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SHORT PAPERS

Chromosome banding patterns and the origin of the B genome in wheat

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SUMMARY

The distribution of heterochromatic regions in the chromosomes of diploid, tetraploid and hexaploid wheat shows that the B genome possesses characteristic large blocks. Though analyses of probable B genome donors indicate that *Aegilops speltoides* has a pattern of distribution of heterochromatin nearest to the B genome chromosomes, a polyphyletic origin of tetraploid wheat seems more plausible.

1. INTRODUCTION

Cultivated wheat, *Triticum aestivum* ($2n = 6x = 42$), is a hexaploid containing three different genomes, namely AA, BB, DD, and is assumed to have arisen as an amphiploid during the course of evolution. Of these three genomes, it is fairly certain that the A and D genomes have been contributed by *Triticum boeoticum* (or its derivative *T. monococcum*) and *Aegilops squarrosa* respectively (Sears, 1969). The source of the B genome is still uncertain. Originally, this genome was attributed to *Agropyron triticeum* (McFadden & Sears, 1944) but later on to *Aegilops speltoides* (Sarkar & Stebbins, 1956). Observations on (*a*) the spikelet morphology (Sarkar & Stebbins, 1956), (*b*) the chromosome morphology, especially the satellited chromosomes (Riley, Unrau & Chapman, 1958), (*c*) the suppressive action of this genome on the 5B effect on chromosome pairing at meiosis in hexaploid wheats (Riley *et al.* 1958), (*d*) nuclear DNA content (Rees & Walters, 1965), and (*e*) the acid phosphatase electrophoretic patterns (Jasaka & Jasaka, 1970) are supportive evidences for *Ae. speltoides* being the donor of the B genome. On the other hand, (*a*) the morphological appearance of amphidiploids derived from diploid *Triticum* and *Ae. speltoides* (Sears, 1969), (*b*) the pairing behaviour of *Ae. speltoides* in wheat hybridis in the absence of the suppressor for pairing (Kimber & Athwal, 1972), and (*c*) the seed protein electrophoretic profiles (Johnson, 1972) cast doubt on *Ae. speltoides* as the donor of the B genome.

Recently many chromosome banding techniques have become available to identify individual chromosomes and the distribution of heterochromatin in a genome (Paris Conference, 1972). Although these techniques have been exploited to a great extent in mammalian cytology, they have been applied in plant materials only to a limited extent, in view of the technical difficulties in making cell wall-free chromosome preparations (Vosa & Marchi, 1972; Natarajan & Natarajan, 1972). Though much progress in the standardization of these techniques for plant material is needed, it was possible to characterize individual chromosomes of rye (*Secale cereale*) (Sarma & Natarajan, 1973)

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and the possibilities were pointed out of identifying rye chromosomes in a *Triticale* genome or in substitution lines. During this study (Sarma & Natarajan, 1973) it was observed that in tetraploid wheat, which was used as a parent in the *Triticale*, there were some characteristic banding patterns in their chromosomes. This prompted us to exploit these techniques to see if they would be of any help in identifying the B genome donor of the present day polyploid wheats.

2. MATERIALS AND METHODS

In the present study, seed material obtained from various sources was used, as listed in Table 1.

Colchicine (0·2 %, for 2 h) treated root tips were fixed in acetic alcohol (1:3) overnight, hydrolysed in 0·1 N-HCl for 5 min at 60 °C, and squashed in a drop of 45 % acetic acid. The preparations were frozen on dry ice, the cover-glasses flipped off and then washed in 95 % alcohol twice for 5 min before air-drying. These preparations were denatured in saturated aqueous barium hydroxide or 0·07 N-NaOH solution for 5 min in room temperature, washed thoroughly in running water, reassociated in 2 × SSC (0·3 M-NaCl + 0·03 M trisodium citrate) at 66 °C, before staining with Giemsa solution (2 % for 10 min at pH 7·0).

Table 1. *Sources of seed material*

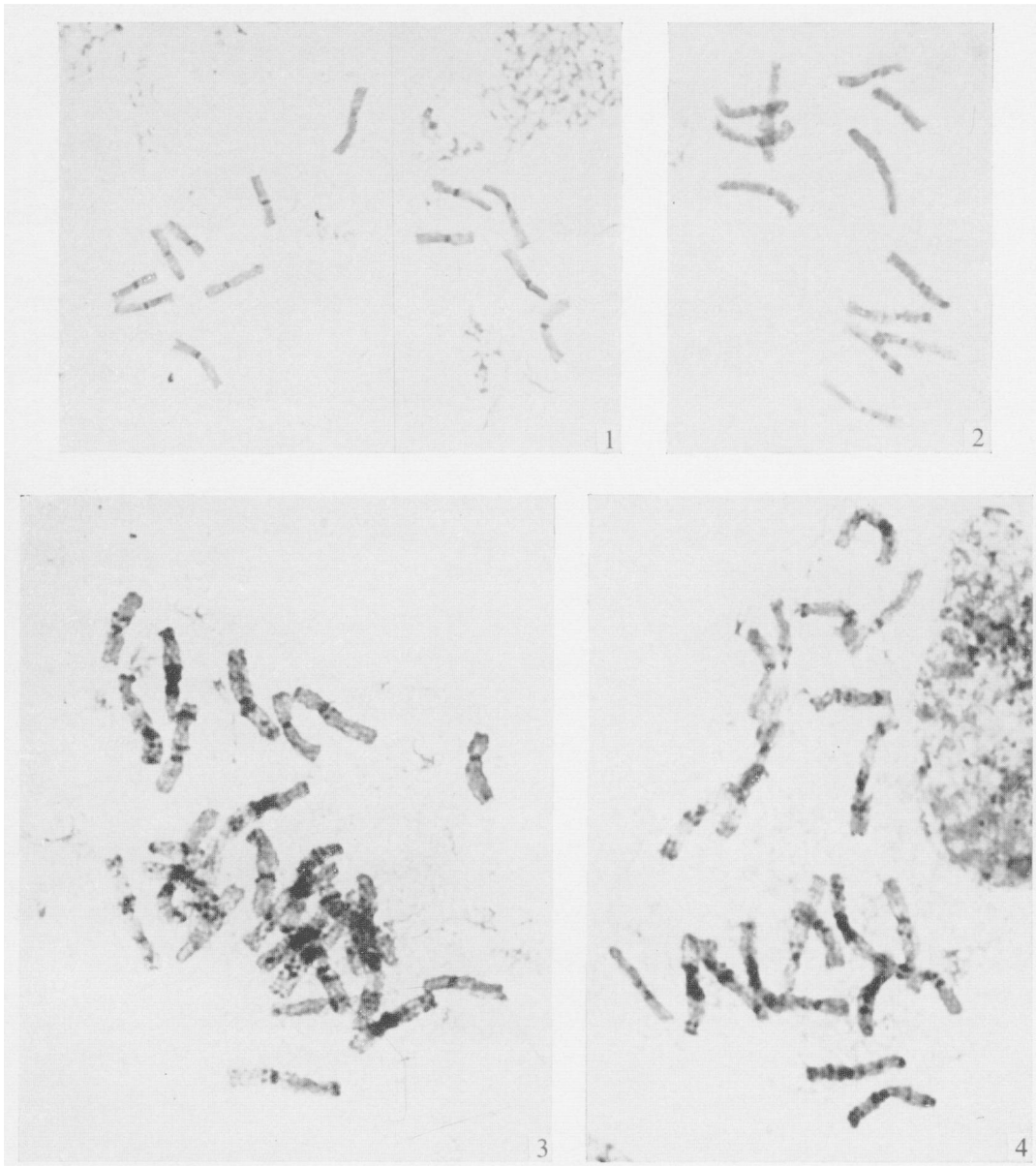
Triticum	Genomes	No. and origin of specimens studied*
T. monococcum L.	AA	3a, c, e
T. dicoccum Schübl.	AA BB	3c, d, e
T. aestivum L.	AA BB DD	2d, e
Aegilops		
Ae. squarrosa L.	DD	2a, b
Ae. speltoides Tausch.	BB	4a, b, c, d
Ae. bicornis (Forsk.) Jaub. et Sp.	—	2a, b
Ae. sharonensis	—	2a, b
Ae. longissima (Schw. et Muschl.) Eig.	—	2a, b

* a, Plant Breeding Institute, Cambridge; b, University of California (Riverside); c, Indian Agricultural Research Institute, New Delhi; d, Weibull's Plant Breeding Institute, Landskrona; e, Royal College of Agriculture, Uppsala.

3. RESULTS AND DISCUSSION

By the technique employed here (i.e. C-banding) one can recognize all the constitutive heterochromatic regions in the chromosomes, which bind heavily to Giemsa stain after denaturation and reassociation. In the species studied the following general types of C-banding were recognized: (1) small centromeric blocks, (2) large centromeric blocks, (3) telomeric blocks, and (4) intercalary blocks which may be present as solid or dispersed, but distinct dot-like bodies. The distribution of these different types of C-banding in the species studied is presented in Fig. 11.

All the chromosomes of *T. monococcum* have a small block of heterochromatin near the centromeric region (Fig. 1). *Ae. squarrosa*, which is the contributor of the D genome to the hexaploid wheat, has three different types of chromosomes, with regard to the distribution of heterochromatin (Figs. 2, 11). All the chromosome pairs have only small amounts



Figs. 1–10. Distribution of heterochromatin in the chromosomes of *Triticum* and *Aegilops* species. In some figures, all the chromosomes in the genome are not shown. 1, *T. monococcum*; 2, *Ae. squarrosa*; 3 and 4, *T. dicoccum*.

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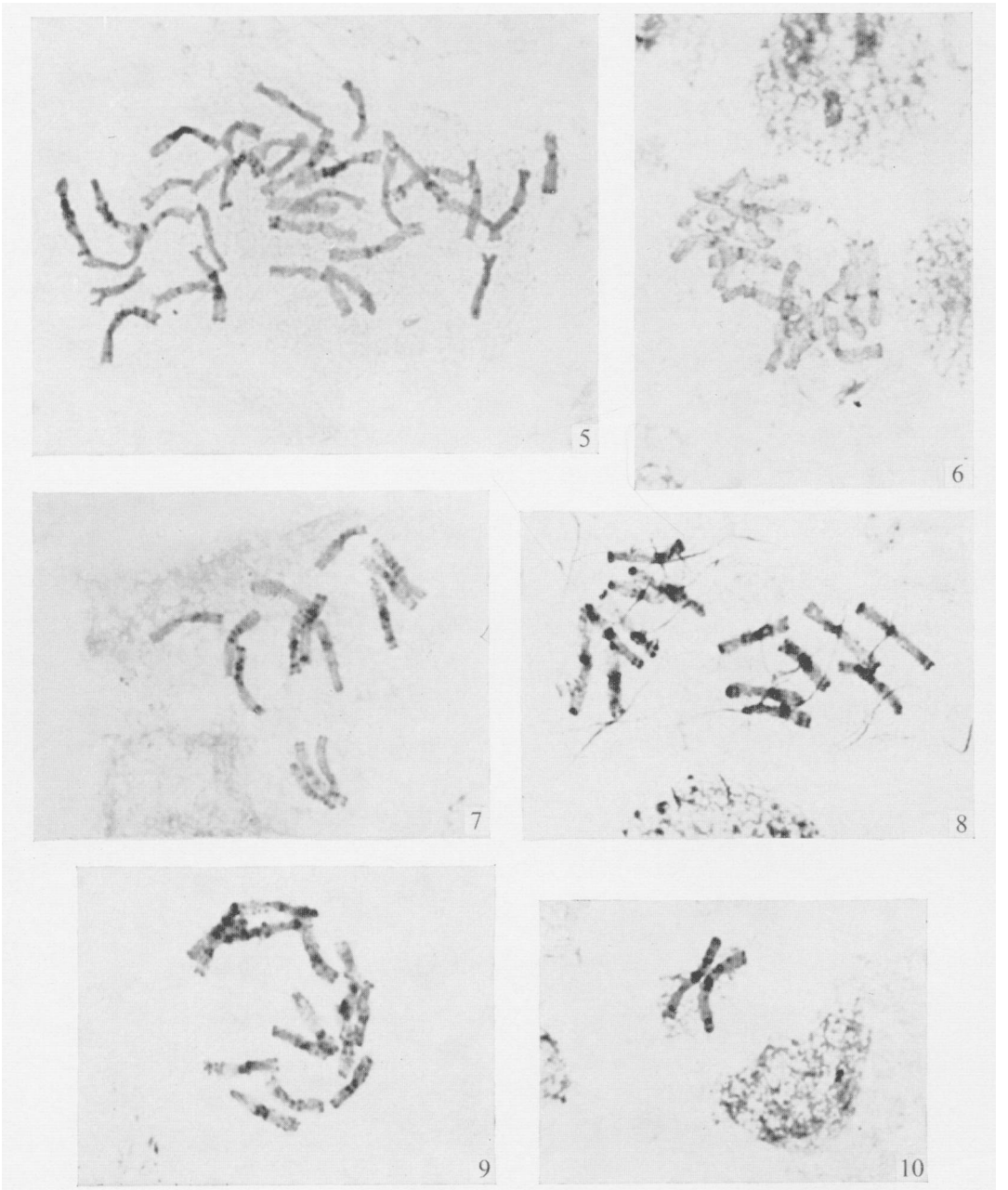

Figs. 5–10. 5, *T. aestivum;* 6, *Ae. bicornis*; 7, *Ae. longissima*; 8, *Ae. speltoides*; 9, *Ae. sharonensis*; 10, an homologous pair of chromosomes in *T. dicoccum* showing identical distribution of heterochromatin as well as somatic association.

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of heterochromatin, but the distribution, in the form of centromeric, telomeric or dot-like intercalary heterochromatin, differs among them.

In *T. dicoccum* (Figs. 3, 4, 11), there are two major groupings with regard to the C-band pattern. One group of chromosomes contains only a small amount of heterochromatin, and resembles the chromosomes of *T. monococcum*, the proposed donor of the A genome. It should be pointed out that not all the chromosomes of this group are similar to those of *T. monococcum*. Some of them have telomeric and intercalary heterochromatin, resembling chromosomes of *Ae. squarrosa*. Thus, the A genome contributor may not be identical

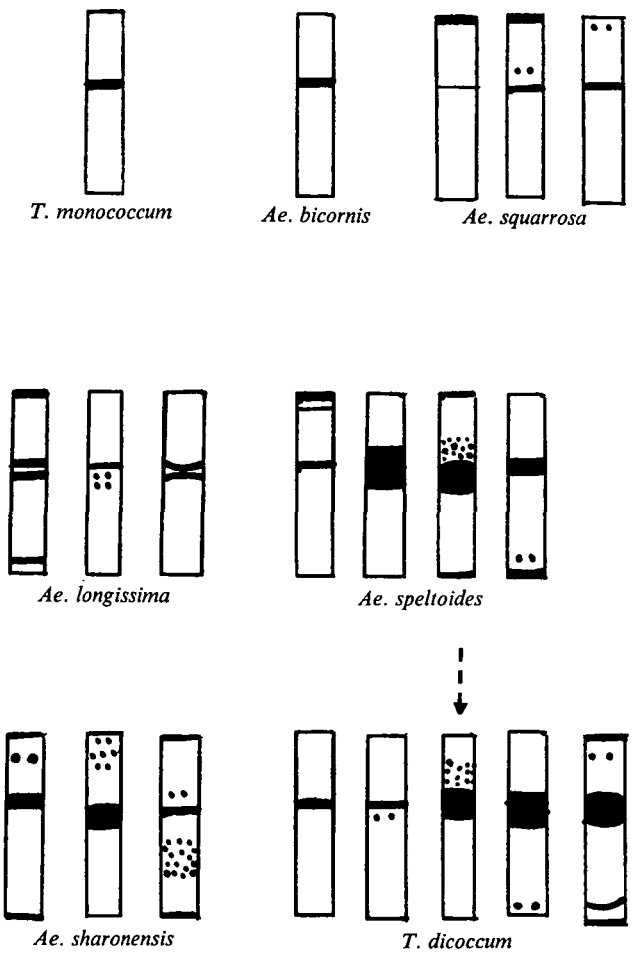

Fig. 11. Diagrammatic presentation of different patterns of distribution of heterochromatin in the chromosomes of *Triticum* and *Aegilops* species. Figures are not drawn to scale.

with the present-day *T. monococcum*, or there may exist biotypes in this species with variations in C-band distribution, other than the type described here. However, it is fairly certain that the chromosomes of this genome do not contain much heterochromatin.

The second group of chromosomes in *T. dicoccum* contains large blocks of heterochromatin around the centromere (Figs. 3, 4). In addition, they have telomeric and intercalary heterochromatin (Fig. 11). This group of chromosomes must have been contributed

by the B genome donor. Hexaploid wheat has also these chromosomes, and in addition seven more pairs with small amounts of heterochromatin (Fig. 5), resembling the chromosomes of *Ae. squarrosa*. This indicates that there has been no major change in the pattern of B genome chromosomes from the tetraploid to the hexaploid level.

The presence of large blocks of heterochromatin and their distribution along the B genome chromosomes make it possible to check which of the probable or suggested B genome donors have a similar pattern of distribution. The chromosomes of four species studied are presented in Figs. 6–9 and 11. *Ae. bicornis* has only one small heterochromatic block at the centromere and one of the telomeres (Fig. 6) and *Ae. longissima* has some more bands, which have no resemblance to the B genome however (Fig. 7). Hence, neither of these two species can be the contributor of this genome. The chromosomes of *Ae. speltoides* (Figs. 8, 11) from all the four sources examined have very large blocks of heterochromatin around the centromere. In addition, they have telomeric and intercalary heterochromatin. The pattern of distribution of heterochromatin in this species is similar, but not identical to that found in the B genome chromosomes. *Ae. speltoides* and the B genome of tetraploid and hexaploid wheats contain four pairs of chromosomes with large blocks of centromeric heterochromatin. But there are some differences in the distribution of intercalary and telomeric heterochromatin (Fig. 11). The diffuse dot-like intercalary heterochromatin seems to be present more in the B genome than in the chromosomes of *Ae. speltoides*. In addition, the pair of chromosomes carrying distinct dot-like heterochromatin in *Ae. speltoides* has telomeric heterochromatin on both arms, whereas it is present only in the short arm of that chromosome pair in the B genome. There are some additional intercalary heterochromatic segments in the B genome, which are absent in *Ae. speltoides*. The predominant dot-like distribution of heterochromatin in the B genome chromosomes resembles the very characteristic pattern present in *Ae. sharonensis*. Though *Ae. speltoides* also has such a distribution in a few chromosomes, this is not as prominent as in the B genome of the polyploid wheats and in *Ae. sharonensis* (Figs. 5, 9). From this limited study some tentative conclusions can be reached. (1) The distribution of heterochromatin in the B genome of the polyploid wheats resembles more closely that of *Ae. speltoides* than others. This supports the earlier evidence on spikelet and chromosome morphology (Sarkar & Stebbins, 1956; Riley *et al.* 1958). (2) Additional patterns, such as diffuse dot-like distribution of heterochromatin, which is characteristic of *Ae. sharonensis*, indicates that *Ae. speltoides* may not be the sole B genome donor. Hybridization and selection between different amphidiploids with common A genome and varying B genomes, at the centre of origin, might have led to the present-day tetraploid wheat. The B genome chromosomes, themselves, might have changed extensively and it may not be possible at the present time to pinpoint a single diploid species as a donor of B genome. Since the B genome has a large amount of heterochromatin, and spontaneous chromosomal aberrations are known to occur preferentially at these regions (Natarajan & Ahnström, 1969, 1973), repatterning of these chromosomes may have occurred during evolution. By denaturation and reassociation studies it has been shown that polyploid wheats have a major component of their DNA in the form of repetitive sequences (Mitra & Bhatia, 1973). From studies on other organisms it is known that the repetitive sequences are concentrated in the heterochromatic regions and attraction between similar sequences may be the basis for common chromocentre formation in the interphase of homologous heterochromatic regions (Natarajan & Schmid, 1971). The phenomenon of somatic pairing has often been observed in polyploid wheats (Fig. 10), especially between homologous chromosomes, with a major attraction at the centromeric region (Feldman, Mello-Sampayo & Sears, 1966). The presence of centromeric heterochromatin in almost all the chromosomes of polyploid wheat should therefore be an important factor leading to somatic pairing of the chromosomes (Rao & Natarajan, 1967).

The present study is of a preliminary nature based on only few varieties of each species, and no definitive conclusions should be drawn based on these observations, as biotype differences are known to occur in wheats (Bhaduri & Natarajan, 1956; Upadhya & Natarajan, 1966; Waines & Kimber, 1973). The results obtained here demonstrate, however, the usefulness of this type of approach to the study of the origin of wheat as well as of other cultivated plants.

Earlier cytological studies were confined to characteristics of arm ratios, satellited chromosomes, and locations of secondary and tertiary constrictions. Additional valuable details of individual chromosomes can be added by the use of the banding techniques. An extensive cytological survey of all the suggested and probable diploid donors as well as of wild wheats near the centre of origin might therefore be rewarding.

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