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Homoeologous pairing of a chromosome from *Agropyron elongatum* with those of *Triticum aestivum* and *Aegilops speltoides*

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1. INTRODUCTION

Common wheat (*Triticum aestivum* L.) is an allohexaploid having forty-two chromosomes ($2n = 6x = 42$) each of which may be classified as belonging to one of three genomes, and one of seven homoeologous groups. The three genomes, A, B and D, each of seven pairs of chromosomes, are derived from the probable diploid ancestors *T. monococcum* (A genome), *Aegilops speltoides* (B genome) and *Ae. squarrosa* (D genome) (McFadden & Sears, 1946; Riley, Unrau & Chapman, 1958). Corresponding chromosomes within each of the three genomes perform similar genetic functions thus giving seven homoeologous groups each of three pairs of chromosomes (Sears, 1954, 1966).

Although functionally related, the homoeologous chromosomes do not normally pair with each other at meiosis, pairing being confined to strictly homologous chromosomes, giving twenty-one bivalents. Chromosome pairing in hexaploid wheat has been shown to be controlled by a genetic activity of chromosome 5B (Okamoto, 1957; Riley & Chapman, 1958). When chromosome 5B is absent pairing at meiosis occurs between homoeologous as well as homologous chromosomes (Riley, 1960; Riley & Kempanna, 1963). It has been shown by Kimber & Riley (1963) that the degree of synapsis between the chromosomes of the hybrids produced by intercrossing the diploid ancestors of hexaploid wheat is equal to that occurring between homoeologous chromosomes of hexaploid wheat in the absence of the effect of chromosome 5B. This confirms that the strictly homologous pairing in hexaploid wheat is controlled by the activity of chromosome 5B and does not arise from changes which have occurred to the structure of the chromosomes during evolution. The activity of chromosome 5B is suppressed in hybrids between hexaploid wheat and *Ae. speltoides* or *Ae. mutica* (Riley, Kimber & Chapman, 1961). The high level of synapsis occurring at meiosis in the twenty-eight chromosome hybrids between *T. aestivum* and either of these two species of *Aegilops* results from associations between homoeologous chromosomes, which includes pairing between wheat and *Aegilops* chromosomes (Riley & Chapman, 1964).

Sears (1954, 1966) demonstrated functional relationships between homoeologous chromosomes by their ability to compensate for deficiencies of chromosomes of the

same homoeologous group, but not of other groups. Recent studies on the substitution of alien chromosomes for chromosomes of hexaploid wheat have shown that alien chromosomes too may be related to particular homoeologous groups of wheat chromosomes. Thus chromosome 6R of *Secale cereale* substituted for chromosomes of wheat homoeologous group 6 only (Riley, 1964; Riley, Kimber & Law, 1964); an *Agropyron elongatum* chromosome was similarly related to homoeologous group 6 (Knott, 1958, 1964; Johnson, 1966), and an *Ae. comosa* chromosome (2M) has been substituted only for chromosomes of wheat homoeologous group 2 (Riley & Chapman, 1966*b*).

In a study of hybrids between *T. aestivum* and *Ae. longissima* it was shown that wheat and alien chromosomes could pair in the absence of chromosome 5B (Riley, Chapman and Kimber, 1959). Although none of the alien chromosomes referred to in the previous paragraph was seen to pair with wheat chromosomes in the presence of chromosome 5B, it is clear that they are very similar in overall genetic activity to their corresponding wheat chromosomes. It therefore seemed possible that wheat-alien chromosome pairing might occur if the activity of chromosome 5B was suppressed.

The following experiment was designed to study the meiotic affinities of wheat chromosomes and the *A. elongatum* chromosome when the activity of chromosome 5B was suppressed by the presence of the *Ae. speltoides* genome.

2. MATERIALS AND METHODS

(i) *Materials*

Details of the three classes of material used in production of *T. aestivum–Ae. speltoides–A. elongatum* hybrids are listed below.

(*a*) *Ditelocentric alien addition line*

This line was a derivative of *T. aestivum* L. emend. Thell. ssp. *vulgare* MacKay var. Thatcher, having twenty-one pairs of wheat chromosomes plus a pair of telocentric chromosomes of *A. elongatum* (Host) Beauv. ($2n = 70$). The *A. elongatum* telocentric chromosome carries a gene or genes for resistance to many races of *Puccinia graminis* var. *tritici*. The derivation of this line from an original cross between Chinese Spring wheat and *A. elongatum* has been described elsewhere (Knott, 1958, 1964; Johnson, 1966).

(*b*) *Chinese Spring ditelocentric lines*

These lines, developed by E. R. Sears, are derivatives of *T. aestivum* L. emend. Thell. ssp. *vulgare* MacKey var. Chinese Spring, having twenty pairs of complete wheat chromosomes, with median or sub-median centromeres, plus one pair of telocentric chromosomes. Thus each line was deficient for one chromosome arm. One ditelocentric line was used to mark each wheat chromosome.

(*c*) Aegilops speltoides *Tausch*

Plants of *Ae. speltoides* Tausch ($2n = 14$) have seven pairs of chromosomes with median or sub-median centromeres.

(ii) *Techniques*

Counts of root-tip chromosomes were made from temporary preparations using the Fuelgen staining method after treatment in mono-bromonaphthalene.

Chromosome configurations at first metaphase of meiosis were examined in permanent squashes of pollen mother cells fixed in acetic-alcohol and stained by the Fuelgen technique. The staining was supplemented by mounting the pollen mother cells in propionic orcein.

(iii) *Experimental procedure*

Twenty-one different ditelocentric lines of the variety Chinese Spring, in which each of the twenty-one pairs of chromosomes was represented in turn by being telocentric, were crossed with the line in which the *A. elongatum* chromosome was represented as a ditelocentric addition. Chromosome counts of root-tip cells of the hybrid seedlings confirmed that each plant possessed forty-three chromosomes, of which two were telocentric. One of the telocentric chromosomes was a wheat chromosome from Chinese Spring, the other was the *A. elongatum* telocentric chromosome. At meiosis in these plants there were twenty bivalents in which homologous wheat chromosomes were paired, one heteromorphic bivalent in which the wheat telocentric paired with its complete homologue and one univalent which was the *A. elongatum* telocentric chromosome. These plants were used as the female parents in crosses with *Ae. speltoides*.

Counts of chromosomes in root-tip cells of seedlings grown from the seed obtained from the crosses with *Ae. speltoides* permitted selection of plants having twenty-nine chromosomes of which two were telocentric. The chromosome complement of these twenty-nine-chromosome hybrids consisted of twenty complete wheat chromosomes, a wheat telocentric, seven *Ae. speltoides* chromosomes and the *A. elongatum* telocentric. Permanent slides of pollen mother cells at first metaphase of

Table 1. *Tabulation of the set of twenty-nine-chromosome hybrids produced, designated by the wheat chromosome represented as a telocentric*

Homoeologous group	Genome		
	A	B	D
1	*	*	*
2	*	*	*
3	*	*	–
4	–	*	*
5	*	*	*
6	*	*	*
7	*	–	*

* Hybrids produced.
– Hybrids not produced.

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meiosis were prepared from the twenty-nine-chromosome plants. Among the collection of hybrids produced all the wheat chromosomes, except 3D, 4A and 7B, were represented by telocentrics (Table 1).

3. RESULTS

(i) *Configurations involving telocentric chromosomes*

Meiotic cells were examined to determine the pairing behaviour of the telocentric chromosomes in relation to each other and to other chromosomes in the cell. The complete meiotic configuration was not evaluated in every cell examined but cells were classified into four categories:

(*a*) Both telocentrics unpaired.
(*b*) One telocentric paired, one unpaired.
(*c*) Both telocentrics paired in different configurations.
(*d*) Both telocentrics paired in the same configuration.

In cases where one telocentric only was paired (category *b*) an attempt was made to decide whether the wheat or the *A. elongatum* telocentric was involved. The *A. elongatum* telocentric was smaller than most of the wheat telocentrics and could frequently be recognized by this feature.

In cases where both telocentrics were paired in the same configuration the exact configuration was recorded in order to determine the synaptic relationship between the arms of the wheat and *A. elongatum* chromosomes represented by the telocentrics.

(ii) *Observations of behaviour of telocentric chromosomes*

The overall frequency with which the *A. elongatum* telocentric chromosome was involved in pairing was low (4·8 % of cells examined) compared with the frequencies of pairing of wheat telocentrics (51·1 % of cells examined) (Table 2). There were also differences in the frequencies of pairing of both wheat and *A. elongatum* telocentrics in the material examined. Pairing of wheat telocentrics ranged from 34·0 % to 71·3 % and pairing of the *A. elongatum* telocentric from 0·0 % to 10·8 % (Table 2). Any attempt to interpret these differences must be approached with caution since no determination was made of overall chromosome pairing in the cells examined, and the hybrids were not grown in a controlled environment. The main distinction, which is clear, is that the *A. elongatum* telocentric paired much less frequently than any of the wheat telocentrics in all hybrids.

The most critical cells observed were those in which both telocentrics were involved in pairing, but because of the low frequency of pairing of the *A. elongatum* telocentric such cells were rare (Table 2). When the wheat telocentrics were for group 6 chromosomes, there were twenty-two cases in which both telocentrics were paired, and in four of these the wheat and *A. elongatum* telocentrics were involved in the same configuration (Plate I *a*). For all other wheat telocentrics, only twenty-five cases were observed in which both telocentrics were paired and in no case were

the wheat and *A. elongatum* telocentrics involved in the same configuration (Plate I*b*). In these results, meiotic pairing was only demonstrated between the *A. elongatum* chromosome and those of group 6, which were already known to have similar genetic activities. Thus the relationships observed when homoeologous affinity was recognized at meiosis coincided with the predictions made on the basis of genetic results.

Table 2. *Frequencies of pairing of the two telocentric chromosomes in all cells examined*

		Per cent cells in which telocentrics paired			
				Both telocentrics	
Wheat telocentric	Cells examined	Wheat telocentric	*A. elongatum* telocentric	Different configurations	Same configuration
1A	100	56·0	4·0	2·0	0
1B	54	50·0	3·7	1·9	0
1D	102	51·0	3·9	2·0	0
2A	93	48·4	0	0	0
2B	102	56·9	6·9	2·9	0
2D	100	34·0	1·0	0	0
3A	100	50·0	4·0	1·0	0
3B	100	37·0	6·0	2·0	0
3D	—	—	—	—	—
4A	—	—	—	—	—
4B	100	51·0	7·0	3·0	0
4D	100	49·0	3·0	2·0	0
5A	100	50·0	5·0	1·0	0
5B	100	47·0	5·0	2·0	0
5D	100	39·0	3·0	1·0	0
6A	157	71·3	10·8	8·9	1·3
6B	137	43·1	1·5	0·7	0
6D	110	60·9	8·2	6·4	1·8
7A	—	—	—	—	—
7B	100	59·0	5·0	3·0	0
7D	100	56·0	5·0	2·0	0
Total	1855	Average 51·1	Average 4·8		

It was also observed that when the *A. elongatum* telocentric was involved in the same configuration as either 6A or 6D telocentrics, the *A. elongatum* and wheat telocentrics were in every case at opposite ends of a trivalent (Plate I*a*). Consequently the arms represented in the wheat telocentrics were not equivalent to the arm in the *A. elongatum* telocentric. This also implies that the 6A and 6D arms employed were equivalent to each other.

No case was found in which wheat telocentric 6B was involved in the same configuration as the *A. elongatum* telocentric, although a few cells were observed in which both telocentrics were close together but not paired. The rates of pairing of the telocentrics for 6B and the *A. elongatum* chromosome were rather low in the cells examined, and only one cell was seen in which both telocentrics had paired; in this case they were in different configurations (Table 2). However, since telocentric 6B was involved in pairing it must have been with other group 6 chromosomes, so that it is likely that the *A. elongatum* telocentric could be found in the same configuration if sufficient cells were examined.

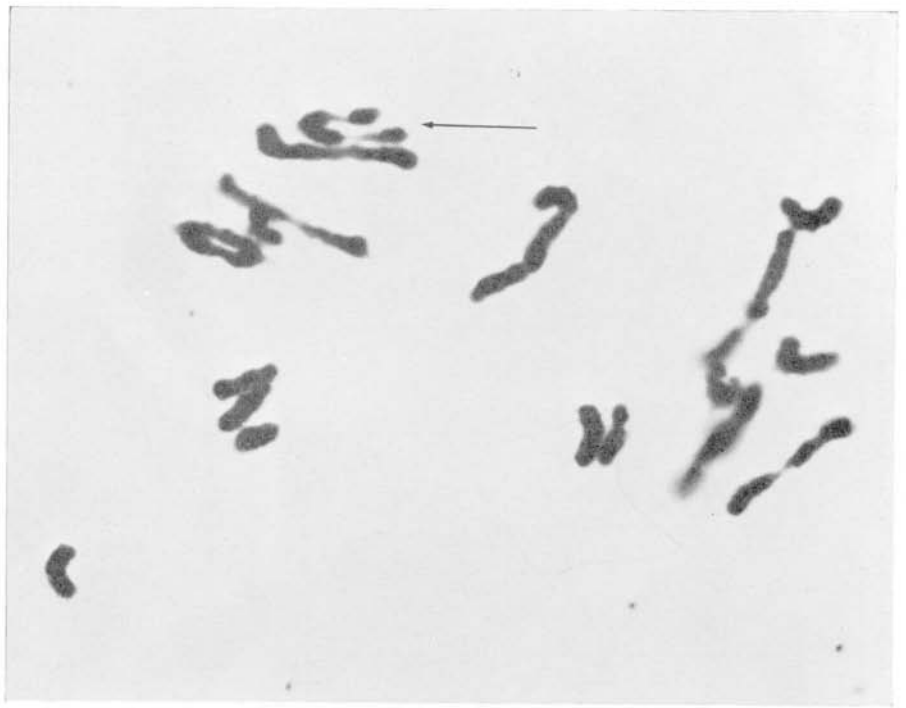
A total of 404 cells was examined in which the wheat telocentric was for a group 6 chromosome, and in twenty-two of these both telocentrics were involved in pairing. A total of 1451 cells were examined in which the wheat telocentric was not of group 6, and in twenty-five of these cells both telocentrics were paired. With the reservation that the hybrids were not grown under controlled conditions, there is an indication that the rate of pairing of the telocentrics was higher when the wheat telocentrics were for group 6 chromosomes, particularly 6A or 6D, than when they were for other chromosomes. Since the *A. elongatum* telocentric and telocentrics 6A and 6D were shown to be for non-homoeologous arms, the hybrids having telocentrics 6A or 6D would have the missing wheat chromosome arm compensated by the *A. elongatum* telocentric. All other hybrids lacked one arm of a wheat chromosome, for which there was no compensation. This may account for the more frequent pairing of telocentrics which was observed when 6A and 6D were present as telocentrics.

4. DISCUSSION

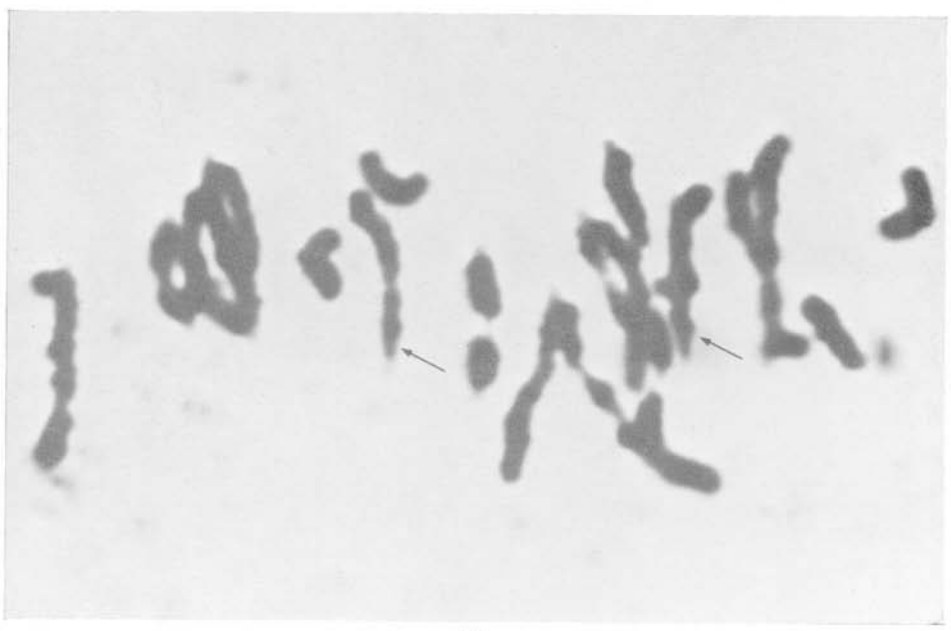
(i) *Pairing and genetic equivalence*

Riley & Chapman (1966*a*) discussed the degree of correspondence between differential affinity, measured by the frequency of chromosome pairing, and genetic equivalence, as indicated by nullisomic-tetrasomic compensation experiments in *T. aestivum*. They showed that in homoeologous group 5, pairing affinity was greater between 5B and 5D than between 5A and either 5B or 5D, though this was not reflected in differential genetic equivalence as indicated by nullisomic-tetrasomic combinations within homoeologous group 5. It was postulated that tests of differential affinity could be a more sensitive indicator of homoeology than nullisomic-tetrasomic compensation tests, or alternatively that the compensation and affinity tests measured different aspects of chromosome relationships.

There are some risks in making comparisons between pairing experiments and compensation tests. Thus, in observations on pairing, the behaviour of telocentric chromosomes is studied, and it is not known whether the rate of pairing of telocentrics is the same as for the corresponding complete chromosomes. The hybrids in which observations on pairing are made often have chromosome deficiencies, and are either haploids or F_1 hybrids between hexaploids and diploids. On the other hand in tests for genetic compensation the plants usually differ from hexaploid



(*a*)



(*b*)

Photographs of two cells from twenty-nine-chromosome hybrids at first metaphase of meiosis.

(*a*) The *A. elongatum* telocentric and wheat telocentric 6A pairing in the same configuration at opposite ends of a trivalent. The trivalent is marked with an arrow.

(*b*) The *A. elongatum* telocentric and wheat telocentric 5A pairing in different configurations in two heteromorphic pairs marked with arrows.

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wheat only in the replacement of one chromosome by another. The chromosomes acting as substitutes may either come from within a wheat genome, or be from other species as in the case of alien substitution. In either case it is usual to assess the ability of complete chromosomes to act as substitutes for the missing chromosomes. However the criteria by which genetic compensation is assessed are often quite superficial, depending on visual comparisons between phenotypes. Since these criticisms apply equally to experiments involving alien chromosomes and those involving only chromosomes within the hexaploid wheat genome it is reasonable to compare the results using alien chromosomes with those involving only wheat chromosomes.

In the experiment described in this paper it was clear that the *A. elongatum* telocentric paired much less frequently (4·8% average) than wheat telocentrics (51·1% average). On the other hand the genetic compensation provided by the whole *A. elongatum* chromosome when acting as a substitute for 6A or 6D has been shown to be remarkably good. When the grain yield and flour quality of the variety Thatcher were compared with substitution lines in which chromosome 6A was replaced by the *A. elongatum* chromosome, some of these lines were equal to Thatcher. Also, pollen in which chromosome 6A was replaced by the *A. elongatum* chromosome was competitively equal in fertilizing ability to euploid wheat pollen (Knott, 1964). Thus, in this case the genetic equivalence of the wheat and *A. elongatum* chromosomes appears to be greater than their pairing affinity would indicate, favouring the view that pairing affinity is not necessarily directly related to overall genetic equivalence. However, comparison of these results with similar experiments with chromosomes of *Secale cereale* reveals another aspect of the problem. Riley (1964) reported that rye chromosome 6R could substitute for chromosomes of wheat homoeologous group 6 but that the fertility of substitution plants was low. In an experiment in which a telocentric for rye chromosome 6R was present in *T. aestivum–Ae. speltoides* hybrids similar to those used in the experiment described above, the rye telocentric was never seen to pair with any other chromosome (Riley & Kimber, 1966). In this case the poorer genetic compensation of the rye chromosome and its failure to pair with wheat chromosomes, compared with the better compensation and pairing of the *A. elongatum* chromosome, apparently indicates some parallel between overall genetic equivalence and pairing affinity.

There need be no conflict between the results of these two similar experiments if it is assumed that pairing affinitites and genetic equivalence are two different aspects of the relationships between chromosomes. The evolution of these two different aspects may occur at different rates, as would appear to be the case for the *A. elongatum* chromosome and its related wheat chromosomes, where pairing affinities have diverged further than the factors controlling genetic equivalence. This situation could develop if pairing were to depend on contact between a limited number of specific chromosome regions (Riley & Chapman, 1966*a*). It could be expected that gradual changes in a number of these regions would be more easily tolerated by a diploid organism than any changes affecting unique and important

physiological processes. It is therefore not surprising to find that where genetic equivalence is obviously reduced, as in the case of the rye chromosome, pairing affinities have also diverged even further.

SUMMARY

1. Complex hybrids were produced having twenty-nine chromosomes, consisting of one telocentric and twenty complete chromosomes of *T. aestivum* ($2n = 6x = 42$), seven complete chromosomes of *Ae. speltoides* ($2n = 2x = 14$) and one telocentric chromosome derived from *A. elongatum* ($2n = 10x = 70$). The presence of the *Ae. speltoides* genome permitted pairing between homoeologous chromosomes at meiosis and the behaviour of the two telocentric chromosomes was observed.

2. The *A. elongatum* chromosome was seen to pair with chromosomes homoeologous to those of group 6. There was no evidence that it paired with chromosomes of any other group.

3. When the *A. elongatum* telocentric and those of 6A and 6D occurred in the same configuration it was evident that the telocentrics 6A and 6D were for corresponding chromosome arms, and the *A. elongatum* telocentric for the opposite arm.

4. The average rate of pairing was much lower for the *A. elongatum* telocentric than for wheat telocentrics. Previous studies had indicated very good genetic compensation of the *A. elongatum* chromosome for chromosomes 6A and 6D. It was therefore indicated that genetic equivalence and pairing affinity were not closely related in this case. Some implications of this are discussed.

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