Theor Appl Genet (1992) 85: 197–204

The location of major genes and associated quantitative trait loci on chromosome arm 5BL of wheat

H. Miura *, **B. B. Parker** and **J. W. Snape**

Cambridge Laboratory, John Innes Centre for Plant Science Research, Norwich NR4 7UJ, UK

Received March 3, 1992; Accepted March 24, 1992
Communicated by G. Wenzel

Summary. Chromosome 5B of bread wheat is known to carry two major genes giving rise to genetic disorders, *Ne1* for hybrid necrosis and *Vg* for winter variegation. Additionally, in many european winter wheat varieties this chromosome is represented in a translocated form, with 5BL-7BL, 5BL-7BS chromosomes rather than the normal 5B and 7B forms of the standard variety Chinese Spring. Genetic analysis has been carried out to map these genes and the translocation break point, and to investigate their pleiotropic effects or those of linked quantitative trait loci (qtl) for economically important characters. This was facilitated by the development of single chromosome recombinant lines between a normal and translocated karyotype, and growing these in field experiments over two seasons. There was differential segregation in favour of the translocated karyotype in the population of recombinant lines. Linkage analysis revealed that the two morphological markers and the isozyme locus *Ibf-B1* were located on the long arm of 5B with a gene order of: breakpoint – *Ne1* – *Vg* – *Ibf-B1*. Analysis of quantitative characters using these genes as landmarks showed pleiotropic effects of *Ne1* or effects of tightly linked qtl on most of the quantitative characters related to grain yield. An additional qtl determining spikelet and grain number/ear appeared to be linked to the centromere. Effects on ear emergence time were associated with both *Ne1* and *Vg*, and these interacted with environments. Similarly, effects on plant height were associated with *Ne1* and *Vg*. In addition, there was a further unlocated locus (loci) for height acting independently of the markers.

Key words: Wheat – Chromosome 5BL – Major genes – Quantitative trait loci

Introduction

The production of a comprehensive genetic map of wheat describing the locations of major gene loci and of quantitative trait loci (qtl) is required for advancing techniques of genetic analysis and for improving the precision of genetic manipulation in breeding programmes. Sax (1923) suggested the basic idea for studying qtls through linkage with markers, and this was developed further by Thoday (1961). This approach has great potency with the present ability to develop comprehensive maps using biochemical and molecular markers, as demonstrated by recent applications for the identification of qtl in tomato (Zhuchenko et al. 1978; Tanksley et al. 1982; Weller et al. 1988; Paterson et al. 1988, 1991), maize (Stuber et al. 1987) and wheat (Law 1966, 1967; Snape et al. 1985).

In bread wheat, *Triticum aestivum*, the availability of aneuploid lines has led to the development of cytogenetical techniques which facilitate the examination, chromosome by chromosome, of genetic variation for quantitative characters (Kuspira and Unrau 1957; Law, Snape and Worland 1987). Following the establishment of the chromosomal locations of major genes and qtl, further manipulation is facilitated by the use of homozygous recombinant lines for individual chromosomes (Law 1966, 1967). By combining comprehensive genetic maps of individual chromosomes with single chromosome recombinant lines, detailed and precise locations of qtl, and their primary and pleiotropic effects, can be established.

The long arm of chromosome 5B is known to carry major gene loci affecting two genetic disorders, *Ne1* for

---

* Present address: Dept of Crop Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan

Correspondence to: J.W. Snape

hybrid necrosis and *Vg* for winter variegation, where the dominant alleles prevent the growth of normal healthy plants; as well as *Kr1* controlling interspecific crossability (Worland et al. 1987). Therefore, detailed genetical analysis of qtl on chromosome 5B is of importance in breeding programmes for developing adapted wheats without problems of necrosis or winter variegation; and also for studies of the transfer of interspecific and intergeneric variation into wheat. In this paper we describe how single-chromosome recombinant lines were developed between the UK winter wheat variety 'Hobbit sib' and a substitution line of 'Chinese Spring' (CS) chromosome 5B into 'Hobbit sib', and were then used to map these markers relative to each other and to the translocation break-point, and to locate a number of qtl for agronomically important characters.

Materials and methods

Development of recombinant lines

Homologous single chromosome recombinant lines were developed for chromosome arms 5BL and 7BL from crosses between 'Hobbit sib', a semi-dwarf winter wheat, and the inter-varietal chromosome substitution line of 'Hobbit sib' in which chromosome arms 5BL and 7BL had been replaced by their homologues from 'CS'. The recipient variety 'Hobbit sib' does not have a normal 5B and 7B karyotype but rather has a translocated karyotype with 5BL-7BL and 5BS-7BS chromosomes. This complication made it impossible to substitute the complete 5B chromosome of 'CS' into 'Hobbit sib', using the conventional procedure described by Law and Worland (1973) and an alternative procedure was used.

From an initial cross between 'Hobbit sib' monosomic 5BL-7BL plants as female and 'CS' as male, modifications were required to maintain the 'CS' 5BL and 7BL chromosome arms in a non-recombined state. This was done by selection for a trivalent at metaphase-I of meiosis in pollen mother cells in the progenies of each generation of backcrossing to the 'Hobbit sib' monosomic 5BL-7BL line. Following five backcrosses, this procedure gave plants carrying the 5BL and 7BL arms of 'CS' in a hemizygous condition in a reconstituted 'Hobbit sib' genetic background. The disomic 'double arm' substitution, which had a normal karyotype, 'Hobbit sib' (CS 5BL, 7BL), was then derived from selfing monosomic plants containing a trivalent and selecting for plants with twenty-one bivalents.

To develop the recombinant lines this substitution line was first crossed with the recipient variety 'Hobbit sib' to give an F_1 hybrid in which only the 5BL and 7BL arms were heterozygous, but as a translocation heterozygote. Thus, these F_1 progeny formed a quadrivalent at meiosis giving six types of gametes, of which only two contain balanced combinations of chromosome arms of 5B and 7B, while the remaining four types contain duplication-deficiency situations for each of the arms of 5B and 7B. The F_1s were then backcrossed as male parents to the 'Hobbit sib' monosomic 5BL-7BL line. In the resulting progeny, monosomic plants were again selected by cytological examination of meiotic chromosomes in pollen mother cell preparations. As expected, two types of monosomics were extracted; those in which the chromosome composition consisted of 20 bivalents plus one 5BL-7BL univalent, and those with 19 bivalents plus one trivalent of the composition 7BL-7BS:7BS-5BS:5BS-5BL. All of these plants contained the 5BL and 7BL chromosome arms in the hemizygous condition, and were products of recombination between the 'Hobbit sib' and 'CS' homologues. On selfing these individuals, disomic plants (one or two) were selected by cytological examination of their progeny, all of which contained twenty-one bivalents at meiosis.

In total, 71 different homozygous disomic recombinant plants, with duplicates for five, were extracted, then grown to maturity and allowed to self-pollinate. The seed from these disomic plants was then used to assess their genotype for the marker genes and for karyotype; and also to assess the effect of the homologous chromosome variation on quantitative traits.

In addition to the homozygous recombinant lines, non-recombinant parental lines were developed by crossing the original substitution line and 'Hobbit sib' to the 'Hobbit sib' monosomic 5BL-7BL line. Monosomic and then, subsequently, disomic plants were extracted. These plants were selfed to produce two groups of lines homozygous for the non-recombined 'CS' 5BL and 7BL chromosome arms. Together with the duplicate disomic lines, these parental lines can provide a test of the genetic segregation in the backgrounds of the 'Hobbit sib' monosomic line and the substitution line caused by possible residual variation other than that determined by chromosome 5B. These parental controls also provide an estimate of the whole-chromosome difference between the homologous parental chromosomes for quantitative traits.

Classification of the recombinant lines at marker loci

The recombinant lines were tested for allele composition at four marker loci – *Ne1*, *Vg*, *Kr1* and *Ibf-B1*, known to reside on chromosome arm 5BL. However, classification for *Kr1*, by assessing crossability with *Hordeum bulbosum*, produced ambigous results, and these data will, therefore, not be considered here.

Hybrid necrosis results from the effects of two dominant genes, *Ne1* on 5BL and *Ne2* on 2BS (Tsunewaki 1960). The 'CS' 5B carries the dominant gene at the *Ne1* locus, and the chromosome substitution line 'Hobbit sib' ('CS' 5BL, 7BL), and consequently, exhibits hybrid necrosis (Worland, personal communication). The recombinants segregated for this character when grown in field experiments, and the individual homozygous recombinant lines and the parental controls were easily distinguished in terms of their genotypes at the *Ne1* locus by the appearance or non-appearance of necrotic leaves.

Winter variegation exhibits itself as achlorotic patches on leaves when plants are grown under cool conditions. These symptoms are produced by an interaction between the cytoplasm and nuclear genes, and are particularly pronounced in alloplasmic lines of wheat having the cytoplasm of *Aegilops umbellulata* with a single dominant nuclear gene, *Vg*, present on the long arm of chromosome 5B (Worland and Law 1983). 'Hobbit sib' exhibits the phenomenon whilst 'CS' does not. The presence or absence of *Vg* was tested for by crossing each recombinant line, as male parent, onto a 'CS'/*Ae. umbellulata* alloplasmic line, and growing three F_1 plants from each cross under cool conditions. In lines carrying the 'Hobbit sib' *Vg* allele, uniform, characteristic symptoms of chlorosis and dwarfing were observed.

A biochemical marker, iodine-binding factor, is controlled by genes on the long arms of the homoeologous group 5 chromosomes (Liu and Gale 1989). The 'Hobbit sib' 5B and 'CS' 5B chromosomes produce different banding patterns for the *Ibf-B1* locus. Using the techniques described by Liu and Gale (1989), isoelectric focusing of extracts from the endosperm-half of mature grains of each recombinant line was carried out and the discrete banding pattern of each line was determined as to whether it carried the $Ibf\text{-}B1^-$ allele of CS or the $Ibf\text{-}B1^+$ allele of 'Hobbit sib'.

In addition to these three marker genes, the difference in chromosome structure was used as a cytological marker. Using the C-banding technique each recombinant line was characterized for whether it carried a translocated or normal karyotype (see Fig. 1).

Assessment of quantitative characters

Two field experiments were conducted at Cambridge, UK in the 1985/86 and 1988/89 growing seasons. Seventy-six homozygous recombinants, made up of five lines in duplicate and 66 single lines, plus five lines of each of the parental controls, were grown in four replicate blocks in the 1985/86 experiment and in five replicates in the 1988/89 experiment. Within each replicate, a genotype was represented by a single row plot of 11 plants, spaced 10 cm apart within rows and 30 cm between rows. During growth and at maturity a range of agronomic characters was measured on each plot. At maturity four random leading tillers were taken from each plot and used for the evaluation of single tiller yield components. The remainder of each plot was harvested and threshed to determine plot yields. The data were transformed to values per plant or per tiller for analysis.

Results

Genotypic classification and linkage estimation

C-banding studies suggested that the translocation break-point is probably centromeric (Fig. 1). Of the 71 unique recombinant lines studied, 46 lines were identified as having the translocated 5BL-7BL, 5BS-7BS karyotype of 'Hobbit sib' and the remaining 25 lines to have the normal 'CS' karyotype of 5BL-5BS, 7BL-7BS. These segregation frequencies gave a significant deviation from the expected 1:1 ratio, suggesting that there has been gametic or genotypic selection in favour of genotypes carrying the translocated karyotype.

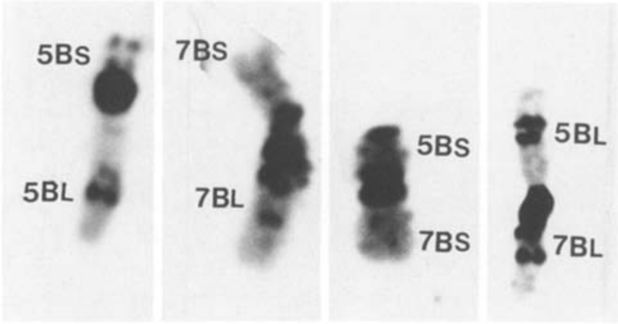

Fig. 1. C-banded karyotypes of the normal 5B and 7B, and translocated 5BS-7BS, 5BL-7BL, chromosomes of the single chromosome recombinant lines

All 71 recombinant lines were also unambiguously classified for their genotype at the *Ne1*, *Vg* and *Ibf-B1* loci. The parental genotypes are 7BLarm – *ne1* – *Vg* – *Ibf-B1*$^{+}$ ('Hobbit sib') and 5BSarm – *Ne1* – *vg* – *Ibf-B1*$^{-}$, and of the sixteen expected genotypic classes only nine were represented. The smallest observed class of 7BLarm – *ne1* – *vg* – *Ibf-B1*$^{+}$ are likely to be the double recombinants between *Ne1*, *Vg* and *Ibf-B1*, and the absence of the alternative class 5BSarm *Ne1* – *Vg* – *Ibf-B1* would also suggest that this is a double recombinant. The recombination distances between the centromere, *Ne1*, *Vg*, and *Ibf-B1* were calculated from the data and the gene order established (Fig. 2). The isozyme locus *Ibf-B1*$^{+}$ is obviously positioned distally on the long arm since it segregates independently of the centromere. Consequently, the gene order on 5BL must be centromere – *Ne* – *Vg* – *Ibf-B1*.

Analysis of quantitative characters

Non-recombinant parental controls. Analyses of variance of the differences between and within the non-recombinant parental controls, together with their mean performance in 1985/86 and 1988/89, for nine representative agronomic characters are shown in Table 1. In interpreting the results from the two seasons, the prevailing climatic conditions during these experiments must be considered. In the 1985/86 season dry weather throughout the autumn delayed germination and seedling emergence was uneven. Subsequently, severe cold weather in January and February caused frost damage and early spring growth was affected. In the 1988/89 experiment, on the other hand, the conditions were fairly good with mild weather from early spring until harvest. These climatic differences resulted in large phenotypic differences for certain characters. For example, data on ear emergence time clearly showed that flowering was delayed in the 1986 season relative to 1989, there being over 16 days difference between the seasons.

In assigning genetic effects to particular chromosomes, using either chromosome substitution lines or single chromosome recombinant lines, problems of background variation can lead to spurious differences. These can, however, be detected by using appropriate controls.

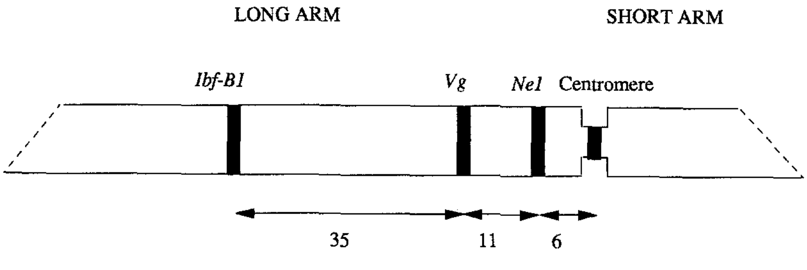

Fig. 2. Partial genetic map of chromosome arm 5BL of wheat

Table 1. Analyses of variance of differences between the parental groups and mean performance in the 1985/86 and 1988/89 experiments

1985/86 experiment Item	df	Mean squares Ear emergence time (days)	Plant height (cm)	Yield/ plant (g)	Grain wt/ear (g)	Fertile tillers/ plant	Spikelet no./ear	Grain no./ear	50 grain wt. (g)	Biomass/ tiller (g)
Parental groups	1	8.333***	18.75***	1,555**	3.818***	–	1.291	25	1.7063***	9.013**
Between lines within groups	7	2.220	3.66	43	0.066	–	0.401	79	0.0201*	0.678*
Error	24	0.722	5.97	94	0.071	–	0.688	56	0.0046	0.199
		Mean phenotype								
'Hobbit sib'		19.2[a]	77.2	46.4	3.26	–	19.9	68.5	2.390	6.54
'Hobbit sib' (CS5BL)		20.9	74.8	23.6	2.14	–	19.3	65.6	1.636	3.72
1988/89 experiment		Mean squares								
Parental groups	1	33.61***	322.7***	2,356**	13.46***	175.8**	5.607*	1,148	4.5763***	53.06**
Between lines within groups	8	0.90	5.1	28*	0.16	10.0***	1.204	243**	0.0329*	1.72***
Error	36	1.30	5.4	10	0.07	1.5	0.791	47	0.0119	0.15
		Mean phenotype								
'Hobbit sib'		2.7[a]	80.4	28.7	3.44	12.8	21.9	68.8	2.500	6.54
'Hobbit sib' (CS5BL)		5.1	73.1	8.8	1.94	7.4	20.9	59.3	1.627	4.11

[a] Days from June 1st

Significance levels: * $P<0.05$, **$P<0.01$, ** $P<0.001$

In this study, therefore, the variation between the replicate lines within parental groups and the variation between the duplicate lines of the recombinants were used to provide a measure of differences ascribable to gene effects other than those carried by the substituted 5BL, 7BL chromosomes. The background genetic variation was significant for both 50 grain weight and biomass/tiller in both experiments, and for yield/plant and fertile tillers/plant in the 1988/89 experiment. This indicates that for these characters the genetic backgrounds of both the 'Hobbit sib' monosomic 5BL, 7BL line and the 'Hobbit sib' ('CS' 5BL, 7BL) substitution line contained some residual heterozygosity. However, over and above this residual genetic variation, differences between means of the parental groups were significant for most of the recorded characters in the two experiments. This implies that there is allelic variation between the long arms of chromosomes 5B and 7B of 'CS' and the homologues from 'Hobbit sib'. In 'Hobbit sib', the 'CS' 5BL, 7BL arms appear to cause a later ear emergence time and a general loss of plant vigour. Most striking is that the homozygous chromosome arm substitutions seem to be associated with negative effects on yield components, with decreased grain weight/ear, fertile tillers/per plant and 50 grain weight, which, together, lead to a drastic net effect of reduced plant yield, associated with the development of necrotic symptoms.

Recombinant lines: variation between genotypic classes. To examine whether the differences found between the parental groups were associated with segregation of the markers on 5BL or with independent loci, the variation between all the lines and between the marker groups was examined (Table 2). The between-groups variation was highly significant for all characters recorded in both seasons. This indicates that the genetical differences are associated with the segregation of the markers on the long arm of chromosome 5B. Indeed, for plant yield and most of its components, more than 90 per cent of the genetical variation could be accounted for by the between-genotypic classes variance, suggesting that those characters must be primarily under the pleiotropic control of the individual markers or of very closely linked loci.

The significance of the within-genotypic classes variation suggests that there is also genetical variation which is independent of the markers. Some of this variation may be due to segregation of background genes responsible for the variation detected previously from the analysis of the parental groups (Table 1). For plant height and spikelet number/ear in the 1988/89 experiment, however, about half or more of the genetic variation (as estimated from the expected mean squares) was accounted for by the within-genotypic classes variation, which was greater than the background chromosome variation. A similar pattern was observed in the 1985/86 experiment. This

Table 2. Analyses of variance of differences between the recombinant lines in the 1985/86 and 1988/89 experiments

1985/86 experiment		Mean squares								
Item	df	Ear emergence time	Plant height	Yield/ plant	Grain wt/ear		Spikelet no./ear	Grain no./ear	50 grain wt.	Biomass/ tiller
Between lines	70	2.30**	28.3***	624***	1.60***		2.58***	167***	0.438***	3.30***
Bet. genotypic classes	8	5.58***	148.1***	4,616***	12.21***		10.60***	708***	3.545***	24.75***
Bet. lines within classes	62	1.88**	12.8***	117*	0.23**		1.54***	98**	0.037***	0.57**
Error	210	1.08	5.1	70	0.11		0.56	51	0.016	0.30

1988/89 experiment Item	df	Mean squares								
		Ear emergence time	Plant height	Yield/ plant	Grain wt/ear	Tiller no/plant	Spikelet no./ear	Grain no./ear	50 grain wt.	Biomass/ tiller
Between lines	70	3.44***	54.9***	378***	2.80***	27.6***	3.69***	189***	0.928***	6.58***
Bet. genotypic classes	8	19.92***	235.0***	3,053***	22.46***	209.2***	13.18***	1,013***	7.765***	55.16***
Bet. lines within classes	62	1.40***	31.7***	32**	0.26***	4.1**	2.47*	84**	0.046***	0.80***
Error	280	0.56	3.6	20	0.12	2.4	0.54	42	0.020	0.28

Significance levels: * $P = 0.05-0.01$, ** $P = 0.01-0.001$, *** $P < 0.001$

suggests that there is a further height and a spikelet number gene(s) which must be segregating independently of the marker loci on the long arm of chromosome 5B. For ear emergence time the relative magnitude of the between- and within-classes variation varied over the two seasons.

From examination of the means of the nine genotypic classes (Table 3), it was confirmed that the substitution of the 'CS' 5BL, 7BL chromosome arms resulted in a later ear emergence time and a general loss of plant vigour. Additionally, as compared to the parental values for yield/plant in Table 1, there were two genotypic classes which transgressed the lower yielding parent, 'Hobbit sib' (CS 5BL, 7BL): 7BLarm – *Ne1* – *vg* – *Ibf-B1*$^{-}$ and 5BSarm – *Ne1* – *Vg* – *Ibf-B1*$^{+}$. This implies gene dispersion for at least two loci controlling this character on this arm.

Recombinant lines: marker allele associations. To examine further the genetic variation detected between the 71 recombinant lines, the data can be partitioned into between- and within-class variation for the alternative alleles at each marker locus in turn. To take into account any interaction with karyotype differences, the recombinant lines were separated into the translocated 5BL-7BL and the normal 5BL-5BS karyotype groups, and then analyzed. Table 4 shows the mean differences, and it is expected that a significant difference between the alleles implies a pleiotropic effect of the locus or the effect of closely linked qtl. On the other hand, if the residual variation is significant then at least some of the variation must be due to other segregating loci.

The results for ear emergence time in the 1989 season indicated that associated with both the *Ne1* and *Vg* alleles was an effect on delaying ear emergence. However, in the 1986 season the allelic differences were less, suggesting an interaction with climatic conditions. An effect on plant height was associated with *Ne1* in the lines having the translocated 5BL-7BL karyotype. This is probably a pleiotropic effect of *Ne1* or an effect of a closely linked locus on restricted stem elongation and ear length. In the lines with the 5BL-5BS karyotype the effect was not detected in both seasons, although a comparison of the 5BSarm – *Ne1* – *Vg* – *Ibf-B1*$^{+}$ and 5BSarm – *Ne1* – *vg* – *Ibf-B1*$^{+}$ genotypes shows its presence (Table 3). In addition, analysis of variance for this character indicated significant residual variation after removal of variation associated with all four markers (data not shown), suggesting a further height gene(s) segregating independently of the markers on the long arm of chromosome 5B.

For the characters related to grain yield and biomass, close agreement between the 1985/86 and 1988/89 experiments was obtained. *Ne1* is thus closely linked to qtl mediating these effects, or more likely, in combination with *Ne2*, has a pleiotropic effect on most yield characters. In the 46 recombinant lines with the 5BL-7BL karyotype the yield reduction in the *Ne1* allelic class was ascribable to a reduction in all of the component characters, grain weight/ear, fertile tillers per plant, spikelet number/ear, grain number/ear and 50 grain weight. In the 25 lines with the normal 5BL-5BS karyotype the effect of *Ne1* on plant yield was correlated with the effects on grain weight/ear, fertile tillers per plant and 50 grain weight. Thus the complementary epistatic interac-

Table 3. Mean phenotypes of each of the nine genotypic classes in the 1985/86 and 1988/89 experiments

Genotypic class	No. of lines	Ear emergence time (days)	Plant height (cm)	Yield/ plant (g)	Grain wt/ear (g)	Fertile tillers/ plant	Spikelet no./ear	Grain no./ear	50 grain wt. (g)	Biomass/ tiller (g)
1985/86 experiment										
7BL, *ne1*, *Vg*, *Ibf-B1*$^+$	24	19.7[a]	77.0	44.8	3.32	–	20.6	73.4	2.264	5.72
7BL, *ne1*, *Vg*, *Ibf-B1*$^-$	14	19.4	77.7	46.7	3.27	–	20.4	73.0	2.244	5.70
7BL, *ne1*, *vg*, *Ibf-B1*$^-$	5	19.4	78.9	43.8	3.05	–	19.8	67.9	2.247	5.35
7BL, *Ne1*, *vg*, *Ibf-B1*$^-$	2	20.1	74.8	16.4	1.58	–	17.7	54.0	1.465	3.24
7BL, *ne1*, *vg*, *Ibf-B1*$^+$	1	18.5	79.0	40.0	3.34	–	20.6	79.6	2.180	5.79
5BS, *Ne1*, *vg*, *Ibf-B1*$^+$	10	20.4	73.8	21.4	2.12	–	19.9	65.8	1.607	4.00
5BS, *Ne1*, *Vg*, *Ibf-B1*$^+$	2	20.9	70.1	17.1	2.06	–	20.3	66.1	1.558	3.91
5BS, *ne1*, *Vg*, *Ibf-B1*$^+$	2	19.9	72.6	36.1	3.04	–	20.8	72.9	2.078	5.32
5BS, *Ne1*, *vg*, *Ibf-B1*$^-$	11	20.0	74.3	22.1	2.10	–	19.9	65.8	1.603	4.02
1988/89 experiment										
7BL, *ne1*, *Vg*, *Ibf-B1*$^+$	24	3.2	78.7	26.3	3.40	11.84	21.8	67.4	2.519	6.35
7BL, *ne1*, *Vg*, *Ibf-B1*$^-$	14	3.0	79.0	25.8	3.39	11.86	21.4	66.6	2.543	6.30
7BL, *ne1*, *vg*, *Ibf-B1*$^-$	5	2.9	79.7	25.8	3.31	12.56	21.2	66.1	2.500	6.08
7BL, *Ne1*, *vg*, *Ibf-B1*$^-$	2	5.0	72.6	5.9	1.41	8.10	18.9	48.7	1.450	3.37
7BL, *ne1*, *vg*, *Ibf-B1*$^+$	1	3.4	76.6	26.9	3.06	13.32	21.1	64.9	2.360	5.84
5BS, *Ne1*, *vg*, *Ibf-B1*$^+$	10	4.7	74.0	9.3	1.91	7.27	20.7	57.9	1.641	4.03
5BS, *Ne1*, *Vg*, *Ibf-B1*$^+$	2	3.8	72.0	6.9	1.92	6.63	21.2	57.5	1.690	4.10
5BS, *ne1*, *Vg*, *Ibf-B1*$^+$	2	3.5	74.6	22.2	3.02	11.03	21.5	65.8	2.300	5.71
5BS, *Ne1*, *vg*, *Ibf-B1*$^-$	11	4.1	75.4	8.8	1.95	7.61	21.1	58.3	1.673	4.14

[a] Days from June 1st

Table 4. Phenotypic differences between allele classes for each genetic marker in the two karyotype groups, 5BL-7BL and 5BL-5BS, in the 1985/86 and 1988/89 experiments

Genetic		Ear emergence time (days)		Plant height (cm)		Yield/plant (g)		Grain weight/ear (g)		Fertile tillers/plant	
		5BL-7BL	5BL-5BS	5BL-7BL	5BL-5BS	5BL-7BL	5BL-5BS	5BL-7BL	5BL-5BS	5BL-7BL	5BL-5BS
ne1–Ne1	1985/86	−0.55	0.35	2.7*	−1.0	28.7***	14.7**	1.70***	1.10***		
	1988/89	−1.91**	−0.84*	6.3**	0.1	20.2***	13.3***	2.04***	1.10***	3.86***	3.65***
Vg–vg	1985/86	0.15	0.24	−0.6	2.6**	9.0**	5.4*	0.58***	0.54*		
	1988/89	−0.39*	−0.74*	1.3	−1.5	5.1***	5.5***	0.59*	0.55*	0.31	1.38
Ibf-B1$^+$–	1985/86	0.20	−0.39	−0.6	1.2	1.4	−1.0	0.20	−0.13		
Ibf-B1$^-$	1988/89	0.04	−0.29	0.0	1.6	2.4	−1.9	0.20	−0.12	0.23	0.11

Genetic marker		Spikelet no./ear		Grain no./ear		50 grain wt. (g)		Biomass/tiller (g)	
		5BL-7BL	5BL-5BS	5BL-7BL	5BL-5BS	5BL-7BL	5BL-5BS	5BL-7BL	5BL-5BS
ne1–Ne1	1985/86	2.8***	0.9**	19.3**	7.0	0.789***	0.477***	2.43**	1.32**
	1988/89	2.7***	0.6	18.2***	7.7	1.071***	0.640***	2.92***	1.62**
Vg–Vg	1985/86	1.2***	0.6**	8.0*	3.7	0.232**	0.212**	0.83	0.60
	1988/89	1.1***	0.4	5.5***	3.6	0.308***	0.338**	0.96***	0.82*
Ibf-B1$^+$–	1985/86	0.6*	0.2	2.6	1.0	0.091	−0.065	0.34	0.16
Ibf-B1$^-$	1988/89	0.6	0.2	2.5	0.7	0.084	0.068	0.36	−0.14

Significance levels: * $P = 0.05–0.01$, ** $P = 0.01–0.001$, ***$P < 0.001$

tion of these necrosis genes acts to prevent healthy growth and results in stunted phenotypes with a drastic reduction in plant yield.

In the lines with the normal 5BL-5BS karyotype, an effect on grain weight/ear was associated with *Ne1* and the centromere, caused by a difference in spikelet number and thus grain number/ear. About half of the genetical variation for spikelet number/ear remained after removal of the variation ascribable to segregation of the markers. Thus, it is possible that in addition to *Ne1* a qtl affecting

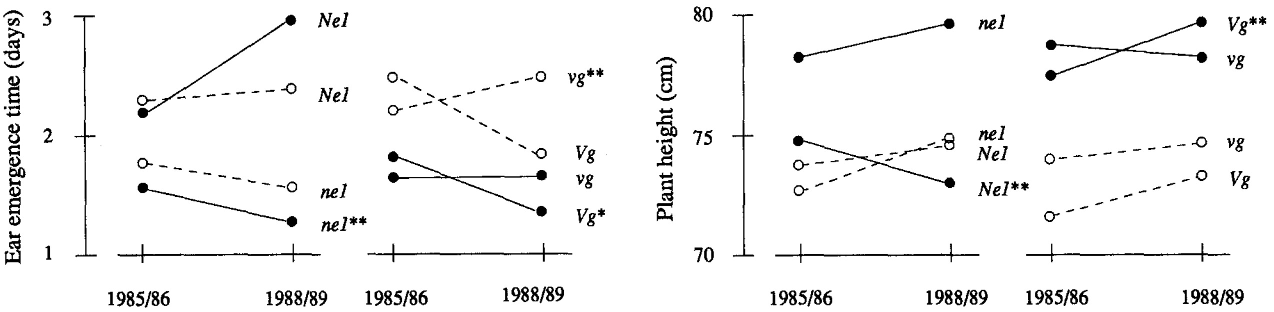

Fig. 3. Changes in means of marker allele groups over seasons for recombinants having the normal karyotype (0- - -0) or the translocated karyotype (●—●). Significant interactions: *, $P = 0.05–0.01$; **, $P = 0.01–0.001$

spikelet number is present, being tightly linked with the centromere. However, this 'spikelet number' gene is probably epistatic to the *Ne1* locus since no significant effect was detected in the alternative karyotype group having the *ne1* allele.

Allele × season interaction

Since there were contrasting climatic conditions between the two seasons, it is of interest to determine whether genotype × environment interactions occur and if they are attributable to particular alleles. The results of a two-way ANOVA showed that significant allele × seasons interactions were concentrated on ear emergence time and plant height. Figure 3 illustrates the response pattern for these characters.

For ear emergence time, the response patterns were similar in both the karyotype groups, although the difference between the *Ne1* allele classes was small in the 1986 season and large in the 1989 season. An interaction associated with the *Vg* locus was caused by an association of the *Vg* allele with an effect on accelerating flowering by about half a day under the favourable conditions of the 1989 season. For plant height, allele × season interactions at the *Ne1* and *Vg* loci were significant only in the 5BL-7BL karyotype group. The height reduction caused by *Ne1* was dependent on environmental conditions, being greater in the 1989 season. The *Vg* allele promoted stem elongation in the more favourable season while the *vg* allele appeared relatively insensitive to environmental variation.

Discussion

In terms of genetical analysis in wheat, the present investigations demonstrate that single-chromosome recombinant lines are an effective tool for examining qtl linkage and the pleiotropic effects of markers. Significantly in the present case, the majority of effects on yield characters would appear to represent net biological effects due to pleiotropy. As discussed by Weller et al. (1988), effects associated with morphological markers are generally much larger than those associated with physical, isozyme and RFLP markers. Two other studies, of chromosome 5A by Snape et al. (1985), and chromosome 2D by Worland et al. (1988), also indicate that a relatively large number of marker-associated effects at morphological loci can be explained by pleiotropic effects of the markers themselves. Hence, it is likely that many quantitative characters of economic importance in wheat include such major gene components of genetic variation.

On the other hand, the studies of Snape et al. (1985) also provided evidence that marker-associated effects on yield components can also be due to linkage of qtl to markers rather than to pleiotropy, and they located two yield genes on chromosome 5A, either side of the Q locus for ear morphology. In the present experiments, similar marker-linked qtl were implicated in the control of spikelet number and grain number/ear. When such qtl have alleles in dispersion between the two parental homologues, it is possible to give rise to recombinant lines whose phenotypes transgress the non-recombinant classes, as shown here for yield. Similarly, by crossovers between the markers, recombinants which lack the undesirable alleles at *Kr1* and *Ne1* on chromosome 5B and Q on chromosome 5A have been isolated, showing the power of the single-chromosome recombinant approach as a method of chromosome engineering in wheat.

In the present studies qtl, both associated with and independent of the markers, were found for plant height, and, generally, height seems to be affected by most genetic factors which regulate development, morphology or vigour (e.g, Law and Worland 1973). Therefore, the allelic variation at other loci, in addition to the pleiotropic effects of *Ne1* and *Vg*, could be responsible for the wide range of whole chromosome effects of 5BL. If this is true, alternative alleles at these loci may act in an epistatic manner, releasing or inhibiting genetic variation at other loci. In addition these may affect the plasticity of the phenotype in response to environmental variation as shown by the significant allele × season interactions. In this case, the variation independent of the markers will have consequences for breeding strategies for modifying height with an appropriate level of plasticity.

The disturbed segregation ratio for karyotype groups indicates differential transmission in favour of the translocated structure, implying a selection pressure either at the gametic or genotypical level. Additionally, there appeared to be a yield advantage for this karyotype when the means of the different non-necrotic genotypic groups are examined (Table 3). Together, these effects may explain the widespread occurrence of this translocation in european winter wheat germplasm.

An estimate of the number of qtl for each character was not determined here using available software such as MAPMAKER/QTL (Paterson et al. 1988), because of the limited numbers of genetic markers available, and their obvious and large pleiotropic effects. Work using additional markers is, however, in progress with this material using RFLPs, to enable a more detailed map of the chromosome arms to be constructed. Nevertheless the present map does provide genetic landmarks on which such an analysis can focus, as well as highlighting the position of the centromere of 5B on the genetic map. This will enhance studies to combine the physical and genetic map of this chromosome.

References

Kuspira J, Unrau J (1957) Genetic analysis of certain characters in common wheat using whole chromosome substitution lines. Can J Plant Sci 37:300–326

Law CN (1966) The location of genetic factors affecting a quantitative character in wheat. Genetics 53:487–498

Law CN (1967) The location of genetic factors controlling a number of quantitative characters in wheat. Genetics 56:445–461

Law CN, Snape JW and Worland AJ (1987) Aneuploidy in wheat and its uses in genetic analysis. In: Lupton FGH (ed) Wheat breeding: its scientific basis. Chapman and Hall, London New York, pp 71–108

Law CN, Worland AJ (1973) Aneuploidy in wheat and its uses in genetic analysis. Plant Breed Inst Annu Rep 1972:25–65

Liu CJ, Gale MD (1989) *Ibf-1* (Iodine-binding factor), a highly variable marker system in the *Triticeae*. Theor Appl Genet 77:233–240

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127:181–197

Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560

Snape JW, Law CN, Parker BB, Worland AJ (1985) Genetic analysis of chromosome 5A of wheat and its influence on important agronomic characters. Theor Appl Genet 71:518–526

Stuber CW, Edwards MD, Wendel JF (1987) Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop Sci 27:639–648

Tanksley SD, Media-Filho H, Rick CM (1982) Use of naturally-occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49:11–25

Thoday JM (1961) Location of polygenes. Nature 191:368–370

Thompson JN Jr, Thoday JM (1979) Quantitative genetic analysis. Academic Press, London

Tsunewaki K (1960) Monosomic and conventional analyses in common wheat. III. Lethality. Jpn J Genet 35:71–75

Weller JI, Soller M, Brody T (1988) Linkage analysis of quantitative traits in an interspecific cross of tomato (*Lycopersicon esculentum* × *Lycopersicon pimpinellifolium*) by means of genetic markers. Genetics 118:329–339

Worland AJ, Law CN (1983) Cytoplasmic variation in wheat. Plant Breed Inst Annu Rep 1982:79–80

Worland AJ, Gale MD, Law CN (1987) Wheat genetics. In: Lupton FGH (ed) Wheat breeding: its scientific basis. Chapman and Hall, London New York, pp 129–171

Worland AJ, Petrovic S, Law CN (1988) Genetic analysis of chromosome 2D of wheat. II. The importance of this chromosome to Yugoslavian varieties. Plant Breed 100:247–259

Zhuckenko AA, Korol AB, Andryuschenko VK (1978) Linkage between loci of quantitative characters and marker loci. Genetika 14:771–778