THE STRANDEDNESS OF MEIOTIC CHROMOSOMES FROM *ONCOPELTUS*

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ABSTRACT

Meiotic chromosomes were isolated from male *Oncopeltus fasciatus* by dissecting the testes under insect Ringer's solution and spreading the living cells on the Langmuir trough. After being dried by the critical point method, preparations were examined under the electron microscope. Chromosomes at all stages of prophase prove to be multistranded. A significant increase in the number of parallel 250 A fibers in the chromosomes occurs between zygotene and diakinesis. Parallel folding, rather than true multistrandedness, is interpreted as the mechanism responsible for this observed increase in multistrandedness. It has not been possible to determine whether the multistrandedness observed at leptotene represents true multistrandedness or is the result of parallel folding. Apparent multistrandedness is lost at metaphase when the 250 A fibers of the chromosomes become coiled more tightly. In preparations isolated by these methods, no structures other than the 250 A chromosome fibers are visible in the chromomeres, which appear as regionally coiled or folded areas of the fibers along the arm of the chromosome.

INTRODUCTION

Recent investigations have shown (34) that the preservation of meiotic chromosome structure obtained by the Langmuir trough–critical point method (13, 17) is good enough to permit direct identification of the stages of meiosis under the electron microscope. In the organism used for these studies, *Oncopeltus fasciatus*, the milkweed bug, it has been possible not only to assign a nucleus to prophase I, metaphase I and II, or telophase I and II (prophase II does not occur in *Oncopeltus*), but also to identify the substages of meiotic prophase. *Oncopeltus* has proved to be peculiarly suited to the isolation method used, allowing clean separation of chromosomes in all meiotic stages.

As noted in a number of previous reports (11, 12, 24, 32, 33) chromosomes prove to be made up of fibers averaging approximately 250 A in diameter when isolated by this technique, loosely coiled and extended at interphase and more or less tightly coiled or folded at metaphase. The quality of preservation of meiotic nuclei from *Oncopeltus* permits a more detailed investigation of the arrangement of the 250 A fibers as meiotic prophase proceeds. In the present study, particular attention has been given to the process of chromosome condensation in prophase, in an effort to work out the "strandedness" of the chromosome, and the structure of the chromomere.

MATERIALS AND METHODS

The testes of a last instar male milkweed bug (*Oncopeltus fasciatus*) were dissected under insect Ringer's solution and transferred to a clean glass slide, in a drop of fresh Ringer's solution. The drop was drawn off and replaced several times to remove contaminating oil droplets from the fat tissues of the insect. Because the follicles of the testes are arranged in parallel they can be cut transversely to remove the

region in which mature spermatozoa occur. This region appears as a glistening, silvery mass at the ends of the testicular follicles toward the vas deferens. The part of the testis containing mature sperm was excised and discarded, and the remainder rinsed again several times to remove any free spermatozoa. This was done because spermatozoa spread more rapidly on the Langmuir trough than cells at earlier stages of meiosis; if these are not removed very few earlier stages can be obtained.

Most of the liquid surrounding the testes was then drawn off and the testes ground lightly between a second glass slide and the first. Both slides were dipped through the surface of a Langmuir trough containing distilled water. The resulting film was compressed until surface wrinkles were just visible in reflected light. The compressed film was picked up by touching a carbon-Formvar coated grid to the surface and "fixed-stained" by floating over 2% uranyl acetate for 8 to 10 min.

Grids were then loaded into a plastic carrier under 70% alcohol, dehydrated, and transferred to amyl acetate according to the following schedule:

70% ethyl alcohol: 1 min

95% ethyl alcohol: 1 min

100% ethyl alcohol: two 5 min, one 10 min change

100% amyl acetate: two 5 min, one 10 min change.

Grids were then transferred to a pressure vessel and dried by the critical point method (1). Grids were examined under a Hitachi HU 11A electron microscope. For this report contrast reversals of the electron microscope plates used for Figs. 1 to 4 and 8 were made on Kodak Panatomic X to provide a finished print in which fibers appear light against a dark background. This technique, similar to the usual procedure for shadowed preparations, provides a print in which the individual fibers are more easily distinguished.

RESULTS

The aggregation of the 250 A chromosome fibers into strands is first apparent as the spermatogonial interphase passes into leptotene (Figs. 1 and 2). The aggregation of strands of 250 A fibers, not visible in isolated premeiotic interphase nuclei, was taken as the onset of leptotene in this study (34). At this time, the sex chromosomes are clearly visible as two bodies within the nucleus in which the 250 A fibers are tightly coiled or folded. In favorable micrographs, the sex chromosomes can be seen to be double at this stage (34). In early leptotene nuclei the first strands to appear are composed of a number of 250 A fibers lying more or less parallel to each other. The surrounding areas of the nucleus contain 250 A fibers with no apparent order (Fig. 2). Early prophase I stages contain lateral 250 A fibers which lie in regions distant from the main axial complexes (34). As condensation of the strands progresses and synapsis occurs, fewer fibers are seen in the nuclear areas surrounding the strands (Figs. 3 and 4.) In many regions of the nucleus the 250 A fibers can be seen to lie parallel to each other for distances of up to several microns (Fig. 5).

In many regions, the parallel fibers of the axial arrays are dispersed sufficiently so that an approximate count can be made. It must be emphasized that the number counted is an approximation at best, because in even the most favorable micrographs the 250 A fibers are superimposed or distorted to an extent. The histograms in Figs. 6 and 7 summarize counts of 37 presynaptic chromosomes and 40 postsynaptic bivalents from 32 nuclei.

From Fig. 6 it appears that there is a lower limit of twelve fibers in the chromosome at the time when organized strands are first visible at leptotene. The cut off observed may represent one of two possibilities. Either strands with less than twelve fibers are not recognizable against the background of unorganized fibers at early leptotene, or the chromosome contains at least twelve fibers at the time it first condenses from spermatogonial interphase. A probit transformation of the data in Fig. 6 would thus seem appropriate in order to determine the mean and variance on a normal distribution. This then allows the application of a t test between the presynaptic and postsynaptic (zygotene, Fig. 7) data. Since the number of fibers is expected to double at synapsis, the actual number counted across both strands of the synapsed homologues provides a method for evaluating the accuracy of the counts obtained. Such a t test, having doubled the presynaptic scores, shows the means not to differ significantly ($t_{(49)} = 0.0281$; $P = 0.9$). Even if untransformed data are used ($t_{(49)} = 1.452$; $P =$ 0.2 to 0.1) there is no significant difference. There is no reason to doubt, therefore, that the strand number doubles between presynaptic chromosomes and postsynaptic bivalents.

A further increase in strandedness is noted after synapsis (Fig. 7). The means of zygotene and diakinesis are significantly different ($t_{(21)} = 5.896$; $P = 0.001$). Counts across chromosomes at zygotene, pachytene, and diakinesis were made across

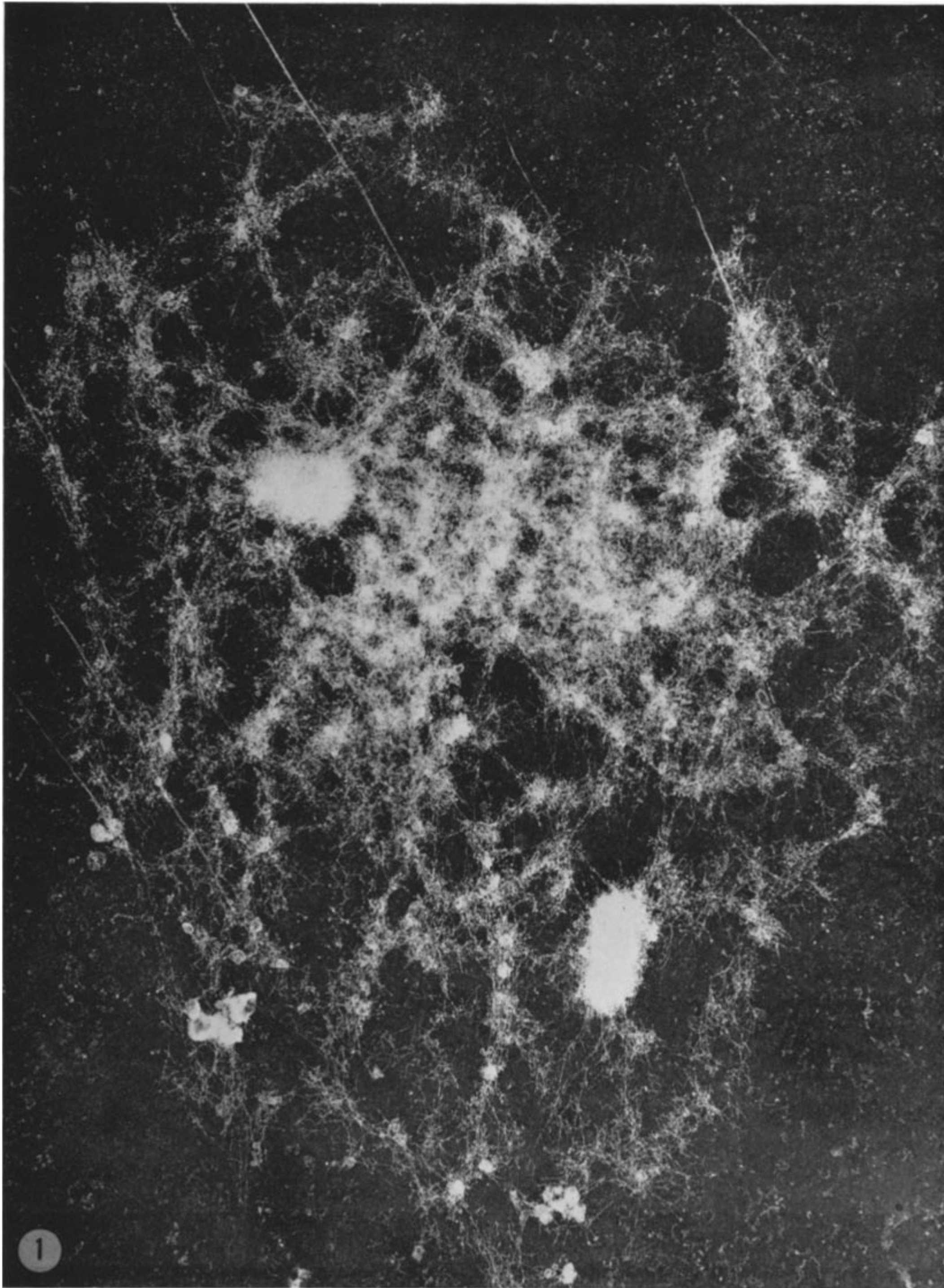

FIGURE 1 Early leptotene nucleus isolated from male *Oncopeltus* by the Langmuir trough-critical point method. Contrast has been reversed in this figure. Aggregation of 250 A chromosome fibers into strands is just visible. The sex chromosomes appear as clumps of heterochromatin. × 7600.

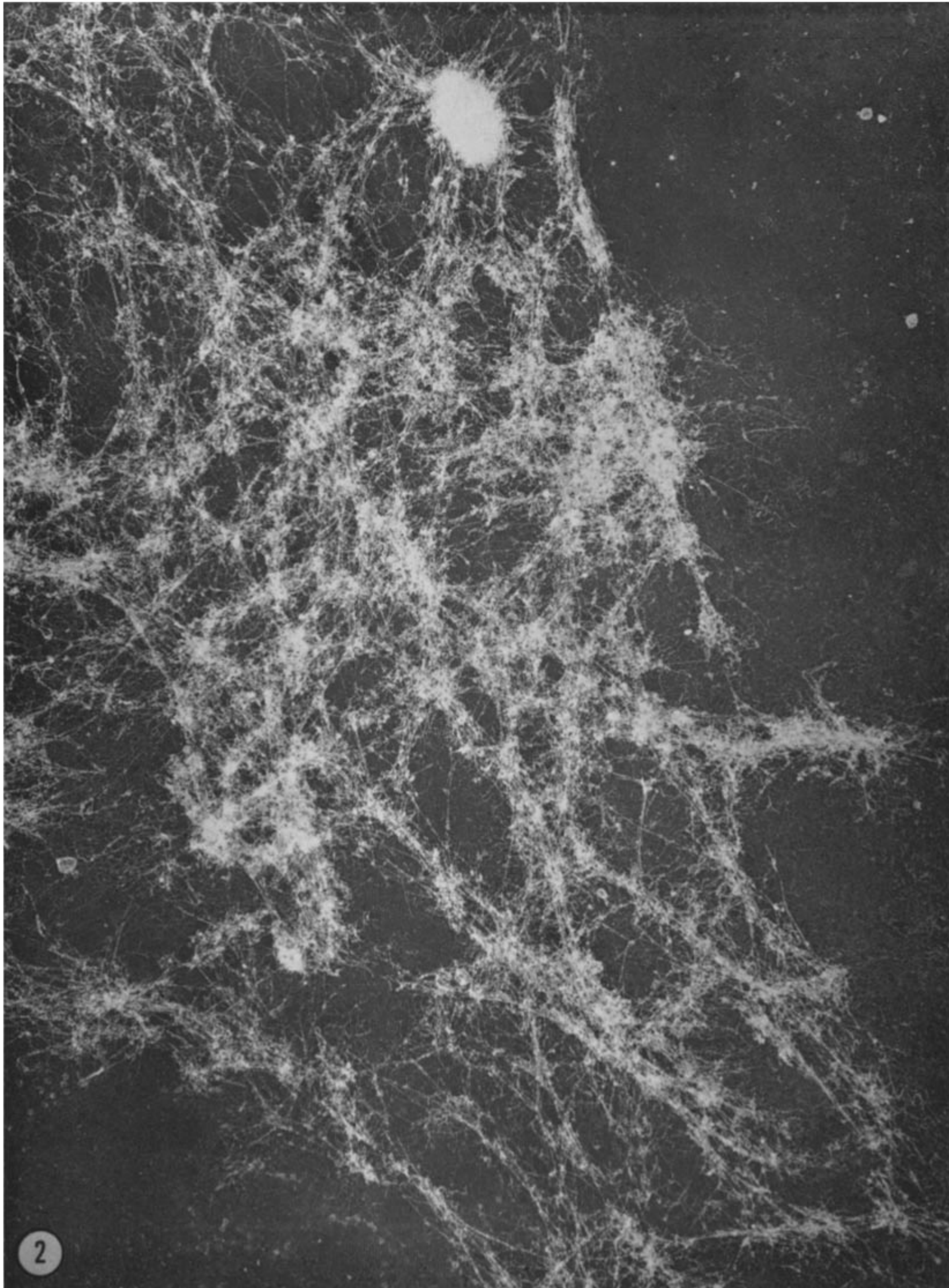

FIGURE 2 Leptotene nucleus in which the aggregation or condensation of 250 A fibers into chromosome strands is more advanced than in Fig. 1. Only 1 sex chromosome of the X-Y pair is visible in this micrograph. × 5000.

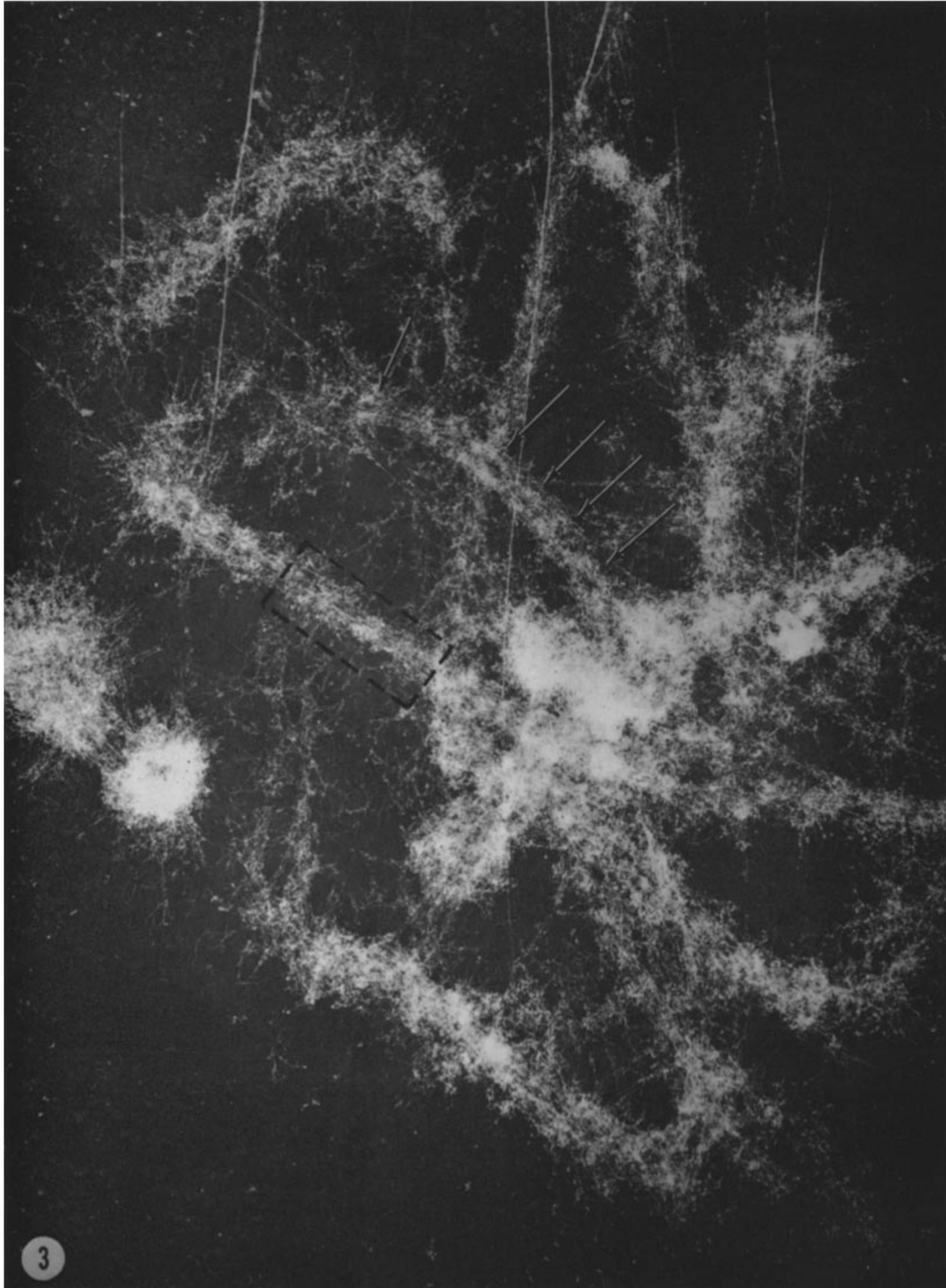

FIGURE 3 Zygotene nucleus. Synapsis of the homologues has occurred. Differentiation of the coiling pattern into chromomeres (arrows) is visible in some regions. The area enclosed by dotted lines appears at a higher magnification in Fig. 5 *c* with contrast reversed. × 9400.

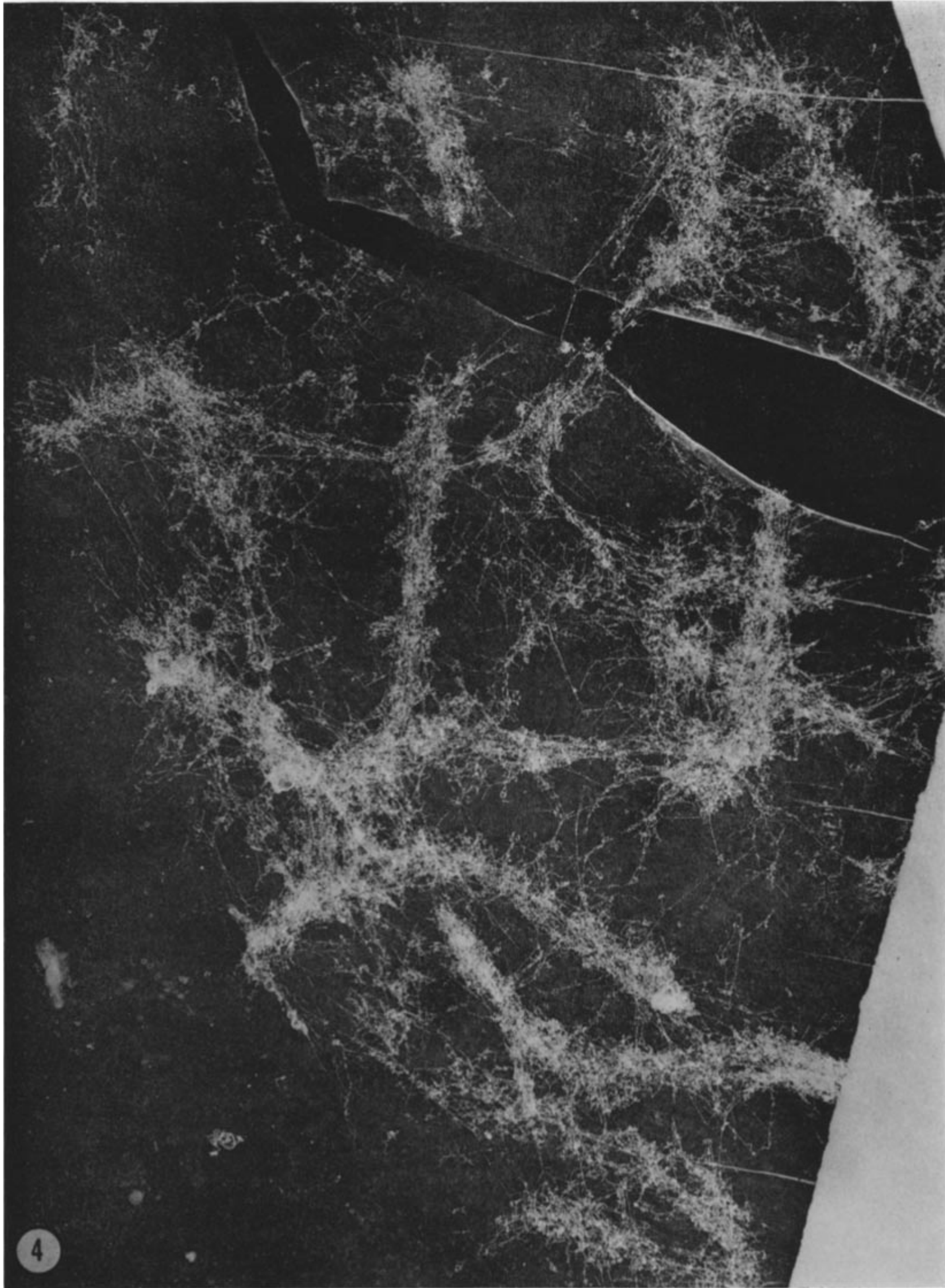

FIGURE 4 Pachytene nucleus. Aggregation of fibers from peripheral strands is nearly complete. × 7800.

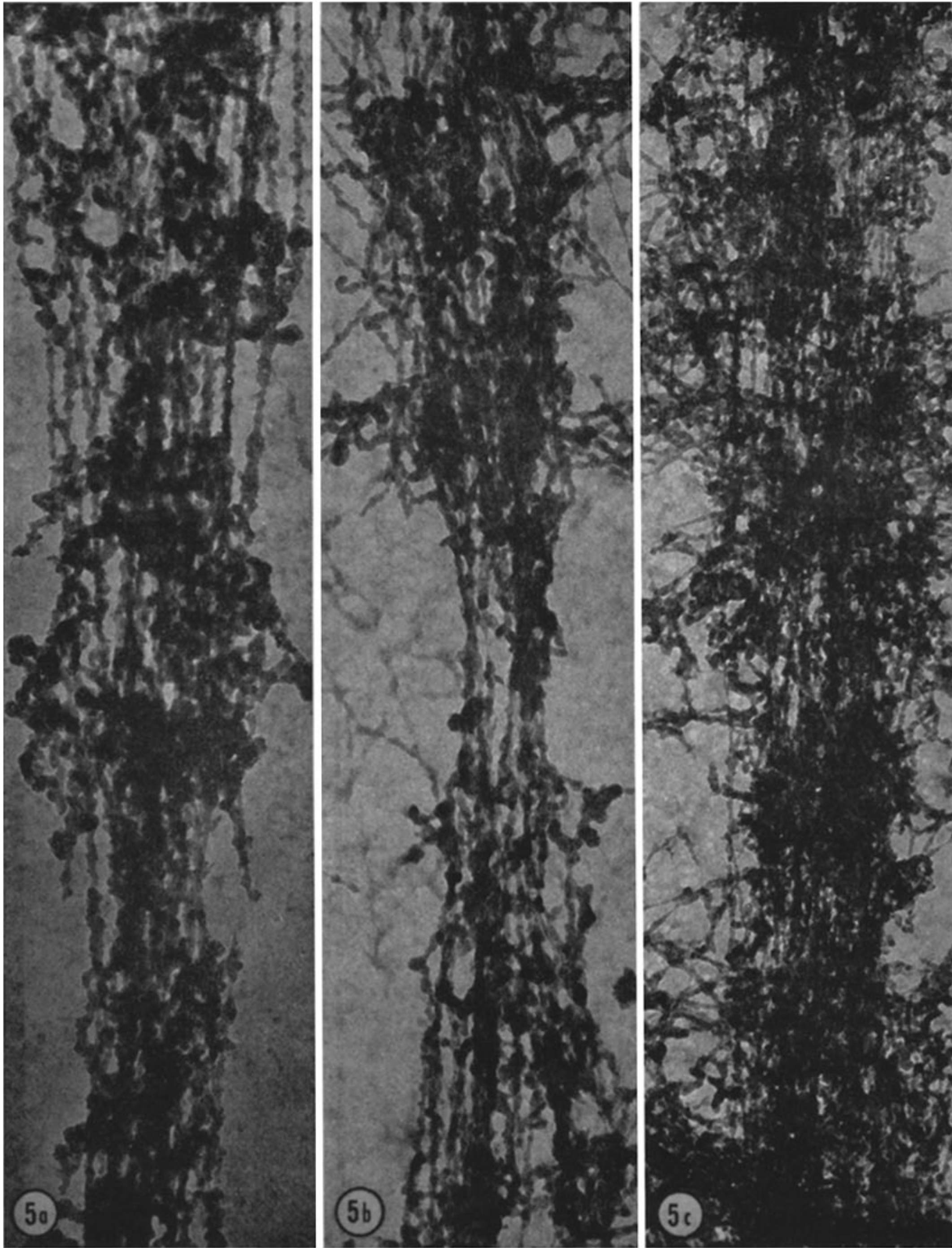

FIGURE 5 Unsynapsed chromosomes are shown in Fig. 5 *a* and *b*; synapsed homologues in Fig. 5 *c*. The chromosome pair in *c* is a part of the nucleus shown in Fig. 3. Fig. 5 *a*, × 79,000; Fig, 5 *b* × 70,000; Fig. 5 *c* × 54,000.

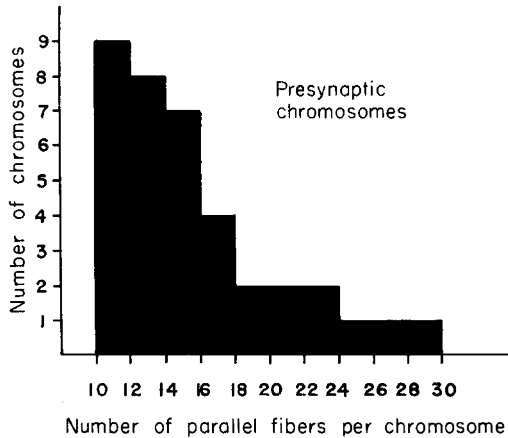

FIGURE 6 Histogram summarizing counts of parallel fibers in 37 presynaptic chromosomes from *Oncopeltus*.

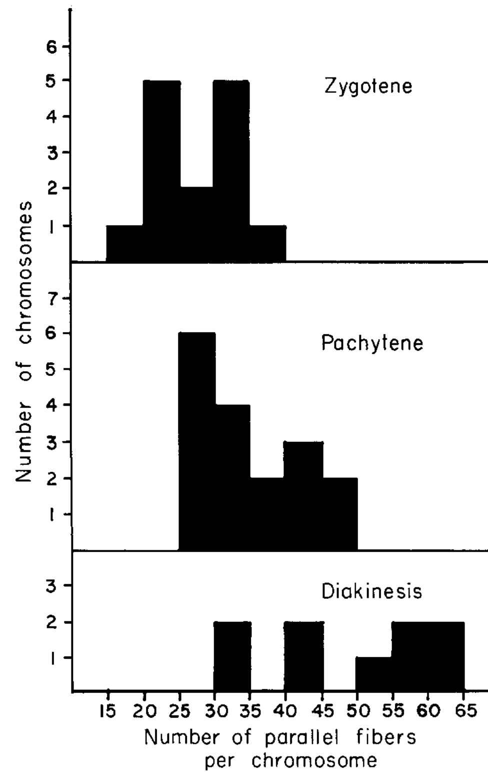

FIGURE 7 Histogram summarizing counts of parallel fibers in 40 postsynaptic bivalents from *Oncopeltus*.

both homologues at paired regions. In *Oncopeltus*, parallel organization of the chromosome fibers can still be distinguished at diakinesis (34). The data available for diakinesis are limited because this stage is obtained less frequently in the isolated preparations. Diplotene is represented by a "diffuse" stage in *Oncopeltus* (30, 34), in which the chromosomes uncoil into a state resembling interphase. Consequently, no counts are available for this stage of prophase. The variation in number of strands along the length of one chromosome arm in each stage would be of significant interest for this study. However, it has not yet been possible to obtain a sufficient number of nuclei with arms that can be counted in more than one region. The shortening of chromosomes into the metaphase condition after diakinesis causes a disappearance of the parallel orientation of the 250 A fibers. At this stage, chromosomes appear as masses of irregularly coiled or folded fibers (34). Indeed, this type of irregular folding is typical of chromosomes of both meiotic metaphases and mitotic metaphase as well (Fig. 8).

The synapsed homologues at pachytene show a pattern of coiling or condensation of the 250 A fibers in certain regions which would be described as chromomeres in the light microscope (Figs. 3 and 4). However, the paired nature of these regions is indistinct in micrographs taken under the electron microscope in which the 250 A fibers are clearly resolved. The gross pattern of chromomeres is much more readily visible on the fluorescent screen of the electron microscope in an underfocus image than in a micrograph close to focus, in which contrast is much reduced. In isolated preparations, no structures other than 250 A fibers can be observed in the chromomeres. Thus, differentiation of the chromosome arm into chromomeres appears to depend upon regionally coiled or folded areas of fibers along the arm of the chromosome, as originally proposed by Ris (21).

DISCUSSION

It is not possible to determine the degree to which the spreading forces of the trough distort the structure of chromosomes. However, when direct comparisons are made between classical light microscope preparations and chromosomes isolated by the trough technique, the degree of correspondence is very precise (34), a fact which indicates that severe distortion of the chromosomes is not caused by the spreading forces of the trough. The existence of fibers in areas of the nucleus peripheral to the axial complexes in early

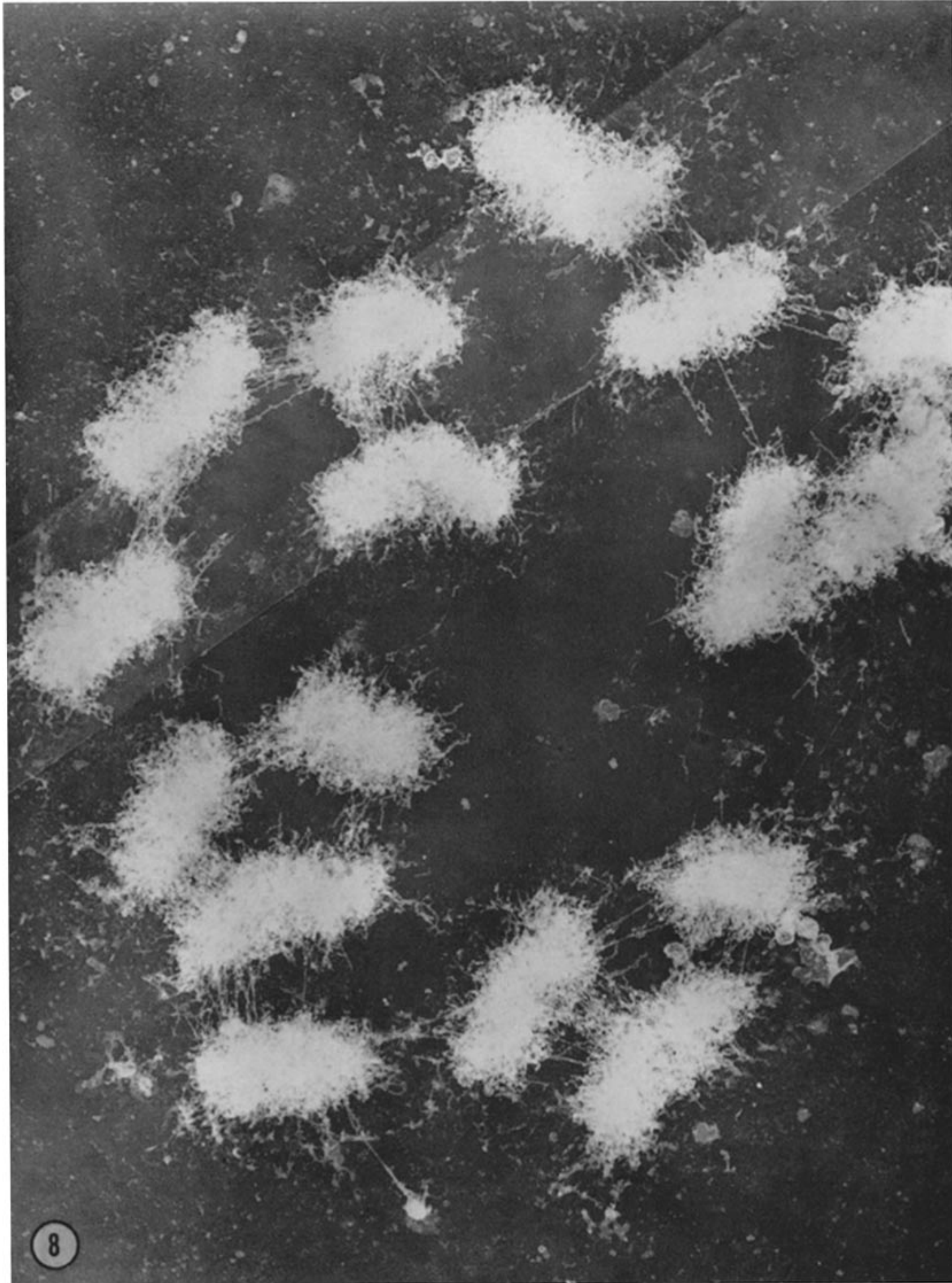

FIGURE 8 Mitotic metaphase from *Oncopeltus*. This group, in which all 16 chromosomes appear, shows the coiling pattern typical of chromosomes at metaphase of meiosis I and II or mitosis. × 18,000.

prophase nuclei must be considered in this context. As mentioned earlier, the number of peripheral fibers decreases as the chromosomes become more condensed through prophase. It is hard to imagine that the forces of the trough act differentially on chromosomes at early and late prophase. Therefore the occurrence of fibers in peripheral areas in leptotene nuclei is considered to be a reflection of the basic condensation process rather than a result of the technique.

Reports of the ultrastructure of chromosomes based on isolated preparations have been limited in number, even though attempts to isolate these structures for electron microscopy extend back to at least 1942 (9). It is interesting to note that in many cases in which the techniques used have permitted resolution of the substructure of condensed chromosomes, the chromosome has been described as multistranded. Ris, in particular, has strongly supported this interpretation of the structure of the chromosome (22, 23). On the other hand, the work of MacGregor and Callan (19), demonstrating a single fiber remaining in the side loops of isolated lampbrush chromosomes after digestion with ribonuclease, provides evidence for the existence of a single strand or fiber as the structural axis of the chromosome. In a complementary study Gall (14), analyzing the kinetics of breakage of the loops and axes of lampbrush chromosomes by deoxyribonuclease, reached the same conclusion. Lafontaine and Ris (18), however, have reported that the side loops of this type of chromosome are multistranded in isolated preparations.

Light microscopists have most frequently interpreted the chromosome as multistranded. In their extensive review of the literature of light microscopy dealing with the problem of strandedness, Kaufmann et al. (16) conclude that results from this source eliminate the possibility of a single stranded chromosome. In a more recent electron microscope study (25), in which a report is given of reconstruction of the three-dimensional configuration of mitotic chromosomes from thin sections of *Tradescantia*, Kaufmann and his coworkers report that these chromosomes are multistranded and reaffirm their earlier interpretation of chromosome structure. Trosko and Wolff (28) have also determined the chromosome to be multistranded under the light microscope, after digestion by trypsin, and in their review of the literature similarly point out the preponderance of evidence from light microscopy for the multistranded chromosome. In an interesting study using phase-contrast time lapse photography in living endosperm tissue, Bajer (2) was able to demonstrate division of chromosomes into half-chromatids at anaphase and telophase.

Evidence from several other sources has not settled the question of strandedness to the satisfaction of all investigators. While Taylor (27), in his study of isotope incorporation into replicating chromosomes, assumes that a single-stranded chromosome fits the observed phenomena best, isolabeling, as pointed out by Peacock (20), is best explained if chromosomes are assumed to be multistranded. Chromosome aberrations induced by irradiation present a similar case. The logical and simple explanation for most induced chromosome or chromatid aberrations is based on the assumption that the chromosome is single stranded. But here again a series of subchromatid aberrations exists (10, 26, 31) which support the multistranded hypothesis.

Several reports from microbial genetics have presented very strong evidence for a single-stranded chromosome in organisms with prokaryotic nuclei. Cairns (5), in an analysis of the mass per unit length of bacteriophage DNA, found a ratio which indicated that the DNA is in the form of a single Watson-Crick double helix. Further, Baldwin and Shooter (3), studying the melting curve of hybrid DNA from *E. coli*, demonstrated a pattern expected for DNA in which the subunits are the polynucleotide strands of a single Watson-Crick double helix. However, a number of physical studies of DNA isolated from microorganisms (4, 6–8, 15) have indicated that in *E. coli* and *Pneumococcus* the conserved unit in replication is a Watson-Crick double helix rather than a single nucleotide strand, and that the "interphase" DNA in these organisms is four-stranded in terms of single nucleotide chains.

It is significant that the multistranded hypothesis has met with only limited acceptance, except in the case of the polytene chromosomes of certain dipterans, which have a special configuration which is not directly applicable to cells in general. The inference to be taken from the lack of acceptance is that the cited works which support multistrandedness are faulty in techniques or interpretation, or at best that an alternate explanation is possible. The major difficulty in proposing multistrandedness is the necessity of invoking a mecha-

nism which causes a multiple number of identical DNA-protein molecules to act as a unit in replication, mutation, recombination, and segregation. When compared to the simplicity provided by the single-stranded model, theoretical systems designed to fit multistrandedness to these chromosome functions are complicated and suffer from a complete lack of experimental evidence for any of the systems proposed. Point mutations and crossing over, in particular, are difficult to account for in a multistranded chromosome. Nevertheless, a number of models have been advanced which can account for at least replication (20, 28) and crossing over (29) in a multistranded chromosome.

There is no question that the chromosomes isolated from *Oncopeltus* by the techniques used in this study are multistranded in appearance. It is not possible to rigorously distinguish, however, between two alternative possibilities to account for the observed multistrandedness: true multistrandedness, involving a multiple number of identical DNA molecules with associated protein, or a form of coiling which results in the back-and-forth folding of a single fiber over distances of several microns, giving the appearance of multistrandedness. It should be noted that the latter hypothesis is rejected by Kaufmann et al. (16).

As the bivalents pass from zygotene to pachytene and diakinesis, the strandedness is seen to increase. If breakage and fusion are assumed to account for recombination, aggregation of multiple fibers must be complete at synapsis to permit complete exchanges of chromatids to occur. Thus a further increase in true multistrandedness after zygotene-pachytene would not be expected. The increase observed (Fig. 7) is taken to mean that, at some point after zygotene, parallel folding of fibers does occur in *Oncopeltus* to cause an apparent increase in multistrandedness. The basic question is thus reduced to distinguishing whether or not the observed multistrandedness at leptotene and zygotene is a result of parallel folding. Since parallel folding is inferred as the mechanism accounting for the increase in strandedness after zygotene, it seems reasonable to assume that it operates to produce the multistrandedness observed at leptotene. However, the accumulated evidence for true multistrandedness is at least sufficient to merit serious consideration of this alternate possibility.

Whether or not the multistrandedness observed at zygotene represents true multistrandedness or parallel folding, any model of recombination must take into account this feature of chromosome structure. This arrangement would prevent any close pairing of individual homologous fibers and would require an additional mechanism operating over relatively long distances to permit precise breakage and exchange to occur.

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