Theor Appl Genet (1985) 69:263-270

THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1985

Intrachromosomal mapping of the nucleolar organiser region relative to three marker loci on chromosome 1B of wheat (*Triticum aestivum*)

J. W. Snape, R. B. Flavell, M. O'Dell, W. G. Hughes and P. I. Payne
Plant Breeding Institute, Trumpington, Cambridge, CB2 2LQ, England

Received April 19, 1984; Accepted May 28, 1984
Communicated by F. Mechelke

Summary. Restriction enzyme digestion of the ribosomal RNA genes of the nucleolar organisers of wheat has revealed fragment length polymorphisms for the nucleolar organiser on chromosome 1B and the nucleolar organiser on 6B. Variation between genotypes for these regions has also been demonstrated. This variation has been exploited to determine the recombination frequency between the physically defined nucleolar organiser on 1B (designated *Nor1*) and other markers; two loci, *Glu-B1* and *Gli-B1* which code for endosperm storage proteins and *Rf3*, a locus restoring fertility to male sterility conditioned by *T. timopheevi* cytoplasm. *Gli-B1* and *Rf3* were located on the short-arm satellite but recombine with the nucleolar organiser giving a gene order of *Nor1 – Rf3 – Gli-B1*. *Glu-B1* is located on the long arm of 1B but shows relatively little recombination with *Nor1*, which is, in physical distance, distal on the short arm. This illustrates the discrepancy between map distance and physical distance on wheat chromosomes due to the distal localisation of chiasmata. The recombination between *Nor1* and *Rf3* indicates that, contrary to previous suggestions, fertility restoration is not a property of the nucleolar organiser but of a separate locus.

Key words: Intrachromosomal mapping – Nucleolar organiser region – *Triticum aestivum*

Introduction

In bread wheat (*Triticum aestivum*) the chromosomal locations of marker genes can be established by cytogenetical techniques (Law and Worland 1973). By such means an extensive catalogue of genes has been built up (McIntosh 1973). However, because of the scarcity of multiple marker stocks, most data on the intrachromosomal locations of loci relate only to specific arms or to linkage distances from the centromere, rather than to associated loci. The development of biochemical techniques for the analysis of isozyme polymorphisms (Hart 1979) and for endosperm protein subunits (Payne et al. 1982, 1983) has now resulted in a great increase in the potential number of loci available for chromosome mapping in wheat. In addition, structural polymorphisms are being recognised for regions of chromosomes by the use of restriction endonucleases and these can also be used.

This paper presents data gained from combining these approaches for mapping genes on chromosome 1B. This chromosome is becoming better characterised since it carries two loci coding for endosperm storage proteins, one on the long arm and the other on the short arm (Payne et al. 1982, 1983). Both loci are highly polymorphic and therefore eminently suitable for mapping studies. The short arm of this chromosome is also the site of the nucleolar organiser region (*Nor*). The ribosomal RNA genes which make up this region have long been shown to vary in copy number between varieties (Flavell and Smith 1974) but recent work has also shown that there is structural variation in the DNA between varieties expressed as fragment length polymorphisms following restriction enzyme digestion (Flavell 1983). This allows the nucleolar region to be mapped as a gene locus designated *Nor1*. Since the locus can be mapped physically by observing the nucleolar constriction in metaphase chromosomes and also by in situ hybridisation (Miller et al. 1980; Appels et al. 1980) in cytological preparations this also allows a comparison of physical and genetic maps to be made. Previously this has only been possible for chromosome

1B of wheat by using deletion stocks (Payne et al. 1984).

A further locus for study on the short arm of chromosome 1B is *Rf3*, which restores normal pollen production in cytoplasmic-male-sterile lines conditioned by *T. timopheevi* cytoplasm (Tahir and Tsunewaki 1969). It has been speculated that this property may be a function of the nucleolar region (Tsunewaki 1982). Using appropriate genetic stocks this hypothesis has been tested. A further novel feature of the present investigation is that inbred recombinant lines, rather than the more conventional F_2 or backcross generations of a cross are used as the segregating material under study.

Materials and methods

Genotypes

Random recombinant F_6 lines developed by single seed descent from the crosses 'Maris Huntsman'×'Bezostaya 1' (MH×B) – 23 lines, and 'Maris Huntsman'×'Maris Ranger' (MH×MR) – 54 lines, were used for linkage estimation in this study. The varietal characteristics for the loci under study are shown in Table 1. Such inbred material was used in preference to conventional F_2 or backcross generations for two reasons. Firstly, the classification of plant genotype necessitated destructive sampling for each character separately and this required large amounts of grain. Secondly the examination of each of the characters is labour intensive and time consuming, so that fewer progenies could be examined than in most mapping studies. Hence examination of predominantly homozygous populations simplified genotypic classification.

To develop the recombinant lines, F_1 progeny between the parental varieties were produced and selfed to produce the F_2. Three generations of single seed descent, where the plants were cultivated under crowded fast growing conditions, were practised from F_2 to F_5. F_5 plants were then grown to produced bulk F_6 families where each F_6 family was derived from a different F_2 plant. The genotypic constitution of each F_5 parent for each locus was then elucidated using samples of this F_6 grain.

Methods of genotypic classification

a) Examination of restriction enzyme digest fragment length polymorphisms (RFLP's) for ribosomal RNA genes. The varieties used exhibit RFLP's for the ribosomal (r)RNA genes of the nucleolar organiser regions of chromosomes 1B and 6B. Each inbred line could be characterised for its respective parental patterns for these rDNA multigene families. To facilitate this classification the DNA of seedlings grown in the dark for 4 d was extracted and purified using a procedure communicated by Thanh Huynh of Stanford University, California, USA.

Shoots from 5 seedlings of each family were homogenised in a buffer solution of 15% sucrose, 50 mM Tris-HCl, 50 mM Na_3 EDTA and 0.25 M NaCl, pH 8.0. After centrifugation, the crude nuclear/chromatin pellet was resuspended in 0.4 ml of the above ice-cold buffer which lacked the NaCl. 1 μl of diethylpyrocarbonate was added, followed by 5 μl of 20% SDS. After heating at 70 °C for 10 min, 50 μl of 5 M potassium acetate was added, the solution shaken vigorously and then cooled for 30 min on ice and the precipitate removed by centrifugation. DNA in the supernatant was precipitated by adding two volumes of ethanol and then recovered by centrifuging. The DNA was redissolved in buffer to which was added 10 μg of RNAase per ml of buffer. After incubation at 37 °C for 30 min the solution was extracted twice with phenol saturated with 10 mM Tris-HCl, 10 mM EDTA, pH 8.0. The aqueous phase was brought to 0.2 M sodium acetate and the DNA precipitated with two volumes of ethanol. This pellet was dissolved in 10 mM Tris-HCl, 1 mM Na_3 EDTA, pH 8.0.

Restriction enzyme digestion with ECoRI and BamHI (Bethesda Research Laboratories) was carried out on 3 μg of each DNA sample at 37 °C for 16 h. The reaction was terminated by the addition of 25 mM Na_3 EDTA, 0.1% glycerol and 0.01% SDS. The digested DNA was fractionated by electrophoresis in 1% agarose gels. The ribosomal DNA fragments were then detected by transferring the DNA to nitrocellulose (Southern 1975) and hybridising with the plasmid pTa71 which contains an rDNA repeat unit from wheat in the vector pACYC/184 (Gerlach and Bedbrook 1979). Radioactive labelling of the plasmid with ^{32}P was carried out as described by Rigby et al. (1977).

b) Examination of endosperm proteins. Allelic variation at the endosperm storage protein loci *Glu-B1* and *Gli-B1* was determined by sodium dodecyl sulphate, polyacrylamide gel elec-

Table 1. Varietal alleles for the loci studied

Character analysed	Locus	Method of phenotypic classification	Alleles present		
			'Maris Huntsman'	'Bezostaya 1'	'Maris Ranger'
High molecular weight glutenin sub-units	*Glu-B1*	SDS-PAGE	*Glu-B1d* (bands 6+8)	*Glu-B1c* (bands 7+9)	*Glu-B1a*[a] (band 7)[b]
ω-gliadins	*Gli-B1*	SDS-PAGE	*Gli-B1α* (bands A+C)	*Gli-B1β* (band B)	*Gli-B1γ* (band D)
Ribosomal RNA genes	*Nor1*	Restriction enzyme digestion fragment length polymorphism	*Nor1a*	*Nor1b*	*Nor1c*[c]
Cytoplasmic male sterility fertility restoration	*Rf3*	Test crosses to *T. timopheevi* alloplasmic lines	*Rf3*	*rf3*	*rf3*

[a] Payne and Lawrence (1983)
[b] See Fig. 3
[c] See Fig. 1

trophoresis (SDS-PAGE). Proteins from three grains of each line were extracted together and fractionated by the method of Laemmli (1970) as modified by Payne et al. (1981). *Gli-B1* is a complex locus coding for ω-gliadins, γ-gliadins and low molecular weight subunits of glutenin (Payne et al. 1984) but only the ω-gliadins were used as markers in these genetical studies. *Glu-B1* encodes high molecular weight subunits of glutenin.

c) Classification at the restorer locus. The individual inbred lines were classified as carrying the restoring (*Rf3*) or non-restoring allele (*rf3*) by test crosses to *T. timopheevi* alloplasmic lines of two varieties, 'Maris Templar' and 'Maris Ranger'. These male-sterile genotypes had been developed by recurrent backcrossing to the Bison[10] A-line (Livers and Heyne 1962; Hughes 1984). Two plants of each line were crossed as pollen parent to each male-sterile genotype. From these crosses, three F_1 progeny from each cross combination were grown to maturity in a glasshouse and all ears enclosed in cellophane bags prior to anthesis. F_1's produced from a line homozygous for the dominant restoring allele would be heterozygous and therefore male-fertile and self-fertile, whilst F_1's produced from a non-restoring genotype would be homozygous for the non-restoring allele and hence male-sterile and self-sterile.

Method of linkage estimation

The frequency of parental and recombinant genotypes for two loci linked by a recombination frequency p, in any generation derived by recurrent selfing from an F_1, was given by Haldane and Waddington (1931).

If the frequencies of the following genotypes in any generation are:

AABB, aabb $= C_n$
AAbb, aaBB $= D_n$
AABb, AaBB, Aabb, aaBb $= E_n$
AB · ab $= F_n$
Ab · aB $= G_n$

then the frequencies in the n + 1 generation are given by the recurrance equations

$$C_{n+1} = C_n + \tfrac{1}{2} E_n + \tfrac{1}{4} (1-p)^2 F_n + p^2 G_n$$
$$D_{n+1} = D_n + \tfrac{1}{2} E_n + \tfrac{1}{4} p^2 F_n + \tfrac{1}{4} (1-p)^2 G_n$$
$$E_{n+1} = \tfrac{1}{2} E_n + \tfrac{1}{4} (2p-2p^2)(F_n + G_n)$$
$$F_{n+1} = \tfrac{1}{2} (1-p)^2 F_n + \tfrac{1}{2} p^2 G_n$$
$$G_{n+1} = \tfrac{1}{2} F_n + \tfrac{1}{2} (1-p)^2 G_n$$

As selfing proceeds the frequencies of parental and recombinant homozygotes increases and the frequencies of heterozygotes decreases. When selfing is advanced to complete homozygosity (the $F\infty$ generation) the population consists of homozygotes in the frequencies:

$$C\infty = \frac{1}{2(1+2p)}$$

$$D\infty = \frac{p}{(1+2p)}$$

From these frequencies the maximum likelihood value of p can be obtained from the equation: $p = \frac{N_R}{N_p}$

where

N_R = observed number of recombinant genotypes
N_p = observed number of parental genotypes

This has a standard error of

$$\left[\frac{P_2 (1+2p)^2}{N_R (1+4p) - 4N_p p^2}\right]^{1/2}$$

In the present experiment the data consisted of genotype frequencies in the F_5 generation. Thus to calculate maximum likelihood recombination frequencies, expected F_5 genotypic frequencies were calculated using the above recurrance relationships and equated to the observed frequencies using a general optimising routine (Ross 1980), following the procedure discussed by Gale et al. (1983). This was achieved using a computer programme written by R. A. Kempton and P. J. Stoehr of the Plant Breeding Institute, Cambridge. Standard errors of these estimates could not be calculated in this way and approximate standard errors were derived using the $F\infty$ equation given above.

Results

Genotypic classifications

a) Ribosomal DNA polymorphisms. Figures 1a and b show the restriction enzyme digest profiles for the rDNA of the nucleolar organiser regions for the parental varieties and for a representative sample of the single seed descent lines. These are the combined profiles for the region on 1B (*Nor1*) and on 6B (designated *Nor2*). The differences in RFLP between *Nor1* and *Nor2* within a genotype occur because of variation in lengths of the spacer DNA between the transcribed units. This is illustrated by the organisation of the rDNA gene shown in Fig. 2. This variation is due to differences in the number of copies of a small repeated sequence in these regions (Flavell 1983; Appels and Dvorak 1983). Variation between genotypes in 1B and 6B patterns is also caused by differences in these intergenic regions and this enables allelic variation between the parental genotypes to be recognised.

Prior to classification of the single seed descent lines for their *Nor1* patterns, the restriction fragments of each of the nucleolar organisers of the parents were distinguished. For the cross MH × MR this was possible by comparing the profiles of the parental lines with those of the genotypes 'Chinese Spring', 'Cappelle-Desprez' and the single chromosome substitution line 'Chinese Spring' ('Cappelle-Desprez 6B'). This substitution line differs in banding pattern from 'Chinese Spring' only at the *Nor2* region and hence one of the five 'Cappelle-Desprez' bands could be classified as originating from *Nor2.* Maris Ranger is a variety derived from a 'Cappelle-Desprez' cross and it has inherited the 'Cappelle-Desprez' *Nor1* and *Nor2* banding patterns. Consequently the single seed descent lines could be identified as carrying the 'Cappelle-Desprez' *Nor1* or *Nor2* pattern or the different patterns of 'Maris Huntsman' – Fig. 1a. 'Bezostaya' produces a

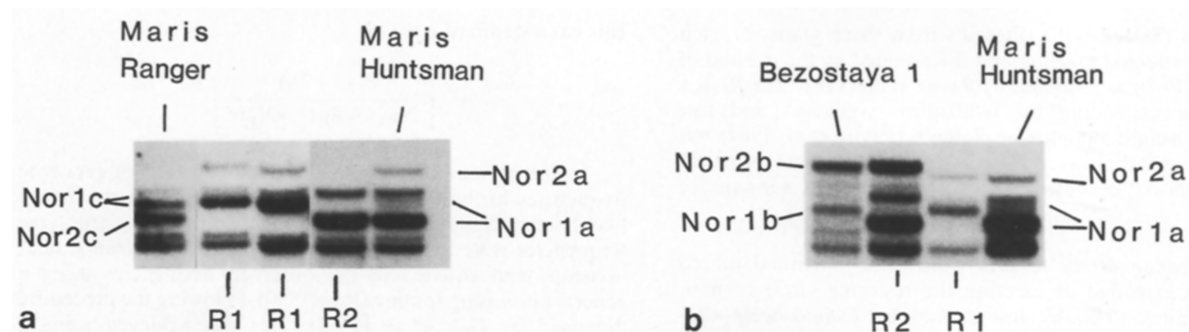

Fig. 1. Autoradiographs obtained from probing the rDNA fragments of *Nor1* and *Nor2* following digestion with Eco RI and Bam HI restriction endonucleases for the parental varieties and a representative sample of single seed descent lines of the crosses 'Maris Ranger'×'Maris Huntsman' (**a**) and 'Bezostaya 1'×'Maris Huntsman' (**b**). Single seed descent lines labelled R1 have the *Nor1* allele of 'Bezostaya 1' or 'Maris Ranger' and the *Nor2* allele of 'Maris Huntsman'. Lines labelled R2 have the *Nor1* allele of 'Maris Huntsman' and the *Nor2* allele of 'Bezostaya 1' or 'Maris Ranger'

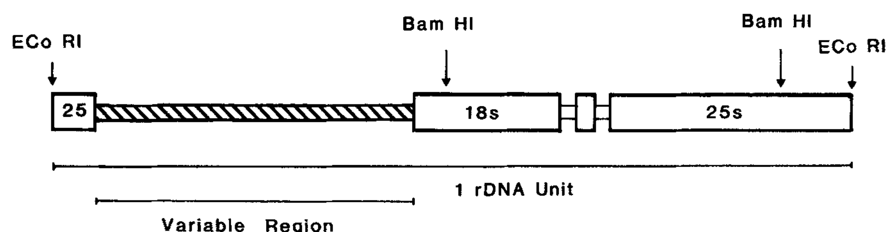

Fig. 2. Organisation of rDNA showing restriction sites and the region producing RFLP between *Nor1* and *Nor2* and between varieties

restriction profile different from both 'Maris Huntsman' and 'Maris Ranger'. However, from a knowledge of the 'Maris Huntsman' *Nor1* pattern the MH×B lines were easily classified into their respective parental types – Fig. 1b. In both crosses no variants other than the respective parental patterns were observed in any of the lines indicating that these multiple gene regions are inherited as a block with no apparent intralocus recombination.

Due to variation in the quality of the autoradiographs not all the single seed descent lines were unambiguously classified. In total, 4 of the MH×B and 14 of the MH×MR lines could not be classified and these are omitted from the linkage analysis. Of the remaining lines, however, a clear 1:1 segregation of parental patterns was observed (Table 2).

b) Endosperm proteins. Figure 3 shows the SDS-PAGE profiles of a sample of single seed descent lines for the two crosses examined. The 1B chromosome of 'Maris Huntsman' encodes two major proteins which correspond to bands labelled 6+8 at the *Glu-B1* locus and ω-gliadin bands A+C at the *Gli-B1* locus. In contrast the 1B chromosome of 'Bezostaya 1' encodes bands 7+9 and B, whilst 'Maris Ranger 1B' encodes bands 7 and D. The other bands present represent segregation at the homoeologous loci on chromosomes 1A and 1D (Payne et al. 1982).

All but one of the single seed descent lines were unambiguously classified for their genotype at both loci. The observed segregation frequencies of parental alleles in each cross were not significantly different from the expected 1:1 ratio (Table 2) and the frequency of heterozygotes was not significantly different from the frequency of 1/16 expected for the F_5 generation. In addition, as Fig. 3 illustrates, recombinants between the *Glu-B1* and *Gli-B1* loci were present in both crosses.

c) Fertility restoration. In the F_1 progeny derived from crosses between the individual single seed descent lines

Table 2. Observed genotype frequencies for each allele at each locus

Cross	Genotype	Locus			
		Glu-B1	*Nor1*	*Rf3*	*Gli-B1*
MH×B	'M. Huntsman'	12	10	8	7
	'Bezostaya'	9	9	12	13
	Heterozygotes	2	–	3	2
	Unclassified	–	4	–	1
MH×MR	'M. Huntsman'	21	19	27	24
	'M. Ranger'	32	21	23	29
	Heterozygotes	1	–	–	1
	Unclassified	–	14	4	–

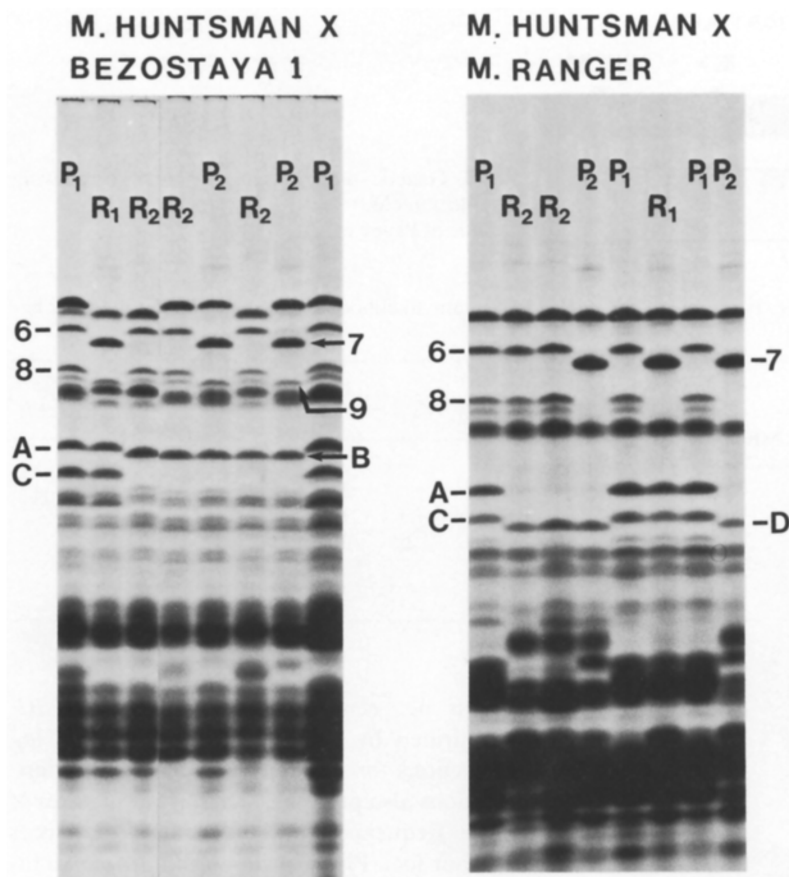

Fig. 3. Analysis by SDS-PAGE of endosperm proteins from the F_6 single seed descent lines of 'Maris Huntsman' × 'Bezostaya 1' and 'Maris Huntsman' × 'Maris Ranger'. The chromosome 1B encoded high molecular weight (HMW) glutenin subunits of each parent have been labelled by numbers and ω-gliadins coded by 1B, by letters. 'Maris Huntsman' parental types with 6+8, A+C are labelled P_1, and 'Bezostaya 1' or 'Maris Ranger' types with 7+9 and B or 7 and D are labelled P_2. Recombinants with 'Bezostaya 1' or 'Maris Ranger' HMW subunits and 'Maris Huntsman' ω-gliadins are labelled R_1 and those with 'Maris Huntsman' HMW subunits and 'Bezostaya 1' or 'Maris Ranger' ω-gliadins, as R_2

and the *T. timopheevi* alloplasmic testers, clear differences in the frequencies of seeds set were observed and plants were easily classified as sterile or fertile by eye. For most lines a consistent classification within crosses and across the testers was obtained so that it was possible to unambiguously classify lines as being homozygous for the restoring allele of 'Maris Huntsman' or the nonrestoring allele of either 'Bezostaya 1' or 'Maris Ranger'. However, progeny from crosses of three lines of MH × B segregated for sterility indicating that their F_5 parents were heterozygous for this locus. Four lines of the MH × MR cross could not be classified because of the failure to obtain test cross seed. In both crosses, as with the other loci examined, a clear 1 : 1 segregation of parental homozygotes was obtained.

Linkage estimation

The complete genotypic classification at all four loci is shown in Table 3. The two crosses were tested for heterogeneity on the completely classified data and no differences between them was detected ($\chi^2_{[15]}=7.4$, $P=0.8$–0.9). Consequently for linkage estimation the data were combined over crosses.

Maximum likelihood estimates of recombination frequencies were calculated between each pair of loci and map distances derived using the Kosambi mapping function, (Kosambi 1943). These are given in Table 4. Previous analysis of the *Glu-B1* and *Gli-B1* loci by Payne et al. (1984) has established that these are only loosely linked. The map distance of 36 cM obtained here suggests a closer linkage, although because of the large error due to the small number of lines, this is not significantly different from the estimate of 66 cM given by Payne et al. Also the map distance of 24 cM between *Glu-B1* and *Nor1* is very similar to the 22 cM obtained by Payne et al. (1984). This close linkage between *Glu-B1,* which is on the long arm and the nucleolar organiser, which is distal on the short arm, illustrates the discrepancy between recombination maps and physical maps highlighted previously for 1B by Payne et al. (1984) and by Dvorák and Chen (1983) for chromosome 6B.

Deletion mapping of 1B by Payne et al. (1984) has shown that *Gli-B1* is distal to *Nor1* and thus located on the satellite of the short arm. This locus is relatively closely linked with *Rf3* indicating that this locus must also be located on the satellite.

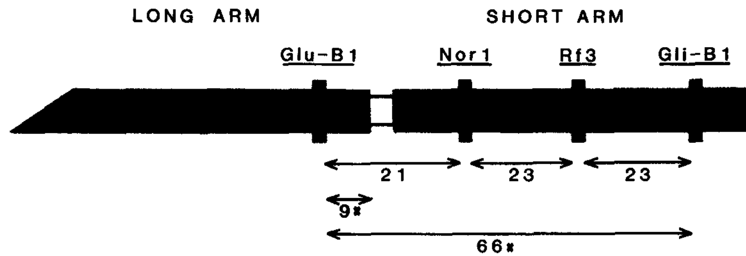

Fig. 4. Genetic map of chromosome 1B showing distances in cM.
* Date of Payne et al. (1984)

Table 3. Complete genotypic classification of the F_5 single seed descent plants

Genotypes				No. of lines		Combined
Glu-B1	*Nor1*	*Rf3*	*Gli-B1*	MH×B	MH×MR	
H	H	H	H	3	7	10
H	H	H	B	1	2	3
H	H	B	H	0	0	0
H	H	B	B	3	3	6
H	B	H	H	1	2	3
H	B	H	B	0	0	0
H	B	B	H	0	1	1
H	B	B	B	1	2	3
B	H	H	H	0	4	4
B	H	H	B	0	1	1
B	H	B	H	0	0	0
B	H	B	B	1	1	2
B	B	H	H	0	1	1
B	B	H	B	0	3	3
B	B	B	H	0	3	3
B	B	B	B	3	6	9
H	–	B	B	0	1	1
H	–	H	H	1	1	2
H	–	H	B	0	1	1
H	–	B	H	0	1	1
B	–	–	B	0	1	1
B	–	H	B	0	3	3
B	–	B	H	0	2	2
B	–	B	B	2	2	4
B	–	H	H	0	2	2
H	H	H/B	B	1	0	1
B	B	H/B	B	1	0	1
H/B	–	H	H	1	0	1
H	B	B	H/B	1	0	1
B	B	H/B	H	1	0	1
H/B	H	H	–	1	0	1
B	B	B	H/B	1	0	1
H	H	–	H	0	1	1
H/B	H	H	H/B	0	1	1
B	B	–	B	0	2	2

H = 'Maris Huntsman' homozygote
B = 'Bezostaya 1' or 'Maris Ranger' homozygote
H/B = heterozygote

A recombination frequency of 22.6% was obtained between *Nor1* and *Rf3*. Thus contrary to the suggestion of Tsunewaki (1982), restoration of male sterility is not a function of the nucleolar organiser but of a separate closely linked locus.

A comparison of the recombination frequencies between the three loci on the short arm indicates the gene order to be: centromere – *Nor1* – *Rf3* – *Gli-B1*. This was confirmed by calculations of maximum log likelihood functions for different possible gene orders. These calculations also produced combined estimates of recombination frequencies and hence map distances between the four loci. Figure 4 shows the gene map incorporating these values, expressed in cM. Recombination in the satellite region produced a combined map distance of 46 cM compared to only 21 cM in the proximal region between *Glu-B1* and *Nor1*. This again illustrates the high frequency of chiasmata occurring in the satellite region of 1B.

Table 4. Maximum likelihood recombination frequencies between loci

Combination	Recombination frequency (%)	Map distance (cM)
Glu-B1 – Nor1	22.5 ± 9.3	24
Glu-B1 – Rf3	34.1 ± 8.5	42
Glu-B1 – Gli-B1	30.8 ± 7.4	36
Nor1 – Rf3	22.6 ± 9.2	24
Nor1 – Gli-B1	36.8 ± 13.5	47
Rf3 – Gli-B1	22.1 ± 6.4	24

Discussion

The present study has shown how the approaches of classical genetics and molecular biology can be combined to produce genetic maps of wheat chromosomes. The use of RFLP's opens up the possibility of analysing allelic variation without the necessity for phenotypic variability, thereby dramatically increasing the number of markers available for mapping studies. Further, by using the techniques of in situ hybridisation (Hutchinson et al. 1981) in parallel with such investigations, knowledge of the physical maps of chromosomes could be correspondingly enhanced.

An interesting feature of the RFLP profiles of the *Nor* loci is the high degree of homogeneity within the arrays of repeated genes clustered within a *Nor*. The length heterogeneity in the spacer regions between the adjacent rDNA repeat units is extensive within wheat but only a few forms are predominant in a given *Nor*

(Flavell, unpublished). This homogeneity implies that the mechanisms which maintain it, unequal crossing over, gene conversion and possibly, selection, must predominate over the mechanisms which reduce it, namely, mutation and meiotic recombination between homologues. This suggests that recombination within the *Nor* locus is rare even though it is a large locus often containing more than 1,000 genes or 10^7 base pairs.

The difference between the physical and genetic maps of chromosome 1B of wheat poses the question of what regulates the distribution of chiasmata in a chromosome arm. It is interesting to note that on the chromosome arms of wheat already shown to exhibit distal crossing-over (6B, Dvorák and Chen 1983; and 1B this paper) there is a major block of heterochromatin around the centromere and also a smaller amount around the *Nor*. The reduction of crossing over in heterochromatic regions containing tandem arrays of repeated sequences is consistent with the hypothesis that recombination is initiated preferentially at or near to sites containing specific kinds of sequences, which are absent from the tandem arrays of repeats. However, in *Drosophila* the presence of telomeric heterochromatin has been shown to suppress recombination resulting in the localisation of chiasmata further away from the heterochromatic regions (Yamamoto and Miklos 1978; Miklos and Nankivell 1976). If this is the case in wheat then for the chromosomes of the B genome, all of which contain blocks of proximal heterochromatin, recombination would be expected to be distal. However, for the chromosomes of the A and D genomes with much less pericentric heterochromatin the effect of distal localisation should not be so pronounced.

The speculation by Tsunewaki (1982) that the nucleolar organiser on chromosome 1B was involved in determining restoration of fertility to cytoplasmic male sterility was based on evidence from several mapping studies. In addition to *Rf3*, Tsunewaki (1974) located *Rfu1* restoring fertility to *Aegilops umbellulata* cytoplasm and *Rfo3* restoring fertility to *Ae. ovata* cytoplasm on 1B. Mukai and Tsunewaki (1979) located the restorer locus *Rfv1* to *Ae. kotschyi* cytoplasm on the satellite of chromosome 1B. This multiplicity of restorer genes could be due to allelic variation at a single locus. A locus restoring fertility to *Ae. caudata* cytoplasm has also been located on chromosome 6B, the site of the other nucleolar organiser in wheat (Tsunewaki 1974). Evidence in *Nicotiana* by Burns and Gerstel (1981) also showed a relationship between restoration of fertility to cytoplasmic male sterility and a nucleolar organiser chromosome.

The evidence from the present study, however, indicates that in wheat, at least for chromosome 1B, the relationship is coincidental rather than causal. Furthermore, evidence from Hamawaki and Mukai (1980) showed that at least two genes *Rf3* and *Rfv1*, occupy different loci on chromosome 1B. In addition, other chromosomes of the wheat genome carry restorer genes (Tsunewaki 1982) but do not have nucleolar organiser regions. Thus in wheat, the nucleolar organiser region of 1B would not appear to be directly involved in fertility restoration although a regulatory function cannot be rule out.

Acknowledgements. The authors are grateful to Mr P. J. Stoehr and Mr R. A. Kempton for estimating the maximum likelihood recombination values and to Miss L. A. Sitch and Miss. B. B. Parker for technical assistance.

References

Appels R, Dvorák J (1982) The wheat ribosomal DNA spacer region: its structure and variation in populations and amongst species. Theor Appl Genet 63:337–348

Appels R, Gerlach WL, Dennis ES, Swift H, Peacock WJ (1980) Molecular and chromosomal organisation of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma 78:293–311

Burns JA, Gerstel DU (1981) Role of nucleolar chromosomes in anther restoration of male-sterile tobacco. J Hered 72:413–418

Dvorák J, Chen K-C (1984) Distribution of nonstructural variation between wheat cultivars along chromosome arm 6Bp: evidence from the linkage map and physical map of the arm. Genetics 106:325–333

Flavell RB (1982) Recognition and modification of crop plant genotypes using techniques of molecular biology. In: Vasil IK, Scowcroft WR (eds) Plant improvement and somatic cell genetics. Academic Press, London New York, pp 277–291

Flavell RB (1983) Chromosomal variation in Crop Plants. In: Owen LD (ed) Genetic engineering: applications to agriculture. Rownan and Allanheld, Totowa, USA pp 15–25

Flavell RB, Smith DB (1974) Variation in nucleolar organiser rRNA gene multiplicity in wheat and rye. Chromosoma 47:327–334

Gale MD, Law CN, Chojecki AJS, Kempton RA (1983) Genetic control of α-amylase production in wheat. Theor Appl Genet 64:309–316

Gerlach WL, Bedbrook JR (1979) Cloning and characterisation of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1886

Haldane JBS, Waddington CH (1931) Inbreeding and linkage. Genetics 16:357–374

Hamawaki H, Mukai Y (1980) Telomeric mapping of the fertility-restoring gene *Rfv1* against *Aegilops variabilis* cytoplasm in wheat (Abstr). Jpn J Genet 55:453

Hart GE (1979) Genetical and chromosomal relationships among the wheats and their relatives. Stadler Symposium 1979, vol 3. University of Missouri, USA, pp 9–23

Hughes WG (1984) Monosomic analysis of the restoration of fertility in male-sterile wheat (in preparation)

Hutchinson J, Flavell RB, Jones J (1981) Physical mapping of plant chromosomes by in situ hybridisation. In: Setlow J, Hollaender A (eds) Genetic engineering, vol III. Plenum Press, New York, pp 207–222

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

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

Livers R, Heyne EG (1962) Annu Wheat Newslett 9: 10–13
McIntosh RA (1973) A catalogue of gene symbols for wheat. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp. University of Missouri, Columbia Mo, pp 893–937
Miller TE, Gerlach WL, Flavell RB (1980) Nucleolus organiser variation in wheat and rye revealed by in situ hybridisation. Heredity 45: 377–382
Miklos GMG, Nankivell RN (1976) Telomeric satellite DNA functions in regulating recombination. Chromosoma 56: 143–167
Mukai Y, Tsunewaki K (1979) Basic studies on hybrid wheat breeding. 8. A new male sterility-fertility restoration system in common wheat utilizing the cytoplasms of *Aegilops kotschyi* and *Ae. variabilis*. Theor Appl Genet 54: 153–160
Payne PI, Corfield KG, Holt LM, Blackman JA (1981) Correlations between the inheritance of certain high-molecular weight subunits of glutenin and bread making quality in progenies of six crosses of bread wheat. J Sci Food Agric 32: 51–60
Payne PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetical studies on the high molecular weight subunits of wheat glutenin. 3. Telocentric mapping of the subunit genes on the long arms of the homoeologous group 1 chromosomes. Theor Appl Genet 63: 129–138
Payne PI, Holt LM, Hutchinson J, Bennett MD (1984) Development and characterisation of a line of bread wheat, *Triticum aestivum,* which lacks the short-arm satellite of chromosome 1B and the *Gli-B1* locus. Theor Appl Genet 68: 327–334
Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1, Glu-B1* and *Glu-D1* which code for the high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 2: 29–35
Rigby PNJ, Dieckman M, Rods C, Berg P (1977) Labelling DNA to high specific activity by nick translation with DNA polymerase I. J Mol Biol 113: 237–251
Ross GJS (1980) Maximum likelihood programme. Rothamstead Exp Statn. Harpenden, England
Southern EM (1980) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98: 503–517
Tahir CM, Tsunewaki K (1969) Monosomic analysis of *Triticum spelta* var 'duhamelianum' a fertility restorer for *T. timopheevi* cytoplasm. Jpn J Genet 44: 1–9
Tsunewaki K (1974) Monosomic analysis of two restorers to *Ae. caudata* and *Ae. umbellulata* cytoplasms. Jpn J Genet 49: 425–433
Tsunewaki K (1982) Monosomic analysis on the fertility restoration by *Triticum aestivum* cv. 'Chinese Spring' against *Aegilops ovata* cytoplasm. Jpn J Genet 57: 513–525
Yamamoto M, Miklos GLG (1978) Genetic studies on heterochromatin in *Drosophila melanogaster* and their implications for the functions of satellite DNA. Chromosoma 66: 71–78