Theor Appl Genet (1987) 73:516–522

TAG THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1987

Chromosome pairing in a *Lolium temulentum* × *Lolium perenne* diploid hybrid with a low chiasma frequency

G. Jenkins and M. J. Scanlon

Department of Agricultural Botany, School of Agricultural Sciences, U.C.W., Penglais, Aberystwyth, Dyfed SY23 3DD, UK

Received August 4, 1986; Accepted October 14, 1986
Communicated by R. Riley

Summary. Despite an average difference of about 50% in DNA amount, homoeologous chromosomes pair effectively at first metaphase in the diploid interspecific hybrid between *Lolium temulentum* and *Lolium perenne*. However, in the presence of accessory B chromosomes and "diploidising genes" pairing at metaphase I is severely reduced. Reconstruction of serial electron micrographs through pollen mother cell nuclei show that synaptonemal complexes are formed at pachytene between not only homoeologous but also non-homologous chromosome segments resulting in multivalent formation. These associations are largely ineffective in terms of chiasma formation and degenerate at late pachytene. It is highly probable that the pairing determinants exercise their control on chromosome pairing largely by prohibiting the siting of crossovers in homoeologously paired chromosome segments.

Key words: *Lolium* – Hybrid – Synaptonemal complex – Crossing over – Meiotic pairing

Introduction

The two species *Lolium perenne* and *Lolium temulentum* are closely related diploid ryegrasses (both 2n = 14) which differ by about 50% in nuclear 2C DNA amount (Hutchinson et al. 1979). This difference is manifested in the interspecific hybrid as a large disparity in size between homoeologous chromosomes which, nevertheless, pair effectively at first metaphase of meiosis. Chromosome reconstruction from serial electron micrographs has shown that regularity of pairing at metaphase is in fact mirrored by effective synaptonemal complex formation between homoeologous chromosomes during pachytene (Jenkins 1985a). In the presence of accessory B chromosomes and of "diploidising genes" homoeologous association at first metaphase in the diploid hybrid is, in sharp contrast, severely reduced with a commensurate increase in the number of univalents (Evans and Macefield 1973; Taylor and Evans 1977).

The object of the present work was to use the same electron microscope technique to monitor the progress of pairing during meiotic prophase in the diploid hybrid containing these pairing determinants in order to elucidate the means by which B chromosomes and "diploidising genes" exert their effect on chromosome pairing behaviour.

Materials and methods

Lolium perenne E5 (2n = 2x = 14 + 2B), a genotype derived from Lp19 (Taylor and Evans 1977), and *Lolium temulentum* Ba3081 (2n = 2x = 14), a genotype obtained from the Welsh Plant Breeding Station, were grown in pots in an unheated greenhouse. Although the species are closely related the former has a 2C DNA amount of 4.16 pg and the latter 6.23 pg, a difference of about 50% (Hutchinson et al. 1979). The two species were crossed, the F_1 embryos cultured and the growing seedlings transplanted into pots in an unheated greenhouse according to the method of Evans and Macefield (1973).

Emerging inflorescences at approximately the required stage of meiotic development were sampled and one of the three anthers from each floret was dissected out, squashed in aceto-carmine and viewed under bright-field illumination of a light microscope in order to determine more precisely the meiotic stage. If an anther was found to be at metaphase I of meiosis, the two remaining anthers of the same floret were transferred to Carnoy's fixative and then to 70% ethanol two to three days later. Twelve fixed anthers from 6 spikes of the plant used in this study were each squashed in aceto-carmine

and 20 pollen mother cells at metaphase I per slide were scored for chiasma and bivalent frequencies.

If an anther was found to be at zygotene or pachytene of meiosis, the remaining two in the floret were prepared for ultrastructural analysis under the electron microscope. Each anther was sunk in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 4 h at room temperature. After primary fixation the anthers were immediately dehydrated in a graded ethanol series followed by en bloc staining in 1% ethanolic phosphotungstic acid (PTA) for 16 h at 4 °C. This was followed by washing in ethanol and propylene oxide and infiltration with Spurr's standard low viscosity resin (Spurr 1969), which was polymerised overnight at 70 °C. The method of cutting, examining and photographing sections through the anthers is as given in Jenkins (1985 a) except that the electron microscope negatives were printed onto 24.0 × 30.5 cm Agfa-Gevaert Rapitone P1-4 paper which was processed in an Agfa-Gevaert Rapidoprint DD6400 processing unit. The tracing and length measurements of synaptonemal complexes (SCs) and their elements within reconstructed nuclei are also as previously described except that in the two diplotene nuclei the long axes of chromosomes were followed instead, since by this stage the SCs had degenerated and were discontinuous. The volumes of the chromosomes in these two cells were measured using the same computer equipment by summing the area of chromatin belonging to each chromosome from every section and multiplying the total by the average section thickness. The controlling programme was written by Dr. J. S. Heslop-Harrison of the Plant Breeding Institute, Cambridge.

In the present work the chromosomes of five hybrid nuclei were reconstructed. Two of the cells are at diplotene. Since the other three nuclei contain unpaired axial elements they may be assigned to the zygotene stage of meiosis which, by definition, is the stage during which chromosomes begin and complete pairing. However, SC formation is highly irregular and the chromosomes are much shorter than expected when compared with the total lengths of chromosomes from other hybrid nuclei at zygotene with a similar % pairing (Table 1), even if allowance is made for reasonable length variation during this stage. Despite the incompleteness of pairing, therefore, the three nuclei are considered to be at mid or late pachytene depending on the position of the total chromosome length measurement in the ranking of Table 1. It follows that these nuclei display some degree of pairing failure and highlight the inadequacies of the distinction between zygotene and pachytene based simply upon the progress of chromosome pairing.

Results

First metaphase

The mean chiasma frequency estimated from 20 pollen mother cells in each of 12 anthers from the hybrid plant investigated was 1.6 per pollen mother cell, with a range of from 0.8 to 2.9 per anther. The mean bivalent

Table 1. The meiotic stage, chromosome constitutions, total lengths and % pairing of chromosomes in 19 nuclei from Jenkins 1985 a (1), Jenkins 1985 b (2), Jenkins 1986 (3) and the present work (4), ranked according to decreasing chromosome length. Total length is for the diploid hybrid complement with the exception of the estimates for Lp19 and the pachytene nucleus of the triploid which represent *Lolium perenne* only. The length estimates in the polyploid nuclei have been derived by simple division of the total lengths for each cell. % pairing is calculated as the length of lateral components involved in SC formation expressed as a % of the total length of lateral components per nucleus. % pairing does not take into account whether or not pairing is homologous. Note that three of the nuclei in the present work have been placed in mid and late pachytene according to their position in the ranking of total lengths and not by the % pairing they display

Genotype	Ploidy	No. Bs	Meiotic stage	Total length (μm)	% pairing	Where described
Ba3081 × Lp10	2x	0	early zygotene	844.04	15	1
Ba3081 × Lp10	2x	0	early zygotene	835.87	22	1
Ba3081 × Lp10	3x	2	early zygotene	708.59	39	2
Lp19	2x	1	late zygotene	587.18	91	1
Ba3081 × Lp10	4x	0	late zygotene	545.67	88	3
Ba3081 × Lp10	2x	0	pachytene	536.84	100	1
Ba3081 × Lp10	2x	0	pachytene	523.05	97	–
Ba3081 × Lp10	2x	0	pachytene	495.82	99	1
Ba3081 × Lp10	2x	0	pachytene	495.77	100	1
Ba3081 × E5	2x	2	?	468.77	60	4
Ba3081 × Lp10	2x	0	pachytene	453.51	100	1
Ba3081 × Lp10	2x	0	pachytene	444.51	100	1
Ba3081 × Lp10	3x	2	pachytene	436.92	100	2
Ba3081 × E5	2x	2	?	308.49	32	4
Ba3081 × E5	2x	2	?	285.45	21	4
Ba6092 × Lp19	4x	4	late pachytene	283.97	43	3
Ba6902 × Lp19	4x	4	late pachytene	260.36	80	3
Ba3081 × E5	2x	2	diplotene	125.97	0	4
Ba3081 × E5	2x	2	diplotene	84.64	0	4

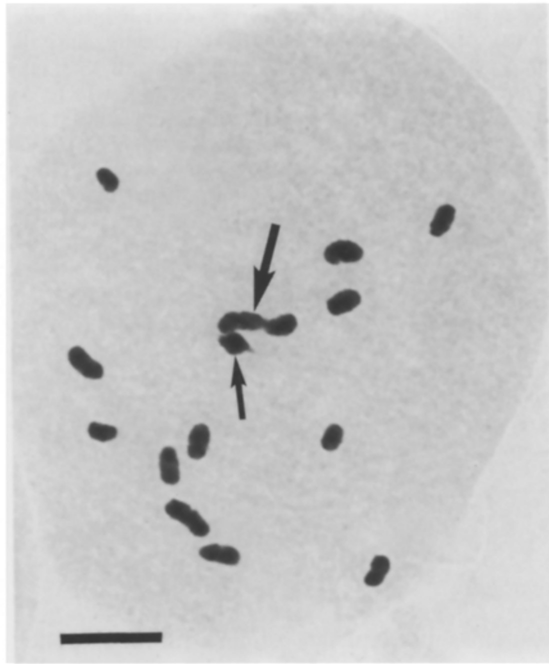

Fig. 1. Light micrograph of a typical pollen mother cell at metaphase I of meiosis from the diploid hybrid containing B chromosomes and "diploidising genes". Note the very low chiasma frequency, the single heteromorphic rod bivalent (large arrow) and the B chromosome bivalent (small arrow). Bar represents 10 μm

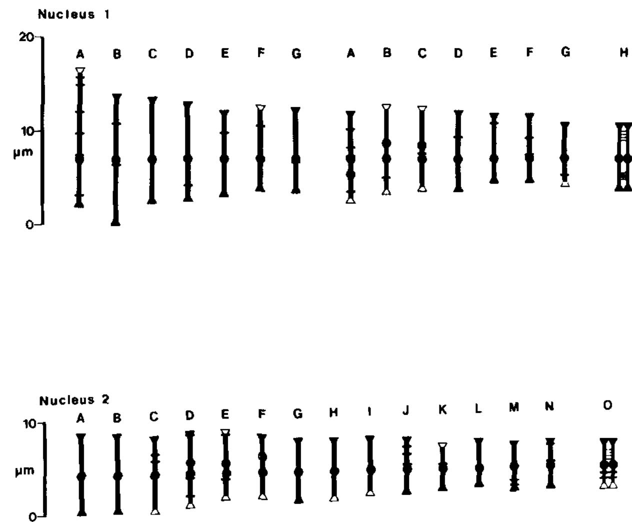

Fig. 2. Line diagrams of the chromosomes of the two diplotene nuclei. Chromosome axes are represented by thick lines, SC by crosshatching, centromeres by large filled circles, recombination nodules by small filled circles, nucleolar organising regions by open circles, telomeres attached to the nuclear envelope by filled triangles and unattached telomeres by open triangles and SC fragments by crossbars. In nucleus 1 the two haploid sets of seven chromosomes are separated and have been labelled A to G for reference purposes only and not to indicate homoeology. H represents the B chromosome bivalent. In nucleus 2 the chromosomes (A–N) are ranked according to length. 0 represents the B chromosome bivalent. Note the considerable difference in length between the chromosomes of nucleus 1 and nucleus 2 even though the 2 cells occupied adjacent positions in the same anther

frequency was 1.5, with a range of 0.8 to 2.6 per anther. The two B chromosomes form a bivalent in all the cells analysed. A typical cell is shown in Fig. 1. The low frequencies in this hybrid are due to the action of B chromosomes and also to "diploidising genes" from E5, both of which are known to suppress homoeologous pairing at first metaphase in *Lolium* hybrids (Evans and Macefield 1973; Taylor and Evans 1977).

Diplotene

The chromosomes of two diplotene nuclei were reconstructed fully and are shown diagrammatically in Fig. 2. The nuclei were judged to be at diplotene since their chromosomes were very short and condensed (Table 1) and the SCs (with the exception of those of the B chromosomes) had degenerated into small fragments seemingly randomly scattered in the chromatin (Fig. 3).

Nucleus 1 shows 14 univalents representing the entire A chromosome complement of the hybrid. Three nucleolar organising chromosomes were active. Since the three organising chromosomes are the largest members of both haploid sets, these three chromosomes could be assigned unequivocally to *L. perenne.* It was then possible to resolve the overlap in size and morphology between the two haploid sets and thereby

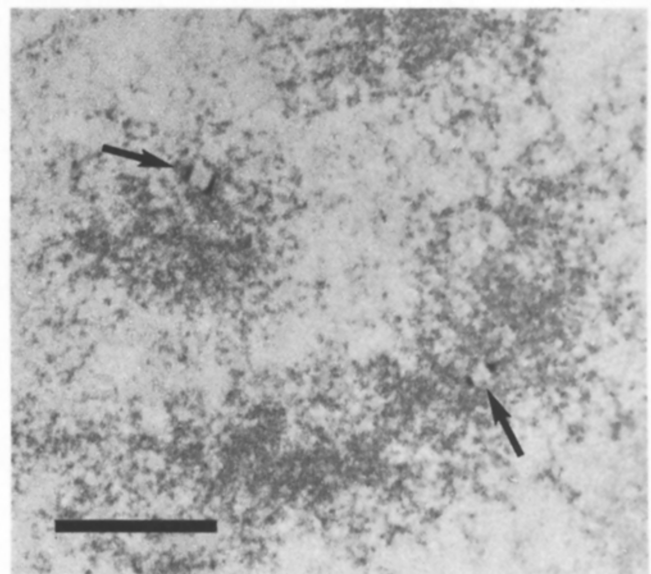

Fig. 3. Electron micrograph showing small SC fragments (arrows) in transverse section and embedded in the chromatin of diplotene chromosomes. Bar represents 1 μm

separate all the chromosomes belonging to *L. perenne* and *L. temulentum.* The total axis length of the *L. perenne* chromosomes was 53.3 μm and that of *L. temulentum* 72.7 μm, a difference of 36%. This difference corresponds closely to the estimate of 39% between homoeologous lateral components of the two species in a triploid hybrid (Jenkins 1985b). Both estimates are consistently lower than the 50% difference in nuclear DNA amount between the two parental species (Hutchinson et al. 1979). Together, the two sets of results show that chromosome length in these hybrids in not directly proportional to the DNA amount contrary to that reported in other plant species (Anderson et al. 1985). That the difference in length is not directly proportional to the DNA difference may be due to differential condensation of the two chromosome sets in the hybrid (Jenkins 1985a). Indeed, a difference of only 28% between the total volumes of *L. perenne* chromosomes (19.8 μm^3) and *L. temulentum* chromosomes (25.3 μm^3) at diplotene in this hybrid indicates that the chromatin itself is differentially compacted.

The conclusion assumes, of course, that there is no intraspecific variation in DNA amount within the *Lolium* species, that is to say that the 50% interspecific difference reported by Hutchinson et al. (1979) is characteristic and consistent. Such intraspecific variation cannot be ruled out. The DNA difference between *L. perenne* and *L. temulentum* genotypes reported by Rees and Jones (1967) is very much lower (about 35%) than that given by Hutchinson et al. (1979).

Identification of the two haploid sets of chromosomes also allowed the relative spacial distribution of chromosomes to be investigated. If each centromere is given a three-dimensional coordinate based on its mid-point position in the reconstructed nucleus, it is possible to separate the centromeres belonging to the two haploid sets by a single plane (Fig. 4a). To clarify, the plane may be rotated and viewed in such a way that it appears as a straight line which separates both complements of centromeres (Fig. 4b). Side-by-side genome separation or complement fractionation occurs in other hybrids e.g. *Rubus procerus* × *R. laciniatus* (Bammi 1965), *Hordeum vulgare* × *Secale africanum* (Finch et al. 1981), and also in non-hybrid species such as *Zea mays* and Tuleen 346 barley (Bennett 1983). It is not possible to assess the significance of genome separation in the *Lolium* hybrid as it has only been demonstrated in one cell and modelling has shown that 20% of meiocytes would show such separation by chance when 2n = 14 (Bennett 1983). Furthermore, the two genomes are separated only with respect to centromeres. Whole chromosomes (including telomeres) are seemingly randomly distributed throughout the nucleus at this stage.

The B chromosome bivalent (H) is readily identified by its relatively small size and by the fact that it is the only association of two chromosomes with some SC retained and a recombination nodule (Fig. 5).

Nucleus 2 shows 14 univalents (A to N) representing the entire A chromosome complement of the hybrid. Although three nucleolar organising chromosomes were active it was not possible to separate the two haploid parental sets as the relative differences in length between chromosomes were much smaller. This precluded an analysis of the relative sizes of the two genomes and the spacial distribution of centromeres.

The two B chromosomes (O) are joined by a small stretch of SC.

Pairing and SC formation between homoeologues in these two diplotene nuclei are completely absent. The is consistent with observations at metaphase I, at which stage many pollen mother cells have 14 univalents.

Late pachytene

The chromosomes of two late pachytene nuclei were reconstructed fully and are shown diagrammatically in Fig. 6.

Nucleus 1 has the following features:

1) Four bivalents (A to D) which are probably homoeologous and contain short stretches of SC in mainly interstitial regions. The centromeres in A and B are grossly mispaired with non-centromeric chromatin. Bivalent A organises the nucleolus and has a single lateral component break and a recombination nodule. Bivalents C and D have perfectly matched centromeres

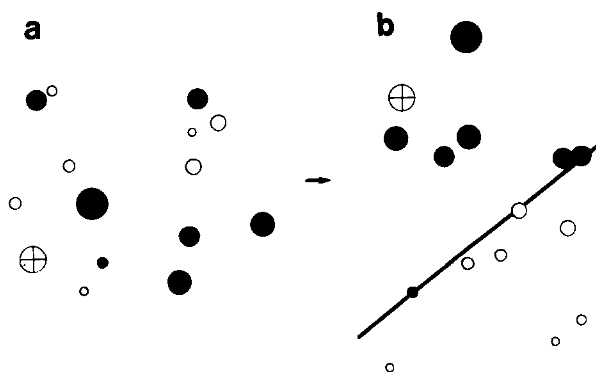

Fig. 4. a Centromere distribution in diplotene nucleus 1 viewed from the plane of sectioning. Filled circles represent mid-point positions of *L. temulentum* centromeres, open circles those of *L. perenne* and the open circle with a cross that of the B chromosome bivalent. Circle size indicates depth in the nucleus, smaller ones being deeper. **b** Rotation of **(a)** such that centromeres belonging to each haploid set are separated by a straight line

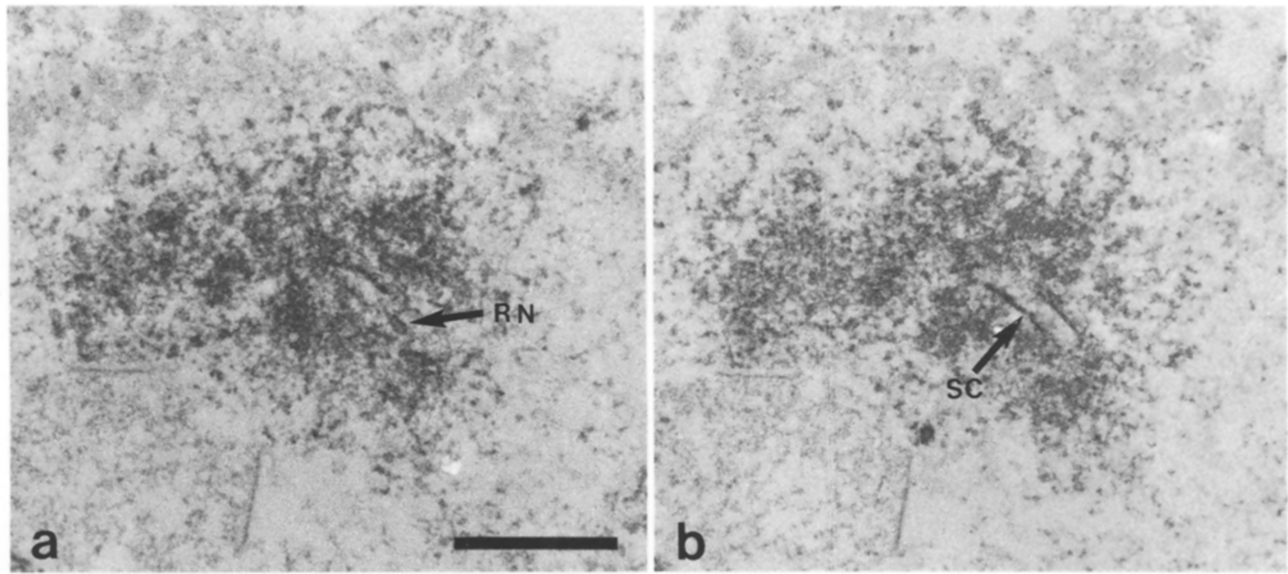

Fig. 5a, b. Consecutive electron micrographs from diplotene nucleus 1 showing synaptonemal complex (SC) of the B chromosome bivalent in longitudinal section and containing a recombination nodule (RN). Bar represents 1 μm

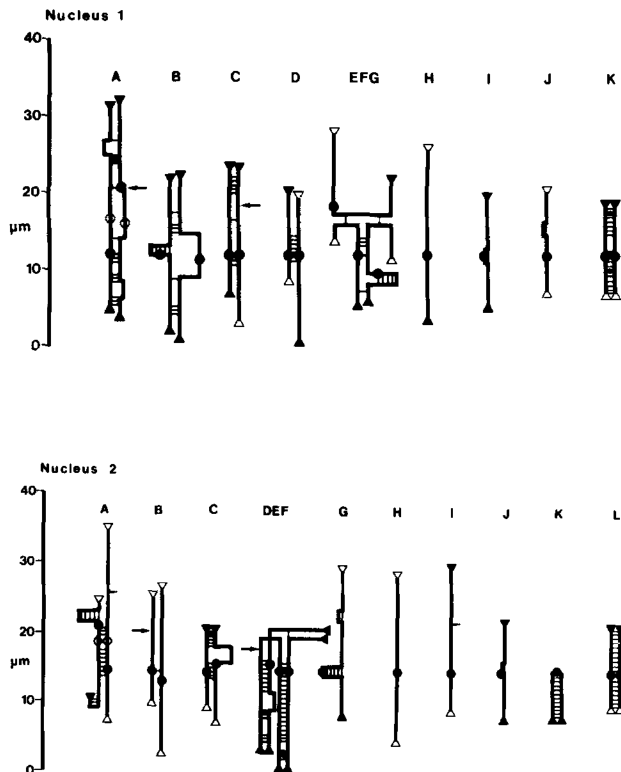

Fig. 6. Line diagrams of the chromosome pairing configurations of the two late pachytene nuclei. Lateral components are represented by thick lines, short stretches of non-homologous SC by broken lines and lateral component breaks by arrows. All other symbols are as described in Fig. 2

and display considerable disparity in lateral component length between homoeologues and C has a single lateral component break. 2) A "trivalent" (EFG). Two chromosomes are joined by substantial stretches of SC but each exchanges pairing partners in favour of synapsis with the same arm of another chromosome. Two centromeres are mispaired, one being involved in a fold-back loop. 3) Three univalents (H to J), two of which contain short fold-back loops. 4) The B bivalent (K) identified by its relative size and its regular and extensive SC which contains two recombination nodules.

Nucleus 2 has the following features:

1) Two bivalents (A and C) probably comprising homoeologues of different lengths. Both chromosomes of bivalent A organise the nucleolus, one having two fold-back loops and the other a small stretch of non-homologous SC with chromosome I. Bivalent C possesses a distally located recombination nodule. B probably does not constitute a bivalent since the broken lateral component is joined to the other only by a single short stretch of SC involving a centromere. 2) A "trivalent" (DEF). Two putative homoeologues are paired together with a long SC containing a distal recombination nodule. They both exchange pairing partners in favour of a completely non-homologous chromosome. Non-homologous pairing in this multiple is quite extensive and actually incorporates a re-

combination nodule. One lateral component exhibits a single breakage. 3) Five univalents (G to K), three of which have fold-back loops involving the centromeres. I is paired non-homologously with a chromosome in bivalent A. 4) The B bivalent (L) identified by the same criteria used in nucleus 1. In this case, however, SC formation is complete and uninterrupted from telomere to telomere.

Clearly at this stage SCs are formed between both homoeologous and non-homologous chromosome segments. However, the paucity of chiasmate associations at first metaphase and diplotene establishes that most of the SCs are not effective in terms of chiasma formation.

Mid pachytene

Although all axial cores and SCs were traced, the karyotype was not completely reconstructed due to the complexity and irregularity of pairing and the discontinuity and breakage of lateral components. However, it was possible to discern the following features:

1) Extensive but incomplete SC formation occurring preferentially at the telomeres (14 of the 28 A chromosome telomeres are synapsed in pairs). 2) Regular and complete SC formation with matched centromeres between the two B chromosomes. 3) Irregular SCs. All the A chromosome centromeres are mispaired with non-centromeric chromatin, several of which are included in fold-back loops. 4) Multiple associations. Three and four centric fragments are paired together, respectively, into a "trivalent" and a "quadrivalent".

Extensive pairing is permitted between homoeologous and non-homologous chromosome segments at this stage. It may be inferred from observations at later stages that these SCs do not support genetic exchange.

Discussion

At first metaphase and diplotene of meiosis in the diploid hybrid containing B chromosomes and "diploidising genes" pairing is virtually absent between homoeologous chromosomes. However, this does not accurately reflect chromosome pairing behaviour during meiotic prophase since at pachytene there is extensive SC formation between both homoeologous and non-homologous chromosome segments which results in the formation of multivalents. The presence of multiple configurations during meiotic prophase is consistent with similar observations in the triploid and tetraploid *Lolium* hybrids (Jenkins 1985 b, 1986) and provides conclusive proof that diploidisation at metaphase by B chromosomes and "diploidising genes" on the A chromosomes is not achieved by preventing synapsis of homoeologous or even non-homologous chromosomes at zygotene.

Since multivalents are not observed at metaphase I or at diplotene it follows that they are transformed at an earlier stage during meiotic prophase. This correction may be achieved in two ways:

1) The SCs of multivalents may be reassembled into bivalents prior to pachytene or at least before crossing over has occurred. This means of exclusive bivalent formation was clearly demonstrated in the diploid *Lolium* hybrid without pairing determinants (Jenkins 1985 a), the triploid hybrid (Jenkins 1985 b) and allohexaploid wheat (Hobolth 1981; Jenkins 1983). The extent to which this mechanism is employed in the present hybrid is difficult to ascertain as the zygotene stage of meiosis was not investigated. However, the greater number of multiple configurations seen at mid pachytene as compared with late pachytene may indicate that there is some element of transformation at least during pachytene and maybe even earlier. 2) The multivalents may be corrected upon entry into diplotene because of an absence of crossing over. Some multivalents in the present material persist until late pachytene by which time the period of crossing over almost certainly has passed. Along with the bivalents, they then degenerate at diplotene resulting in a high frequency of univalents. Since crossovers are likely to consolidate pairing association (Rasmussen and Holm 1979) it is concluded that most of the SCs formed during pachytene between both homoeologous and non-homologous chromosome segments are not effective in terms of chiasma formation. Non-homologously paired segments would not be expected to participate in genetic exchange due to dissimilarity between juxtaposed DNA molecules. This is clearly demonstrated in haploid organisms e.g. barley (Gillies 1974) in which extensive SC formation occurs between non-homologous chromosomes but does not support crossing over. Homoeologously paired segments, however, would be much more alike and would have the potential to cross over. It follows that B chromosomes and "diploidising genes" probably exert their effect by preventing crossing over within these segments. Indeed, if these determinants are absent homoeologous chromosomes will pair and form seven bivalents with chiasma frequencies not unlike one of the parents (Jenkins 1985 a).

The mechanism by which the pairing determinants influence the siting of crossovers is not known. Detailed information of this nature could be obtained from a molecular study of chromosome pairing and recombination.

Acknowledgements. The authors wish to thank Prof. Hugh Rees for his help with the manuscript, Dr. Gareth Evans for providing the plant material, Mr. Derek Fallding and the EM Unit (Zoology) for technical assistance, Dr. Pat Heslop-Harrison for the computer programme and the AFRC for financial support (grants AG2/82 and LRG/21).

References

Anderson LK, Stack SM, Fox MH, Chuanshan Z (1985) The relationship between genome size and synaptonemal complex length in higher plants. Exp Cell Res 156:367–378

Bammi RK (1965) "Complement fractionation" in a natural hybrid between *Rubus procerus* Muell. and *R. laciniatus* Willd. Nature 208:608

Bennett MD (1983) The spacial distribution of chromosomes. In: Brandham PE, Bennett MD (eds) Kew Chromosome Conference II. Allen and Unwin, London, pp 71–79

Evans GM, Macefield AJ (1973) The effect of B chromosomes on homoeologous pairing in species hybrids. 1. *Lolium temulentum* × *Lolium perenne*. Chromosoma 41:63–73

Finch RA, Smith JB, Bennett MD (1981) *Hordeum* and *Secale* mitotic genomes lie apart in a hybrid. J Cell Sci 52: 391–403

Gillies CB (1974) The nature and extent of synaptonemal complex formation in haploid barley. Chromosoma 48: 441–453

Hobolth P (1981) Chromosome pairing in allohexaploid wheat var. Chinese Spring. Transformation of multivalents into bivalents, a mechanism for exclusive bivalent formation. Carlsberg Res Commun 46:129–173

Hutchinson J, Rees H, Seal AG (1979) An assay of the activity of supplementary DNA in *Lolium*. Heredity 43:411–421

Jenkins G (1983) Chromosome pairing in *Triticum aestivum* cv. Chinese Spring. Carlsberg Res Commun 48:255–283

Jenkins G (1985a) Synaptonemal complex formation in hybrids of *Lolium temulentum* × *Lolium perenne* (L.). 1. High chiasma frequency diploid. Chromosoma 92:81–88

Jenkins G (1985b) Synaptonemal complex formation in hybrids of *Lolium temulentum* × *Lolium perenne* (L.). 2. Triploid. Chromosoma 92:387–390

Jenkins G (1986) Synaptonemal complex formation in hybrids of *Lolium temulentum* × *Lolium perenne* (L.). 3. Tetraploid. Chromosoma 93:413–419

Rasmussen SW, Holm PB (1979) Chromosome pairing in autotetraploid *Bombyx* females. Mechanism for exclusive bivalent formation. Carlsberg Res Commun 44:101–125

Rees H, Jones GH (1967) Chromosome evolution in *Lolium*. Heredity 22:1–18

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26: 31–43

Taylor IB, Evans GM (1977) The genotypic control of homoeologous chromosome association in *Lolium temulentum* × *Lolium perenne* interspecific hybrids. Chromosoma 62: 57–67