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Wheat mutants permitting homoeologous meiotic chromosome pairing

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SUMMARY

Plants of *Triticum aestivum* ($2n = 6x = 42$) ditelocentric $5B^L$ were treated with EMS in order to produce mutations in the 5B system by which meiotic pairing between homoeologous chromosomes is normally prevented. To check for the occurrence of mutation *T. aestivum* ditelo-$5B^L$ plants were pollinated with rye (*Secale cereale* $2n = 14$) and meiosis was examined in the resulting hybrids.

Wheat–rye hybrids were scored for the presence of mutants when the wheat parents were either the EMS-treated wheat plants, or their selfed derivatives, or their progenies obtained after pollination with untreated euploid individuals.

Mutants were detected by each of these procedures and mutant gametes were produced by the treated ditelocentric plants with frequencies between 1·5 and 2·5 %, but there were differences between the mutants in the extent to which homoeologous pairing occurred in the derived wheat-rye hybrids. The differences may have resulted from the occurrence of mutation at different loci or to different extents at the same locus.

Two mutants, Mutant 10/13 and Mutant 61, were fixed in the homozygous condition. Mutant 10/13 was made homozygous both in the $5B^L$ ditelocentric and in the euploid conditions but these genotypes regularly formed 21 bivalents at meiosis, and there was no indication of homoeologous pairing although the mutant 10/13 gave rise to homoeologous pairing in wheat-rye hybrids.

1. INTRODUCTION

A genetic activity of chromosome 5B of the bread wheat (*Triticum aestivum* $2n = 6x = 42$) is responsible for the restriction of chromosome pairing at meiosis to fully homologous partners (Riley & Chapman, 1958; Riley, 1960; Riley & Law, 1965). When this activity is removed, or suppressed genetically, homoeologous chromosomes, which are genetically and evolutionarily related chromosomes of different genomes, will pair with each other. The effect of chromosome 5B on meiotic chromosome pairing is, therefore, responsible for the meiotic stability and disomic inheritance of hexaploid wheat.

The long arm alone of the chromosome ($5B^L$) is responsible for the prevention of homoeologous pairing. This was shown from the behaviour of haploid plants which had either the complete euhaploid complement of 21 chromosomes or which were deficient for chromosome 5B or for its long arm or its short arm separately (Riley & Law, 1965). Chromosome pairing at meiosis in the deficiency of the short arm

resembled that in the euhaploid, whereas pairing in the deficiency of the long arm resembled that in 20-chromosome haploids lacking the entire chromosome.

A major restriction in the study and exploitation of this sytem is that the pattern of pairing can only be modified by the presence or absence of the entire chromosome or its long arm. Although it has been assumed that the effect derives from the activity of a single locus, allelic variation is very rare in nature. Nakajima (1952, 1956) has described the meiotic behaviour of a *T. aestivum* × *Secale cereale* hybrid with a much higher level of chromosome pairing than its sibs. It seems reasonable to conjecture that this hybrid arose from a fertilization in which the wheat gamete carried a mutant condition permitting homoelogous pairing. Apparently, therefore, allelic variation can occur in the system controlling such pairing. An additional example of the spontaneous origin of allelic variation occurred in this laboratory in a progeny of ten hybrids from the cross *T. aestivum* var. Chinese Spring and *S. cereale*. The hybrids from a single pollination segregated to give the ratio 5 with to 5 without homoeologous pairing, indicating that the wheat parent apparently had been heterozygous at a locus affecting pairing. Unfortunately it was not possible to recover the mutant allele and no controlled study of allelic variation in this system has yet been made.

Attempts have therefore been made to isolate induced mutants so that the analysis of the formal genetics of the 5B system could be completed. Such mutants would also provide genotypes for use in practical work on wheat improvement and for the investigation of the causal basis of the alternative functional conditions leading to entirely homologous or to homologous and homoeologous meiotic pairing. This paper describes work directed towards the isolations of mutants and a subsequent paper (Wall, Riley & Gale, 1971) will describe the genetics of one mutant more fully. The mutants for which selection was practised were those in which the genetic regulation of meiotic chromosome pairing had been modified to permit homoeologues to pair.

2. MATERIAL

The wheat parental plants used in this work were all derivatives of *Triticum aestivum* L. emend. Thell ssp. *vulgare* Mackey variety Chinese Spring ($2n = 6x = 42$) in which either chromosome 5B was complete and disomic or in which this chromosome was represented disomically by the telocentric for its long arm (ditelo ($5B^L$), the short arm being entirely absent (Fig. 1*a*)). The rye parental plants were *Secale cereale* L. variety Petkus. *Aegilops longissima* Schwein. and Musch. ($2n = 14$) was also used.

3. METHODS

The mutagen used was ethyl methanesulphonate (EMS). Samples of 50 dry seeds of *T. aestivum* ditelo-$5B^L$ were soaked in 50 ml of 1·0 or 0·5 % aqueous solutions of EMS for periods of either 16 or 24 h at room temperature. Immediately following this treatment the seeds were washed in running tap water for 15 min. The treated seeds were then planted out in seed trays in conditions of continuous light and at a constant temperature of 20 °C. The aim was to use treatments that resulted in 50 %

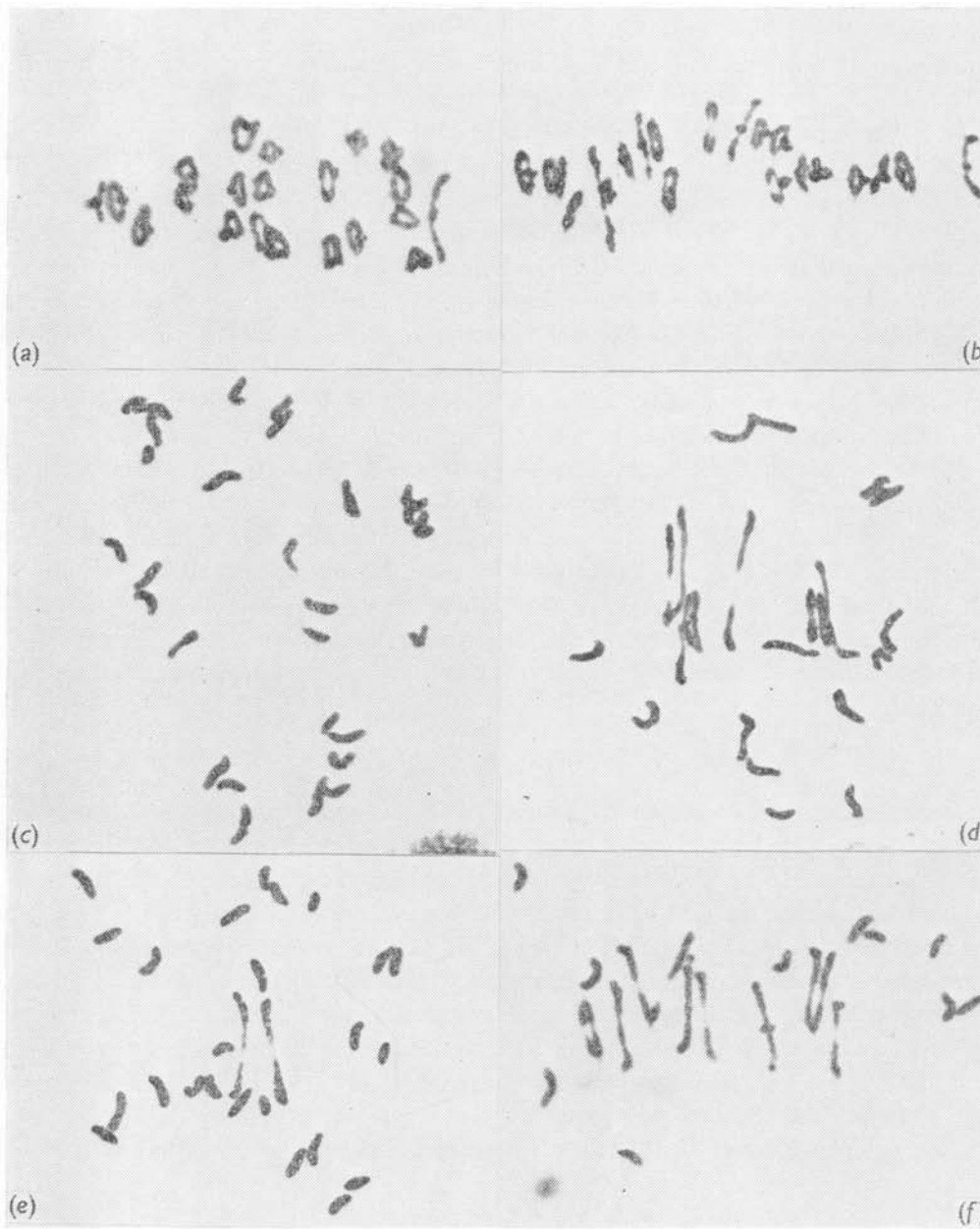

Fig. 1. First metaphase of meiosis in pollen mother cells of *T. aestivum*, *T. aestivum* × *S. cereale* hybrids and *T. aestivum* × *Ae. longissima* hybrids. (*a*) *T. aestivum* ditelo-5B^{L}, 21 bivalents, 5B^{L} bivalent extreme left. (*b*) *T. aestivum*, ditelo-5B^{L} Mutant 10/13, 21 bivalents, 5B^{L} bivalent eight from left. (*c*) *T. aestivum* ditelo-5B^{L} × *S. cereale* non-mutant, 1 bivalent and 26 univalents. (*d*) *T. aestivum* ditelo-5B^{L} Mutant 10/13 × *S. cereale*, 2 trivalents, 4 bivalents and 14 univalents. (*e*) *T. aestivum* ditelo-5B^{L} × *Ae. longissima* non-mutant, 2 bivalents and 24 univalents. (*f*) *T. aestivum* ditelo-5B^{L} Mutant 10/13 × *Ae. longissima*, 2 trivalents, 6 bivalents and 10 univalents.

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lethality before or immediately following germination, since this was expected to give the highest rate of mutation.

The initial scoring, to detect the occurrence of mutation, was carried out on *T. aestivum* × *S. cereale* hybrids using temporary aceto-carmine squashes of anthers with pollen mother cells at first metaphase of meiosis. Normally in these hybrids there is a very low level of chromosome pairing at meiosis (Fig. 1*c*). By contrast, in otherwise similar hybrids, but lacking chromosome 5B, pairing is considerably increased (Riley, 1960). The distinction in the meiotic behaviour of wheat-rye hybrids with and without chromosome 5B is so obvious that it can be detected at low-power (× 60) on the light microscope. The purpose of the initial sieve of potential mutants was to detect hybrids that, although still carrying $5B^L$, resembled hybrids lacking chromosome 5B in meiotic pairing. This system removed the need to search for homoeologous meiotic pairing at the 42-chromosome level in *T. aestivum* where detection would have been made much more difficult.

When hybrids appeared to have higher than normal levels of chromosome pairing other anthers from the same plants were fixed in acetic-alcohol and stained by the Feulgen procedure. First metaphase of meiosis was then scored on permanent slides of anther squashes. The same methods were used for the staining and squashing of all the materials from which scores were taken to record meiotic behaviour.

Determinations of somatic chromosome constitutions were made from squashes of root-tips that had been pretreated with mono-bromonaphthalene and stained by the Feulgen procedure.

4. SPONTANEOUS VARIATION IN THE 5B SYSTEM

Before an attempt was made to induce mutation in the system restricting meiotic chromosome pairing to full homologues, an assessment was made of the frequency of such variants in an untreated wheat stock. One spike on each of 230 plants of *T. aestivum* Chinese Spring euploid was pollinated with *S. cereale* Petkus and a second spike was bagged to ensure self-pollination. The progeny obtained by self-pollination were intended to be used in the recovery of any variants with unusual meiotic pairing that might be revealed from the behaviour of *T. aestivum* × *S. cereale* hybrids.

Altogether 990 wheat-rye hybrids derived from the 230 wheat parents were examined at meiosis and of these 988 had extremely low levels of chromosome pairing. The mean chromosome pairing of two plants typical of this majority is shown in Table 1 (plants 26/3 and 82/5). This is the pattern of pairing normally to be expected in wheat–rye hybrids.

In the other two hybrids, 92/3 and 179/2 (Table 1), bivalents ranged from one to six per cell, trivalents from zero to three per cell and there was an occasional quadrivalent. This pattern of pairing resembles that in wheat–rye hybrids lacking chromosome 5B (Riley, 1960) so that it may be inferred that modification had occurred in the 5B system contributed by the wheat gamete to the hybrids.

One of these hybrids (179/2) had only 27 chromosomes and, although chromosome 5B was not structurally recognizable in this material, it was assumed that deviation from the usual pattern of meiotic pairing arose from the absence of this chromo-

some. The other hybrid (92/3) with higher meiotic pairing had 28 chromosomes but the presence of chromosome 5B could not be confirmed. The wheat gamete involved in the fertilization from which this hybrid arose, therefore, either carried a mutant allele removing the restriction on homoeologous pairing or was simultaneously deficient for chromosome 5B and disomic for a compensating homoeologue.

Table 1. *Mean chromosome pairing at first metaphase of meiosis in hybrids from the cross* T. aestivum × S. cereale (*30 cells per plant*)

Hybrid	Chrom. no.	Mean per cell				
		Univ.	Biv.	Triv.	Quad.	Chiasmata
26/3	28	27·80	0·10	—	—	0·10
82/5	28	26·50	0·75	—	—	0·75
92/3	28	14·75	4·55	1·25	0·10	8·65
179/2	27	15·10	4·15	1·00	0·15	7·20

The two deviant hybrids were derived from different parental plants of *T. aestivum*. There were fourteen sibs of the deviant hybrid 92/3 derived from the same cross but all had the normal low level of pairing. There was one hybrid sib of hybrid 179/2 and this also had the normal low pairing. In addition it was not possible to detect a genetic condition leading to higher levels of meiotic pairing when the selfed progeny of the *T. aestivum* parents of these hybrids were checked in crosses with *S. cereale*. The breakdown in the mechanism preventing homoeologous pairing that is assumed to have occurred was therefore a property only of hybrids 92/3 and 179/2 and possibly of the wheat gametes from which they arose. Apparently the abnormal genetic condition was not transmitted from the wheat parents concerned, so that in two of 990 wheat eggs or in the derived zygotes, genetic changes occurred affecting homoeologous pairing, that could be detected in wheat–rye hybrids. One of these events certainly involved the origin of aneuploidy and the other could have been mutation involving allelic substitution or structural change too slight to be detected cytologically.

This survey confirmed that spontaneous change can occur in the genetic system by which meiotic chromosome pairing is normally restricted in wheat. However, such change is relatively infrequent and to improve the probability of isolating genetic variants in the 5B system it was necessary to induce mutation.

5. DIRECT DETECTION OF MUTATION

Seeds of *T. aestivum* ditelocentric $5B^L$ were treated with EMS and survivors of those treatments that resulted in about 50 % lethality were grown on and pollinated with *S. cereale* pollen. Altogether, 75 wheat female parents gave rise to 206 *T. aestivum* × *S. cereale* hybrids which were grown in a glasshouse at about 20 °C. Chromosome pairing in the 28-chromosome, $5B^L$ telocentric hybrids was checked at first metaphase of meiosis. There was a low level of pairing with only rare bivalents in 199 hybrids (Table 2). Three 27-chromosome hybrids (9/1, 72/17 and 83/11), all

lacking the telocentric marking chromosome $5B^L$, had much higher levels of pairing, with from one to eight bivalents and zero to six trivalents per cell (Table 2). This pattern is characteristic of that of *T. aestivum* × *S. cereale* hybrids lacking chromosome 5B, and the absence of the telocentric indicated that chromosome 5B was not present in these three hybrids.

One hybrid (9/2) had 28 chromosomes but the $5B^L$ telocentric was replaced by a small centric fragment which was presumed to be a breakage product of the telocentric. The hybrid had a level of pairing at meiosis close to that of some 5B-deficient hybrids. From this it is concluded that the region of chromosome 5B normally responsible for the prevention of homoeologous pairing had been deleted and if this were so it would suggest that the effective region is not immediately adjacent to the centromere.

Table 2. *Mean chromosome pairing at first metaphase of meiosis in hybrids between EMS-treated* T. aestivum *ditelocentric* $5B^L$ *and* S. cereale *(20 cells per plant)*

Hybrid	Chrom. no.	$5B^L$	Mean per cell				
			Univ.	Biv.	Triv.	Quad.	Chiasmata
5/10	28	Present	27·80	0·10	—	—	0·10
28/10	28	Present	27·70	0·15	—	—	0·15
82/12	28	Present	27·10	0·45	—	—	0·45
9/1	27	Absent	15·50	3·70	1·30	0·05	7·35
72/17	27	Absent	19·30	2·50	0·90	—	4·60
83/11	27	Absent	17·30	3·85	0·60	0·05	5·60
9/2	28	Fragment	19·40	3·40	0·60	—	4·95
5/12	28	Present	21·10	2·40	0·70	—	3·80
8/12	28	Present	14·70	5·20	0·90	0·05	8·70
81/15	28	Present	13·60	4·75	1·35	0·15*	9·90

* 0·05 as quinquavalent.

Three hybrids (5/12, 8/12 and 81/15) had 28-chromosomes, including an apparently normal telocentric $5B^L$, and yet displayed higher than normal levels of meiotic pairing (Table 2). Pairing was similar to that in hybrids deficient for chromosome 5B and the breakdown in the normal limitation of pairing was probably due to a mutation in the 5B system of pairing regulation. Mutation may have occurred at a critical locus on 5B or at an inhibitor locus anywhere in the genotype that interacted with the 5B activity. Irrespective of their precise nature, the mutants detected in this limited experiment suggested that, with the EMS treatment employed, mutation rate in the overall 5B system was quite high at about 1·5 %. Of course it is recognized that small deletions may have been misinterpreted as gene mutations in these instances but in the present material these alternatives would be indistinguishable. Since EMS is less likely to cause deletion it is probable that the changes observed arose by allelic substitution.

All three mutant hybrids, 5/12, 8/12 and 81/13, were derived from different wheat parents and were therefore of independent origin. The wheat–rye hybrids were sterile so that the mutant condition could not be recovered from them. Con-

sequently an attempt was made to recover the mutants from the progenies obtained by self-pollinating the *T. aestivum* parents of the mutant hybrids.

Eight plants were grown from each of these three progenies. All 24 plants were checked at meiosis and found to have regular bivalent formation and all were pollinated with *S. cereale* pollen to give 24 wheat–rye hybrid families all of which contained at least five plants. Altogether 159 wheat–rye hybrid plants were checked at meiosis and all had low levels of pairing like normal wheat–rye hybrids. The mutant conditions had not, therefore, been transmitted to the selfed progenies and it was presumed that the EMS-treated parental plants had been sectorial for the mutant states which had been transmitted only to the hybrids in which they were detected.

In spite of the failure to recover and retain a mutant the initial exploration provided a guide to the methods that could be used in the isolation of mutants. Clearly the EMS treatment was highly mutagenic and a different breeding procedure would increase the probability of holding mutants in the 5B system.

6. INDIRECT DETECTION OF MUTATION

The inflorescences of the ditelocentric $5B^L$ parents, from which wheat–rye hybrids 5/12, 8/12 and 81/15, were derived, were apparently sectorial for mutant conditions affecting meiotic pairing since only one hybrid was mutant in the progeny of each. The progeny sizes were respectively 6, 6 and 4, and, of course, the occurrence of other mutant sectors might have gone undetected in wheat–rye hybrid families derived from other EMS-treated parents. To examine this possibility progenies were grown, each of six M_2 plants, that had been derived by selfing eight EMS-treated M_1 parents. All 48 M_2 plants were pollinated with rye and 42 families of wheat–rye hybrids resulted. There was very low pairing at meiosis in all 209 hybrids examined in these families and no evidence that their ditelocentric 5B wheat parents had been mutant in the 5B system nor that the EMS treated plants had carried mutant sectors.

A larger progeny of 27 M_2 plants derived, by selfing, from EMS-treated ditelocentric 5B plant 10, was also grown and each plant was pollinated by rye to give 662 hybrids. Meiotic chromosome pairing was scored in 133 of these with the results indicated in Table 3. There were high-pairing hybrids, presumably with homoeologous pairing, in 12 families, while 14 families contained low-pairing hybrids only. One family (10/1) contained hybrids with high and with low pairing, but those with high pairing all had 27-chromosomes and were deficient for the $5B^L$ telocentric so that the M_2 wheat parent was apparently monotelocentric $5B^L$. Consequently this plant and its family of hybrids with rye will be discounted from any further consideration.

The occurrence of 28-chromosome, $5B^L$ telocentric, wheat–rye hybrids with homoeologous pairing indicates that mutation had apparently occurred in EMS-treated, $5B^L$ ditelocentric, plant 10. Its selfed M_2 progeny contained plants which, on the basis of the sometimes limited families derived from rye pollinations, were

diagnosed as having been homozygous non-mutant, heterozygous, or homozygous mutant. Where segregation occurred the heterozygous status of the M_2 parent was clearly determined, assuming no misclassification, but the family size was often too small for homozygosity in the M_2 parents to be diagnosed with certainty. Nevertheless there was an excess of homozygous non-mutant M_2 parents. The selfed spike from which the M_2 progeny of EMS-treated plant 10 were derived was probably sectorial for the mutant condition, but even so the deficit of mutant homozygotes relative to heterozygotes cannot readily be explained although it may have arisen from a selective advantage of the non-mutant condition. A major difficulty was the possibility of misclassification in this generation because of the effects of temperature variation in the glasshouse.

Table 3. *Segregation of hybrids with low or high pairing at first metaphase of meiosis in families derived from crosses with* S. cereale *of plants in the selfed M_2 progeny of EMS-treated plant 10*

	Pairing			Pairing	
M_2 parent	Low	High	M_2 parent	Low	High
10/3	10	0	10/23	2	0
10/5	3	5	10/24	2	2
10/11	3	1	10/25	4	2
10/12	2	3	10/26	3	1
10/13	0	11	10/27	4	0
10/14	0	1	10/29	3	0
10/15	2	0	10/30	1	3
10/16	3	0	10/31	1	0
10/17	6	0	10/32	1	4
10/18	8	0	10/35	4	1
10/19	6	0	10/34	3	1
10/20	5	0	10/33	7	0
10/21	1	0	10/1	2	6*
10/22	6	0	Total	92	41

* 27-chromosome hybrids deficient for $5B^L$.

Only the wheat–rye family 10/13, containing 11 hybrids, suggested that the M_2 parent from which it was derived might have been homozygous for the mutant condition and there was uncertainty about this because of environmental variation.

In wheat–rye family 10/13 there were differences between individual hybrids in the level of meiotic pairing (Table 4). Some hybrids (10/13/12, 10/13/5 and 10/13/6) had chiasma frequencies approaching those in hybrids deficient for 5B, while in others pairing was considerably lower. This variation raised doubts about the homozygosity of M_2 plant 10/13 so it was necessary to test its progeny derived by self-pollination.

For this purpose 35 M_3 plants derived from 10/13 were grown and pollinated with rye. A total of 350 wheat–rye hybrids derived in this way were grown and 176 of these were examined at meiosis mostly in a controlled environment chamber held at 20 °C with continuous light (Table 5). The majority of hybrids displayed high

Table 4. *Mean chromosome pairing at first metaphase of meiosis in hybrids between* T. aestivum *ditelocentric* $5B^L$ *10/13, an* M_2 *derivative of EMS-treated plant 10, and* S. cereale. (*All had 28-chromosomes including* $5B^L$ (*20 cells per plant*))

Hybrid 10/13	Mean per cell				
	Univ.	Biv.	Triv.	Quad.	Chiasmata
12	18·55	3·80	0·55	0·50	5·50
5	19·10	3·85	0·40	—	5·35
6	19·05	3·95	0·35	—	5·10
1	21·20	2·80	0·40	—	3·85
11	21·30	3·05	0·20	—	3·55
14	21·65	2·95	0·15	—	3·40
8	22·05	2·75	0·15	—	3·35
13	22·60	2·55	0·10	—	2·90
7	24·20	1·60	0·20	—	2·00
2	24·40	1·80	—	—	1·90
4	24·40	1·80	—	—	1·90

Table 5. *Segregation of hybrids with low or high pairing at first metaphase of meiosis in families derived from crosses with* S. cereale *of plants in the selfed* M_3 *progeny of plant 10/13*

M_3 parent 10/13	Pairing		M_3 parent 10/13	Pairing	
	Low	High		Low	High
1	0	3	19	1	4
2	2	3	20	0	6
3	0	6	21	3	1
4	0	4	22	1	5
5	0	7	23	4	2
6	4	3	24	1	4
7	3	2	25	4	0
8	0	9	26	3	5
9	0	5	27	5	1
10	1	7	28	1	4
11	5	1	29	0	3
12	4	4	30	3	4
13	3	2	31	2	5
14	1	2	32	0	1
17	0	4	33	1	4
18	0	6	34	1	2
			35	4	0
			Total	57	119

pairing approaching that of wheat-rye hybrids lacking chromosome 5B but there were also some with much lower pairing. Some family sizes were too small for judgements to be made about the status of their wheat parents, but, ignoring those with less than four hybrid plants, the segregation indicated that the M_3 had segregated into homozygous mutant and non-mutant and into heterozygous categories. Apparently, therefore, the M_2 plant 10/13 was heterozygous.

The wheat–rye hybrid family derived from M_3 plant 10/13/8 consisted of nine plants all of which had high pairing (Fig. 1*d*; Table 6). Subsequent testing of the selfed progeny of M_3 plant 10/13/8 showed it to have been homozygous for the mutant condition affecting meiotic pairing. A homozygous mutant line was thus derived and was designated *Mutant 10/13* (Fig. 2).

Table 6. *Mean chromosome pairing at first metaphase of meiosis in hybrids between* T. aestivum *ditelocentric* $5B^L$ *10/13/8, an* M_3 *derivative of EMS-treated plant 10, and* S. cereale. (*All had 28-chromosomes including* $5B^L$ (*20 cells per plant*))

Hybrid 10/13/8	Mean per cell				
	Univ.	Biv.	Triv.	Quad.	Chiasmata
9	20·55	3·10	0·35	0·05	4·20
7	21·35	2·60	0·45	—	3·60
3	21·45	2·75	0·35	—	3·45
2	21·85	2·85	0·15	—	3·20
8	22·35	2·30	0·35	—	3·15
5	22·88	2·19	0·25	—	2·75
4	22·95	2·45	0·05	—	2·60
10	23·80	1·95	0·10	—	2·20
6	24·25	1·80	0·05	—	1·90

Table 7. *Mean pairing at first metaphase of meiosis in hybrids between non-mutant and Mutant 10/13 forms of* T. aestivum *ditelocentric* $5B^L$ *and* Ae. longissima (*20 cells per plant*)

T. aestivum ditelo-$5B^L$ parent	Mean per cell				
	Univ.	Biv.	Triv.	Quad.	Chiasmata
Non-mutant	27·60	0·20	—	—	0·20
Non-mutant	25·76	1·00	0·08	—	1·16
Mutant 10/13	18·20	4·50	0·20	0·05	5·40
Mutant 10/13	12·20	6·40	0·60	0·30	9·75
Mutant 10/13	11·00	6·60	0·70	0·40*	10·90
Mutant 10/13	8·15	8·55	0·65	0·20	12·05

* One association of five.

7. MUTANT 10/13 × *AEGILOPS LONGISSIMA*

Although there was distinctly higher meiotic chromosome pairing in hybrids between Mutant 10/13 and *S. cereale* than in normal wheat–rye hybrids it was considered necessary to check that the use of Mutant 10/13 genuinely permitted chromosomes to pair that were normally precluded from doing so in such hybrids. It should be realized that *S. cereale* had been used in the recognition of mutants primarily because it crossed readily with wheat and at the same time gave rise to hybrids in which disturbances could be detected in the normal restriction of chromosome pairing, but the differences between mutant and non-mutant forms was not large. Consequently a test was needed in which the distinction between

the normal operation, and ineffectiveness, of the $5B^L$ system would be readily apparent. For this purpose comparisons were made between hybrids of *T. aestivum* ditelocentric $5B^L$ non-mutant, or mutant 10/13, with *Aegilops longissima* ($2n = 14$). This test was chosen because of the large differences in the level of meiotic chromosome pairing in hybrids of *T. aestivum* × *Ae. longissima* with and without chromosome 5B (Riley, Chapman & Kimber, 1959).

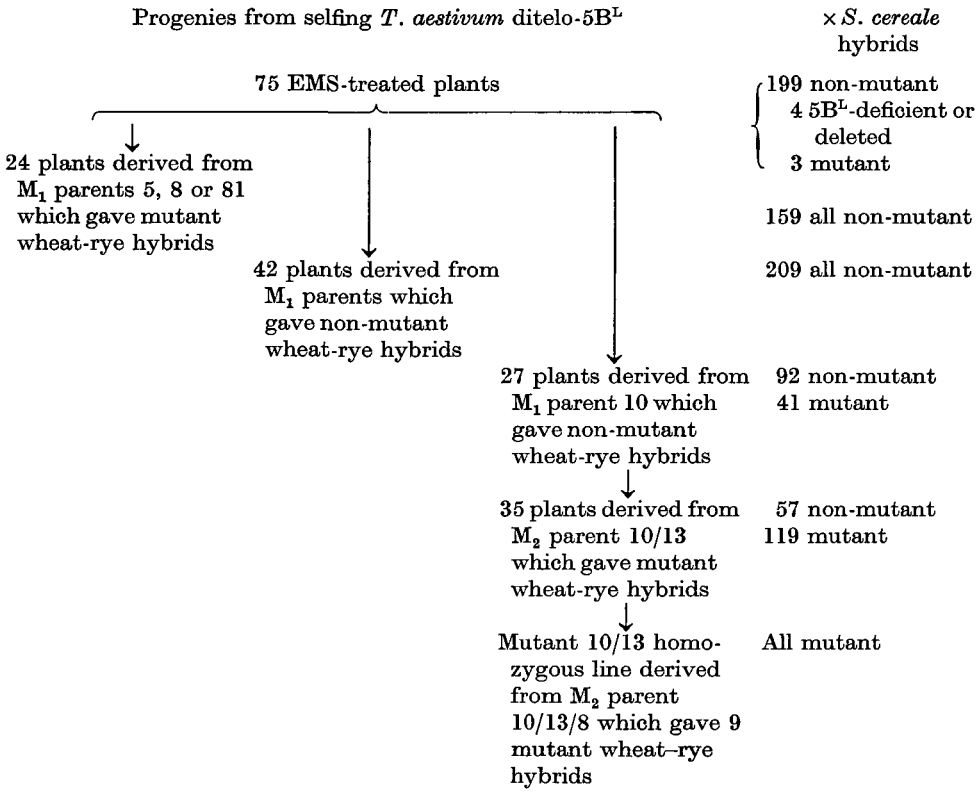

Fig. 2. Diagram to illustrate the breeding system used in the testing and isolation of Mutant 10/13.

Non-mutant plants of *T. aestivum* ditelocentric $5B^L$ and plants of Mutant 10/13 also ditelocentric $5B^L$ were therefore crossed with *Ae. longissima* and the resulting hybrids examined at meiosis. There was very little chromosome pairing in hybrids derived from non-mutant parents, indeed there was a mean of no more than 1·16 chiasmata per cell (Fig. 1*e*). Whereas in hybrids derived from Mutant 10/13 pairing was much higher with mean chiasma frequencies ranging from 5·40 to 12·05 per cell (Fig. 1*f*; Table 7).

The difference between the two types of hybrid with *Ae. longissima* emphasized the marked effect on meiotic pairing of Mutant 10/13. This effect, like that arising from chromosome $5B^L$ deficiency, is more readily demonstrated in hybrids with *Ae. longissima* than with *S. cereale*.

8. MUTANT 10/13 HOMOZYGOUS IN DITELOCENTRIC $5B^L$

Meiotic chromosome pairing was scored at first metaphase in a number of plants of *T. aestivum* ditelocentric $5B^L$ homozygous for the mutant 10/13 condition. The scores from two typical Mutant 10/13 plants are shown in Table 8 together with the score for a non-mutant control. Mutant 10/13 plants never had any multivalents in the way that might have been expected in genotypes in which there was reduced meiotic isolation of homoeologues (Fig. 1*b*). There may have been some reduction in chiasma frequency in Mutant 10/13 but the experiment was inadequate for this to be determined unequivocally.

Table 8. *Mean pairing at first metaphase of meiosis in* T. aestivum *ditelocentric* $5B^L$ *non-mutant and Mutant 10/13 (20 cells per plant)*

	Mean per cell		
Genotype	Univ.	Biv.	Chiasmata
Non-mutant	0·20	20·90	43·35
Mutant 10/13	—	21·00	39·80
Mutant 10/13	0·40	20·80	38·20

The short arm of chromosome 5B ($5B^s$) promotes meiotic chromosome pairing and was absent from the ditelocentric $5B^L$ Mutant 10/13 examined. It seemed possible that the presence of $5B^s$ in Mutant 10/13 might increase pairing to a level at which multivalents would occur due to the association of homoeologues, so the mutant condition was made homozygous in a euploid chromosome complement.

9. MUTANT 10/13 IN A EUPLOID CHROMOSOME COMPLEMENT

T. aestivum ditelocentric $5B^L$ and homozygous for the Mutant 10/13 condition was pollinated by *T. aestivum* euploid to give derivatives heteromorphic (telocentric $5B^L$ and complete) in the 5B pair and heterozygous for the mutant state. These F_1 derivatives were self-pollinated and an F_2 progeny was grown in which plants with euploid chromosome constitutions – that is, without $5B^L$ telocentrics – were selected. The genotypes of these F_2 plants were tested in hybrids obtained by pollinating them with rye. Ten wheat–rye families were grown and 112 hybrids in these families were scored at meiosis for the presence of the normal or mutant phenotype. Eight families segregated, containing hybrids with low and others with high levels of pairing, so their *T. aestivum* parents were diagnosed as having been heterozygous. Two families, one containing 15 hybrids and the other 13 hybrids, were entirely high pairing and their *T. aestivum* parents were diagnosed as having been homozygous for the mutant 10/13 condition. Selfed progeny were available from these plants so Mutant 10/13 was established in the euploid chromosome complement.

Euploid Mutant 10/13 homozygotes were entirely regular at meiosis. Many plants of this genotype were examined but there were no multivalents although these

might have been expected in a genotype with a reduced barrier to homoeologous pairing (Table 9). Apparently the effect of Mutant 10/13 on meiotic pairing was only detectable in hybrids like those with *S. cereale* or *Ae. longissima* in which there were no fully homologous partner chromosomes. In terms of its effect on meiosis in *T. aestivum*, Mutant 10/13 could be regarded as being neutral to selection. However, such alleles are not common in nature so it may be that there are other disadvantageous phenotypic effects that either do not disturb meiosis or do so under environmental conditions different from those used in the present work.

Table 9. *Mean pairing at first metaphase of meiosis in* T. aestivum *euploids non-mutant and Mutant 10/13 (30 cells per plant)*

	Mean per cell		
Genotype	Univ.	Biv.	Chiasmata
Non-mutant	0·07	20·97	48·73
Mutant 10/13	0·07	20·97	47·60

10. ISOLATION OF MUTANT HETEROZYGOTES

When EMS-treated plants were crossed with rye to detect the occurrence of pairing mutations the mutant condition was lost because of the sterility of the resulting wheat–rye hybrids. Searching for pairing mutants in the progeny obtained by selfing EMS-treated plants increased the labour because of segregation. A further breeding system was consequently examined in an attempt to avoid these difficulties. In this system plants of *T. aestivum* ditelocentric $5B^L$ were treated with EMS and subsequently pollinated with untreated euploid *T. aestivum*. The products of this cross were potentially heterozygous for mutant conditions and heteromorphic for chromosome 5B, with one complete and one telocentric member. These plants were pollinated with rye and the resulting hybrid families examined at meiosis for deviation from the usual very low level of chromosome pairing indicating the occurrence of mutation. Of course single locus heterozygotes would be expected to produce wheat–rye hybrid families segregating in a 1 : 1 ratio. The progeny derived by selfing plants whose heterozygous status had been detected in wheat–rye hybrid families could then be searched for homozygotes.

Applying this system, 233 heteromorphic plants were obtained from the cross EMS-treated ditelocentrics × euploid. All had 42 chromosomes with one telocentric and 118 of them were pollinated with rye to give hybrid families. From these 118 families 854 wheat-rye hybrids were scored for the level of chromosome pairing at first metaphase of meiosis. In all, except four, of the families the hybrids had the very low level of pairing expected in normal wheat–rye hybrids with only occasionally a bivalent, and with mean chiasma frequencies never exceeding 0·80 per cell in the plants scored in detail. In the other four families there were plants with unusually high pairing, with from one to six bivalents, up to two trivalents, and occasionally a quadrivalent. One of these families (112) contained six plants

all with 27 chromosomes and it was assumed that chromosome 5B was deficient (Table 10).

Family 53 contained four 28-chromosome wheat–rye hybrids two of which had the usual low level of pairing while the other two had distinctly higher pairing. Although the pairing in these plants, which had mean chiasma frequencies of 2·75 and 3·45 cell, was not as high as that in hybrids deficient for chromosome 5B it seems likely that 5B heteromorphic plant 53 was heterozygous for a mutant condition (Table 10).

Table 10. *Mean chromosome pairing at first metaphase of meiosis in hybrids from the crosses* (*EMS-treated* T. aestivum *ditelo-5B*^{L} × T. aestivum *euploid*) × S. cereale (*20 cells per plant*)

		Mean per cell				
Hybrid	Chrom. 5B	Univ.	Biv.	Triv.	Quad.	Chiasmata
1/8	Telocentric	27·60	0·20	—	—	0·20
1/6	Telocentric	27·00	0·50	—	—	0·50
112/3	Absent	16·20	4·05	0·90	—	6·55
112/4	Absent	14·55	4·50	0·95	0·15	7·90
53/3	Telocentric	22·80	2·45	0·10	—	2·75
53/7	Telocentric	21·75	2·60	0·35	—	3·45
6/10	Complete	25·20	1·40	—	—	1·40
6/8	Complete	24·80	1·45	0·10	—	1·50
6/2	Complete	24·65	1·60	0·05	—	1·70
6/4	Telocentric	23·95	1·95	0·05	—	2·05
6/7	Complete	24·10	1·80	0·15	—	2·10
6/1	Telocentric	22·55	2·35	0·25	—	2·85

Family 6 had six plants, all with 28 chromosomes, including 5B^{L} telocentric in two and 5B complete in four plants. All six hybrids had a level of meiotic chromosome pairing higher than that in normal hybrids, but lower than that expected in the deficiency of chromosome 5B (Table 10). Consequently heteromorphic plant 6 was probably heterozygous for a mutant condition affecting meiotic pairing, but like that carried by plant 53 its effect on pairing was less profound than that arising from the absence of chromosome 5B or from the presence of Mutant 10/13.

The fourth variant family (61) contained hybrids with levels of pairing comparable with standard wheat–rye hybrids and others with chiasma frequencies close to, but still somewhat below, those of 5B-deficient hybrids (Table 11). It seems probable that the heteromorphic plant 61 was heterozygous for a mutant in the system regulating homoeologous pairing and that the variation in pairing in the wheat–rye family arose from environmental variation in a mutant genotype in which there was an incomplete breakdown in the isolation of homoeologues. Only two of the hybrids in family 61 carried the 5B^{L} telocentric and both of these were in the low pairing category.

In an attempt to retain the mutant condition present in the apparently heterozygous plant 61, its progeny obtained by selfing was grown and pollinated by rye.

Wheat–rye hybrid progenies were obtained from 12 selfed derivatives of plant 61. A total of 96 hybrids in these families was examined at meiosis. Segregation occurred into high- and low-pairing categories which had chiasma frequencies like those of the corresponding segregants among the hybrids of plant 61. The low-pairing hybrids had mean chiasma frequencies less than 0·76 and the high pairing hybrids had mean chiasma frequencies between 2·23 and 4·70, in the plants scored.

Table 11. *Mean chromosome pairing at first metaphase of meiosis in family 61 from the cross (EMS-treated* T. aestivum *ditelo-5B^L* × T. aestivum *euploid)* × S. cereale *(20 cells per plant)*

Hybrid 61	Mean per cell			
	Univ.	Biv.	Triv.	Chiasmata
4*	27·80	0·10	—	0·10
2	27·40	0·30	—	0·30
6*	27·20	0·40	—	0·40
8	25·60	1·20	—	1·20
9	23·55	2·15	0·05	2·25
1	21·50	3·25	—	3·45
3	21·80	2·95	0·10	3·50
7	21·25	3·00	0·25	3·70
5	20·45	3·40	0·25	4·45

* Telocentric 5B^L present.

Table 12. *Segregation in crosses between members of the selfed progeny of* T. aestivum *plant 61* × S. cereale *for high and low pairing at meiosis and for the complete or telocentric state of chromosome 5B^L*

Plant 61 progeny parent	Low pairing		High pairing	
	5B complete	Telo. 5B^L	5B complete	Telo. 5B^L
1	3	0	2	0
2	0	1	0	3
3	12	0	0	0
4	0	6	0	0
5	4	2	0	0
7	12	0	0	0
9	1	2	1	0
11	1	2	0	0
15	13	0	7	0
17	0	0	2	1
18	1	2	4	1
20	10	3	0	0
Total	57	18	16	5

The segregation of hybrids, with high or low pairing and with the complete chromosome 5B or telocentric 5B^L, is shown in Table 12. Considered overall the ratio was 75 low : 21 high pairing and 73 complete : 23 telocentric 5B. Apparently, although a high-pairing mutant condition was transmitted to the selfed progeny of plant 61 it was not inherited in the 1 : 1 ratio expected if mutation had occurred

at a single locus. Also, as expected because of the poorer competitive ability of pollen carrying the telocentric relative to that with the complete 5B, the telocentric was less frequent than the complete chromosome in the progeny of plant 61. Because of the confirmed mutant status of this material it was subsequently named 'Mutant 61'.

The status of the selfed derivatives of plant 61 can be inferred from the status of wheat–rye hybrid progenies to which they gave rise, although some progenies contained too few individuals for them to be an accurate guide to the genotypes of their wheat parents. Seven wheat–rye progenies were entirely non-segregating and low pairing, eight segregated and one was non-segregating and high pairing. If plant 61 had been heterozygous at a single locus and the alleles had segregated at random into its selfed progeny a 1:2:1 ratio would have been expected in these three categories of wheat–rye families. The observed segregation was consistent with plant 61 having been heterozygous at two loci with duplicate effects, such that only wheat–rye hybrids carrying mutant alleles at both loci had higher than normal pairing. However the segregation in the initial wheat–rye family derived from plant 61 was not consistent with this hypothesis.

Table 13. *Mean chromosome pairing at first metaphase of meiosis in hybrids from the cross* T. aestivum *Mutant 61* × Ae. longissima (*20 cell per plant*)

	Mean per cell				
Hybrid	Univ.	Biv.	Triv.	Quad.	Chiasmata
61/4	17·45	5·05	0·02	—	5·55
61/3	17·10	5·45	0·01	0·01	6·40
61/6	15·20	5·85	0·30	0·05	6·95
61/5	15·50	5·60	0·30	0·10	7·25

On the basis of the series of wheat–rye families only plant 61/17 appeared to have been homozygous for the mutant 61 condition but tests on the selfed progeny of this plant demonstrated that it too was heterozygous. Subsequently, although the mutant condition of family 61 was confirmed by the higher than normal pairing of *T. aestivum* × *Ae. longissima* hybrids (Table 13), further work was concentrated on Mutant 10/13 because the lack of clarity in the inheritance of Mutant 61 and because of its lesser effect as judged by the level of meiotic chromosome pairing in Mutant 61 × rye hybrids.

The three mutants recognized in plants from the cross EMS-treated ditelocentric $5B^L$ × euploid wheat had increases in the level of pairing in wheat–rye hybrids but the level did not attain that of 5B-deficient hybrids. Nevertheless three mutants were observed among the 118 gametes examined, which is a mutation rate of about 2·5 % compared with a rate of about 1·5 % in the work described earlier.

11. DISCUSSION

Okamoto (1962, 1966) has already shown that mutants can be recognized in *T. aestivum* that disrupt the normal isolation of homoeologous chromosomes at meiosis. These mutants were detected following X-ray treatments but none was retained so that no evidence was provided on the chromosomal location of the loci involved. Okamoto's investigation of the 5B problem by induced mutation was, apparently, the first attempt to study meiotic chromosome pairing in this way. Most other induced mutants affecting meiotic pairing have been isolated fortuitously in general studies of mutation.

In the present work, mutants with increased levels of homoeologous pairing were isolated with frequencies of between 1·5 and 2·5% among the gametes examined from EMS-treated germinating seeds. Although this frequency is high it is lower than that observed by Okamoto (1962) following X-irradiation of almost mature wheat. This higher frequency may have resulted from the greater numbers of deletions resulting from X-ray treatment or from the reduced somatic competition to which mutant cell lines were exposed, when mutation occurred immediately before meiosis.

From the present work it is clear that mutants affecting homoeologous meiotic pairing can be isolated and retained in wheat. However, somewhat tedious systems are required for their recognition and for the progeny-testing required to establish homozygosity. The need to use wheat–rye hybrids to determine the genotypes of the wheat parents makes this work extremely laborious. In the present work, at all stages of the programme, meiosis was examined in 2935 wheat–rye hybrids, which is probably a larger number of hybrids than has been reported on in the entire literature on the cytogenetics of the wheat–rye combination.

The chromosomal location of Mutant 10/13 is discussed in a subsequent paper (Wall, Riley & Gale, 1971) but it is appropriate at this stage to allude to one characteristic of this genotype. Although in hybrids between Mutant 10/13 and either *S. cereale* or *Ae. longissima* there is considerable homoeologous chromosome pairing at meiosis, there is no evidence that homoeologues pair in homozygotes for the mutant condition in *T. aestivum* irrespective of whether 5B is represented by the long telocentric or the complete chromosome. Apparently, therefore, Mutant 10/13 had no effect on the meiosis of *T. aestivum*. Consequently it might be expected that such genetic variants would suffer no selective disadvantage in wheat and would therefore be found in normal wheat stocks. However, if they occur at all they are not common, so it may be that there are pleiotropic effects which place them at a selective disadvantage.

There were several reasons for attempting to select and fix mutants giving homoeologus pairing. One was to determine whether a single locus on $5B^L$ is implicated in the normal restriction of this pairing. Another use for the variants was to be in breeding exercises of several kinds. Both of these aims have been or are being realized with the exploitation of Mutant 10/13.

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