INVESTIGATION OF HOMOLOGOUS CROSSING OVER AND SISTER CHROMATID EXCHANGE IN THE WHEAT *NOR-B2* LOCUS CODING FOR rRNA AND *GLI-B2* LOCUS CODING FOR GLIADINS

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ABSTRACT

Recombination was investigated within the *Nor-B2* locus of wheat chromosome *6B* that contains several thousand of the 18S-5.8S-26S rRNA (rDNA) repeated units. Additionally, recombination was assessed for several chromosome regions, in arm *6Bq* between the centromere and the *B2* locus (awn suppressor) and in arm *6Bp* between the centromere and *Nor-B2*, between *Nor-B2* and a distal C-band and between *Nor-B2* and *Gli-B2* coding for gliadins. The experimental design permitted the distinction between crossing over between homologous chromosomes and exchange between sister chromatids. No homologous crossing over within the *Nor-B2* locus was found in a sample of 446 chromosomes, but one exchange with the attributes of unequal sister chromatid exchange was identified. The molecular characteristics of this presumed sister chromatid exchange indicate that the spacer variants present in the *Nor-B2* locus are clustered. No homologous recombination was detected within the distal *Gli-B2* locus containing repeated genes coding for gliadin seed-storage proteins. Both arms of chromosome *6B* showed low crossing-over frequency in the proximal regions. The distance from the centromere to *Nor-B2* was only from 0.3 to 2.2 cM although it accounts for about two-thirds of the metaphase chromosome arm, which shows a great distortion of the metaphase map of the arm. The level of homologous recombination within the *Nor-B2* locus is lower than in the chromosome region immediately distal to it. Whether it is comparable to that in the chromosome region proximal to it could not be determined. Recombination frequencies of different pairs of chromosome *6B* in all but one interval paralleled the frequencies of their metaphase I pairing: Lower pairing at metaphase I was paralleled by lower crossing-over frequency. This relationship indicated that reduced metaphase I pairing between *6B* chromosomes from different populations is due to impaired crossing-over and not due to precocious chiasma terminalization.

IN plants and animals genes coding for the 18S-5.8S-26S(28S) rRNA genes (rDNA) are tandemly arranged in one or several chromosomal loci. These gene arrays were shown to be stably inherited in yeast, Xenopus, Drosophila, man, wheat and barley (REEDER *et al.* 1976; PETES 1980; COEN, THODAY and

DOVER 1982; BONCINELLI *et al.* 1983; RANZANI, BERNINI and CRIPPA 1984; DVOŘÁK and CHEN 1984; SAGHAI-MOROOF *et al.* 1984; SNAPE *et al.* 1985). In the tribe Triticeae the rRNA genes are conserved, but the external spacers separating the gene units from each other ("spacers") have been rapidly evolving (APPELS and DVOŘÁK 1982a,b; DVOŘÁK and APPELS 1982). Unequal exchanges (the term "crossing over" will be used here only for homologous reciprocal meiotic exchanges) within and between chromosomes have been postulated to play a vital role for the maintenance of some degree of sequence homogeneity in repeated nucleotide sequence arrays (TARTOF 1975; SMITH 1976). Only a few attempts have been made to determine the frequencies of homologous crossing over and sister chromatid exchanges (SCEs) in the rDNA of plants and animals. The data available for Drosophila indicate that homologous crossing over rarely occurs in rDNA (COHEN, THODAY and DOVER 1982; BONCINELLI *et al.* 1983). Although the Drosophila rDNA is in heterochromatin of the sex chromosomes and, thus, may have unusual crossing-over characteristics, available data indicate that crossing-over frequency may be low also in human rDNA (RANZANI, BERNINI and CRIPPA 1984) and wheat rDNA (DVOŘÁK and CHEN 1984; SNAPE *et al.* 1985).

The present study was undertaken with the primary objective of obtaining more extensive data on the frequency of crossing over between homologous, and sister chromatid, rDNA in bread wheat (*Triticum aestivum* L., $2n = 6x = 42$). The wheat genotypes employed had two major rDNA loci in the *B* genome, *Nor-B1* on chromosome *1B* and *Nor-B2* on chromosome *6B*, and a minor rDNA locus (*Nor-D1*) on chromosome *5D* (APPELS and DVOŘÁK 1982a). This paper focuses on the *Nor-B2* locus. A secondary objective of the study was to determine the frequencies of crossing over in the regions of chromosome *6B* that flank the *Nor-B2* locus or the centromere and to compare them with that within the *Nor-B2* locus.

MATERIALS AND METHODS

Genetic stocks: The study utilized *6B* chromosomes from cultivars Timstein (T), Hope (H) and Cheyenne (C) substituted in a disomic state in Chinese Spring (CS). The nomenclature and constitution of chromosomal variants used in the experimental design is summarized in Table 1. The allelic variation among the *6B* chromosomes of Chinese Spring, Timstein, Hope and Cheyenne is described in Table 2.

Experimental design: Two closely related designs were used. In the experiments involving Timstein chromosome *6B* (hereafter *T6B*) and Hope chromosome *6B* (hereafter *H6B*), disomic substitutions were crossed with Chinese Spring double ditelosomic *6B* (Figure 1). The layout of the experiment involving Cheyenne chromosome *6B* (hereafter *C6B*) was similar, except that disomic substitution *C6B*(*CS6B*) was crossed with Chinese Spring ditelosomic *6Bp* (Figure 2). In both designs the F_1 generation plants were testcrossed as males with monotelosomic *6Bp* in order to isolate a population of BC_1F_1 testcross progeny with chromsome *6B* in a monosomic state. In the design outlined in Figure 1, the BC_1F_1 progeny that resulted from regular chromosome disjunction were either monosomic (with one bibrachial *6B* chromosome), double monotelosomic (with one *p* and one *q* telosome) or monotelodisomic (Figure 1). Only the plants that were monosomic or double monotelosomic were self-pollinated to produce the BC_1F_2's (Figure 1). In the similar design outlined in Figure 2, only the monotelosomic or monosomic BC_1F_1 plants were self-pollinated to produce BC_1F_2's (Figure 2).

TABLE 1

Terminology and description of the chromosomal stocks

Stock	Chromosome constitution
Chinese Spring (CS)[a]	21 pairs of CS chromosomes
Chinese Spring monosomic *6B*[a]	20 pairs of CS chromosomes plus a single CS chromosome *6B*
Chinese Spring[a] ditelosomic *6Bp*	20 pairs of CS chromosomes plus a pair of CS telosomes *6Bp* (=*6BS*)
Chinese Spring[b] double ditelosomic *6B*	20 pairs of CS chromosomes plus a pair of CS telosomes *6Bp* and a pair of CS telosomes *6Bq* (=*6BL*)
Chinese Spring[c] monotelosomic *6Bp*	20 pairs of CS chromosomes plus a single CS telosome *6Bp*
Disomic substitution[d] T*6B*(CS*6B*)	20 pairs of CS chromosomes plus a pair of Timstein chromosomes *6B*
Disomic substitution[d] H*6B*(CS*6B*)	20 pairs of CS chromosomes plus a pair of Hope chromosomes *6B*
Disomic substitution[e] C*6B*(CS*6B*)	20 pairs of CS chromosomes plus a pair of Cheyenne chromosomes *6B*
Monotelodisomic *6Bp*	20 pairs of CS chromosomes plus a heteromorphic chromosome pair comprised of Timstein, Hope or Cheyenne chromosome *6B* and CS telosome *6Bp*
Double telotrisomic *6B*	20 pairs of CS chromosomes plus a Timstein or Hope chromosome *6B* and two CS telosomes, *6Bp* and *6Bq*

[a] SEARS (1954). Provided by E. R. SEARS, University of Missouri, Columbia.
[b] SEARS and SEARS (1979). Provided by E. R. SEARS.
[c] Developed by crossing CS ditelosomic 6Bp with CS monosomic 6B and selecting a monotelosomic progeny.
[d] SEARS (1953). Provided by E. R. SEARS.
[e] MORRIS *et al.* (1966). Provided by R. MORRIS, Department of Agronomy, University of Nebraska, Lincoln.

It should be noted that because of selection of BC_1F_1 plants that had only a single *6B* chromosome, homologous recombination could occur only in the F_1 generation. Because the monosomic state of *6B* precluded recombination in the BC_1F_1, each BC_1F_2 family should be monomorphic. If a family segregated, it was due to either a SCE that occurred in the BC_1F_1 or an error in the determination of the chromosome constitution of a BC_1F_1 plant.

Analysis of recombination: To obtain BC_1F_1 testcross plants in a monosomic state (or double monotelosomic, which is equivalent) for *6B*, the F_1 monotelodisomics and double telotrisomics had to be used as males in the crosses. If the cross is made in this direction and only the monosomics, monotelosomics or double monotelosomics from the testcross are used in recombination analyses, biased estimation of recombination is obtained (DVOŘÁK and MCGUIRE 1981). In the testcross involving F_1 monotelodisomics, the bias is due to exclusion from fertilization of most of the nullisomic and a portion of the monotelodisomic pollen resulting from the nondisjunction. The monotelodisomic pollen that was not excluded from fertilization results in monotelodisomic plants that are excluded by choice from analyses. For example, Chinese Spring telosome *6Bp* pairs at metaphase I (MI) with Cheyenne chromosome *6B* in only 60% of the pollen mother cells (PMCs) (DVOŘÁK and MCGUIRE 1981; CROSSWAY and DVOŘÁK 1984). If each univalent is incorporated into the nucleus of only 1 of 4 of the resulting microspores (SEARS 1953), the two univalent chromosomes will generate 9 of 16 nullisomic microspores and 1 of 16 monotelodisomic microspores. Thus, in the *CS6Bp/C6B* heterozygote, 25% of microspores ($^1/_{16}$ of 40%) are expected to be either nullisomic or mono-

TABLE 2

Description of gene loci on chromosome *6B* of cultivars Chinese Spring, Timstein, Hope and Cheyenne used in the study.

Locus	Arm location	Description	Genotype of chromosome			
			CS6B	*T6B*	*H6B*	*C6B*
B2	*q*	Dominant suppressor of awns	*B2*	*b2*	*b2*	*b2*
Ctr	*p* and *q*	Centromere. Terminal position in the Chinese Spring telosomes and median in the bibrachial Timestein, Hope and Cheyenne chromosomes	*Ctr-Telo*	*Ctr-M*	*Ctr-M*	*Ctr-M*
Nor-B2	*p*	Nucleolus-organizing region. Polymorphism is in the lengths of *TaqI* spacer fragments of the rDNA (APPELS and DVOŘÁK 1982a).	*Nor-B2CS*	*Nor-B2T*	*Nor-B2H*	*Nor-B2C*
C-band	*p*	Interstitial C-band *6Bp23* (GILL 1985) in the satellite. It is present in the Timstein, Hope and Cheyenne, but is indiscernable in Chinese Spring (DVOŘÁK and CHEN 1984).	*C-band*$^-$	*C-band*	*C-band*	*C-band*
Gli-B2	*p*	Locus containing genes coding for gliadins. Polymorphism is in the electrophoretic migration of specific proteins.	*Gli-B2CS*	*Gli-B2T*	*Gli-B2H*	*Gli-B2C*

The loci are arranged in a descending order beginning with the most distal locus in the *q* arm (*B2*) and ending with the most distal locus in the *p* arm (*Gli-B2*).

telodisomic. Since this represents a substantial fraction of the microspores, the percentage of recombination needs to be adjusted by adding estimated number of nullisomic and monotelodisomic gametes to the nonrecombinant class (assuming all nondisjunction is due to lack of crossing over). This adjusted recombination percentage (RP_{adj}) can be calculated for a testcross according to the following formula (J. DVOŘÁK, unpublished results):

$$RP_{adj} = \frac{100b}{n\left(1 + \frac{a}{160 - a}\right)},$$

where a is the percentage of MI pairing failure of the telosome with a specific bibrachial homologue, b is the number of recombinant chromosomes and n is the total number of chromosomes investigated.

If a double telotrisomic is used as a male in a testcross, the recombination estimates are also biased upward. While nondisjunction caused by simultaneous absence of crossing over in both arms is relatively rare, due to multiplicative probability of such an event (for data see DVOŘÁK and MCGUIRE 1981), a specific telosome will be unpaired in a similar percentage of PMCs as in a corresponding monotelodisomic plant. If one of the two telosomes fails to cross over, the disjunction of the resulting bivalent occurs normally, and the unpaired telosome is incorporated into only one-quarter of the microspores. In only one-half of the cases this telosome will reach the same nucleus as its partner telosome. Thus, of the resulting microspores $1 - (¼ \times ½)$, which is 7 of 8,

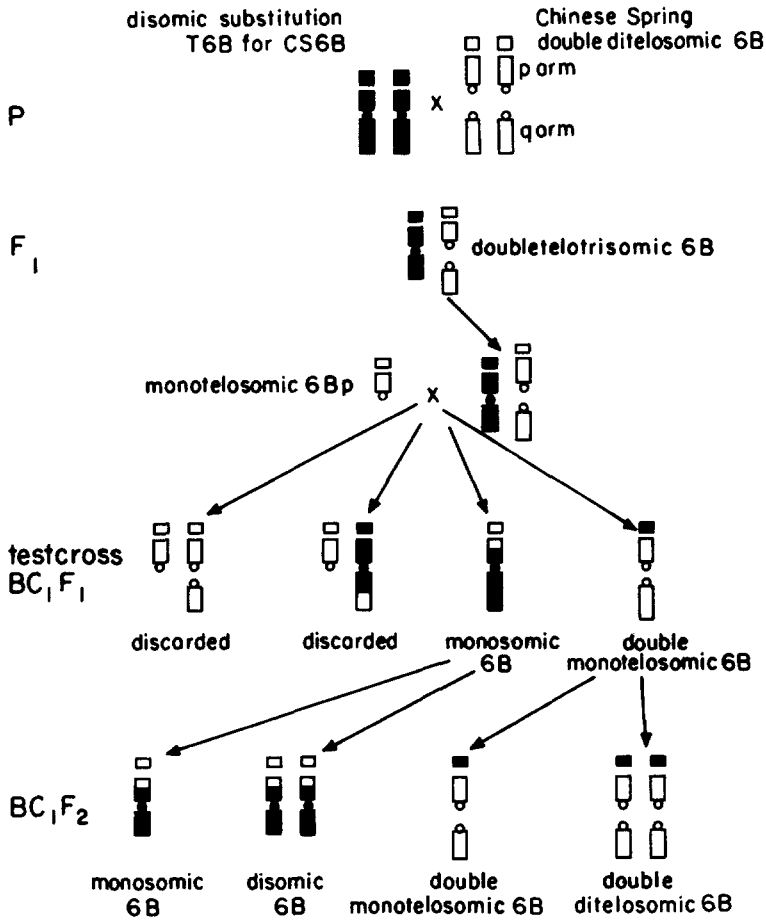

FIGURE 1.—Experimental procedure that was used in the investigation of recombination between Chinese Spring chromosome *6B* and the Timstein or Hope chromosome *6B*. The Chinese Spring chromatin is white, and that of Timstein (or Hope) is black. All plants had an additional 20 pairs of Chinese Spring chromosomes, which are not shown. BC_1F_1 plants resulting from nondisjunction are not shown since they were excluded from analyses.

will be monotelosomic or monotelodisomic. Since most of these gametes are excluded from fertilization, and those that are not result in plants that are excluded by choice from recombination analyses, the estimated recombination is biased upward. The recombination percentage in a testcross can be adjusted (RP_{adj}) for this bias (a simultaneous pairing failure of both telosomes will not be considered, because it is negligibly low) according to the following formula (J. DVOŘÁK, unpublished results):

$$RP_{adj} = \frac{100b}{n\left(1 + \frac{7a}{800 - 7a}\right)},$$

where a, b and n have the same meaning as above.

DNA preparation and analysis: Two tillers from 1-month-old plants were used for isolating DNA following the procedure detailed by APPELS and MORAN (1984). Gen-

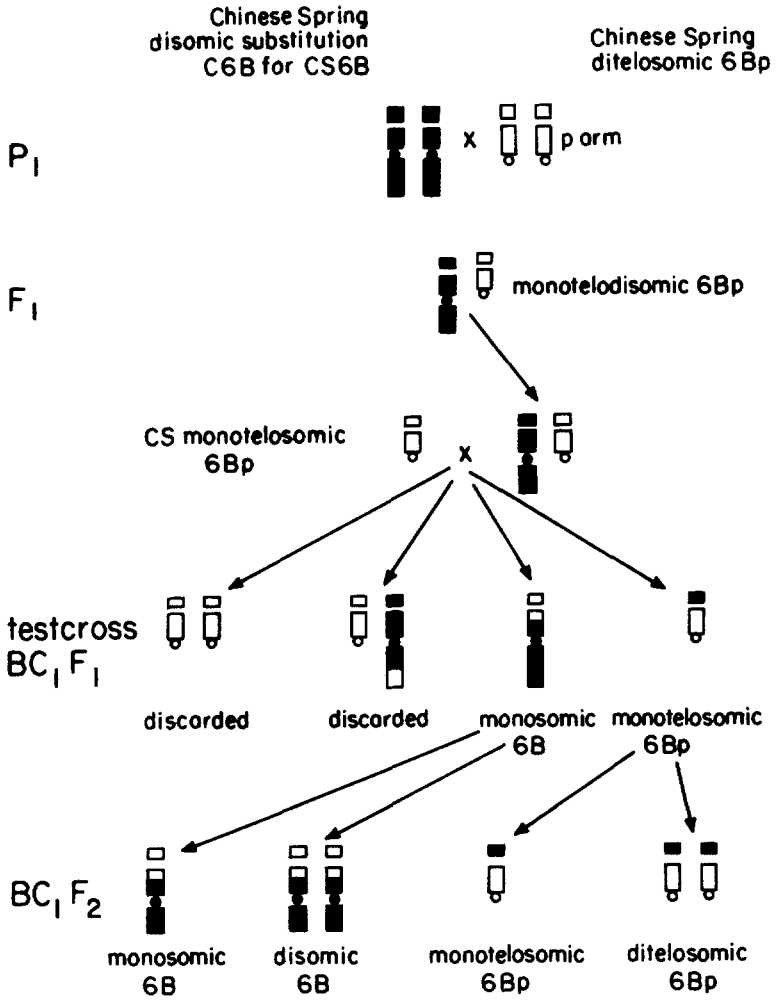

FIGURE 2.—Experimental procedure that was used in the investigation of recombination between Chinese Spring chromosome arm *6Bp* and its Cheyenne homologue. The Chinese Spring chromatin is white, and that of Cheyenne is black. All plants had an additional 20 pairs of Chinese Spring chromosomes, which are not shown. BC_1F_1 plants resulting from nondisjunction are not shown since they were excluded from analyses.

erally 0.5–1 mg of DNA was recovered and frozen as a stock solution of approximately 1 $\mu g/\mu l$ in TE buffer (0.001 M EDTA, 0.01 M Tris-HCl, pH 8.0). Samples for analysis (10 μg) were incubated with the restriction enzyme *Taq*I at 65° in a standard buffer (6 mM $MgCl_2$, 6 mM Tris-HCl, pH 8.0, 70 mM NaCl, 100 μg/ml BSA) at 65° for 1 hr. The *Taq*I restriction endonuclease was prepared from 50-g aliquots of *Thermus Aquaticus* using the procedures described by GREENE *et al.* (1978). Digested samples were electrophoresed in 1% agarose gels containing Tris-HCl-borate buffer, such that the bromophenol blue marker migrated 18 cm. The segment of the gel from the origin to 12 cm was set up to transfer the DNA to Gene-screen (New England Nuclear) following manufacturer's instructions. The filter was then baked for 2 hr at 80° under vacuum and was prehybridized in PH buffer (3 × SSC, 50% formamide, 0.1% SDS, 0.1% polyvinylpyrrolidone, 0.1% BSA, 0.1% Ficoll, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA)

for 2–3 hr at room temperature. Hybridization of the filters was carried out in PH buffer with 0.01 μg/ml ^{32}P-labeled pTA 250.4 insert DNA (specific activity ca. 10^7 cpm/μg) at 37° for approximately 10 hr. The plasmid contains the 2.8-kb *Taq*I fragment of the spacer separating the 18S and 26S rRNA genes subcloned by APPELS and DVOŘÁK (1982a) from pTA 250 containing the entire rDNA repeated unit (GERLACH and BEDBROOK 1979). Following hybridization, the filters were washed at room temperature in 2 × SSC, 0.1% SDS, which was initially 65°, for approximately 5 hr, with three changes of the wash solution.

C-banding analysis: Root tips were taken from 2-day-old seedlings germinated in dark and were pretreated in distilled water at 2° for 18 hr. They were fixed in 45% glacial acetic acid for 12 hr, treated with 10% pectinase (Sigma) for 15 min and stored in distilled water at 4° for 24–48 hr. The meristem was then cut off and squashed in 55% acetic acid. Slides were frozen on dry ice and the cover glass was removed. The chromosomes were C-banded as described by DVOŘÁK and APPELS (1982) and were mounted in Permount.

Gliadin electrophoresis: One-half of the endosperm of a single seed was homogenized with a pestle and mortar in the presence of acid-washed sand. The resulting powder was transferred into a 1.5-ml microfuge tube and was extracted with 0.2 ml of 1.5-M dimethylformamide, 20% sucrose. The sample was centrifuged for 2 min, and 0.02 ml was electrophoretically fractionated in a 6% polyacrylamide vertical-slab gel utilizing an aluminum lactate buffer system, pH 3.1 (LAFIANDRA and KASARDA 1985).

RESULTS

rDNA characterization: Spacers separating the tandemly arranged 18S-26S rRNA gene units in wheat rDNA loci contain internal arrays of tandemly arranged subrepeats that have a modal length of 133 base pairs (bp). *Taq*I recognition sites flank the internal repeated arrays of the rDNA spacers (APPELS and DVOŘÁK 1982a), and digestion of genomic DNA with *Taq*I provides a convenient means of assessing variation in the number of the 133-bp subrepeats that form the array and, thus, in the length of the spacer. An additional source of polymorphism in the *Taq*I fragment length may be elimination of a recognition site by mutation, or creation of a new one.

If CS genomic DNA is digested with *Taq*I endonuclease and the respective blot is probed with pTA 250.4, six bands 0.6, 1.7, 2.7, 2.8, 3.1 and 3.5 kb appear. The 0.6- and 1.7-kb bands come from rDNA on chromosome *5D*; 2.7- and 2.8-kb bands come from rDNA on chromosome *6B*; and the latter two come from rDNA on chromosome *1B* (APPELS and DVOŘÁK 1982a; DVOŘÁK *et al.* 1984). Since the four parents used here differed only in chromosome *6B*, the assignment of the restriction fragments to chromosomes in BC_1F_2 was unambiguous and was in full agreement with the assignment of restriction fragments to chromosomes previously reported for Hope, Timstein and Cheyenne by APPELS and DVOŘÁK (1982a). Disomic substitutions T*6B*(CS*6B*) and H*6B*(CS*6B*) were missing most, but not all, of the 2.7- and 2.8-kb fragments. Disomic substitution T*6B*(CS*6B*) had an additional major fragment 3.9-kb long, and H*6B*(CS*6B*) had an additional major fragment 2.4-kb long. Disomic substitution C*6B*(CS*6B*) had a 2.8-kb fragment (Figure 3).

Analysis of recombination: Timstein *6B*: A total of 232 BC_1F_1 chromosomes from the cross of disomic substitution T*6B*(CS*6B*) × CS double ditelosomic *6B* were investigated through analyses of the BC_1F_2 families. (A family

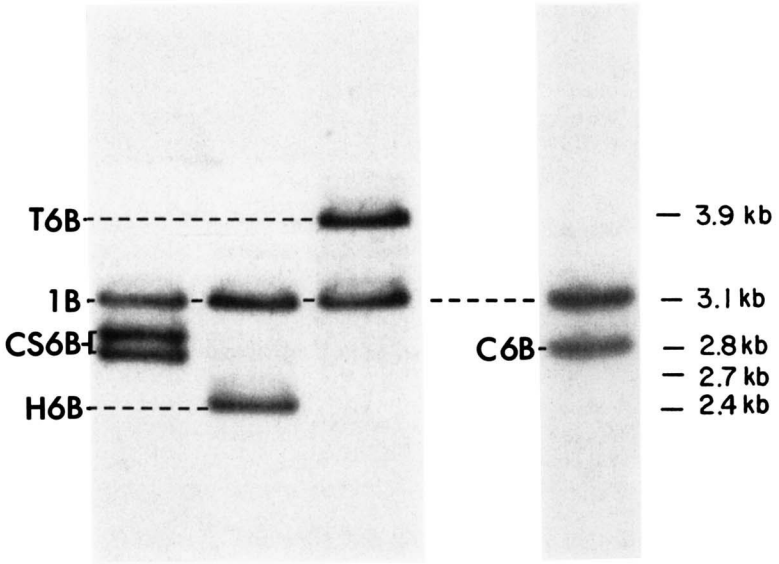

FIGURE 3.—rDNA phenotypes of parents used in the analysis of the *Nor-B2* locus on chromosome *6B*. *Left to right:* Chinese Spring, disomic substitution (DS) of Hope *6B* for Chinese Spring *6B*, DS Timstein *6B* for Chinese Spring *6B*, and DS of Cheyenne *6B* for Chinese Spring *6B*. Genomic DNAs were digested with *Taq*I and were electrophoresed, and Southern blot was probed with a 2.8-kb *Taq*I fragment of the wheat rDNA spacer.

consists of siblings produced by self-pollination of a single BC_1F_1 plant.) In this BC_1F_1 population, 80 chromosomes were bibrachial and 152 were double telocentric (Table 3), which corresponds to a 1:2 ratio instead of the expected 1:1 ($\chi^2 = 26.8$; $P < 0.01$). The numbers of recombinant individuals recovered (Table 3) was, unfortunately, insufficient to locate the factor responsible for the distortion of the transmission.

Recombination between the centromere (*Ctr*) and *Nor-B2* and between *Ctr* and *B2* was 0.9% and 5.2%, respectively (Table 4). From these estimates the adjusted recombination percentage (RP_{adj}) was calculated to take into account the bias due to the use of double telotrisomic as pollen parent in the testcross. Since the *6Bp* arm pairs poorly with *T6B*, whereas the *6Bq* arm pairs well (DVOŘÁK and MCGUIRE 1981), the difference between the *RP* and RP_{adj} was meaningfully large only for the *p* arm (Table 4). The map distance (cM) was calculated from the RP_{adj} using the mapping function (SUZUKI, GRIFFITHS and LEWONTIN 1981). The distance between *Nor-B2* and *Gli-B2* is not reported because the *Gli-B2T* was difficult to distinguish from *Gli-B2CS* in the hemizygous state.

To determine crossing-over frequency in an interval of known physical length distal to the *Nor-B2* locus (see DISCUSSION), recombination was estimated between *Nor-B2* and the *C-band*, which is between the *Gli-B2* and *Nor-B2* loci (DVOŘÁK and CHEN 1984), in a population of 70 chromosomes randomly selected from the total of 233 chromosomes. Of these 70 chromosomes, two were recombinant, both had CS centromere and Timstein *C-band*. Recombi-

TABLE 3

Numbers of the BC_1F_1 generation *6B* chromosome arms with specified genotypes from the crosses of Chinese Spring double ditelosomic *6B* (DDT) or ditelosomic *6Bp* (DT) with disomic substitutions T*6B*(CS*6B*), H*6B*(CS*6B*) and C*6B*(CS*6B*) with CS monotelosomic *6Bp*

Cross	*p* arm			No. of chromosome arms	*q* arm		No. of chromosome arms
	Ctr	*Nor-B2*	*Gli-B2*		*Ctr*	*B2*	
CS DDT*6B* ×	CS	CS	?	145	CS	CS	145
T*6B*(CS*6B*)	CS	CS	?	6	CS	T	6
	CS	T	?	1	CS	CS	1
	T	T	?	74	T	T	74
	T	T	?	5	T	CS	5
	T	CS	?	1	T	CS	1
Total				232			232
CS DDT*6B* ×	CS	CS	CS	63	CS	CS	63
H*6B*(CS*6B*)	CS	CS	H	3	CS	CS	3
	CS	H	CS	1	CS	CS	1
	CS	CS	CS	3	CS	H	3
	H	H	H	50	H	H	50
	H	H	CS	14	H	H	14
	H	H	H	1	H	CS	1
Total				135			135
CS DT*6Bp* ×	CS	CS	CS	8			
C6B(CS*6B*)	CS	CS	C	2			
	CS	C	C	1			
	C	C	C	59			
	C	C	CS	8			
	C	CS	CS	1			
Total				79			

nation between the *Nor-B2* and *C-band* for this chromosome pair was 2.9 ± 1.9%, which is equivalent to RP_{adj} of 2.0 ± 1.7% and 2.0 ± 1.7 cM.

Hope *6B*: A total of 135 BC_1F_1 chromosomes were investigated for recombination between *Ctr* and *B2* in the *q* arm and *Ctr*, *Nor-B2* and *Gli-B2* in the *p* arm. Of this total, 65 chromosomes were bibrachial, whereas 70 were double monotelosomic. The segregation of the centromere in the BC_1F_1 fitted the expected 1:1 ratio ($\chi^2 = 0.75$; $0.1 < P < 0.5$). The 2.7 cM obtained for the distance between the *Ctr* and *B2* was significantly lower than the estimated 5.2 cM for the same two loci of the *CS6B/T6B* chromosome pair (Table 4).

Cheyenne *6B*: Of the total 79 BC_1F_1 chromosomes (Table 3), 48 were previously reported by DVOŘÁK and CHEN (1984) and 31 were newly analyzed chromosomes. The distance between *Ctr* and *Nor-B2* was 2.2 cM and between *Nor-B2* and *Gli-B2* was 11.0 cM (Table 2). The estimated distance for the *Ctr* to *Nor-B2* interval was significantly greater than those obtained for *CS6B/T6B* and *CS6B/H6B* chromosome pairs. The distance between *Nor-B2* and *Gli-B2* was not significantly different from that obtained for the *CS6B/H6B* chromosome pair.

TABLE 4

Recombination percentage (*RP*), recombination percentage adjusted for nondisjunction (RP_{adj}) and RP_{adj} corrected for the mapping function (cM) between the specified loci of chromosome *6B*

Cross	% of PMCs in which *6Bp* telosome paired[a]	*Ctr Nor-B2*			*Nor-B2 Gli-B2*			% of PMCs in which *6Bq* telosome paired[a]	*Ctr B2*		
		RP	RP_{adj}	cM	*RP*	RP_{adj}	cM		*RP*	RP_{adj}	cM
DDT *6B* × T*6B*(CS*6B*)	48.2	0.9 ± 0.6	0.5 ± 0.5	0.5 ± 0.5a[b]				93.0	5.2 ± 1.5	5.0 ± 1.4	5.2 ± 1.5a
DDT *6B* × H*6B*(CS*6B*)	37.7	0.7 ± 0.7	0.3 ± 0.5	0.3 ± 0.5a	14.0 ± 3.1	10.2 ± 2.6	11.4 ± 2.7a	79.7	3.0 ± 1.5	2.6 ± 1.4	2.7 ± 1.4b
DT *6Bp* × C*6B*(CS*6B*)	61.0	2.5 ± 1.8	1.9 ± 1.6	1.9 ± 1.6b	12.7 ± 3.8	9.5 ± 3.4	10.5 ± 3.5a				

[a] From DVOŘÁK and MCGUIRE (1981).
[b] Means followed by the same letter are not significantly different from each other.

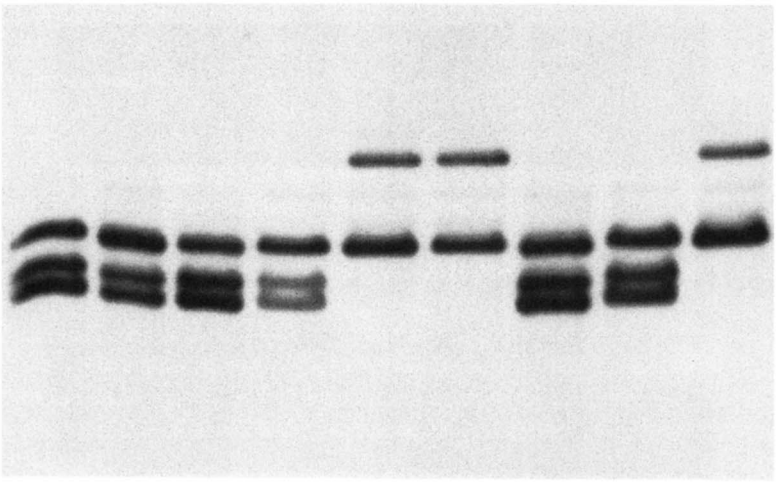

FIGURE 4.—A sample of the BC_1F_2 progeny analyzed in the *CS6B/T6B* experiment showing their rDNA phenotypes. Plants 1, 3, 7 and 8 from left are homozygous for *Nor-B2*; the remainder are hemizygous.

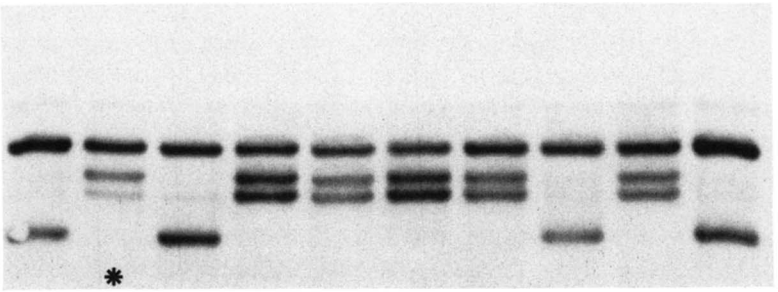

FIGURE 5.—A sample of the progeny analyzed in the *CS6B/H6B* experiment showing their rDNA phenotypes. *From left:* plants 3, 4 and 6 are homozygous for *Nor-B2*, whereas 1, 2, 5, 7, 8, 9 and 10 are hemizygous. The asterisk denotes the sample that appears to have had an unequal SCE in a *CS6B* chromosome.

Recombination within the *Nor-B2* locus: If recombination would occur within the *Nor-B2* locus of the Chinese Spring and Timstein chromosome pair, the resulting chromosome would show a composite profile consisting of bands characterizing the parental genotypes shown in Figure 3. All 232 chromosomes in the *T6B* experiment showed either Chinese Spring or Timstein patterns (Figure 4); the same result was obtained for the 135 BC_1F_1 *6B* chromosomes derived from the *H6B* experiment (Figure 5).

A profile expected from recombination between *Nor-B2CS* and *Nor-B2C* would be less clear since the loci have the 2.8-kb band in common (see DVOŘÁK and CHEN 1984). A recombinant would resemble a *Nor-B2CS/Nor-B2C* heterozygote. However, since both parents had the same *1B* chromosome, the 3.1-kb *Taq*I fragment originating from the *1B* chromosome was used as a reference in comparing optical densities of the *Taq*I fragments originating from the *6B* chromosome. This, and cytological examination of the BC_1F_1 siblings, facili-

tated unequivocal discrimination between accidental heterozygotes and potential rDNA recombinants. Analysis of the 31 BC_1F_1 chromosomes yielded no recombinant. The 48 BC_1F_1 chromosomes included in Table 3 from data by DVOŘÁK and CHEN (1984) also did not show any homologous recombinant. Combining all data in Table 3, no homologous recombination in the *Nor-B2* locus occurred in the sample of 446 chromosomes.

*Taq*I profiles for all BC_1F_1 chromosomes were visually examined for variation in the relative intensity of the bands, with the objective of detecting an unequal SCE; autoradiograms of ten BC_1F_2 individuals, each from a different family, were also scanned quantitatively with a densitometer (for examples, see Figure 6). No evidence for unequal SCE was obtained in the *CS6B/T6B* and *CS6B/C6B* experiments.

A single plant in a BC_1F_2 family in the H*6B* experiment showed a clear unequal exchange within the *6B* rDNA (Figure 5). This hemizygous plant had the *Taq*I bands characterizing the *Nor-B2CS*, but the number of the 2.7-kb restriction fragments was reduced relative to the 2.8-kb variant by about 50% (Figure 6). Thirty-eight siblings of this BC_1F_2 plant were analyzed for *Taq*I restriction pattern. Thirty-six had a normal CS rDNA profile (either monosomic or disomic), and two were *6B* nullisomics. The gliadin profile analyzed in five other siblings of this family showed a normal CS profile. Four additional BC_2F_2 siblings were analyzed by C-banding, and they were double monotelosomics with CS C-band pattern in the *6Bp* telosome. The genotype of the double monotelosomic BC_1F_1 plant parental to this BC_1F_2 family was deduced to be *B2 Ctr-Telo Nor-B2CS C-band-Gli-B2CS*. The lack of recombination of flanking markers and segregation of rDNA in BC_1F_2 indicated that the unusual rDNA pattern resulted from an unequal exchange that occurred between sister chromatids in the BC_1F_1 generation. Combining all three experiments, the estimated frequency of unequal SCE was 1 of 446.

Recombination in the *Gli-B2* locus: The one-dimensional gliadin electrophoretic profile of CS differed from that of H*6B*(CS*6B*) in two bands (see Figure 7). The band specified by a single arrow in the Chinese Spring profile is absent in the *Gli-B2H*. Conversely, the band specified by a double arrow in the profile of disomic substitution H*6B*(CS*6B*) is absent in the profile of CS. If recombination would occur between the *Gli-B2CS* and *Gli-B2H*, the resulting genotype would show either both or neither of the bands.

Two individuals for each BC_1F_2 family were electrophoretically analyzed. None of the 135 families showed a gliadin pattern that would indicate that homologous crossing over occurred in F_1.

The difference between the CS and C*6B*(CS*6B*) gliadin electrophoretic profiles was previously described by DVOŘÁK and CHEN (1984). CS differs from the C*6B*(CS*6B*) by presence of two extra bands. Combined data of DVOŘÁK and CHEN (1984) and those obtained here failed to detect any recombination between the *Gli-B2C* and *Gli-B2CS* in 79 BC_1F_1 chromosomes. Combining all data, no homologous recombination was detected in the *Gli-B2* locus among 214 chromosomes.

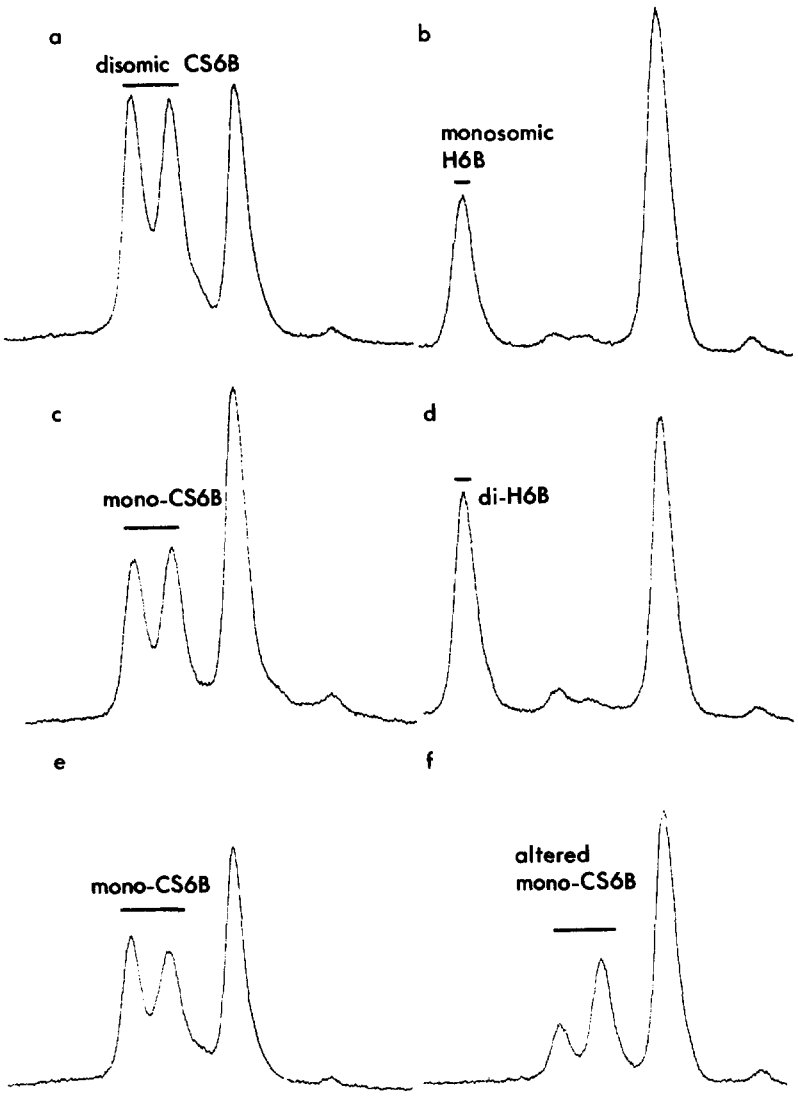

FIGURE 6.—Densitometer tracings of rDNA phenotypes from the *CS6B/H6B* experiment. Peaks that are below the horizontal lines are rDNAs that are at the specified *Nor-B2* locus. Note that the absence of the Chinese Spring *Nor-B2* allele does not result in loss of all copies of the rDNA variants characterizing the *Nor-B2CS* compound allele. The chromosomal location of these remaining copies has not been determined.

DISCUSSION

Among the 446 *6B* chromosomes that were assayed, only one with nonparental rDNA pattern was found. This chromosome resulted from an apparent unequal SCE, not from crossing over between homologues. Preceding studies, albeit based on smaller samples, also failed to detect homologous crossing over in either *Nor-B2* or *Nor-B1* (DVOŘÁK and CHEN 1984; SNAPE *et al.* 1985).

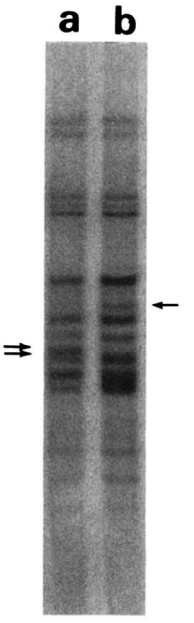

FIGURE 7.—Electrophoretic profiles of gliadins in disomic substitution of Hope *6B* for Chinese Spring *6B* (a) and Chinese Spring (b). The single and double arrows designate bands in which the two lines differ.

Thus, wheat rDNA loci, although consisting of several thousand repeated gene units (FLAVELL and O'DELL 1976) are transmitted as single Mendelian factors, such as rDNA in Drosophila and other organisms (COEN, THODAY and DOVER 1982; BONCINELLI *et al.* 1983; RANZANI, BERNINI and CRIPPA 1984).

An intriguing question is whether this low level of recombination is expected from the amount of DNA in the rDNA loci and the frequency of crossing over in the surrounding chromatin. The wheat haploid chromosome complement contains 18.1 pg of DNA (BENNETT 1972). From this figure and relative chromosome arm lengths (DVOŘÁK, MCGUIRE and MENDLINGER 1984), it was calculated that the *6Bp* arm contains 0.49 pg of DNA. Using average molecular mass of 6.5×10^5 daltons per kilobase, 1 pg of wheat DNA equals 9.26×10^5 kb, and the *6Bp* arm of Chinese Spring contains 4.53×10^5 kb.

Chinese Spring *Nor-B2* was estimated to contain 5500 rRNA genes (FLAVELL and O'DELL 1976). This amounts to 4.9×10^4 kb, assuming that the average length of the repeated gene units is 9 kb (GERLACH and BEDBROOK 1979). From the above-derived figure of 4.53×10^5 kb per the *6Bp* arm, the calculated DNA duplex in *Nor-B2CS* represents 11% of the DNA of the arm.

From the optical density of *Taq*I bands originating from the *Nor-B2* locus in disomic substitutions C*6B*(CS*6B*), H*6B*(CS*6B*), and T*6B*(CS*6B*), it is inferred that *Nor-B2C* contains approximately 3000 rRNA genes, *Nor-B2H* contains 3000 genes and *Nor-B2T* contains 2000 genes. Hence, Cheyenne, Hope and Timstein rDNA represent about 6%, 6% and 4% of the Chinese Spring chromosome arm, respectively.

The physical distance between the centromere and the secondary constriction equals 68% of the Chinese Spring *6Bp* chromosome arm (DVOŘÁK and CHEN 1984). Since it is not clear exactly where the constriction occurs within the array of rDNA units, the safest assumption is to place it in the middle of the *Nor-B2* locus. In that case the *Nor-B2* locus would occupy 5.5% of the arm on each side of the constriction, and the region proximal to the *Nor-B2* locus would represent 62.5% of the arm. By averaging the three estimates in Table 2, this region is 1.0-cM long. If crossing over would be as frequent in the *Nor-B2* locus as in the proximal region, the calculated lengths in centimorgans of the *Nor-B2* locus would be 0.10, 0.10 and 0.06 for *CS6B/C6B*, *CS6B/H6B*, and *CS6B/T6B* heterozygotes, respectively. Although the failure to find any homologous recombinant in the 446 progeny of these heterozygotes is compatible with these estimates, the numbers of chromosomes sampled are still below the sample size needed to have a high likelihood of finding a recombinant if the crossing-over frequencies are as low as estimated.

The region between the secondary constriction and the C-band represents 14% of the chromosome arm (DVOŘÁK and CHEN 1984) , and if 5.5% is subtracted to account for rDNA distal to the constriction, the region would be 8.5% of the chromosome arm. The crossover frequency in this region was estimated for two heterozygotes, *CS6B/C6B* and *CS6B/T6B*. In the *CS6B/C6B* heterozygote the region was 10.2 ± 4.3 cM (calculated from 12.2% recombination reported by DVOŘÁK and CHEN 1984). In the *CS6B/T6B* heterozygote the region was 2.0 ± 1.6 cM. Because the physical length of the distal region is similar to the *Nor-B2* locus in the three heterozygotes investigated, the crossover frequency in the *Nor-B2* locus must be considerably lower than in the region immediately distal.

In a sample of 63 individuals derived by single-seed descent from two F_2 populations, SNAPE *et al.* (1985) did not find any recombination in the *Nor-B1* locus on chromosome arm *1Bp*. The *1Bp* arm is only slightly shorter than the *6Bp* arm (DVOŘÁK, MCGUIRE and MENDLINGER 1984); the *Nor-B1* locus is in a similar place relative to the centromere and telomere as is the *Nor-B2* locus in the *6Bp* arm, and the *1Bp* arm shows a linkage map distortion similar to the *6Bp* arm (SNAPE *et al.* 1985). Since the numbers of gene copies at the *Nor-B1* locus are not dramatically greater than at the *Nor-B2* locus, the failure to find recombination within the *Nor-B1* locus among 66 chromosomes is expected if crossing over in the *Nor-B1* locus is similar to that in the *Nor-B2* locus.

COEN, THODAY and DOVER (1982) did not find any rDNA variant that could be unequivocally attributed to homologous recombination in ten pairs of lines of *D. melanogaster* analyzed after 200 generations of maintenance in the laboratory. BONCINELLI *et al.* (1983), however, reported one *X*-chromosome recombinant in a sample of only 96 chromosomes. A number of recombinants at the *X*-chromosome *bb* locus were obtained by SCHALET (1969). He estimated the homologous recombination frequency in the *X*-chromosome rDNA to be 0.4%. Both BONCINELLI *et al.* and SCHALET's data indicate that the frequency of homologous recombination in Drosophila rDNA is higher than in wheat.

In contrast to our failure to find any homologous crossing over, we did identify one unequal SCE. At face value, this places the frequency of SCEs within the *Nor-B2* locus at 1 of 446. It is likely, however, that not all SCEs are so unequal as to significantly alter the number of gene copies, and thus, the frequency may be higher. The frequency of SCE in the rDNA locus of yeast was estimated to be 10% or greater (PETES 1980). Thus, wheat may have a lower frequency of SCE than yeast by about one order of magnitude. The reasons for this difference, if it exists, are unknown.

Chinese Spring *Nor-B2* contains two equally frequent rDNA variants that differ by one having an extra 133-bp repeat in the spacer (APPELS and DVOŘÁK 1982a). The fact that the observed SCE resulted in loss of about one-half of the copies of one variant, but the copy number of the other variant was unchanged, indicates that the two variants are clustered in the *Nor-B2* locus. The same observation was reported for the organization of the rDNA variants in the Drosophila *Y* chromosome (COEN and DOVER 1983) and *X* chromosome (SHARP, GHANDI and PROCUNIER, 1983), although PELLEGRINI, MANNING and DAVIDSON (1977) concluded that, on the basis of electron microscopic data, two specific 28S rRNA gene variants can also be interspersed.

The SCE could have occurred in a somatic cell or a meiocyte. In the former case the BC_1F_1 plant was expected to be chimeric, and as a result, more than one offspring was likely to acquire the altered rDNA. In the latter case the gamete with the new rDNA variant was expected to be unique and should not have occurred among the siblings of the specific BC_1F_2 plant. Of 38 siblings, none had the altered rDNA profile, suggesting that the SCE occurred during meiosis. A high frequency of SCEs in rDNA occurring in meiosis was demonstrated directly by tetrad analysis in yeast (PETES 1980). Whether this type of exchange precedes the normal homologous recombination as suggested by PETES (1980), and is, thus, physiologically distinct from it, or whether it is equivalent to classical homologous crossing over remains to be determined.

Neither homologous recombination nor a SCE were detected in *Gli-B2*, the other locus of repeated genes that was investigated here. Hence, the gliadin genes on chromosome *6B* must form a tightly linked locus. To our knowledge, there is no report of recombination in this locus. This contrasts with the gliadin genes in chromosome *1B*; these are in two loci 28.1-cM apart (GALILI and FELDMAN 1984).

The present study provided further evidence of the severe distortion of the linkage map of the *6Bp* arm originally observed in a small sample of chromosomes derived from the *CS6B/C6B* pair (DVOŘÁK and CHEN 1984). Larger samples of chromosomes derived from the *CS6B/H6B* and *CS6B/T6B* chromosome pairs showed a virtual absence of crossing over between the centromere and the *Nor-B2* locus, even though this chromosome region comprises two-thirds of the length of the chromosome arm at metaphase. This distortion occurred irrespective of whether a telosome for only one arm or telosomes for both *6B* arms were present. Since SEARS (1972) showed that the use of a monotelodisomic in the determination of linkage underestimates recombination in the proximal regions, the *6B* map may, in reality, not be as extremely

distorted as indicated by the present results. Nevertheless, SNAPE *et al.* (1985) reported a similar distortion of the linkage map of chromosome *1B*, even though bibrachial chromosomes were employed. Although data for other chromosomes of the *B* genome are meager, they indicate that this distortion may occur in other chromosome arms. The *B2* locus in the *6Bq* arm shows a tight linkage with the centromere [present data and FU and SEARS (1973)], but physically it is quite far from it (GIORGI 1981). The *Ph1* locus in the *5Bq* arm is also tightly linked to the centromere, but physically it is in the middle of the arm (JAMPATES and DVOŘÁK 1986).

DVOŘÁK and MCGUIRE (1981) reported chromosome pairing frequencies at MI of Chinese Spring telosomes *6Bp* and *6Bq* with the Hope, Cheyenne and Timstein homologues (= heterohomologues) relative to the pairing of the Chinese Spring telosomes with the Chinese Spring homologous chromosome (= euhomologues). The MI pairing of the heterohomologous chromosome pairs was reduced. The F_1 genotypes that were used in the present study were identical to those originally used by DVOŘÁK and MCGUIRE. The recombination estimates obtained here can, therefore, be employed to determine whether precocious chiasma terminalization or impaired crossing over was responsible for the reduced MI pairing between these specific heterohomologous chromosome arms. If the former were true, there should be no relationship between the MI pairing frequency and crossing-over frequency, whereas if the latter were true, there should be a positive one. Except for the recombination in the distal interval *Nor-B2* to *Gli-B2*, there was a parallel between the level of MI pairing of a specific heterohomologous chromosome pair and the level of recombination (Table 4). Additionally, agreement also occurred between MI pairing and recombination in the interval *Nor-B2* to C-band (data not tabulated in Table 4). Chinese Spring telosome *6Bp* paired with the Timstein heterohomologue in 48.2% of the PMCs and with the Cheyenne heterohomologue in 61.0% of the PMCs. In the former heterohomologous chromosome pair, the linkage between the two loci was 2.0 ± 1.6 cM, whereas in the latter pair, it was 10.2 ± 4.3 cM. Recombination estimates for the interval where this relationship did not hold (*Nor-B2* to *Gli-B2* in the *CS6B/C6B* heterozygote) are based on only a small sample and, hence, are subject to a large sampling error. In fact, if the original estimate of 20.0 ± 5.3% (n = 55) recombination in this interval for the *CS6B/C6B* chromosome pair (DVOŘÁK and CHEN 1984) would be used, the agreement would be complete. Although more data are needed, the findings nevertheless provide a first indication that the failure of MI pairing between wheat heterohomologues is due to impaired crossing over and not to precocious terminalization of chiasmata during the transition from diakinesis to MI.

The linkage distance between the centromere and the *B2* locus in the *6Bq* arm was estimated here to range from 2.6 to 5.0%. Both estimates are higher than that of 0.87% reported by FU and SEARS (1973). Both experimental designs involved using a telosome for determining the distance. FU and SEARS used a monotelodisomic F_1, whereas a double telotrisomic was used here. Additionally, they used the monotelodisomic as a female, whereas here the double

telotrisomic was used as a male for the reasons explained earlier. It is difficult to envision a reason why the same telosome should yield three to five times higher recombination in a double telotrisomic than in a monotelodisomic. Present data for intervals *Ctr* to *Nor-B2* and *Nor-B2* to *Gli-B2* did not show reduced recombination in the monotelodisomic relative to double telotrisomic (compare *T6B/CS6B* and *H6B/CS6B* with *C6B/CS6B*). The bias caused by using the double telotrisomic as a male was largely corrected for by using the formula presented earlier. FU and SEARS, however, used a chromosome *6B* that had a foreign telomere. This is, in our view, the most plausible cause of the discrepancy in the recombination estimates.

This discrepancy between our data and those of FU and SEARS (1973) and the variations among recombination estimates (Table 4) illustrate an important point for recombination analyses in wheat; namely, that different heterohomologous pairs of the same chromosome arm can show widely different recombination in the same interval. We believe that these differences reflect the degree of molecular homology of wheat heterohomologous chromosomes, perhaps in frequency and distribution of recombination initiating sites and homology in noncoding DNA. Regardless of the reason, the practical consequence is that there may be manifold differences among the estimates of recombination in a specific interval.

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