for the storage proteins of barley. *Abs XIII int. Bot. Congress*, Australia, 1981.

NETSVETAEV, V. P. 1978. (The author's translation of) Mapping of loci Hrd in chromosome 5 of barley with the help of reciprocal translocations. *Biologischeskie Osmovy Ratseonalnogo Ispolzovaniya Jivotnogo i Rastitelnogo Myra*, USSR, Riga, "Zinatne", 1978, pp. 145–146.

ORAM, R. N., DOLL, H. AND KØIE, B. 1975. Genetics of two storage protein variants in barley. *Hereditas*, *80*, 53–58.

PAYNE, P. I., HOLT, L. M., WORLAND, A. J. AND LAW, C. N. 1982. 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. *Theor. appl. Genet.*, *63*, 129–138.

SHEWRY, P. R., ELLIS, J. R. S., PRATT, H. M. AND MIFLIN, B. J. 1978*a*. A comparison of methods for the extraction and separation of hordein fractions from 29 barley varieties. *J. Sci. Fd. Agr.*, *29*, 433–441.

SHEWRY, P. R., PRATT, H. M. FINCH, R. A. AND MIFLIN, B. J. 1978*b*. Genetic analysis of hordein polypeptides from single seeds of barley. *Heredity*, *40*, 463–466.

SHEWRY, P. R., PRATT, H. M. AND MIFLIN, B. J. 1978*c*. Varietal identification of single seeds of barley by analysis of hordein polypeptides. *J. Sci. Fd. Agr.*, *29*, 587–596.

SHEWRY, P. R., PRATT, H. M., FAULKS, A. J., PARMAR, S. AND MIFLIN, B. J. 1979. The storage protein (hordein) polypeptide pattern of barley (*Hordeum vulgare* L.) in relation to varietal identification and disease resistance. *J. natn. Inst. agric. Bot.*, *15*, 34–50.

SHEWRY, P. R., AUTRAN, J-C., NIMMO, C. C., LEW, E. J-L. AND KASARDA, D. D. 1980*a*. N-terminal amino acid sequence homology of storage protein components from barley and a diploid wheat. *Nature, Lond.*, *286*, 520–522.

SHEWRY, P. R., FAULKS, A. J., PICKERING, R. A., JONES, I. T., FINCH, R. A. AND MIFLIN, B. J. 1980*b*. The genetic analysis of barley storage proteins. *Heredity*, *44*, 383–389.

SHEWRY, P. R., FIELD, J. M., KIRKMAN, M. A., FAULKS, A. J. AND MIFLIN, B. J. (1980*c*) The extraction, solubility and characterization of two groups of barley storage polypeptides. *J. Exp. Bot.*, *31*, 393–407.

SOLARI, R. M. AND FAVET, E. A. 1971. Polymorphism in endosperm proteins of barley and its genetic control. In R. Nilan (ed.) *Barley Genetics II* Washington University Press, Pullman, WA, USA. pp. 23–31.

SOZINOV, A. A., NETSVETAEV, V. P., GRIGORYAN, E. M. AND OBRAZTSOV, I. S. 1978. Mapping of Hrd locuses in barley (*Hordeum vulgare* L. emed. Vav et Bacht). *Genetika*, *14*, 1610–1619 (translated into English in *Soviet Genetics*, 1979, *14*, 1137–1147).

SOZINOV, A. A. AND POPERELYA, F. A. 1980. Genetic classification of prolamines and its use for plant breeding. *Ann. Technol. agric.*, *29*, 229–245.

TORP, J., JENSEN, J. P. AND JØRGENSEN, J. 1978. Powdery mildew resistance genes in 106 northwest European spring barley varieties. *Arsskr. K. Vet.-LandbKøjsk*, 75–102.

WIBERG, A. 1974. Sources of resistance to powdery mildew in barley. *Hereditas*, *78*, 1–40.