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The position of a locus on chromosome 5B of *Triticum aestivum* affecting homoeologous meiotic pairing

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SUMMARY

An investigation was made of the chromosomal position of the mutant locus, in Mutant 10/13 of *Triticum aestivum* ($2n = 6x = 42$), affecting homoeologous chromosome pairing at meiosis. In hybrids between Mutant 10/13 and rye (*Secale cereale* $2n = 14$), homoeologous chromosomes frequently pair at meiosis although normally, in wheat–rye hybrids, this happens infrequently.

The association of the mutant condition with chromosome 5B was determined by (i) the absence of segregation in hybrids obtained when Mutant 10/13 monosomic 5B was pollinated by rye; (ii) the occurrence of trisomic segregation for pairing behaviour in 28-chromosome wheat–rye hybrids, obtained from 5B trisomic wheat parents with two 5B chromosome from a non-mutant and one from a mutant parent; (iii) the absence of segregation for pairing behaviour in the 29-chromosome wheat–rye hybrids obtained from the same trisomic wheat parents.

The alternative pairing behaviours segregated independently of the centromere when wheat plants that were simultaneously heteromorphic, $5B^L$ telocentric/5B complete, and heterozygous for the Mutant 10/13 state, were pollinated by rye. The alternative chromosome-pairing patterns segregated to give a ratio not different from 1:1, so that the association of homoeologous pairing with Mutant 10/13 probably derived from the occurrence of mutation at a single locus on $5B^L$. In the disomic heteromorphic state, $5B^L$ was 91 map units in length.

Trisomic wheats with two complete 5B chromosomes and one $5B^L$ telocentric, that were also heterozygous for the Mutant 10/13 condition, were pollinated by rye. Among the resulting 28-chromosome hybrids there was a 2:1 segregation of hybrids with low pairing:high (homoeologous) pairing and also of hybrids with complete 5B:telocentric $5B^L$. However, there was no evidence of linkage in this trisomic segregation. All the 29-chromosome hybrids from this cross had low pairing and it could be concluded that the single mutant allele, in Mutant 10/13, was recessive. In the trisomic condition, relative to a simplex situation, $5B^L$ was 33·05 map units in length.

The critical locus on $5B^L$ was designated *Pairing homoeologous*. The normal dominant allele was symbolized *Ph* and the recessive allele, in Mutant 10/13, *ph*.

The prevention of homoeologous pairing by the activity of a single locus makes the evolution of the regular meiotic behaviour of *T. aestivum* more readily comprehensible.

1. INTRODUCTION

Meiotic pairing between homoeologous chromosomes of different genomes of bread wheat (*Triticum aestivum* $2n = 6x = 42$) is normally prevented by a genetic activity of the long arm of chromosome 5B (Riley & Chapman, 1958; Riley, 1960; Riley & Law, 1965). It has been assumed that the activity is determined at a single locus on $5B^L$ but the assumption has not been tested because no allelic variation was available. Partly to complete the analysis of the formal genetics of the $5B^L$ system, attempts have been made to isolate allelic variants (Okamoto, 1966; Riley, Chapman & Belfield, 1966; Wall, Riley & Chapman, 1971). An ethyl methanesulphonate (EMS)-induded variant, Mutant 10/13, isolated by Wall *et al.* (1971) has an effect on the meiotic chromosome pairing in *T. aestivum* × *S. cereale* hybrids approaching that in similar hybrids deficient for chromosome 5B. It was presumed that homoeologous chromosomes paired in Mutant 10/13 × *S. cereale* hybrids and that mutation has occurred in the 5B system of pairing regulation. Whether the mutant locus was on chromosome 5B, or at a suppressor locus elsewhere, was not known. The purpose of the present work was to determine the chromosomal position of the mutant locus.

2. MATERIALS

The wheat parents used were all in the variety Chinese Spring of *Triticum aestivum* L. emend. Thell. ssp. *vulgare* Mackey ($2n = 6x = 42$). The Mutant 10/13 forms were ditelocentric for the long arm of chromosome 5B and homozygous for the mutant condition. The other forms used were euploid or had 44 chromosomes and were tetrasomic for chromosome 5B, or were 41-chromosome 5B or 3B monosomics.

The rye material used was *Secale cereale* L. variety Petkus ($2n = 14$).

3. METHODS

Mutant 10/13 does not display a distinctive pattern of meiotic pairing in *T. aestivum* but gives higher than normal pairing in *T. aestivum* × *S. cereale* hybrids. Consequently *T. aestivum* × *S. cereale* hybrids were used to diagnose the genotypes of wheat parents and the patterns of segregation to which wheat parents could give rise.

Somatic chromosome constitutions of this material were determined on Feulgen-stained squashes of root-tips that had been pretreated with mono-bromonaphthalene and fixed in glacial acetic acid. Meiotic pairing patterns were determined at first metaphase from permanent squashes of anthers fixed in acetic-alcohol and stained by the Feulgen procedure.

4. MONOSOMIC ANALYSIS

Since chromosome 5B is known to play a large part in the genetic limitation of homoeologous meiotic pairing in wheat, it was an obvious preliminary to enquire whether the genetic change in Mutant 10/13 was also associated with that chromo-

some. For this purpose monosomic analysis, which is frequently used in genetic studies of wheat, was employed. In the way used here, monosomic analysis relates genetic effects to particular chromosomes by the distortion of segregation ratios that occur when the locus being studied is in the hemizygous rather than the heterozygous condition. Distortions of ratios arise because monosomic chromosomes are only transmitted to about 25 % of egg cells but are transmitted through about 96 % of the functioning pollen grains. In the present instance use was made only of disturbed segregations through eggs.

Table 1. *Segregation of wheat–rye hybrids with low (no homoeologous) or high (homoeologous) meiotic chromosome pairing from 5B monosomic wheat parents*

	Low pairing		High pairing	
Monosomic analysis	5B present	5B- deficient	5B present	5B- deficient
T. aestivum mono-5B × *S. cereale*	6	0	0	24
T. aestivum Mutant 10/13 mono-5B × *S. cereale*	0	0	22	35

Normal, non-mutant, forms of *T. aestivum* monosomic for chromosome 5B were pollinated by *S. cereale*. As expected, 24 of the resulting wheat–rye hybrids had 27 chromosomes, lacking 5B, while only 6 hybrids had 28 chromosomes including 5B. When checked at meiosis all the 27-chromosome hybrids had the high pairing expected when 5B is deficient while the 28-chromosome hybrids had low pairing (Table 1). Thus the restriction of high pairing – that is, of homoeologous pairing – was shown, by monosomic analysis, to be determined by chromosome 5B.

T. aestivum monosomic 5B was pollinated by *T. aestivum* ditelocentric $5B^L$ Mutant 10/13 and, in the derivatives of the cross, plants were selected with 41 chromosomes including one $5B^L$ telocentric. The selected plants were monosomic for chromosome 5B, the sole representative of this chromosome being the long arm telocentric derived from Mutant 10/13. These $5B^L$ monosomics were pollinated by *S. cereale* and 57 wheat–rye hybrids were obtained. There were 22 hybrids with 28 chromosomes, including the $5B^L$ telocentric, and 35 with 27 chromosomes, lacking the $5B^L$ telocentric. At meiosis all of these hybrids, irrespective of whether $5B^L$ was present or not, had high levels of chromosome pairing. There was no segregation.

T. aestivum monosomic 3B was pollinated by *T. aestivum* ditelocentric $5B^L$ Mutant 10/13 and, in the derivatives of the cross, plants were selected that had 41 chromosomes including one $5B^L$ telocentric. The selected plants were monosomic for chromosome 3B, the sole representative of this chromosome having been derived from Mutant 10/13. The chromosome 5B pair was heteromorphic, telocentric-complete. The 3B monosomics were pollinated by *S. cereale* and 44 wheat–rye hybrids were obtained. There were 12 hybrids with 28 chromosomes carrying 3B, and 32 hybrids with 27 chromosomes lacking 3B. At meiosis the hybrids segregated into 16 with high pairing and 28 with low pairing (Table 2). Moreover the mutant condition derived from Mutant 10/13 segregated independently of chromosome 3B.

The absence of segregation for low- and high-pairing phenotypes in wheat–rye hybrids in which the wheat parent was monosomic for $5B^L$ derived from Mutant 10/13 showed that, provided this chromosome was present, the level of pairing corresponded to that occurring in the absence of chromosome 5B. Consequently the property of causing high pairing in wheat–rye hybrids, that distinguishes Mutant 10/13, was shown to be determined by $5B^L$.

Table 2. *Segregation for the presence or absence of chromosome 3B and for high (homoeologous) or low (no homoeologous) meiotic pairing in the cross* (T. aestivum *mono-3B* × *Mutant 10/13*) *mono-3B* × S. cereale

	Pairing		
Chromosome 3B	Homoeologous	No homoeologous	Total
Present	5	7	12
Absent	11	21	32
Total	16	28	44

5. DISOMIC TELOCENTRIC MAPPING

Since monosomic analysis had shown the mutant allele in mutant 10/13 to be on the $5B^L$ telocentric, it seemed possible that the locus concerned might be mapped relative to the centromere using the principles and procedures exploited by Sears (1966) and Law (1966). In the present work the method used was to produce hybrids between *T. aestivum* Mutant 10/13 and *T. aestivum* euploid. Such hybrids were heteromorphic complete/telocentric, for chromosome 5B and heterozygous for the mutant condition. The heteromorphic heterozygotes were pollinated with rye and the resulting wheat–rye hybrids scored for the presence of the complete or the telocentric chromosome 5B and for the normal low level of meiotic pairing or for the homoeologous pairing characteristic of the mutant.

A total of 154 wheat–rye hybrids was produced from the heteromorphic and heterozygous wheat parents. These segregated for the presence of 5B complete or telocentric and for homoeologous or no homoeologous pairing in the frequencies shown in Table 3. In neither instance was there a significant deviation from the expected 1:1 ratio nor was there a significant deviation from independent segregation of the chromosomal condition and meiotic pairing type. This pattern of segregation showed that the hypothesis could be sustained that the alternative meiotic pairing behaviours were determined by the presence of alternative alleles at a single locus. The locus has been designated *pairing homoeologous* (*Ph* or *ph*). No evidence was provided from this work to show that the *ph* allele of Mutant 10/13 is linked to the 5B centromere although monosomic analysis had shown the allele to be on $5B^L$. The percentage recombination was $50{\cdot}65 \pm 4{\cdot}03$.

To determine the genetic length of the long arm of chromosome 5B, the numbers of chiasmata, in the heteromorphic bivalent associating the long arm telocentric and the complete chromosome, were determined at first metaphase in the hetero-

morphic heterozygotes previously discussed (Table 4). There were 182 chiasmata in $5B^L$ in 100 cells scored, so the total map length of the arm is estimated to be 91 map units under these genetic and environmental circumstances. It was not unreasonable to have observed, therefore, that the *Ph* locus, although on $5B^L$, segregated independently of the centromere. Ideally, intermediate markers were required to map the *Ph* locus, but none are available so the alternative of trisomic mapping was examined.

Table 3. *Segregation from the cross* (T. aestivum *Mutant 10/13 $5B^L$ ditelo.* × *euploid*) × S. cereale

	Pairing		
5B state	Homoeologous	No homoeologous	Total
Complete	40	46	86
Telocentric	30	38	68
Total	70	84	154

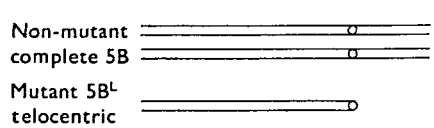
Segregation	Expected	χ^2	D.F.	*P*
No homoeol.:homoeol. pairing ...	1:1	1·27	1	0·5–0·3
Complete 5B:telocentric $5B^L$ ...	1:1	2·10	1	0·2–0·1
Linkage		0·03	1	0·9–0·8
Total	1:1:1:1	3·40	3	0·5–0·3

Table 4. *Numbers of chiasmata in the long arm of chromosome 5B in plants of* T. aestivum *derived from the cross Mutant 10/13 ditelocentric $5B^L$ × euploid*

Chiasmata in $5B^L$	No. of cells	Total chiasmata
0	2	0
1	22	22
2	68	136
3	8	24
Total	100	182

6. TRISOMIC TELOCENTRIC MAPPING

It was not possible to map the position of the *Ph* locus relative to the centromere when 5B was disomic. However, if the chromosomes were made trisomic the apparent tightening of linkage might make mapping possible. The experimental situation was achieved by making the cross *T. aestivum* ditelocentric $5B^L$ Mutant 10/13 × *T. aestivum* tetrasomic 5B. The products of the cross were 43-chromosome trisomics, with two complete 5B chromosomes and one $5B^L$ telocentric with the following structure:



These trisomics were pollinated with rye and 28-chromosome hybrids, to which only one 5B chromosome had been transmitted, were scored for the presence of the complete or the telocentric 5B and for high or low homoeologous pairing at meiosis.

In this situation, which corresponds to a trisomic testcross, the products of one crossover between the mutant locus and the centromere of one chromosome, in this case the telocentric, will give an apparent 0·22 probability of recovery of recombination products. Two crossovers will give a recombination value of 0·33, three will give 0·39 and complete independence will be at 0·44. In the disomic condition any number of chiasmata will give 0·50. These recombination figures are those that will occur when the chiasmata between the marker and the centromere are formed at random between the three long arms of 5B present. Similarly the figures assume random recovery of the trisomic meiotic products.

Table 5. *Numbers of chiasmata in the long arm of chromosome 5B in trisomic plants of* T. aestivum *derived from the cross tetrasomic 5B × Mutant 10/13 ditelocentric 5B^L*

		Chiasmata in 5B^L	
5B^L telo.	No. cells	Per association	Total
Unpaired	17	?	?
Bivalent	5	1	5
Bivalent	14	2	28
Bivalent	2	3	6
Trivalent	21	1	21
Trivalent	35	2	70
Trivalent	6	3	18
Total	100	—	148

An attempt to find the limiting recombination frequency in the trisomic was made using the total numbers of chiasmata scored in the long arm, 5B^L, in trisomic pollen mother cells. Table 5 shows that 148 chiasmata in 5B^L were scored in 100 cells; however, in 17 cells the telocentric chromosome was univalent and the two complete 5B chromosomes were paired in a bivalent, but this bivalent could not be distinguished from the other 20 bivalents. If it is assumed that the frequencies of chiasmata in these bivalents correspond to those in the heteromorphic bivalents, a total of 23·35 chiasmata is obtained for the 17 cells making an overall total of 171·35, which is similar to the 182 observed in the disomic (Table 4), as required by classical cytology.

Now, in 17 cells 5B^L telocentric was unpaired and thus could not give any recombinants. In 21 cells 5B^L was paired with a single complete 5B and, since 28-chromosome progeny were selected so that only the meiotic products from chromosomes in the bivalent would be transmitted, the situation will correspond to that of a disome, giving 0·50 recombination frequency for any number of chiasmata. The remaining 62 cells were trivalents. Those with one chiasma, 21 cells, will consist only of recombinations between the telocentric and a complete chromosome, giving a

mean recombination frequency of $0{\cdot}22 \times \frac{3}{2}$ or 0·33. Similarly the trivalents with two chiasmata in $5B^L$ will include all combinations except double crossovers between the complete chromosome, thus $0{\cdot}33 \times \frac{9}{8}$ or 0·375. Trivalents with three chiasmata will give a mean recombination value of 0·4039. On the basis of chiasma formation in the pollen mother cells, the limiting observable recombination between the centromere and the distal tip of the telocentric will be:

Telocentric at MI	Observed frequency	Mean recombination (P)	Total
Unpaired	0·17	0	0
Paired in bivalent	0·21	0·5000	0·1050
Paired in trivalent	0·21	0·3333	0·0700
	0·35	0·3750	0·1313
	0·06	0·4039	0·0242
Expected limiting recombination	—	—	0·3305

The phenotypes of 187 wheat–rye hybrids derived from the trisomics are listed in Table 6. The segregation of the non-mutant:mutant meiotic pairing pattern did not deviate from a 2:1 ratio, so that this evidence was not in conflict with that previously obtained, indicating that the *Ph* locus is on $5B^L$. In addition, the segregation of complete:telocentric 5B chromosomes did not deviate from the expected 2:1 ratio. However, there was no evidence of linked segregation of the alternative structures of 5B and the associated pairing pattern, so that this attempt to map the *Ph* locus also failed.

Table 6. *Segregation from the cross* (T. aestivum *Mutant 10/13 5B^L ditelo.* × *tetrasomic 5B*) × S. cereale

	Pairing		
5B state	Homoeologous	No homoeologous	Total
Complete	53	79	132
Telocentric	19	36	55
Total	72	115	187

Segregation	Expected	χ^2	D.F.	P
No homoeol.:homoeol. pairing ...	2:1	2·25	1	0·2–0·1
Complete 5B:telocentric $5B^L$ ...	2:1	1·29	1	0·3–0·2
Linkage		0·71	1	0·5–0·3
Total	4:2:2:1	4·25	3	0·3–0·2

A curious anomaly arose in this experiment in that, although the percentage of recombination in long arm of 5B was predicted to be 33·05, on the basis of the frequency of chiasmata, the observed percentage recombination between *Ph* and the centromere was $47{\cdot}59 \pm 3{\cdot}66$. A simple explanation of this anomaly is not possible, but it should be pointed out that chiasma frequencies were scored in pollen mother cells while recombination was observed in the products of megaspore

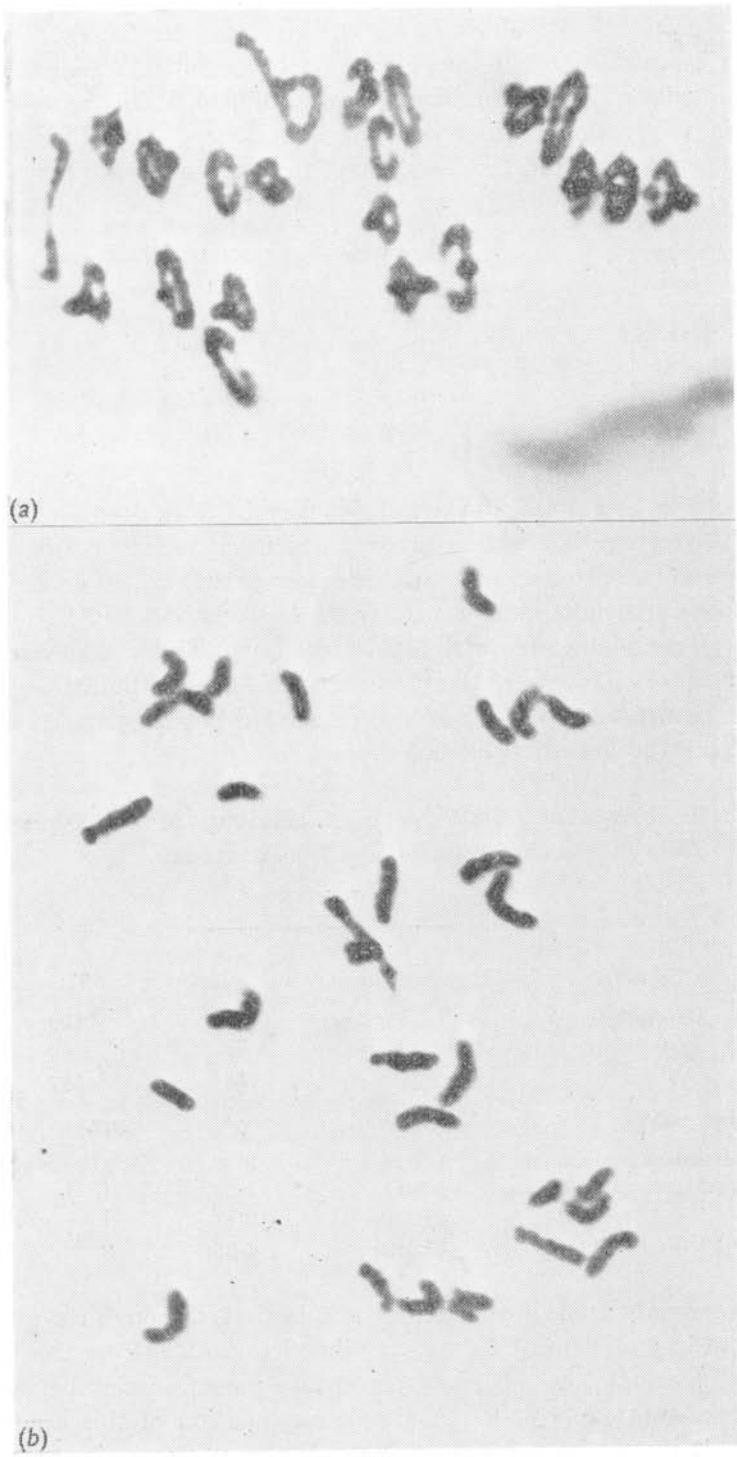


Fig. 1. For legend see facing page.

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mother cells. If recombination were lower on the male than on the female side, the discrepancy would be accounted for. Alternatively, in the estimation of the genetic length of $5B^L$ it was assumed that there was no chromatid interference. This is a reasonable assumption but if wrong the frequency of crossing over would be inflated relative to the estimate. The occurrence of chromatid interference is unlikely, so that differences in recombination frequencies on the male and female sides may well be the explanation of the excess of the observed over the estimated rates.

7. DOMINANCE RELATIONSHIPS

Since the mutant allele in Mutant 10/13 is not expressed, even when homozygous, in *T. aestivum* (Wall *et al.* 1971) and its presence is only revealed in hybrids with *S. cereale* or other diploid species, information about its dominance relationship can only be obtained from similar hybrids. Trisomics with one mutant allele and two non-mutant alleles at the *Ph* locus were obtained from the cross Mutant 10/13 × tetrasomic 5B. The trisomics were pollinated with rye and 29-chromosome hybrids that were disomic for chromosome 5B were selected.

If the mutant allele, causing homoeologous pairing, had been dominant a ratio of 2 mutant:1 non-mutant pairing would have been expected among the 29-chromosome hybrids. By contrast, if the normal allele, preventing homoeologous pairing, had been dominant all 29-chromosome hybrids would have been without homoeologous pairing, in the absence of double reduction.

Meiosis was examined in 27 hybrids with 29 chromosomes, all of them possessing one complete and one telocentric $5B^L$. All had a low level of meiotic chromosome pairing, similar to that found in the presence of a normal 5B chromosome, although all showed the single 5B bivalent (Fig. 1). From this it was deduced that the mutant allele was recessive to the normal allele present in standard plants of *T. aestivum* Chinese Spring. The normal allele was therefore designated *Pairing homoeologous* (*Ph*) and the mutant allele *pairing homoeologous* (*ph*).

These results from the 29-chromosome hybrids confirmed that the *Ph* locus is on chromosome 5B since if it had been on any other chromosome there would have been a 1:1 segregation of plants with phenotypes associated with the presence of the *Ph* or the *ph* alleles.

8. DISCUSSION

This work has demonstrated that homoeologous meiotic chromosome pairing in wheat–rye hybrids is prevented by the activity of the *Ph* locus on chromosome 5B of wheat. The evidence that the mutant *ph* allele is on chromosome 5B derives

Fig. 1. Photomicrographs of first metaphase of meiosis in (*a*) *T. aestivum* trisomic 5B ($2n = 6x = 42+1$) derived from the cross Chinese Spring ditelo – $5B^L$ Mutant 10/13 × Chinese Spring tetra – 5B. The trivalent, upper centre, consists of one long-arm telocentric and two complete 5B chromosomes. (*b*) 29-chromosome *T. aestivum* × *S. cereale* hybrid in which the wheat parent was a trisomic of the type illustrated in (*a*). There are 27 univalents and one bivalent which associates a long arm telocentric and a complete chromosome 5B.

from (i) monosomic analysis, (ii) trisomic inheritance in 28-chromosome, 5B-monosomic, wheat–rye hybrids, and (iii) the absence of segregation in 29-chromosome, 5B-disomic, wheat–rye hybrids. It is reasonable to presume that the *Ph* allele is also responsible for the restriction of meiotic pairing to fully homologous chromosomes in *T. aestivum*. This activity was first detected by Riley & Chapman (1958) and its subsequent study has depended entirely on the comparison of plants with or without the chromosome or its long arm (Riley, 1960; Riley & Law, 1965; Riley & Chapman, 1967).

The position of the locus on $5B^L$ was not determined but it is apparently distally located on this genetically long arm. The eventual determination of its precise position depends upon the availability of an intermediate marker and none are available at present.

The present evidence is not inconsistent with the notion that there are several loci on $5B^L$ all of which must be active for the prevention of homoeologous meiotic pairing. However, this requires an elaboration of hypothesis that is unnecessary to account for the observations. The simpler explanation is that the *Ph* locus is alone responsible for the normal restriction of pairing in *T. aestivum*, which gives the regularity of meiotic segregation, disomic segregation and the high fertility, that characterize this hexaploid species. The suggestion that the present activity of chromosome $5B^L$ of *T. aestivum*, affecting meiotic pairing, arose by mutation subsequent to the incorporation of the chromosome in polyploid wheat (Riley, Kimber & Chapman, 1961) is also much more acceptable if mutation occurred at a single locus.

The *ph* allele of Mutant 10/13 is apparently active since, although demonstrated to be recessive in the present trisomic segregation test, and giving a level of meiotic pairing in wheat–rye hybrids little short of that occurring when chromosome 5B is absent, it nevertheless prevents homologous–homoeologous multivalent formation when homozygous in *T. aestivum*. This suggests that some of the activity of the normal product of the locus is retained by the product of the *ph* allele. Clearly mutants with greater deviations from normality could arise and it may be that they would have certain advantages for practical breeding work and also, perhaps, in the functional analysis of the system, but the Mutant 10/13 effect has been quite adequate for the study of segregation and for attempts to be made to map the *Ph* locus.

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