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A Reassessment of the Course of Evolution of Wheat

(speciation/synapsis/hybrids/polyploid/chromosomes)

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ABSTRACT **Chromosome pairing in hybrids involving *Triticum aestivum* and new accessions of *T. speltoides*, and in an amphiploid of these species, indicates that *T. speltoides* can no longer be considered to be the donor of the B genome of the polyploid wheats. This necessitates a reconsideration of the genome relationships and evolutionary processes that gave rise to cultivated wheats.**

The evolutionary processes and the species involved in the origin of wheat (*Triticum aestivum*, L.; 2n = 6x = 42) have been the subject of intensive study for several decades. It is now well accepted that a representative of the diploid wheats contributed the A genome that is found in both the tetraploid and hexaploid species (1). The D genome, found only in the hexaploid and not in the tetraploid species, was donated by *T. tauschii* (*Aegilops squarrosa*) (2–4).

The donation of the B genome to the tetraploid wheats, from which it was contributed to the hexaploid forms, has been variously ascribed to *Agropyron triticeum* (3), *T. bicorne* (*Ae. bicornis*) (5), and *T. speltoides* (*Ae. speltoides*) (6–8). In recent years *T. speltoides* has come to be widely accepted as the source of the B-genome. A conclusive test of this hypothesis through a demonstration of chromosome homology or nonhomology has unfortunately not been possible, because *T. speltoides* suppresses the regulatory activity of wheat chromosome 5B and thereby permits pairing not only of homologues but also of homoeologues (related chromosomes).

The general acceptance of *T. speltoides* as the B-genome donor was based on four kinds of evidence: Morphological evidence adduced by Sarkar and Stebbins (7) and karyotypic, synaptic, and geographical evidence gathered by Riley, Unrau, and Chapman (8). Sears (9) has indicated that some of the evidence may not be as conclusive as was earlier assumed. For one thing, a synthetic amphiploid of *T. speltoides* x *T. monococcum* does not resemble tetraploid wheat very closely (5). Also, the karyotypic evidence supporting *T. speltoides* (8) is based on the close similarity of two pairs of large-satellited chromosomes in *T. speltoides* with two in the polyploid wheats. However, the difference between *T. speltoides* and other related and excluded forms is only the absence of a very small piece of chromatin in the distal region of the satellite on one of the pairs of chromosomes. Waines and Kimber (unpublished) have established that there is variation in the satellite condition found in *T. monococcum*, and thus, it is possible that similar variation may exist in relatives of *T. speltoides*.

The synaptic evidence has also been questioned (9). Kimber (10) crossed *T. speltoides* x *T. longissimum* (*Ae. sharonensis*) and backcrossed twice to *T. speltoides*, selecting against the special ability of *T. speltoides* to cause homoeologous pairing. He then crossed to *T. aestivum* and found that in the low-pairing segregates, only 2.9 bivalents per cell were formed. As Sears (9) points out, this extent of pairing is lower than would be expected if *T. speltoides* were the donor of the B genome, for about 87% of the chromosome complement of the *speltoides*-like parent must actually have come from *T. speltoides*.

Riley and Chapman (11), after a quantitative study of the pairing affinities of the long arms of the chromosomes of homoeologous group 5, stated that chromosomes 5B of *T. aestivum* and the corresponding chromosome (5S) of *T. speltoides* ". . .would show little or no meiotic pairing were the 5B activity not suppressed" (i.e., if only homologous pairing would occur). Generalizing from 5B and 5S to the entire B and *speltoides* genomes leads to the conclusion that, since there would be no synapsis, *T. speltoides* could not be the donor of the B genome.

Recent studies (12) of the electrophoretic banding pattern of seed proteins do not support *T. speltoides* as the donor of the B genome. Thus, the geographical distribution of *T. speltoides* is the only remaining unquestioned piece of evidence contributing to its acceptance as the B-genome donor.

Because of the unique chromosome-pairing patterns produced in hybrids with *T. speltoides*, it is not possible to investigate its synaptic relationships satisfactorily. However, Dover and Riley (13) have found variation in the mechanism affecting pairing affinity in hybrids of *T. aestivum* x *T. tripsacoides* (*Ae. mutica*), and they speculate that if similar variation exists in *T. speltoides* this would add weight to the evidence that this species is the donor of the B genome. In this contribution such variation is described, and from this and other evidence it is concluded that it is improbable that *T. speltoides*, or any of its currently known relatives, could be the donor of the B genome.

MATERIALS

The availability of new collections of *T. speltoides* has allowed a greater range of variation to be investigated than with the previously examined accessions. In particular, eleven new accessions from the University of California Riverside Collection given to G. K. by Dr. J. G. Waines have been crossed with *Triticum aestivum* 'Chinese Spring' and examined cytologically.

The morphology of these accessions all corresponded to *T. speltoides* and provided no evidence of introgression from *T. longissimum* or *T. bicorne*. In addition, they all had two

pairs of chromosomes with large satellites in their somatic cells, in common with all other accessions of *T. speltoides* reported so far.

An amphiploid between *T. aestivum* and *T. speltoides*, which was made by R. S. A. was also examined cytologically. This amphiploid was produced from an F_1 hybrid between *T. speltoides* and *T. aestivum* 'Chinese Spring', in which chromosome 5B was represented by a pair of telocentric chromosomes for the long arm and to which had been added, monosomically, chromosome A of *T. umbellulatum* (*Ae. umbellulata*). The F_1 did not contain chromosome A of *T. umbellulatum*, which is normally transmitted to only 25% of the female gametes, but, of course, did carry a telocentric chromosome 5BL. This 28-chromosome plant was treated with colchicine, and a 54-chromosome amphiploid was derived in which 26 chromosomes, including the telocentric 5BL, were present disomically and two chromosomes were present monosomically. Thus, the amphiploid contained at least one representative of all the chromosomes in the F_1 hybrid. The *T. speltoides* used in this cross had previously been crossed with *T. longissimum* (*Ae. sharonensis*), but had been backcrossed with *T. speltoides* twice and self-pollinated twice. In a total of over twenty other crosses with this line that had been examined cytologically, the chromosome pairing was always recognized by the high frequency of multivalent formation previously thought characteristic of hybrids with *T. speltoides*. It is assumed, therefore, that the differences in the cytological behavior of this hybrid and its amphiploid, to be described in this paper, are the result of a mutation or deletion of the well-documented genetic system of *T. speltoides* epistatic to that located on the long arm of chromosome 5B of *T. aestivum*.

RESULTS

The 11 F_1 hybrids between *T. aestivum* and the accessions of *T. speltoides* from the U. of California collection could be divided into three groups on the basis of their cytological behavior. There was one group of seven accessions (G412, G712, G834, G1039, G1080, G1089, and G1272) in which meiosis was characterized by a high degree of synapsis and the presence of many trivalents and quadrivalents (Fig. 1*A* and Table 1). A second group, with but a single representative (G366), had an intermediate pattern where multivalents were rare and an average of 5.2 bivalents per cell (all rod) was found (Fig. 1*B* and Table 1). The third group (G1064, G1167, and G1316) had very little chromosome pairing at meiosis (Fig. 1*C*, *D* and Table 1).

It is significant that heteromorphic bivalents were easily observed at meiosis in the intermediate- and low-pairing (Fig. 1*B* and *D*), as well as in the high-pairing types where they are expected. This result shows that at least some of the pairing observed in the low and intermediate types was not between homologous chromosomes, but presumably between homoeologous chromosomes. Some of the consequences of this observation will be discussed later.

A detailed analysis of the F_1 hybrid that gave rise to the amphiploid was not made; however, it was observed that bivalent formation was minimal, being less than one bivalent per cell. An analysis of 20 cells of the colchicine-induced amphiploid is given in Table 1, and a cell with 25 ring bivalents, one ditelocentric rod bivalent, and two univalents is shown in Fig. 1*E*. One of the univalents was probably a satellited chromosome, and the other was a small chromosome with a submedian centromere. The average of 3.2 univalents per cell are accounted for by the two chromosomes that are present monosomically, and by bivalent failure. An average of 1.2 univalents per cell as a result of bivalent failure is directly comparable to the frequency of bivalent failure observed in the euploid *T. aestivum* (14). Consequently, we conclude that the chromosome pairing in this amphiploid is homologous pairing, and that the genetic mechanism on chromosome 5B is functioning normally, neither suppressed nor enhanced by any system introduced by the *T. speltoides* chromosomes.

DISCUSSION

Three points have been demonstrated in the data presented in this paper: first that variation occurs in the genetic mechanism in *T. speltoides* that affects chromosome pairing in hybrids with that species; second, that in hybrids with *T. aestivum*, where either low or intermediate frequencies of pairing are observed, homoeologous chromosome pairing is not completely suppressed; and third, in an amphiploid between *T. aestivum* and *T. speltoides*, where the activity of chromosome 5B of *T. aestivum* was unchanged and normal homologous chromosome pairing took place, synapsis between the chromosomes of *T. aestivum* and *T. speltoides* could not be observed.

The variation in *T. speltoides* of the factors affecting chromosome pairing in hybrids is similar to that recorded by Dover and Riley (13) in *T. tripsacoides*, but differs in that only three classes were observed compared to the four in that species. It is probable that further examination would reveal other variation in *T. speltoides*.

The presence of heteromorphic bivalents in the hybrids with either low or intermediate frequencies of pairing is of some significance, for it demonstrates that homoeologous chromosomes can pair in these hybrid situations. Since the pairing of homoeologous chromosomes (which, according to Riley (15), ". . . can therefore be regarded as being highly heterozygous chromosomes. . .") is not precluded, then it must be concluded that the pairing of homologous chromosomes is not precluded either. Since the demonstrable chromosome affinity in the low and intermediate types of hybrids is well below that expected if the chromosomes of *T. speltoides* were homologous to the B genome of *T. aestivum*, then *T. speltoides* cannot reasonably be regarded as the donor of the B genome.

The striking feature of the amphiploid derived from a low-pairing F_1 is the average of 25.2 bivalents per cell from a maximum possible of 26. From this it is apparent that the normal pairing of homologous chromosomes is in no way impaired. In the twenty cells analyzed, only two multivalents (both quadrivalents) were observed; in each case they were open, chain-type configurations. Consequently, it can again be concluded that *T. speltoides* shows little, if any, homology for the chromosomes of the B genome of *T. aestivum*. Similar conclusions concerning the genomic relationships of *T. tripsacoides* could be made if there were regular bivalent formation in amphiploids between low-pairing *T. tripsacoides* types and *T. aestivum*.

T. longissimum and *T. bicorne* can similarly be excluded as potential donors of the B genome, as their hybrids with polyploid wheat are characterized by a very low frequency of pairing (8), although an intermediate pairing type of *T. longissimum* has just been described by Mello-Sampayo

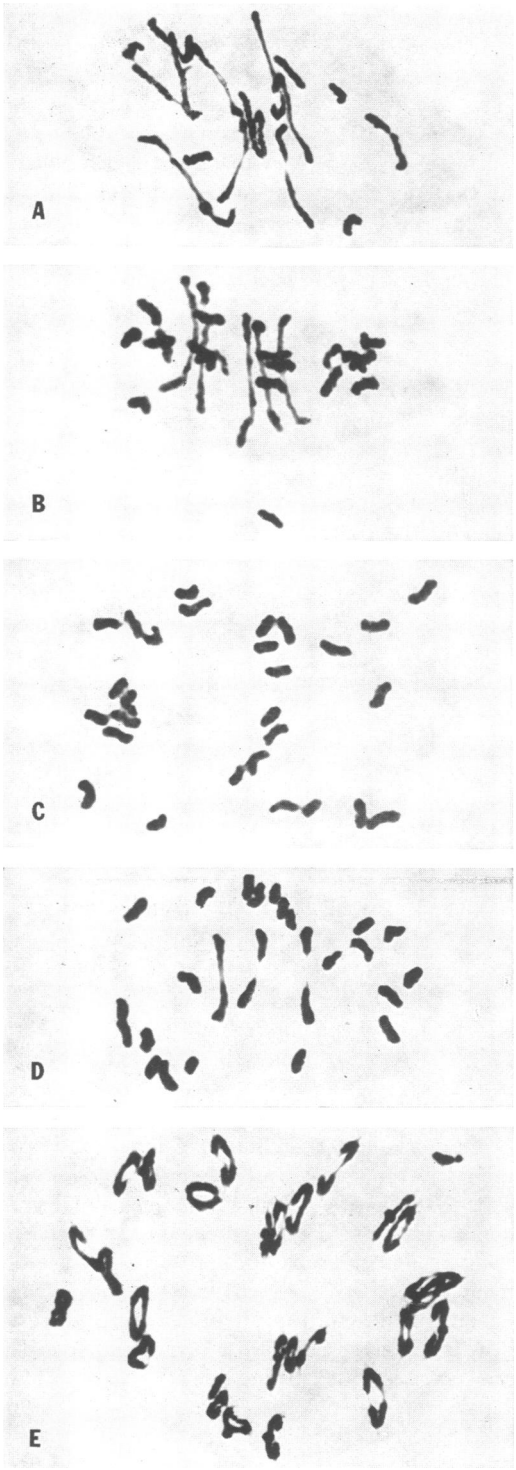

FIG. 1. Meiosis in hybrids and an amphiploid involving *Triticum aestivum* and *T. speltoides*. (*A*) F_1 hybrid with a high frequency of homoeologous chromosome pairing. (*B*) F_1 hybrid with an intermediate frequency of chromosome pairing. Note the heteromorphic bivalents. (*C*) F_1 hybrid with a low frequency of homoeologous chromosome pairing. (*D*) A cell with four bivalents in the low-pairing type of hybrid. Note the two heteromorphic bivalents. (*E*) Amphiploid of a low-pairing F_1 hybrid. In this plant, two chromosomes were represented monosomically and one pair was telocentric. This cell, with normal homologous chromosome pairing, has 25 ring bivalents, one ditelocentric bivalent, and two univalents. Magnification of all cells ×1200.

(16). Since *T. speltoides* shows almost complete chromosome pairing with both *T. longissimum* or *T. bicorne* (17), it is possible to infer that *T. speltoides* cannot be the B-genome donor, for if its chromosomes are homologous to those of *T. longissimum* and *T. bicorne*, then they cannot be homologous to any of the chromosomes of polyploid wheats. However, there is reason to question the homology of *T. speltoides* with the other diploids, in that only high-pairing *T. speltoides* has thus far been used in the crosses with them.

The recognition of the heteromorphic bivalents in the hybrids leads to some indication of the function of the genes regulating pairing in the diploid itself. Since homoeologous chromosome pairing is not completely prohibited in any of the hybrids, it must be concluded that homologous chromosome pairing is not precluded either. The regular bivalent formation in the amphiploid indicates that the genetic system causing the lowest pairing frequency in the F_1 does not inhibit homologous chromosome pairing. Similarly, since the diploid is a regular bivalent-forming species, it can be concluded that the genetic variation observed in these hybrids does not affect the synapsis of homologous chromosomes. Therefore, the variations observed must reflect the frequency with which homoeologous chromosomes synapse. Thus, variation in these genetic mechanisms in the diploids must be of little or no consequence.

Since it is improbable that *T. speltoides* is the donor of the B genome, it is obvious that the process of identification of the progenitor must be intensified again. No entity that has been described morphologically and examined cytologically will fit more than one or two of the criteria required. It is possible to make some predictions concerning the putative donor species:

(*a*) It probably will be morphologically similar to the *T. speltoides*, *T. bicornis*, *T. longissimum* group of species; however, it would be unwise, in view of the evidence of Johnson and Hall (12), and also the studies of Waines (21) to exclude the diploid wheats from consideration.

(*b*) It should exhibit a geographical distribution that overlaps that of diploid wheat. This again does not preclude other diploid wheats from consideration.

(*c*) All its chromosomes should have submedian centromeres, and one chromosome should show an arm ratio of 2.0:1–2.5:1, like chromosome 5B. Two pairs should have large satellites.

(*d*) Genetically, the individual chromosomes should correspond well to the homoeologous grouping of *T. aestivum* (18).

It is not necessary, or even probable, that all of these characteristics will be recognized, for both the B-genome donor and the B genome of the polyploids have perhaps undergone considerable evolutionary change since the initial hybridization.

What are the most likely sources of the B genome? Several possibilities may be considered.

a. The donor of the B genome was some as-yet-undiscovered form of *T. speltoides*. This is improbable, for if the diploid were a mere form of *T. speltoides*, its chromosomes would be unlikely to differ substantially from those of *T. speltoides*, which are demonstrably not homologous to the B genome of *T. aestivum*.

b. Tetraploid wheat is an autopolyploid of the A-genome diploid. This too is improbable, for crosses between the tetraploid and diploid wheats result in relatively low chromo-

TABLE 1. *The mean and range of chromosome pairing observed in hybrids and an amphiploid of Triticum aestivum x T. speltoides*

Material	I*	II Rod	II Ring	III	IV	V	VI	Number of cells
CS x *T. speltoides* F_1	6.8	5.0	1.8	1.8	0.5	—	0.05	20
G 1272	3–13	1–9	0–5	0–4	0–2		0–1	
CS x *T. speltoides* F_1	16.7	5.2	—	0.03	—	—	—	30
G 366	14–20	4–7		0–1				
CS x *T. speltoides* F_1	26.6	0.7	—	—	—	—	—	50
G 1316	20–28	0–4						
CS x *T. speltoides* Amphiploid	3.2	3.9	21.3	—	0.1	—	—	20
A18-7-1	2–6	1–9	16–25		0–1			

* I, univalent; II, bivalent; III, trivalent; IV, quadrivalent; V, pentavalent; and VI, hexavalent.

some pairing, with multivalents rare (19). The low pairing rate and the infrequent multivalents must also preclude the possibility of much repatterning of the A and B genomes in the tetraploid, with the repatterning being confined within the homoeologous groups.

c. A hybrid B-genome donor resulting from hybridization of two diverse diploids immediately before the cross with the diploid wheat that produced (with chromosome doubling) the tetraploid wheats. This is improbable, for if the hybrid B-genome plant were to be fertile, it must have arisen from types with similar or identical genomic constitution. Under these circumstances the B-genome donor would be genetically heterozygous but genomically homozygous, and would thus correspond to an easily identifiable diploid.

d. The tetraploid wheats were polyphyletic in origin; that is, two or more amphiploids originated as a result of hybridization between diploid wheat and other species. Intercrossing of these amphiploids would cause relatively little change in the constitution of the A genome, but would allow considerable repatterning of the B genome, with the consequent difficulty, or impossibility, of recognizing the original contributors. Thus, under these circumstances, the B genome of the polyploid forms would never correspond exactly, or even approximately, with any diploid analyzer. This process is already recognized in the Triticinae (20) and must, therefore, be seriously considered in the development of any evolutionary hypothesis concerning the polyploid wheats.

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