MECHANISMS OF PAIRING IN RYE AND TRITICALE

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Julian Bruce Thomas

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JULIAN BRUCE THOMAS

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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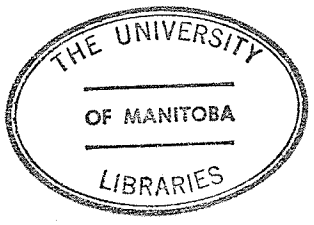

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FOREWORD

This thesis is written in the paper style, specified in the 1976 Plant Science Thesis Preparation Guide. It contains four manuscripts. The first, entitled "The genomic origin of the unpaired chromosomes in triticale", was published in the Canadian Journal of Genetics and Cytology, volume 18, pages 687 to 700. The second, entitled "A bouquet-like attachment plate for telomeres in leptotene of rye revealed by heterochromatin staining" was published in Heredity, volume 36, pages 155 to 162. The third, entitled "The effect of colchicine on chromosome pairing", was published in the Canadian Journal of Genetics and Cytology, volume 19, pages 231 to 249. The fourth paper, entitled "The effect of chromosomes 1B and 6B on nucleolus formation and first metaphase chiasma frequency in hexaploid triticale" is currently under review by the Canadian Journal of Genetics and Cytology.

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ABSTRACT

Triticale plants, aneuploid for chromosomes 1B and 6B were used to investigate the pattern of nucleolus formation in hexaploid triticale. Active chromosome movement within the meiotic nucleus was investigated using both nucleoli and telomeric heterochromatin as interphase and prophase markers for the chromosomes. Chromosomes of triticale which were unpaired at first meiotic metaphase (MI) were identified as to their genome of origin by use of differential staining of telomeric rye heterochromatin and telocentric chromosomes.

Nucleolus formation in hexaploid triticale was restricted to chromosomes 1B and 6B of the wheat genome. Complete fusion of all nucleoli occurred between late premeiotic interphase and early zygotene in rye, in triticale and in hybrids between durum wheat and hexaploid triticale (pentaploid triticale). During nucleolus fusion in rye and triticale a chromocenter was formed out of all or most of the telomeric rye heterochromatin. Formation of the chromocenter occurred on an annular shaped region of the nuclear membrane, adjacent to the tapetum/archesporium interface. Inhibition of chromosome pairing following injection of a 0.03% solution of colchicine into the boot

triticale is probably unrelated to any of the principal control points of meiotic pairing that have so far been described in the Triticinae. Instead it was suggested that chiasma formation in triticale tends to be more terminal than it is in rye. Since chiasmata are unlikely to be formed in heterochromatin, the result would be a low frequency of chiasmata in those rye chromosomes with the most terminal heterochromatin.

INTRODUCTION

Triticale (X Triticosecale Wittmack) refers to the hybrid complex derived from the crossing of members of the wheat genus (Triticum L.) with the rye genus (Secale L.) (Gustafson, 1976). Triticale is believed to have certain potential as a cereal crop in its own right (Hulse and Spurgeon, 1974; Zillinsky, 1974; Zillinsky and Borlaug, 1971). In general, triticales suffer from four reproductive disorders. These are meiotic irregularity, partial sterility, endosperm shrivelling and premature sprouting of the embryo (Gustafson, 1976; Zillinsky and Borlaug, 1971). The principal symptoms of meiotic instability are the presence of univalents at first meiotic metaphase and the high frequency of aneuploids in general populations of triticale (Kaltsikes, 1974; Scoles and Kaltsikes, 1974; Tsuchiya, 1974). This thesis deals with the topic of meiotic instability in triticale and general mechanisms of pairing in the Triticinae.

REVIEW OF LITERATURE

General Cytology of Triticale

The Chromosomes of Rye and Wheat

In a general way the karyotypes of rye and wheat are similar. Chromosomes of the A and B genomes are mostly median or submedian (Giorgi and Bozzini, 1969) whereas most of the rye chromosomes are submedian or subterminal (Bhattacharyya and Jenkins, 1960; Heneen, 1962). The nucleolus organizing chromosomes are satellited close to the short arm telomeres in both species. There are two pairs of prominent satellites in wheat (AABB), and one pair in rye (RR). Following inclusion into triticale, the prominent secondary constriction of the main rye satellite becomes indistinct. This may have led to some confusion in the karyotype analysis of triticale (Merker, 1973; Shigenaga and Larter, 1971). The major distinction between wheat chromosomes and rye chromosomes is in their size. Per chromosome, the rye genome contains about 40% more DNA than the wheat genome (Kaltsikes, 1971). This size differential is maintained in triticale, where the rye chromosomes are conspicuously larger than most wheat chromosomes (Merker, 1973; Pieritz, 1970; Shigenaga and

Larter, 1971). This is in accordance with the conclusions of Pegington and Rees (1970) that the DNA content was linearly related to both chromosome mass and volume, and that none of these characters was disturbed through incorporation into a polyploid. In one long established triticale line however, the DNA content of the nucleus was significantly below the sum of the two parental cultivars (Kaltsikes, 1971).

Particular segments of wheat and rye chromosomes can be differentially stained with what are known as "C" band techniques (Gill and Kimber, 1974a; Sarma and Natarajan, 1973; Verma and Rees, 1974). "C" band techniques were discovered when it was found that the centromere and other areas could be specifically stained with Giemsa, after procedures modified from those used in the in situ hybridisation of DNA and RNA (Arrighi and Hsu, 1971, Pardue and Gall, 1970; Yunis et al., 1971). Because areas stained by these techniques were known to be constitutive heterochromatin on other criteria, it was logical to call them "C" bands (Cooper and Hsu, 1972; Hsu, 1971; Hsu and Arrighi, 1972; Schweizer, 1973). "C" bands around the centromeres of mouse chromosomes contain highly repetitive DNA sequences that are homologous to the light (A-T rich) satellite of mouse DNA (Jones, 1970; Pardue and Gall, 1970). Because of the association between repetitious DNA and the

"C" bands it was proposed that the technique depended on the staining of preferentially re-annealed repetitious DNA. However, in addition to repetitive DNA, constitutive heterochromatin may carry a large proportion of unique sequences (Comings, 1973; Ockey, 1973). Also *in situ* re-annealing of DNA is rapid in all segments of the chromosome, although "C" bands may be fastest (de la Chapelle *et al.*, 1973; Comings, 1973). The weight of evidence is that the final basis of "C" banding is some protein-DNA interaction specific to constitutive heterochromatin (Comings, 1973; Comings *et al.*, 1973).

Large heterochromatic blocks occupy up to 11 out of the 14 telomeres of the rye genome (Darvey and Gustafson, 1975; Merker, 1975; Vosa, 1974). These large terminal segments in rye are late replicating relative to the median and proximal euchromatin, to chromatin around the centromeres and to the chromatin of B chromosomes (Ayonoadu and Rees, 1973; Darlington and Haque, 1966; Lima-de-Faria and Jaworska, 1972). Wheat chromosomes lack the large terminal blocks of heterochromatin that are characteristic of rye chromosomes (Gill and Kimber, 1974b). However, in both wheat and rye chromosomes many small intercalary bands are found, including variable staining of the centromere. In addition a band adjacent to the nucleolus organising regions of chromosomes 1B, 6B and 1R stains with Giemsa.

The function of the large terminal blocks of heterochromatin found in rye is unknown. A large quantity of highly repetitive DNA has been isolated from the DNA of rye (Ranjekar et. al., 1974) and it is probable that this repeated DNA is located in the terminal "C" bands. Wild species of rye tend to have less DNA per chromosome and/or smaller terminal bands than those found in cultivated rye (Bennett et al. 1977).

Nucleolus Formation in Triticale

In octoploid (8x) triticale, Shkutina and Khvostova (1971) found four bivalents attached to the nucleoli in diakinesis, and a maximum of seven to eight nucleoli per root tip cell. In hexaploid (6x) triticale, they found only two bivalents attached to the nucleoli in diakinesis, but in particular 6x derivatives of octoploid x hexaploid crosses, they found three bivalents attached to the nucleolus. Darvey found that only two pairs of organisers were active in root tip cells of primary hexaploid triticales (Darvey, 1973) but that in the case of Chinese Spring x Imperial rye (8x), the 6R chromosome also formed nucleoli in addition to the activity of the wheat chromosomes 1B, 6B and 5D (Darvey, 1972; Driscoll, pers. comm.). No nucleolar activity of the principal nucleolus organising chromosome of rye, chromosome 1R has been demonstrated in triticale.

Meiotic Cytology

The Leptotene Bouquet

The leptotene bouquet is formed when points at which the chromosomes are attached to the nuclear membrane, become concentrated on a restricted area of the nuclear membrane (attachment plate of Kaufmann, 1925); in most cases this grouping of attachment sites involves the telomeres (Wilson, 1934). At some point in time, the regions of the chromosome adjacent to this attachment plate can become orientated parallel to each other and perpendicular to the attachment plate (Marengo, 1949), or they may acquire an appearance of "whirling" around the attachment plate (Hiraoka, 1941).

Synapsis usually begins in the chromosome regions close to the attachment plate and spreads along the polarised threads toward the interior of the nucleus (Darlington, 1937; Hiraoka, 1941; Rasmussen, 1976; Wilson, 1934). Since the bouquet is usually recognised from the characteristically polarised chromosomes, it should be remembered that there is some polarisation in all interphase nuclei. Because centromeres are drawn to the pole at anaphase, the nuclei begin interphase with the centromeres closely grouped at the polar end of the nucleus and the telomeres loosely grouped at the equatorial end (Rabl orientation, Rieger *et al.*, 1968). In wheat, this polarisation commonly persists from one mitosis to the next (Darvey, 1972).

In the presence of a leptotene bouquet in both Psilotum (Hiraoka, 1941) and Onoclea (Marengo, 1949), the entire leptotene nucleus was displaced toward the cell margin on the side of the attachment plate. Thus there is a mechanical interaction between the nucleus and the cytoplasm associated with the bouquet. When a centriole is present in the cytoplasm, it is found close to the attachment plate of the bouquet (Wilson, 1934). A variety of changes may occur in the astral activity around the centriole, which correlate with polarisations of chromosomes within the prophase nucleus. In mantids, the attachment plate of the leptotene bouquet to the nuclear membrane was formed close by an active centriole complex in the cytoplasm (Hughes-Schrader, 1943). After the aster disappeared in mid-prophase, bouquet polarisation was lost. When asters reappeared in diplotene, telomeres were again attracted toward them. Consequently, the bivalents in the nucleus were polarised toward the asters in the cytoplasm by their telomeres. Polarisation lapsed as the nuclear membrane dissipated at diakinesis. At the same time the centromeres became active and began to orient the bivalents for meiotic metaphase. Perhaps, during the meiotic cycle the telomeres have an ability to interact with a spindle-like system in the cytoplasm but only through association with the nuclear membrane.

Centromere-like activity (neocentric activity) of particular telomeres has been found in the meiosis of rye. In certain inbreds of rye, particular telomeres have the ability to polarise part or all of the chromosome to one pole or the other during either first or second meiotic metaphase (Prakken and Muntzing, 1942; Rees, 1955). During root tip divisions these telomeres were inactive (Prakken and Muntzing, 1942). Therefore, neocentric activity is probably unique to the metaphases of meiosis. Incomplete degradation of membrane attachments after meiotic prophase could allow the telomeres to retain properties normally concerned with the processes of chromosome pairing and so behave like centromeres on the meiotic spindle (Ostergren and Prakken, 1946).

Meiotic Cytology of Cereals

The premeiotic interphase is initiated by a G1 hold of the asynchronous archesporium (Bennett et al., 1973). The premeiotic interphase can be divided into three segments based on the staining and appearance of the interphase nuclei. Pollen mother cells (PMC) in the first stage of the premeiotic interphase (S1) contain large nuclei that stain poorly with acetocarmine (Bennett et al., 1973). During the second period of the premeiotic interphase (S2) the nuclei are quite round and stain darkly with acetocarmine. The last period of the premeiotic interphase (S3)

was reported by Bennett and coworkers (Bennett and Smith, 1972; Bennett et al., 1971, 1973) to be the period of DNA replication (premeiotic "S"). PMC in the premeiotic interphase contain one to several nucleoli and at some point in the meiotic cycle, the average number of nucleoli per PMC is reduced to one by a process of active nucleolus fusion (Bennett et al., 1973). Bennett et al. (1973) assigned the period of nucleolus fusion to the S3 period. Other workers (Darvey, 1972; Darvey et al., 1973; Roupakias and Kaltsikes, 1977a) assigned nucleolar fusion to leptotene. The number of nucleoli per PMC nucleus is easily determined whereas the visual criteria of leptotene are descriptive and subject to individual differences in interpretation (Bennett, pers. comm.). It therefore appears that the S3 period has been assessed as leptotene by some workers and as interphase by others.

The description of meiosis codified by Wilson (1934) is applied to the cereals (Bennett et al., 1973). When chromosome threads first appear they are single (leptotene). Later on, double stretches appear, sometimes connected to pairing forks (zygotene). After synapsis is completed, it remains stable for a certain period (pachytene) and then breaks down (diplotene). After the bivalents open out, the localisation of chiasmata can be seen. From late diplotene onward, the distribution of chiasmata is usually heavily proterminal. Because chromatids do not become visible

until first anaphase, diplotene in cereals is not visibly four stranded. Consequently it is not known for certain whether extensive terminalisation of chiasmata occurs in the cereals. Fu and Sears (1973) reported good correlation between diplotene chiasmata and genetic recombination for two heteromorphic bivalents in bread wheat; however by first meiotic metaphase (MI) there were insufficient chiasmata to account for the number of crossovers. Chromosomes involved in these experiments were heterozygous for a terminal, nonhomologous translocation. If all chiasmata were formed proximally to the breakpoint, then at least some chiasmata must have terminalised for the complete length of the translocation. Perhaps chiasmata terminalise more easily through nonhomologous chromatin.

Sears (1972) found that there was a decreased tendency for the formation of proximal crossovers in the pairing of telocentrics, compared to the pairing of the whole chromosome in wheat. The same result was reported in cotton (Endrizzi and Kohel, 1966). Therefore, it is unlikely that the centromere is an important pairing site in wheat, because crossover on one side of the centromere is enhanced by synapsis on the other side.

Symptoms of Meiotic Disturbance in Triticale

A variety of premetaphase disturbances have been reported in triticale (Scoles and Kaltsikes, 1974). Most

reports are hard to evaluate because of the failure to compare with parental varieties.

Shkutina and Khvostova (1971) reported that an octoploid triticale which was unstable in the premeiotic mitoses tended to form more than one nucleolus at diakinesis, in contrast to a stable octoploid which formed only one nucleolus. Pachytene is usually normal in triticale, although some chromosomes may remain at pachytene while others pass on to diplotene in certain isolated cases (Stutz, 1962; Shkutina and Khvostova, 1971). Diplotene and diakinesis are frequently less clear in triticale than they are in wheat (Tsuchiya, 1970; c.f. Fu and Sears, 1973). At diakinesis chromosome pairing is practically complete (Lelley, 1974; Tsuchiya, 1970). The first consistent sign of meiotic disturbance in triticale is the appearance of numbers of rod bivalents and univalents at MI (Scoles and Kaltsikes, 1974). Because pairing at diakinesis is normal, it is probable that most of the pairing failure arises from the desynapsis of existing bivalent associations before MI. Whether these bivalents never formed chiasmata, or whether they did form chiasmata and then lost them is unknown (Thomas and Kaltsikes, 1974a).

Genomic Distribution of Pairing Failure in Triticale

The genomic origin of the chromosomes which were unpaired at MI has been the subject of several studies.

Muntzing (1957) suggested that univalents present in octoploid triticales were mostly rye chromosomes because lines with many univalents reverted to wheat. Other studies have identified the chromosomes that were missing in spontaneous aneuploid plants of triticale. Larter et al. (1968) reported that wheat chromosomes 1B and 6B were never deficient in hexaploid triticale. Pieritz (1970) found that in two octoploid triticales, most aneuploids were deficient for rye chromosomes. However, Shigenaga et al. (1971), Merker (1973) and Weimarck (1974) all found that wheat and rye chromosomes contributed to aneuploidy in proportion to the genomic makeup of the triticale.

Other studies have examined the univalents at MI. From comparison of the range of univalent lengths in polyhaploid Rosner to the range of univalent lengths in monosomic 1B Rosner, Larter and Shigenaga (1971) concluded that both wheat and rye chromosomes could appear as univalents. Thomas and Kaltsikes (1972) showed that within the genome of triticale, there was a group of chromosomes which was less likely to pair than the rest of the complement. Subsequently, by use of "C" banding it was shown to be rye chromosomes which failed to pair at MI (Lelley, 1975; Thomas and Kaltsikes, 1974b; see also Lelley, 1976)

Control of Chromosome Pairing in the Triticinae

Homoeologous Pairing

Although wheat contains three sets of closely related chromosomes (genomes, Sears, 1966) there is usually not much pairing between them. Pairing usually occurs between strict homologues so that wheat is genetically a diploid. The major determinant of this disomic behaviour of wheat is a gene system located distally on the long arm of chromosome 5B (Wall et al., 1971). When chromosome 5B is deficient, pairing may take place between nonhomologous chromosomes (Okamoto, 1957; Riley, 1960; Riley and Chapman, 1958; Riley and Law, 1965; Sears and Okamoto, 1958). Most of this nonhomologous pairing occurs between chromosomes that are genetically equivalent or homoeologous (Riley and Kempanna, 1963; Sears, 1966).

Two theories have been advanced to explain the action of $5B^L$. Riley (1968) suggested that $5B^L$ cut short the time available for effective pairing so that only the most efficient pairing partners (in other words homologues) would have time to pair. However, Bennett et al. (1974a) reported that various wheat genotypes, with and without $5B^L$, had the same duration of prophase stages. This was also true of other genotype comparisons with different levels of homoeologous pairing. Feldman and Avivi (1973) reported that $5B^L$ suppressed somatic association between

homoeologous chromosomes in root tip cells of wheat. By placing homoeologues but not homologues at a distance from one another in the premeiotic interphase, $5B^L$ could diminish the chance of effective pairing between homoeologues in meiotic prophase (Feldman, 1966, 1968). $5B^L$ appears to change the properties of microtubules and related systems, especially the mitotic spindle, perhaps through biochemical modification of microtubule subunits (Avivi and Feldman, 1973; Avivi et al., 1970ab). On the other hand, two independant investigations have failed to detect any evidence for somatic association between full homologues in root tip cells of wheat (Darvey and Driscoll, 1972b; Dvorak and Knott, 1973).

Various other gene systems affect the level of homoeologous pairing in wheat and its hybrids (Dover and Riley, 1972ab; Driscoll, 1972; Dvorak, 1972; Mello-Sampayo and Canas, 1973; Miller and Chapman, 1976).

The pairing of chromosome sets derived from Secale montanum Guss. (R^m) and cultivated rye (S. cereale L.) (R^c) was inhibited by the presence of the genomes of both hexaploid and tetraploid wheats (Miller and Riley, 1972; Riley and Miller, 1970; Thomas and Kaltsikes, 1971). Riley and Miller (1970) interpreted this as an inhibition of homoeologous pairing between the partly related genomes of R^c and R^m through the action of $5B^L$. However, this

effect is not one sided. An increase in the dosage of rye chromosomes led to an increase in homoeologous pairing among wheat genomes when these were in the polyhaploid condition (Lelley, 1976; Miller and Riley, 1972). At least some of this effect was replaceable by chromosome $5R^{s}$ alone (Miller and Riley, 1972). Consequently, there is bilateral control of pairing in triticale. Rye chromosomes promote homoeologous pairing among wheat chromosomes, whereas wheat chromosomes inhibit pairing among rye chromosomes (Lelley, 1976).

Two doses of $5B^{L}$ allowed homologous chromosomes to pair without disturbance in bread wheat, whereas four and six doses led to asynapsis and bivalent interlocking (Feldman, 1966). Two doses of $5B^{L}$ are probably optimal for the pairing of wheat homologues in triticale, but this same dose rate could be superoptimal for the pairing of rye homologues (Riley and Miller, 1970; Thomas and Kaltsikes 1971). Chromosome $5B^{L}$ could therefore be a cause of pairing failure in triticale. However, $5B^{L}$ pairing failure is asynaptic (Feldman, 1966) whereas pairing failure in triticale is desynaptic (Lelley, 1974; Tsuchiya, 1970).

Colchicine Induced Pairing Failure

Colchicine is a potent inhibitor of chromosome pairing in wheat (Dover and Riley, 1973; Driscoll et al., 1967), and other species (Barber, 1942; Dermen, 1938; Levan, 1939; Shepard et al., 1974; Walker, 1938). Colchicine is best known as a mitotic poison. It achieves its effect

on mitosis through binding to a small, soluble dimer protein that is a subunit of microtubules (Borisy and Taylor, 1967ab; Bryan, 1972; Shelanski and Taylor, 1967; Taylor, 1965; Wilson, 1970; Wilson and Friedkin, 1967). Binding of colchicine to the protein prevents polymerisation of microtubules from the free dimer (tubulin); consequently processes that depend on the growth of microtubules are sensitive to colchicine, whereas processes that employ established microtubule structures are much less sensitive to colchicine (Margulis, 1973). Binding of colchicine to the soluble protein is slow at low temperatures, but is greatly speeded up at 37°C; following unbinding of colchicine the tubulin will not bind colchicine a second time so that the reaction seems to be irreversible (Borisy and Taylor, 1967a; Bryan, 1972; Wilson, 1970; Wilson and Friedkin, 1967).

Colchicine sensitivity is generally assumed to indicate a role for microtubules in the inhibited process (Margulis, 1973). However, a colchicine binding protein distinct from tubulin was associated with the colchicine inhibition of chromosome pairing in leptotene and early zygotene of lily (Hotta and Shepard, 1973; Shepard et al., 1974); at the same time a particular DNA binding protein failed to associate with the nuclear membrane in the presence of colchicine (Hotta and Shepard, 1973).

In wheat, colchicine seems to block the presynaptic juxtaposition of homologues, since colchicine had no effect on the interarm pairing of an isochromosome (Dover and Riley, 1973; Driscoll and Darvey, 1970). Dover and Riley (1973) concluded that the colchicine sensitive period in wheat occurred at or shortly after the last premeiotic mitoses.

Temperature Dependant Pairing Failure

Chromosome 5D in bread wheat stabilises chromosome pairing against extremes of temperature (Bayliss and Riley, 1972a). In the absence of 5D, temperatures below 15^{o}C block the synapsis of wheat chromosomes almost completely. At 20^{o}C, the sensitive period occurs about 36 hours before MI (Bayliss and Riley, 1972b). According to Bennett et al. (1971) this would place the sensitive period during the premeiotic interphase, prior to the "S" period. Interaction of 5D deficiency and low temperature is distinct from the effect of colchicine, because unlike colchicine, this combination strongly inhibits the interarm pairing of an isochromosome (Bayliss and Riley, 1972a).

Meiotic Duration and Pairing Efficiency

Bennett and coworkers (Bennett et al., 1971; Bennett and Kaltsikes, 1973) have suggested that there is considerable difference in total meiotic duration, between the slow meiosis of rye and the fast meiosis of wheat and triticale.

This might account for the pairing difficulties of the slow pairing rye genome in the fast meiosis of triticale. However, Bennett et al. (1974a) and Bayliss and Riley (1972b), both concluded that manipulation of major gene systems that control chromosome pairing in wheat, and its hybrids, produced no important change in the times required to complete the different meiotic stages. Also, there was no relationship between the level of chromosome pairing in various triticales, and the time they took to complete their meiotic development (Roupakias and Kaltsikes, 1977ac). Furthermore, the duration of meiosis in triticale was not necessarily less than the duration observed in the rye parent (Roupakias and Kaltsikes, 1977b).

Chiasma Localisation

In the Triticinae, each pair of chromosome arms carries one or less commonly two chiasmata at MI. Chiasmata are generally terminal in position. Consequently, homologues generally pair as relatively unconstricted ring bivalents. A radically different pattern of chiasma distribution was reported in a selection from an interspecific rye cross (Jones and Rees, 1964).

In the normal way, each bivalent forms two chiasmata, one in each arm. In the distributional variant, some bivalents had three or even four chiasmata while others had none and so formed univalents at MI. Despite

the presence of these univalents, chiasma frequencies were roughly normal (Jones, 1967; Jones and Rees, 1964). At the same time as the regular distribution of chiasmata among bivalents disappeared, so did the preponderance of terminal chiasmata, so that chiasmata occurred at high frequency in the median and proximal segments as well (Jones and Rees, 1964). Genetic studies indicated that the unusual meiotic phenotype was polygenically controlled (Jones, 1967).

A similar association of characters exists in general populations of rye. Over a large number of rye genotypes, variation in chiasma frequency between bivalents was positively correlated with the proportion of chiasmata that were nonterminal (Jones, 1974ab). Consequently, the overall control of chiasma localisation appears to be a whole cell phenomenon. Jones (1974a) argued that this finding also indicated that chiasmata were not formed sequentially, from initiation sites close to the telomere.

Another type of distributional variant has been described in Hypochoeris radicata (Parker, 1975). In this case, normal MI phenotype is four bivalents. In the variant three out of four bivalents were normal but the fourth and smallest bivalent was highly desynaptic. This variant was conditioned by a single, recessive gene. In addition, chiasma frequency in the normal bivalents was

higher when the fourth bivalent desynapsed into a pair of univalents, than when it carried chiasmata and persisted until MI. The distribution of chiasmata is thus controlled both at the level of the entire cell, and at the level of the individual bivalent. What these control mechanisms might be is unknown.

Conclusion

Meiotic chromosome pairing is a complex process. This is especially true of polyploids, where pairing chromosomes differentiate between homoeologous chromosomes in favor of homologous pairing partners. When chromosome sets from a lower ploidy level are incorporated together in a plant of higher ploidy, it is not surprising if the meiotic system should maybe reflect this evolutionary discontinuity, through a certain level of meiotic disturbance.

THE GENOMIC ORIGIN OF THE UNPAIRED CHROMOSOMES IN TRITICALE

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GENOMIC ORIGIN OF THE UNPAIRED CHROMOSOMES IN TRITICALE

Abstract

Differential staining of telomeric rye heterochromatin and telocentric chromosomes were used to identify chromosomes which were unpaired at first meiotic metaphase of hexaploid triticale (X Triticosecale Wittmack). Both approaches showed that it was the rye chromosomes which were seen as univalents. Differences in the rate of pairing from triticale to triticale were mostly explained by variation in the pairing of the rye genome. Within the rye genome, chromosome arms with telomeric heterochromatin showed much lower pairing rates than chromosome arms lacking heterochromatin. Wheat telocentrics and heterochromatin-free rye telocentrics, which showed intermediate levels of pairing failure (65-90%), had mostly terminal chiasmata. On the other hand, rye telocentrics with large heterochromatic bands on the telomeres had mostly nonterminal chiasmata and very low pairing (5-35%). It is concluded that the presence of heterochromatin on certain telomeres of rye chromosomes, blocks the formation of terminal chiasmata and this results in desynapsis and univalents at first meiotic metaphase.

Introduction

Since the first studies of meiosis in triticale (X Tritisosecale Wittmack), it became clear that certain chromosomes were not paired at first metaphase (Muntzing, 1957). The genomic origin of these chromosomes has been the subject of several studies which concluded that the unpaired chromosomes belonged either predominantly to rye (Larter et al., 1968; Muntzing, 1957; Pieritz, 1970; Sanchez-Monge, 1958) or to wheat and rye in proportion equal to their contribution to the genomic make-up of the triticale studied (Larter and Shigenaga, 1971; Merker, 1973; Shigenaga et al., 1971; Weimarck, 1974).

With the exception of the work of Larter and Shigenaga (1971), the genomic origin of the unpaired chromosomes has been inferred from the study of the progeny of plants exhibiting reduced pairing, rather than the study of the univalents themselves. Recognizing the uncertainties involved in extrapolating from the frequency of the various aneuploids to the genomic origin of the unpaired chromosomes which gave rise to them, we undertook the study of the univalents themselves at first metaphase. Our first report (Thomas and Kaltsikes, 1972) showed that a group of chromosomes, possibly those of rye, were less likely to pair than the rest of the complement. In our second report (Thomas and Kaltsikes, 1974), by differentially staining the telomeric heterochromatin of the rye

chromosomes, we showed that, in one triticale line and some hybrids, it was the rye chromosomes which failed to pair at MI. Furthermore, we postulated that it was the telomeric heterochromatin which prevented the rye chromosomes from pairing. In this report, we present further evidence that it is the heterochromatic parts of the rye chromosomes which do not pair at MI of triticale by utilizing two approaches: (1) differential staining of heterochromatin (C-banding) in a group of triticale lines which differ widely in chromosome pairing; and (2) the use of telocentrics for the production of wheat or rye heteromorphic bivalents, the pairing of which can be followed during meiosis.

Material and Methods

The pedigree and pairing characteristics of the lines used in the C-banding experiment are given in Table I.

Several telocentrics were collected from the triticale cv Rosner; those in the disomic condition were test-crossed with either Stewart 63, a cultivar of durum wheat (Triticum turgidum L. var. durum), or the hexaploid wheat (T. aestivum L. em. Thell) cv. Anza to ascertain the genomic origin of the telocentric chromosomes. The identification of the particular chromosome involved was obtained through the criteria listed in Table II. All telocentrics

were backcrossed once to their parental line. Monotelodisomic progeny of the backcross were scored for the overall pairing and for the pairing and distribution of chiasmata in the heteromorphic bivalent. A scheme of bivalent categories was established in order to classify heteromorphic bivalents for the distribution of chiasmata (Fig. 1). The first bivalent category was the "touch and go" pairing category with the co-orientated homologues widely separated by a fine thread, part of which may be invisible in the light microscope. The second category showed a pronounced constriction at the chiasma, but the connection was generally substantial and the homologues were not widely separated. The third category was a strict rod bivalent with no constriction or swelling visible at the chiasma. The fourth and sixth categories showed, respectively, short and long chromosome segments distal to the chiasma. The fifth and seventh categories showed two chiasmata with, respectively, small and large loops between the chiasmata. All observed bivalents fitted into these categories. Obviously, there was some overlap between the categories but the classification scheme proved to be easy to use and effective.

Although the general outline of the C-banding technique was reported previously (Thomas and Kaltsikes, 1974), the procedure is given here in an easy step-by-step form (Table III). The exact optimum conditions needed to

produce good contrast between euchromatin and heterochromatin differed from genotype to genotype, and, within each genotype, they depended on the time that the preparations had been in storage. Therefore, conditions, for staining should be selected by preliminary trials. Table IV shows the technique used with the various materials employed in the present study.

In general, contrast can be heightened by steeping the slides in 45% acetic acid adjusted to pH = 1.0 with hydrochloric acid. Compared with an increase in the duration of $Ba(OH)_2$, pre-treatment with acid does minimal damage to the chromosomes.

Slides stored for long periods (3 months or more) need stringent conditions to achieve contrast between euchromatin and heterochromatin. However, chromosomes of aged preparations were more resistant to the destructive effects of $Ba(OH)_2$ and high temperature used to achieve comparable levels of contrast. If the temperature of the saline sodium citrate (2 x SSC) buffer is raised over 60° C, all chromatin rapidly loses its ability to stain; prolonged staining in Giemsa results in staining of the nucleolus. Over-treatment with $Ba(OH)_2$ results in irregular swelling of the chromosomes and irregular staining. During staining a scum is sometimes picked up from the surface of the staining solution. Cells beneath this scum tend to stain pink rather than blue. If the

staining of the cells is good, the scum may be removed by briefly dipping the slides in 70% ethanol. Over-stained slides can also be improved by partially destaining with ethanol. The destaining can be accelerated by slightly acidifying the ethanol with acetic acid.

Results and Discussion

Triticale, Its Parents and Its Parental Genomes

Compared to its two parental species, triticale has an irregular meiosis with many univalents and rod bivalents observed at MI (Table I). A general idea of the pairing of the two parental genomes in triticale was obtained from progenies of the first backcrosses of triticale to durum (AABBR) and to rye (ABRR). In practice, there is some homoeologous pairing of wheat chromosomes in ABRR. However, since homoeologous bivalents are most frequently rod bivalents (Table V), we can best gauge the pairing of the rye genome from ring bivalents alone. Wheat chromosomes in the AABBR hybrids behaved normally in the presence of the R genome, whereas rye chromosomes in ABRR hybrids did not show full pairing when accompanied by the A and B genomes (Fig. 2).

C-Banding Experiment

Rye chromosomes can be selectively stained in somatic cells of triticale by means of C-banding techniques. These procedures bring out the large blocks of terminal

heterochromatin which are present on many of the telomeres of rye chromosomes. When PMC's of rye, wheat and triticale were stained in this way (Figs. 3, 4), it was possible to distinguish between wheat chromosomes (unbanded) and rye chromosomes (showing terminal bands at MI).

Chromosome pairing was separated into its wheat and rye components in four out of the seven triticales examined (Table VI). In these four triticales, the rye genome invariably showed a lower pairing frequency than did the wheat genome. Furthermore, most of the variation in overall pairing from triticale to triticale was attributable to differences in pairing of the rye chromosomes. The level of overall pairing agreed closely with pairing data obtained from acetocarmine squashes of the same material (entries listed as overall pairing/indirect method in Table VI) which means that the analyses were accurate. Clumping of the MI preparations following the C-banding procedure made it impossible to analyse the pairing in three of the seven triticales directly. In these cases, an indirect analysis was used as follows:

The distribution of heterochromatin was recorded on metaphase chromosomes which lay clear of all other chromosomes. Since univalents are generally easier to identify than ring bivalents, it follows that univalents and bivalents will not be recorded in their true proportion, i.e., bivalents underestimated. For example, rod bivalents

might be considered as a random sample from the population of rod bivalents but not from the chromosome population as a whole. Chromosomes carrying terminal heterochromatic bands (i.e., rye chromosomes) dominated the rod bivalent and univalent categories but wheat chromosomes were present mostly as ring bivalents (Table VII). In the Stewart Prolific triticale (6A190) there was a mean of 15.14 ring bivalents per cell (Table VI). However, in this triticale only 18.75% of ring bivalents were banded (Table VII); 18.75% of 15.14 ring bivalents is 2.84, which provides an estimate of the number of ring bivalents formed by rye chromosomes in this triticale. If this procedure is repeated for bivalents and univalents, the overall pairing can be partitioned to the wheat and rye genomes indirectly. When the estimated numbers of chromosomes in these three categories are added, their total should approximate 14, which is the number of rye chromosomes in triticale. In fact, for the seven triticale examined, the mean number of rye chromosomes estimated was 15.04. The sampling was clearly slightly biased in favor of rye chromosomes. Comparison of the partition of chromosome pairing according to the direct and indirect methods (Table VI) showed that while the estimated number of rye univalents was approximately correct, the number of ring bivalents and rod bivalents was generally too high.

Oversampling of rye bivalents suggested that they might occupy a more peripheral position on the metaphase plate than their wheat counterparts. A similar phenomenon was noted in rye where there was a significant tendency ($x^2_6 = 18.43$, $p \leq 0.01$) for rod bivalents to occupy peripheral positions in the squash (Table VIII).

These two phenomena may have a common basis in the length of the inter-centromere distance. In rye this would be greatest for rod bivalents because of greater metaphase stretch. In triticale it would be greater for rye bivalents compared to similar bivalents of wheat simply because the chromosomes are longer.

Whatever the origin of this slight bias, its effect on the estimated pairing was small. Among the four triticales for which direct estimates were available, comparisons show that the overall pairing rates of two genomes were both overestimated by the indirect method by only 0.007 chiasmata per chromosome (Table VI). Compared to the difference in pairing rate between wheat and rye chromosomes, this much bias is trivial and, anyway, had no effect on the difference between the two genomes.

Since the bias was small and had no serious effects on estimated pairing rates the analysis was extended. Instead of grouping all rye chromosomes together, each of the possible bivalent types was treated as a separate category. By distinguishing between chromosomes with no

terminal bands, one terminal band, and two terminal bands, the pairing rates of rye telomeres that carry heterochromatin, rye telomeres that lack heterochromatin, and wheat telomeres can be estimated. Over the pairing range encountered in these triticales, most of the variation in overall pairing was accounted for by variation in the pairing of heterochromatic rye telomeres (Fig. 9). By the same token, the pairing rates of wheat telomeres and rye telomeres without heterochromatin remained relatively constant. At the low end of the pairing range, pairing failure began to affect nonheterochromatic telomeres as well (Fig. 9). Similar indications were noted in aceto-carmine squashes of MI of the lowest pairing triticale examined (Tetra-prelude x OD289 - 998.2). Metaphase plates of this triticale contained a number of rod bivalents that were unusually short for triticale. Presumably these short rod bivalents represented the smaller chromosomes of wheat. Even so, this does not alter our main conclusion. Over the pairing range normally encountered in triticale, most pairing failure is associated with the arms of rye chromosomes that carry heterochromatin at the telomere (Fig. 9).

The Pairing of Telocentric Chromosomes

Another approach to the problem of the genomic origin of univalents involves the use of telocentrics. The pairing of heteromorphic rye bivalents can be compared

with that of heteromorphic wheat bivalents. Telocentrics of chromosomes 1B and 6B were easily identified from root tip squashes. Other telocentrics were genomically identified by crossing the telosomics to tetraploid or hexaploid wheat and examining meiosis in the resulting hybrid (Figs. 5, 6; Table II). Two telocentrics were identified as rye chromosomes from the presence of large heterochromatic bands on the telomeres at MI (Table II).

Among wheat telocentrics, pairing varied from 65% to 100% (Table IX). Among the rye telocentrics, certain arms showed very low levels of pairing (between 10 and 35%). All other rye telocentrics fell within the same range of pairing frequency as the wheat telocentrics. When the rye telocentrics were stained for heterochromatin at MI, it was found that all four chromosomes with abnormally low levels of pairing (t105, t103, 34-B, $1R^S$) carried large heterochromatic bands (Figs. 7, 8; Table IX). Rye telocentrics which showed more or less normal pairing rates had no heterochromatin (Figs. 7, 8), with the exception of telo 3R in Rosner, on which a small terminal band was observed at MI (this was also confirmed from root tip squashes stained for heterochromatin). The long arm of chromosome 1B also carries a minor heterochromatic band on the telomere. However, compared to the four strongly heterochromatic arms, these two telos were considered as essentially nonheterochromatic (Table IX).

Among the telocentrics the presence of large terminal blocks of heterochromatin was strongly associated with pairing failure at MI (Table IX). However, all rye telocentrics with large intensely staining bands were relatively short.

Ignoring the four strongly heterochromatic rye telocentrics, a significant relationship was found between the position of metaphase chiasmata and the pairing rate of the telocentric. The distribution of chiasmata was recorded as the frequency distribution of the seven bivalent types of Fig. 1 in Table IX. These bivalent types were given a value from one through to seven and were multiplied by their respective frequency to calculate the mean bivalent type for each telocentric, which is given as the terminalization score in Table IX. For the 19 nonheterochromatic telocentrics, there was a significant correlation between percentage pairing and the terminalization score (Spearman's $R_S = +0.600$, 16 d.f., $p < 0.01$), meaning that, if the pairing rate of a telocentric was low, then chiasmata were generally single and terminal in position.

The heterochromatic telomeres did not conform to this relationship. Despite their low pairing, these telocentrics showed an increased tendency for proximal localisation of chiasmata (Fig. 9; Table X). In other words, they were deficient in terminal chiasmata (Table IX)

i.e. in the heterochromatic regions at the telomere. Deficiency of chiasmata in heterochromatic regions has also been observed in other species (Brown, 1949; Fox et al., 1973; John and Lewis, 1965; Klasterska et al., 1974). Perhaps in triticale there is a tendency for chiasmata to occur close to the telomere. This will be critical only where there are large blocks of heterochromatin at the telomere since no chiasmata can form in these regions. If this view is correct, then the distribution of chiasmata in particular chromosomes should be more terminal in low pairing triticales than it is in high pairing triticales or in wheat or rye.

Acknowledgments

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Table I

Chromosome pairing in wheat, rye and triticale

Genotype	Bivalents		Univalents	Chiasmata per chromosome	No. of cells examined
	Ring	Rod			
Triticum turgicum var. *durum*					
Stewart 63	13.15	0.83	0.05	0.969	75
Cocorit	11.60	2.28	0.24	0.910	45
Other	13.37	0.59	0.08	0.976	100
Triticum aestivum					
Anza	19.59	1.41	0.00	0.966	45
Glenlea	20.28	0.72	0.00	0.983	25
Secale cereale					
Prolific	6.29	0.70	0.02	0.949	160
Gazelle	6.61	0.39	0.00	0.972	150
x *Triticosecale*					
T. turgidum x *S. cereale*	18.39	2.49	0.24	0.935	100
Tetra-Thatcher x Prolific	17.86	2.96	0.36	0.922	105
Tetra-Rescue x Prolific	17.21	3.59	0.40	0.905	135
Stewart 63 x Prolific 278-9	17.14	3.57	0.64	0.901	100
Stewart x Prolific 6A190	14.86	5.22	1.86	0.832	88
Rosner	14.57	5.77	1.31	0.831	200
Stewart 63 x OD289 65-4	14.40	5.50	2.20	0.817	100
Tetra-Prelude x OD289 998-2	13.16	6.43	2.82	0.780	100

Table II

Criteria for identification of particular telocentrics in Rosner and other triticales

Telocentric	Criterion			
	Karyotype	Crossed to Anza	Crossed to Stewart 63	Banding at MI
Wheat				
telo $1B^L$ Rosner	Feulgen	-	-	-
telo $6B^L$ Rosner	Feulgen	-	-	-
telo $1B^S$ Rosner	Feulgen	-	-	-
telo $6B^S$ Rosner	Feulgen	-	-	-
telo 16 Rosner	-	BIV**	-	-
telo 32 Rosner	-	BIV	-	-
telo 35 Rosner	-	BIV	-	-
telo 38 Rosner	-	-	BIV	-
telo 43 Rosner	-	BIV	-	-
telo 48 Rosner	-	BIV	-	-
telo 101 St '63 x OD289-326.5	-	BIV	-	-
telo 102 T4N x OD289-381.3	-	BIV	-	-
Rye				
telo $1R^S$ Rosner	-	NO BIV	-	Giemsa
telo 3R Rosner	Leishman's	NO BIV	NO BIV	Giemsa
telo 12* Rosner	-	NO BIV	-	Giemsa
telo 31* Rosner	-	-	NO BIV	Giemsa
telo 34A Rosner	-	NO BIV	-	Giemsa
telo 46* Rosner	-	NO BIV	NO BIV	Giemsa
telo 34B Rosner	-	-	-	Giemsa
telo 47 Rosner	-	NO BIV	-	Giemsa
telo 103 St '63 x OD289-130.1	-	NO BIV	-	Giemsa
telo 104 Line-110	-	NO BIV	-	Giemsa
telo 105 Line-110	-	-	-	Giemsa

*All provisionally identified as $7R^S$. **BIV & NO BIV indicates the formation, or lack, of bivalents in the hybrid.

Table III

General outline of the "C" band technique for staining chromosomes at first meiotic metaphase

1. Fix spikes in Carnoy's II for 24 hours and refrigerate in 70% ethanol until required.
2. Clean slides by steeping in glacial acetic acid and then in 95% ethanol. Air dry.
3. Macerate and squash anthers in 45% acetic acid and select first meiotic metaphase stages by phase contrast observation.
4. Freeze slides with dry ice, flick off coverslips and air dry. Store for 3 weeks or more.
5. Steep slides in 45% acetic acid adjusted to pH 1.0 with HCl for 3 hours (optional).
6. Treat slides with saturated $Ba(OH)_2$.
7. Flush out $Ba(OH)_2$ with running deionised water.
8. After several rinses in deionised water transfer to 2 x SSC (saline sodium citrate) and place in an oven set between 50°C and 60°C.
9. After the temperature of the SSC attains oven temperature, remove slides, wash in deionised water and stain in 2% Giemsa at pH 6.8.

Table IV

Details of representative "C" banding schedules adapted for particular materials

Genotype	Approximate age of slides (weeks)	45% Acetic acid + HCl to pH 1.0	Saturated $Ba(OH)_2$ (in M)	Temperature of 2 x SSC
Rosner	3	none	6	52° C
T. turgidum x *S. cereale*	12	none	16	60° C
T. durum "Stewart '63" x *S. cereale* "Prolific" 278.9	12	none	17	60° C
T. aestivum (AABB) 'tetra-Prelude" x *S. cereale* "2D289" 998-2	12	3 h	12	60° C
S. cereale "Prolific"	6	none	8	57° C

Table V

Frequencies of ring and rod bivalents in the meiosis of wheat-rye hybrids

	Ring bivalents	Rod bivalents	No. of cells examined
Thatcher x Prolific (3 hybrids)	4	133	56
Chinese Spring x UC90 (2 hybrids)	2	44	50

Table VI

The genomic distribution of chromosome pairing in seven triticale lines

Line	Chromosome pairing	Method of estimation	Cells examined	Pairing characteristics: Bivalents: Ring	Pairing characteristics: Bivalents: Rod	Pairing characteristics: Univalents	Chiasmata per chromosome	Chromosome no. (2n)
1. *T. turgidum* x *S. cereale*	Overall	Direct*	13	17.92	3.00	0.16	0.925	42.00
		Indirect	100	18.39	2.49	0.24	0.935	42.00
	Wheat	Direct	13	13.00	1.00	0.00	0.964	28.00
		Indirect	100	12.79	0.77	0.01	0.971	27.12
	Rye	Direct	13	4.92	2.00	0.15	0.846	14.00
		Indirect	100	5.60	1.72	0.23	0.869	14.87
2. Thatcher (AABB) x *S. cereale* (Prolific)	Overall	Direct	27	17.85	2.96	0.38	0.920	42.00
		Indirect	105	17.86	3.00	0.28	0.922	42.00
	Wheat	Direct	27	13.00	0.96	0.07	0.963	28.00
		Indirect	105	12.93	0.31	0.02	0.988	26.50
	Rye	Direct	27	4.85	2.00	0.30	0.836	14.00
		Indirect	105	4.93	2.69	0.26	0.810	15.50
3. Rescue (AABB) x *S. cereale* (Prolific)	Overall	Indirect	135	17.21	3.59	0.39	0.905	42.00
	Wheat	Indirect	135	12.69	0.69	0.03	0.973	26.80
	Rye	Indirect	135	4.52	2.90	0.36	0.786	15.20

Table VI (Continued)

	Line	Chromosome pairing	Method of estimation	Cells examined	Pairing characteristics: Bivalents: Ring	Pairing characteristics: Bivalents: Rod	Pairing characteristics: Univalents	Chiasmata per chromosome	Chromosome no. (2n)
4.	Stewart 63 x *S. cereale* (Prolific) 278-9	Overall	Direct	140	17.22	3.43	0.76	0.902	42.00
			Indirect	100	17.11	3.57	0.64	0.900	42.00
		Wheat	Direct	140	12.96	1.01	0.07	0.962	28.00
			Indirect	100	12.60	0.72	0.06	0.971	26.70
		Rye	Direct	140	4.26	2.42	0.63	0.781	14.00
			Indirect	100	4.51	2.85	0.58	0.776	15.30
5.	Stewart 63 x *S. cereale* (Prolific) 6A190	Overall	Indirect	28	15.14	5.10	1.50	0.842	42.00
		Wheat	Indirect	28	12.30	1.56	0.30	0.934	28.02
		Rye	Indirect	28	2.84	3.54	1.21	0.660	13.97
6.	Stewart 63 x *S. cereale* (OD289)	Overall	Direct	12	14.66	5.17	2.33	0.821	42.00
			Indirect	100	14.40	5.50	2.20	0.817	42.00
		Wheat	Direct	12	12.66	1.25	0.16	0.949	28.00
			Indirect	100	11.88	1.41	0.30	0.936	26.88
		Rye	Direct	12	2.00	3.92	2.17	0.604	14.00
			Indirect	100	2.52	4.09	1.90	0.568	15.12
7.	Prelude (AABB) x *S. cereale* (OD289)	Overall	Indirect	100	13.16	6.43	2.82	0.780	42.00
		Wheat	Indirect	100	10.35	2.82	0.35	0.881	26.69
		Rye	Indirect	100	2.81	3.61	2.47	0.603	15.31

Table VI (Continued)

*Direct: Pairing analysis based on Giemsa-stained PMC in which all chromosomes could be seen.

Indirect: Overall pairing from acetocarmine squashes apportioned to wheat and rye genomes on the basis of the banding characteristics of those chromosomes which lay clear of the clumped MI configuration.

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Table VII

The distribution of terminal heterochromatin in ring bivalents, rod bivalents and univalents in seven hexaploid triticales

Type of chromosomes association	Triticales ranked according to the mean pairing shown in Table VI						
	1	2	3	4	5	6	7
Ring bivalents							
Chromosomes with:							
2 terminal bands	12	8	13	32	0	9	2
1 terminal band	27	34	33	66	15	52	23
0 terminal band	89	110	129	274	65	287	92
% banded/ total	30.47	27.63	26.28	26.34	18.75	17.53	21.36
Rod bivalents							
2 terminal bands	19	12	36	57	7	34	21
1 terminal band, hetero-chromatic arms unpaired	38	39	90	113	25	111	37
1 terminal band, hetero-chromatic arms paired	6	2	8	7	2	9	2
0 terminal band	28	6	32	45	15	53	47
% banded/ total	69.23	89.83	80.72	79.73	69.39	74.40	56.07

Table VII (Continued)

Type of chromosomes association	Triticales ranked according to the mean pairing shown in Table VI						
	1	2	3	4	5	6	7
Univalents							
2 terminal bands	18	28	70	116	17	100	74
1 termainal band	23	17	75	70	12	105	33
0 terminal band	1	6	13	21	7	32	15
% banded/ total	97.62	88.24	91.77	89.85	80.56	86.49	87.70

Table VIII

Frequency distribution of positions of rod bivalents along the MI plate of cells of Prolific rye[1]

Position	1	2	3	4	5	6	7
Frequency	36	22	12	15	24	21	31

[1]To construct this table the chromosome associations along the metaphase plate were numbered 1 through 7 from left to right. If a particular position was occupied by a rod bivalent it was counted and entered in the table.

Table IX

Number and location of chiasmata on the heteromorphic bivalent involving wheat or rye telocentric chromosomes

Telocentric	Percent pairing	Type of bivalent[1] 1	2	3	4	5	6	7	Terminalization score	Chiasmata/cell	Cells examined	Heterochromatic
Wheat telocentrics												
$1B^L$	93	4	5	35	9	2	1	2	3.19	34.37	100	(+)[2]
$6B^L$	80	13	12	16	2	-	-	-	2.16	34.51	100	-
t38	98	-	-	12	26	9	1	1	4.04	33.68	100	-
t43	100	1	2	23	15	6	2	1	3.66	33.84	50	-
t101	100	-	-	3	15	7	-	-	4.16	32.20	25	-
$6B^S$	65	3	4	35	3	-	-	-	2.84	33.18	65	-
$1B^S$	92	4	10	31	1	-	-	-	2.63	33.66	50	-
t102	94	10	9	20	7	1	-	-	2.57	33.80	50	-
t48	100	-	4	28	13	4	-	1	3.42	34.52	50	-
t32	99	8	10	30	2	-	-	-	2.52	34.35	100	-
t35	98	-	2	8	24	9	3	4	4.30	34.30	100	-
t16	74	16	7	14	-	-	-	-	1.94	33.10	50	-

Table IX (Continued)

Telocentric	Percent pairing	Type of bivalent[1] 1	2	3	4	5	6	7	Terminal-ization score	Chiasmata/ cell	Cells examined	Hetero-chromatic[2]
Rye telocentrics												
t105	17	-	-	4	1	-	-	-	3.20	33.48	50	+
t103	34	2	2	12	1	-	-	-	2.71	31.56	50	+
34-B	5	-	-	3	-	-	-	-	3.00	33.48	62	+
$1R^s$	12	-	-	6	-	-	-	-	3.00	33.90	50	+
34-A	91	27	25	40	9	1	-	-	2.33	34.72	112	-
12	88	16	12	14	1	1	-	-	2.07	34.18	50	-
31	88	15	12	16	1	-	-	-	2.07	35.48	50	-
46	94	18	14	14	1	-	-	-	1.96	36.41	50	-
t104	94	2	17	23	4	1	-	-	2.68	34.90	50	-
47	96	2	5	9	16	9	2	4	3.74	34.55	100	-
3R	73	8	12	7	6	1	-	-	2.41	34.76	50	(+)

[1]The seven types of bivalents are described in Materials and Methods and are shown diagrammatically in Fig. 1.

[2]+ indicates strongly heterochromatic, (+) indicates weakly heterochromatic, - indicates absence of significant heterochromatin.

Table X

Average terminalization in four arbitrary pairing ranges of triticale telocentric chromosomes

Pairing (%)	Terminalization score	Type of telocentric
95 - 100% (7 telos)	3.70	nonheterochromatic
90 - 95% (6 telos)	2.56	nonheterochromatic
65 - 90% (6 telos)	2.25	nonheterochromatic
0 - 35% (4 telos)	2.98	heterochromatic

Figure 1. Diagram of the seven types of heteromorphic bivalents.

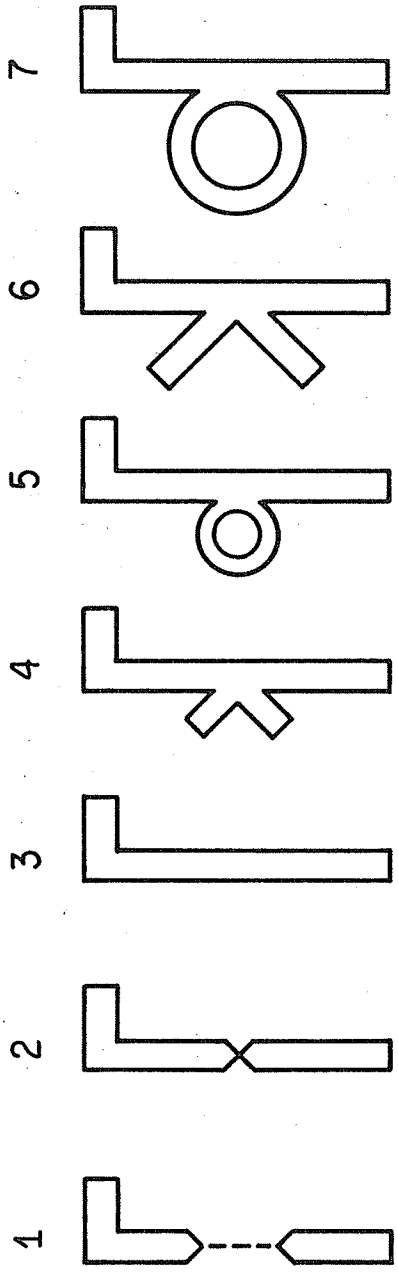
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Figure 2. Frequencies of ring bivalents in the parents of triticale and their backcrosses to it. AABB: 3 varieties AABBR: 3 crosses; ABRR: 3 crosses, 2 or more hybrids per cross; RR two varieties, 6 plants per variety. Data on the vartical axis are unpooled mean percentages.

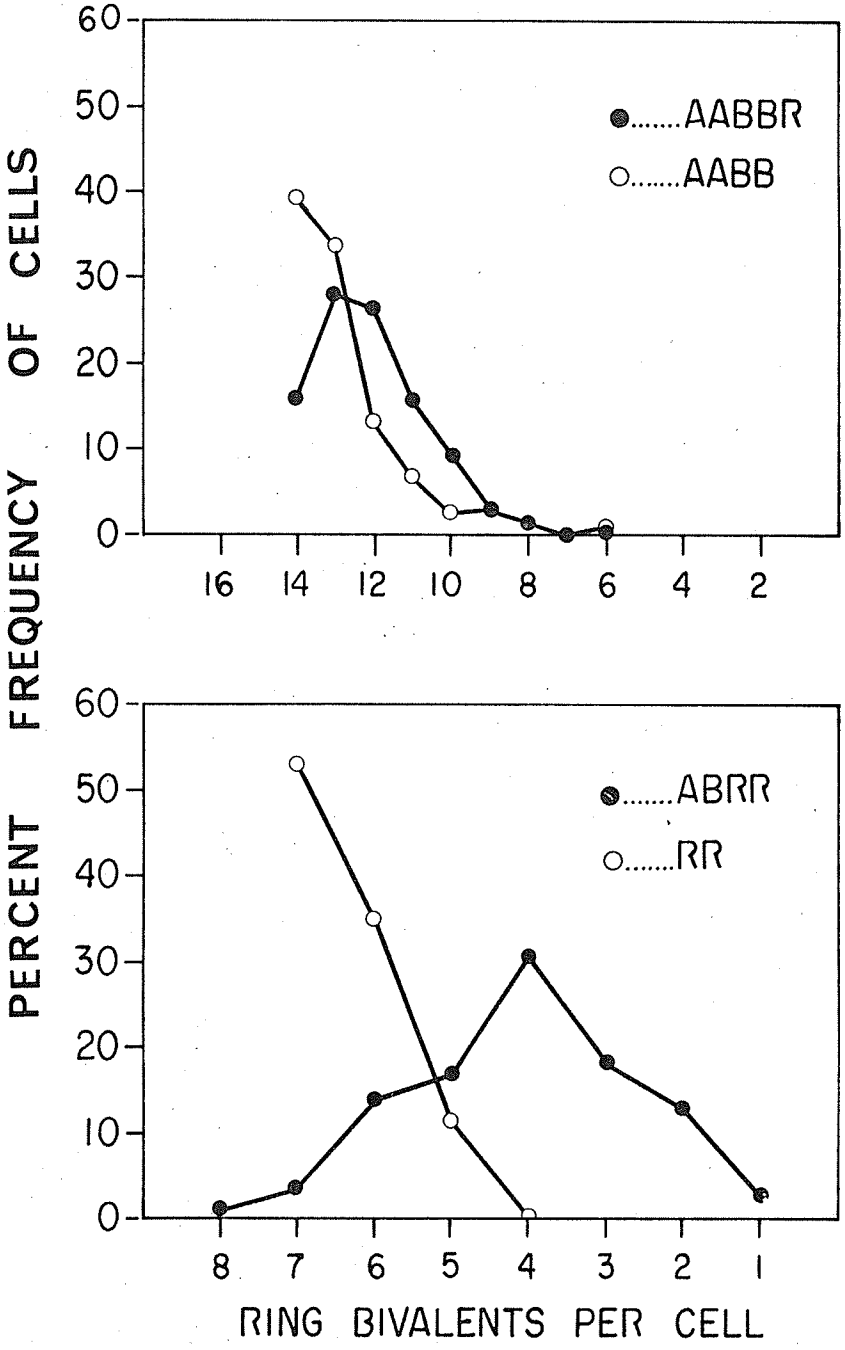
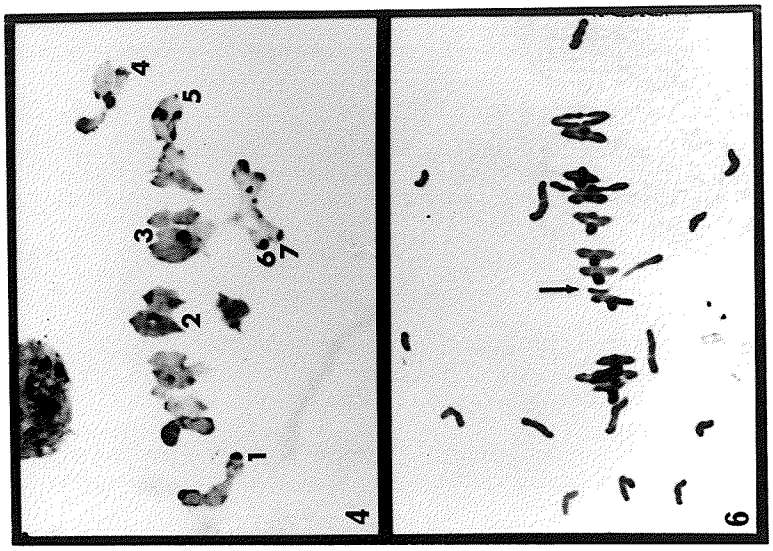

PERCENT FREQUENCY OF CELLS
60
50
40
30
20
10
0
AABBR
AABB
16 14 12 10 8 6 4 2
ABRR
RR
8 7 6 5 4 3 2 1
RING BIVALENTS PER CELL

Figure 3. Giemsa stained MI of rye showing seven heterochromatic bivalents. Bar is 20 um.

Figure 4. Giemsa stained MI of triticale with seven heterochromatic rye bivalents, shown by numbers 1 to 7. Magnification as Figure 3.

Figure 5. MI in Stewart '63 x Rosner telocentric 31 showing unpaired telocentric chromosome (arrow). Bar is 25 um.

Figure 6. MI in telocentric stock 102 x Anza, showing heteromorphic bivalent (arrow). Magnification as Figure 5.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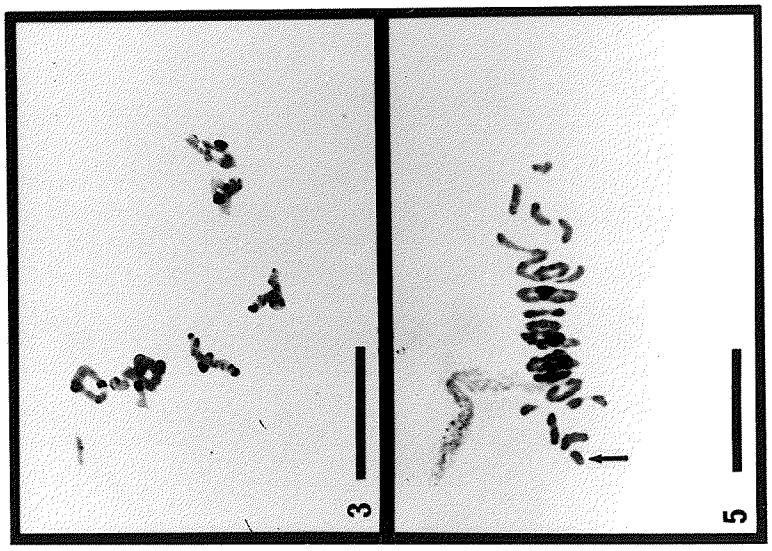
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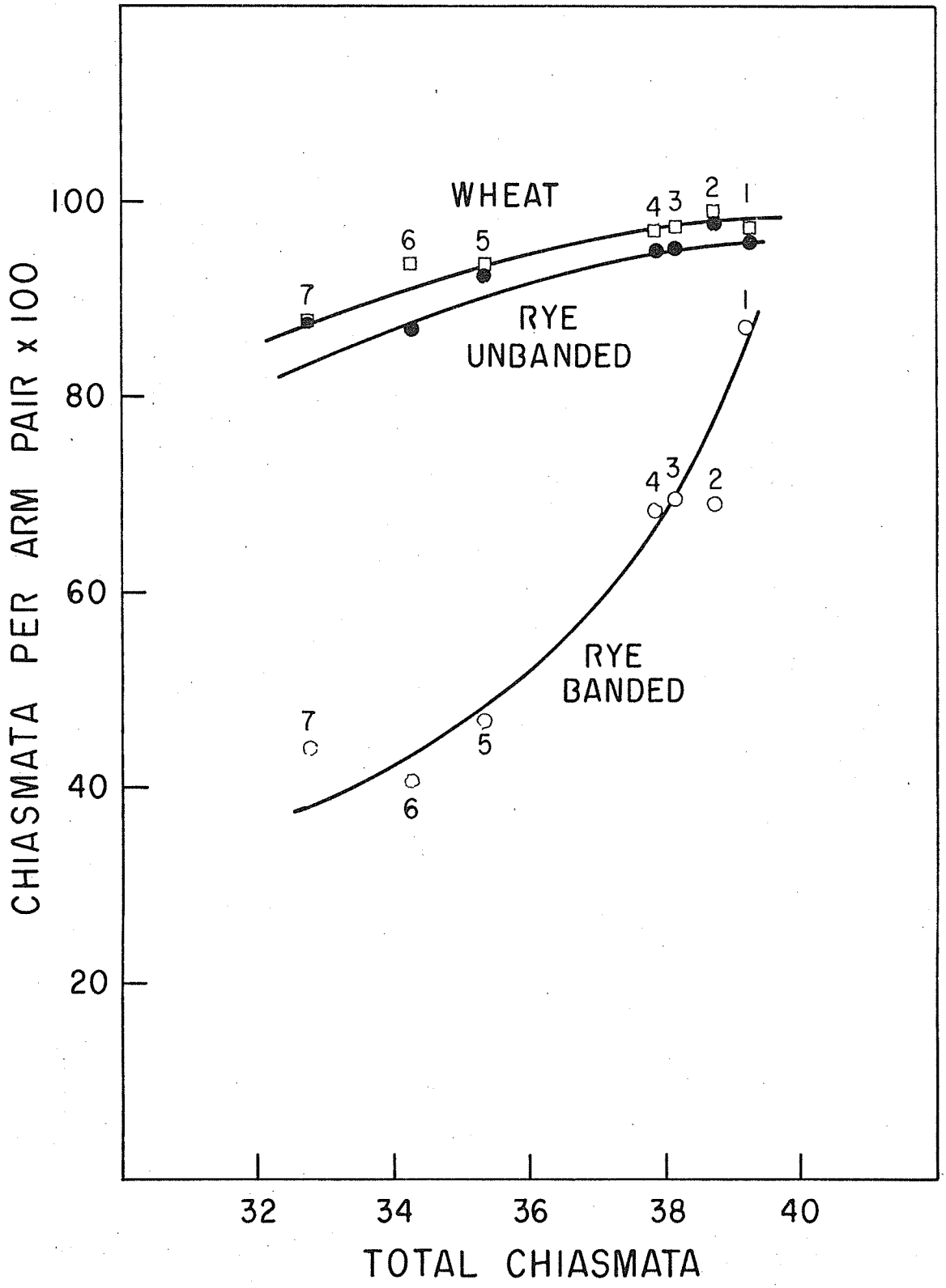
Figure 7. MI in telocentric 34-A x Rosner. Arrow points to the paired, unbanded telocentric chromosome. Note the band present on the opposite arm of the intact homologue. Bar is 20 um.

Figure 8. MI in telocentric 34-B x Rosner. Arrows point to the banded telo which is unpaired, and to the intact homologue which is also univalent. Magnification as Figure 7.


8


7

Figure 9. Pairing rates, in triticale, of wheat and rye chromosomes with (banded) and without (unbanded) heterochromatin. Data from indirect analysis of Table VI. Horizontal axis shows total chiasmata per cell.


WHEAT
RYE
UNBANDED
RYE
BANDED
CHIASMATA PER ARM PAIR x 100
TOTAL CHIASMATA
100
80
60
40
20
32
34
36
38
40
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7

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A BOUQUET-LIKE ATTACHMENT PLATE FOR TELOMERES IN LEPTOTENE OF RYE REVEALED BY HETEROCHROMATIN STAINING

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A BOUQUET-LIKE ATTACHMENT PLATE FOR TELOMERES IN LEPTOTENE OF RYE REVEALED BY HETEROCHROMATIN STAINING

Abstract

Using a "C" banding technique the behaviour of the heterochromatic telomeres of rye (Secale cereale L.) was followed throughout the meiotic cycle. Marked aggregation of these telomeres occurred during leptotene concurrently with nucleolar fusion. A conspicuous chromocentre was formed at the nuclear membrane, often in association with the single nucleolus. Telomere groupings at later stages, which derived from this chromocentre, included nonheterochromatic telomeres which indicated that the association did not depend on the presence of heterochromatin.

This aggregation of the telomeres at the nuclear membrane makes leptotene in rye comparable to published reports of the leptotene bouquet, even though characteristic polarisation of the chromosome segments close to the bouquet attachment plate was not seen in rye. Because telomere aggregation is often associated with nucleolar fusion, it is concluded that similar attachments

are formed when nucleoli fuse at meiosis of hexaploid wheat, although, because of the absence of heterochromatic telomeres in wheat, no chromocentres are observed.

Introduction

Formation of the leptotene bouquet is believed to occur when points at which the chromosomes are attached to the nuclear membrane become concentrated on a restricted area of the nuclear membrane (attachment plate of Kaufmann, 1925); in most cases this grouping seems to involve the telomeres (Wilson, 1934). At some point in time the regions of the chromosome adjacent to this attachment plate may become oriented parallel to one another and perpendicular to the attachment plate (Marengo, 1949), or they may acquire a "whirling" appearance around the attachment plate (Hiraoka, 1941).

Synapsis is usually reported to begin in the chromosome regions close to the attachment plate and to spread along the polarised threads toward the interior of the nucleus (Darlington, 1937; Hiraoka, 1941; Wilson, 1934). Since the bouquet is usually recognised from the characteristically polarised chromosomes, it should be borne in mind that there is some degree of polarisation in any interphase nucleus. Because the centromeres are drawn to the pole at anaphase, the nuclei begin interphase with the centromeres closely grouped at the polar end of the

nucleus and with the telomeres grouped at the other end (Rabl orientation) (Rieger et al., 1968).

Absence of typical bouquet polarisation does not preclude the possibility that the telomeres do become tightly grouped in early prophase. Among the grasses the telomeres seem to be important in determining the choice of a pairing partner and the patterns of synapsis (Burnham et al., 1972; Kasha and Burnham, 1965; Michel and Burnham, 1969). Perhaps the grouping of the telomeres is an essential feature of leptotene in these species in spite of the absence of an easily recognised bouquet polarisation.

The species Secale cereale L. (rye) possesses large heterochromatic blocks located on most of the telomeres of its seven pairs of chromosomes. Since a specific stain now exists for this type of heterochromatin (Sarma and Natarajan, 1973) and since the amount of intercalary heterochromatin is small, any clustering of the telomeres into a bouquet-like arrangement should be very easy to detect. Consequently, we examined pollen mother cells (PMC) of rye stained by this method to see if aggregation of the terminal heterochromatin occurred at any point in the meiotic cycle.

Materials and Methods

Inflorescences of Secale cereale L. (cv. Prolific and a bulk population of fall rye) were fixed in Carnoy's II

for 1 to 24 hours and then placed in 70% alcohol. Columns of tapetal and pollen mother cells (PMC) from one anther per floret were extruded into acetocarmine to determine the meiotic stage. The other two near synchronous anthers from the floret were then squashed in 45% acetic acid. These latter slides were stained using a standard "C" type heterochromatin schedule (Thomas and Kaltsikes, 1974). The stain used was either Giemsa or Leishman's.

Results

The most useful criterion of meiotic sequence lies in the developmental gradients that occur along the spikes of rye. These pass from old in the centre spikelets to young in the spikelets at the base and apex. Anthers in which the archesporium was non-mitotic but the tapetum was highly mitotic were in pre-meiotic interphase (Figs. 1 and 2). Sometime after the tapetum was synchronised (the tapetal nuclei became uniform in size and non-mitotic) PMC entered leptotene (Figs. 3 and 4). The frequency of nucleoli per PMC fell during leptotene such that by zygotene there was only one nucleolus in each cell. The tapetum in all cases divided synchronously during zygotene. Zygotene (Figs. 5-8) was followed by pachytene (Figs. 9-11) which was followed by typical diplotene (Fig. 12) with no sign of a schizotene stage or a diffuse stage. Consequently the overall sequence of meiotic development in rye

(Figs. 1-13) was found to agree with that described for hexaploid wheat (Triticum aestivum L.) by Bennett et al. (1973) except for the question of the period of nucleolar fusion. In this respect we agreed with Darvey and co-workers who concluded that nucleolar fusion does occur during leptotene of hexaploid wheat (Darvey, 1972; Darvey et al., 1973). In contrast, Bennett (Bennett et al., 1973; Bennett, quoted in Shepard et al., 1974) reported that nucleolar fusion occurs prior to leptotene in hexaploid wheat.

Since the slides stained for heterochromatin were pre-classified for their meiotic stage, cyclic change in the distribution of the telomeric heterochromatin was assessed without bias. During the pre-meiotic interphase the terminal "C" bands were either distributed in one half of the nucleus in association with the nucleoli (Fig. 1), or were distributed right across the flattened nucleus (Fig. 2). These two arrangements were probably equatorial and polar views of a persistent Rabl orientation established at anaphase of the last pre-meiotic mitosis. In many cells a dark staining diffuse area was also noted in addition to the intensely staining and condensed telomeres. Where the telomeres were distributed in one half of the nucleus this dark staining region was restricted to the opposite half (Fig. 1). This bipolar appearance was also noted in PMC in early meiotic prophase (compare Fig. 1 with Figs. 3 and 4), and in interphase

tapetal cells (Figs. 14 and 15) and in somatic cells generally. Since in all these cases the dark staining diffuse region of the nucleus was found to lie opposite the interphase telomeres we believe that it represents that part of the nucleus that was formed from the polar area. Anaphase convergence of the proximal regions into the pole would result in a high density of euchromatin around the centromeres. The orientation of sister nuclei within the binucleate tapetal cells also suggested that the interphase centromeres were located in this dark staining diffuse area. Immediately following the synchronous tapetal mitosis most nuclei were arranged with their telomere region facing the sister nucleus, and with the dark staining diffuse area orientated away from their sister nucleus and toward the pole position (Fig. 14). The circular distribution of nuclear orientations within the binucleate tapetal cells shows that just after the synchronous tapetal mitosis the dark staining diffuse area lay almost exclusively within the polar quadrant (Fig. 16). Strong association between the pole of the cell and this area of the nucleus immediately following anaphase confirms the idea that it indicates the position of the centromeres within the interphase nucleus. Later on (by first meiotic metaphase) the orientation of this "centromere area" was nearly random relative to the location of the sister nucleus (Figs. 15 and 16). Since the

anaphase orientation of the nucleus was not conserved some rotation of the nucleus probably occurred during interphase.

As the PMC passed through the beginning of meiotic prophase there was marked aggregation of the heterochromatic telomeres in the same area of the nucleus that was occupied by the telomeres in interphase (Figs. 3 and 4). This aggregation resulted in the formation of a single large chromocentre and was accompanied by progressive fusion of the nucleoli so that in the end each PMC exhibited one very prominent heterochromatic body, often closely associated with the single nucleolus and located at the nuclear margin (Figs. 5 and 6). Although association between the nucleolus and the chromocentre was noted in most cells it was not present in all (Fig. 5). Injection of a 0.1% solution of colchicine into the boot three days before fixation blocked the formation of the chromocentre in rye. The chromocentre persisted well into the period of chromosome synapsis (Fig. 7), but later on it began to break up before the beginning of pachytene (Figs. 8-12). However telomere groupings were found in many pachytene nuclei (Figs. 9 and 10) and in diplotene most nuclei showed close end-to-end associations between two or more bivalents (Fig. 12). Non-heterochromatic telomeres were found both in telomere groups at pachytene (Fig. 10) and in the end-to-end assocations of diplotene.

Statistical analysis of 26 end-to-end associations of two, three or four telomeres at diplotene, in a plant which exhibited 10 heterochromatic telomeres, showed that heterochromatic and non-heterochromatic telomeres participated in these associations as expected on the basis of 10 heterochromatic telomeres to four non-heterochromatic. Therefore it is likely that non-heterochromatic telomeres were also included in the chromocentre that was formed earlier.

Discussion

The Attachment Plates in Rye and Wheat

Heterochromatin staining reveals that during early meiotic prophase the telomeres of rye chromosomes acquire a remarkable property that results in their becoming tightly grouped at the nuclear membrane. It could be argued that rye is a special case and that the chromocentre is an example of non-specific groupings of heterochromatic loci (Walters, 1970). However, the presence of non-heterochromatic telomeres in the telomere groupings of pachytene and diplotene suggest that it was indeed telomeres which aggregated and not merely the associated heterochromatin. This tight grouping of the telomeres at leptotene is comparable to the attachment plate in other, more obvious bouquets. On the other hand, the typical bouquet appearance with parallel or spiral arrangement of

the near terminal chromosome segments was not seen in rye. Consequently, without the terminal heterochromatin as a marker for the chromosome ends the grouping of the telomeres might well have gone unnoticed. Since the chromosomes of wheat lack this pronounced heterochromatin it is interesting to note that some parallels exist between the cryptic bouquet in rye and the process of nucleolar fusion in wheat.

In hexaploid wheat there are two pairs (1B and 6B) of conspicuously satellited chromosomes (Gill et al., 1963) which are the most active among the nucleolus organising chromosomes (Crosby, 1957; Longwell and Svihla, 1960); their nucleolus organising regions (NOR's) are probably exclusively responsible for the maximum of four large nucleoli that is usually found in wheat (Darvey and Driscoll, 1972).

In the course of meiosis the mean number of nucleoli per cell is reduced to one before or during leptotene (Bennett et al., 1973; Darvey, 1972; Darvey et al., 1973). This common nucleolus lies adjacent to the nuclear margin (Bennett et al., 1973). Subsequently two bivalents are generally seen attached to the single nucleolus at diakinesis (Darvey, 1972) which shows that during leptotene all four NOR's are brought to a common region of the nucleus, adjacent to the nuclear membrane. Secondary constrictions are close to the short-arm telomeres in both

wheat (Gill et al., 1963) and rye (Heneen, 1962) so perhaps the short-arm telomeres have a role in nucleolar fusion. Strong association between the fused rye nucleolus and the telomere attachment plate was noted earlier. It is plain that the strange behaviour of wheat nucleolus organisers, whereby both homologous and non-homologous nucleoli all fuse together, can be explained if we assume that wheat chromosomes participate in an attachment plate similar to that noted in rye. Inclusion of the telomeres on $1B^S$ and $6B^S$ in such a bouquet could bring all four NOR's sufficiently close together to guarantee complete nucleolar fusion close to the nuclear membrane.

Chromosome Association and the Bouquet

What could be the significance of the leptotene attachment plate in rye. One obvious possibility is that it represents a process whereby homologous chromosomes locate one another prior to detailed synapsis. Since chromosome association in wheat x triticale (unpublished) and formation of the bouquet (in rye) are both inhibited by colchicine and since both occur in the same general period of meiosis they seem to be different aspects of the same process. Formation of the bouquet seems to be achieved by an organising centre (pole determinant) in the cytoplasm of the PMC adjacent to the tapetum. The same organising centre may also be concerned with the control of pore development. Dover (1973) noted several lines of

evidence that indicated a connection between pore abnormalities and unusual pairing conditions in the cereals. High levels of homoeologous pairing were associated with multi-pore pollen whereas colchicine induced asynapsis was associated with poreless pollen. Formation of the pore in Sorghum, another grass (Christensen and Horner, 1974) occurred at the four points of contact between the quartet and the inner wall of the tapetum. This is the same region of the cytoplasm which was associated with the formation of the heterochromatic aggregate. These considerations suggest that the control of chromosome pairing is integrated into a much wider scheme of developmental control which also regulates the axes of meiotic division and the normal development of pollen (Dover, 1972, 1973). It also seems likely that this overall control is regulated by a colchicine sensitive organising centre located in the PMC between the nucleus and the tapetum. Whereas Dover concluded that pore formation and chromosome pairing were both inhibited by colchicine in G_1 (Dover, 1972; Dover and Riley, 1973), our data as well as those of other authors (Barber, 1942; Darvey, 1972; Dermen, 1938; Levan, 1939; Shepard et al., 1974; Walker, 1938) indicate that colchicine can act on pairing, and possibly pollen pore formation, as late as G_2/leptotene.

Formation of the Attachment Plate

With these ideas in mind it is worth pointing out some features that have been reported concerning both the telomeres and the formation of the leptotene bouquet. In the presence of a leptotene bouquet in both Psilotum (Hiraoka, 1941) and Onoclea (Marengo, 1949) the entire leptotene nucleus was displaced toward the cell margin on the side of the attachment plate. Thus there seemed to be some mechanical interaction between nucleus and cytoplasm associated with the bouquet. In general, where there is a centriole present in the cytoplasm it is found close to the attachment plate of the bouquet (see esp. Hughes-Schrader, 1943; Wilson, 1934). Hughes-Schrader (1943) reported a series of changes in astral activity around the centriole that correlated with the polarisation of chromosomes within the prophase nucleus. Initially the attachment plate of the leptotene bouquet was formed close by an active centriole complex (aster) in the cytoplasm, but after the aster disappeared in mid-prophase, bouquet polarisation was lost. However, when the asters reappeared in diplotene the telomeres regained their ability to polarise the bivalents toward the aster. Finally polarisation by the telomeres lapsed about the time that the nuclear membrane was dissipated (diakinesis). At the same time the centromeres became active and began to orient the bivalents for meiotic metaphase. These results suggest that during the meiotic cycle the telomeres

might have the ability to interact with a spindle-like system in the cytoplasm but only through association with the nuclear membrane. In fact centromere-like activity (neocentric activity) of particular telomeres has also been found in the meiosis of rye. In certain inbred lines of rye these telomeres are able to polarise part or all of the chromosomes to one pole or the other, but only during the two meiotic divisions (Prakken and Muntzing, 1942; Rees, 1955). Incomplete removal of membrane material from the telomeres after meiotic prophase could allow the telomeres to retain properties normally concerned with formation of the bouquet attachment plate, and to behave like single centromeres on the meiotic spindle (Ostergren and Prakken, 1946).

Bennett et al. (1974) reported that bundles of 20 nm fibres appear within the PMC nucleus during pre-meiotic interphase of hexaploid wheat. Some of these bundles were found in the narrow gaps between the chromosomes and the nuclear membrane. The authors suggested that this material could be a specific structure involved in meiotic interaction between the nuclear membrane and the chromosomes.

Other Meiotic Systems

In summary, detection of a cryptic bouquet stage in rye and its inferred existence in wheat suggests that bouquet arrangements may be more widespread than has been

suspected hitherto. It is possible that the formation of an attachment plate in leptotene corresponds directly to the colchicine sensitive pairing activity that has been detected in wheat.

However, despite the emphasis that we have placed on telomeres in the present discussion, in different meiotic systems other parts of the chromosome could be drawn to the attachment plate. Furthermore, since colchicine sensitive activity is not essential for synapsis provided chromosome segments are close enough to one another (Driscoll and Darvey, 1970; Driscoll et al., 1967) it follows that a leptotene attachment plate might be redundant in species exhibiting intense somatic association. These simple considerations may explain much of the divergence between different accounts of meiosis (cf. McClung, 1927 Wenrich, 1917).

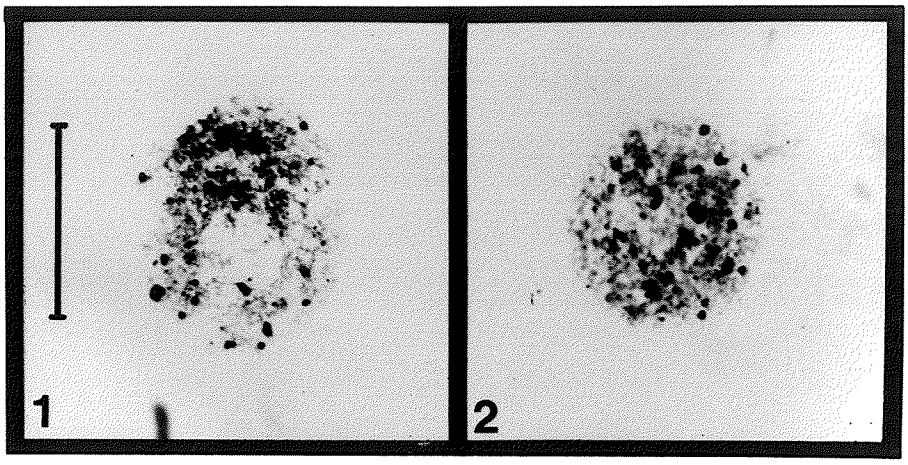
Acknowledgments

Financial assistance from the International Development Research Centre and the National Research Council of Canada, both of Ottawa, is gratefully acknowledged. We would also like to thank Dr. Norman Darvey for discussing with us the ideas in this paper, and Mr. Graham Scoles for scoring the data in Fig. 16.

Figures 1 and 2. PMC, pre-meiotic interphase (bar on Fig. 1 is 25 μm).

Figures 3 and 4. PMC, leptotene nucleolar fusion

Figures 5 and 6. PMC, early zygotene (Fig. 5: arrows indicate chromocentres (stained) and nucleoli (unstained); bar is 50 μm. Fig. 6: bar is 200 μm). (Figs. 1-5 are fall rye bulk, stained with Giemsa; Fig. 6 is Prolific, stained with Leishman s. Figs. 2-4 are same magnification as Fig. 1.)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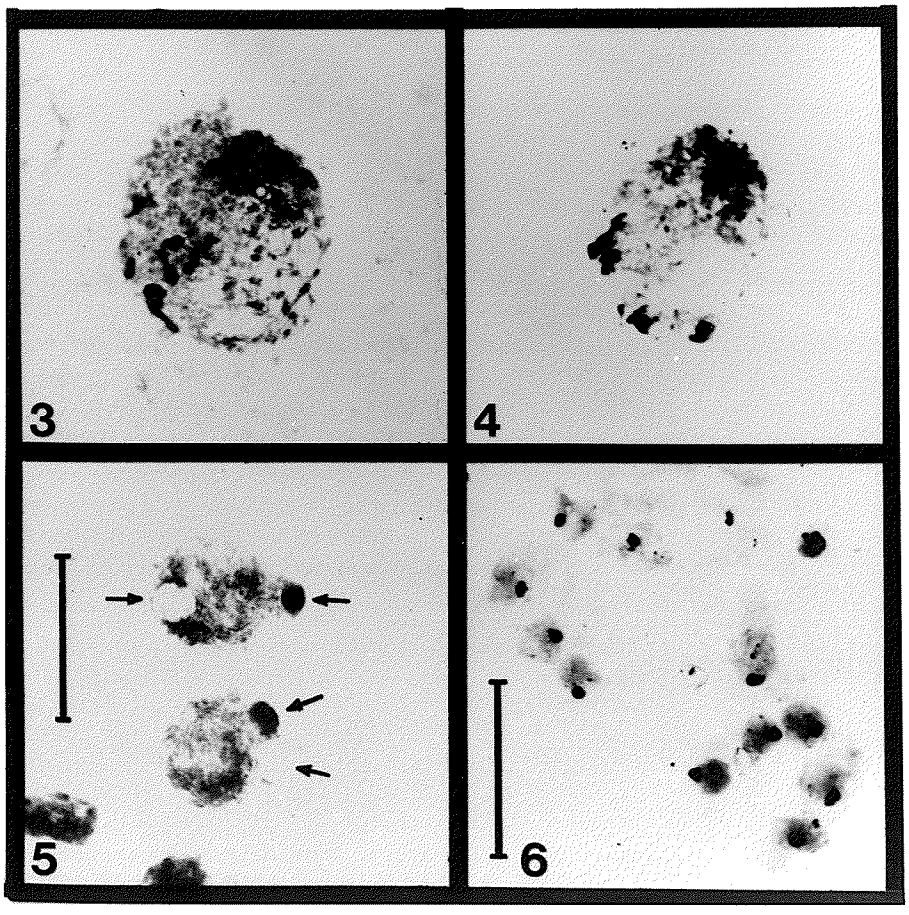
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Figure 7. PMC, mid zygotene.

Figure 8. PMC, late zygotene.

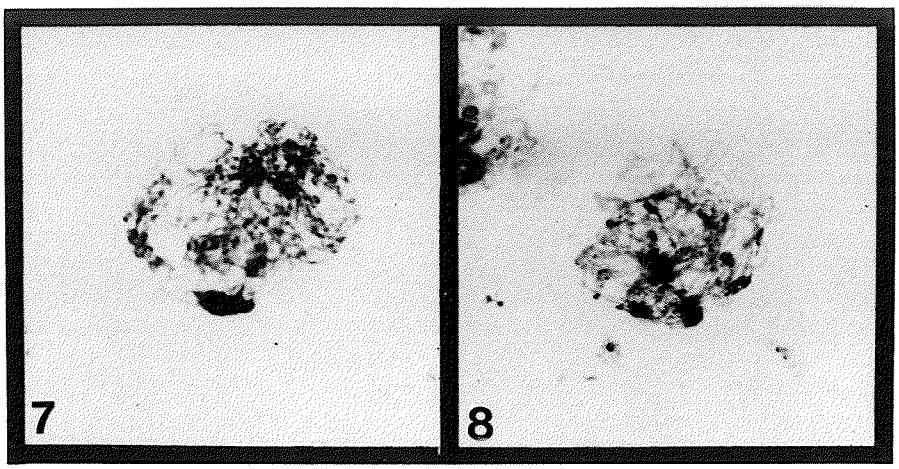
Figure 9. PMC, early pachytene.

Figure 10. PMC, mid pachytene (arrows with open heads indicate non-heterochromatic telomeres, arrows with solid heads indicate heterochromatic telomeres).

Figure 11. PMC, late pachytene.

Figure 12. PMC, diplotene (this cell contains five ring bivalents with a band on both ends and two ring bivalents with a band on one end only).

Figure 13. PMC, first meiotic metaphase (this cell contains five ring bivalents of which three have a band on one side only and two have a band on both sides; of the two rod bivalents, one has bands on both the paired and unpaired arms and the other has a band only on the unpaired arm. This latter band shows clear heteromorphism between the homologues, the top chromosome carries no prominent heterochromatin at all (arrowed). (Figs. 7 and 12 are Prolific, rest are fall rye bulk; Fig. 13 is stained with Leishman's and has the same magnification as Fig. 5 (Plate I) rest are stained with Giemsa and have same magnification as Fig. 1.)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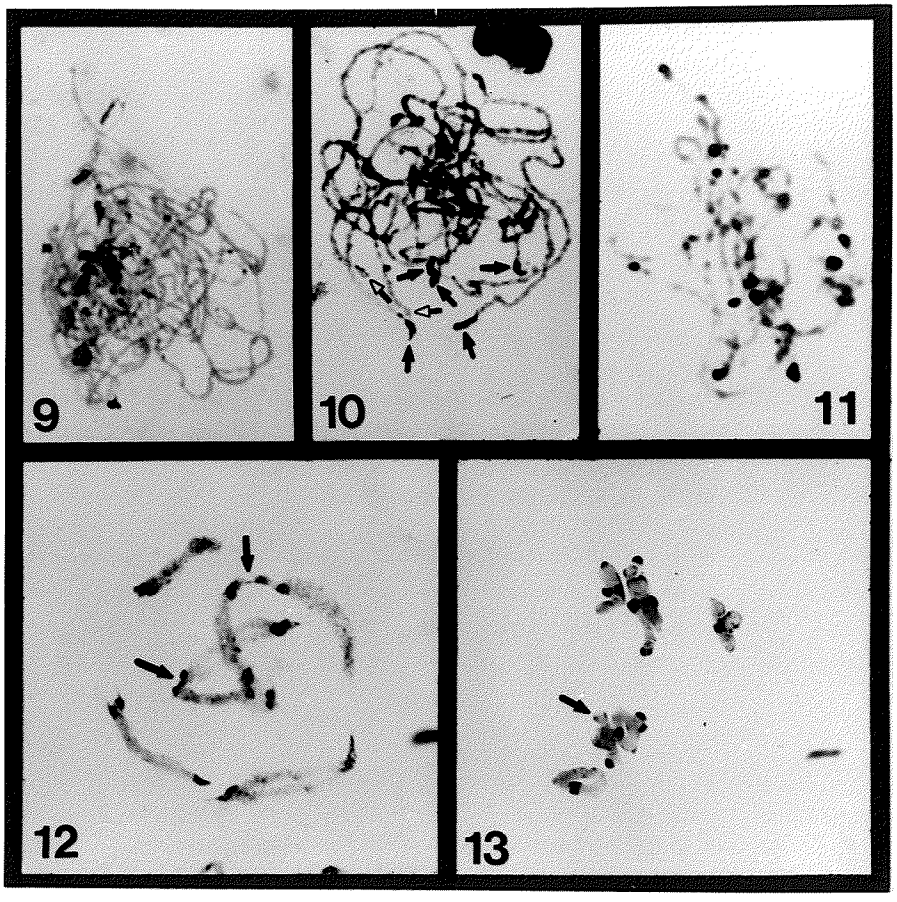
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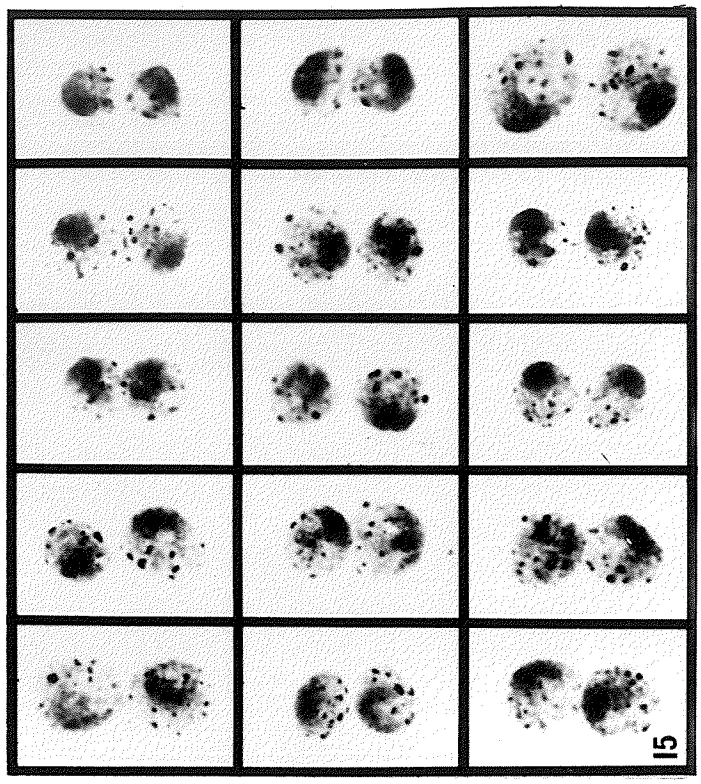
Figure 14. Random sample of interphase binucleate tapetal cells from an anther still containing cells in the synchronous tapetal division. (Bar is 20 μm).

Figure 15. Random sample of binucleate tapetal cells from an anther in first meiotic metaphase. (Magn. same as Fig. 14. This anther was probably 24-36 hours later in development than that illustrated in Fig. 14. Both anthers were from the same spikelet of fall rye and both are stained with Giemsa.)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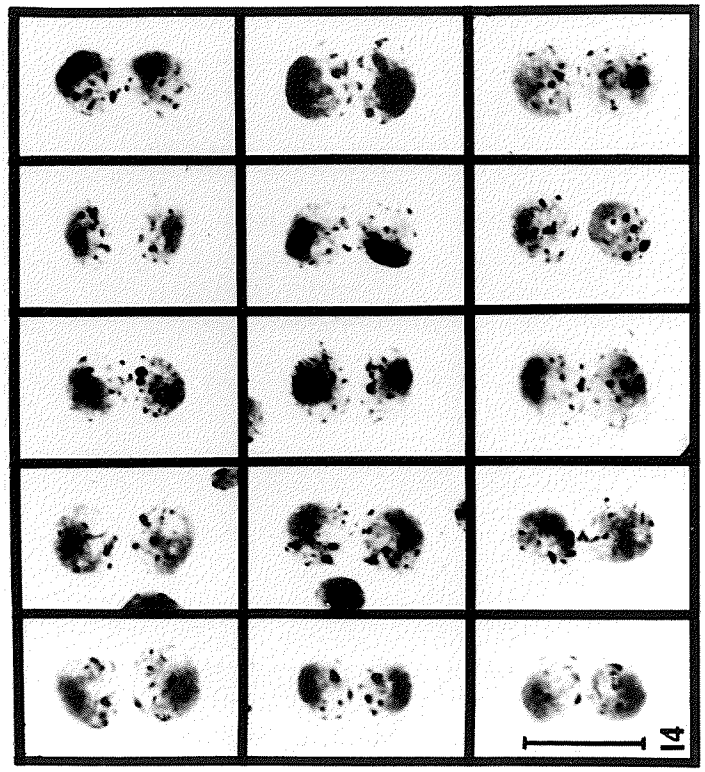
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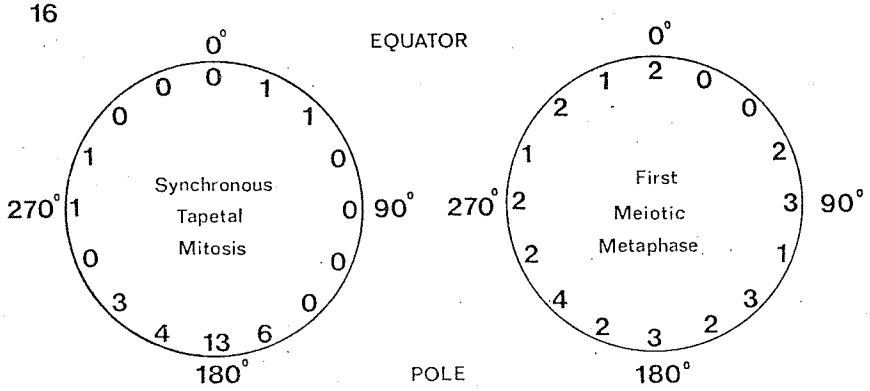

FIG. 16.—The circular distribution of nuclear orientations in binucleate tapetal cells at two different stages of development. (Nuclear orientation represents the angle of the dark staining diffuse area about the nuclear centre. Estimates to the nearest sixteenth of a clockwise revolution were made from the intersection of the nuclear margin with the axis joining the two nuclear centres. These data were obtained from the photomicrographs in figs. 14 and 15 by an independent observer after the photos were coded and randomised.)

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THE EFFECT OF COLCHICINE ON CHROMOSOME PAIRING

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THE EFFECT OF COLCHICINE ON CHROMOSOME PAIRING

Abstract

Beginning at 120 hours prior to first metaphase of meiosis (MI) a 0.03% aqueous solution of colchicine was injected into the boot of pentaploid (hexaploid triticale x tetraploid wheat) hybrids developing at 20° C ± 1 under continuous illumination. Colchicine applied 40 h or less prior to MI had no effect on chromosome pairing, while its application 40 h or more prior to MI induced a steady decline, culminating in a 40% reduction in chromosome pairing at about 80 h from MI. Between 48 and 35 h before MI (late premeiotic interphase to early zygotene) meiocytes underwent a period of active nucleolar fusion. The time, therefore, at which the colchicine-sensitive aspects of chromosome pairing were completed coincided with the completion of nucleolar fusion. From comparison with other findings, it was concluded that there is a colchicine-sensitive bouquet stage which appears in leptotene and early zygotene; this bouquet is responsible for active nucleolar fusion and final close association between homologous chromosomes.

Introduction

In hexaploid wheat there are two pairs of conspicuously satellited chromosomes (Gill et al., 1963). These two pairs (1B and 6B) correspond to chromosomes SAT 1 and SAT 2 of tetraploid wheat (Giorgi and Bozzini, 1969) and they are also prominent in hexaploid triticale (Merker, 1973; Shigenaga and Larter, 1971). Chromosomes 1B and 6B are the most active among the nucleolus organising chromosomes of wheat (Crosby, 1957; Longwell and Svihla, 1960), and their nucleolus organising regions are probably exclusively responsible for the maximum of 4 large nucleoli found in common wheat (Darvey and Driscoll, 1972) and the maximum of four nucleoli found in tetraploid wheat and hexaploid triticale (Thomas and Kaltsikes, in preparation).

In hexaploid wheat, cells in the premeiotic interphase contain one to several nucleoli (Bennett et al., 1973; Darvey, 1972). In the course of meiosis, the average number of nucleoli per cell is reduced to one by a process of active nucleolar fusion. Active nucleolar fusion occurs before or during leptotene (Bennett et al., 1973; Darvey, 1972; Darvey et al., 1973) such that by late zygotene and pachytene there is only one nucleolus

per cell which lies adjacent to the nuclear membrane (Bennett et al., 1973). Subsequently, two bivalents are seen attached to the single nucleolus at diplotene and diakenesis (Darvey, 1972) which means that during nucleolar fusion all four nucleolus organisers are brought to a common region of the nucleus, adjacent to the nuclear membrane.

Active nucleolar fusion is therefore a singular process on two counts. Firstly, it cannot be simply dismissed as being related to synapsis, because homologous and non-homologous nucleoli all fuse together. Secondly, the process is highly efficient which suggests that it reflects an important activity of the meiotic nucleus.

Because the nucleolus organising regions are close to the short arm telomeres in both the principal nucleolus organising chromosomes, it follows that the process of nucleolar fusion could be accounted for by the formation of a bouquet. By drawing the telomeres together, a bouquet stage would bring all four nucleolus organising regions close enough tegether to guarantee complete nucleolar fusion close to the nuclear membrane. Bouquet formation has been described in cultivated rye, which is a close relative of wheat (Thomas and Kaltsikes, 1976a).

Colchicine-sensitive pairing activity in wheat and its relatives is believed to represent an association process that brings homologous chromosomes into close

proximity as a preliminary to synapsis and crossing over (Darvey, 1972; Driscoll and Darvey, 1970; Driscoll et al., 1967; Dover and Riley, 1973). Because nucleolar fusion in wheat (Darvey, 1972) and bouquet formation in rye (Thomas and Kaltsikes, 1976a) were both sensitive to colchicine, Thomas and Kaltsikes (1976a) suggested that both active nucleolar fusion and bouquet formation were visible aspects of colchicine-sensitive pairing behaviour. This report describes experiments that examine the relationship between active nucleolar fusion, colchicine-sensitive pairing activity, and the meiotic cycle in hybrids between durum wheat and hexaploid triticale.

Materials and Methods

The plants used in this study were pentaploid hybrids obtained by crossing durum wheat (*Triticum turgidum* L. em. Bowden cv. Stewart '63) with hexaploid triticale (x *Triticosecale* Wittmack) cv. Rosner and University of Manitoba accession 6A391 [*T. aestivum* L. em Thell. cv. Tetra-Prelude (AABB) x *Secale cereale* L. cv. Prolific]. Material was maintained as vegetative clones, and grown at 20° C under constant illumination. Only 35 chromosome plants were used and the karyotype was repeatedly checked from the meiotic pairing relationships. At MI these plants show full pairing of wheat genomes (AABB) accompanied by 7 rye univalents (R). Only one case of a somatic change was detected from 14 II + 7 I to 13 II + 1 iso + 8 I. This plant was discarded.

All observations pertaining to tapeta and to premeiotic and meiotic archesporia were made on cells obtained by extruding the columns of tapeta and archesporia into acetocarmine stain. Gentle tapping of a supported cover glass then separated the tapeta into sheets and the archesporia into cylindrical columns. Using this technique there was no possibility of confusion between wall cells, tapetal cells and archesporial cells.

Meiotic intervals were timed by a procedure described by Bennett et al. (1971). Three to five spikelets were sampled from the central region of one side of the spike, through a door cut in the leaf sheathes, and these were placed in Carnoy's fixative. The door was replaced with adhesive tape and the entire spike was allowed to continue its development. After a measured interval the entire spike was fixed. Stages present on one side of the spike at the time of sampling were compared with stages present in the spikelets on the other side at the time of spike fixation. Data on meiotic timing represent observations from a total of 1,712 florets from 207 spikes.

Data from the timing experiments were interpreted according to logical criteria that take into account the developmental gradients that exist within the spike. These gradients are such that oldest stages are found in the central spikelets with younger stages toward the base and apex. The most precise criterion was found to be a "two oldest florets criterion" which assumes that the most advanced floret at the time of sampling was at the same point in development as the most advanced floret encountered at the time of fixation. However, other criteria are also possible (Bennett et al., 1971).

The meiotic cycle was timed in relation to a sequence of nine recognisable events that were considered as fixed points in the cycle. The cycle began with the

cessation of mitoses in the archesporium and ended with MII (Table I). The durations of the eight developmental segments between the nine events were estimated from the raw data by least squares procedures as outlined by Roupakias and Kaltsikes (1977).

The time course of nucleolar fusion relative to MI was determined in the following way. The average number of nucleoli per meiocyte (80-150 cells sampled over 2 to 3 anthers per floret) was determined from spikelets, sampled according to the timing procedure described above. The sampled spike was then fixed after a measured time interval. The floret with the highest level of nucleolar fusion (lowest average number of nucleoli) at the time of spikelet sampling was then compared with the most advanced floret on the other side of the spike at the time the entire spike was fixed. If the development within the spike indicated that the floret with the highest level of nucleolar fusion would have not yet reached MI this observation was graphed in Figure 1 as the symbol <. However if this floret would have reached MI in the time interval, or passed it, then the level of nucleolar fusion was graphed as the symbols = or > respectively.

The procedure outlined by McLeish and Sunderland (1961) with a hydrolysis time of 7 minutes was used for the estimation of DNA content with a Barr and Stroud GN2

integrating microdensitometer.

In order to study the effect of colchicine on chromosome pairing, 0.03% aqueous solutions of colchicine were injected into the boot (sheath of the flag or last leaf which contains the spike) for varying periods before fixation. Colchicine was injected upwards, from below the spike, to fill the entire boot so that colchicine emerged at the junction of the flag leaf and its sheath. Excess solution drained from the injection hole leaving the exterior of the spike wet with colchicine. The concentration of 0.03% was selected from preliminary trials as being low enough to avoid killing the spike quickly but still high enough to give a high level of pairing inhibition.

Colchicine did not penetrate the interior of the florets directly. To assess the rate at which colchicine penetrated to the anthers, a study was made of the time required for C-mitoses to appear in the anther filament, the tapetum and the mitotic archesporium of primary and secondary florets of injected spikes.

The number of pairs of chromosome arms which carried chiasmata (X), and the number of paired chromosomes (B) were recorded at MI at different intervals after injection of colchicine, from anthers of the primary and secondary florets. Because the plants were maintained in a constant environment, all trends in pairing must represent an

effect of injection. The injection of water alone had no effect on B or X at MI between 24 and 72 hours after injection. The ploidy state of the tapetal cells at MI was also recorded.

Chromosome pairing is a two-step process (Darvey, 1972; Driscoll and Darvey, 1970). First, in the association step, homologous chromosomes must find one another; and second, in the synapsis/chiasma formation step, they synapse and may form chiasmata. According to Darvey (1972), only the association step is sensitive to colchicine. Therefore to properly assess the effect of colchicine on chromosome pairing a distinction must be made between the number of chromosomes which associate with their homologue (P) and the number of chromosomes which continue beyond the association step to form chiasmata. Consequently, the MI raw data on chromosome pairing have to be analyzed in such a way that the effect of colchicine, if any, on either or both steps of chromosome pairing can be assessed. To do so, the following formula (given as formula 11 in the Appendix) was developed to relate P (the maximum number of chromosomes which complete the association step of chromosome pairing) to Xg (total sum of pairs of chromosome arms paired), Bg (total number of paired chromosomes) and Z (number of cells examined):

$$P = \frac{Xg^2 + (Xg - Bg)Z}{(2Xg - Bg)Z}$$

Results and Discussion

Active Nucleolar Fusion

Pollen mother cells (PMC) in the premeiotic interphase contained one to four nucleoli (Fig. 2). At early stages of leptotene, nuclei contained one to three nucleoli (Figs. 3 and 4). At diakinesis, two pairs of nucleolus organisers were found attached to the nucleolus (Fig. 5).

Leptotene lasted 8 to 9 h, from about 45 hours before MI to about 37 hours before MI (Tables I and II). Reduction in the number of nucleoli occurred during this same period of the meiotic cycle (Fig. 1). About 48 hours before MI there were 2.0 to 2.2 nucleoli per nucleus. This value declined steadily until about 35 hours before MI there was only one single nucleolus per nucleus.

Bouquet formation occurred during leptotene/late nucleolar fusion in hexaploid triticale (nucleolar fusion = 1.5 to 1.2, Figs. 7 and 8), just as it did in rye (Thomas and Kaltsikes, 1976a). In rye, formation of the bouquet occurred around the margin of a circle, laid out on the surface of the nucleus (Fig. 9). This locus of aggregation was orientated outward, toward the exterior of the

PMC column, i.e. toward the archesporium/tapetum interface (Fig. 10).

In the hybrids between durum wheat and hexaploid triticale, zygotene lasted 16-17 h from about 37 hours before MI until about 20 hours before MI (Tables I and II). In many cases synapsis was first observed around the nucleolus (Fig. 11).

PMC of Stewart '63 x 6A391, with an average of 1.5 nucleoli per nucleus (about 40 hours before MI; Fig. 6), contained the full 4C amount of DNA (Table III), whereas PMC from anthers showing the last asynchronous mitoses in the tapetum (mitotic index of tapetum = 1 to 2%; about 58 hours before MI; Table I), contained DNA values intermediate between 2C and 4C (Table III).

Therefore the process of nucleolar fusion begins just prior to leptotene, occurs concurrently with bouquet formation in rye and triticale, ends in zygotene and does not seem to be associated with the S period (c.f. Bennett et al., 1973; Darvey et al., 1973).

The Effect of Colchicine

Enough colchicine to inhibit mitoses quickly penetrated the anther after injection (Table IV). The minimum time for C mitoses to appear in the anther filament was about 2 hours, and by 6 hours colchicine was present in mitotically active concentration within the anthers of

more than 50% of florets (Table IV). No cases were observed where mitoses were inhibited in the tapetum but were not inhibited in the archesporium or in the anther filament. Therefore colchicine must have entered the anther along the anther filament and the premeiotic archesporium was as accessible to colchicine as the tapetum.

Nucleolar fusion was inhibited by colchicine. For example, one floret at the synchronous mitosis (Figs. 12 and 13), normally expected to show only one nucleolus per nucleus (Table I; Fig. 1), in the presence of colchicine contained 57 nucleoli in 40 cells (nucleolar fusion = 1.43).

C-mitoses and polyploid nuclei were observed in the anther filament (Figs. 14 and 15), the tapetum (Figs. 12, 13 and 16), the archesporium (Fig. 17), the anther wall, and somatic cells generally. However the spindle of the first and second meiotic metaphases were never observed to undergo inhibition, even up to 5 days after injection with 0.03% colchicine (Figs. 18 and 19). Chilling entire spikes of durum wheat and hexaploid triticale in iced water for up to 3 days, also inhibited the various somatic and premeiotic spindles, but failed to inhibit the spindles of first and second meiotic metaphase. It appears that during the meiotic cycle, there is some change in the spindle or its precursors that makes the

spindle less susceptible to inhibition by both cold water and colchicine.

Interlocked ring bivalents and proximal chiasmata were both found in MI preparations affected by colchicine. All the cases of bivalent interlocking, observed at MI (Figs. 20 and 21), occurred 35 to 50 hours after colchicine injection (Table V) and the most striking cases of proximal chiasmata (Fig. 22) were observed in the same interval (Table V). Two cases of bivalent interlocking were observed at diakinesis, 66 hours (Fig. 6) and 96 hours after colchicine injection. No cases of interlocking were observed prior to 35 hours after injection or in water controls, although we have observed them in untreated preparations of Rosner. Darvey (1972) reported that colchicine did not induce bivalent interlocking in the hexaploid wheat variety Chinese Spring.

The number of paired chromosomes (B), and the number of pairs of chromosome arms with chiasmata (X) were both strongly inhibited by colchicine, some considerable time after injection (Table V).

Application of the Two-Step Model of Chromosome Pairing

It is believed that colchicine specifically inhibits the association process whereby chromosomes locate their pairing partner (Darvey, 1972; Dover and Riley, 1973; Driscoll and Darvey, 1970). If this is true, then in

the absence of colchicine and other factors which inhibit chromosome association, the number of chromosomes which locate a pairing partner (P) should remain constant even through the number of chiasmata (X) and the number of paired chromosomes (B) fluctuate widely; for the AABB genomes, the maximum possible value of P is the number of chromosomes which comprised these genomes, i.e. 28. The observed relationship between B and X for MI pairing data of the AABB genomes is shown in Figure 23. These pairing data were taken in a range of different circumstances but always in the absence of colchicine. With P set at 28, the B solution of the model (formula 5) (see Appendix) gave a good fit to these MI pairing data. Therefore this single (maximum) value of P was adequate to relate B and X over a wide range of pairing levels. In addition, a single value of P was sufficient to fit the model to data of homoeologous chromosome pairing in Triticum aestivum x Agropyron intermedium hybrids (P = 37.52; Gaul, 1958) and to homologous pairing in inbred rye (P = 14; Lamm, 1936; Prakken, 1943). (Gaul's (1958) formula also gave a good fit for these two sets of data. Nonetheless, in the Appendix we show that it contains an incorrect term.) Consequently, these usual types of pairing failure do not interfere with the ability of chromosomes to locate their pairing partner. However, the model could not be fitted to pairing data of

the AABB genomes, taken in the presence of colchicine (Fig. 23). Instead the number of paired chromosomes (B) was lower than expected. Since B and X are observations, the fault must lie in the assumption that P = 28. In fact, no single value of P was adequate to relate B and X over the whole pairing range. Instead, P can be calculated using formula 11 (see Appendix).

There was a change in the calculated value of P as a function of time after colchicine injection (Fig. 24). The effect of colchicine on P was characteristic and large. Between 24 and 40 hours after injection there was little change in the number of chromosomes associated from the maximum value of 28. Between 45 and 80 hours after injection the number of chromosomes associated declined steadily from 28 chromosomes to a minimum of about 12 chromosomes (about 40% of homologous pairs associated). After 80 hours there was little further change in P.

If P is the number of chromosomes which have passed the association step and are potentially capable of forming chiasmata, then X/P represents the efficiency with which they do so. Colchicine also inhibited the efficiency of chiasma formation. However in contrast to its effect on P, the effect on X/P was small, without any obvious forward limit and variable in relation to the magnitude of the response (Fig. 24). This shows that the model

embodied in formula 11 is well able to analyse chromosome pairing into two components which behave very differently with respect to colchicine.

The Forward Limit of Colchicine Sensitivity

Ploidy changes in the tapetum can be used to relate the time at which an anther was first penetrated by colchicine to its effect on chromosome pairing (Dover, 1972; Dover and Riley, 1973).

Anthers containing only binucleate tapeta were first penetrated by colchicine after the synchronous tapetal division. Meiotic intervals that could have been affected by colchicine are late zygotene, pachytene and diplotene/diakinesis (Tables I and V). Anthers containing uninucleate tapeta of uniform size must have been first penetrated by colchicine after the asynchronous divisions in the tapetum were arrested, but before the synchronous division. Meiotic intervals relevant to this period are the late pre-meiotic interphase, leptotene and early zygotene (Tables I and V). Uninucleate tapeta containing nuclei of non-uniform ploidy must have been penetrated by colchicine before the asynchronous mitoses in the tapetum came to a halt. This covers the major part of the pre-meiotic interphase (Tables I and V).

The greatest part of the response of chromosome pairing to colchicine occurred during the period between tapetum synchronisation and the synchronous tapetal mitosis (Table V). Furthermore, the first group of anthers showing uninucleate-4C tapeta showed essentially no response to colchicine. Therefore, chromosome pairing must have become resistant to colchicine before the middle period of zygotene.

The earliest indication of the inhibition of pairing was found in PMC first affected by colchicine about 37 hours before MI (see footnote of Fig. 24). However, there was also a colchicine-induced delay in the rate of meiotic development (Table VI). This means that the PMC took longer to reach MI when colchicine was present than when it was absent. If the PMC at the end of nucleolar fusion were delayed for two hours by an injection of colchicine about this time (Table VI) then the point at which colchicine-sensitive pairing activity was completed would coincide exactly with the point at which active nucleolar fusion was completed: 35 hours before MI. In any event, colchicine-sensitive pairing activity finished no earlier than the end of leptotene (Table I; Figure 24) and no later than the middle of zygotene (see above).

Anthers with uninucleate-4C + 8C tapeta showed only minor decreases in pairing over the minimum observed in anthers containing only uninucleate-4C tapeta (Table V).

Therefore, the reduction in pairing achieved by exposure of the late premeiotic interphase, leptotene and early zygotene to colchicine was not much increased by additional exposure of the pre-meiotic interphase.

General Discussion

Buss and Henderson (1971) using locusts and Dover and Riley (1973) using wheat and wheat hybrids, both claimed that the pre-meiotic mitosis was the last point at which the arrangement of chromosomes could be influenced experimentally before synapsis. However, in our opinion, the data presented by Buss and Henderson (1971) do not support their contention that chromosome entanglements leading to bivalent interlocking are unresolvable after the premeiotic mitosis. If entanglements remained unresolved because of high temperature treatments at the pre-meiotic mitosis, then interlocking should have persisted until the last cells to divide mitotically at high temperatures passed MI. The authors stated that it took 7 to 8 days at normal temperatures for cells in the pre-meiotic "S" period to reach MI (Buss and Henderson, 1971, para. 4, line 3). Last gonial mitoses would therefore require 8 to 9 days minimum to reach MI. However, bivalent interlocking disappeared from MI 5 to 6 days after the return to normal temperatures in locusts subjected to two different regimes of normal to high to normal temperature shifts (Buss and Henderson, 1971, Fig. 2). Therefore, it can be concluded that in this

material there is a specific stage of meiosis during which chromosome entanglements become unsnarled, but that this stage not only comes after the pre-meiotic mitosis but after the premeiotic "S" period as well.

In the present material it was demonstrated that the first measurable effect of colchicine on pairing occurred roughly half way between the final archesporial mitosis and first meiotic metaphase. Colchicine exerted a profound effect on the pairing of homologues when applied during late premeiotic interphase and early meiotic prophase, with pairing becoming resistant to colchicine as late as early zygotene. Early zygotene also marked the end of nucleolar fusion. Bouquet formation (Thomas and Kaltsikes, 1976a; Bowman and Rajhathy, 1977) and nucleolar fusion both demonstrate that there is movement of chromosomes within the leptotene nucleus and that this movement is sensitive to colchicine.

Thomas and Kaltsikes (1976a) suggested that bouquet formation is universal in the Triticinae and that it is a colchicine-sensitive process whereby homologous chromosomes locate on another prior to synapsis. As expected, there is also formation of a bouquet in triticale which confirms that wheat and rye have similar meiotic processes.

Colchicine-sensitive pairing activity in the wheat x triticale pentaploids was also comparable to the situation in lily. In lily, chromosome pairing was sensitive

to colchicine during leptotene but became resistant to colchicine in zygotene (Shepard et al., 1974) although its main effect was reported to be inhibition of chiasma formation (Hotta and Shepard, 1973).

It seems likely that examination of fine structure in leptotene cells will reveal a bouquet in wheat, and will show how the recognition of homologous chromosomes is achieved.

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Appendix

A Two-Step Model of Chromosome Pairing

The Association Step: P

In a bivalent-forming diploid with $2n$ isobrachial chromosomes, the number of pairs of homologous chromosomes completing the association step is defined as P/2, P being an even integer; where $P \leq 2n$. $2n - P$ is the number of pre-association univalents, i.e. chromosomes which fail to pair because they do not become intimately associated with their homologue. This association is the step which is considered to be highly sensitive to colchicine.

The Synapsis and Chiasma Formation Step: X

With P/2 homologous pairs associated, there are P sites for chiasma formation since each pair of chromosomes can form chiasmata in either arm. If there are X first (see Gaul, 1958) chiasmata per cell ($X \leq P$), then the average probability of each site forming a chiasma is X/P. (A first chiasma indicates that the chromosome arm in question is paired. Of course, more than one chiasma may be present in the particular chromosome arm

but for the present purpose these are unimportant.) The model assumes that each potential site for chiasma formation has an identical probability (X/P) of actually doing so.

The Model

It is not possible to work from the above probabilities directly since P is unknown. Instead the question is asked, how many chromosomes (B) are expected to pair for given values of P and X and then resolve the relationship for P instead of B. The assumption of an equal probability of chiasma formation for all associated chromosomes is satisfied by supposing that the X chiasmata and the P - X absences of chiasmata are discrete events numbered 1 through P, which are randomly combined without replacement into P/2 pairs. Numbers 1 to X are associated with chiasmata and numbers X + 1 to P are associated with the absence of chiasmata. A pair of numbers from between 1 and X corresponds to a ring bivalent, a pair of numbers from between X + 1 and P corresponds to a pair of univalents while a mixed pair corresponds to a rod bivalent. The number of ways P individuals can be combined into P/2 pairs is:

$$\frac{P!}{[(P/2)!]2^{P/2}} \qquad (1)$$

Since each set of pairs, or cell, contains P/2 pairs, the total number of pairs of associated chromosomes is:

$$\frac{P(P!)}{(P/2!)\,2^{(P/2)+1}} \qquad (2)$$

If there are X chiasmata per cell, then the total number of bivalents, both ring and rod, present in all these pairs is:

$$\frac{X(X-1) + 2X(P-X)}{2} \qquad \frac{(P-2)!}{(((P-2)/2)!)\,2^{(P-2)/2}} \qquad (3)$$

The probability of an associated chromosome being paired is therefore given by dividing equation (3) by equation (2) which simplifies to:

$$\frac{X(2P - X - 1)}{P(P - 1)} \qquad (4)$$

Since there are P associated chromosomes per cell, the total number of paired chromosomes per cell can now be written:

$$B = \frac{X(2P - X - 1)}{P - 1} \qquad (5)$$

Solving for P gives:

$$P = \frac{X^2 + X - B}{2X - B} \qquad (6)$$

This is similar to Gaul's formula (Gaul, 1958) lacking only his correction for the calculation of P from data summed over Z cells. In fact, the definitions of B and X in Gaul's formula are different from those in (6) since he defined them as sums, whereas B and X in (6) are defined only in relation to a single cell or to mean

values. Retaining for the moment the present definitions, the number of paired chromosomes per cell as given in (5) is:

$$B = \frac{X(2P - X - 1)}{P - 1}$$

The number in Z cells is therefore:

$$ZB = ZX\left[\frac{2P - X - 1}{P - 1}\right] \qquad (6a)$$

Solution for P gives:

$$P = \frac{ZX^2 + ZX - ZB}{2ZX - ZB} \qquad (7)$$

If X and B are redefined according to Gaul (1958), then:

$$Xg = ZX \qquad (8)$$

$$Bg = ZB \qquad (9)$$

Substitution of these into (7) gives:

$$P = \frac{\frac{1}{Z}Xg^2 + Xg - Bg}{2Xg - Bg} \qquad (10)$$

This is the same as:

$$P = \frac{Xg^2 + (Xg - Bg)Z}{(2Xg - Bg)Z} \qquad (11)$$

This is different from the formula Gaul (1958) provided for the calculation of P from sum data which was:

$$P = \frac{Xg^2 + Xg - Bg}{(2Xg - Bg)Z} \qquad (12)$$

It appears that his modification is incorrect since simulation with equations (11) and (12) showed that the value of P given by (12) changed with Z, independently of any change in the pairing trend. On the other hand,

equation (11) gave the same result for P with mean data (when Z = 1) as it did with sum data (when Z = number of cells scored).

Assumptions and Applications

The prediction of the relationship between B and X based on this model rests on two assumptions. The first assumption is the particular value given to P. In the absence of factors which inhibit chromosome association, P should equal 2n. As we have shown in the text, in the absence of colchicine, and for a homogenous set of chromosomes such as those of the AABB genomes of tetraploid wheat or of the RR genomes of diploid rye, P = 2n accurately relates B and X over a wide range of pairing levels.

The second assumption is that all chromosomes which associate will form chiasmata with the same efficiency. Therefore, deviation from the expectation based on P = 2n is expected where some chromosomes are more likely to pair than others. This condition is found in triticale where rye chromosomes are less likely to pair than wheat chromosomes (Thomas and Kaltsikes, 1976b). Consequently, the relationship between B and X deviates significantly from expectation (Thomas and Kaltsikes, 1972). In this case, deviation probably does not indicate a lack of chromosome association, because, judging from diakinesis,

all the chromosomes of triticale can locate their homologue (Tsuchiya, 1970; Lelley, 1974).

Deviation of pairing data from the predictions of the model is also expected, following the injection of colchicine. In this case, deviations reflect a significant inhibition of the calculated value of P following the injection. However, calculation of P from B and X in the presence of colchicine, is only justified if B and X conform to the 2n expectations when colchicine is not applied.

Table I

Sequence of meiotic events measured in hours from MI in two durum x 6x triticale pentaploids

Developmental Stage	Material	
	Stewart '63 x 6A391	Stewart '63 x Rosner
G1 hold of PMC nearly complete. Mitotic index of asynchronous archesporium - 0.5-1%	-73.79 ± 1.280*	-87.08 ± 1.554
G1 hold of tapetum nearly complete. Mitotic index of asynchronous tapetum 1-2%	-58.06 ± 0.996	-60.76 ± 1.719
Leptotene begins	-46.61 ± 1.023	-44.81 ± 1.6739
Zygotene begins	-37.28 ± 0.987	-36.59 ± 1.437
Synchronous mitosis in tapetum	-26.17 ± 0.944	-29.98 ± 1.443
Zygotene ends	-20.97 ± 1.229	-19.54 ± 1.664
Pachytene ends	- 5.04 ± 1.134	- 5.63 ± 1.430
MI	0.00	0.00
MII	4.42 ± 0.996	4.57 ± 1.311
Total Duration - Meiotic Cycle	78.21 ± 1.280	91.65 ± 1.435
Duration - Synchronous Tapetal Cycle	31.89 ± 1.070	30.78 ± 1.608

*Standard error. The number of degrees of freedom associated with the standard errors was 10 for Stewart '63 x 6A391 and 9 for Stewart '63 x Rosner.

Table II

Duration of meiotic intervals measured in hours in two AABBR pentaploids

Meiotic Stage	Material	
	Stewart '63 x 6A391	Stewart '63 x Rosner
Premeiotic interphase	27.18 ± 1.565*	42.26 ± 2.049
Leptotene	9.33 ± 1.115	8.22 ± 1.996
Zygotene	16.31 ± 1.374	17.05 ± 1.823
Pachytene	15.93 ± 1.243	13.91 ± 1.516
Diplotene to MI	5.04 ± 1.134	5.63 ± 1.430
MI to MII	4.42 ± 0.996	4.57 ± 1.311

*Standard error.

Table III

DNA values in the PMC at five points in the meiotic cycle of Stewart 63 x 6A391 (AABBR)

Description of meiotic stage	Approximate time to MI (h)	DNA amount (arbitrary values)
Final mitoses in the archesporium	74.00	1.44*
Final asynchronous mitoses in the tapetum	58.00	2.27*
Nucleolar fusion	40.00	2.98ns
Synchronous tapetal mitoses	26.00	2.92ns
Pachytene	13.00	3.05

Each replicate was measured against the 2C value of root tips of Stewart 63.

Each value was tested against the one following it by a one-tailed t-test: ns = not significant; * = $p<0.05$.

Table IV

Penetration of colchicine into the anthers following injection into the boot of Stewart '63 x 6A391

Hours after injection	Percentage florets with C mitoses in*		
	Filament	Tapetum	Archesporium
1	0 (100)**	0 (41)	0 (9)
3	24 (114)	11 (37)	10 (10)
6	60 (97)	73 (40)	74 (19)
9	69 (119)	60 (20)	-
12	77 (114)	86 (36)	100 (13)
24	98 (96)	-	-

*Threshold concentration for C mitoses in filament of explanted anthers within 3 hours was 0.005 - 0.007%.

**Figures in parentheses are total numbers of florets scored with mitoses in particular tissues.

Table V

Chromosome Pairing in Anthers Classified by Ploidy Changes in Tapetum Following Injection of 0.03% Colchicine

Binucleate-2C Diakinesis Diplotene Pachytene Late Zygotene				Mononucleate-4C* Mid & Early Zygotene Leptotene Late Premeiotic Interphase (s3/s2)**						Mononucleate-Ploidy Uncertain				Monucleate-4C/8C Mid and Early Premeiotic Interphase (s2/s1)**			
G	B	X	T	G†	B	X	T	I	P	G	B	X	T	G	B	X	T
0.03% Colchicine				0.03% Colchicine						0.03% Colchicine				0.03% Colchicine			
R	28.00	24.92	36.75	R	28.00	25.20	48.00	-	-	A	14.64	11.68	70.50	A‡	15.92	13.12	72.50
R	27.92	26.28	23.50	A‡	27.96	25.52	30.92	-	-	A	14.12	11.80	73.00	A	15.24	12.80	96.88
A	27.92	25.56	30.83	A	27.84	25.72	40.83	-	-					R	11.92	9.35	108.00
A	27.92	25.84	38.92	R	27.76	25.56	48.33	-	-					R	11.36	9.08	78.17
A	27.85	25.81	24.88	R	27.68	26.28	36.00	-	-					A	11.16	9.76	85.50
A	27.84	25.56	43.75	R‡	27.64	25.12	30.58	-	-					R‡	9.92	7.64	73.50
A	27.80	26.56	29.33	R	27.64	23.96	44.50	-	-					R	9.20	7.00	84.00
A	27.60	25.36	24.67	R	27.56	25.72	36.25	-	-								
R	27.56	25.80	23.50	A	27.52	24.76	57.00	-	-								
				R	27.48	24.24	36.58	-	-								
Water (Controls)				R	27.48	25.36	60.00	-	-								
				A	27.40	24.36	36.75	I	-								
R	28.00	26.32	70.00	R	27.28	23.92	44.50	I	-								
R	27.96	26.68	60.00	R	27.16	25.12	42.33	-	-								
R	27.92	25.96	23.50	R	27.00	24.12	42.67	I	P								
R	27.92	26.48	23.50	R	26.68	24.27	43.50	I	-								
R	27.92	26.00	48.50	R	26.64	24.16	39.00	-	-								
A	27.85	25.56	24.17	A	26.40	22.60	45.58	-	-								
A	27.84	26.20	24.33	A	25.84	21.72	51.00	-	-								
A	27.84	25.56	49.00	A	25.52	23.00	49.00	-	-								
A	27.76	25.72	48.33	A	25.40	22.30	45.58	-	-								
A	27.76	24.40	70.50	A	25.14	21.92	54.00	-	-								
A	27.64	25.52	51.00	A	24.44	21.12	42.42	-	-								
R	27.52	25.88	72.00	R	24.08	21.20	48.00	-	P								
				R	23.84	20.68	48.00	I	P								
				R	23.33	20.33	48.00	-	-								
				A	23.29	19.39	54.00	-	-								
				A	23.08	19.67	45.50	-	-								
				A	22.55	19.59	47.75	I	-								
				A	22.53	19.71	51.00	-	-								
				A	21.37	17.93	60.30	-	-								
				R	20.00	16.68	54.67	-	-								
				A	18.50	14.05	54.00	-	-								
				A	17.88	14.24	60.25	-	-								
				R	17.36	14.24	60.00	-	-								
				A	16.76	13.44	61.75	-	-								
				R	16.04	12.57	56.00	-	-								
				R	15.76	12.36	60.42	-	-								
				R	14.44	11.64	66.00	-	-								
				A	14.24	12.12	66.00	-	-								
				A	13.85	11.35	61.58	-	-								
				R	12.85	9.96	72.33	-	-								
				A	12.67	9.96	96.67	-	-								
				R	12.60	10.16	67.50	-	-								
				R	12.12	9.24	78.17	-	-								

Sample sizes 20-30 cells except in 5 cases which had 5-15.

*G is genotype, B is number of paired chromosomes, X is chiasmate arm pairs, T is time in hours after injection, I indicates bivalent interlocking, and P indicates proximal chiasmata.

**s3, s2 and s1 are divisions of the premeiotic interphase proposed by Bennett et al. (1973).

†Two different genotypes were used: A stands for Stewart '63 x 6A391 and R stands for Stewart '63 x Rosner.

‡Earliest florets in each genotype with relevant ploidy change.

Table VI

Estimates (in hours) of the colchicine-induced delay of MI incurred at two fixed points in the meiotic cycle

	Synchronous division cycle of tapetum			
	Synchronous mitosis		G_1 synchronisation	
Observation	x 6A391	x Rosner	x 6A391	x Rosner
Hours after colchicine injection for first observed ploidy change (from Table V)	30.92	30.58	72.50	73.50
Minimum time for colchicine penetration (from Table IV)	- 2.00	- 2.00	- 2.00	- 2.00
Normal time of occurrence before MI (from Table I)	-26.17	-29.98	-58.06	-60.76
Delay	+ 2.75	- 1.40	+12.44	+10.74
Mean	+0.68		+11.59	

No anthers containing tetraploid PMC at MI were obtained up to 120 h after injection for Stewart '63 x 6A391. Therefore the delay incurred at the last pre-meiotic mitoses is probably greater than 2 days. After 6 days post injection, meiotic stages were unrecognisable. Meiotic delay increased progressively with time after injection. A reasonable figure for the delay following injection about 40 h before MI, would be 2 hours.

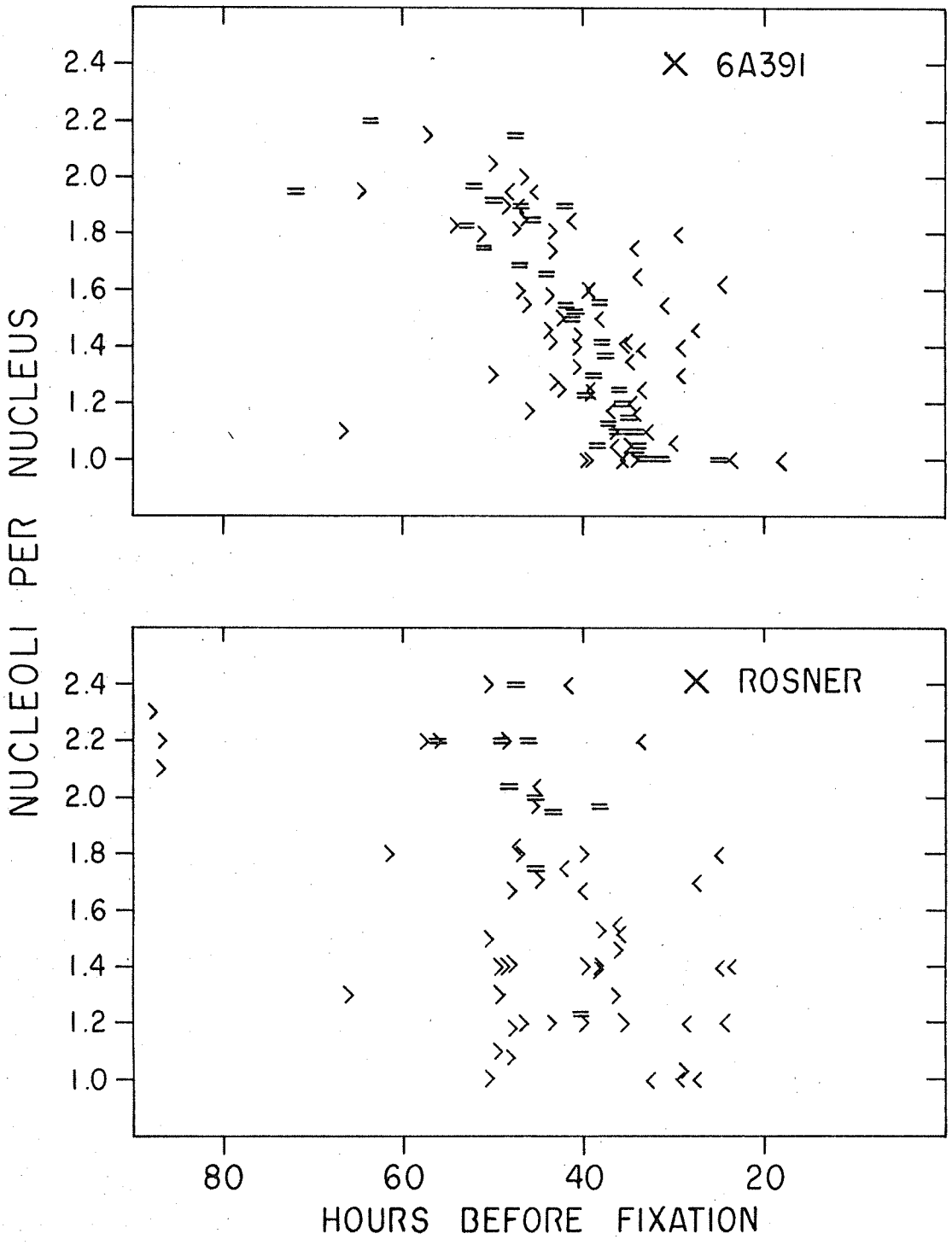

6A391
ROSNER
NUCLEOLI PER NUCLEUS
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
80
60
40
20
HOURS BEFORE FIXATION

Figure 2. PMC of Stewart '63 x 6A391 in early premeiotic interphase. Stained with acetocarmine plus iron mordant. Three nucleoli are visible. Bar is 25 um.

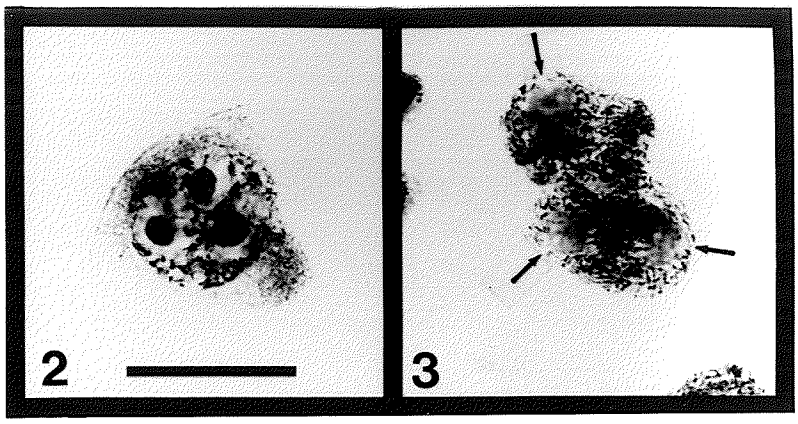
Figure 3. PMC of Stewart '63 x 6A391 in early leptotene. Arrows mark position of nucleoli within the unsquashed nuclei. Nuclear detail was better preserved in briefly fixed material without squashing and so all timing work was done using such preparations. The lower nucleus clearly contains two nucleoli and the chromosomal threads of leptotene are visible around the margin of both nuclei. Stained with acetocarmine. Magnification as Figure 2.

Figure 4. PMC of Stewart '63 in early leptotene. Two nucleoli are visible (arrows) and the chromosomal threads of leptotene appear as a tangled web after squashing the nucleus. Stained with acetocarmine and photographed in negative phase contrast. Bar is 10 um.

Figure 5. Normal diakinesis of Stewart '63 x Rosner. A quadrivalent (IV) and a bivalent (II) are attached to the nucleolus at 10 o'clock. One univalent (I) is inserted through the IV, another is visible beside the nucleolus at 9 o'clock (I). The remaining univalents are paired among themselves in a synaptic knot (S), visible at 5 o'clock. Magnification as Figure 2.

Figure 6. Diakinesis in Stewart '63 x Rosner, 66 hours after colchicine injection. One bivalent (II) and either three or two univalents (I) are attached to the triangular nucleolus in the center of the cell. There is extensive asynapsis (6 II + 23 I) and the synaptic knot (see Figure 5) is also abolished. A rod bivalent is inserted through a ring bivalent at 2 o'clock (arrow) and two ring bivalents are interlocked at 7 o'clock (arrow). Magnification as Figure 2.

Figure 7. Leptotene PMC of (Triticum aestivum cv. Tetra Prelude (AABB) x Secale cereale cv. 0D289) no. 998.2, stained for C bands. Nucleolus fusion = 1.5 to 1.2. Aggregation of heterochromatic telomeres is in progress. Bar is 50 um.


2
3

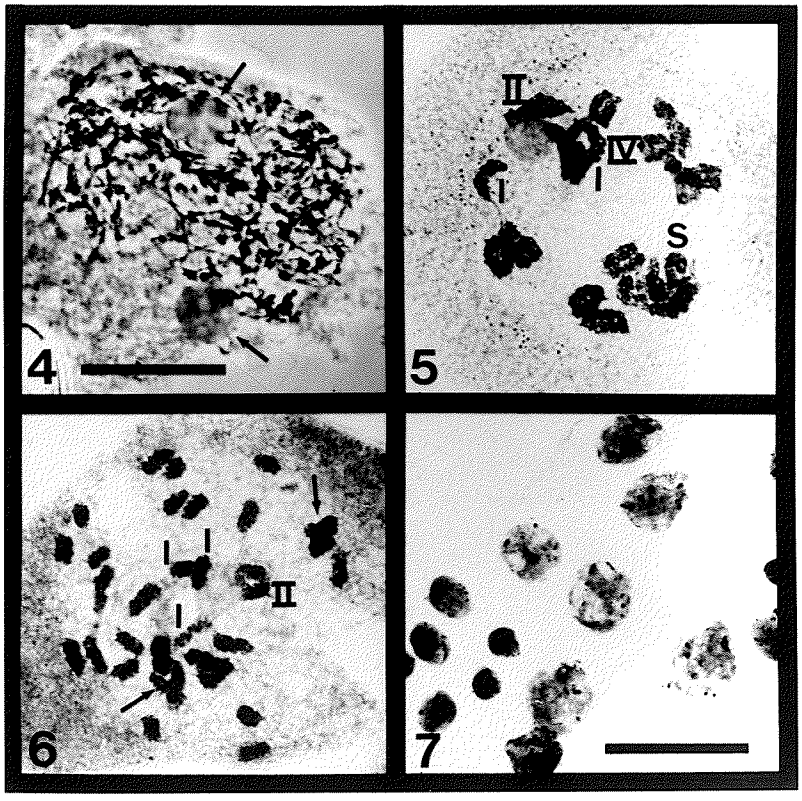
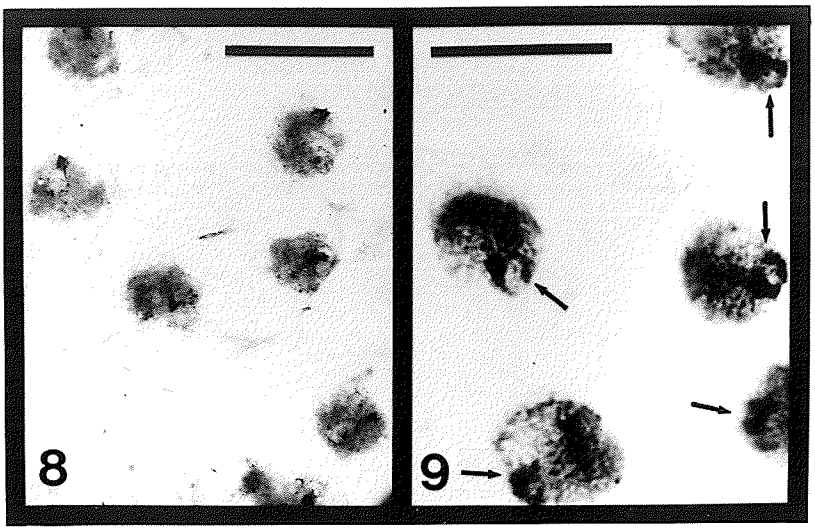

II
IV
I
S
4
5
II
6
7

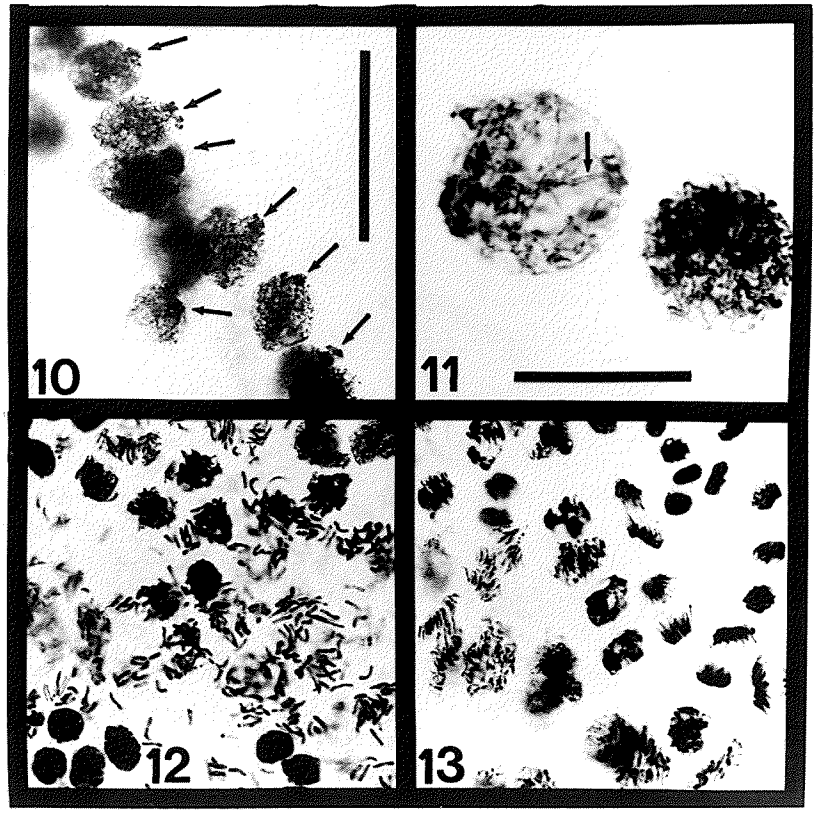
Figure 8. Leptotene PMC of (Triticum turgidum, turgidum group x Secale cereale) 6A445, stained for C bands. Nucleolus fusion = 1.5 to 1.2. The aggregation of heterochromatic telomeres is clearly visible. Bar is 50 um.

Figures 9 and 10. PMC of S. cereale stained with aceto-carmine at leptotene/zygotene. Figure 9. Surface view of intact PMC column. Figure 10. Optical section through intact PMC column, focussed on the outer margin of column. The aggregation of telomeres occurs on an annular area of the nuclear membrane (Figure 9, arrows) that is orientated toward the exterior of the PMC column, i.e. toward the archesporium/tapetum interface (Figure 10, arrows). These relationships were not seen in preparations stained with C banding procedures. Figure 9: bar is 20 um. Figure 10: bar is 50 um.

Figure 11. Two PMC of Stewart '63 x 6A391 in leptotene/zygotene, nucleolus fusion = 1.2. A stretch of synapsed chromosomes is visible, running on the surface of the single transparent nucleolus of the larger nucleus (arrow). Unsquashed and stained with acetocarmine. Bar is 25 um.

Figures 12 and 13. Tapeta of Stewart '63 x 6A391 undergoing synchronous tapetal mitosis in the presence (Figure 12) and absence (Figure 13) of colchicine. Figure 12 is 6 hours after the injection of 0.03% aqueous colchicine. Unsquashed and stained with acetocarmine. Magnifications as Figure 8.


8
9


10
11
12
13

Figures 14 and 15. Mitoses in the filament of Stewart '63 x 6A391 in the presence (Figure 14) and absence (Figure 15) of colchicine. Figure 14 is 24 hours after the injection of 0.03% aqueous colchicine. Stained with acetocarmine. Magnification as Figure 17.

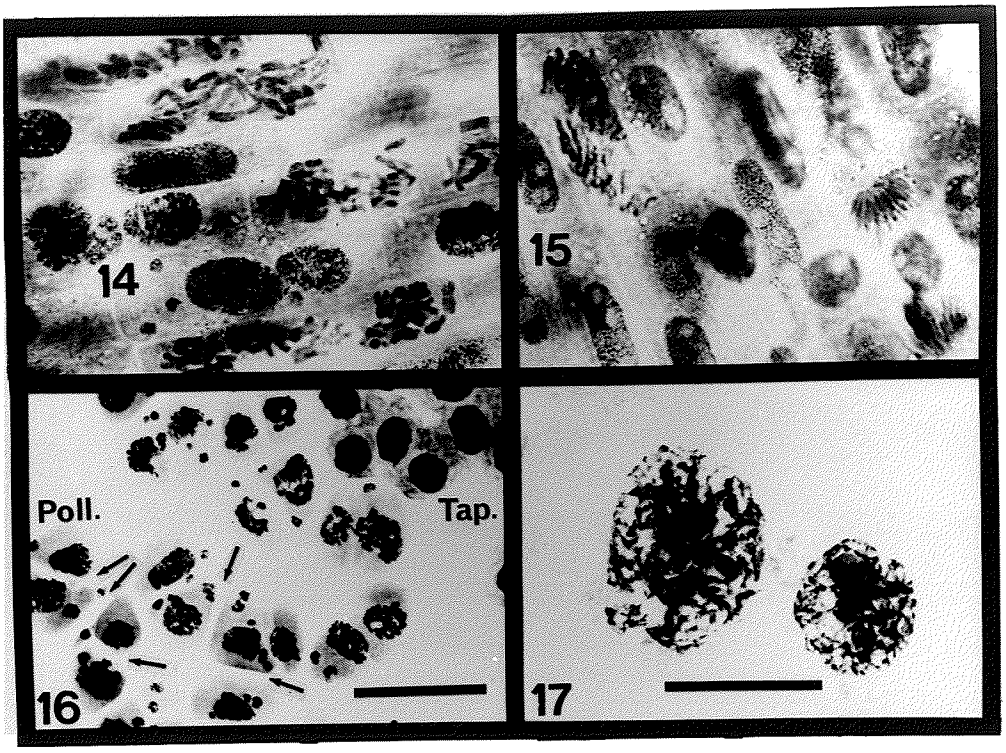
Figure 16. Tapetal cells and young pollen from Stewart '63 x 6A391, 56 hours after injection of 0.03% aqueous colchicine. Tapetal cells on the right (Tap.) are uninucleate whereas PMC have divided giving rise to young pollen on the left (Poll.) with micronuclei and cleavages (arrows) still visible. Bar is 50 um.

Figure 17. Pentaploid and decaploid PMC of Stewart '63 x 6A391 at pachytene, 120 hours after injection of 0.03% aqueuos colchicine. Bar is 50 um.

Figures 18 and 19. PMC of Stewart '63 x 6A391 at MI in the presence (Figure 18) and absence (Figure 19) of colchicine. Figure 18 is 66 hours after colchicine injection. Normal pairing at MI (Figure 19) shows the pentaploid nature of the hybrids (13 ring bivalents, 1 rod bivalent and 7 univalents). Some considerable time after colchicine injection, chromosome pairing became strongly inhibited (Figure 18: 4 ring bivalents, 1 rod bivalent and 25 univalents). Nonetheless the bivalents which are present with the colchicine are clearly stretched and orientated by spindle activity (arrows). This was true of all first and second metaphases examined between 1 and 120 hours after injection of 0.03% aqueous colchicine. Figure 18: magnification as Figure 17. Figure 19: bar is 25 um.

Figures 20 and 21. PMC of Stewart '63 x Rosner at MI, 44 hours after injection of 0.03% aqueous colchicine. Ring quadrivalent (IV) interlocked with three ring bivalents (II). The clearest case is at 12 o'clock. Figure 21: bar is 10 um. Figure 20: magnification as Figure 21.

Figure 22. PMC of Stewart '63 x Rosner at MI, 48 hours after injection of 0.03% aqueous colchicine. Proximal chiasmata (arrows). Magnification as Figure 17.


14
15
Poll.
Tap.
16
17

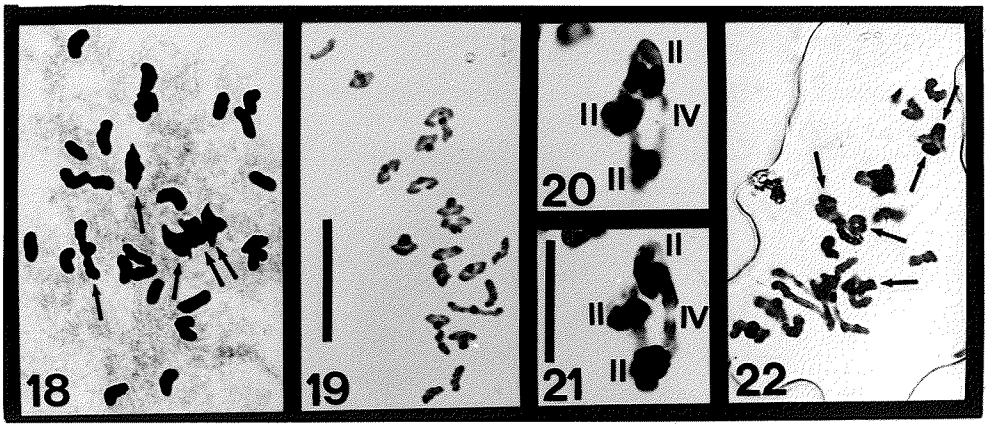

18
19
II
II
IV
II
20
II
II
IV
II
21
22

Figure 23. The relationship between the number of chiasmata (X) and the number of paired chromosomes (B) at MI in the presence (▲) and absence (□) of colchicine. The solid line is the predicted relationship $B=(2PX-X^2-X)/(P-1)$, when P=28 and X is the observed number of chiasmata. Pairing data in the absence of colchicine were pooled from the following genotypes; AABB: Stewart '63, Cocorit '71, 4x segegates of Cocorit x Triticum aestivum; AABBR: Stewart '63 x Rosner, Stewart '63 x 6A391 normal and with high temperature induced pairing failure (6 hours at 35°C); AABBD: Stewart '63 x Anza; AABBDR: (Tetra Thatcher x rye) x Anza, (Stewart x Prolific) x Anza, (Stewart '63 x Prolific) x Anza, Rosner x Anza, triticale 110 x Anza; AABBDE: (Stewart x Agropyron elongatum) x Anza. Variability about the lower end of the observed pairing range in the absence of colchicine is attributable to small sample sizes. Pairing data in the presence of colchicine were pooled from all the pairing data of Stewart '63 x Rosner and Stewart '63 x 6A391 taken after injection of 0.03% aqueous colchicine.

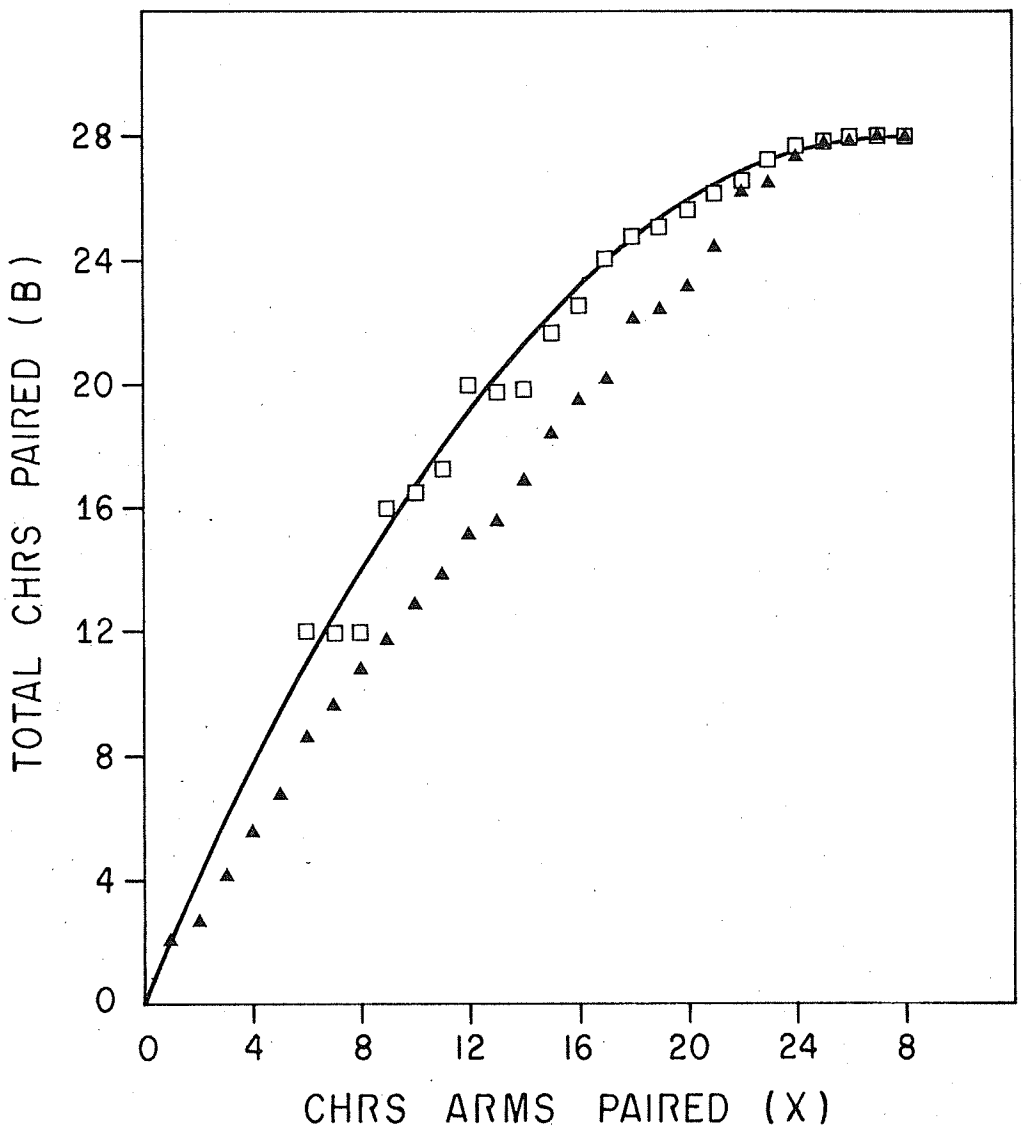
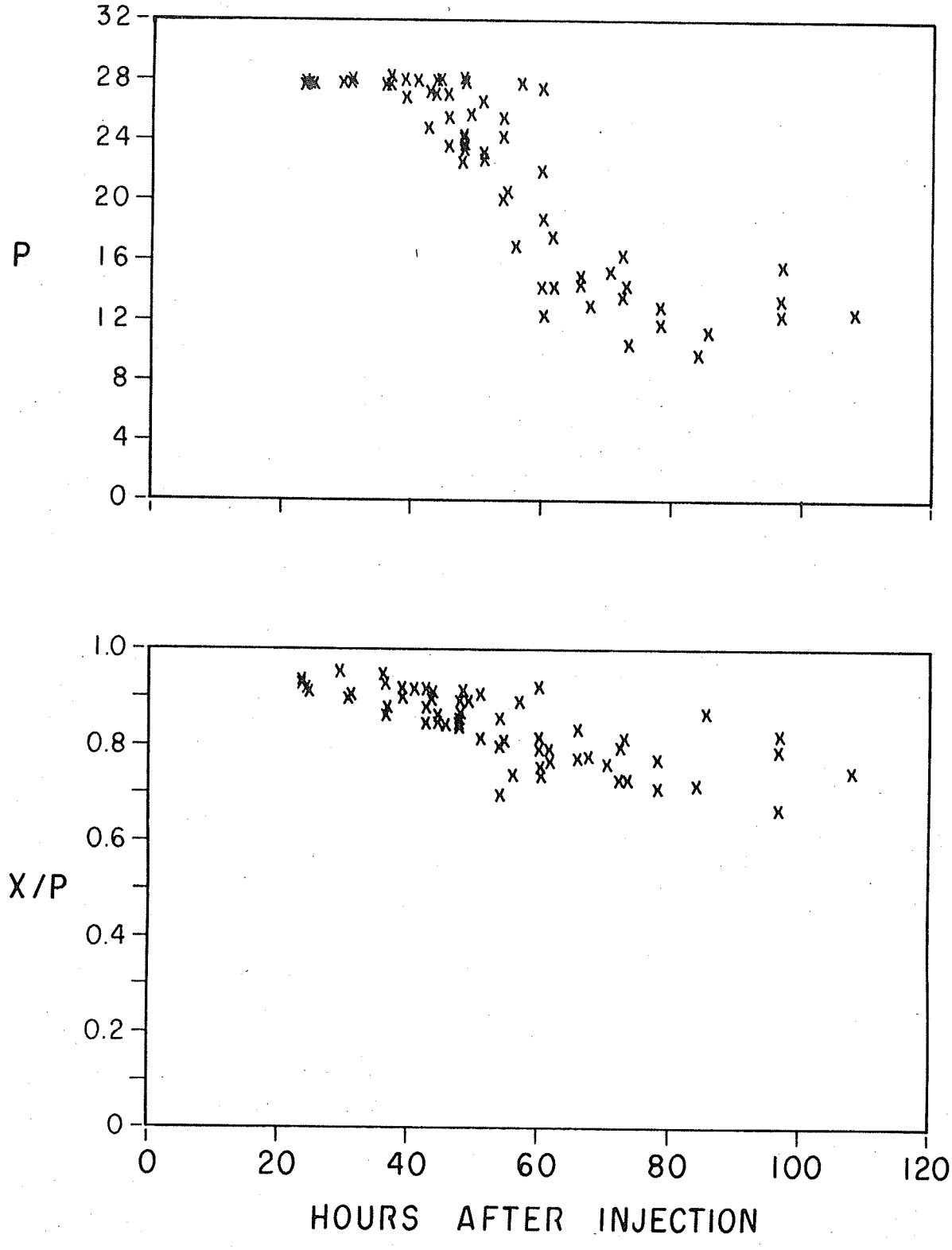

TOTAL CHRS PAIRED (B)
28
24
20
16
12
8
4
0
0
4
8
12
16
20
24
8
CHRS ARMS PAIRED (X)

Figure 24. The inhibition of MI chromosome pairing as a function of time after colchicine injection. The upper graph shows the change in the calculated value of P, and the lower graph the change in the value of X/P. P was calculated from the mean data as $P=(X^2+X-B)/(2X-B)$, where X is the mean number of paired chromosome arms and B is the mean number of paired chromosomes. Each point represents a sample of about 25 cells. The mean value of P for the 12 water injected controls was 27.91 ± 0.0434. The first spike that gave a value of P that was significantly below 27.91 was injected with colchicine 39 hours before fixation. An allowance of 2 hours for the minimum penetration lag of 0.03% aqueous colchicine (Table IV) corrects this to 37 hours. Between 40 and 80 hours after injection of colchicine, the relationship between P and time is visibly linear. Linear regression of all points between 40 and 80 hours is $P=46.67-0.452T$ ($P < 0.001$). Extrapolating to P=27.91 indicates that P began to suffer reduction by colchicine on average 41.54 hours after colchicine injection. An allowance of 5 hours for the median penetration lag of colchicine (Table IV) corrects this to 36.54 hours. The mean value of X/P for the 12 water injected controls was 0.0928 ± 0.0069.


P
32
28
24
20
16
12
8
4
0
X/P
1.0
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
HOURS AFTER INJECTION

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THE EFFECT OF CHROMOSOMES 1B AND 6B ON NUCLEOLUS FORMATION AND FIRST METAPHASE CHIASMA FREQUENCY IN HEXAPLOID TRITICALE

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THE EFFECT OF CHROMOSOMES 1B AND 6B ON NUCLEOLUS FORMATION AND FIRST METAPHASE CHIASMA FREQUENCY IN HEXAPLOID TRITICALE

Abstract

Chromosomes 1B and 6B were solely responsible for nucleolus formation in root-tip cells of hexaploid triticale (X Triticosecale Wittmack). Depending on the line of triticale involved, either 1B or 6B organised larger nucleoli. Chromosomes 1B in Rosner and 6B in line 125 both reduced the frequency of chiasmata at first meiotic metaphase (MI), whereas both 6B and 1B in line 110 and 6B in Rosner all had no such effect. The 1B chiasma suppressor in Rosner (1) was located on the short arm ($1B^S$) together with the most active organizer of root tip nucleoli, (2) had greatest effect when the two nucleolus-organizing arms were present in the form of an isochromosome, and (3) had an effect that was statistically independent of low-temperature conditions which also reduced chiasma frequency at MI. A model of pairing failure in triticale was proposed to show that effective pairing of rye chromosomes may be obstructed when fusion of the wheat nucleoli occurs at the time of bouquet formation in triticale.

Introduction

Hexaploid triticale generally has two pairs of active nucleolus organising chromosomes (Darvey, 1973; Shkutina and Khvostova, 1971). There are three pairs of prominently satellited chromosomes in the parents of hexaploid triticale, the tetraploid wheat parent having two (Giorgi and Bozzini, 1969) and the diploid rye parent having one (Balkandschiewa and Mettin, 1974; Heneen, 1962). The two wheat chromosomes are the nucleolus organising chromosomes 1B and 6B (Crosby, 1957; Flavell and O'Dell, 1976; Flavell and Smith, 1974; Gill et al., 1963 Larsen and Kimber, 1973; Longwell and Svihla, 1960), and the single rye chromosome is 1R (Balkandschiewa and Mettin, 1974; Darvey and Gustafson, 1975). In hexaploid triticale the satellites of 1B and 6B remain distinct, but the satellite of 1R fuses with the rest of the short arm (Merker, 1973; Shigenaga and Larter, 1971). Consequently, the number of prominently satellited nucleolus organising chromosomes in hexaploid triticale agrees with the observed maximum number of nucleoli per nucleus.

Chromosome 1B may also influence the low first metaphase pairing of rye chromosomes that is typical of hexaploid triticale. When chromosome 1B was lost from Rosner, or substituted with 1D in a raw amphiploid, the number of univalents present at MI was reduced (Larter,

pers. comm.; Shigenaga, unpublished).

This study was undertaken to examine the influence that chromosomes 1B and 6B have on nucleolus formation and MI chiasma frequencies in Rosner and two other lines of hexaploid triticale.

Materials and Methods

Genotypes used in the present study include two cultivars each of rye (Secale cereale L.), tetraploid wheat (Triticum turgidum L. em. Bowden) and hexaploid wheat (T. aestivum L. em. Thell.)(Table I). In addition, Tetra Prelude, the extracted tetraploid form (2n=4x=28= AABB) of Prelude hexaploid wheat (Kaltsikes et al., 1969) was used, together with five hexaploid triticale lines (X Triticosecale Wittmack)(Table I) and various 1B and 6B aneuploids derived from three of these lines (Merker, 1973; Shigenaga et al., 1971)(Table II).

To study nucleoli, root tips were prepared according to Rattenbury's (1952) schedule. Nuclei containing the maximum number of nucleoli were photographed and nucleolus diameters were measured from the projected negatives. Volumes were estimated by treating nucleoli as prolate spheroid (volume = $4/3\ \pi\ ab^2$, where a was the major and b the minor radius of the nucleolus). To eliminate cell-to-cell variation, the percentage contribution of each nucleolus to total nucleolar volume was calculated.

Pollen-mother-cell (PMC) material was collected in the early boot stage from plants grown either in the greenhouse or in the growth cabinet (10 or 20°C, constant illumination). Spikes were fixed in Carnoy's II, stored in

70% ethanol and stained with acetocarmine. The term chiasma frequency is used to indicate the frequency with which a pair of homologous chromosome arms were joined by chiasmata. Chiasma frequency per cell is therefore twice the number of ring bivalents plus the number of rod bivalents. Chiasma frequency per chromosome is the frequency of chiasmata per cell divided by twice the number of homologous pairs of chromosomes present in the stock.

Results and Discussion

Nucleolus Formation in Wheat, Rye and Triticale

The maximum numbers of nucleoli that were observed in tetraploid wheat and hexaploid triticale indicated that there are only two active pairs of nucleolus-organising chromosomes in these two species (Table I). In contrast, hexaploid wheat seems to have three active pairs of nucleolus organisers and rye has one (Table I). In addition to the nucleolus organisers on chromosomes 1B and 6B, chromosomes 1A, 6A and 5D of hexaploid wheat have all been reported to carry nucleolus organisers (Crosby, 1957; Darvey and Driscoll, 1972). In the absence of the D genome, the AABB genomes of hexaploid wheat cultivar Prelude had the same level of nucleolar activity as durum wheat (Table I). Therefore the most likely location of the third active active organiser in root tip cells of bread wheat is chromosome 5D (Crosby, 1957; Darvey and Driscoll, 1972; Flavell and O'Dell, 1976). In the hexaploid triticale Tetra Prelude x Prolific, the rye genome of Prolific has been substituted for the D genome of Prelude (Thomas and Kaltsikes, 1972). Therefore, comparison of Prelude with Tetra Prelude x Prolific contrasts the nucleolar activity of the D genome with that of the rye genome, against a similar genetic background of the A and B genomes. Com-

pared to the D genome of hexaploid wheat, the rye genome of hexaploid triticale was totally inactive in nucleolus formation (Table I). This conclusion rests on the assumption that both chromosomes 1B and 6B remain active in nucleolus formation in hexaploid triticale. To examine this assumption, nucleolus activity was investigated in plants of triticale that were aneuploid for chromosomes 1B and 6B.

Pure 6B nullisomics were not established in either line 110 or in Rosner. One case of a 6B nullisomic was identified in Rosner but the seedling died after producing only one root. Many other seedlings died during germination of monosomic 6B progenies in both Rosner and line 110. It is probable that these nonviable seedlings were in fact the nullisomics. Nevertheless, a line of 6B nullisomics was established in line 110. However, this line was not simply nullisomic but was simultaneously monotelotrisomic and subsequently ditelotetrasomic for a short telocentric chromosome. The telocentric carried a small, indistinct satellite and at MI was found in heteromorphic trivalents with two subterminal chromosomes that were visibly one of the largest pairs of chromosomes in the cell. These nulli 6B monotelotrisomics were at least as vigorous as the normal 6B monosomics of the same line. Therefore the substitution of only one telo gave considerable compensation for the 6B deficiency. Large size of the intact chromosome and the presence of a small, indistinct satel-

lite identify this telocentric as belonging to the rye genome (Merker, 1973; Shigenaga and Larter, 1971). The high arm ratio and the small satellite further restrict this identification to one of chromosomes 4R, 5R or 6R (Darvey and Gustafson, 1975; Shigenaga and Larter, 1971). Finally the strong substituting ability of the telo for chromosome 6B shows that it belongs to the sixth homoeologous group (Sears, 1966). Based on this evidence the telo was identified as chromosome $6R^{s}$.

When either chromosome 1B or 6B was placed in the monosomic and then the nullisomic or long-arm ditelosomic condition, the mean number of nucleoli per cell was successively reduced (Table II). Therefore, both of these chromosomes must be highly active in nucleolus formation in hexaploid triticale (Table I). When chromosome 6B was found in the trisomic condition in Rosner, there was a corresponding increase in both the mean number of nucleoli per cell, and in the maximum number of nucleoli (Table II). This confirms the nucleolus-organising activity of 6B in triticale, and shows that additional nucleolus organisers are detectable in triticale if they are active.

When either of chromosomes 1B or 6B was deficient, the maximum number of nucleoli per nucleus was in some cases greater than could be accounted for by the number of 1B and 6B organisers present (Table II). This additional activity was greatest in the substituted 6B nullisomics of

line 110 (Table II), which suggests that the organisers may have been located on the short arm of chromosome 6R. However, even in this case the induced activity did not restore mean nucleolus frequency to the level of the disomic plants (Table II), so that compared to 1B or 6B, this additional activity was minor.

In octoploid triticale, additional activity above that observed in bread wheat has been recorded (Darvey, 1972; Shkutina and Khvostova, 1971). This nucleolus-organising activity also appears to come from chromosome 6R (Darvey, 1972; Driscoll, pers. comm.). Nucleolar and karyotype analysis of rye itself indicates that there is only one pair of active nucleolus-organising chromosomes present (Table I; Balkandschiewa and Mettin, 1974; Bhattacharyya and Jenkins, 1960; Darvey, 1972; Heneen, 1962). This chromosome appears to be the smallest of the complement, which is chromosome 1R (Darvey and Gustafson, 1975). Therefore, while 6R appears to be inactive in rye, 1R appears to be inactive in triticale.

In conclusion, the wheat chromosomes 1B and 6B are almost exclusively responsible for the formation of nucleoli in hexaploid triticale. However when dosage of 1B or 6B is reduced, additional nucleolus organisers may become active. The most likely sources of this induced activity are chromosome 6R or perhaps 1A (Crosby, 1957).

The Balance Between 1B and 6B Nucleolus Activity

Although the maximum number of nucleoli per cell should reveal the number of active nucleolus organizers present in triticale, it does not reveal quantitative differences in nucleolar activity (percent contribution to total nucleolar volume) among the organizers. In root-tip cells of euploid (6x) triticale there are only two pairs of active nucleolus organizers: the 1B pair and the 6B pair. Consequently, in root-tip cells that contain four nucleoli, each nucleolus represents the activity of a single 1B or 6B organizer. Triticales are generally homozygous. Therefore in an inbred triticale or in an amphiploid, the two 1B organizers should be identical to one another and so should the 6B pair. Therefore, for a single line of triticale, genetic variation in nucleolus-organizing activity should only occur between the 1B and 6B organizers. If the 1B and 6B organizers have widely different activities, then out of the four nucleoli, the two larger nucleoli will be the product of the more active pair and the two smaller nucleoli will be the product of the less active pair (Figure 1). Variation in the difference of the sum volume of the two larger nucleoli and the two smaller nucleoli will therefore reflect the extent of inequalities in nucleolus-organizing activity between the 1B pair and the 6B pair (Figure 1).

This difference in the sum of the volume of the two larger nucleoli and the two smaller nucleoli was greatest in

Rosner, least in line 110 and intermediate in line 125 Table III). Consequently, there appears to be considerable variation in the size of nucleoli produced between the 1B and 6B organizers in triticale.

Chromosomes are most easily recognised at metaphase, but nucleolus organisers are only active during telophase, interphase and prophase. Consequently, direct association of particular nucleoli with particular chromosomes in order to determine if 1B or 6B carries the more active organizer, is not easily done. Instead, an indirect approach was used, based on two assumptions. Firstly, it was assumed that the relative activity of individual 1B and 6B organisers was the same in monosomics 1B and 6B as it was in the euploid. Secondly it was assumed that the low level of nucleolus formation by minor organisers which was observed in monosomics 1B and 6B could be neglected (Table II). The maximum number of nucleoli that is possible in an individual cell of monosomic 1B or 6B is therefore 3. In such cells, each individual nucleolus will again reflect the activity of a single 1B or 6B organiser. Consider the case of a 1B monosomic from a line in which the 1B organiser produces larger nucleoli than the 6B organiser. A cell with 3 nucleoli will ideally contain 1 large nucleolus and 2 smaller nucleoli. In practise the 2 smaller nucleoli will not have the same volume exactly and so the 3

nucleoli will rank: large, medium and small. The large nucleolus will be the product of the single 1B nucleolus organizer, whereas the small nucleolus will be the product of 1 of the 2 6B organizers. The nucleolus of intermediate volume, therefore, will be the product of the remaining 6B organizer. Now consider the case of a 6B monosomic from the same line. Cells with three nucleoli will, ideally, contain 2 large nucleoli and 1 small. In practise, they will again rank: large, medium and small. The large nucleolus will again be the product of a 1B organizer and the small nucleolus will again be the product of a 6B organizer. The remaining nucleolus organizer, however, is no longer 6B but 1B. Therefore, the nucleolus of intermediate volume in monosomic 6B is produced by a 1B organizer, and vice versa (Figure 1). The volume of the intermediate nucleolus in monosomic 1B is now compared with the volume of the intermediate nucleolus in monosomic 6B (Figure 1). Since the 1B organizer was most active, it is expected that the 6B monosomic will produce the largest intermediate nucleolus. Conversely, if it were found that the 1B monosomic produced the largest intermediate nucleolus, the conclusion would be that the 6B organiser was most active. This test can be applied equally well to absolute and relative nucleolar volume.

In Rosner, chromosome 1B contributed more to the

nucleolar volume than did chromosome 6B (Figures 2 and 3; Table III). In line 110, this position was reversed and the 6B organiser had a greater relative activity than the 1B organiser (Table III). Furthermore, the absolute difference between 1B and 6B was significantly greater in Rosner than in line 110 (Table III). This confirms the assumption that the large within-cell variation in nucleolus volume in euploid Rosner reflects a great difference in activity between the 1B and 6B organizers.

In conclusion, the nucleolus organizers of chromosomes 1B and 6B vary in the contribution that they make to total nucleolar volume. Since this variation was found both within and between varieties of triticale, it is probable that some chromosomes may carry more active organizers than their homologues. Similar variation was found in wheat, both within and between homologues, for the number of cistrons specifying ribosomal RNA (rRNA) (Flavell and O'Dell, 1976; Flavell and Smith, 1974). On the other hand, there may not be a simple relationship between cistron number and nucleolar volume (Flavell and O'Dell, 1976) or between nucleolar volume and rRNA output for that matter. Nevertheless, nucleoli are the product of nucleolus organisers and so the volume of the nucleolus that is produced is clearly one measure of an organiser's activity.

Chiasma Frequencies in 1B and 6B Aneuploids

The five dosage series of the nucleolus-organising chromosomes were scored for chiasma frequency at MI (Table IV). Significant promotion of chiasma frequency per chromosome occurred when chromosome 1B was removed from Rosner and when chromosome 6B was removed from line 125 (Table IV). At 20°C, nullisomic 1B Rosner had significantly more chiasmata per cell than euploid or trisomic 1B Rosner, even though it had fewer chromosomes (Table V); therefore, promotion of chiasma frequency at MI cannot be explained merely by the removal of a pair of chromosomes with a low number of chiasmata. In fact both the long and the short arms of chromosomes 1B and 6B show high frequencies of chiasmata in triticale (Thomas and Kaltsikes, 1976a).

Removal of chromosome 6B from Rosner, and either of chromosomes 1B or 6B from line 110 had no effect on chiasma frequency per chromosome at MI (Table IV).

Among the 1B aneuploids of Rosner, chiasma frequency per chromosome was promoted in both ditelosomic $1B^{L}$ and nullisomic 1B (Table IV). Therefore it was the short arm of chromosome 1B which was responsible for lowering chiasma frequency at MI.

A single plant of Rosner that carried the two short arms of chromosome 1B as an isochromosome (monoiso-

monosomic $1B^S$) showed a frequency of chiasmata that was more than $t_{0.025}$ standard deviations below the mean of other plants of Rosner, examined at Manitoba under roughly comparable conditions (Table VI). The sampling variation represented by this standard deviation is considerable since it covers two observers, observations in the growth cabinet at $20^{\circ}C$, observations in the greenhouse taken on at least four different occasions, and plants isolated from at least seven different bulk populations of Rosner. Assuming the variances to be equal, the frequency of chiasmata per chromosome in monoisomonosomic $1B^S$ was significantly below the frequency in disomic 1B Rosner (Table VI). This indicates an effect of position for the $1B^S$ inhibitor, such that the two short arms reduced chiasma frequency more when they were attached to the same centromere than when they were attached to separate chromosomes.

1B and Low Temperature

In addition to the effects of 1B and 6B dosage on the frequency of chiasmata at MI, chiasma frequency is also affected by variation in temperature (Bayliss and Riley, 1972; Boyd, Sisodia and Larter, 1970). Consequently, the effect of 1B to reduce chiasma frequency might be more pronounced at temperatures that induce asynapsis than it is at normal temperatures (c.f. Bayliss and Riley, 1972). However, although high 1B dosage and low temperature (10°C *vs.* 20°C) both decreased chiasma frequency in Rosner (Table IV; Table V) there was no positive interaction between the two factors. Instead the nullisomics and the long arm ditelosomics (i.e. low 1B dosage) both showed a *lower* frequency of chiasmata per cell and large plant to plant variation at 10°C relative to the disomics (Table V). Even when the nullisomics and ditelosomics were eliminated from the analysis of variance because of their large standard deviations at 10°C (Table V) there was no evidence of synergism between high 1B dosage and low temperature to reduce chiasma frequency per chromosome at MI (Table VII).

Between 20°C and 10°C, the percentage of cells in which 1B paired as a trivalent declined by 9% (in trisomic 1B) and as a ditelobivalent by 16% (in ditelosomic $1B^{L}$). In hexaploid triticales grown at normal temperatures, low

chiasma frequencies are restricted to the chromosomes of the rye genome, and chromosome 1B has among the highest frequencies of chiasmata in the wheat genome (Thomas and Kaltsikes, 1974, 1976 ; c.f. Larter and Shigenaga, 1971). Consequently, pairing failure at 10°C must have affected both the wheat and the rye chromosomes as well.

Figure 4 compares chiasma frequency in Rosner between 10°C and 30°C with the chiasma frequencies observed in bread wheats over the same range (data compiled from Tables IV and V, from Bayliss and Riley (1972) and from Boyd et al. (1970)). There is a broad temperature optimum for chiasma frequency in Rosner, comparable to that observed in bread wheats but at a lower level (Figure 4). In the absence of chromosome 5D, the hexaploid wheat variety Chinese Spring shows extensive pairing failure at temperatures below 20°C (Bayliss and Riley, 1972; Riley 1966). Hexaploid triticales lack the 5D chromosome that buffers chromosome pairing against low temperatures in Chinese Spring but in most cases they should carry a corresponding gene on chromosome 5A which stabilises chromosome pairing against low temperatures in tetraploid wheats (Riley and Hayter, 1967). In addition, there are probably analogous genes in rye, which would also be present in triticale. Since chiasma frequency in Rosner is stable over a wide range of temperatures, albeit at a lower level than is observed in bread wheats, it is

unlikely that pairing failure in triticales grown at normal temperatures occurs because of reduced activity of genes of the 5A and 5D type. This is also evident from studies of meiotic prophase. In nulli 5D tetra 5A Chinese Spring, grown at low temperatures, pairing failure is asynaptic (Bayliss and Riley, 1972). In triticales grown at normal temperatures, pairing failure is desynaptic (Lelley, 1974; Tsuchiya, 1970). Since Rosner is no exception to this rule, chromosome 1B probably acts to increase the extent of desynapsis, up to MI. Absence of synergism between high 1B dosage and low temperature to decrease chiasma frequency at MI, indicates that the two factors operate additively on two different phases of pairing.

Asynaptic pairing failure in wheat also occurs following the injection of colchicine (Dover and Riley, 1973; Driscoll and Darvey, 1970; Driscoll et al., 1967; Thomas and Kaltsikes, 1977) and in the presence of high dosage of chromosome 5B (Feldman, 1966, 1968). This makes it unlikely that pairing failure in triticale occurs through one of these meiotic mechanisms either.

Table I. Nucleolar frequencies in root-tip cells of wheat, rye and hexaploid triticale

Material	Number of nucleoli per nucleus						
	Frequency of cells with indicated number						Mean and standard error
	1	2	3	4	5	6	
Diploid rye							
Snoopy	283	116	-	-	-	-	1.29 ± 0.022
Prolific	210	80	-	-	-	-	1.27 ± 0.026
Tetraploid wheat							
Stewart '63	96	203	77	22	-	-	2.06 ± 0.040
Cocorit '71	81	172	123	32	-	-	2.26 ± 0.042
Tetra Prelude	78	184	115	27	-	-	2.22 ± 0.041
Hexaploid wheat							
Anza	63	159	131	44	6	3	2.46 ± 0.048
Prelude	37	123	146	67	26	3	2.83 ± 0.053
Hexaploid triticale							
Stewart '63 x Prolific	73	190	115	25	-	-	2.23 ± 0.040
Tetra Prelude x Prolific	64	178	135	21	-	-	2.28 ± 0.039
Rosner	70	186	110	41	-	-	2.30 ± 0.043
Line 110	50	203	134	35	-	-	2.36 ± 0.039
Line 125	66	176	127	43	-	-	2.36 ± 0.043

Table II. Nucleolar frequencies in root-tip cells of 1B and 6B aneuploids of hexaploid triticale

Material	Number of nucleoli per nucleus						
	Frequency of cells with indicated number						Mean and
	1	2	3	4	5	6	standard error
Line 110 - Nulli 1B	107	93	1	-	-	-	1.47 ± 0.036
- Mono 1B	157	183	68	-	-	-	1.78 ± 0.035
- Disomic	50	203	134	35	-	-	2.36 ± 0.039
- Mono 6B	157	193	64	-	-	-	1.78 ± 0.034
- Nulli 6B*	174	210	17	2	-	-	1.62 ± 0.029
Rosner - Ditelo $1B^L$	216	197	2	-	-	-	1.48 ± 0.025
- Mono 1B	103	213	85	-	-	-	1.96 ± 0.034
- Disomic	70	186	110	41	-	-	2.30 ± 0.048
- Tri 6B	52	148	126	61	14	-	2.59 ± 0.046
- Mono 6B	106	191	83	-	-	-	1.94 ± 0.036
- Ditelo $6B^L$	136	164	-	-	-	-	1.55 ± 0.029
Line 125 - Disomic	66	176	127	43	-	-	2.36 ± 0.043
- Mono 6B	114	186	103	2	-	-	1.98 ± 0.037
- Nulli 6B	193	197	4	-	-	-	1.52 ± 0.026

* These plants were simultaneously monotelotrisomic or ditelotetrasomic for a short telocentric chromosome, probably $6R^S$.

Table III. The influence of genotype on the distribution of nucleolar volume within the nucleus of root-tip cells of hexaploid triticale

		Rosner	Line 125	Line 110
Percentage difference in volume between the larger pair and the smaller pair of nucleoli in disomics[1]		37.52 ± 3.58[2]	26.49 ± 2.96	19.80 ± 3.97
		Rosner > Line 110 ($p < 0.01$)		
Percentage volume of intermediate sized nucleolus in monosomics[1]	Mono 6B	33.03 ± 0.862	31.89 ± 0.275	32.64 ± 0.652
	Mono 1B	27.08 ± 0.872	- -	34.72 ± 0.606
		In Rosner: Mono 6B > Mono 1B ($p < 0.005$)		
		In Line 110: Mono 6B < Mono 1B ($p < 0.05$)		

[1]These variables are explained in the text

[2]Standard error of the mean

Table IV. Mean numbers of chiasmata per chromosome in 1B and 6B aneuploids of three triticale lines.

Line	Aneuploid Stock	Mean and Standard Error	Number of Plants Scored	Analyses of Variance			
				Source of Variation	df.	MSx10^6	F
Rosner	Tri 1B	0.801 ± 0.0132	8	Aneuploids	4	12249	30.01***
	Di 1B	0.831 ± 0.0050	8	Error	33	408	
	Mono 1B	0.886 ± 0.0055	8				
	Nulli 1B	0.891 ± 0.0050	8				
	Ditelo $1B^L$	0.880 ± 0.0132	6				
Rosner	Di 6B	0.847 ± 0.0085	8	Aneuploids	2	1627	$1.72^{ns.}$
	Mono 6B	0.852 ± 0.0087	4	Error	17	946	
	Ditelo $6B^L$	0.823 ± 0.0141	8				
Line 110	Di 1B	0.870 ± 0.0011	5	Aneuploids	2	47	$0.27^{ns.}$
	Mono 1B	0.875 ± 0.0077	6	Error	12	177	
	Nulli 1B	0.875 ± 0.0052	4				
Line 110	Di 6B	0.872 ± 0.0041	4	Aneuploids	2	27	$0.21^{ns.}$
	Mono 6B	0.868 ± 0.0072	4	Error	9	128	
	Nulli 6B/ telotri $6R^S$	0.873 ± 0.0052	4				
Line 125	Di 6B	0.819 ± 0.0070	10	Aneuploids	2	5059	12.46***
	Mono 6B	0.859 ± 0.0052	12	Error	27	406	
	Nulli 6B	0.862 ± 0.0060	8				

*** Significant at the 0.001 level. ns. Not significant

Table V. Mean numbers of chiasmata per pollen mother cell in nullisomics, monosomics, disomics, trisomics and long arm ditelosomics of chromosome 1B in the hexaploid triticale cultivar Rosner grown at two temperatures

Aneuploid stock	Temperature			
	20°C		10°C	
	Mean and standard error	Number of plants scored	Mean and standard error	Number of plants scored
Ditelosomic $1B^{L}$	36.08 ± 0.540	6	30.18 ± 1.061	8
Nullisomic 1B	35.59 ± 0.198	8	28.57 ± 1.645	7
Monosomic 1B	35.42 ± 0.219	8	30.91 ± 0.470	8
Disomic 1B	34.91 ± 0.242	8	30.84 ± 0.295	12
Trisomic 1B	33.63 ± 0.334	8	29.88 ± 0.537	7

At 20°C nullisomic > disomic ($p < 0.05$) and nullisomic > trisomic ($p < 0.001$) among others. At 10°C disomic > trisomic ($p < 0.05$).

Table VI. Mean numbers of chiasmata per chromosome in nullisomics, disomics and a short arm monoisomonosomic of chromosome 1B in the hexaploid triticale cultivar Rosner

Aneuploid stock	Mean and standard error	Number of plants scored	Environment
Nullisomic 1B (20 pairs)	0.891 ± 0.0050	8	Growth cabinet 20°C
	0.900	1	Greenhouse
	0.877	1	Greenhouse
Disomic 1B (20 pairs + 1B pair)	0.864 ± 0.0080	10	Greenhouse
	0.847 ± 0.0085	8	Growth cabinet 20°C
	0.831 ± 0.0058	8	Growth cabinet 20°C
	0.840 ± 0.0131	5	Greenhouse
	0.855 ± 0.0045	38	Greenhouse
Monoisomonosomic $1B^S$ (20 pairs + iso $1B^S$)	0.783*	1	Greenhouse

*This observation was 2.584 standard deviations below the pooled average of all data of disomic 1B Rosner. Assuming equality of variance this difference is significant ($p < 0.025$, 68 d.f.).

Table VII. Analysis of variance of chiasmata per chromosome among 1B aneuploids of Rosner[1] grown at two temperatures

Source of variation	df	MS x 10^6	F value
Chromosome 1B (1B)	2	22911	36.58***
Temperature (T)	1	126431	201.89***
Interaction (1B x T)	2	603	0.96^{ns}
Error	45	627	

[1]Monosomics, disomics and trisomics only.

***Significant at the 0.001 probability level.

[ns]Not significant.

NUCLEOLUS ORGANISING CHROMOSOMES

MOST ACTIVE PAIR

LEAST ACTIVE PAIR

NUCLEOLAR ASYMMETRY IN DISOMICS

VOLUME DIFFERENCE OF INTERMEDIATE NUCLEOLUS BETWEEN MONOSOMICS

MOST ACTIVE IS DEFICIENT

LEAST ACTIVE IS DEFICIENT

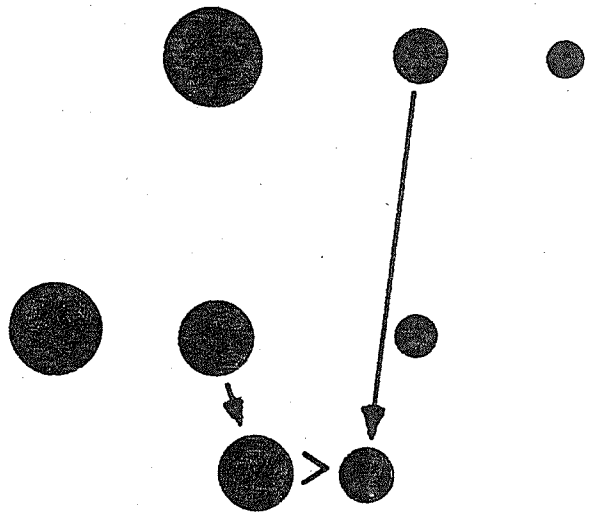
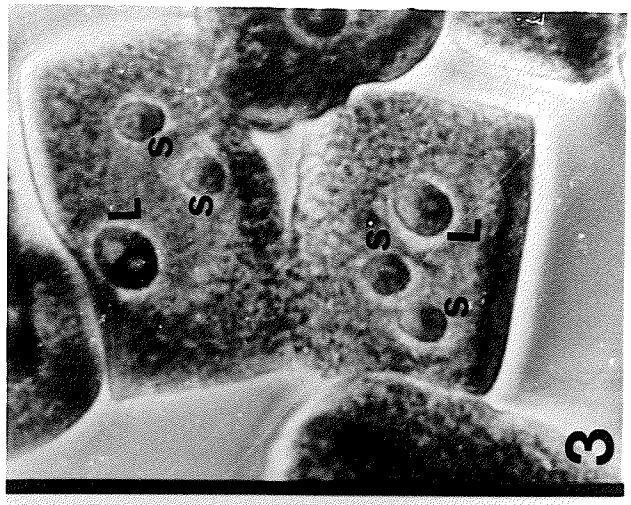


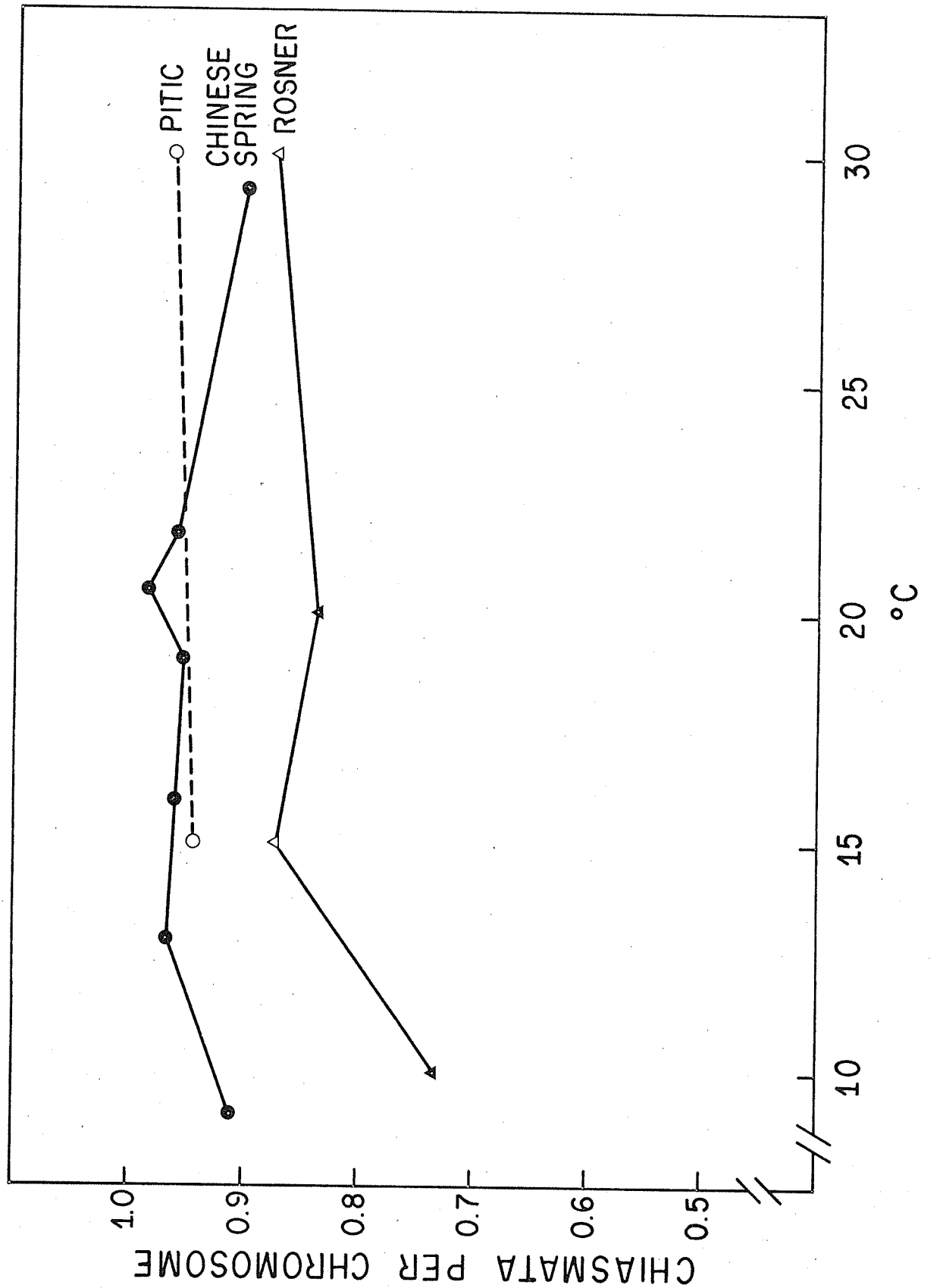
Figure 2. Nucleoli in root tip cells of monosomic 6B Rosner. In cells with three nucleoli, there is a tendency for two nucleoli to be visibly larger (L) than the other one (s). Bar is 20 um.

Figure 3. Nucleoli in root tip cells of monosomic 1B Rosner. In cells with three nucleoli there is a tendency for two nucleoli to be visibly smaller (s) than the other one (L). Same magnification as Figure 2.


S
L
S
S
L
S
3


S
L
L
2

Figure 4. Frequency of chiasmata per chromosome at MI in PMC of bread wheat and hexaploid triticale between 10°C and 30°C. The frequency of chiasmata was computed as the number of rod bivalents plus twice the number of ring bivalents divided by 42 for all data. Black circles are data of Bayliss and Riley (1972), clear circles and triangles are data of Boyd et al. (1970) and black triangles are data of the present study. Pitic and Chinese Spring are bread wheats and Rosner is hexaploid triticale.


PITIC
CHINESE SPRING
ROSNER
CHIASMATA PER CHROMOSOME
1.0
0.9
0.8
0.7
0.6
0.5
10
15
20
25
30
°C

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GENERAL DISCUSSION

A Model of Pairing Failure in Triticale

Why should deficiency for chromosomes 1B and 6B in certain cases promote chromosome pairing in hexaploid triticale? Chromosomes 1B and 6B are both important nucleolus-organising chromosomes in triticale. The chiasma inhibitor present on chromosome 1B Rosner was located on the same arm as the nucleolus organiser ($1B^S$). This $1B^S$ organiser was shown to produce larger nucleoli than the $6B^S$ organiser, at least in root-tip cells. Furthermore, when the two arms of $1B^S$ were attached together on a single isochromosome, the frequency of chiasmata was lowered by even more than when they were present on separate chromosomes. In root-tip cells of wheat, attachment of two nucleolus organisers to a single isochromosome increases the probability of fusion between the homologous nucleoli (Darvey and Driscoll, 1972).

Pairing effects of other nucleolus-organising chromosomes can be cited. Among trisomics of rye, the lowest rate of trivalent formation and lowest frequency of chiasmata per cell were recorded when the active nucleolus-organising chromosome 1R was trisomic (Balkandschiewa and

Mettin, 1974). Lowest rates of trivalent formation were also found in monotelotrisomics of barley and primary trisomics of pearl millet, when the trisomic chromosome carried an active nucleolus organiser (Sayed, 1973; Manga 1976). Chromosome 1B carries the most active nucleolus organiser in meiocytes of Chinese Spring (Darvey, 1972), and deficiency for this same chromosome appeared to promote homoeologous pairing by about one chiasma per cell in hybrids between Chinese Spring and Aegilops variabilis (Driscoll, pers. comm.). All these findings are consistent with the hypothesis that increases in (1) the number of active nucleolus organisers, (2) total nucleolar volume and (3) the preexisting level of nucleolar fusion between nucleoli can act to lower chiasma frequency in a variety of pairing situations.

Chromosome pairing in the Triticinae appears to go through an important bouquet stage during leptotene that brings all the telomeres to a small specific area of the nuclear membrane (Thomas and Kaltsikes 1976a, 1977). In bouquet-forming species, synapsis is usually reported to begin close to the bouquet and to proceed toward the interior of the nucleus (Darlington, 1937; Hiraoka, 1941; Wilson, 1934). There is also reason to think that in the cereals, synapsis begins at or close to the telomere (Burnham et al., 1972; Kasha and Burnham, 1965; Michel and Burnham, 1969; Tabata, 1963).

Because nucleolus-organising regions are generally located a short distance from the short-arm telomeres of nucleolus-organising chromosomes (Lima-de-Faria, 1976), all nucleoli of the PMC become joined in a single fusion nucleolus as the telomeres aggregate at the attachment plate on the nuclear membrane (Thomas and Kaltsikes, 1976a, 1977). In the case of iso $1B^S$, a high preexisting level of nucleolar fusion in somatic cells (Darvey and Driscoll, 1972) should increase the tendency toward nucleolus fusion in the meiocytes.

The fusion nucleolus associates so strongly with the telomere attachment plate that the nucleolus appears to partially extrude from the nucleus (Bennett et al., 1973; Darvey, 1972; Roupakias and Kaltsikes, 1977a). Lima-de-Faria (1976) has pointed out that the nucleolus organisers are preferentially located adjacent but never very close to the short arm telomere. This segment, very close to the short arm telomere, was described as the "blocked region" for the location of the nucleolus organiser (Lima-de-Faria, 1976). A shift in the position of the nucleolus organiser into the blocked region, close to a telomere, would bring the fusion nucleolus into the center of bouquet formation. Thus, if the kind of bouquet that is observed in rye and triticale (Thomas and Kaltsikes, 1976a, 1977) is widespread, terminal nucleolus organisers would intrude the nucleolus

into the centre of the bouquet, and so disrupt regular meiosis. Selection pressures favoring regular meiosis would therefore select against terminal nucleolus organisers.

Even so, factors which increase the rate of nucleolus fusion, the size of the final fusion nucleolus, or its proximity to the attachment plate, could influence the progress of synapsis from initiation points (telomeres) at the bouquet. For instance, a pair of synapsing chromosome arms might happen to pass on either side of the fusion nucleolus even though their homologous telomeres were closely associated at the bouquet. Under these conditions, synapsis in the subterminal segment would be prevented, leaving only the most terminal segment correctly paired during early zygotene. Depending on the length of the terminal segment that was synapsed, chiasmata could still be formed in this region. Thus, among telocentrics of triticale, (with the exception of heterochromatic telocentrics), positive correlation existed between frequency of univalency at MI and increasing terminal localisation of chiasmata (Thomas and Kaltsikes, 1976b).

Lowest chiasma frequencies in triticale were invariably found in those arms of the rye genome that carried large blocks of terminal heterochromatin (Thomas and Kaltsikes, 1974, 1976b). Furthermore, when heterochromatin was deleted from the telomere, chiasma frequency

was promoted (Merker, 1976; Roupakias and Kaltsikes, pers. comm.). Hoever, despite their very low pairing, heterochromatic telocentrics failed to show the expected terminal chiasmata. Instead, chiasmata were significantly ($p < 0.01$) more proximal in the heterochomatic telocentrics than they were in the next highest pairing category (Thomas and Kaltsikes, 1976b). This finding can probably be explained by the fact that chiasmata do not generally form in heterochromatin (for refs. see Thomas and Kaltsikes, 1976b). Consequently, if a pair of heterochromatic chromosome arms were blocked from further synapsis by the nucleolus, then they would be unable to form chiasmata both in the nonsynapsed subterminal euchromatic segment, and in the synapsed but heterochromatic terminal segment.

Thomas and Kaltsikes (1976a) reported that the bouquet began to break up by mid zygotene, while synapsis was still in progress. Loss of association between the telomeres and the nucleolus would allow those regions that were physically separated by the nucleolus to complete their synapsis to the high level that is observed in pachytene and diplotene. However, if this late synapsis were ineffective for the formation of chiasmata, then heterochromatic arms that were blocked by the nucleolus would lack chiasmata and would desynapse at MI.

This hypothesis is presented as a preliminary account of why heterochromatic chromosome arms of rye fail to pair in triticale, and it emphasises the events of meiotic prophase, when the structure of the MI bivalents is determined.

CONCLUSION

By use of heterochromatin staining it was shown that the unpaired chromosomes in triticale were mostly rye chromosomes. Low chiasma frequencies in triticale were restricted to chromosome arms of the rye genome which carry large blocks of the characteristic rye heterochromatin at the telomere. Low pairing of these heterochromatic chromosome arms in heteromorphic bivalents was associated with an anomalous, slightly procentric distribution of chiasmata.

Active fusion of nucleoli in early meiotic prophase coincided approximately with leptotene in each of rye, triticale and durum x triticale pentaploids. Bouquet formation was found to occur at leptotene in both rye and triticale, at least among the rye chromosomes. Injection of 0.03% aqueous colchicine into the boot of durum x triticale pentaploids during late premeiotic interphase and leptotene inhibited chromosome pairing at diakinesis and first meiotic metaphase. Therefore, active nucleolar fusion, bouquet formation and sensitivity of chromosome pairing to colchicine all occurred in the same general period of the meiotic cycle between the middle of the pre-

meiotic interphase and early zygotene.

Nucleolar frequencies were studied in root-tip cells of triticale, including 1B and 6B aneuploids, and in wheats and ryes. Chromosomes 1B and 6B were the only nucleolus organisers active in root-tip cells of hexaploid triticale under normal conditions. Deficiencies for chromosomes 1B and 6B promoted chromosome pairing in certain cases and had no effect in others.

It was concluded that there is an important step in the meiotic pairing of chromosomes, which takes the form of a colchicine-sensitive bouquet stage, and that this stage may be part of the process of homologue recognition. From consideration of these and other findings it was suggested that pairing failure in triticale is not strictly a problem of homologue recognition, nor is it due to super or suboptimal activity of the pairing control systems present on the chromosomes of homoeologous group 5. Instead, effective pairing of rye chromosomes may be obstructed when fusion of the wheat nucleoli occurs at the time of bouquet formation in triticale.

Ultrastructural studies of the basis of bouquet formation, and study of the relationship between chiasma distribution and overall pairing rates for particular chromosomes in wheat, rye and triticale are recommended for further study.

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APPENDIX

Table 1. The distribution of heterochromatin among telomeres participating in end-to-end associations* at early diplotene in a rye plant with 10 heterochromatic and 4 euchromatic pairs of telomeres.

Number of telomere pairs present in the end-to-end association		
2	3	4
HH# 6	HHH 2	HHHH 1
HE# 14	HHE 2	
EE 1		

	Total H	Total E
Observed	39	19
Expected@	41.42	16.57

* Level of proximity between telomeres considered to represent end-to-end association indicated by arrows on Figure 12, page 79 (bouquet paper).

\# H and E indicate presence of heterochromatic or euchromatic telomere pairs respectively in the end-to-end association.

@ Expectation based on the ratio of 10 heterochromatic telomere pairs to 4 euchromatic. Only cells in which all 14 telomere pairs could be identified were analysed for end-to-end associations.

Unweighted Least Squares Estimation of Meiotic Intervals

The raw data was consolidated as follows. Suppose the duration of the interval from the synchronous tapetal mitosis (STM) to the end of pachytene (EP) was observed to take more than 21 hours but less than 23 hours then its duration is reasonably estimated to be 22 hours. Equations were then set up to express the observed intervals in terms of the intervals whose durations were to be estimated by least squares procedures. For instance, to determine the sequence of meiotic events in hours from first meiotic metaphase (MI) STM to EP was written as follows:

$$1(\text{STM to MI}) - 1(\text{EP to MI}) = 22.$$

In this way a system of equations was built up for the available observations. Coefficients of the equation system together with their signs were taken as the X matrix and the corresponding vector of observations as the Y vector. The least squares solution of the desired intervals is as follows:

$$b = (X'X)^{-1}X'Y.$$

The standard errors of these estimates are the square roots of the corresponding variance on the leading diagonal of the variance covariance matrix.

The variance covariance matrix is:

$$(X'X)^{-1}s^2$$

where

$$s^2 = ((Y'Y) - b'X'Y)/df.$$

where df. is the number of degrees of freedom which is the number of observations minus the number of solutions.

The Extended Analysis of Pairing Rates in Triticale

This procedure is the extension of the procedure used to generate the indirect analysis tabulated in Table VI, page 40 (genomic origin paper). In triticale 4, there were 17.11 ring bivalents, 3.57 rod bivalents and 0.64 univalents per pollen mother cell (Table VI). In the same triticale, chromosomes entirely without bands constituted respectively 73.65% of ring bivalents, 20.27% of rod bivalents and 10.14% of rod bivalents (Table VII). Therefore, it is estimated that there were 12.60 ring bivalents, 0.72 rod bivalents and 0.06 univalents per pollen mother cell (PMC), entirely without heterochromatin in this triticale (73.65% of 17.11 ring bivalents is 12.60 etc.). Chromosomes with a single major terminal heterochromatic band constituted respectively 17.74% of ring bivalents and 33.81% of all univalents. Among the rod bivalents, 50.90% were single banded in the unpaired arm and 3.15% were banded in the paired arm. Therefore it is estimated that chromosomes with one prominent terminal band paired as 3.04 ring bivalents, 1.82 rod bivalents paired in the non-heterochromatic arm, 0.11 rod bivalents paired in the heterochromatic arm and 0.22 univalents. Finally, the chromosomes with two major terminal heterochromatic bands constituted 8.6% of ring bivalents, 25.67% of rod bivalents and 56.03% of univalents. This amounts

to 1.47 ring bivalents, 0.92 rod bivalents and 0.36 univalents per PMC.

This breakdown of the pairing allows the calculation of a chiasma frequency for chromosomes which have no bands, for the euchromatic chromosome arm of chromosomes with a single terminal band and for the pairing of chromosome arms which carry prominent heterochromatin. These three values are taken to represent the pairing efficiency of the wheat genome as a whole, the pairing efficiency of the chromosome arms of the rye genome which lack heterochromatin and the pairing efficiency of the chromosome arms of the rye genome which carry prominent heterochromatic bands at the telomere respectively. They were calculated as follows:

Wheat chromosomes: $(2 \times 12.60 + 0.72)/$
$(2 \times (12.60 + 0.72) + 0.06)$
$= \underline{0.971}$,

Rye chromosome arms
without heterochromatin: $(3.04 + 1.82)/$
$(3.04 + 1.82 + 0.11 + 0.22/2)$
$= \underline{0.957}$,

Rye chromosome arms
with heterochromatin: $(3.04 + 0.11 + 2 \times 1.47 + 0.92)/$
$((3.04 + 1.82 + 0.11 + 0.22/2) +$
$2 \times (1.47 + 0.92) + 0.36))$
$= \underline{0.686}$.

These data were calculated for each triticale in turn and plotted against overall chiasma frequency for the whole chromosome complement to give Figure 9, page 58.