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Equivalence of the A genome of bread wheat and that of *Triticum urartu*

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SUMMARY

Lines of *Triticum aestivum* Chinese Spring ($2n = 6x = 42$) which were ditelocentric or doubly ditelocentric, in turn, for the 14 chromosomes of the A and B genomes were pollinated by *Triticum urartu* ($2n = 14$). The behaviour of the marked telocentric chromosomes was scored in the 14 distinct hybrids obtained from these pollinations. In 6 of the hybrids in which different A genome chromosomes were marked by telocentrics there were from 50 to 80% of the pollen mother cells in which the telocentrics were paired. In the seven hybrids in which different B genome chromosomes were marked the telocentrics were never paired. It was concluded that the genome of *T. urartu* matched very closely the A genome of hexaploid wheat and that it did not correspond, as had been proposed by Johnson, to the B genome. The pairing behaviour of the 14 *T. aestivum* × *T. urartu* hybrids was compared with earlier results obtained from hybrids between *T. aestivum* and *T. boeoticum*. It was proposed that the higher trivalent frequencies seen in the *T. boeoticum* hybrids could be due to homoeologous pairing and that the genotype of *T. boeoticum* has the capacity partly to suppress the activity of the *Ph* locus of chromosome 5B of wheat, as a result of which homoeologous pairing is normally prevented.

1. INTRODUCTION

Knowledge of the course of the evolution of the bread wheat of commerce (*Triticum aestivum* $2n = 6x = 42$) to its present cytogenetic structure is not only of intrinsic interest, it is also necessary for the determination of the kinds of genetic manipulation that may be employed in practical breeding programmes. An important part of the evolutionary advance in *Triticum* has been the change first from diploidy to tetraploidy and subsequently to hexaploidy. The genome structures at the three levels of polyploidy are indicated by the genome symbols:

diploid ($2n = 2x = 14$)	AA
tetraploid ($2n = 4x = 28$)	AABB
hexaploid ($2n = 6x = 42$)	AABBDD

In *Triticum* a genome is a set of seven chromosomes either representing the haploid complement of distinct diploid species or acquired, in duplicate, at each advance in polyploidy.

The origins of the genomes of hexaploid wheat have recently been discussed extensively (see Riley, 1965; Kimber, 1973, 1974; Johnson, 1975) and a review is

unnecessary in this paper. It is sufficient to point out that the evidence is strong that the A genome was contributed by a diploid form of *Triticum* such as *T. boeoticum*, and that the D genome was derived from *Aegilops squarrosa*.

However uncertainty still surrounds the source of the B genome. Certain evidence has been adduced in favour of the notion that the B genome was contributed by *Aegilops speltoides* (for summary see Riley, 1965). Subsequent evidence was collected which was interpreted as implying that the B set of chromosomes could not have been derived from *Ae. speltoides* but no alternative source was suggested (see Kimber, 1974).

However, more recently Johnson (1975) has claimed that the B genome was contributed to tetraploid and hexaploid wheat by *Triticum urartu* ($2n = 14$), a diploid form said to be widely distributed in several Middle East countries. This claim was based on the comparisons of the banding patterns obtained following the electrophoretic separation of albumin proteins extracted from seeds. Johnson pointed out that there were apparently several protein bands in extracted albumins of the two tetraploid species *T. dicoccoides* and *T. araraticum* which were also present in the albumin of *T. urartu* but not in that of *T. boeoticum* the presumptive A genome donor. He concluded that the distinctive proteins present in the tetraploids, but not in *T. boeoticum*, must be produced by the activities of genes contributed by the B genome donor and thus that *T. urartu* could be the source of the B genome.

While electrophoretic evidence can be very effective at demonstrating the absence of a phylogenetic relationship it is less dependable at showing phylogenetic proximity unequivocally. Consequently it is necessary to test the validity of Johnson's proposal by other methods. This paper reports work showing that the chromosomes of *T. urartu* correspond to those of the A genome of *T. aestivum* and not to the B genome.

2. PLANT MATERIALS

The aim of this work was to observe the relative frequencies with which A or B genome chromosomes pair at meiosis in *T. aestivum* × *T. urartu* hybrids, using hybrids in which specific chromosomes were marked by being telocentric.

The form of *Triticum urartu* Tum. used throughout was obtained from the N. I. Vavilov Institute for Plant Industry, Leningrad, U.S.S.R., under the Leningrad designation K33870. This form has all the distinguishing characters ascribed to *T. urartu* by Johnson (1975) except that it has white and not red coloured grains. Disc electrophoresis of glutenin proteins carried out by our colleague Mr J. B. Smith, revealed *T. urartu* to have the band pattern described by Johnson (1975).

The bread wheat lines used were all in the species *Triticum aestivum* ssp. *vulgare* variety Chinese Spring. Lines of Chinese Spring were used in which, in turn, all of the chromosomes of the A and B genomes were marked by being doubly ditelocentric. These lines were produced by Dr E. R. Sears, University of Missouri, U.S.A., and were kindly supplied by him. The cytogenetic structure of these lines is that they possess 44 chromosomes of which 20 pairs are in the normal condition with median or sub-median centromeres while one chromosome pair is represented by four

telocentrics. Each arm of this chromosome is represented by two telocentrics. Thus, except for the duplication of the centromere region the structurally marked chromosome was present in normal dosage. Such doubly telocentric lines were used where the structurally modified chromosome was either 2*A*, 3*A*, 4*A*, 5*A*, 7*A*, 1*B*, 2*B*, 3*B*, 4*B*, 5*B*, 6*B* or 7*B*.

The lines in which chromosomes 1*A* and 6*A* were marked were ditelocentric for only one arm of the chromosome, the other arm being entirely deficient. For 1*A* the telocentric for the long arm was present, for 6*A* the telocentric for the short arm was present.

Provision was made for some comparisons in the current experiments between the behaviour of marked telocentric chromosomes in *T. aestivum* × *T. urartu* hybrids and in *T. aestivum* × *T. boeoticum* hybrids. Lines of Chinese Spring doubly ditelocentric for chromosomes 2*A*, 3*A* and 5*A* were pollinated with *T. boeoticum* Boiss. ($2n = 14$). The products of these pollinations were handled in a way similar to that used with the hybrids derived from *T. urartu* pollinations.

3. METHODS

The Chinese Spring telocentric lines were pollinated with *T. urartu*. The grains that developed as a result of these pollinations were removed 16 days after the pollination. The embryos were excised from the grains and cultured on standard nutrient medium, and when the resulting juvenile hybrid plants were large enough to handle they were transferred to soil in pots and grown until maturity in a controlled environment cabinet at 20 °C with continuous light.

Anthers from the 29- or 28-chromosome hybrids were fixed in 3:1 absolute alcohol:glacial acetic acid and stained by the Feulgen procedure. Permanent slides were prepared from anthers with pollen mother cells at first metaphase of meiosis from every hybrid. The slides were examined for the participation of the telocentric chromosomes in pairing associations: that is in bivalents or trivalents. So that the results could be expressed quantitatively, 30 first metaphase cells from a hybrid of each type were scored for the number of bivalents and trivalents present and for the involvement of the telocentric chromosomes (Tables 1 and 3).

4. RESULTS

Hybridization between *T. aestivum* and *T. urartu* was achieved without difficulty. Altogether 25 ears of the Chinese Spring lines were pollinated with the pollen of *T. urartu*. From these 216 embryos were taken into culture and 176 hybrid plants established. Hybrids were established in which, in turn, every chromosome of the A and B genomes of *T. aestivum* was marked by the telocentric condition. However, in the case of hybrids in which chromosomes 1*A* and 6*A* were marked there was only one telocentric present. By contrast in the other 12 combinations both arms of the marked chromosome were simultaneously telocentric.

Examination of first metaphase of meiosis in the hybrids showed that telocentrics were never present in a bivalent or trivalent when a chromosome in the B genome

was marked by the telocentric condition (Table 1). By contrast telocentrics which marked A genome chromosomes were very frequently in bivalents and trivalents (Plate 1(i)). This is overwhelmingly strong evidence that the chromosomes of *T. urartu* correspond closely with the chromosomes of the A genome of *T. aestivum* and that they have no close affinity with the chromosomes of the B genome.

Table 1. *Chromosome pairing at first metaphase of meiosis in 29-chromosome* T. aestivum × T. urartu *hybrids in which, in turn, every chromosome of the* T. aestivum *A and B genomes was marked by having both arms telocentric (30 cells per hybrid)*

Chromosome telocentric	Mean pairing (range in brackets)			% cells with telocentrics paired in		
	Univ.	Biv.	Triv.	Biv.	Triv.	Total
1*A**	17·27 (16–22)	5·37 (3–6)	0	83	0	83
2*A*	18·17 (15–23)	4·87 (3–7)	0·37 (0–1)	43	37	80
3*A*	18·30 (16–22)	4·60 (2–6)	0·50 (0–1)	37	50	87
4*A*	18·07 (15–23)	5·47 (3–7)	0	0	0	0
5*A*	20·20 (16–25)	4·20 (2–6)	0·13 (0–1)	50	13	63
6*A**	19·00 (16–24)	4·50 (2–6)	0	50	0	50
7*A*	17·93 (16–21)	4·83 (3–6)	0·47 (0–1)	37	47	84
1*B*	19·13 (17–23)	4·93 (3–6)	0	0	0	0
2*B*	19·20 (15–21)	4·90 (4–7)	0	0	0	0
3*B*	20·33 (17–25)	4·33 (2–6)	0	0	0	0
4*B*	18·67 (17–21)	5·17 (4–6)	0	0	0	0
5*B*	18·80 (15–21)	5·10 (4–7)	0	0	0	0
6*B*	18·53 (15–23)	5·23 (3–7)	0	0	0	0
7*B*	19·13 (17–21)	4·93 (4–6)	0	0	0	0

* 28-chromosome hybrids with only one arm of the marked chromosome telocentric.

Chapman & Riley (1966) had earlier carried out a test analogous to this on the relationships of the chromosomes of the form of *T. boeoticum* known as *T. thaoudar* (Reut.) Schiem. to those of the A and B genomes of Chinese Spring. In this test however the marked chromosome was represented by a single telocentric, so that

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one arm was entirely deficient. These earlier results are shown in Table 2. From this it will be seen that there was an equally clear contrast in the frequency of involvement in pairing of A genome and B genome chromosomes. This implies that the pairing affinities of the chromosomes of *T. urartu* or *T. boeoticum* with the chromosomes of *T. aestivum* are essentially similar. This is further confirmed by the pattern of pairing at first metaphase in *T. aestivum* × *T. boeoticum* hybrids with either chromosome 2*A*, 3*A* or 5*A* marked by being telocentric for both arms (Table 3, Plate 1 (ii)). Pairing in these hybrids was comparable with that in the corresponding *T. aestivum* × *T. urartu* hybrids (Table 1).

Table 2. *Chromosome pairing at first metaphase of meiosis in 28-chromosome* T. aestivum × T. boeoticum *hybrids in which, in turn, every chromosome of the* T. aestivum *A and B genomes was marked by being telocentric for one and deficient for the other arm (50 cells per hybrid) (after Chapman & Riley, 1966)*

Chromosome telocentric	Mean pairing (range in brackets)			% cells with telocentrics paired in		
	Univ.	Biv.	Triv.	Biv.	Triv.	Total
1*A*	17·62 (13–24)	5·04 (2–7)	0·10 (0–1)	64	2	66
2*A*	17·80 (13–24)	4·80 (2–7)	0·20 (0–1)	62	8	70
3*A*	16·38 (12–21)	5·30 (2–8)	0·34 (0–2)	50	8	58
4*A*	19·48 (14–23)	4·20 (1–7)	0·04 (0–1)	48	0	48
5*A*	20·06 (14–24)	3·82 (2–7)	0·10 (0–2)	66	0	66
6*A*	18·90 (14–22)	4·36 (3–7)	0·10* (0–1)	52	2	54
7*A*	18·18 (14–23)	4·70 (1–7)	0·14 (0–1)	60	0	60
1*B*	16·26 (10–20)	5·24 (3–9)	0·42 (0–2)	0	0	0
2*B*	19·22 (14–24)	4·30 (1–7)	0·06 (0–1)	2	0	2
3*B*	16·80 (13–22)	5·30 (3–7)	0·20 (0–2)	2	0	2
4*B*	19·68 (16–24)	4·10 (2–6)	0·04 (0–1)	0	0	0
5*B*	21·46 (18–26)	3·06 (1–5)	0·14 (0–1)	2	0	2
6*B*	22·18 (16–26)	3·88 (1–6)	0·02 (0–1)	0	0	0
7*B*	18·58 (13–23)	4·56 (2–7)	0·10 (0–1)	0	0	0

* 0·02 quadrivalents.

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There is one anomalous result among those recorded in Table 1. This relates to the *T. aestivum* × *T. urartu* hybrid in which supposedly both arms of chromosome 4*A* were present in the telocentric condition. Three hybrids of this combination were available. All had 29 chromosomes which included two telocentrics but the telocentrics were never involved either in a bivalent or a trivalent. It seems likely that this behaviour arose as a result of an error in the maintenance of stocks and that the parent used, which should have been doubly ditelocentric for chromosome 4*A*, was in reality telocentric for a chromosome of either the B or the D genome.

Table 3. *Chromosome pairing at first metaphase of meiosis in 29-chromosome* T. aestivum × T. boeoticum *hybrids in which, in turn, chromosomes 2A, 3A and 5A were marked by having both arms telocentric (30 cells per hybrid)*

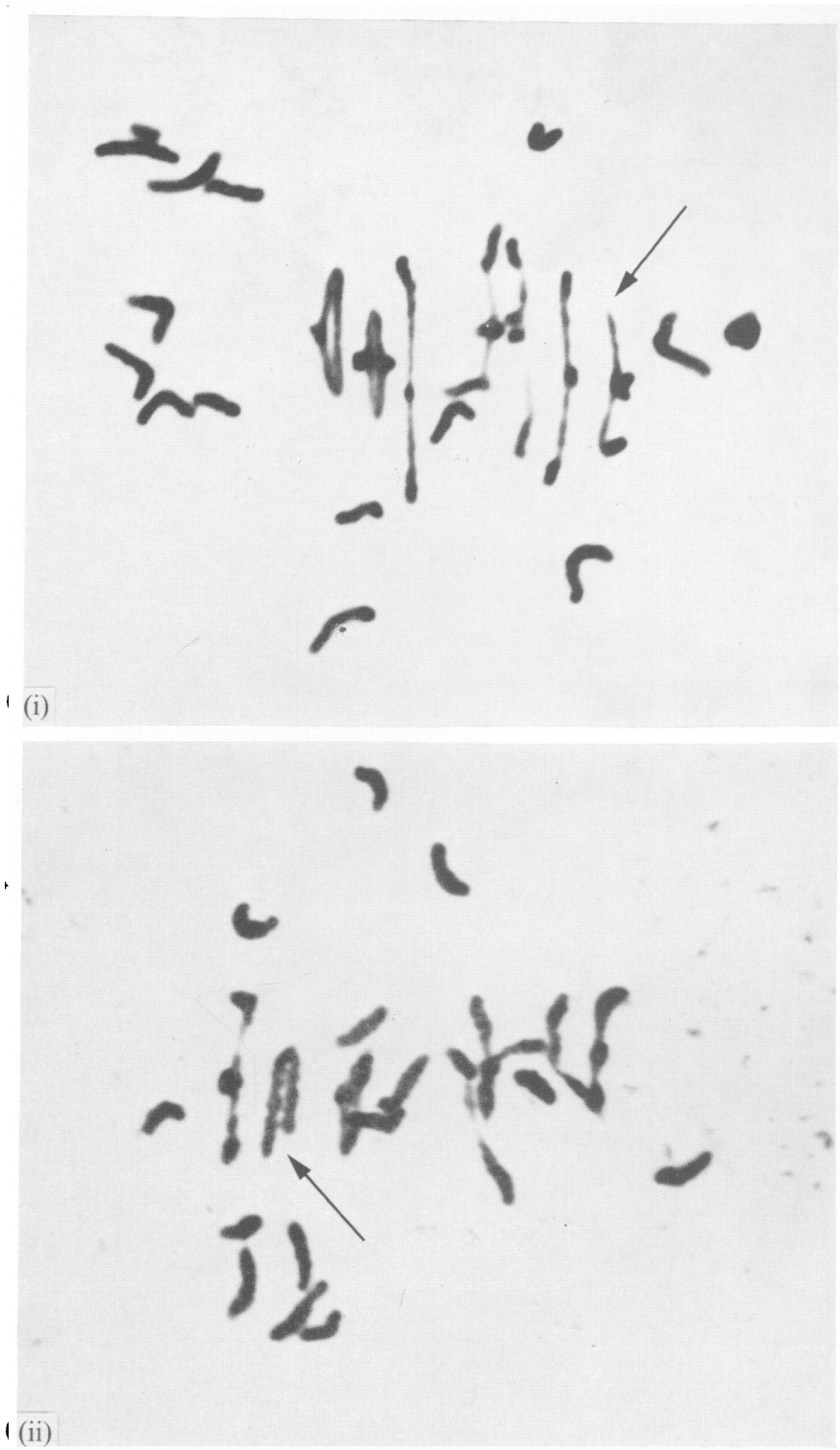
Chromosome telocentric	Mean pairing (range in brackets)			% cells with telocentrics paired in		
	Univ.	Biv.	Triv.	Biv.	Triv.	Total
2*A*	17·67 (13–21)	4·47 (3–6)	0·80 (0–3)	30	57	87
3*A*	19·13 (18–23)	4·53 (3–7)	0·27 (0–1)	50	17	67
5*A*	19·33 (14–25)	4·63 (2–6)	0·13 (0–1)	87	3	90

5. DISCUSSION

Genomes

It is clear that *T. urartu* is not the source of the B genome of the polyploid wheats. It may, however, more nearly match the A genome of the polyploids than any other diploid *Triticum*. This would account for the possession of apparently similar species of albumin molecules in *T. urartu* and in *T. dicoccoides* and *T. araraticum* as displayed by Johnson (1975). Indeed on the basis of this evidence it appears that the B genome donor should be sought among diploid species with albumin proteins that migrate under Johnson's conditions to the region between 6·0 and 8·5 cm from the origin, since neither *T. boeoticum* nor *T. urartu* display bands in this area according to figure 4 of Johnson's (1975) paper but they are present in the tetraploid species.

It is a considerable time since Sarkar & Stebbins (1956) and Riley, Unrau & Chapman (1958) suggested that the evidence then available could be interpreted to mean that the B genome of wheat was derived from *Ae. speltoides*. Subsequently evidence has been adduced in support of, Rees & Walters (1965), or against, Kimber & Athwal (1972), the conclusion that *Ae. speltoides* could have been involved. This issue is still unresolved but it may be noted that while occasional items of positive evidence support the notion of the participation of *Ae. speltoides* there is no positive evidence of an alternative B genome parent. Indeed recent evidence shows that the large subunit of fraction I protein, which is determined by chloroplast DNA and therefore inherited maternally, is similar in *T. aestivum* to that in *Ae. speltoides* but not to that in any other related diploid species thus far examined (Chen, Gray & Wildman, 1975).



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Homoeologous chromosome pairing induced by T. boeoticum

In *T. aestivum* × *T. urartu* hybrids the only trivalents formed were those in which two telocentrics participated (Table 1). They therefore resulted from pairing between fully homologous A genome chromosomes one of which was represented by two telocentric fractions.

By contrast in *T. aestivum* × *T. boeoticum* hybrids there were trivalents which did not include the telocentric chromosomes (Table 3). Moreover there were trivalents in *T. aestivum* × *T. boeoticum* hybrids in which the marked A genome chromosomes were represented by only one telocentric (Table 2). Some of these trivalents also did not include the marked chromosome. When these trivalents were first observed, Chapman & Riley (1966) concluded that they were indicative of interchange heterozygosity. Moreover since trivalents, rather than quadrivalents, were formed it was also concluded that the interchanges must have been between A and B genome chromosomes of Chinese Spring rather than within the A genome. Separate evidence showed the D genome of Chinese Spring to be unmodified structurally (Riley & Chapman, 1960). So the trivalents were thought to involve an A genome chromosome from *T. boeoticum* and an *A-B* and a *B-A* chromosome from Chinese Spring.

However, the interchange hypothesis is shown to be incorrect by the absence of non-telocentric containing trivalents in *T. aestivum* × *T. urartu* hybrids. Such hybrids would be heterozygous for the *A-B* interchanges just like the *T. aestivum* × *T. boeoticum* hybrids if the interchanges were present in Chinese Spring.

In addition to the differences in trivalent formation between the *T. boeoticum* and the *T. urartu* hybrids, two other differences must also be explained. First, in the *T. boeoticum* hybrids telocentrics marking B genome chromosomes were occasionally observed in bivalents, but this did not occur in *T. urartu* hybrids. Secondly in the Chinese Spring × *T. boeoticum* hybrid with chromosome 6*A* telocentric a quadrivalent was observed (Table 2). It seems possible from these differences that the genotype of *T. boeoticum* may be able, in part, to over-ride the normal suppression of homoeologous meiotic chromosome pairing that is caused in *T. aestivum* principally by the activity of the *Ph* locus on chromosome 5*B* (see Riley, 1974). If the genotype of *T. urartu* lacked this capacity, the contrast in meiotic pairing between the two sets of hybrids would be explained.

According to this hypothesis the extra trivalents in the *T. boeoticum* hybrids would be the result of homoeologous association. Moreover, since there were four representatives of every homoeologous group in the hybrids, occasional quadrivalents could be expected. Additionally, all chromosomes would be expected to display some homoeologous pairing, so it could be anticipated that B genome telocentrics might pair with either A or D genome chromosomes.

PLATE 1

First metaphase of meiosis in 29-chromosome hybrids. (i) *T. aestivum* × *T. urartu* with 2 telocentrics marking chromosome 5*A*. There are 7 bivalents, one of which (arrowed) incorporates chromosome 5*A*, and 15 univalents; (ii) *T. aestivum* × *T. boeoticum* with 2 telocentrics marking chromosome 2*A*. There are 3 trivalents, one of which (arrowed) incorporates both 2*A* telocentrics, 3 bivalents and 14 univalents.

If this explanation is correct, genetic variation affecting homoeologous pairing has been recognized in diploid *Triticum* that is analogous to differences reported within some *Aegilops* species (Mello-Sampayo, 1971; Dover & Riley, 1972; Dvorak, 1972; Larsen & Kimber, 1973). Of course the present work does not permit the assertion that the different effects on chromosome pairing of one form of *T. urartu* and one of *T. boeoticum* will be constant for all genotypes of these taxa. There is need to broaden the evidence by the inspection of more *T. aestivum* × diploid *Triticum* hybrids. Moreover the further investigation that is now indicated will attempt to assess whether the A genome incorporated in polyploid *Triticum* exercises an effect on the control of meiotic pairing which is comparable with that of the *T. urartu* or *T. boeoticum* forms used in the present work.

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