Genet. Res., Camb. (1988), **51**, *pp.* 5–12 *With 5 text-figures Printed in Great Britain*

Mapping and biochemical analysis of *Hor 4 (Hrd G)*, a second locus encoding B hordein seed proteins in barley (*Hordeum vulgare* L.)

PETER R. SHEWRY[1],* SAROJ PARMAR[1], JULIAN FRANKLIN[1] AND RODGER WHITE[2]

AFRC Institute of Arable Crops Research, Department of Biochemistry[1] and Statistics[2], Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, U.K.

(*Received 7 June 1987 and in revised form 22 August 1987*)

Summary

The hordein polypeptide controlled by the *Hrd G (Hor 4)* locus in Elgina and derived lines was purified by preparative isoelectric focusing. The amino acid composition was similar to those of the major B hordeins encoded by the *Hor 2* locus. Genetic analysis confirmed that *Hor 4* is located proximally to *Hor 1* on the short arm of chromosome 5. It is speculated that *Hor 4* arose by translocation of genes from *Hor 2*, possibly in an ancestor of Elgina.

1. Introduction

Hordein, the alcohol-soluble (or prolamin) storage protein fraction of barley grain, consists of three distinct groups of polypeptides called B, C and D hordeins (see Shewry, Williamson & Kreis, 1987 for a discussion of their structures). D hordein is a minor component, accounting for 1–2% of the total fraction, and consists of only one or two polypeptides (Shewry & Miflin, 1982; Shewry *et al.* 1983). Genetic and molecular analyses show that it is encoded by a single gene or small multigene family located at the *Hor 3* locus on the long arm of chromosome 5 (Blake, Ullrich & Nilan, 1982; Shewry *et al.* 1983; Forde, 1985; Bunce *et al.* 1986). In contrast, the B and C hordein groups are highly polymorphic mixtures of polypeptides encoded by complex multigenic loci.

C hordein accounts for about 10–20% of the total fraction, and there is extensive genotypic variation in the numbers, molecular weights and isoelectric points of the component polypeptides that are separated by two-dimensional electrophoresis (Shewry *et al.* 1980*b*, 1985*a*). Despite this polymorphism the individual polypeptides have a high degree of structural homology (Shewry, Lew & Kasarda, 1981; Brandt *et al.* 1981) and are all encoded by a multigene family (probably 20–30 copies) at the *Hor 1* locus on the short arm of chromosome 5 (Oram, Doll & Køie, 1975; Shewry *et al.* 1978, 1980*b*, 1985*a*; Doll & Brown, 1979; Jensen *et al.* 1980).

B hordein is the quantitatively major group, and between 8 and 16 major polypeptides together with a number of minor ones can be separated by 2-D electrophoresis (Faulks, Shewry & Miflin, 1981). Although initial studies indicated that, like C hordein, the polypeptides formed a single homologous group encoded by one locus (designated *Hor 2* and located distally to *Hor 1* on the short arm of chromosome 5) (Oram *et al.* 1975; Shewry *et al.* 1978, 1980*b*; Doll & Brown, 1979; Jensen *et al.* 1980), the situation now appears to be more complex. Recent chemical analyses have shown that some of the minor polypeptides are structurally distinct, and these have been called γ-type hordein (Shewry *et al.* 1985*b*). Also, analysis of cDNA clones derived from endosperm-specific mRNAs has shown the presence of two major sub-families of B hordein-related cDNAs (and therefore genes) and several minor groups (Forde *et al.* 1981; Kreis *et al.* 1987).

Detailed genetic analyses by Netsvetaev and co-workers in the USSR have also shown the existence of at last two additional loci encoding B hordein polypeptides (*Hrd F* and *Hrd G*), which are linked to *Hor 1* and *Hor 2* (Netsvetaev & Sozinov, 1982, 1984). The precise nature of the polypeptides encoded by *Hrd F* is not known, but it has been speculated that they are either one of the major B hordein subfamilies identified by cDNA cloning (Shewry & Miflin, 1982) or γ-type hordeins (Shewry & Parmar 1987). In the present paper we present studies of the *Hrd G* locus, which we have re-designated *Hor 4* in line with the nomenclature for hordein genes that is generally accepted in Western Europe and the USA. These studies confirm the linkage relationships reported by Sozinov & Netsvetaev (1984), and show that the polypeptide encoded by this locus is related structurally to the major B hordeins encoded by *Hor 2*.

* Corresponding author.

2. Methods

(i) *Plant materials*

Seed of P12/1 to P12/6 was supplied by Dr V. P. Netsvetaev (All Union Breeding and Genetics Institute, Odessa, USSR) and of doubled monoploid lines from a cross between Magnum and Goldspear (called M × G 5 and M × G 34) by Dr W. Powell (Scottish Crop Research Institute, Pentlandfield, Scotland). Bomi was from a stock derived from Risø National Laboratory (Roskilde, Denmark) and maintained at Rothamsted. All lines were multiplied in the glasshouse at Rothamsted.

(ii) *Extraction of hordein fractions for electrophoresis*

Samples (2 g) of milled grain were stirred for 2 × 1 h at 20 °C with 20 ml volumes of 50% (v/v) aq. propan-1-ol containing 2% (v/v) 2-mercaptoethanol and 1% (v/v) acetic acid. The bulked supernatants were mixed with 2 volumes of 1·5 M-NaCl and stood at 4 °C overnight. The precipitated hordein was removed by centrifugation and reduced and pyridylethylated as described by Friedman *et al.* (1970). Hordein fractions were also extracted from single seeds as described by Shewry *et al.* (1983).

(iii) *Electrophoresis*

The one-dimensional (1-D) sodium dodecylsulphate polyacrylamide gel electrophoresis system was modified from Laemmli (1970) as described by Bunce, White & Shewry (1985). The 2-D isoelectric focusing/SDS–PAGE system was as described by Rahman, Shewry & Miflin (1982).

(iv) *Purification of the* Hor 4 *protein*

A 70 g sample of milled grain was defatted with water-saturated butan-1-ol and then extracted with 700 ml of 70% (v/v) aq. ethanol for 1 h at 20 °C. The hordein was precipitated by the addition of 2 volumes of 1·5 M-NaCl followed by standing overnight at 4 °C. After reduction and pyridylethylation an aliquot of the hordein (300 mg) was dissolved in 15 ml of 6 M urea and separated by preparative isoelectric focusing (pH range 7–9) in granulated gel in the presence of 6 M urea (Field *et al.* 1982). Zones were eluted with 0·1 M acetic acid and monitored by SDS–PAGE. Fractions containing the *Hor 4* protein were dialysed against distilled water, lyophilized and then dissolved in 50% (v/v) propan-1-ol and precipitated by the addition of 2 vol 1·5 M-NaCl to remove ampholyte. This was repeated twice and the protein finally lyophilized. The amino acid composition was determined after hydrolysis using an LKB 4400 amino acid analyser. The $S\Delta_n$ index of Cornish-Bowden (1981) was calculated assuming that each protein had an M_r of 37000, corresponding to 321 residues.

(v) *Genetic analysis*

Crosses were made between plants grown in the glasshouse at Rothamsted. F_2 seeds from one plant from the cross Bomi × P12/3, and four plants each from the crosses (M × G 34) × P12/3 and P12/3 × (M × G 5) were scored for hordein alleles. Gene linkage was tested for by χ^2 tests and calculated as described by Jensen & Jorgensen (1975). Map distance was calculated using the Kosambi function (Jensen & Jorgensen, 1975). Genotype frequencies from different samples were pooled if homogeneous ($P > 0{\cdot}05$) in contingency χ^2 tests. This was the case with the four individual plants of crosses 2 and 3 and with the pooled data from these plants. The χ^2 for homogeneity (D.F. = 1) of the recombination fractions in crosses 2 and 3 were 0·46 for *Hor 2* × *Hor 4*, 2·54 for *Hor 1* and *Hor 2* and 0·27 for *Hor 1* × *Hor 4* (in all cases $P > 0{\cdot}05$). The data from cross 1 were not homogeneous with those from crosses 2 and 3.

3. Results and Discussion

(i) *Electrophoretic and chemical analysis of the* Hor 4 *gene product(s)*

Netsvetaev & Sozinov (1984) identified *Hrd G (Hor 4)* as controlling the presence of two polypeptides in the B hordein region on starch gel electrophoresis of the cv. Elgina. These polypeptides were not apparently present in other cultivars, and have not been reported by workers in other laboratories.

Dr Netsvetaev provided us with six homozygous lines from his P12 population, derived from the cross Nutans 244 × Elgina. These represent three pairs of lines isogenic for *Hor 1* and *Hor 2*, but differing in the absence or presence of the *Hor 4* protein. SDS–PAGE analyses of these are shown in Fig. 1. Two of the lines, P12/3 and P12/4, clearly differed in the presence of an additional band in the B hordein region of the former (arrowed in Fig. 1, track f). This corresponds to the product of the *Hor 4* locus. Two-dimensional analysis of these lines using isoelectric focusing followed by SDS–PAGE (Fig. 2*a*, *b*) showed that the *Hor 4* product had a higher isoelectric point (pI) than the major B hordeins present in these lines. The other four lines (P12/1, P12/6, P12/2 and P12/5) had the same B hordein pattern on SDS–PAGE, and no additional protein corresponding to the *Hor 4* product was observed (Fig. 1, tracks a–d). However, 2-D analysis showed that additional proteins were also present in P12/6 (Fig. 3*b*) and P12/5 (not shown), but co-migrated with B hordeins on SDS–PAGE.

The unusually high pI of the *Hor 4* protein compared with those of the major B hordeins in P12/3 was

https://doi.org/10.1017/S0016672300023879 Published online by Cambridge University Press

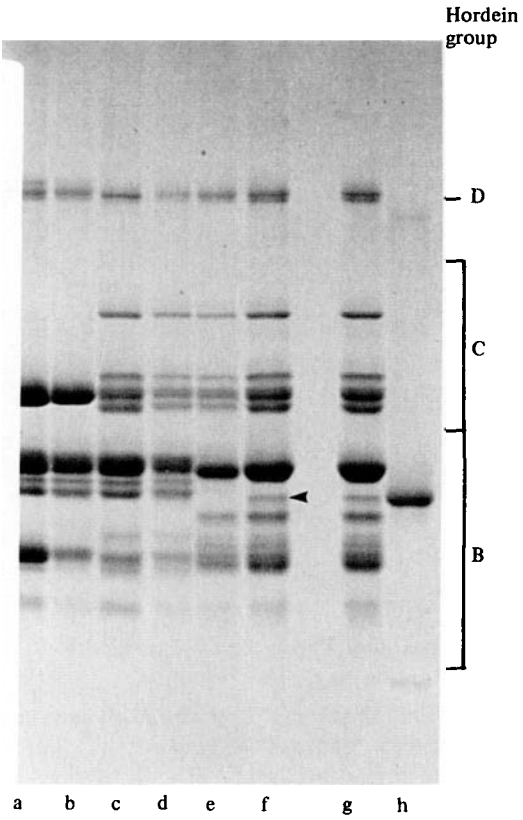

Fig. 1. SDS–PAGE of hordein fractions from six homozygous lines produced from the cross Nutans 244 × Elgina (tracks a–g) and of the *Hor 4* protein purified from P12/3 (track h). P12/1 (track a) and P12/6 (track b), P12/2 (track c) and P12/5 (track d); P12/4 (track e) and P12/3 (tracks f and g) are pairs of lines that have the same *Hor 1* (C hordein) and *Hor 2* (B hordein) alleles, but differ in the absence or presence respectively of an additional protein encoded by *Hor 4* in the B hordein region. This is arrowed in track f, but is not resolved from the major B hordeins in tracks b and d. B, C and D indicate the major groups of hordein polypeptides.

exploited in the purification of small amounts of the protein from this line by preparative isoelectric focusing (Fig. 1, track h). The amino acid composition of this fraction (Table 1) was similar to those of total (Table 1) and purified (Kreis *et al.* 1983) B hordeins, with high glutamate + glutamine (33.6 mol %) and proline (20.5 mol %). The higher content of basic amino acids (lysine, arginine and histidine) (5·2 mol % compared with 5·0 mol % in total B hordein) is probably not sufficient to account for the high pI of the *Hor 4* protein. A lower content of acidic amino acids could also contribute, but the amidation level of the glutamic and aspartic acid residues recovered after acid hydrolysis was not determined in the present study due to lack of purified protein. Although cysteine was not determined precisely, it was clearly present. Statistical comparison of amino acid compositions of the *Hor 4* protein and the B hordein fractions in Table 1 using the $S\Delta_n$ index of Cornish-Bowden (1981) gave a value of 0·07, indicating a high degree of homology. A small amount (< 1 mg) of the *Hor 4* protein was also subjected to automated Edman degradation. Only traces of amino acids were released in 5 cycles of degradation, indicating that it was probably N-terminally blocked (P. R. Shewry, E. J-L. Lew & D. D. Kasarda, unpublished results). It is similar in this respect to the major B hordeins (Shewry *et al.* 1980*a*). The γ-type hordeins are not N-terminally blocked (Shewry *et al.* 1985*b*) and have slightly different amino acid compositions, with either less proline or less glutamate + glutamine than the typical B hordeins and the *Hor 4* protein (see Kreis *et al.* 1983).

From these studies it was concluded that the *Hor 4* protein is closely related to the major B hordeins encoded by the *Hor 2* locus.

(ii) *Mapping of the* Hor 4 *locus*

Crosses were made between P12/3, Bomi and two doubled monoploid lines produced from a cross between Magnum and Goldspear (M × G 5 and M × G 34). The *Hor 4* protein was only present in P12/3. SDS–PAGE separations of hordein fractions from the lines are shown in Fig. 4, and their alleles at the *Hor 1* and *Hor 2* loci are given in Table 2.

P12/3 was initially crossed with Bomi to determine the linkage relationship of the *Hor 4* protein with the major B hordein (*Hor 2*) polypeptides. F2 seeds were scored for the presence or absence of the *Hor 4* protein, with no attempt to distinguish between homozygous present and the two classes of heterozygote differing in gene dosage in the triploid endosperm. The presence of the *Hor 4* protein was, therefore, treated as a dominant allele. The seeds were also scored for co-dominant alleles at the *Hor 2* locus, also without distinguishing between the two classes of heterozygote. The results (Tables 3 and 5) showed 14·18 ± 2·22 % recombination between *Hor 4* and *Hor 2*. Two-dimensional analysis of a homozygous recombinant F_2 seed from this cross (Fig. 2*d*) confirmed that the *Hor 4* protein from P12/3 (arrowed in Fig. 2*b*, *d*) was present together with the B hordeins (encoded by *Hor 2*) from Bomi (cf. Fig. 2*c*, *d*). Because Bomi and P12/3 have the same allele at the *Hor 1* (C hordein) locus (Table 2, Figs. 2 and 4), it was not possible to determine the linkage between *Hor 1* and *Hor 4*. We therefore performed additional crosses between P12/3 and two lines (M × G 5, M × G 34) with different *Hor 1* and *Hor 2* alleles. The results presented in Tables 4 and 5 clearly demonstrate that *Hor 4* is located proximally to *Hor 1*. The linkage relationships between *Hor 4* and some other loci on the short arm of chromosome 5 are summarized in Fig. 5.

The recombination percentages between *Hor 1* and *Hor 4* in crosses 2 and 3 (1·81 % and 3·63 %) are consistent with those reported by Netsvetaev & Sozinov (1984) (2·56 ± 0·70, 0·70 ± 0·41 and 1·19 ± 0·29

https://doi.org/10.1017/S0016672300023879 Published online by Cambridge University Press

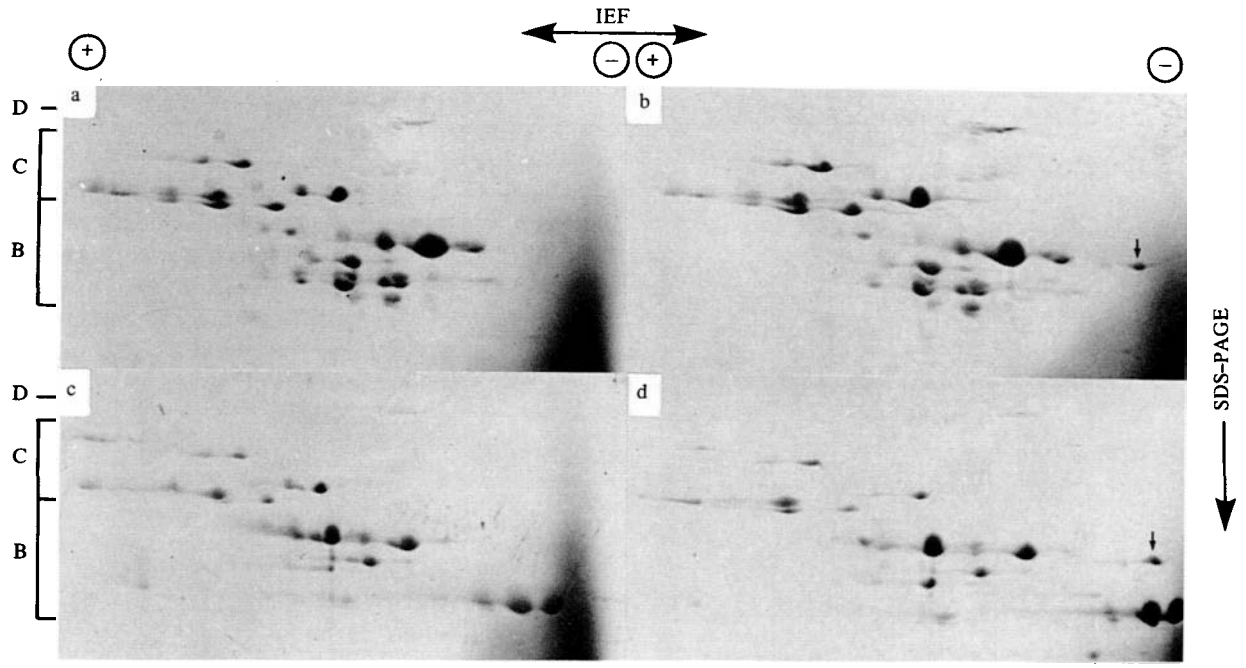

Fig. 2. 2-D isoelectric focusing (pH range 3·5-10)/SDS–PAGE of hordein fractions from a, P12/4; b, P12/3; c, Bomi and d, a homozygous recombinant seed from the cross Bomi × P12/3. The protein controlled by *Hor 4* is indicated by arrows in b and d. B, C and D indicate the major groups of hordein polypeptides.

Table 1. *Comparison of the amino acid compositions (expressed as mol %) of the* Hor 4 *protein and total B hordein*

	Hor 4 protein	Total B[a] hordein		*Hor 4* protein	Total B[a] hordein
Asp[b]	2·2	1·4	Met	0·9	0·6
Thr	1·0	2·1	Ile	4·2	4·1
Ser	5·7	4·7	Leu	6·8	7·0
Glu[b]	33·6	35·4	Tyr	2·8	2·5
Pro	20·5	20·6	Pro	5·4	4·8
Gly	3·6	1·5	His	1·5	2·1
Ala	3·1	2·2	Lys	1·5	0·5
Cys	[c]	2·5	Arg	2·2	2·4
Val	5·0	5·6			

[a] Results reported for total hordein of cv. Julia by Shewry *et al.* (1980*a*).
[b] Include the amides Asn and Gln respectively.
[c] Cysteine was present but could not be determined accurately.

Table 2. *Genotypes of parents of the crosses*

	Hor 1	Hor 2	Hor 4
P12/3	Pr	Ca	+
Bomi	Pr	Ze	–
M × G 5	Ma	Ze	–
M × G 34	Ma	Ze	–

The allelic designations follow Johansen & Shewry (1986).

in three crosses). However, the recombination percentages observed between *Hor 1* and *Hor 2* in crosses 2 and 3 are considerably greater than that observed in cross 1, and than most previously published values. The latter mostly fall between 6% and 15% (see Shewry & Miflin, 1982), and Jensen (1986) has calculated a map distance of 7·7 cM (7·64% recombination) on the basis of the lower estimates. Our results are, however, again consistent with those of Netsvetaev & Sozinov (1984), who reported recombination percentages between *Hor 1* and *Hor 2* of 8·02 ± 1·58 in the cross Union × Elgina, and 17·37 ± 2·34 in the cross Nutans 244 × Elgina. The latter cross was the origin of the P12 lines used in the present study. Taken together, our results and those of Netsvetaev & Sozinov (1984) indicate that recombination between *Hor 1* and *Hor 2* is strongly influenced by the genotype of lines crossed with Elgina and P12/3. Also, this effect is independent of the direction of the cross, since crosses 2 and 3 were essentially reciprocal, with P12/3 as the male and female parent respectively.

https://doi.org/10.1017/S0016672300023879 Published online by Cambridge University Press

Table 3. *Frequency in the progeny of cross 1 (Bomi × P12/3) of genotypes resulting from pairwise combinations of alleles at the* Hor 2 *and* Hor 4 *loci*

		Hor 2			
		ZeZe	ZeCa	CaCa	Totals
Hor 4					
+	+	18	142	65	225
+	−				
−	−	41	12	3	56
Totals		59	154	68	281

Table 4. *Frequency in the progeny of crosses 2 ((M × G 34) × P12/3) and 3 (P12/3 × (M × G 5)) of genotypes resulting from combinations of alleles at the* Hor 1, Hor 2 *and* Hor 4 *loci*

Hor 1	*Hor 2*	*Hor 4*	Cross 2	Cross 3	Total, 2+3
MaMa	ZeZe	+ + + −	1	1	2
MaMa	ZeZe	− −	50	32	82
MaMa	CaZe	+ + + −	2	1	3
MaMa	CaZe	− −	33	17	50
MaMa	CaCa	+ + + −	0	1	1
MaMa	CaCa	− −	3	3	6
MaPr	ZeZe	+ + + −	29	22	51
MaPr	ZeZe	− −	0	1	1
MaPr	CaZe	+ + + −	143	87	230
MaPr	CaZe	− −	2	3	5
MaPr	CaCa	+ + + −	37	25	62
MaPr	CaCa	− −	0	2	2
PrPr	ZeZe	+ + + −	0	2	2
PrPr	ZeZe	− −	0	0	0
PrPr	ZeCa	+ + + −	29	16	45
PrPr	ZeCa	− −	1	0	1
PrPr	CaCa	+ + + −	69	28	97
PrPr	CaCa	− −	0	0	0
Totals			399	241	640

(iii) *General discussion*

Our results show that the additional polypeptide present in the cultivar Elgina and derived lines such as P12/3 is related structurally to the major B hordeins encoded by the *Hor 2* locus. They also confirm the report of Netsvetaev & Sozinov (1984) that the controlling locus, which we have designated *Hor 4*, is located proximally to *Hor 1* on the short arm of chromosome 5. It is of interest that neither *Hor 4* nor its polypeptide product has been identified in any other barley cultivar. This could be because allelic variants of the polypeptide that co-migrate with the major B hordeins are present. A second possibility, which seems more probable in view of the extensive studies of hordein genetics that have been carried out using a range of lines and analytical procedures, is that the *Hor 4* locus has arisen in an ancestor of

https://doi.org/10.1017/S0016672300023879 Published online by Cambridge University Press

Table 5. *Linkage χ^2, probability values, recombination percentages and map distances for the loci segregating in crosses 1–3*

	Cross	Linkage (χ^2 D.F. = 1)	Probability of independence	Recombination percentage (±S.E.)	Map distance in cM (±S.E.)
Hor 2 × Hor 4	1	61·50	< 0·001	14·18 ± 2·22	14·58 ± 2·42
	2	78·68	< 0·001	20·25 ± 2·22	21·49 ± 2·65
	3	35·32	< 0·001	24·82 ± 3·14	27·22 ± 4·17
	2+3	113·44	< 0·001	21·99 ± 1·82	23·60 ± 2·26
Hor 1 × Hor 2	2	137·23	< 0·001	19·08 ± 1·58	20·10 ± 1·85
	3	50·21	< 0·001	22·96 ± 2·25	24·82 ± 2·85
	2+3	184·90	< 0·001	20·49 ± 1·30	21·77 ± 1·56
Hor 1 × Hor 4	2	204·68	< 0·001	1·81 ± 0·67	1·81 ± 0·67
	3	109·55	< 0·001	3·63 ± 1·22	3·64 ± 1·23
	2+3	313·96	< 0·001	2·52 ± 0·63	2·52 ± 0·63

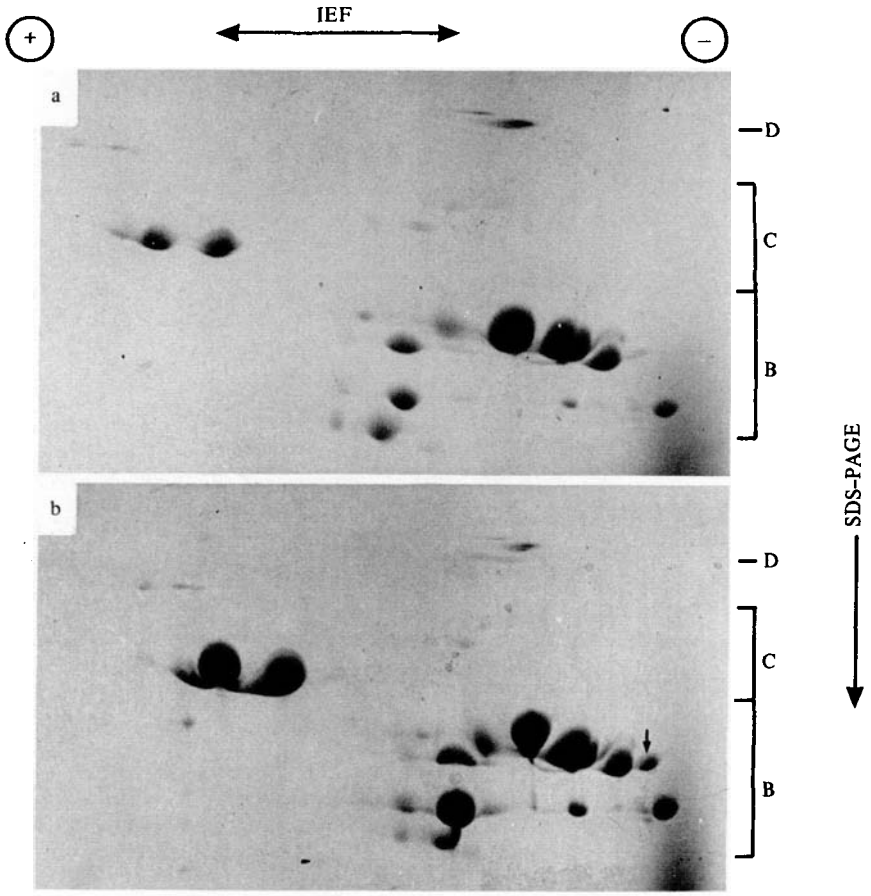

Fig. 3. 2-D isoelectric focusing (pH range 3·5–10)/SDS–PAGE of hordein fractions from a, P12/1 and b, P12/6. The protein controlled by *Hor 4* is indicated by the arrow in b. B, C and D indicate the major groups of hordein polypeptides.

Elgina. It should be possible to distinguish between these two possibilities by molecular analysis using a cloned cDNA or gene derived from the *Hor 4* locus. In either case it is almost certain that the locus was derived from *Hor 2* by translocation of one or a small number of genes. The mechanism for this is not known, but it is significant that the short arm of chromosome 5 contains a number of loci encoding structurally related hordein storage proteins (*Hor 1, Hor 2, Hrd F*), which are interspersed with loci conferring resistance to powdery mildew and rusts (see Fig. 5 and Jensen, 1986). The latter may also be multigenic and derived from one or more ancestral loci. This suggests that a number of structural rearrangements have occurred. The presence of such a rearrangement in Elgina and P12/3 could be related to the unusually high recombination values observed in crosses with some genotypes (i.e. negative chromosome interference).

https://doi.org/10.1017/S0016672300023879 Published online by Cambridge University Press

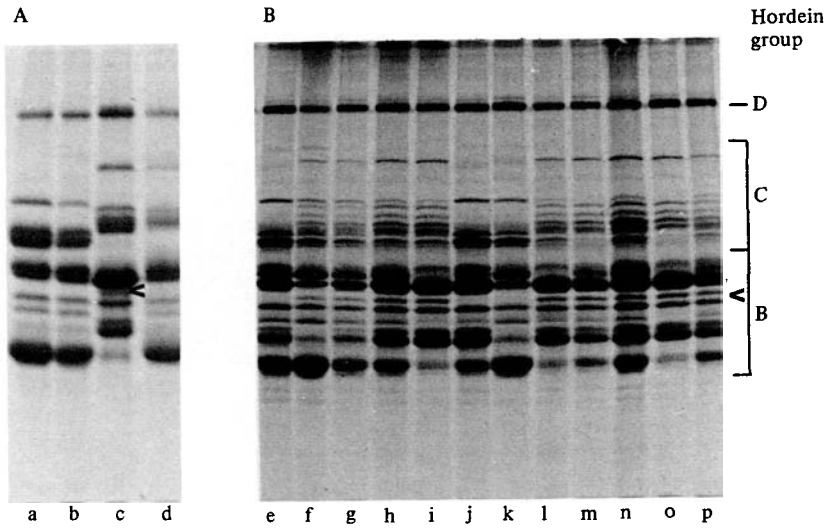

Fig. 4. SDS–PAGE of hordein fractions from single seeds of the parents of crosses 1, 2 and 3 (part A) and random F2 progeny from cross 2 (part B). a, M × G 5; b, M × G 34; c, P12/3 and d, Bomi. The protein controlled by *Hor 4* is indicated by arrows in tracks c and p. B, C and D indicate the major groups of hordein polypeptides.

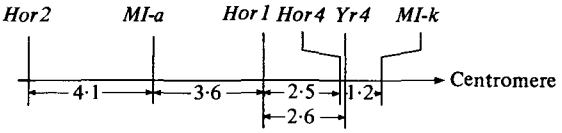

Fig. 5. The linkage relationships between *Hor 4* and some other loci on the short arm of barley chromosome 5. The linkage between *Hor 1* and *Hor 4* is based on the present paper. The rest of the map is based on Jensen (1986). Map distances are in centimorgans (cM).

We are grateful to Dr V. P. Netsvetaev (Odessa, USSR) and Dr W. Powell (Pentlandfield, Scotland) for supplying seeds and to Mrs S. J. Smith for amino acid analyses. We are also indebted to Dr J. Jensen (Risø, Denmark) for discussions and for providing a copy of his computer program for calculating recombination, and to Dr Angela Karp for discussions and critical comments.

References

Blake, T. K., Ullrich, S. E. & Nilan, R. A. (1982). Mapping of the *Hor 3* locus encoding 'D' hordeins in barley. *Theoretical and Applied Genetics* **63**, 367–371.

Brandt, A., Ingversen, J., Cameron-Mills, V., Schmitt, J. M. & Rasmussen, S. K. (1981). Molecular aspects of storage protein synthesis in barley endosperms. In *Barley Genetics*, vol. IV. Edinburgh: University of Edinburgh Press.

Bunce, N., White, R. P. & Shewry, P. R. (1985). Variation in estimates of molecular weights of cereal prolamins by SDS–PAGE. *Journal of Cereal Science* **3**, 131–142.

Bunce, N. A. C., Forde, B. G., Kreis, M. & Shewry, P. R. (1986). DNA restriction fragment length polymorphism at hordein loci: application to identifying and fingerprinting barley cultivars. *Seed Science and Technology* **14**, 419–429.

Cornish-Bowden, A. (1981). Interpretation of amino acid compositions. *Trends in Biochenical Sciences* **6**, 217–219.

Doll, H. & Brown, A. D. H. (1979) Hordein variation in wild (*Hordeum spontaneum*) and cultivated (*H. vulgare*) barley. *Canadian Journal of Genetics and Cytology* **21**, 391–404.

Faulks, A. J., Shewry, P. R. & Miflin, B. J. (1981). The polymorphism and structural homology of storage polypeptides (hordein) coded by the *Hor 2* locus in barley (*Hordeum vulgare* L.). *Biochemical Genetics* **19**, 841–858.

Field, J. M., Shewry, P. R., Miflin, B. J. & March, J. F. (1982). The purification and characterization of homologous high molecular weight storage proteins from grain of wheat, rye and barley. *Theoretical and Applied Genetics* **62**, 329–336.

Forde, B. G., Kreis, M., Bahramian, M. B., Matthews, J. A., Miflin, B. J., Thompson, R. D., Bartels, D. & 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.

Forde, J. (1985). The molecular biology of the high molecular weight glutenin subunits of wheat. Ph.D. Thesis, CNAA, U.K.

Friedman, M., Krull, L. H. & Cavins, J. F. (1970). The chromatographic determination of cystine and cysteine residues as S-β-(4-pyridylethyl) cysteine. *Journal of Biological Chemistry* **245**, 3868–3871.

Jensen, J. & Jorgensen, J. H. (1975). The barley chromosome 5 linkage map. 1. Literature survey and map estimation procedure. *Hereditas* **80**, 5–16.

Jensen, J., Jorgensen, J. H., Jenson, H. P., Giese, H. & Doll, H. (1980). Linkage of the hordein loci *Hor 1* and *Hor 2* with the powdery mildew resistance loci *M1-k* and *M1-a* on barley chromosome 5. *Theoretical and Applied Genetics* **58**, 27–31.

Jensen, J. (1986). Co-ordinator's Report. Chromosome 5. *Barley Genetics Newsletter* **16**, 32–34.

Johansen, H. B. & Shewry, P. R. (1986). Recommended designations for hordein alleles. *Barley Genetics Newsletter* **16**, 9–11.

Kreis, M., Shewry, P. R., Forde, B. G., Rahman, S. & Miflin, B. J. (1983). Molecular analysis of a mutation conferring the high lysine phenotype on the grain of barley (*Hordeum vulgare*). *Cell* **34**, 161–167.

Kreis, M., Williamson, M. S., Forde, J., Clark, J., Buxton, B., Pywell, J., Marris, C., Gallois, P. & Shewry, P. R. (1987). Structure and regulation of seed protein genes in barley. *Plant Physiology and Biochemistry* **25**, 291–302.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 681–684.

Netsvetaev, V. P. & Sozinov, A. A. (1982). Linkage studies of genes *G1e1* and *HrdF* in barley chromosome 5. *Barley Genetics Newsletter* **12**, 13–18.

Netsvetaev, V. P. & Sozinov, A. A. (1984). Location of a hordein G locus, *HrdG*, on chromosome 5 of barley. *Barley Genetic Newsletter* **14**, 4–6.

Oram, R. N., Doll, H. & Køie, B. (1975). Genetics of two storage protein variants in barley. *Hereditas* **80**, 53–58.

Rahman, S., Shewry, P. R. & Miflin, B. J. (1982). Differential protein accumulation during barley grain development. *Journal of Experimental Botany* **33**, 717–728.

Shewry, P. R., Pratt, H. M., Finch, R. A. & Miflin, B. J. (1978). Genetic anaysis of hordein polypeptides from single seeds of barley. *Heredity* **40**, 463–466.

Shewry, P. R., Field, J. M., Kirkman, M. A., Faulks, A. J. & Miflin, B. J. (1980*a*). The extraction, solubility and characterization of two groups of barley storage polypeptides. *Journal of Experimental Botany* **31**, 393–407.

Shewry, P. R., Faulks, A. J., Pickering, R. A., Jones, I. T., Finch, R. A. & Miflin, B. J. (1980*b*). The genetic analysis of barley storage proteins. *Heredity* **44**, 383–389.

Shewry, P. R., Lew, E. J.-L. & Kasarda, D. D. (1981). Structural homology of storage proteins coded by the *Hor 1* locus of barley (*Hordeum vulgare* L.). *Planta* **153**, 246–253.

Shewry, P. R., Finch, R., Parmar, S., Franklin, J. & Miflin, B. J. (1983). Chromosomal location of *Hor 3*, a new locus governing storage proteins in barley. *Heredity* **50**, 179–189.

Shewry, P. R., Bunce, N. A. C., Kreis, M. & Forde, B. J. (1985*a*). Polymorphism at the *Hor 1* locus of barley (*Hordeum vulgare* L.). *Biochemical Genetics* **23**, 389–402.

Shewry, P. R., Kreis, M., Parmar, S., Lew, E. J.-L & Kasarda, D. D. (1985*b*). Identification of γ-type hordeins in barley. *FEBS Letters* **190**, 61–64.

Shewry, P. R. & Miflin, B. J. (1982). Genes for the storage proteins of barley. *Qualitas Plantarum. Plant Foods for Human Nutrition* **31**, 251–267.

Shewry P. R. & Parmar S. (1987). The *Hrd F* (*Hor 5*) locus encodes γ-type hordeins. *Barley Genetics Newsletter* **17**. In press.

Shewry, P. R., Williamson, M. S. & Kreis, M. (1987). Effects of mutant genes on the synthesis of storage components in developing barley endosperms. In *Mutant Genes that Affect Plant Development* (ed. H. Thomas), pp. 95–118. Cambridge: Cambridge University Press.