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Somatic Association of Telocentric Chromosomes Carrying Homologous Centromeres in Common Wheat[1]

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Summary. Measurements of distances between telocentric chromosomes, either homologous or representing the opposite arms of a metacentric chromosome (complementary telocentrics), were made at metaphase in root tip cells of common wheat carrying two homologous pairs of complementary telocentrics of chromosome 1 *B* or 6 *B* (double ditelosomic 1 *B* or 6 *B*). The aim was to elucidate the relative locations of the telocentric chromosomes within the cell. The data obtained strongly suggest that all four telocentrics of chromosome 1 *B* or 6 *B* are spacially and simultaneously co-associated. In plants carrying two complementary (6 B^S and 6 B^L) and a non-related (5 B^L) telocentric, only the complementary chromosomes were found to be somatically associated. It is thought, therefore, that the somatic association of chromosomes may involve more than two chromosomes in the same association and, since complementary telocentrics are as much associated as homologous, that the homology between centromeres (probably the only homologous region that exists between complementary telocentrics) is a very important condition for somatic association of chromosomes. The spacial arrangement of chromosomes was studied at anaphase and prophase and the polar orientation of chromosomes at prophase was found to resemble anaphase orientation. This was taken as good evidence for the maintenance of the chromosome arrangement — the Rabl orientation — and of the peripheral location of the centromere and its association with the nuclear membrane. Within this general arrangement homologous telocentric chromosomes were frequently seen to have their centromeres associated or directed towards each other. The role of the centromere in somatic association as a spindle fibre attachment and chromosome binder is discussed. It is suggested that for non-homologous chromosomes to become associated in root tips, the only requirement needed should be the homology of centromeres such as exists between complementary telocentrics, or, as a possible alternative, common repeated sequences of DNA molecules around the centromere region.

Introduction

Chromosomes are arranged in a specific manner in somatic cells of common wheat *Triticum aestivum*. Feldman, Mello-Sampayo and Sears (1966), Feldman (1966) and Avivi, Feldman and Bushuk (1969) presented evidence which clearly demonstrated that homologous chromosomes were closely associated with each other in cells of the root meristem.

It was initially stated by Feldman, Mello-Sampayo and Sears (1966) that the somatic association was not as regular as meiotic pairing and that more than two chromosomes could be associated at the same time. This association was probably due primarily to a permanent attraction between the centromeres of homologous chromosomes and involving their attachment to the nuclear envelope during interphase and prophase.

The role of the centromere in somatic association was mainly inferred from the observation that telocentrics from the opposite arms of chromosomes 2 *D* and 3 *B* were as closely associated as homologous telocentrics of the same arm. Mutual attraction of the chromosomes, which led to somatic association of telocentrics from opposite chromosome arms, appeared to be restricted to homologous centromeres which were independently derived from misdivision of common metacentric chromosomes. It followed that chromosomes would associate in somatic cells if they carried two homologous centromeres. In such a hypothesis, the genetic uniqueness of centromeres (Steinitz-Sears, 1963 and 1966) should ensure attraction exclusively between homologous centromeres.

Further support for this idea was obtained when Avivi, Feldman and Bushuk (1969), while investigating the work of Driscoll, Darvey and Barber (1967) on meiotic asynapsis caused by colchicine, found that this substance and other spindle-suppressing agents disturbed somatic association in wheat.

It appeared that the spindle fibrils which are disrupted by colchicine (Taylor, 1965 and Borisy and Taylor, 1967) might be acting as the physical binding agents which, at critical moments, brought and held together the specific centromeres of two or more chromosomes which thus became somatically associated.

I considered that investigation of the behaviour of other chromosomes in wheat would confirm whether the somatic association was primarily a feature of commonly derived centromeres.

The association of several different telocentric chromosomes from opposite arms of common metacentric chromosomes was consequently examined. The data presented here show that association occurred in every case.

[1] Dedicated to Professor Dr. Marcus M. Rhoades on his 70th birthday.

Material and Methods

Two different strains of *Triticum aestivum* variety Chinese Spring, simultaneously carrying both arms of a common chromosome as separate telocentric units, were kindly supplied by Dr. E. R. Sears. They were double ditelosomic 1 B and 6 B, respectively ($2n = 44$), in which both the short satellite arm and the long arm (1 B^S and 1 B^L or 6 B^S and 6 B^L) of the same chromosome were represented by a pair of homologous telocentrics. In both cases (Figs. 1 and 2), telocentrics for the same chromosome arm (homologous telocentrics) were morphologically identical, whereas those for opposite arms (complementary telocentrics) were distinct from each other.

A third strain, with $2n = 43$ chromosomes (Fig. 3), was added to this series. It carried a pair of complementary telocentrics for chromosome 6 B (6 B^S and 6 B^L) and a telocentric for the long arm of chromosome 5 B (5 B^L). Whole chromosomes 5 B and 6 B were also present in the complement of this strain. Telocentric 5 B^L represents the largest arm of the chromosome complement of wheat and it is clearly distinguishable from either 6 B^S or 6 B^L. This strain was named heterosomic 5 B—6 B (or hetero 5 B—6 B) because of the heteromorphic representation of both chromosome 5 B and chromosome 6 B in the complement.

Seeds were set to germinate in Petri dishes at 22 °C. Roots 1—3 cm long were severed and the tips immersed in tap water at 0 °C for 24 h and then fixed. The aceto-carmine smearing technique was followed.

Evaluation of the somatic association between telocentric chromosomes was carried out on cells in which the chromosomes were distributed in a circle (Figs. 1, 2, and 3). The effective distance between telocentrics was mea-

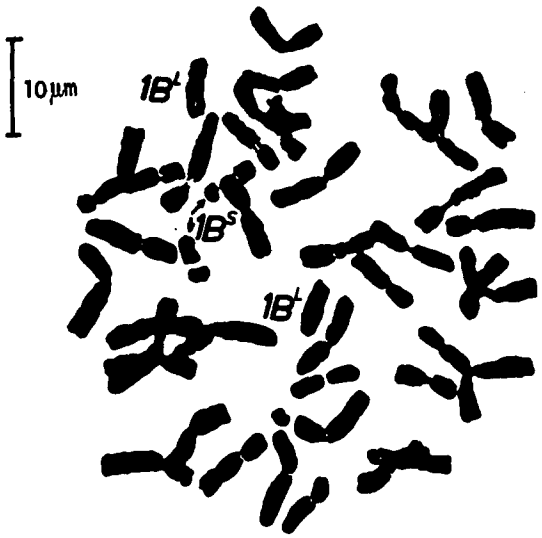

Fig. 1. Cold treated metaphase plate of *Triticum aestivum* double ditelosomic 1 B. Telocentric chromosomes are indicated

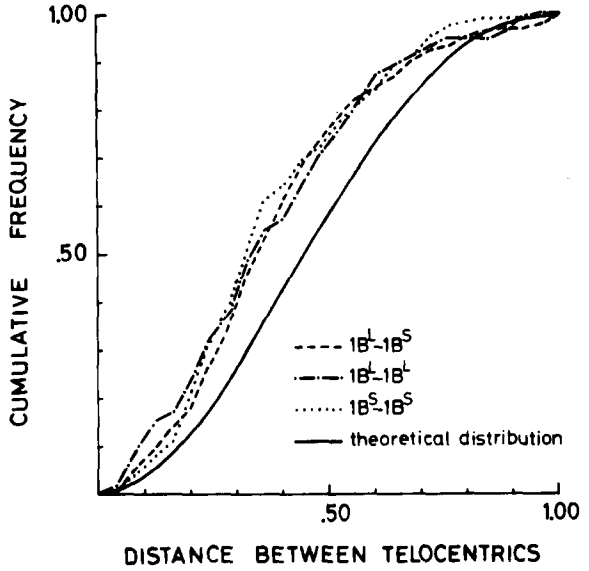

Fig. 1a. Expected and observed cumulative frequencies of distances between two telocentric chromosomes in *T. aestivum* double ditelosomic 1 B

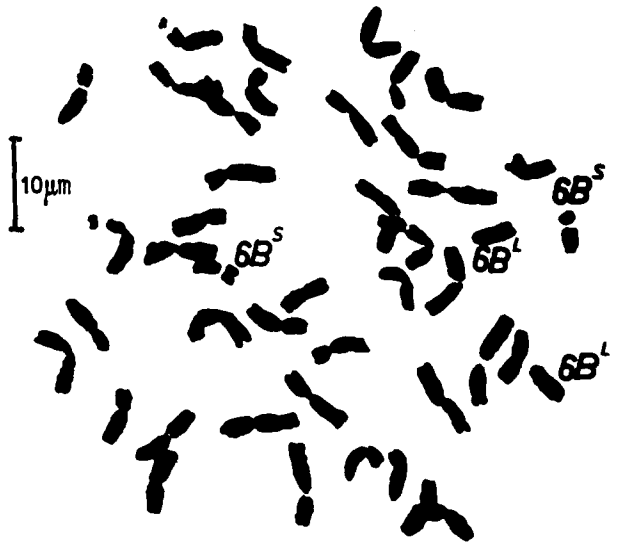

Fig. 2. Cold treated metaphase plate of *T. aestivum* double ditelosomic 6 B. Telocentric chromosomes are indicated

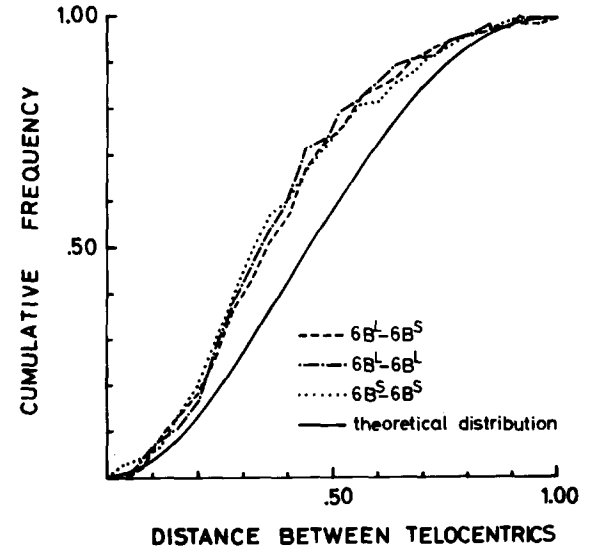

Fig. 2a. Expected and observed cumulative frequencies of distances between two telocentric chromosomes in *T. aestivum* double ditelosomic 6 B

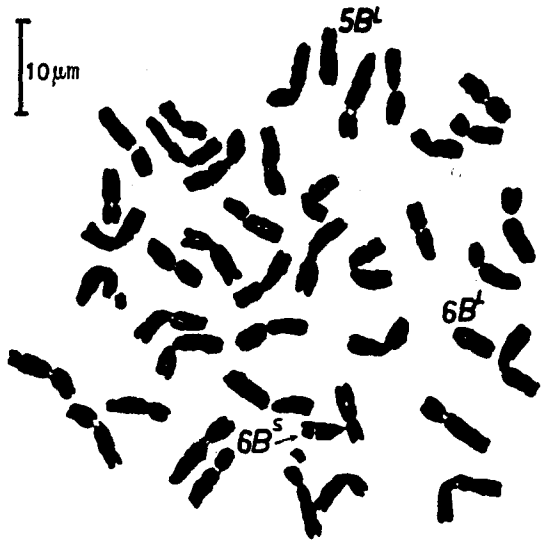

Fig. 3. Cold treated metaphase plate of *T. aestivum* heterosomic 5 *B* — 6 *B*. Telocentric chromosomes are indicated

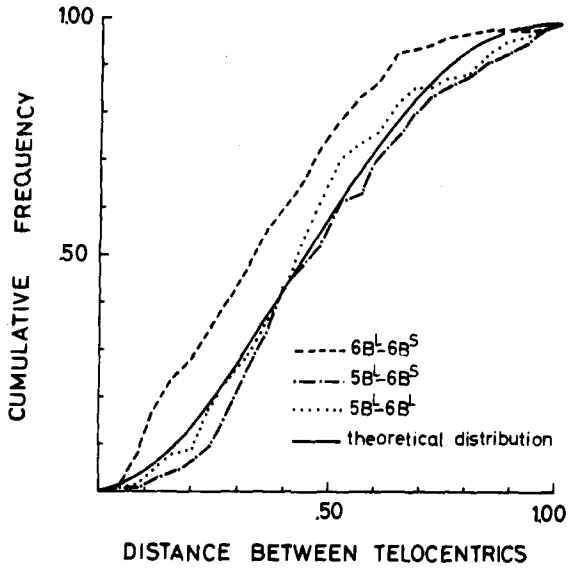

Fig. 3a. Expected and observed cumulative frequencies of distances between two telocentric chromosomes in *T. aestivum* heterosomic 5 *B* — 6 *B*

sured in each cell and divided by the distance between the two chromosomes that were farthest apart. This would compensate for the irregular pressure over the cells. These distances were then plotted in cumulative frequency curves, together with those theoretically ascribed to a pair of randomly distributed chromosomes. The Kolmogorov-Smirnov One-Sample and Two-Sample Tests for goodness of fit (Siegel, 1956) were applied to these curves. The distribution of distances between two randomly positioned chromosomes has been calculated (Feldman, Mello-Sampayo and Sears, 1966). Because the strains carried at least three morphologically recognizable chromosomes, it was also possible to perform direct systematic comparisons within the same cell of distances between two or three different telocentric chromosomes. For this purpose the Wilcoxon Matched-Pairs Signed-Rank Test (Siegel, 1956) was used.

The methods and the statistical tests have been described in detail by Feldman, Mello-Sampayo and Sears (1966).

Normal Chinese Spring plants and others that were ditelosomic for chromosomes 3 *D* and 7 *D* were also used for morphological studies. In this material the Feulgen squash method was used, after fixation with Carnoy 6:3:1.

Results

1. *An Evaluation of the Somatic Association of Chromosomes*

a. *Homologous and complementary chromosomes.* In both double ditelosomic 1 *B* and 6 *B* the mean distance between homologous (1 B^L — 1 B^L, 1 B^S — 1 B^S, 6 B^L—6 B^L and 6 B^S—6 B^S) or complementary (1 B^S—1 B^L, 6 B^S—6 B^L) telocentrics is less than the 0.453 (Table 1) calculated (Feldman, Mello-Sampayo and Sears, 1966) for randomly located chromosomes. In no case did the distance between either homologous or complementary chromosomes show a significant difference when compared two by two (Table 2), even when the significance level was raised to 0.40. The distance between every pair of homologous or complementary telocentrics showed a significant difference from the calculated random distribution (Table 3). The deviation to the left of the theoretical curve can easily be seen for each of the crescent shaped cumulative frequency curves of distances between pairs of telocentrics (Figs. 1a and 2a).

Table 1. *Mean distance between telocentric chromosomes in root tip cells of different strains of Triticum aestivum variety Chinese Spring*

Matched chromosomes	Mean distance*	Variance*	Sample size
Double ditelo 1 *B*			
1 B^S—1 B^S	.364	.039	100
1 B^S—1 B^L	.378	.044	100
1 B^L—1 B^L	.379	.047	100
Double ditelo 6 *B*			
6 B^S—6 B^S	.376	.048	100
6 B^S—6 B^L	.382	.040	100
6 B^L—6 B^L	.372	.039	100
Hetero 5 *B*—6 *B*			
6 B^S—6 B^L	.351	.043	200
6 B^S—5 B^L	.488	.045	200
6 B^L—5 B^L	.452	.048	200

* For two randomly distributed chromosomes, the *mean distance* is equal to .453 and the *variance* .045 (Feldman, Mello-Sampayo and Sears, 1966).

It can be concluded that in both double telosomics 1 *B* and 6 *B*, all four telocentrics derived from the arms of chromosomes 1 *B* and 6 *B* tend to remain associated in a cluster, at a distance from each other that is shorter than that between two randomly located chromosomes.

b. *Complementary and non-related telocentrics.* Hetero 5 *B*—6 *B* plants carry, simultaneously, three

Table 2. *Wilcoxon Matched Pairs Signed-Rank Test for distances between cytologically marked (telocentric) chromosomes (Hypothesis of equality between every two compared distance; two tailed test except when otherwise indicated; sample size is* 100 *expect for hetero* 5 B—6 B *in which case it is* 200)

Distances compared	Probability values
Double ditelo 1 B	
1 B^S—1 B^S/1 B^L—1 B^L	.837
1 B^S—1 B^S/1 B^S—1 B^L	.967
1 B^L—1 B^L/1 B^S—1 B^L	.655
Double ditelo 6 B	
6 B^S—6 B^S/6 B^L—6 B^L	.935
6 B^S—6 B^S/6 B^S—6 B^L	.889
6 B^L—6 B^L/6 B^S—6 B^L	.949
Hetero 5 B—6 B	
6 B^S—5 B^L/6 B^L—5 B^L	.417
6 B^S—6 B^L/6 B^S—5 B^L	.00003*
6 B^S—6 B^L/6 B^L—5 B^L	.00003*

* One tailed test; the alternative hypothesis is that the distance between complementary telocentrics (6 B^S — 6 B^L) is shorter than between non-related telocentrics (6 B^S — 5 B^L) and 6 B^L — 5 B^L).

Table 3. *Kolmogorov-Smirnov One Sample Test applied to distances between cytologically marked (telocentric) chromosomes*

Matched chromosomes	Sample size	D value
Double ditelo 1 B		
1 B^S—1 B^S	100	.248*
1 B^S—1 B^L	400	.591*
1 B^L—1 B^L	100	.188*
Double ditelo 6 B		
6 B^S—6 B^S	100	.208*
6 B^S—6 B^L	400	.171*
6 B^L—6 B^L	100	.251*
Hetero 5 B—6 B		
6 B^S—6 B^L	200	.188*
6 B^S—5 B^L	200	.089
6 B^L—5 B^L	200	.087

* Significant deviation (.05 level) from the theoretical random distribution.

different and perfectly recognizable telocentrics: 5 B^L 6 B^S and 6 B^L (Fig. 3).

When the distances between chromosomes were compared, it was found (Table 2) that those between complementary telocentrics (6 B^S—6 B^L) were significantly (at 0.00003 level) less than those between non-related telocentrics (6 B^S—5 B^L and 6 B^L—5 B^L). The mean distance (Table 1) between complementary telocentrics was 0.351, whereas those between the non-related telocentrics were 0.488 and 0.452, much closer to the random distribution (0.453). As expected (Table 3 and Fig. 3a), the deviation from the random curve is significant for the complementary chromosomes whereas the curves for non-related telocentrics closely follow that of randomly distributed chromosomes.

It is deduced that the complementary telocentrics of chromosome 6 B tend to stay closely associated with each other, which confirms the earlier conclusions for double ditelosomic 6 B. However, in this case it has also been shown that the complementary 6 B^S and 6 B^L telocentrics are randomly positioned relative to a non-related telocentric 5 B^L. Furthermore, these measurements were made on the same cells where the close association was observed.

2. *The Spacial Arrangement of Chromosomes within the Nucleus*

Feldman, Mello-Sampayo and Sears (1966) suggested that the somatic association of chromosomes detected at metaphase by their sensitive statistical method was a residual expression of an intimate association at interphase, which is a stage of little or no chromosome movement (for review, see Comings, 1968).

Direct observation of chromosome arrangement at interphase is not possible in wheat because of their highly despiralized condition. However, the way they are positioned at that stage can be inferred from the highly suggestive configurations they show before and after interphase.

Homologous telocentric chromosomes were seen to be associated at anaphase by their centromeres. In several cases, as the chromosomes proceeded to the poles led by their centromeres, daughter homologous telocentrics were observed being pulled to the same polar regions with their centromeres turned towards each other, as if converging on a common point (Fig. 4). It is therefore very likely that homologous chromosomes end up closely associated by their centromeres at the polar region.

Early in prophase the chromosomes were seen to have a polar orientation. The morphological appearance of prophase nuclei suggests that this arrangement — the so-called Rabl orientation — results from the maintenance of late anaphase configuration. Sometimes, prophase chromosomes were found to be polarly arranged in a truncated cone-shaped configuration (Fig. 5). Homologous telocentrics at prophase also tended to remain closely associated (Fig. 6), suggesting the polar pattern of the chromosome set at anaphase.

The polarized chromosomes at prophase were apparently attached by their centromeres and proximal regions to a peripheral zone of the nucleus, perhaps to the nuclear membrane (Fig. 7).* Favourable cells (Fig. 8) at final prophase also showed a very persistent chromosome polarity only broken at the last moment after the desintegration of the nuclear envelope and spindle formation. In this short transitional state also, the centromeres were seen to be in

* At this stage, chromosome ends were seen also closely associated with the nuclear membrane at the opposite hemisphere and sometimes attached two-by-two, as found by Wagenaar (1968 and 1969) in other materials.

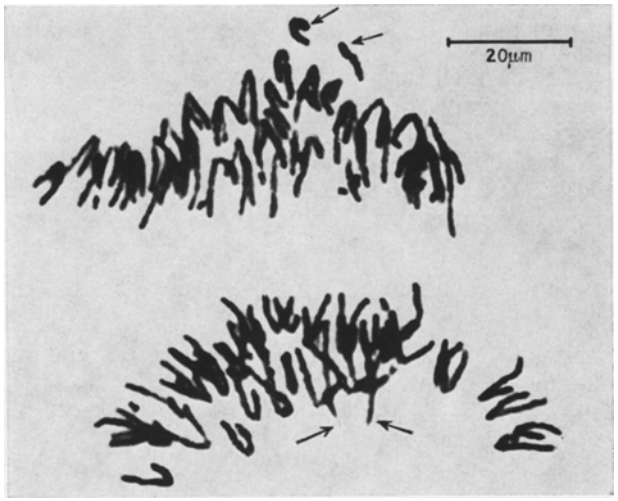

Fig. 4. Anaphase in ditelosomic 3 *D*. Arrows indicate the centromeres of associated telocentric 3 *D* chromosomes

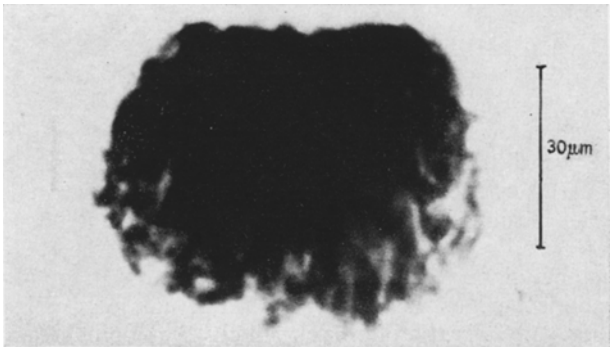

Fig. 5. Anaphase-shaped prophase in normal wheat

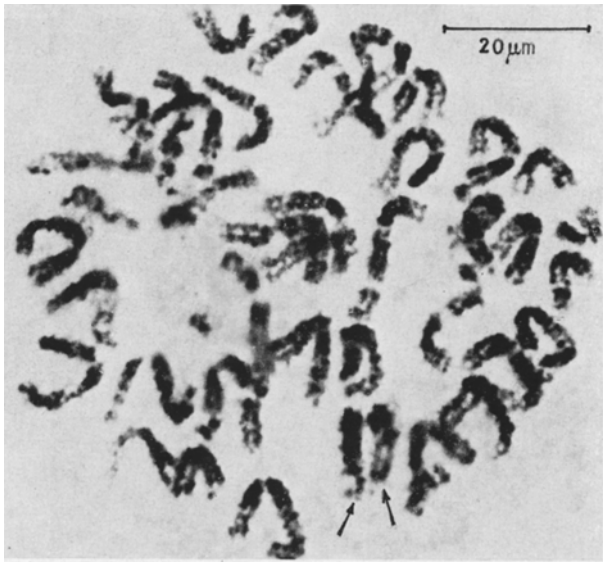

Fig. 6. Prophase in ditelosomic 7 *D*. Arrows indicate the proximal region of two associated 7 *D* telocentrics

Figs. 4 to 6. Mitosis in root tips of *T. aestivum*

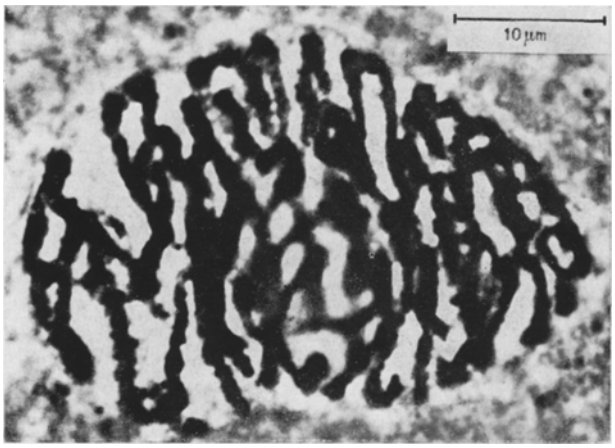

Fig. 7. Contrast phase picture showing centromeres and chromosome ends oriented toward diametrically opposite locations on the inner surface of the nuclear envelope

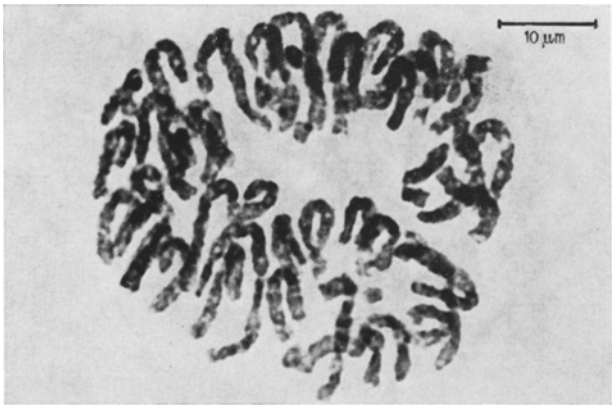

Fig. 8. A similar arrangement at more advanced stage prior to nuclear membrane disruption

Figs. 7 to 8. Middle and late prophase in root tips of *T. aestivum* showing polar orientation of chromosomes

a circular arrangement at the periphery of the nucleus (Fig. 8).

Discussion

The results presented in this paper clearly indicate that, in double ditelosomics, complementary and homologous telocentric chromosomes are associated with each other forming clusters of four chromosomes. Because 1 *B* and 6 *B* are nucleolar chromosomes, a pair of homologous nucleolar telocentrics are involved in double ditelosomics 1 *B* and 6 *B*. The possibility that their direct relation to the nucleolus may be a cause of their association does not rule out the general conclusion that homologous and complementary telocentrics are closely located in the same cell and that this association is independent of the nucleolus. A non-nucleolar pair of homologous telocentrics is carried by these ditelosomics, and they equally belong to the same group-of-four. The equal and simultaneous association of these chromosomes with each other and with their complementary partners eliminates any doubt about the independence of

somatic association. The same independence was also found in chromosomes of *Avena* by Sadasivaiah, Watkins and Rajhathy (1969).

The additional data obtained with heterosomic 5 *B*—6 *B* can be used as a complementary test to study the position of two complementary telocentrics in relation to a third non-related telocentric. This was found to be random.

It is probable that both complementary telocentrics tend to be associated with their normal metacentric partners. The three chromosomes would, in this case, be brought together into a cluster in a similar way to that found in double ditelosomics.

Eventually this association of both complementary telocentrics with the opposite arms of their corresponding metacentric would strengthen rather than loosen their mutual association. It is reasonable to assume that this would not significantly change their spacial relationship with other non-related chromosomes of the complement.

Feldman, Mello-Sampayo and Sears (1966) pointed out the primordial role of centromeres in the somatic association of chromosomes. The centromere is apparently the only homologous part that can be carried in common by two complementary telocentric chromosomes.

Probably in both double ditelosomics 1 *B* and 6 *B*, the complementary telocentrics, coming from relatively stable ditelosomic lines, carry complete, or almost complete, homologous centromeres (Steinitz-Sears, 1966). Their homology would secure genetic specificity to hold them closely associated.

Feldman, Mello-Sampayo and Sears(1966) supported the hypothesis of Metz (1916) and Kitani (1963), that chromosomes are intimately associated at interphase. Our material indicates an anaphase convergence of centromeres of homologous telocentrics. Through this mechanism, homologous centromeres move and become closely attached at sites on the nuclear membrane of telophase nuclei and appear to be held in this position until the next prophase by microtubular material, possibly the chromosomal fibers that keep the centromeres attached to the nuclear membrane (Feldman, 1966). On this assumption, anaphase would be a critical stage in bringing together, by their homologous centromeres, the loosely associated chromosomes of metaphase.

It is therefore not surprising that colchicine and other spindle-disrupting substances affect somatic association (Driscoll, Darvey and Barber, 1967, Avivi, Feldman and Bushuk, 1969, Burgess and Northcote, 1969). These agents would upset in several ways the normal positioning of homologous centromeres in the nuclear membrane, the orderly convergence of centromeres to the poles being commonly disturbed.

Centromere association seems to be very difficult to detect by direct observation at interphase in normal somatic cells. Chauhan and Abel (1968) and Maguire (1967) presented examples of two-by-two association of homologous heterochromatic segments at interphase. If in the first case centromeres as well as proximal regions can be taken as involved in the association, Maguire's maize material showed associated knobs. Evidence was also obtained that chromosomes are somatically associated at the ends (Sved, 1966, Wagenaar, 1969).

These examples suggest that chromosomes are associated during distended stages at regions other than the centromeres. The heterochromatic knobs and ends are points of occasional neocentric activity (Rhoades, 1952, Bajer and Östergren, 1961). This requires temporary spindle fibre attachment. The chromosomes may even be assumed to be loosely coaligned along their entire length (Comings, 1968, Feldman, 1966). The centromere can be considered to be the only part of the chromosome persistently connected by fibres. These are probably involved in transmitting the orderly arrangement of chromosomes from one prophase to the next telophase. The nuclear envelope and spindle are two alternative cell structures which may secure the somatic association of chromosomes.

White (1954) believed that all chromosomes were two-armed. However, since it was found that the centromere was a compound structure (Lima-de-Faria, 1949 a and b, 1950, Tjio and Levan 1950 a and b) and that a telocentric could be viable even when carrying at its kinetic end a sub-unit of the original broken centromere (Müntzing and Lima-de-Faria, 1953 and Lima-de-Faria, 1954), it seemed likely that telocentrics were occasionally formed by the normal processes of transverse breakage at the centromere region. Naturally occurring telocentrics have subsequently been found in several plant and animal species (Tjio and Levan, 1954, Gimenez-Martin, 1962, and Gimenez-Martin and Lopez-Saez, 1966, Battaglia, 1964, John and Hewitt, 1966, and John and Lewis, 1968).

Whenever several telocentrics occur in the same somatic cell, it is likely that homologous or complementary elements will tend to be somatically associated by their homologous centromeres. Centromere attachments between telocentrics were found to occur at metaphase in *Oxalis dispar* (Marks, 1957) and *Cloeon dipterum* (Wolf, 1960), and Bennett (1966) observed two-by-two association between non-satellited telocentrics in mice.* It can be seen that somatic association does not necessarily imply contact or even very close proximity in all cases, so that many more such associations between chromosomes might have been detected had the chromosomes been conveniently identified or the proper statistical methods

* It is also likely that related acrocentric chromosomes may remain associated by their homologous centromeres which occasionally have been found to be fused (Hsu, 1969).

used.* Feldman, Mello-Sampayo and Sears (1966) suggested that the somatic association of chromosomes might persist throughout the life cycle of the individual. In pre-meiotic cells, somatic association of homologous chromosomes would correspond to the spacial arrangement necessary for meiotic pairing and synapsis.

Attraction between homologous centromeres should not be considered sufficient for the meiotic pairing of homologous chromosomes: other homologous parts of the chromosomes, such as the potentially motile sectors (heterochromatic blocks, chromosome ends, etc). might have the same function of bringing the chromosomes close together. However, their functioning might be retarded or minimized until later stages and they would become operative only in sporogenous or gonadal tissues, with full expression at the onset of meiosis (Brown and Stack, 1968).

Using this assumption, the meiotic association of chromosomes would be the integrated effect of somatic attraction at several sites along the chromosomes from the centromere to the ends. It is likely that homologous chromosomes finally come into contact at all these points of mutual attraction shortly before synapsis. Probably only homologous centromeres associate in early generations of cells and this might not be important or necessary for the somatic association of chromosomes in pre-meiotic cells. A rather impressive demonstration of independence between centromeres of complementary chromosomes at the pre-meiotic stages is given by the fact that bivalents of complementary chromosomes in both the double ditelosomics 1 *B* and 6 *B* do not show secondary association in the first division of meiosis (Sears, personal communication).

In a recent article, Darvey and Driscoll (1972) presented extensive data about the relative positions of chromosomes in the root tips of hexaploid wheat. According to their results, the homologous chromosomes 1 *B*, 1 B^L or 6 B^S do not associate in root tips in individuals which were ditelosomic for the long arms of the same or the alternate satellited chromosome. This strongly suggests that, in these tissues, distance relationships between homologous satellited chromosomes, or between telocentrics for their arms, may be disturbed when the genetic background of the cells does not correspond to that of a complement carrying complete members of the alternate nucleolar chromosome. The close association of homologous 6 B^S chromosomes found by the same authors in double ditelosomic 6 *B* material seems to confirm this hypothesis.

This is also shown by our material, and the clustering of homologous and complementary chromosomes in double ditelosomics found by us can hardly have been denied since the positioning of chromosomes was double checked at least for the complementary chromosomes in the hetero 5 *B*—6 *B* strains.

The finding of the primary rôle of the centromere in root meristems sheds new light on the problem of somatic association of chromosomes. In these tissues complementary telocentrics are able to associate by their homologous centromeres, so it is highly probable that other non-homologous chromosomes may also become close to each other provided they have homologous centromeres. Data in animals, with *in situ* hybridization of radioactive nucleic acids with the DNA of chromosomes (Pardue and Gall, 1970, Jones and Robertson, 1970 and McGregor and Kezer 1971), indicated that the region involving the centromere in non-homologous chromosomes may have common sequences of repetitive DNA molecules. It is highly probable that these chromosomes have, at least in the early stages, a tendency to become associated by the centromere region when their repetitive DNA content exceeds the amount needed for their homology to be expressed.

Although wheat chromosomes were not tested for repetitive sequences of DNA, it is possible that some of the non-homologous chromosomes have such homologous centromeres. This could explain the somatic association of non-homologous telocentrics found by Darvey and Driscoll (1972) in their experiments with root tip meristems of wheat.

* It is also very tempting to suggest that many of the centric fusions reported in the literature might have occurred between somatically associated homologous centromeres.

Acknowledgements

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Literature

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