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Giemsa C-Banding and the Evolution of Wheat

(polyploid/chromosomes/heterochromatin/genomes)

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ABSTRACT **The somatic chromosomes of common wheat, *Triticum aestivum* L. (2*n* = 6x = 42), and those of two of its diploid progenitors and *T. speltoides*, have been individually identified by a Giemsa staining technique. In wheat, telocentric chromosomes were used to aid the recognition of individual chromosomes, and an ideogram has been constructed depicting the C-band positions. There is no similarity in the C-banding of chromosomes within a homoeologous group, with the possible exception of group 5. Comparisons of the C-banding of the diploid species *T. monococcum*, *T. speltoides*, and *T. tauschii* with that of the A, B, and D genomes, respectively, in hexaploid wheat corroborate that *T. speltoides* could not be the donor of the B genome to wheat and that *T. monococcum* and *T. tauschii* are the probable donors of the A and D genomes, respectively.**

The Giemsa staining technique for the detection of constitutive heterochromatin in cereal chromosomes has been described by Gill and Kimber (1). The C-bands in cereal chromosomes are usually present in the centromeric area and, additionally, may or may not be present in interstitial or terminal regions or both. There is a characteristic C-banding pattern for the individual chromosomes in somatic metaphases and, thus, also for the species. These features enabled the identification of all of the somatic metaphase chromosomes of rye and also allowed the recognition of rye chromosomes in wheat-rye addition lines (2). Other species for which C-banded somatic karyotypes have been described are *Scilla sibirica* (3), *Allium flavum* (4), and some species of *Anemone* (5). Quinacrine mustard and other compounds can be used to produce differential fluorescence (Q-banding), and Q-banded somatic karyotypes have also been described in a few plant species (3, 4, 6, 7).

A unique situation exists in wheat (*Triticum aestivum* L. 2*n* = 6x = 42), where 41 of the 42 possible telocentric misdivision products are available. In a line with only one chromosome represented by a telocentric, it is possible to determine the banding pattern in a known chromosome arm. By repeating this procedure for all of the available telocentrics, a virtually complete map can be synthesized. Since each chromosome arm is identified by its terminal centromere, the technique allows the unequivocal determination of the C-banded karyotype, as distinct from the inferences drawn by comparing the putative diploids with their polyploid relatives. Further, since the genome and homoeologous relationships of the chromosomes are already known, and were determined independently (8), it is possible to compare the banding patterns of the chromosomes within a homoeologous group and also to describe the characteristics of the chromosomes of a genome. Both of these points are crucial to evolutionary studies in the genus.

Triticum monococcum L. (2*n* = 14) and *T. tauschii* (Coss.) Schmal. (*Aegilops squarrosa*, 2*n* = 14) are widely accepted as the donors of the A and D genomes, respectively, to hexaploid wheat (9). *T. speltoides* (Tausch) Gren. ex Richter (*Ae. speltoides*, 2*n* = 14) was proposed as the donor of the B genome by Sarkar and Stebbins (10) on the basis of morphological evidence and supported by Riley, Unrau, and Chapman (11) on the basis of karyotypic, synaptic, and geographical distribution evidence. However, recent studies on chromosome pairing (12–15), on protein electrophoresis (16), and on the number and size of satellites (17) have disputed this. Since the C-banding technique can reveal details of chromosome structure not previously recognized, accessions of *T. monococcum*, *T. tauschii*, and *T. speltoides* were examined.

MATERIALS AND METHODS

Stocks of the telocentrics in wheat cultivar Chinese Spring were provided by Dr. E. R. Sears. They included double ditelocentric lines (18) for most of the chromosomes; that is, lines that are genetically euploid but with one chromosome represented by two telocentrics of each arm simultaneously. Wherever the arms of a chromosome were of similar size, two separate ditelocentric lines were used to avoid confusion in the recognition of a specific arm. Thus, with the exception of the chromosome arm 7DL, all the arms were available for study.

The technique for C-banding is that previously described by Gill and Kimber (1, 2). The dehydration of the slides was usually limited to 1 hr, and the roots were not left in the glacial acetic acid for more than 1 day. Solutions were generally less than 2 weeks old.

Photomicrographs, all at the same magnification, were made of somatic cells containing the telocentric chromosomes. The telocentrics were cut out of the prints and mounted to produce a karyotype arranged according to the homoeologous classification (Fig. 1). Since the prefixation treatment used leads to different degrees of contraction from root to root and even from cell to cell, the amount of contraction was not uniform. Thus, the arm ratios seen in Fig. 1 are not necessarily those of the complete chromosomes. However, the relative positions of the bands within a chromosome arm are assumed to be constant.

An ideogram of the wheat karyotype was produced by drawing the bands at their correct positions within an arm on a chart in which the correct arm ratio and relative size is represented to scale (Fig. 3). The relative sizes and arm ratios used are those determined by Sears (19) for telophase II of cultivar Chinese Spring, modified, in the case of chromosome 5B, according to the data of Larsen and Kimber (20).

For the C-banding studies in the diploid species, three accessions of *T. speltoides* (G366, G1272, and G1316 of the University of California-Riverside Collection) were used. Two accessions (G1275 and G1276) were used for *T. tauschii* and two (G367 and G495) for *T. monococcum*. The seven individually identified chromosomes in each species were cut out of photomicrographs of somatic cells and mounted to produce the karyotype.

RESULTS

The C-banding of a particular chromosome is believed to be constant for that chromosome. Some of the faint bands are difficult to see in some preparations, particularly when the chromosomes are well contracted. In order to recognize the telocentric chromosomes in wheat, it is sometimes necessary to examine cells in which the chromosomes are too contracted to allow the recognition of all of the bands. In some cases, where the complete chromosome can be recognized easily by its banding pattern (Fig. 2), it is possible to determine the presence of additional bands by an examination of the chromosome when it is not as contracted as is necessary with the telocentrics. The bands drawn in Fig. 3 represent a summation of observations.

Description of the chromosomes of common wheat

A Genome. Centromeric heterochromatin is always present. Interstitial bands are present in chromosomes 4A, 5A, 6A, 7A, and possibly 1A.

1A. Submetacentric. The long arm has a faint terminal band. In some long-arm telos, a faint interstitial band was sometimes seen near the centromere. Coupled with the arm ratio, the banding of 1A is fairly diagnostic.

2A. No interstitial bands in the short or the long arm.

3A. Metacentric. One terminal band in the long arm.

4A. Metacentric. Highly heterochromatic. The α arm has two proximal bands, which in the complete chromosome appear to be fused. The β arm has two faint interstitial bands and a terminal band. The banding is highly diagnostic.

5A. The long arm has a very characteristic interstitial band and the short arm has a terminal band. The banding is highly diagnostic.

6A. The β arm has two very characteristic and highly diagnostic bands near the terminal end.

7A. Metacentric. The short arm has an interstitial band near the centromere.

B Genome. The B-genome chromosomes are the most heterochromatic, and all of them exhibit interstitial bands in the short or the long arm or both. All are easily identified, except chromosome 3B. Chromosome 1B has a medium-sized band at the end of the long arm, the only such chromosome in the whole complement. The nucleolar regions in chromosomes 1B and 6B exhibit large heterochromatic blocks. Centromeric heterochromatin is always present.

1B. The short arm has a series of four bands, of which the one in the nucleolar region is the most prominent. The long arm has one band proximal to the centromeric band, one faint interstitial band, and a fairly large terminal band. This chromosome is easily recognized.

2B. The short arm has a very prominent interstitial band near the centromeric band and two very characteristic bands at the end of the arm. The long arm has only one interstitial band proximal to the centromere. It is easily recognized.

3B. The long arm has two faint bands, one interstitial and one terminal. The short arm has only the terminal band. Although this chromosome is uniquely marked, it is often difficult to recognize as a complete chromosome.

4B. The long-arm telo showed two almost terminal bands, and in some cases two more faint bands were seen, one on each side of the subterminal band. In one whole chromosome, a series of three bands was seen, the middle one being the largest. Banding is highly diagnostic.

5B. Heterobrachial. The short arm has one characteristic terminal and one interstitial band. The centromeric area has the biggest block of heterochromatin in the whole complement. The proximal band seen in the long-arm telo seems to fuse with the centromeric heterochromatin when observed in the whole chromosome. The long arm has also a series of three faint interstitial bands best seen in the complete chromosomes. Banding is highly diagnostic.

6B. The short arm has a large, dark band in the nucleolar region and, in some preparations, a faint proximal band near the centromere. The long arm has two large bands near the centromeric band, a faint interstitial band, and a faint terminal band. Banding is highly diagnostic.

7B. This chromosome has a series of three small bands in the centromeric area.

D. Genome. Centromeric heterochromatin is present in all chromosomes except possibly 1D. The chromosomes 1D, 4D, and 5D exhibit very characteristic, dark, interstitial bands.

1D. It has a diagnostic proximal band in the long arm and a faint terminal band in the short arm. The terminal band was not present in the telocentric. In the complete chromosome long-arm, an additional interstitial faint band was observed. This is the only chromosome that appears to be devoid of centromeric heterochromatin. Banding is highly diagnostic.

2D. Metacentric. The α arm has a faint proximal band, and the β arm has a small terminal band.

3D. Metacentric. The α and β arms exhibit similar and characteristic dark terminal bands.

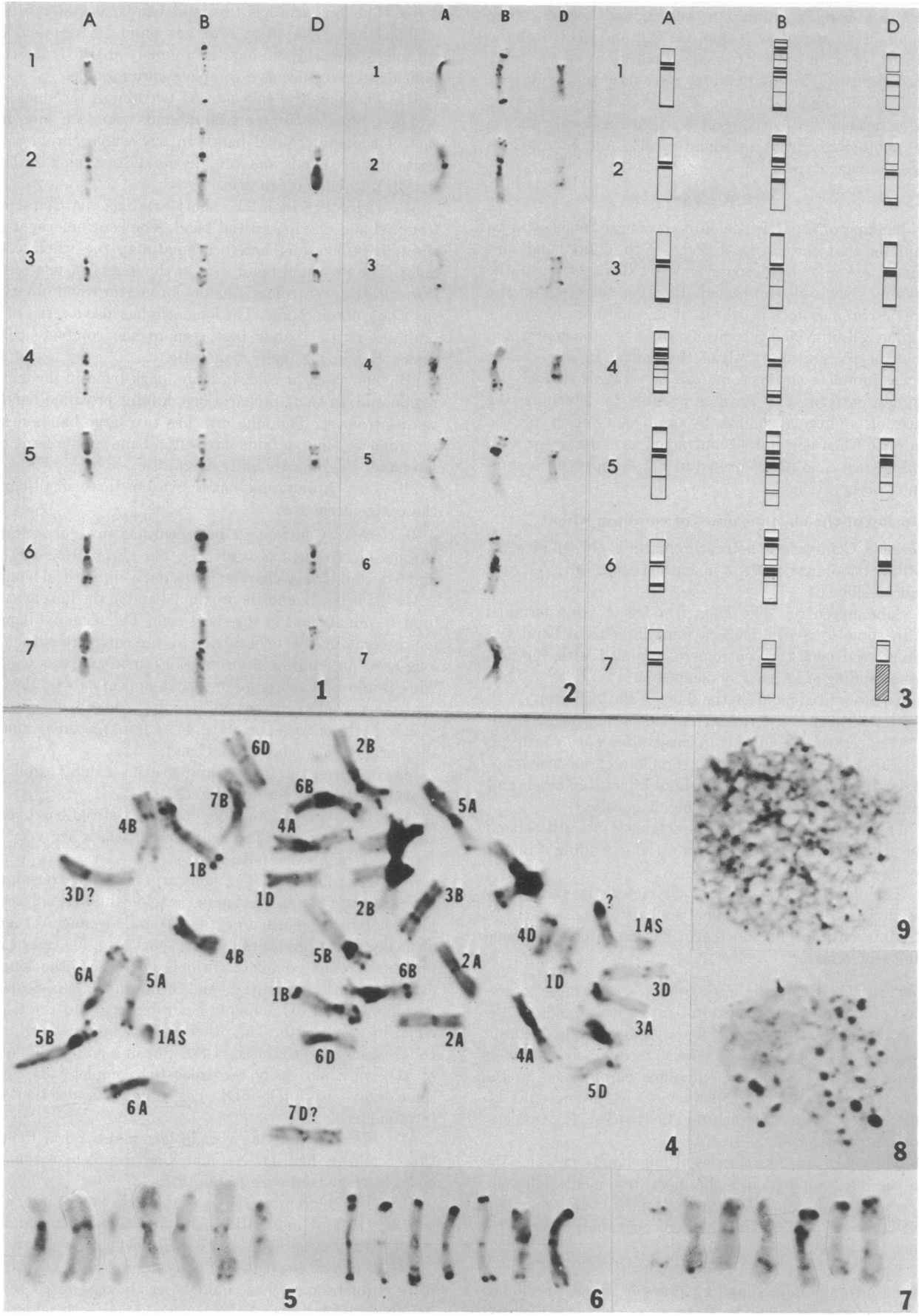
4D. It has a characteristic, dark, interstitial band near the end of the long arm. The centromeric bands are very faint. Banding is highly diagnostic.

5D. Heterobrachial. The short arm has a dark terminal and a centromeric band. The long-arm telo exhibited only centromeric heterochromatin and appeared only as long as the short arm. Because of its characteristic short arm, 5D can be easily matched to the complete chromosome where the long arm exhibits a dark, interstitial band. From the data of Sears (19), it is known that 5D is highly heterobrachial but the telos are equal in arm length. The other chromosome with which 5D could possibly be confused is 4D. But in a double ditelo stock of 4D, we could easily recognize the complete 5D chromosome. Obviously, telo 5DL has suffered a deletion of its terminal part.

6D. Metacentric. Has a small terminal band in the α arm (lower telo in Fig. 1). No matching complete chromosome could be picked out with certainty.

7D. Only telocentric 7DS was available for C-banding. This arm has a faint interstitial band and a terminal band. The whole chromosome could not be identified with certainty.

Fig. 2 shows the cut-out photomicrographs of the complete chromosomes that could be easily recognized by their banding pattern. Fig. 4 shows a good cell with many easily recognizable chromosomes.



FIGS. 1–9. *(Legend appears at bottom of the next page.)*

Descriptions of the chromosomes of diploid species

Most of the *T. speltoides* chromosomes are characterized by large terminal bands (Fig. 6). The C-banding pattern was identical in all the three accessions examined. Centromeric heterochromatin is always present. The interphase nucleus shows characteristic heteropycnotic knobs (Fig. 8). Four pairs of chromosomes have conspicuous terminal bands in both arms, two pairs have terminal bands in one arm, and one pair has no terminal bands. Since there has been discussion about *T. speltoides* being the donor of the B genome to wheat, each of the chromosomes is described individually. All seven chromosomes are identifiable. The arrangement of Fig. 6 does not correspond to the homoeologous grouping.

1. Large terminal bands in both arms and a large interstitial band near the terminal band in the long arm.
2. A large terminal band in the short arm and a smaller terminal band in the long arm. Very faint interstitial bands are present in both arms.
3. A medium-sized terminal band in one arm and a series of three bands distally in the other arm, the central one being the most conspicuous.
4. Large terminal bands in both arms and also faint interstitial bands near the terminal bands in both arms.
5. Medium-sized bands terminally in both arms. No interstitial band.
6. No large terminal bands, but faint terminal and interstitial bands are present.
7. One terminal band in the short arm and two or three faint bands including a terminal band in the long arm.

T. tauschii chromosomes have faint terminal or interstitial bands or both. Centromeric heterochromatin is faintly stained in five or perhaps six chromosomes (Fig. 7). It is probable that chromosomes 1D through 5D can be identified in *T. tauschii*, and the chromosomes in Fig. 7 are arranged so that these are the first five chromosomes from left to right. Chromosome six and seven in Fig. 7 did not correspond to any recognizable chromosomes of the D genome in *T. aestivum*.

T. monococcum chromosomes required 2 hr of dehydration in absolute alcohol to produce good C-bands. The general pattern of C-banding is like that of *T. tauschii* in the absence of large bands and the presence of small terminal or interstitial bands or both. All seven chromosomes exhibited centromeric heterochromatin (Fig. 5), and they could be matched with the chromosomes of the A genome of *T. aestivum*. The chromosomes in Fig. 5 are arranged to correspond, from left to right, with the seven chromosomes of the A genome of *T. aestivum*.

DISCUSSION

The utilization of stocks having specific chromosomes represented by one or two telocentric derivatives has permitted the establishment of a nearly unequivocal C-band karyotype in *Triticum aestivum* (Figs. 1, 2, and 3), with each chromosome identified as to its genomic and homoeologous relationships. The ability to identify each chromosome cytologically should prove very useful in cytological and evolutionary studies in the *Triticinae*.

Although homoeologues are presumably derived from a single ancestral chromosome, there is little similarity in banding pattern within the homoeologous groups in wheat, except possibly homoeologous group 5. The short arms of chromosomes 5A, 5B, and 5D have terminal and dark centromeric bands, and the long arm in each case exhibits a dark interstitial band. The differences among them are in arm ratio of 5A and 5B and in the presence of some extra faint bands in 5B not present in 5A and 5D. Chromosomes 3A and 3D are quite similar except for the terminal band in the short arm of 3D and the intensity of banding. In general, the three chromosomes of a homoeologous group are highly differentiated from each other, with each having its own characteristic banding.

In terms of total heterochromatin per genome in wheat, the B genome is the most heterochromatic, the D genome the least and the A genome slightly more heterochromatic than the D genome. The most heterochromatic chromosomes are 4A, 1B, 2B, 5B, and 6B.

Differences were noted between the telocentric karyotype and the recognizable complete chromosomes. The short arm of the complete chromosome 1D has a faint terminal band that was not visible in the short-arm telocentric (1DS), but there is other evidence that the supposed telo 1DS is actually a fragment, presumably proximal, of 1DL (personal communication from E. R. Sears). In the complete chromosome 5D there is a prominent interstitial band in the long arm which, together with a considerable portion of the euchromatin, is absent in the long-arm telocentric (5DL). It is most probable that the telocentric 5DL stock has suffered a terminal deletion. In Fig. 4 one chromosome (marked with a question mark) has a conspicuous terminal band. This chromosome was easily recognized in several preparations, but no such telocentric was detected, nor was such a chromosome seen in the diploids examined. It is also possible that this band represents a segment deleted in one of the telocentrics, and thus at least three terminal deletions may have taken place in the telocentric stocks. These deletions could give rise to difficulties if those telocentrics were used in mapping experiments. For example, a locus identified with a particular complete chromosome by monosomic analysis could not be allocated to either arm, nor could it be linked with the centromere, if it were distal to the deletion point of the telocentric used in the linkage analysis.

The A genome of the polyploid wheats was derived from the diploid wheats (9), and, as might be predicted, the chromosomes of *T. monococcum* exhibited a great similarity to the chromosomes of the A genome of *T. aestivum*.

The banding pattern of *T. tauschii* and the D genome of *T. aestivum* match well for five of the chromosomes (1D through 5D), with chromosomes 1D, 3D, 4D, and 5D showing complete identity. Interestingly, the band deleted from telocentric 5DL and present in the complete 5D in wheat is also seen in *T. tauschii*. The two remaining chromosomes (at the right of Fig. 7) do not correspond to any of the D-genome

FIGS. 1–9. (*on preceding page*). FIG. 1. Forty-one Giemsa C-banded telocentric chromosomes of wheat arranged according to the homoeologous classification. In cases of α- and β-arm telos, the α arm is in the upper position. FIG. 2. Seventeen identifiable C-banded complete chromosomes of wheat arranged according to the homoeologous classification. FIG. 3. Ideogram of the wheat karyotype showing the relative sizes and positions of the Giemsa C-bands. FIG. 4. Somatic cells of wheat in which 32 chromosomes can be identified by their C-bands and two (1AS) can be recognized as telocentrics. FIGS. 5, 6, and 7. Giemsa C-banded karyotype of *T. monococcum*, *T. speltoides*, and *T. tauschii*, respectively. FIG. 8. Interphase nucleus of *T. speltoides* showing the large heteropycnotic knobs. FIG. 9. Interphase nucleus of *T. aestivum* without large heteropycnotic knobs.

chromosomes of *T. aestivum*; however, chromosome 6D is not highly diagnostic, and the banding pattern of only one arm of 7D is known with certainty.

The banding pattern of the *T. speltoides* chromosomes is so distinct and different from that of the B-genome chromosomes in wheat that, on the basis of the C-banding alone, *T. speltoides* could be eliminated as a possible donor of the B genome to wheat. Amongst the B-genome chromosomes, only 1B has a medium-sized terminal band, and no other bands of the B-genome chromosomes can be matched to the banding pattern of the *T. speltoides* chromosomes. The terminal heterochromatic bands of the *T. speltoides* chromosomes are also conspicuous as large heteropycnotic areas in the interphase nucleus (Fig. 8), whereas similar heteropycnotic areas are not to be observed in the interphase nucleus of *T. aestivum* (Fig. 9). The terminal banding of the T. *speltoides* chromosomes and the heteropycnosis of the interphase nucleus are similar to the banding and interphase heteropycnosis of rye (2), which, like *T. speltoides*, is an out-breeding species.

The C-banding patterns of five (or six) species in the *Triticinae* are now known. The karyotype of *Secale cereale* was established by Gill and Kimber (2), and in this paper the karyotypes of *T. aestivum*, *T. monococcum*, *T. speltoides*, and *T. tauschii* have been demonstrated. The homoeologous relationships of the chromosomes of *T. aestivum* and *S. cereale* are known and are inferred for most of the chromosomes of *T. monococcum* and *T. tauschii*. The homoeologous relationships of the highly characteristic chromosomes of *T. speltoides* are not known at this time. The karyotype and homoeologous relationships of the chromosomes of a sixth species(?) has possibly also been established; that is the diploid that gave rise to the B genome of the polyploid wheats. This is a situation where characteristics of a possibly extinct species (13) can be determined from related species. Clearly, these characteristics can be used in attempts to identify the donor of the B genome to the polyploid wheats.

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