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The recognition of an alien chromosome segment translocated to a wheat chromosome

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1. INTRODUCTION

The bread wheat of commerce is an allohexaploid species with a basic chromosome number of seven. Thus, *Triticum aestivum* has forty-two somatic chromosomes fourteen of which have been derived from a diploid wheat, fourteen from *Aegilops speltoides* and fourteen from *Ae. squarrosa*. The hexaploid species has evolved by two cycles of hybridization and chromosome doubling. First a hybrid was formed between an A genome diploid wheat and *Ae. speltoides*, the donor of the B genome. Chromosome doubling in this hybrid gave rise to the tetraploid wheats which are thus genomically AABB. The second cycle of hybridization and chromosome doubling involved a tetraploid wheat and *Ae. squarrosa* which is the donor of the D genome. *T. aestivum* ($2n = 6x = 42$) can therefore be described genomically as AABBDD.

During the exploitation of the cultivated forms of the genus *Triticum* by man, the species have been parasitized by various forms of pathogen. Plant breeders have manipulated various stocks so that they could combat the damage caused by the pathogens and in recent years it has become obvious that some of the relatives of the cultivated forms had highly desirable disease resistance. Riley & Kimber (1966) have reviewed the attempts at the introduction of alien variation, both when the alien chromosomes are homologous and when they are non-homologous to those of *T. aestivum*.

The introduction of the first alien chromosome segment by Sears (1956) involved the irradiation induced breakage of the appropriate alien chromosome from *Ae. umbellulata* and its translocation to a wheat chromosome. The chromosome of *Ae. umbellulata* from which the segment was translocated has been designated chromosome A by Kimber (1967), and the selected product of Sears' attempts at this manipulation was given the variety name Transfer. By linkage with other genes and also by monosomic analysis Sears (1961, 1963) was able to show that this alien chromosome segment had been translocated to chromosome 6B, that is, the chromosome of the B genome that is in wheat homoeologous group 6.

Sears (1956) originally thought that the alien chromosome segment had been intercalated into the wheat chromosome but the possibility of its transfer as a

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terminal segment was by no means excluded. Later data (Sears, 1963) showed that the alternative of the translocation being terminal was in fact more probable. Any change in the length or arm ratio of chromosome 6B in Transfer relative to the initial variety would, of course, not give any information as to the position of the alien segment. The presence of a constriction in Transfer 6B that is not present in the non-translocated 6B may be indicative of the translocation point but it is necessary to demonstrate that the chromosome material on each side of this point is in some way different.

It is, of course, possible that there is a difference in some physical property between the chromosomes of *Ae. umbellulata* and those of *T. aestivum* that would allow the alien chromosome segment to be detected. The development of the interference microscope which allows the recognition, and in some cases measurement, of very small differences in the relative phase retarding properties of material may make it possible to detect such a difference between the chromosomes of *Ae. umbellulata* and *T. aestivum*. The phase difference, if present at all, between these chromosomes does not necessarily imply any fundamental difference in the chromosome structure of the two species but simply a change in optical properties that may have arisen from one or a combination of causes.

This paper describes attempts to demonstrate such a difference and to obtain visible corroboration of Sears' genetic and cytogenetic data.

2. MATERIALS AND METHODS

(i) *Materials*

Somatic chromosomes were examined in three types of material.

(1) *Ae. umbellulata* Zhuk. a seven-chromosome wild grass of the Mediterranean region. Stocks of this species are maintained at the Plant Breeding Institute and seed were taken from these plants.

(2) *T. aestivum* L. emend. Thell. ssp. *vulgare* variety Chinese Spring ($2n = 6x = 42$). Seed taken from stocks maintained at the Plant Breeding Institute.

(3) *T. aestivum* L. emend. Thell. ssp. *vulgare* variety Transfer ($2n = 6x = 42$). This variety was produced by E. R. Sears by the X-ray induction of a translocation between the brown rust-resistant chromosome A of *Ae. umbellulata* and wheat chromosome 6B. Seed was taken from a stock of this material maintained by the Pathology Section of the Plant Breeding Institute.

(ii) *Methods*

(*a*) *Feulgen technique*

All of the somatic chromosomes examined were from root-tips prefixed in a saturated solution of mono-bromonaphthalene for 3–5 hours at room temperature and then fixed in glacial acetic acid. The Feulgen-stained material was hydrolysed in N hydrochloric acid at 60°C. for 12 min. and then placed in Feulgen reagent. Squashes were made in 45% propionic orcein.

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(*b*) *Interference technique*

The preparation schedule of the material for examination on the interference microscope was designed to be as similar as possible to the Feulgen technique but with the omission of the staining stages. Prefixation, fixation and hydrolysation were identical with the procedures described for the Feulgen technique. The root tips were then placed in fresh sulphur dioxide water and the squashes were made in 45% propionic acid. By the adoption of this technique the material had a very similar consistency to the Feulgen-stained material thus facilitating the production of the squashes but not introducing the complications of stained material into the production of an interference image.

In order to produce an unequivocal image on an interference microscope several conditions must be fulfilled. First, the material examined must not be overlaid, or lying over any other material with a heterogenous optical constitution. Secondly, the portion of the reference beam relevant to the object being examined must be free from material. Third, in this case the chromosomes being examined must be in a horizontal plane and the chromatids must not be relationally twisted. The fulfilment of all these conditions plus other technical difficulties considerably limits the selection of suitable material for examination. Chromosomes separated from compact metaphase groups are often most easily examined.

Photomicrographs were taken on high contrast 35 mm. film. The interference equipment used was a Zeiss Standard Universal Polarizing microscope with transmitted light interference objectives.

3. RESULTS

Karyotype analyses of *T. aestivum* (Morrison, 1953; Sears, 1954; Ray & Swaminathan, 1959; Sasaki, Morris, Schmidt & Gill, 1963; Gill, Morris, Schmidt & Maan, 1963) reveal that all of the chromosomes have essentially sub-median centromere positions. The most heterobrachial chromosome is 5B with an arm ratio of 2·6:1. Whilst it is possible, in particularly favourable material, to recognize this chromosome and probably also the shortest member of the set (6D) centromeric position is of little value in somatic chromosome recognition. Two of the chromosomes (1B and 6B) are satellited and can usually be recognized by this feature. Furthermore, it is possible to distinguish between these two chromosomes by the size of their satellites and their relative arm ratios (Riley, Unrau & Chapman, 1958). Chromosome 1B being the more heterobrachial and having the smaller satellites (Plate 1).

Due to differences in the contraction of the chromosomes during prefixation, the relational coiling of chromatids and the presence of overlying chromosomes, it is not possible to observe chromosomes 1B and 6B clearly in every cell. However, an examination of fifty Feulgen-stained cells of the variety Chinese Spring, in which chromosome 6B could be recognized unequivocally, never showed the presence of a constriction or knob on the long arm. An examination of a similar number of Feulgen-stained examples of chromosome 6B in the variety Transfer led to the

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demonstration of a constriction at a point approximately one-third of the length of the long arm from its distal end (Plate 1). Again, due to variation in the degree of contraction of the chromosomes, this feature was only visible in about 10% of the cells examined. If this constriction does in fact represent the translocation point at which the alien chromosome segment joins the wheat chromosome the position would be completely in agreement with the genetical data of Sears (1963); when he showed that the gene Lr 9, derived from *Ae. umbellulata*, was probably distal to Sr 11 (Timstein stem-rust resistance) which showed 45·1% crossing-over with the centromere in plants without the translocation, if the crossover distance is related to chromosome length.

Constrictions and very narrow heterochromatic regions in other cases of the translocation of alien chromosome segments to wheat have been observed (Kimber, unpublished data; Riley, personal communication). However, the identification of the constriction at a point that is consistent with the genetic data, on a chromosome that is recognizable by other features, does not, in fact, provide proof that the translocated segment is terminal in position. It is necessary to demonstrate that the material on each side of the constriction is different.

Plate 2 is an interference photomicrograph of a group of four unstained chromosomes from a cell in a root tip of the variety Transfer. The longest chromosome has a large satellite and by this feature and the size of the short arm it can be recognized as chromosome 6B. There is a sharp change in the phase-retarding properties of the chromatids of the long arm distal to the point where the constriction was observed in stained material and where a constriction is just recognizable in this particular chromosome.

Since this chromosome is not overlying or overlaid by optically heterogenous material and as the chromatids are demonstrably flat and not twisted, this change in the phase-retarding properties of the chromosome must be a reflexion of a change, however caused, of the concentration of dry matter along the length of the chromosome. Thus it can be shown that the chromosome material on each side of the constriction observed in the stained material differs, at least, in its optical properties.

As was pointed out in the description of the interference microscopy technique several conditions must be met for the production of an unequivocal image. In excess of 100 examples of chromosome 6B in the variety Transfer have been examined and of this total four were observed under unequivocal conditions. In each of these cases a terminal segment of differing optical properties was observed. A similar number of examples of chromosome 6B in the variety Chinese Spring were examined and in ten observed under unequivocal conditions it was not possible to recognize any change in the phase-retarding properties of the distal portion of the long arm.

The magnitude of the phase difference between the Transfer segment and the Chinese Spring chromosome is on the limit of the discriminating power of the interference microscope. In order to recognize a change of this magnitude it is necessary to have the two different phase-retarding materials adjacent to each other. It

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would therefore be very difficult, if not impossible, to observe convincing examples of phase differences between additions of complete alien chromosomes and wheat chromosomes in this material. Furthermore, the magnitude of the phase difference is such that the presence of a segment of such a small difference in optical performance could only be satisfactorily demonstrated in a chromosome that could be identified by another feature. These restrictions on the usefulness of the interference technique do not detract from the validity of the observations in this particular case. In other material, where larger differences in the phase-retarding properties may be demonstrable, it is possible that individual chromosomes may be recognized or isolated segments detected without reliance being placed on alternative diagnostic features.

Whilst chromosome 6B from Transfer was being examined by the interference technique the presence of a small knob on the end of the long arm was detected (Plate 3). Therefore, if the translocated segment is terminal and also is derived from a terminal segment of chromosome A of *Ae. umbellulata* it should be possible to demonstrate the same knob on chromosome A. Chromosome A of *Ae. umbellulata* is the most heterobrachial of the complement and can be easily recognized by the feature (Kimber, 1966). The examination of Feulgen-stained examples of this chromosome in cells with a reduced prefixation period revealed the presence of a knob of similar proportions to that observed distally on the long arm of Transfer chromosome 6B (Plate 5). This knob was also observed in Feulgen-stained Transfer 6B chromosome (Plate 4).

4. DISCUSSION

It was demonstrated, by the observation described in this paper, that in Feulgen-stained material a sub-terminal constriction in the long arm of chromosome 6B of the variety Transfer occurred at a point where there was a discontinuous change in the phase-retarding properties of the chromosome. Further, a terminal knob on the long arm of Transfer 6B was shown also to be present on the long arm of chromosome A of *Ae. umbellulata* from which the translocated segment of Transfer was drived.

Sears (1956) was of the opinion that the line, with the translocated segment, No. 47, which he was later to name Transfer, was intercalated into a wheat chromosome. The alternative of a reciprocal translocation was (Sears, 1963) thought to be more probable and the genetic evidence that he produced at that time showed that the gene Lr 9, that marks the translocation, is distal to Sr 11 (Timstein stem-rust resistance) and B_2 (a major awn inhibitor). The cytological data of the present publication is entirely consistent with this hypothesis.

Bhatia & Smith (1966) by an investigation of the electrophoretic mobility of the leaf and seed proteins of Transfer and Chinese Spring have shown the presence of two proteins in the seed of Transfer that are not present in Chinese Spring and one in Chinese Spring that is not present in Transfer. Also one protein was present in the leaf of Transfer that was not detected in Chinese Spring. The results of Bhatia and Smith are consistent with the introduction of an alien segment and the reciprocal

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loss of a wheat segment and therefore support the conclusions of Sears (1963) and the data of this paper.

In addition to the segment being located distally on the long arm of chromosome 6B of Transfer it was shown that the segment was derived from a terminal piece of the long arm of chromosome A of *Ae. umbellulata*. The translocated segment is some 15–17 % of the total length of chromosome 6B and probably represents about 20 % of the long arm of chromosome A. Therefore Lr 9 must be distally located on the long arm of chromosome A. It is anticipated that further data at present being collected by Kimber and Williams will allow a more precise cytological location of Lr 9 on chromosome A.

The basic change in physical properties that underlies the observed discontinuity in the phase-retarding properties of chromosome 6B of Transfer is unknown. It is possible that there is some basic difference in the chemical constitution of chromosome A, or that a different number of strands of DNA is present. It is also possible that the particular change resulting in the discontinuity is a product of an interaction of the wheat and alien chromosomes with the agent employed in the prefixation procedures. However, it is considered more probable that the difference is a reflexion of a difference in the rate of coiling or the time at which coiling is initiated.

Any change in the packing of material due to differences in the rate or timing of coiling would clearly alter the concentration of dry matter and it is simply the concentration of dry matter that is being demonstrated by the interference technique.

SUMMARY

1. Chromosome 6B of *T. aestivum*, to which a small segment of an *Ae. umbellulata* chromosome had been translocated, was examined both by interference microscopy and in stained preparations.

2. In the stained preparation a constriction was observed in the long arm of chromosome 6B and also a terminal knob that was also observed on chromosome A of *Ae. umbellulata*.

3. These features were also observed by interference microscopy and in addition the portion of the chromosome 6B of Transfer distal to the constriction had different phase-retarding properties.

4. It is concluded that the part of chromosome 6B distal to the constriction is the part of *Ae. umbellulata* chromosome A which carries the gene for the leaf-rust resistance of that species and of the wheat variety into which it was translocated.

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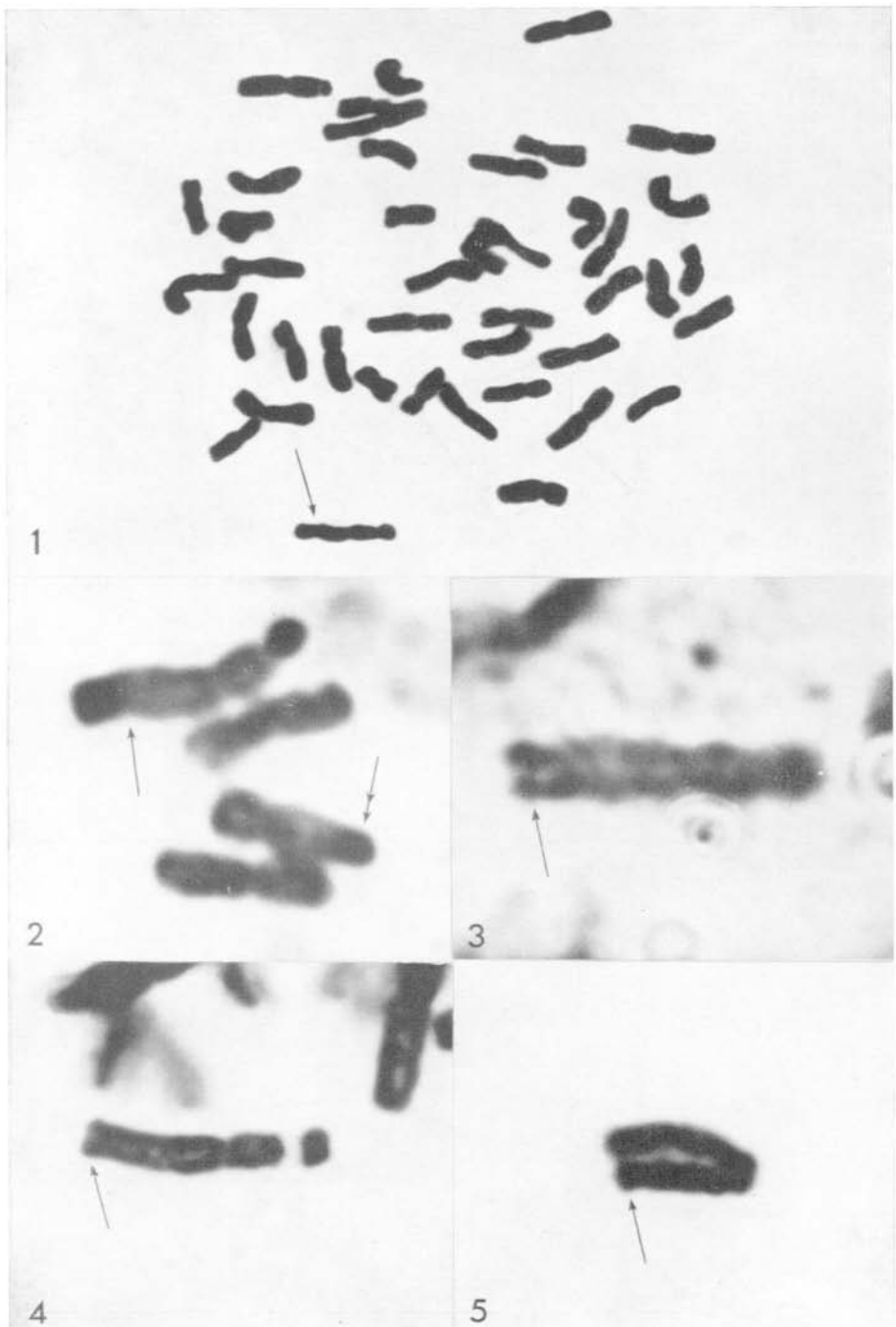

G. KIMBER

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EXPLANATION OF PLATES

Plate 1. Feulgen-stained chromosomes from a root-tip cell of Transfer. The constriction in the long arm of chromosome 6B is arrowed. The other satellited chromosome visible in this cell is 1B.

Plate 2. Interference photomicrograph of chromosome 6B (and others) in a root-tip cell of Transfer. The point of change in the phase-retarding properties of the long arm of 6B is arrowed. The dark area at the right-hand end of the chromosome marked with a double arrow is due to the relational coiling of its chromatids.

Plate 3. Interference photomicrograph of chromosome 6B of Transfer. The knob in the terminal dark segment on the long arm is arrowed.

Plate 4. Feulgen-stained chromosome 6B of Transfer. The knob on the end of the long arm is arrowed.

Plate 5. Feulgen-stained chromosome A of *Ae. umbelullata*. The knob on the end of the long arm is arrowed.

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