CHROMOSOME PAIRING IN TRIPLOID FEMALES OF BOMBYX MORI ANALYZED BY THREE DIMENSIONAL RECONSTRUCTIONS OF SYNAPTONEMAL COMPLEXES.

by

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Chromosome pairing in triploid females of *Bombyx mori* was analyzed by serial sectioning and three dimensional reconstruction of synaptonemal complexes and unpaired lateral components in 11 nuclei covering the period from early to late pachytene. Two distinct phases of chromosome pairing with synaptonemal complex formation were revealed; one phase mediates pairing between homologous chromosomes and is followed by a second phase which is responsible for non-homologous pairing of univalents. Trivalent pairing of three homologues as well as bivalent interlocking appear to be corrected by dissolution and reassembly of the central regions of the synaptonemal complex before the initiation of or during the second pairing phase.

1. INTRODUCTION

A precise pairing of homologous chromosomes during early meiotic prophase is a prerequisite for crossing over, chiasma formation, and regular disjunction. The synaptonemal complex provides the structure which ensures this precise site-to-site matching of homologous chromosomes and the subsequent stabilization of the paired regions. In those cases where pairing has been analyzed by serial sectioning and three dimensional reconstructions of zygotene and pachytene nuclei (2, 6, 7, 15, 21, 25, 28, 56) it is apparent that pairing occurs with great precision. At pachytene, where the result of the

pairing process can be analyzed, irregularities are the exception (11, 28) and a precise alignment of homologous chromosomes the rule.

After the synaptonemal complexes are shed from the bivalents, the association of homologous chromosomes which is essential for a regular disjunction, is mediated by chromatin bridges formed by non-sister chromatid exchanges (10, 37, 39, 52, 53). Investigations in human spermatocytes have shown that small stretches of synaptonemal complex are retained in the bivalents at early metaphase I. This suggests that the synaptonemal complex in some cases continues to play a role in the association

of homologous chromosomes after the majority of complexes is eliminated in early diplotene (23, HOLM and RASMUSSEN, unpublished results).

In the absence of crossing over, substitute mechanisms are required to ensure a regular disjunction of the homologues at anaphase I. The synaptonemal complexes in *Bombyx* oocytes undergo an ultrastructural modification by addition of material to the lateral component. The modified synaptonemal complexes are retained by the bivalents until anaphase I. At anaphase I, the modified synaptonemal complexes are shed from the homologues and remain in the position of the metaphase plate (18, 30, 34).

Several authors (21, 25, 41, 51, 54, 55) have proposed on the basis of ultrastructural or biochemical studies that the pairing of homologous chromosomes occurs in two steps: a primary attraction of homologous chromosomes, followed by stabilization of pairing by the formation of synaptonemal complexes.

Information on the mechanisms underlying the primary attraction and recognition of the homologous chromosomes and their subsequent intimate pairing is confined to the following relatively well-established facts. a) The chromosomes are distributed randomly in the nucleus or are contained in two different nuclei prior to the meiotic prophase (15, 28, 33, 56). b) A lateral component of the synaptonemal complex is organized in each chromosome before or during the pairing (10, 15, 27, 28, 55, 56). c) The ends of all lateral components attach to the nuclear envelope. d) The attachment sites are concentrated within a limited region of the nuclear envelope, the bouquet region (10, 15, 21, 19). e) The homologous chromosomes approach each other to a distance of approximately 200-300 nm (21, 25, 28, 51, 55, 56). f) The synaptonemal complex is completed by the formation of the central region between the lateral components. The central region is formed from starting points near the attachment sites of the lateral components on the nuclear envelope (25, 28) and/or from starting points distributed

along the chromosomes (10, 15, 22). g) The attachment sites of the bivalents with completed synaptonemal complexes move away from the bouquet region and the chromosomes become uniformly distributed in the nucleus (10, 28).

Studies on chromosome pairing in haploid organisms (5, 8, 36, 47) have demonstrated that extensive, presumably non-homologous, chromosome pairing can occur, and in barley may include up to 60% of the lateral components. Synaptonemal complexes between non-homologous chromosomes are morphologically indistinguishable from complexes between homologous chromosomes (8). This supports the view that the specificity involved during primary recognition and/or subsequent formation of the synaptonemal complex can be suppressed, resulting in synapsis between non-homologous chromosome segments.

In the silkworm, *Bombyx mori*, both sexes possess synaptonemal complexes (16, 20, 28) but crossing over and chiasma formation are limited to males (42, 43, 44). Chromosome pairing during early meiotic prophase in diploid oocytes is normal, resulting at pachytene in 28 bivalents each having a continuous synaptonemal complex between the attachment sites on the nuclear envelope (28). In four nuclei at late zygotene five cases of interlocking were observed. Four of these cases involved interlocking of two bivalents, and in the fifth case an unpaired lateral component was trapped between unpaired lateral components of an otherwise completely synapsed bivalent (28, 29). At pachytene, no interlockings were revealed in six completely reconstructed nuclei, thus indicating that a correction of pairing is possible during the transition from zygotene to pachytene. In triploid males and females of *Bombyx mori* more than the haploid number ($n = 28$) of chromosome elements is present at metaphase I and both males and females are sterile due to an unequal distribution of the three chromosome sets at anaphase I (1). Autotetraploid males are also sterile and less than the expected 56 elements are found at metaphase I, while tetra-

ploid females are fertile and the expected 56 chromosome elements are present at metaphase I (45, 50). SEILER (35) showed that a different situation is encountered in triploid intersexual hybrids between tetraploid parthenogenetic females and bisexual diploid males of *Solenobia triquetrella*. In these triploid hybrids the haploid number ($n = 31$) of trivalents is present at metaphase I indicating that the association of homologues persists until anaphase I.

The present paper aims to analyse further details in the process of chromosome pairing, and the possible subsequent correction of pairing, by an examination of triploid females of *Bombyx mori*. The addition of an extra set of chromosomes provides a competition between three homologous chromosomes, as normally only two homologous segments are combined by a synaptonemal complex at a given site. Triploidy allows a clear distinction between the primary recognition of homologues and their intimate pairing through the synaptonemal complex.

2. MATERIALS AND METHODS

The material for this study was kindly provided by the late professor B. L. ASTAUROV, Institute of Developmental Biology, Academy of Sciences USSR, Moscow. The triploid females ($2n = 84$) were obtained from crosses between tetraploid females ($4n = 112$) and diploid males ($2n = 56$). The tetraploid female parents were produced by thermal activation of normal diploid eggs and were maintained as parthenogenetic clones by thermal activation of dissected eggs as described by ASTAUROV (1) and VEREISKAYA (49).

The procedure for removing the ovaries and their subsequent fixation, dehydration, and embedding has previously been described (28). Thick sections (2-4 microns) were cut and examined in the light microscope in order to identify regions in the ovary containing nuclei in the desired stage of development. Series of thin sections were cut, photographed at a primary magnification of 4-5.000 x, and reconstructed as described by GILLIES (6). The pro-

Table I

Classification of 11 completely reconstructed pachytene nuclei 5 from triploid females of *Bombyx mori* according to developmental stage.

Nucleus number ^{a)}	Nuclear vacuole	Bivalents in bouquet ^{b)}	Univalents in bouquet ^{b)}	All three telomeres associated ^{c)}	SC or LC attached to nuclear envelope ^{b)}
1	+	+++	+++	43	+++
2	+	+++	+++	48	+++
3	+	++	++	20	++
4	+	++	++	0	++
5	—	—	+	2	+
6	—	—	++	0	+
7	—	—	++	0	+
8	—	—	+	0	—
9	—	—	+	0	—
10	—	—	—	0	—
11	—	—	—	0	—

a) the nuclei in each group are from one cell cluster with the exception of nucleus 9 which belongs to a separate cell cluster.

b) +++, all; ++, the majority; +, a minor fraction.

c) the lateral component of the telomere of the univalent chromosome is closer than 300 nm to the one lateral component of the synaptonemal complex. Maximal possible number is 56.

jected lengths were measured and the real length calculated using a Hewlett Packard digitizer (HP 9864A) and calculator (HP 9825A).

The results were based on 11 completely reconstructed oocyte nuclei covering the period from early pachytene to late pachytene.

3. RESULTS

3.1 General comments

The temporal sequence of the 11 completely reconstructed oocyte nuclei was established on the basis of the sequential changes in the fol-

lowing cellular and nuclear characters (see Table I): a) The distribution of organnels (Golgi apparatus, mitochondria) in the cell. b) The morphology of the nuclear vacuoles. c) The distribution of the attachment sites of lateral components and synaptonemal complexes on the nuclear envelope. As previously noted (28), only two chromosomes can be identified unequivocally by morphological markers; the longest chromosome, number 1, by a knob which is present from late zygotene to modified pachytene and chromosome 2 by the nucleolus organizer region. TRAUT (48) was able to iden-

Figure 1a

Figure 1a-d. A complete reconstruction of synaptonemal complexes and lateral components of the 84 chromosomes from a triploid *Bombyx* oocyte nucleus (number 1) at early pachytene. The chromosomes are all attached at both ends to the nuclear envelope in a bouquet opposite the nucleolus (N) and the nuclear vacuole (V). In the majority of the cases, two of the three homologous chromosomes have paired with a synaptonemal complex while the third homologue is roughly aligned with the bivalent, either along its entire length (e.g., chromosomes 6 and 20 in Figure a, 9 and 28 in Figure b, 2, 18, and 26 in Figure d) or only at one or both attachment sites on the nuclear envelope (e.g., 3, 4, and 10 in Figure a, 14 and 23 in Figure c). The only exception is chromosome 8 where the bivalent and its homologous univalent are located in different region of the nucleus (bivalent 8 is at the top and univalent 8 at the bottom in Figure c). In the lower left region of the nucleus (Figure c), the unpaired lateral component of chromosome 1 is trapped by stretches of bivalent 1 and by bivalent 14, both with completed synaptonemal complexes. Also chromosomes 11, 22, and 23 (Figure c) are entangled. In four cases, synaptonemal complex formation has presumably started from both telomeres with two different homologous lateral components giving rise to a typical trivalent configuration (chromosome 6 in Figure a, 5 in Figure b, and 1 and 16 in Figure c). Note the two paired chromosome fragments in the lower left region of Figure a. (The underlined numbers designate the chromosomes which are also listed in Tables II and VI. The smaller numbers denote the section in which a particular telomere has been found.)

Figure 1b

tify by light microscopical analysis of pachytene bivalents 6 bivalents including chromosomes 1 and 2. The remaining 26 chromosomes can thus only be classified according to their length at the electron microscopical level. As some of the chromosomes are similar in length and the real length of a particular chromosome at pachytene is found to vary in different nuclei, an unequivocal identification of individual chromosomes other than chromosomes 1 and 2 was impossible. This is illustrated by the ranking of the latter two chromosomes in nuclei 5 to 11 in Table VI, if they were to be identified by length alone. The bivalent chromosome 1 is longer than chromosome 2 in all nuclei except 7 where it is shorter. With regard to the univalent chro-

mosome 1 it is shorter than 2 in nuclei 8 and 10 but otherwise longer. On the other hand, homology is established in most cases unequivocally by synaptonemal complex formation between lateral components of equal length and at early to mid pachytene also by close association of at least one set of the three telomeres.

3.2 Early pachytene (nuclei 1 and 2)

Two nuclei belonging to the same cell cluster were analyzed. The stage was characterized by large nuclear vacuoles in the region opposite the chromosome bouquet, and by smaller multi-layered invaginations of the inner membrane of the nuclear envelope in the region of chromosome attachment. On the cytoplasmic side of

Figure 1c

this region, a large number of mitochondria and Golgi apparatuses had accumulated. The lateral components of the synaptonemal complexes were attached at both ends to the nuclear envelope, the attachment sites being concentrated to a limited region of the nuclear envelope, forming the chromosome bouquet (Figure 1). With few exceptions, the three homologous telomeres were attached approximately 100-200 nm from each other. In most cases, two of the three homologous lateral components had combined into a synaptonemal complex, while the third lateral component was roughly aligned with the bivalent at a distance of about 200-500 nm (Figure 4). Near the attachment sites on the

nuclear envelope the three homologous lateral components occasionally combined into what appeared to be double complexes at low magnification. As seen in Figures 2 and 5, the central regions of the double complexes, and especially those of the synaptonemal complex including the univalent, lack the ladder-like organization characteristic of completed central regions in more advanced nuclei. In seven cases the lateral component of the third homologue was exclusively associated with its homologous bivalent at one end. In three cases the bivalent was not associated at all with the third homologue. If synaptonemal complex formation starts simultaneously from both ends of a

Figure 1d

Figures 2a-h. Electron micrographs of six consecutive sections through a trivalent and a diagrammatic reconstruction of its synaptonemal complex and univalent lateral component. In the lower part of Figures a and b, all three lateral components are parallel but only two are combined into a complex of standard dimensions. The central region between lateral components 1 and 2 (61 nm wide) in Figures a and b and between 2 and 3 in the upper part of Figures b, c, and d has the typical cross striation while the central region between lateral components 2 and 3 in the lower part of Figure b is wider (106 nm) and amorphous (enlargement in Figure g). Note the dense structure in the central region between lateral components 2 and 3 (arrows in Figures c and g).

Figures 2a-f, bar = 0.5 μ m

Figure 2g, bar = 0.2 μ m

Figure 3. A reconstruction of chromosomes 1 from nucleus 4. Synaptonemal complex formation between different lateral components at either end of the three homologues has led to formation of a trivalent, the site of pairing partner exchange being inside the knob. The dashed lines inside the knob indicate that the synaptonemal complex and the lateral components are continuous through the knob but less distinct than when present in the nucleoplasm.

grossly paired set of homologues in such a way that it involves different combinations of lateral components at the two ends, a trivalent is formed (Figures 2 and 3). Twelve such cases of exchange of pairing partners resulting in formation of trivalents were found in the two reconstructed nuclei. One nucleus had 8 trivalents of 28 possibles, (Figure 1) and the other had 4, suggesting that during late zygotene the exchange of pairing partner occurs exclusively between homologous chromosomes. The gross pairing was not affected by the shift of pairing partner during synaptonemal complex formation, as the segments of unpaired lateral components of a trivalent in most cases remain associated with the intimately paired stretches containing synaptonemal complexes (Figure 1).

The lengths of synaptonemal complexes and unpaired lateral components of the two reconstructed nuclei at early pachytene are given in

Table II. In those cases where the bivalent and its homologous univalent were grossly aligned along the entire distance between their attachment sites on the nuclear envelope, the ratios of synaptonemal complex lengths of the bivalents to the lateral component lengths of their homologous univalents were close to one (e.g., chromosomes 2, 6, 17, 18, 20, 26, 28 in Figure 1 and Table II). If a bivalent and its homologous univalent were only partly associated or entirely unassociated, the ratio of synaptonemal complex length to lateral component length of the three homologous chromosomes deviated significantly from one (e.g., 3, 4, 5, 8 in Figure 1 and Table II). In a few cases, the deviation of the ratio from one could be explained by a mechanical stretching of the bivalents or univalents due to entanglement with other chromosomes (e.g., chromosomes 1, 11, 22, 23 in Figure 1 and Table II).

Figure 4. A cross section through two sets of three homologues. In the lower part of the micrograph, the three lateral components are connected with central region material whereas in the upper triplet, the third lateral component is located 190 nm from the synaptonemal complex of the bivalent. In the lower set of three homologues the left hand two lateral components are separated with a central region of 80 nm width whereas the third lateral component is separated with material spanning 100 nm.

SC, synaptonemal complex. LC, lateral component. DSC, double synaptonemal complex
Bar = 0.2 μ m.

Figures 5a-c. Three consecutive sections through the lateral component attachment sites on the nuclear envelope of three homologues at early pachytene. Over a shorter stretch, all three lateral components (Figure a) have approached each other side by side with a distance of 50 nm between them but the central regions are amorphous without the ladder-like organization characteristic of completed synaptonemal complexes in nuclei of later stages. Note the dense structure (arrow in Figure b) in the central region of the synaptonemal complex where the third lateral component diverges from the synaptonemal complex.

Bar = 0.2 μ m.

Figures 6a-c. Reconstruction of the synaptonemal complexes and lateral components of the 84 chromosomes of an early to mid pachytene nucleus (number 3). This reconstruction reveals the first phase in the dispersal of the chromosome bouquet. As compared to the nucleus in Figure 1, the distance between the attachment sites of a bivalent and its third homologous univalent has increased in many cases. Three univalents, chromosomes 6, 8, and 12 in Figure b are completely separated from their bivalents as compared to 1 in the nucleus of Figure 1. In those cases where the three homologous telomeres are still associated on the nuclear envelope, the univalent telomere appears to have moved away from the bouquet together with the bivalent telomere (e.g., chromosomes 10, 13, 22

Figure 6a

and 26 in Figure a). A total of 26 bivalents, one trivalent (number 11 in Figure a), and 24 univalents are found. The remaining 3 univalents and the two homologous chromosomes 12 are accounted for in Figure b as follows: One chromosome 16 and one chromosome 23 have paired non-homologously forming a synaptonemal complex with one chromosome 12. The other chromosome 12 has formed a synaptonemal complex with one chromosome 28 in a non-homologous association. A bivalent formation of two of the three chromosomes 12 has apparently been prevented in this nucleus by the involvement of two chromosomes in non-homologous pairing with chromosomes 16, 23, and 28.

Figure 6b

brane of the nuclear envelope and the smaller vesicles in the region of chromosome attachment, which were present at the previous stage, could no longer be recognized. In both nuclei 26 bivalents with continuous synaptonemal complexes between the attachment sites on the

nuclear envelope were present (versus 20 and 24 in nuclei 1 and 2). There was only 1 trivalent in each nucleus as compared to 4 and 8 in the previous stage. The frequency of the close location of three homologous telomeres as well as the frequency of gross alignment between the bivalents and homologous univalents had declined. Many attachment sites of the synaptonemal complexes had moved away from the bouquet region and in some instances the univalents were left behind in the bouquet (Figures

7b and 17). In other instances, especially in nucleus 3 (Figure 6 and Table I), the univalent homologue remained associated with the bivalent and was moved from the bouquet region together with the bivalent.

As mentioned, one homologous trivalent was found in each of the two nuclei, the lateral component lengths amounting to 5.3, 5.1, 5.9 μm and 8.1, 9.0, 9.2 μm . In the latter trivalent, the chromosomes are marked by a knob and can thus be identified as the three homologues of

Table II

Length in μm of synaptonemal complexes and unpaired lateral components at early zygotene^{a)}.

Nucleus 1					Nucleus 2			
Chromosome	SC(LC ₁)	LC ₂	LC ₃	SC/LC	SC(LC ₁)	LC ₂	LC ₃	SC/LC
1	6.1 ^{b)}	6.7	7.6	0.8	7.2 ^{b)}	7.8	7.2	0.9
2	6.3	5.9		1.1	5.5 ^{b)}	6.1	5.7	0.9
3	6.5	4.9		1.3	5.9 ^{c)}	5.3		1.1
4	6.2	4.3		1.4	5.8 ^{c)}	3.6		1.6
5	6.1 ^{b)}	6.2	5.2	1.2	5.9 ^{b)}	5.6	5.7	1.0
6	5.9 ^{b)}	6.3	5.8	0.9	5.6 ^{b)}	5.0	5.5	1.1
7	5.3	4.8		1.1	5.2	5.2		1.0
8	5.0 ^{c)}	3.6		1.4	5.1	4.7		1.1
9	5.0	4.5		1.1	5.1 ^{b)}	5.1	5.0	1.0
10	5.0	4.9		1.0	4.9	5.0		1.0
11	4.8	5.3		0.9	4.8	4.4		1.1
12	4.8	4.8		1.0	4.6	4.7		1.0
13	4.7	4.0		1.2	4.5	4.4		1.0
14	4.6	4.0		1.2	4.5	4.0		1.1
15	4.6	4.5		1.0	4.4	4.1		1.1
16	4.6 ^{b)}	4.6	4.5	1.0	4.4	4.0		1.1
17	4.4	4.4		0.9	4.3	4.0		1.1
18	4.3	4.0		1.1	4.2	4.4		1.0
19	4.2	3.6		1.2	4.2 ^{b)}	4.1	4.4	0.9
20	4.1	4.3		0.9	4.1	4.2		1.0
21	3.9	3.5		1.1	4.2 ^{b)}	4.0	3.8	0.9
22	3.9	4.4		0.9	3.9	3.0		1.3
23	3.8	3.2		1.2	3.9 ^{b)}	3.8	3.6	1.1
24	3.7	3.4		1.1	3.5	3.5		1.0
25	3.7	3.6		1.0	3.5	3.5		1.0
26	3.7	3.8		1.0	3.3	3.9		0.9
27	3.6	3.5		1.0	3.2	2.9		1.1
28	3.4	3.5		1.0	2.8	2.9		1.0

a) the chromosomes are arranged according to length of the bivalent (average of the three lateral components is used for the trivalents).

b) trivalent (the lengths of the lateral components are given). The SC/LC ratio is calculated using the average of the two lateral components closest in length as the SC length.

c) bivalent and univalent not associated on the nuclear envelope.

chromosome 1 (Figure 4). Five cases of non-homologous pairing were revealed in the two nuclei; one hairpin, two non-homologous associations of two chromosomes, and two non-homologous associations of three chromosomes (Table IV). The non-homologously paired chromosomes were all attached to the nuclear envelope outside the bouquet region. The la-

teral component lengths of the two configurations involving three non-homologously associated chromosomes in nuclei 3 and 4 are 3.8, 6.6, 2.8 μm and 5.3, 5.0, 6.2 μm . Two of the chromosomes of the latter association could be homologous as judged by the similarity in lengths of the lateral components.

Figure 7a

Figures 7a and b. A reconstruction of a mid pachytene nucleus (number 5). Figure a shows the complete diploid complement of 28 bivalents each with a synaptonemal complex spanning their entire length. The bivalents are distributed uniformly in the nucleus in contrast to the univalents in Figure b. The attachment sites of the univalents are still concentrated in one half of the nucleus (the left part in Figure b). The univalents which are not attached to the nuclear envelope with both telomeres in the bouquet region of the nuclear envelope are frequently stretched out (chromosomes 1, 6, 8, and 9) whereas those attached in the bouquet region are recognized by their loop organization. Univalent 23 with a short piece of synaptonemal complex in a foldback paired interstitial segment represents the only example among the 11 reconstructed nuclei of synaptonemal complex formation not initiated at the nuclear envelope.

Figure 7b

Figure 8. Reconstruction of non-homologous association of four chromosomes from nucleus 6 (with lateral component lengths of 3.3, 4.6, 7.4 and 9.2 μm). Note that pairing is complete between the non-homologous chromosome segments except in regions where only one lateral component is available for pairing.

3.4 Mid to late pachytene (nuclei 5-11)

At this stage, nuclear vacuoles were no longer present. The cytoplasmic organelles were uniformly distributed in the cell and the nuclear polarity observed earlier was almost absent. The majority of the bivalents and the non-homologously paired chromosomes had detached from the nuclear envelope and were free in the nucleoplasm.

Trivalents were not found in the seven reconstructed nuclei (numbers 5-11, see Figures 7 and 13 and Table IV). Instead various non-homologous chromosome associations were observed. Four of the nuclei were triploid with 84 chromosomes and three nuclei were hypertriploid with 87 chromosomes (Table IV). Between 25 and 28 bivalents (i.e., maximal number possible) were present in the triploid oocytes, whereas 23, 28, and 30 bivalents were counted in the nuclei with 87 chromosomes.

The number of univalents varied between 18 and 27 in the triploid nuclei. There were 18 or 19 in the nuclei with 87 chromosomes. A number of univalents, 0-4 per nucleus, form hairpins that are paired with a synaptonemal complex by folding back on themselves. Between 2 and 9 associations of two to four non-homologous chromosomes were found per nucleus (Table IV). Examples for these are depicted in Figures 8-12, 14, and 15. In Figure 7b and 13b, the two lateral components of non-homologous bivalents are of different length, and demonstrate that the pairing of non-homologous lateral com-

ponents into synaptonemal complexes does not involve an "adjustment" of lateral component length. Even relatively small differences in length between the two lateral components were reflected by an unpaired segment of lateral component extending beyond the completed synaptonemal complex. It was characteristic of "hairpins" (foldback pairing of a single chromosome) and all other non-homologous associations, that the synaptonemal complexes were continuous from the attach-

Figure 9. Reconstruction of a non-homologous association of three chromosomes (with lateral component lengths of 5.3, 5.0, and 6.2 μm) from nucleus 4.

ment sites on the nuclear envelope to a site of lateral component exchange or to the site of termination of one of the two lateral components of the complex (Figures 8-12). It is likely that synaptonemal complex formation is initiated at the attachment site on the nuclear envelope. In contrast to pairing in trivalents involving only 2 of the 3 lateral components (Figures 2 and 3), the formation of synaptonemal complexes in the non-homologous associations was as complete as possible be-

Figure 11. Reconstruction of an association of one of the chromosomes 1 with a non-homologous chromosome in nucleus 7. The lengths of the synaptonemal complex and the third lateral component of chromosome 1 are 8.5 and 10.1 μm respectively and the lateral component length of the non-homologously paired chromosome is 6.9 μm . The dashed lines inside the knob indicate that the synaptonemal complex and the lateral component are continuous through the knob although the morphology of the components is less distinct.

Figure 10. Reconstruction of synaptonemal complex association formed by three chromosomes in nucleus 9. The similarity in lateral component lengths of the two longer chromosomes (6.7, 6.9, and 3.3 μm) indicates that they are homologous.

Figure 12. Reconstruction of a non-homologous pairing of three chromosomes (with lateral component lengths of 3.8, 5.0, and 7.9 μm) in nucleus 7. Note the completeness of the non-homologous pairing.

tween all three lateral components; unpaired stretches of lateral component being in most cases limited to regions where no pairing partner was available. It cannot be excluded that the associations of 3 chromosomes depicted in Figures 10, 14, and 15 consist of two homologous chromosomes and one non-homologous. It is possible that these non-homologous associations have occurred during the correction of

pairing in a trivalent at early pachytene, as a result of a competition between the two homologues and a non-homologous third chromosome. From Figures 17a-c and Table I it is apparent that the lateral components of univalents have a tendency to remain attached to the nuclear envelope within the bouquet region longer than those of the bivalents.

Figure 13a

Figures 13a and b. A reconstruction of the synaptonemal complexes and lateral components of the 87 chromosomes from a late pachytene nucleus (number 11). Figure a shows 28 bivalents each with a synaptonemal complex from end to end. The bivalents are no longer attached to the nuclear envelope and are evenly distributed throughout the nucleus. The univalents, hairpins (chromosomes 29 and 31), the associations of two non-homologously paired chromosomes (5+13, 6+19, 1+26, 4+15 and 9+27), and the non-homologous association of three chromosomes (3+7+8) have also become detached from the nuclear envelope and are dispersed throughout the nucleus (Figure b). Three dense nodules are present in Figure b; one is associated with a lateral component of the non-homologous synaptonemal complex formed by chromosomes 4 and 15, the second with the lateral component of chromosome 11, and the third one is lying close to the lateral component of the univalent chromosome 16. In all the non-homologously paired chromosomes, unpaired lateral components stretches are found only where no pairing partner is available (e.g., 3+7+8, 5+13, 6+19, and 4+15 in Figure b). N, nucleolus. Kn, knob.

Figure 13b

Figure 14. Reconstruction of an association of three chromosomes in nucleus 9. The similarity in lengths of two of the lateral components (7.1 and 7.8 versus 5.7 μm for the third) indicates that two of the three lateral components might be homologous. This association constitutes one of the few examples of incomplete non-homologous pairing (compare with Figures 9, 10, 11, and 12).

Two to ten nodules with a diameter of approximately 200-300 nm and with an electron density slightly lower than that of the nucleoli were found in four nuclei at mid pachytene and in one at late pachytene (nuclei 6-9 and 11, respectively) (Figures 13b, 14, 15 and 16). The nodules were associated either with homologous or non-homologous synaptonemal complexes, or with unpaired lateral components. Some were also located free in the nucleoplasm. It is not clear whether these nodules are involved in crossing over as suggested by CARPENTER (3) for similar structures in *Drosophila*. If they are, then the lack of crossing over in *Bombyx* females has to be the result of their

malfunction or a block at some other point in the sequence of events leading to crossing over. It should be noted that nodules – when present at all – were observed associated with synaptonemal complexes only in 2-6 instances per nucleus and with lateral components at 1 or 2 sites per nucleus (Table III). The nodules were most frequent in nucleus 7 and as nuclei 6-9 appeared to be in a similar stage of development, the presence of the nodules presumably was restricted to a short interval of the pachytene stage. This may explain why these nodules were not seen in diploid pachytene nuclei (28).

As mentioned, the total number of chromosomes in two of the five mid pachytene nuclei (numbers 8 and 9) and in one of the two nuclei at late pachytene (number 11) was 87 (Table IV). Nuclei 6, 7 and 8 belonged to the same 8-cell cluster, whereas nucleus 9 was from the same individual but from a different cluster. Nuclei 10 and 11 were from the same cluster. Nuclei with 87 chromosomes were thus found in three separate cell clusters in two individuals.

Figure 15. Reconstruction of an association of three paired chromosomes in nucleus 7. The lengths of the lateral components (7.6, 7.5, and 6.2 μm) indicate that two of the involved chromosomes are homologous. Note the dense nodule attached to homologously paired as well as to unpaired lateral components.

Table III

Chromosome associated nodules in pachytene oocyte nuclei.

Nucleus number	Nodule associated with LC of univalent	Nodule associated with LC of bivalent
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	2	0
7	1	6
8	0	3
9	0	2
10	0	0
11	1	0

Two of these clusters also contained nuclei with 84 chromosomes, the expected number for a triploid *Bombyx*. In addition, some of the nuclei analyzed contained between 1 and 3 chromosome fragments less than 2 μm long, (i.e., shorter than chromosome 28) with lateral components. In nucleus 8, one of the fragments was paired into a normal-looking synaptonemal complex with the lateral components of chromosome 2. The supernumerary chromosomes presumably are the result of a failure of sister chromatid separation in one of the preceding mitoses, whereas the fragments possibly result from chromosome breakage. As *Bombyx* has holocentric chromosomes (26), such fragments can be transmitted through several generations.

3.5 Quantitative aspects of chromosome pairing

From the data given in Table V and Va it appears that approximately two-thirds of the lateral component length (68%) was paired into synaptonemal complexes at the end of zygotene as would be expected if lateral components were homologously paired two-by-two, leaving the third set of chromosomes and chromosome segments unpaired. The total complex length at the beginning of pachytene (nuclei 1, 2, 3) was

Figure 16a-c. Three consecutive sections through a dense nodule that is attached to one lateral component of a synaptonemal complex in a bivalent. The diameter of the nodule is approximately 300 nm. The arrows indicate the synaptonemal complex in cross section. Bar = 0.5 μm .

Table IV

Chromosome configurations in triploid pachytene nuclei of *Bombyx mori*.

Nuc- leus	Univa- lents	Biva- lents	Triva- lents	Hair- pins	Non-homologous pairing involving:			Frag- ments	Total chromosome number ^{a)}
					2 chromo- somes	3 chromo- somes	4 chromo- somes		
1	24	24	4	0	0	0	0	2	84
2	20	20	8	0	0	0	0	0	84
3	24	26	1	0	1	1	0	0	84
4	23	26	1	1	1	1	0	0	84
5	16	28	0	4	4	0	0	0	84
6	25	25	0	2	0	1	1	0	84
7	16	27	0	2	3	2	0	0	84
8	19	30	0	0	4	0	0	3	87
9	18	23	0	0	5	3	1	1	87
10	20	26	0	1	2	1	1	0	84
11	16	28	0	2	5	1	0	1	87

a) fragments are not included.

Table V

Variation in length in μm of synaptonemal complexes and lateral components during pachytene in *Bombyx mori*.

Nucleus number	SC length of bivalents	LC length of univalents	SC length of trivalents	SC length of non-homologous associations	LC length of aberrant associations	LC length total	SC length total	Per cent LC paired into SC
1	110	101	24	0	71	391	134	68
2	87	82	42	0	125	381	129	68
3	116	131	5	10	37	401	131	65
4	143	127	8	15	58	470	166	70
5	175	100	0	31	72	519	205	79
6	175	171	0	21	49	569	196	69
7	157	82	0	34	91	487	190	78
8	166	114	0	20	41	488	186	76
9	140	113	0	50	122	515	190	74
10	159	124	0	23	56	498	182	73
11	165	87	0	43	103	519	208	80

131 μm . (The short stretches of the second complex present in the "double complexes" were not included in the total synaptonemal complex length.) In nucleus, 4 the percentage of lateral

components which is paired into synaptonemal complexes had increased to 70% of the total lateral component length. Simultaneously, the length of synaptonemal complexes between

non-homologous chromosome segments increased to $15\mu\text{m}$. In the remaining 7 nuclei, the percentage of lateral components paired into synaptonemal complexes (homologously and non-homologously) amounted to 76% the lowest value being 69% and the greatest 80%. The increase in synaptonemal complex length

from $131\mu\text{m}$ (average of nuclei 1-3) to an average of $194\mu\text{m}$ can be accounted for entirely by the increase in length of synaptonemal complexes between non-homologous chromosome segments.

It thus appears that chromosome pairing with the synaptonemal complex in triploid *Bombyx*

Figure 17a

Figure 17a-c. Diagrammatic drawings of complete reconstructions of nuclei 1, 4, and 10. The black lines represent the synaptonemal complexes and the red lines the unpaired lateral components. The three pictures illustrate the two phases in the dispersal of the chromosome bouquet. In Figure a, both synaptonemal complexes and lateral components have their attachment sites on the nuclear envelope within a limited region. The attachment sites of the synaptonemal complexes in Figure b have shifted so that the paired chromosomes are evenly dispersed throughout the nucleus while about half of the unpaired lateral components are still attached within the bouquet region of the nuclear envelope (the upper left part of Figure b). In Figure c, both synaptonemal complexes and lateral components are evenly distributed in the nucleus.

Kn, knob. N, nucleolus.

Table Va

Comparison of total synaptonemal complex and lateral component length between nuclei 1-4 and 5-11.

	Nuclei 1-4	Nuclei 5-11	t-test
Mean total SC length	140 ± 17	194 ± 10	P < 0.001
Mean total LC length	411 ± 41	514 ± 28	P < 0.001

females occurs in two separate phases, one in which homologous pairing is the rule and a second one, in which univalents pair non-homologously. Between the two phases synaptonemal complexes in trivalents are rearranged to yield homologically paired bivalents and univalents. The first phase or chromosome pairing and synaptonemal complex formation occurs during zygotene and is identical with the pairing in normal diploid organisms. The next phase takes place during early pachytene and corresponds to the non-homologous pairing observed

Figure 17b

in haploids. Analysis of four diploid nuclei in late zygotene (28, 29, and unpublished results) indicates that the chromosome pairing in nuclei with the diploid chromosome number followed the same scheme. In one of these nuclei, 2 homologous chromosomes were unpaired and were located in separate regions in the nucleus whereas in a second, 2 pairs of homologous chromosomes were completely unpaired. In both nuclei, synaptonemal complex formation was complete in the majority of the bivalents. In the two triploid nuclei at early pachytene, 28

homologous associations were revealed, whereas in 8 of the remaining 9 nuclei analyzed, the number of bivalents is less than the expected 28 for those nuclei with 84 chromosomes (nuclei 3, 4, 6, 7) and less than 31 for those with 87 (nuclei 8, 9, 11). The pairing of the remaining homologues could have been prevented either by disturbance of the correction of pairing, by the presence of the extra set of chromosomes or as a result of a failure of primary recognition during early zygotene due to the large number of chromosomes in the triploid.

Figure 17c

It is likely that the two diploid zygotene nuclei revealed the latter situation. In a diploid *Bombyx* female, the second round of chromosome pairing with synaptonemal complex formation ensures the completion of the pairing into bivalents, whereas in triploids, the presence of an extra set of chromosomes disturbs the homologous pairing by competing with the homologues in the nonspecific pachytene pairing.

3.6 Variation in chromosome length during pachytene

Two chromosomes can be identified at

pachytene in *Bombyx mori* by morphological markers. Chromosome 1 is identified by a knob which is readily recognized during pachytene. The knob divides the chromosome into two segments with an average ratio of 1:1.8 (Tables VI and VIa). In most nuclei, the synaptonemal complex or lateral component passed uninterrupted through the knob. It was difficult to determine whether the synaptonemal complex or lateral component was continuous through the knob in a few cases because the synaptic structures were obscured by the material of the knob. It is assumed in the calculation of chromosome lengths that the synaptonemal com-

Table VI

Length (in μm) and segment ratio for chromosomes 1 and 2. Chromosome 1 is divided into two segments by a knob, chromosome 2 by the nucleolus organizer region.

Nucleus number	Chromosome 1				Chromosome 2 ^{a)}				Sum of all lateral components
		Absolute length	Relative length	Segment ratio		Absolute length	Relative length	Segment ratio	
1	lc	6.1 ^{c)}	1.6	2.0	lc	5.9	1.5	1.6	391
	lc	6.7	1.7	1.5					
	lc	7.6					1.6	1.8	
2	lc	7.2 ^{c)}	1.9	2.1	lc	6.2 ^{c)}	1.6	1.1	381
	lc	7.8	2.1	2.1	lc	6.1	1.6	1.2	
	lc	7.3	1.9	2.2	lc	6.1	1.6	1.8	
3	lc	7.9	2.0	1.6	lc	8.3			470
	sc	7.9	2.0	1.8	sc	8.5	2.1	1.8	
4	lc	8.1 ^{c)}	1.7	1.5	lc	9.6	2.1	2.4	
	lc	9.0	1.9	1.7					
	lc	9.2	2.0	1.5	sc	8.7	1.9	2.2	
5	lc	9.6	1.8	2.0	lc	8.9	1.7	2.4	519
	sc	10.7	2.1	1.8	sc	8.3	1.6	2.1	
6	lc	10.6	1.9	1.8	lc	8.7	1.5	1.7	569
	sc	11.3	2.0	2.1	sc	9.0	1.6	1.8	
7	lc	10.1	2.1	1.9	lc	8.1	1.7	1.8	487
	sc	8.5	1.8	1.7	sc	8.9	1.8	2.0	
8	lc	7.9	1.6	1.6	lc	9.7 ^{b)}	2.0	1.2	488
	sc	9.5	1.9	1.9	sc	7.5	1.5	2.3	
9	lc	8.0	1.6	1.8	lc	7.4	1.4	2.1	515
	sc	8.9	1.7	2.0	sc	7.5	1.5	2.3	
10	lc	9.4	1.9	1.7	lc	10.0	2.0	1.4	498
	sc	8.9	1.8	2.0	sc	8.2	1.7	2.0	
11	lc	9.3	1.8 ^{b)}	1.8	lc	7.1 ^{c)}	1.4	1.7	519
	sc	9.7	1.9	1.6	sc	8.9	1.7	2.3	

a) the synaptonemal complex or lateral component of chromosome 2 is in most cases discontinuous in the nucleolus organizer region. The length is combined from the lengths of the two segments.

b) non-homologously paired with a different chromosome.

c) trivalent.

Table VIa.

Comparison of mean length and segment ratio of chromosomes 1 and 2 between nuclei 1-4 and 5-11.

	Mean length and standard deviation		t-test	Mean segment ratio and standard deviation		t-test
	Nuclei 1-4	Nuclei 5-11		Nuclei 1-4	Nuclei 5-11	
Lateral component chromosome 1	7.7 ± 1.0	9.3 ± 1.0	$P < 0.001$	1.8 ± 0.3	1.8 ± 0.1	$P > 0.90$
Lateral component chromosome 2	7.1 ± 1.6	8.5 ± 1.1	$P < 0.001$	1.5 ± 0.5	1.7 ± 0.4	$P > 0.40$
Synaptonemal complex chromosome 1	$7.9 - a)$	9.6 ± 1.0	$0.25 > P > 0.20^{b)}$	$1.8 - a)$	1.9 ± 0.2	$P > 0.70$
Synaptonemal complex chromosome 2	7.9 ± 1.3	8.3 ± 0.6	$0.50 > P > 0.40^{b)}$	1.9 ± 0.3	2.1 ± 0.2	$P > 0.20$

a) only one observation

b) the low number of observations in nuclei 1-4 possibly accounts for the lack of significance.

plexes and lateral components pass straight through the knob. The other chromosome which can be identified unambiguously was chromosome 2 in which the nucleolus organizer region divided the chromosome into two segments with an average ratio of 1:1.8. The synaptonemal complex and the lateral component of chromosome 2 were interrupted at the site of nucleolus attachment in all nuclei analyzed; the distance between the two arms was increasingly larger during pachytene. The interruption of the synaptic structures inside the nucleolus was not included in the calculation of chromosome length.

Table VI shows the lengths of the synaptonemal complex and the lateral component, the segment ratios for chromosomes 1 and 2, and the sum of all paired and unpaired lateral components for each of the 11 nuclei. It appears from Tables VI and VIa that the total length of the lateral components increased from $411 \pm 41 \mu\text{m}$ (average of nuclei 1-4) at the beginning of pachytene to $514 \pm 28 \mu\text{m}$ (average of nuclei 5-11) towards the end of pachytene.

The total lengths of all lateral components differed considerably in the 11 nuclei (Table V and Va); nucleus 6 had the greatest total length of lateral components, being about 50% greater

than that of nucleus 1. It is uncertain whether the 25% increase in mean lateral component length from early to mid pachytene is due to the presence of an extra genome or is a normal feature of development. A less drastic rise in synaptonemal complex length was observed in 4 zygotene and 6 pachytene nuclei from diploid females previously analyzed. The average diploid zygotene length was $195 \pm 8 \mu\text{m}$ and the pachytene length $212 \pm 7 \mu\text{m}$ (28, 29 and unpublished results). A comparison of the two means with a t-test gave a P value between 0.1 and 0.05. The difference is thus not significant.

The data in Table VI also show for some of the nuclei a rough relationship between the length of the synaptonemal complex or the lateral component of chromosomes 1 and 2 and the sum of all lateral components (including those which are paired into synaptonemal complexes). If the total lengths of all lateral components were small, the lengths of the synaptonemal complexes and lateral components of chromosomes 1 and 2 were also small. Compare for example the lengths of chromosomes 1 and 2 in nuclei 1 and 2 with nucleus 6. In several cases though, the opposite situation was encountered: Nucleus 3 has a total lateral component length of $401 \mu\text{m}$ and a lateral component

length for chromosomes 1 and 2 of 7.9 μm and 8.3 μm respectively, whereas the total lateral component length of nucleus 9 is 516 μm with a lateral component length for chromosomes 1 and 2 of 8.9 μm and 7.5 μm respectively, i.e., the 29% difference in total lateral component length between the two nuclei is not reflected in the lengths of chromosomes 1 and 2.

When the segment ratios for each of the two chromosomes were compared in the 11 nuclei an even greater variation in length of synaptonemal complexes and lateral components was observed. The arm ratio for chromosome 1 varies between 1:1.5 and 1:2.2, and for chromosome 2, between 1:1.1 and 1:2.4 (Table VI). On the other hand, a comparison of the segment ratios between nuclei 1-4 (early to mid pachytene) and nuclei 5-11 (mid to late pachytene) of chromosomes 1 and 2 showed no significant differences between the segment ratios of univalents and bivalents (Table VIa).

As indicated by the data in Table IV, the synaptonemal complex length of a bivalent was generally longer than the lateral component length of its homologous univalent, the ratio of synaptonemal complex length to lateral component length being 1.1 ± 0.1 in nuclei 1 and 2. This suggests that lateral components are stretched as a result of their pairing into synaptonemal complexes.

4. DISCUSSION

4.1 Determination of a temporal sequence in nuclear development

The transition from zygotene to pachytene is characterized by the chromosome bouquet, the association of the three homologues, and bivalents or bivalent segments with completed synaptonemal complexes. At early pachytene the bouquet is dissolved and the third homologue separates from its bivalent. At mid to late pachytene the telomeres detach from the nuclear envelope and, towards the end of the stage, the chromosomes are uniformly distributed in the nucleoplasm (28). This study examined 11 nuclei during this period of meiotic prophase. Some temporal changes are thus seen in greater detail, such as variations in the number and position of nuclear vacuoles, the morphology and

spatial arrangement of chromosomes, and the pairing of the lateral components into synaptonemal complexes. The changes in these nuclear components are synchronous only to a limited extent in a nucleus.

4.2 Specificity of chromosome pairing

Generally, studies on chromosome pairing in a wide range of normal diploid organisms provide evidence in favour of a high degree of regularity in the pairing of homologous chromosomes into bivalents with the aid of the synaptonemal complex. (See WESTERGAARD and von WETTSTEIN, 54, GILLIES, 9 for general reviews.) Data based on complete serial reconstructions are available in *Zea mays* (7), yeast (2), *Neurospora* (6), *Ascaris* (12), *Bombyx* (28), and *Sordaria* (56) among others. The expected number of bivalents with synaptonemal complexes spanning the distance between the attachment sites on the nuclear envelope is regularly found thus indicating a perfect homologous pairing. Several cases are known however, where extensive chromosome pairing with synaptonemal complexes occurs between apparently non-homologous chromosome segments. Synaptonemal complexes are readily observed in haploid maize (46, 47), barley (8), *Petunia* and *Antirrhinum* (36), and tomato (18). In haploid barley, up to 60% of the lateral component length is paired into synaptonemal complexes, which are morphologically indistinguishable from complexes between homologous chromosomes (8). In hybrids between tomato and *Solanum*, synaptonemal complexes are formed between homoeologous chromosomes, the pairing considered to be primarily non-homologous (18). GILLIES (7, 10) also described in *Zea mays* a possible non-homologous foldback pairing in nuclei heterozygous for an abnormal chromosome 10, K10. (K10 has an extra chromosome segment attached to the long arm of the normal chromosome 10.). Furthermore, the nature of the pairing in reconstructed nuclei heterozygous for an inversion on chromosome 3 is dependent on whether the abnormal chromosome 10 is present or not. The inverted segment is frequently unpaired in the absence of K10, whereas in the presence of K10 the inverted segment is paired either into a typical inversion

loop, or non-homologously paired with its synaptonemal complex continuous with complexes of the adjacent chromosome segments. The latter two situations were found with equal frequency. A similar situation was reported by SOLARI (38) in mice carrying Searle's translocation, the pairing being primarily homologous in the translocated region giving rise to a quadrivalent (with two autosomes, the X chromosome, and Y chromosome). In 50% of the nuclei analyzed, however, a segment of the X chromosome formed a synaptonemal complex with a segment of one of the autosomes.

It thus appears that intimate chromosome pairing mediated by the synaptonemal complex occurs preferentially between homologous chromosome segments; but in the absence of a homologous pairing partner, or if the homologous matching is impeded by structural rearrangements of one or more chromosomes, the synaptonemal complex is also formed between non-homologous chromosome segments.

The distinction between homologous and non-homologous pairing is particularly clear in triploid organisms, where homologous and non-homologous pairing can be compared in the same nucleus. Previous work on triploid *Lilium* (22, 24), *Allium* (40) and chickens (4) has revealed two characteristics of pairing in triploids which may also be relevant to chromosome pairing in normal diploids: The association of the three homologues with a synaptonemal complex between two of the three lateral components at any place along the chromosome and the short stretches of "double" synaptonemal complex which were observed near the attachment sites of the lateral complexes on the nuclear envelope in triploid chickens (4). Chromosome pairing in triploid females of *Drosophila* constitutes an exception, as lateral components were only present in the two chromosomes paired by synaptonemal complexes and absent in the third homologue (19).

At the start of the transition from zygotene to pachytene in triploid *Bombyx* females, the majority of chromosomes are roughly paired in sets of three, two of the chromosomes having a complete synaptonemal complex and the third lying parallel at some distance. The presence of the double complex near the nuclear envelope

in *Bombyx* shows that a second central region can temporarily attach to a normal complex in a lateral position and combine with the third lateral component. This provides additional evidence that synaptonemal complex formation in *Bombyx* starts exclusively from the attachment sites on the nuclear envelope. These observations are in agreement with earlier reports (4, 40). Zygotene pairing is initiated by the alignment of homologues and requires that the chromosome segments to be paired should be homologous. This first pairing phase thus constitutes the main period for homologous chromosome pairing in diploid organisms and includes cases in which rearranged chromosomes pair homologously (e.g., heterozygous inversions in maize, GILLIES, 7, 10). A complete serial reconstruction of the very long chromosome in an early zygotene nucleus of *Lilium*, with about 25% of the lateral components combined into synaptonemal complexes, illustrates the preponderance of homologous chromosome pairing during zygotene. Only very short stretches that might possibly be non-homologously paired chromosome segments were found (15).

The remarkable decrease in the number of trivalents after the start of pachytene in triploid *Bombyx*, together with the observation that the short stretches of double complex disappear in regions near the nuclear envelope, provides support for the previously postulated mechanism (28) for correction of incompletely paired chromosomes. The conversion of trivalents into bivalents and univalents requires the dissolution of the synaptonemal complex and its reformation between two different lateral components.

After the attraction of homologous chromosomes ceases, the remaining non-homologous chromosome segments form synaptonemal complexes. The extent of this second non-homologous pairing depends upon the degree of synaptonemal complex formation which has been achieved by the preceding homologous pairing.

In haploid nuclei chromosome pairing is of the second, non-homologous type, and probably dependent in extent on the proximity of two initiation points for complex formation, one on each of the two lateral components. In hybrids

where the two sets of chromosomes have approximately the same length and contain sizeable homologous segments, the pairing could be as complete as in normal diploids (18). Pairing can be initiated with the homologous segments and completed by the unspecific non-homologous formation of complexes.

Further experiments are required before the differences in behaviour of the meiotic chromosomes between triploid *Solenobia* and triploid *Bombyx* can be explained (see SEILER, 35, ASTAUROV, 1, VEREISKAYA, 49, and KAWAGUCHI, cited from TAZIMA, 45). The observation that in triploid *Solenobia* the three homologues remain associated until anaphase I (35) suggests a temporal sequence of events in zygotene and pachytene different from that observed in triploid *Bombyx* (45). It is likely that a modification of synaptonemal complexes similar to that of female *Bombyx* (28) occurs in *Solenobia*. In triploid *Solenobia*, the modification may take place at a time when the three homologues are associated and thereby preserves the association of homologues until "chromatin elimination" and disjunction occur in anaphase I. Predominantly female intersexes of triploid *Solenobia* are able to produce eggs which can develop parthenogenetically by a fusion of the nucleus resulting from the second polar nucleus with the daughter nucleus of the first polar nucleus ("Richtungskopulationskern") whereby the triploid chromosome number is re-established.

A different way of preserving the triploid chromosome number is observed in parthenogenetic *Otiorrhynchus* (34), where the first meiotic division is rudimentary and the triploid female thus fully fertile. Also the sex differences in fertility of male and female tetraploid *Bombyx* need further clarification. Especially the determination of the exact time of crossing over is essential for understanding the reported differences in the number of chromosome elements at metaphase I (56 elements in females and less than 56 in males) (50). It is possible that crossing over in tetraploid *Bombyx* males protects the synaptonemal complex from being corrected at early zygotene or alternatively, that the aberrant chromosome associations observed at metaphase I are formed in the second

round of pairing and subsequently preserved until metaphase I by crossing over and chiasma formation.

On the basis of cytological and genetic studies of chromosome pairing in hexaploid *Triticum*, RILEY and CHAPMAN (31), RILEY et al. (32), MELLO-SAMPAYO (17), and FELDMAN (5) concluded that the amount of homoeologous pairing between genetically equivalent chromosomes from different species is inhibited by one or several genes located on the long arm of chromosome 5B. Later, MELLO-SAMPAYO (17) demonstrated that a gene with a similar effect was present on chromosome 3D and arrived at the same conclusion as RILEY and FELDMAN, namely that the primary function of these genes is to inhibit non-homologous pairing. In the absence of the short arm of chromosome 5B, a decrease in synapsis is accompanied by an increase in homoeological pairing and chromosome interlockings. In the light of the results presented in this report, it is conceivable that instead of an inhibition of non-homologous (homoeological) pairing, the genes on chromosome 5B may promote homologous chromosome pairing during zygotene, so that at the onset of pachytene, only a few or no unpaired chromosomes remain. Alternatively, the genes on chromosome 5B may extend the requirement for homology of the chromosomes to be paired into the second pairing phase. The result in both cases is increased homologous pairing. The increase in non-homologous pairing and in the number of chromosome interlockings observed in the absence of the short arm of chromosome 5B (5), may be explained as the result of a deficient system for correction of chromosome pairing.

The present results may also be relevant to the interpretation of the two types of chromosome pairing in *Drosophila* which have been demonstrated by GRELL (13, 14) on the basis of chromosome disjunction in situations where extra chromosomes or chromosome segments are present. The "exchange" pairing which is suggested to involve homologous chromosomes only and the subsequent "distributive" pairing which may involve homologous as well as non-homologous chromosome segments, might thus be a reflection of the two phases (the homolo-

gous and non-homolous phases) of chromosome pairing and of synaptonemal complex formation as described in the present report.

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