# MULTIPLE CORE COMPLEXES IN GRASSHOPPER SPERMATOCYTES AND SPERMATIDS

PETER B. MOENS

From the Department of Biology, York University, Toronto 12, Ontario, Canada

ABSTRACT

At meiotic prophase, the grasshopper *Chorthippus longicornis* has normal synaptinemal complexes inside paired homologous chromosomes. Evidence is presented that short single cores and small multiple core complexes occur inside metaphase I chromosomes. At first anaphase, interphase, and early spermatid stage, large multiple core complexes are located in the cytoplasm. It is speculated that the multiple core complexes have some structural elements in common with the synaptinemal complexes, but that different forms of pairing behavior are exhibited by the different complexes.

## INTRODUCTION

In most plants and animals, paired homologous chromosomes at meiotic prophase have a tripartite axial structure (8). This structure, the synaptinemal complex, has been implicated in the pairing of homologous chromosomes and in genetic and cytological exchange (1,7). The involvement of the complex in genetic exchange was confirmed by studies in *Drosophila melanogaster* (4, 5). The pairing of axial cores of single leptotene chromosomes into a synaptinemal complex of the bivalent was confirmed in *Lilium longiflorum* (6).

A number of insects have aggregates of cores which resemble a stack of synaptinemal complexes and which are referred to as "multiple core complexes." These may occur in association with pachytene bivalents (mosquito oöcytes, 10), or separate from pachytene chromosomes. The latter situation maintains in the diplotene oöcyte nucleus (10), oöcyte nurse cell nucleus (10), pachytene nucleus of spermatocytes, and the spermatid nucleus in *Gryllus campestris* and *G. bimaculatus* (2), the spermatid nucleus of *G. domesticus* (11, 15), spermatocytes and spermatids of *G. Domesticus* (13), the spermatids of *Blaptica dubia* (12), and spermatocytes of *Philaenus spumaris* (3).

The structural similarity of the synaptinemal complexes to the multiple core complexes suggests that a relationship exists between the two. The possibility is the more likely since the two forms occur in the same or similar cells and since they occur at a specific developmental stage. On the other hand, the differences between the synaptinemal complex and the multiple core complex indicates that the relationship cannot be a simple one.

## MATERIALS, METHODS, AND TERMS

Testicular tubules of *Chorthippus longicornis* were fixed in a 2% glutaraldehyde solution in phosphate buffer. The tubules were postfixed in osmium tetroxide and embedded in Epon. Thin sections were stained with uranyl acetate and lead hydroxide.

The tripartite structure found in meiotic prophase bivalents is the *synaptinemal complex* (7). The two densely staining outer ribbons, which are parallel to each other at a distance of about 1000 A, are the *lateral elements*, and the less dense median ribbon is the *central element*. The fine filaments between and perpendicular to the lateral elements are the *transverse filaments*. The dense ribbon is referred to as a *core* where it is not associated with a synaptinemal complex. The periodically banded body which ap-

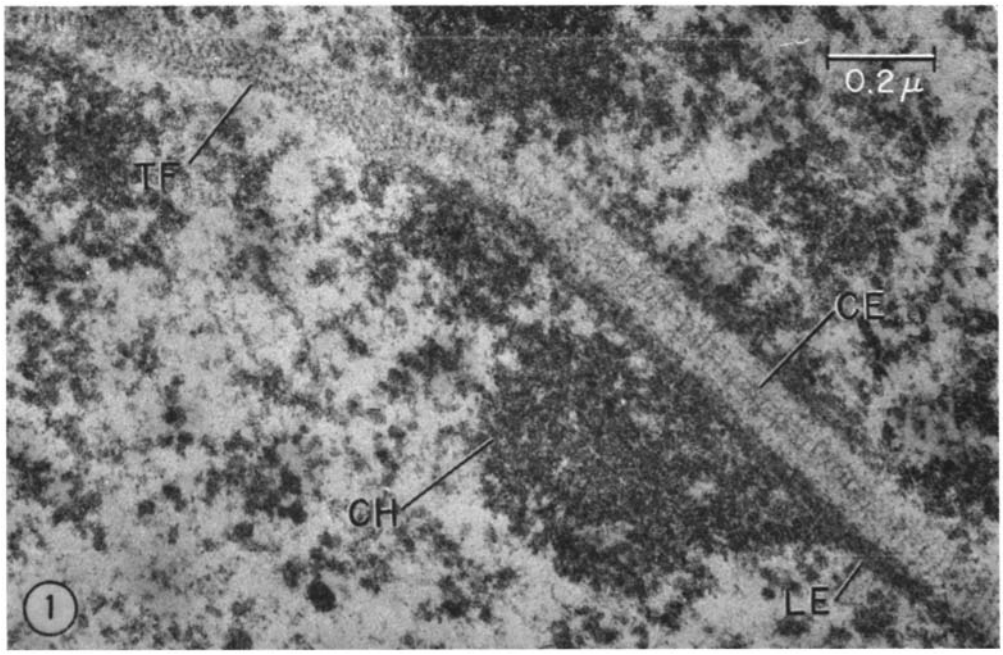


FIGURE 1 The synaptinemal complexes of a bivalent at the pachytene stage of meiotic prophase. The lateral elements (*LE*) are 1000 A apart and between them is the ladder-like central element (*CE*). The transverse filaments (*TF*) which extend from the lateral elements to the central element appear as dots in cross-section where the complex has twisted so that one lateral element is above the plane of section and the other below. The entire structure is surrounded irregularly by chromatin (*CH*). × 70,000.

pears to be an orderly aggregation of several cores is referred to as a *multiple core complex*. The multiple core complexes have structures similar to the transverse filaments and central elements of the synaptinemal complex, and they are referred to as such. The cores of the multiple core complexes prove to be planes of core material, but they are still referred to as cores to avoid the introduction of new terms.

## OBSERVATIONS

The paired homologous chromosomes of cells in the pachytene stage of meiotic prophase contain the usual synaptinemal complexes (Fig. 1). These synaptinemal complexes are similar in most details to the ones described in other insects (2, 3, 10–12). The lateral elements are about 1000 A apart, and the ladder-like central element is located between the lateral elements. The transverse filaments appear as dots in cross section where one lateral element is above the plane of section and the other below (Fig. 1).

At the end of the pachytene stage the synaptinemal complexes lose their axial orientation to the bivalent, their continuity, and their regular structure. Instead, short cores with transverse filaments as shown in the metaphase I chromosome of Fig. 3a are common in postpachytene bivalents. The large bivalent of Fig. 2a has, within the densely stained chromatin, a less dense crescent which contains filaments and some core material. The upper core in Fig. 3a resembles a segment of a lateral element, but it is different in that there are transverse filaments on at least two sides of this core. The lower core of Fig. 3a and the left side core of Fig. 4a′ appear to have filaments radiating in several directions. Periodically arranged agregates of cores were observed at diakinesis and metaphase I (Figs. 2b, 3b). Small aggregates as in Fig. 3b occur regularly. The multiple core complex shown in Fig. 2b is the largest one found in several hundred sections of metaphase I cells. The dense cores are about 1000 A apart, and between them are the central elements and the transverse filaments. The width of each core does not exceed the width of the lateral elements of the synaptinemal complex.

At first anaphase, multiple core complexes were

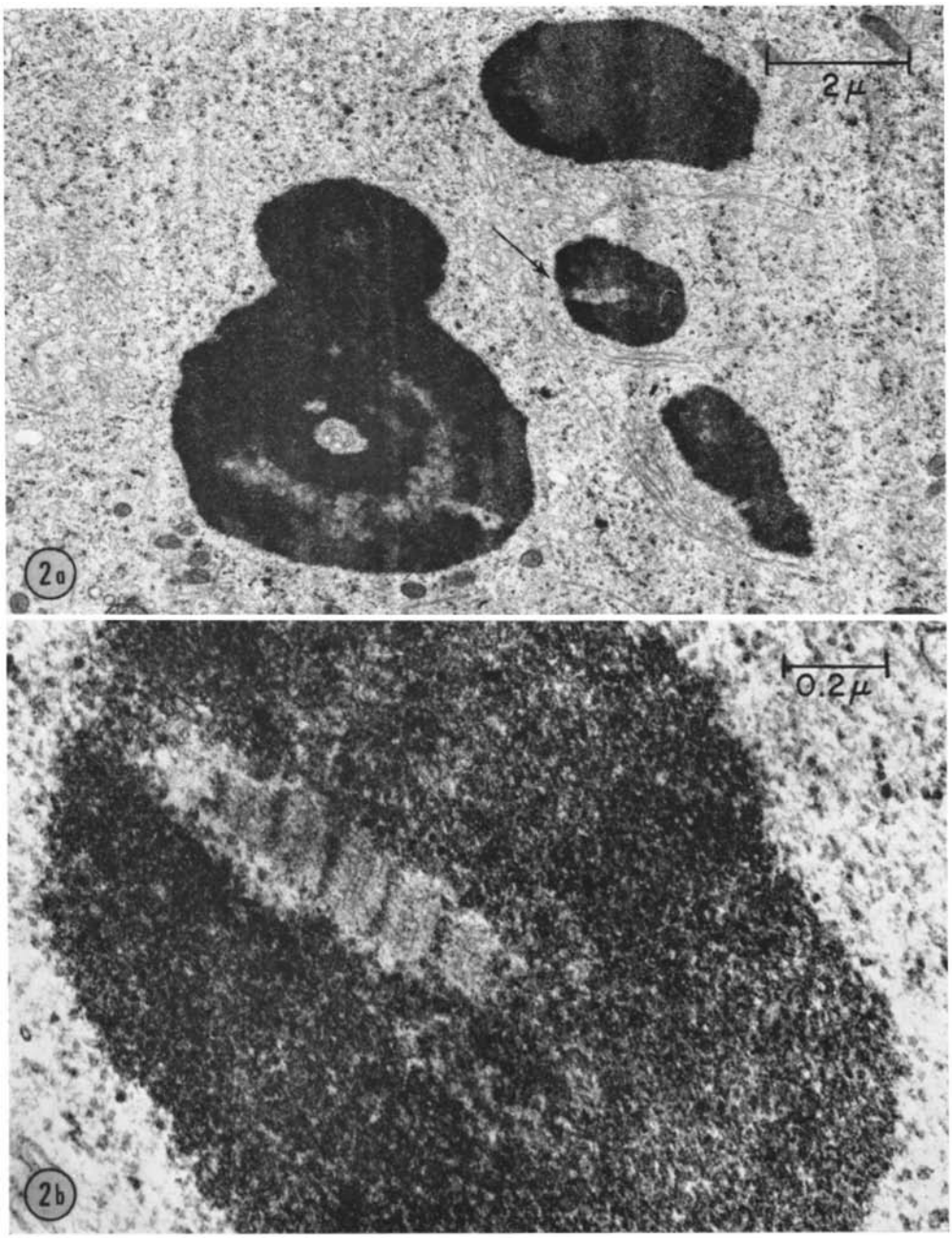


FIGURE 2a Paired chromosomes at first metaphase of meiosis contain disorganized core material as shown in the large bivalent, or multiple core structures as in the bivalent marked by the arrow. The latter bivalent is enlarged in Fig. 2b. × 10,000.

FIGURE 2b A first metaphase bivalent with a multiple core complex. The dark bands or cores are 1000 A apart and central elements lie between the cores. × 72,000.

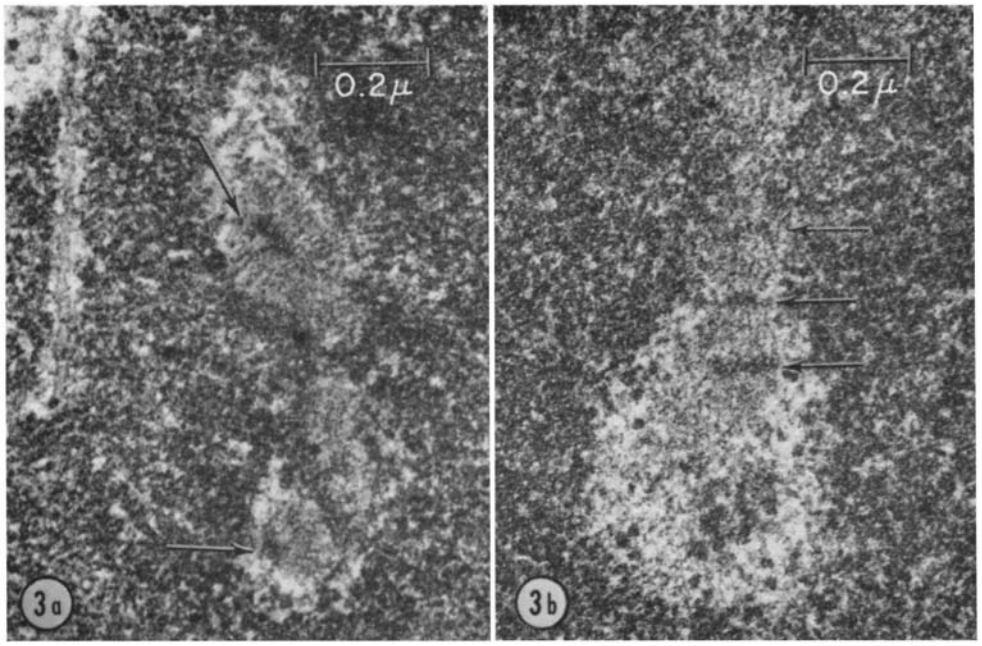


FIGURE 3a Most metaphase I bivalents have short cores with the associated filaments (arrows). × 72,000.

FIGURE 3b A metaphase I bivalent with a small multiple core complex, consisting of three cores which are marked by arrows. × 72,000.

observed in the cytoplasm of the spermatocytes (Figs. 4a, 4b, 4c). Most frequently the multiple complexes were found among the elongate mitochondria stretched between the poles. They are either spindle-shaped or branched as shown in Fig. 6 (spermatid). On the average, one complex was found when about 100 sections of anaphase cells were scanned. 12 different multiple complexes were photographed and several more were encountered but not recorded. The details of a multiple complex at first anaphase can be seen in Fig. 4b. The space between the dense cores is about 1000 A, and each core is about 200 A wide, giving a periodicity of 1200 A. The central elements are well defined in this thick section. Some of the complexes are associated with membranes. The dense mass at the tapered end of the complex occurs regularly in that position and can also be seen in Figs. 5b and 6b. Fig. 4c is included to show the presence of filaments next to a multiple core complex in a zone where the cores are not clearly defined.

After the first meiotic division, during meiotic interphase, the nuclear membrane reforms briefly while the chromosomes remain fairly contracted (Fig. 5a). At this stage the multiple core complexes were observed in the cytoplasm of those cells (Fig. 5a). The frequency of occurrence was somewhat lower than at anaphase I, and a total of nine different multiple core complexes were photographed, two of which were in serial sections. All of these complexes were larger than those seen at metaphase I. The structural details of the multiple core complex at meiotic interphase can be seen in Fig. 5b. The complex is 2 μ long and has 18 bands. It is bounded by membranes and has some less well-organized cores and filaments next to it. Because of the thinness of the section, the central elements are not well defined. At points along the complex, bundles of filaments can be seen to diverge and rejoin again (marked by arrow in Fig. 5c). Where the filaments have diverged, the cores are discontinuous. Eight serial sections, each about 800 A thick, contain this complex, indicating that it is at least 0.5 μ thick. Figs. 5b, 5c, and 5d are electron micrographs of the 4th, 5th, and 6th sections. The dense bands are in register, which means that the bands are, in fact, cross sections through planes

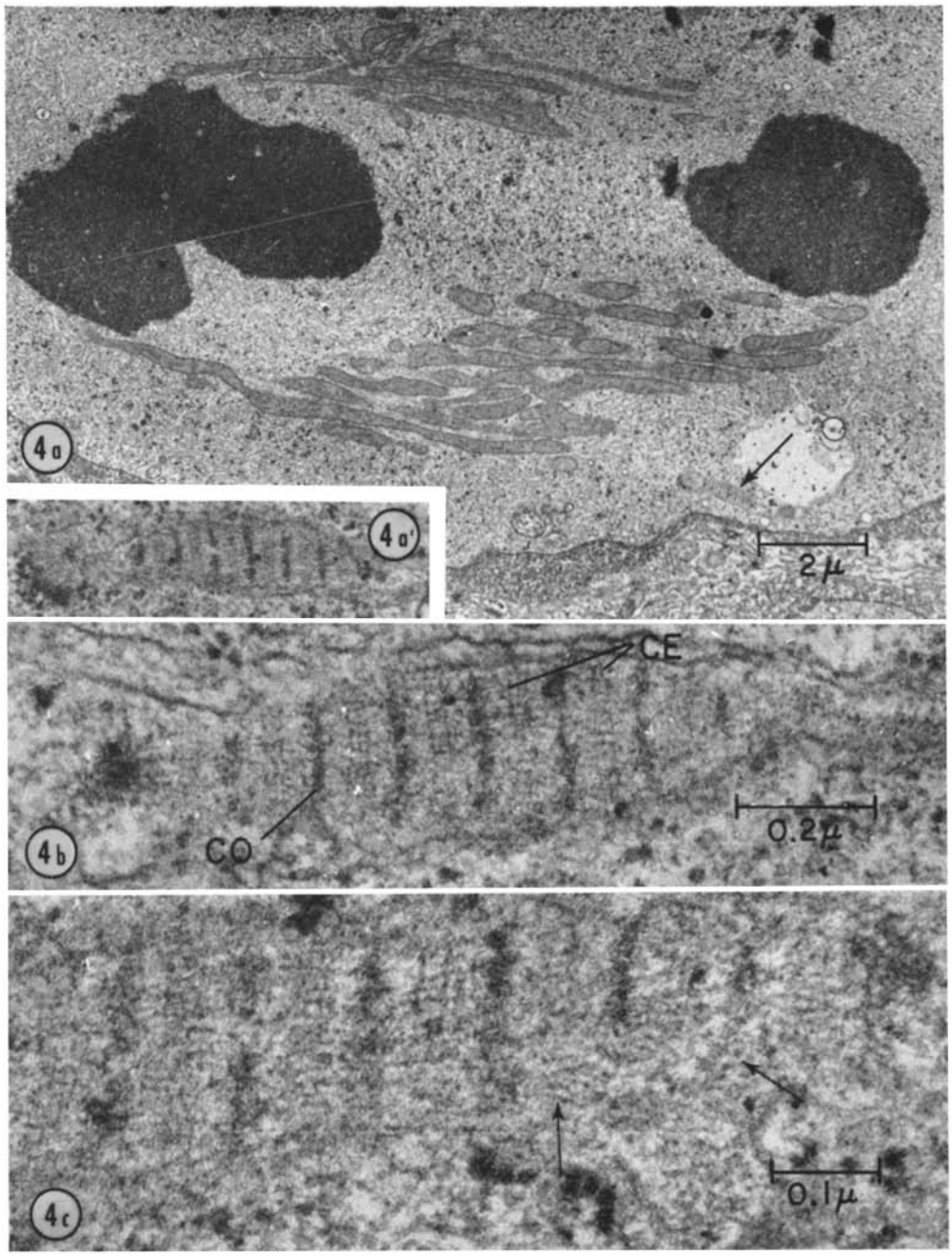


FIGURE 4a At first anaphase, multiple core complexes occur in the cytoplasm of the spermatocyte (arrow). A narrow overexposed strip, caused by a shutter defect, has been taken out of the electron micrograph. × 8,000.

FIGURE 4a′ An enlargement of the complex in Fig. 4a. The small dot to the left has transverse filaments radiating in several directions. × 40,000.

FIGURE 4b A high magnification of a multiple core complex at anaphase I. Due to the thickness of the section, the central elements (*CE*) are well defined. The cores (*CO*) are about 1000 A apart, and each core is 200 A wide, giving a periodicity of 1200 A. Frequently, complexes are spindle shaped like this one, and they have a dense mass of core substance at the tapered end (left side). × 95,000.

FIGURE 4c A thin section of a complex at anaphase I to show the filaments (arrows) associated with the complex. × 150,000.

of core substance. Another multiple complex of which serial sections were available was 8 bands long, and just under 1 $\mu$ in diameter. The three-dimensional structure of the multiple core complex accounts for the observation that frequently the periodicity of the bands was in excess of 1200 A. Sections perpendicular to the dense planes will give the shortest distance between bands; all oblique sections will have larger d istances.

Only two early spermatids with multiple core complexes were found (Fig. 6). The cell shown in Fig. 6a has two multiple core complexes (the only case of two complexes in one section of a single cell): one is branched, and both have a considerable amount of dense material at the end of the complex.

## DISCUSSION

The multiple core complexes are interesting from the point of view that they may have elements in common with the synaptinemal complexes, and may therefore contribute information on the pairing function of synaptinemal complexes in chromosomes at meiotic prophase. This report describes the synaptinemal complex at meiotic prophase and the multiple core complexes at metaphase I, anaphase I, meiotic interphase, and in early spermatids. Although it seems reasonable that these structures are related to each other, only indirect evidence, based on morphological features, is available.

The possibility that the synaptinemal complex and the multiple core complex are related is suggested by their structural similarity, by the fact that they both occur inside the chromatin of meiotic bivalents, and by the fact that they occur shortly after each other at a specific developmental stage of the spermatocyte. The similarities of the two forms in section include the parallel arrangement of densely staining ribbons, the distance between the ribbons, and the structure of the central element. In at least one insect (10) the transition from synaptinemal complex to multiple complex has been recorded. At the onset of diplotene in the mosquito oöcyte, Roth (10, p. 381) reports: "Since the polycomplex (PC) is a sheet of units, each resembling a SC, it is thought that the PC may arise by the aggregation of the synaptinemal complexes. This hypothesis is supported by the observation that units of the polycomplexes have the same dimensions as a SC, are devoid of chromatin, and during early diplonema sometimes are seen closely aligned to a SC still partially coated with chromation (Fig. 6)." SC is the abbreviation used for synaptinemal complex.

The differences between the synaptinemal complex and the multiple complex indicate that a simple relation cannot be involved. The synaptinemal complex has two long, densely staining ribbons paired parallel to each other in essentially a two-dimensional arrangement. Within the pachytene bivalent, this arrangement appears to be the only possible one. In the multiple complex several short ribbons are arranged parallel to each other, and serial sections show the structure to be three-dimensional rather than two-dimensional. Furthermore, the single width of each ribbon in the multiple complex indicates that a simple stacking of short synaptinemal complexes is unlikely. To account for the structure of multiple complexes in the mosquito oöcyte, Roth (10) has proposed that when the synaptinemal complexes leave the bivalent, one-half of each lateral element remains behind. However, no remaining elements were reported nor does the model consider the out-of-phase pairing of extensive sheets of multiple complexes at a later stage. Schin (11) considers the transverse filaments as a basic unit from which either synaptinemal complexes or multiple complexes can be constructed. Transverse filaments with local diameter increments will, when aligned in register, produce the central element (11).

The observation presented here favor the likelihood that small units (possibly filaments) rather than segments of synaptinemal complexes or cores are involved in the assembly of multiple core complexes. Filaments occur at pachytene in the synaptinemal complex, at diakinesis, and in metaphase I chromosomes. They can also be seen along the multiple complex at anaphase I in Fig. 4c. The various shapes of the multiple core complexes further suggest that the additions made to them during anaphase I and during meiotic interphase consist of small units. The multiple complexes which are membrane-bounded are long and narrow, and may be only a few filaments wide (e.g., the center of the complex in Fig. 5d, two sections farther on). Multiple complexes which are free in the cytoplasm are smooth, spindle-shaped bodies, and the one examined in serial sections is nearly ball shaped. The transverse filaments of the multiple complexes have structural features in common with the transverse filaments of the synaptinemal complex so that, when these filaments are aligned, the central element and the distance between cores is the same in both forms

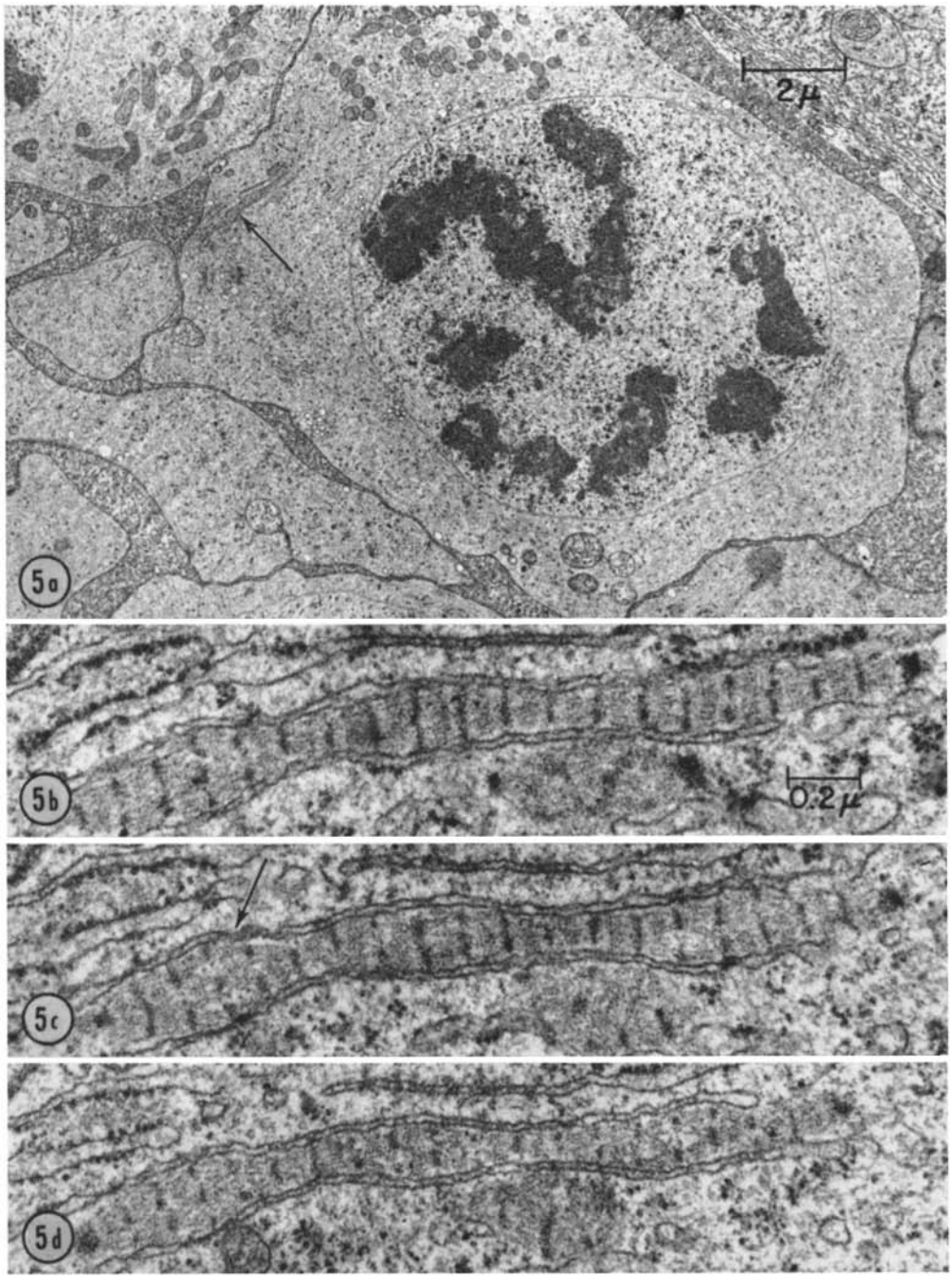


FIGURE 5a During meiotic interphase a nuclear membrane forms, but the chromosomes remain contracted. The multiple core complex (arrow) lies in the cytoplasm. × 7,000.

FIGURES 5b–d Three consecutive sections of eight serial sections of the complex in Fig. 5a. Each section is about 800 A thick, so that the complex is at least 0.5 μ thick. The cores are in register throughout the serial sections so that there are in fact planes of core material. Next to this 18-band complex are some less well-organized filaments and core material. × 50,000.

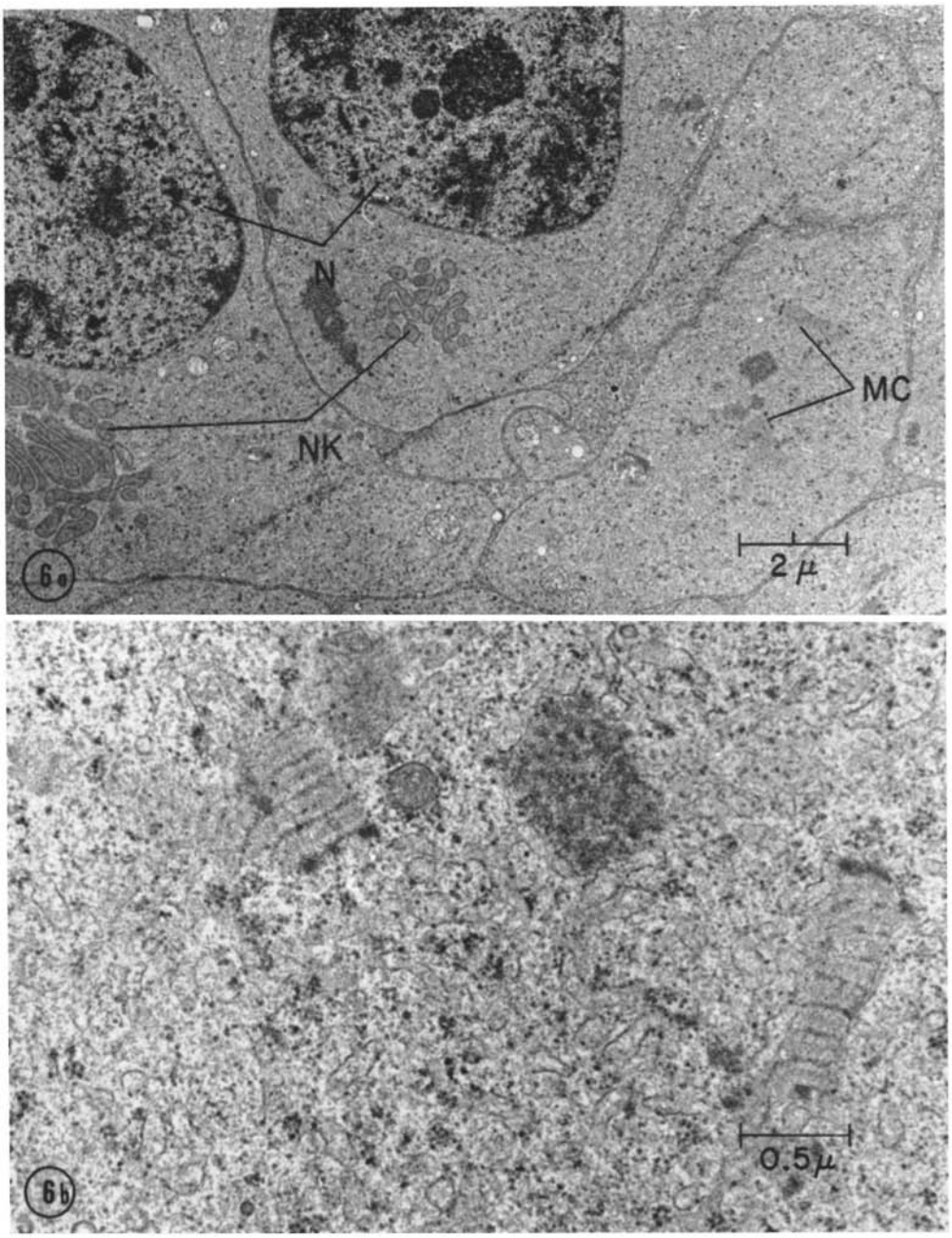


FIGURE 6a The spermatid nuclei (*N*) have an interphase structure, and the cytoplasm contains the initial stages of the nebenkern (*NK*). The cytoplasm of one spermatid has two multiple complexes (*MC*). × 7,700.

FIGURE 6b Enlargement of Fig. 6a. The complex on the left is branched. × 31,000.

of complexes. Either because of structural differences, or because of different physiological conditions, the filaments of the multiple core complexes apparently can undergo associations not found in the synaptinemal complex.

The synaptinemal complex of the pachytene bivalent, which is largely proteinaceous (1, 8, 9), is closely associated with the hereditary material of the spermatocyte, and it is probably essential for meiotic genetic exchange (4, 5, 8). The multiple core complex at anaphase I and later is not associated with the permanent genetic material of the cell. The structures described here differ in this respect from the multiple core complexes described in *Gryllus*. In *G. domesticus*, parallel planes of core material with transverse filaments and central elements between them are associated with the unpaired sex chromosome and the nucleolus at meiotic prophase (13, 14). In *G. campestris* and *G. bimaculatus*, Guénin (2) reports multiple core complexes and extensive masses of filamentous material in association with the nucleolus at pachytene. Sotelo (13) has also reported small, free-lying multiple core complexes at both meiotic divisions at the time when no nuclear membranes were present. The multiple core bodies in the spermatid nuclei of *Gryllus* were originally thought to be associated with the X-chromosome (11, 13), but their occurrence in spermatids without X-chromosomes has ruled out that possibility (15). Wolstenholme and Meyer further show that the multiple complexes, referred to as axial core structures by the authors, have chromosomal material associated with them (15).

It appears that during meiosis and in spermatids of insects, a variety of structures may occur which share morphological characteristics with synaptinemal complexes. The structures all appear to share a pairing function, but different structures from different association. It is likely that the various complexes have some structural elements in common, but that their organization and function are different in each case.

This work was financially supported by the National Research Council of Canada and by the Science Division of York University. Mrs. L. Oostwoud provided technical assistance.

*Received for publication 9 July 1968, and in revised form 14 October 1968.*

## REFERENCES

1. COLEMAN, J. R., and M. J. MOSES. 1964. DNA and the fine structure of synaptic chromosomes in the domestic rooster (*Gallus domesticus*). *J. Cell Biol.* **23**:63.
2. GUÉNIN, H-A. 1965. Observations sur la structure submicroscopique du complexe axial dans les chromosomes meiotiques chez *Gryllus campestris* L. et *G. bimaculatus* de Geer (*Orthopt. gryll.*). *J. Microsc.* **4**:749.
3. MAILLET, P. L., and R. FOLLIOT. 1965. Sur les ultrastructures chromosomiques de la meiose chez *Philaenus spumarius* L. male (*Homoptere cercopidae*). *C. R. Hebd. Seances Acad. Sci. Paris.* **260**:3486.
4. MEYER, G. F. 1960. The fine structure of spermatocyte nuclei of *Drosophila melanogaster*. *Proc. European Regional Conf. Electron Microscopy, Delft.* **2**:951.
5. MEYER, G. F. 1964. A possible correlation between the submicroscopic structure of meiotic chromosomes and crossing over. Third European Regional Conference on Electron Microscopy. M. Titlebach, editor. Publishing House of the Czechoslovak Academy of Sciences, Prague. B461.
6. MOENS, P. B. 1968. The structure and function of the synaptinemal complex in *Lilium longiflorum* sporocytes. *Chromosoma.* **23**:418.
7. MOSES, M. J. 1958. The relation between the axial complex of meiotic prophase chromosomes and chromosome pairing in a salamander (*Plethodon cinereus*). *J. Biophys. Biochem. Cytol.* **4**:633.
8. MOSES, M. J. 1964. The Nucleus and Chromosomes: A Cytological Perspective. *In* Cytology and Cell Physiology. G. Bourne editor. Academic Press Inc., New York. 423.
9. NEBEL, B. R., and E. M. COULON. 1962. Enzyme effects on pachytene chromosomes of the male pigeon evaluated with the electron microscope. *Chromosoma.* **13**:292.
10. ROTH, T. F. 1966. Changes in the synaptinemal complex during meiotic prophase in mosquito oöcytes. *Protoplasma.* **61**:346.
11. SCHIN, K. S. 1965. Meiotische Prophase und Spermatidenreifung bei *Gryllus domesticus* mit besonderer Berücksichtigung der Chromosomenstruktur. *Z. Zellforsch. Mikroskop. Anat.* **65**:481.
12. SOTELO, J. R., and O. TRUJILLO-CENÓZ. 1960. Electron microscope study on spermatogenesis (Chromosome morphogenesis at the onset of

meiosis (Cyte I) and nuclear structure of early and late spermatids.) *Z. Zellforsch. Mikroskop. Anat.* **51**:243.

13. SOTELO, J. R., and R. WETTSTEIN. 1964. Electron microscope study on meiosis (The sex chromosome in spermatocytes, spermatids and oöcytes of *Gryllus argentinus*). *Chromosoma.* **15**:389.

14. SOTELO, J. R., and R. WETTSTEIN. 1965. Fine structure of meiotic chromosomes. *Nat. Cancer Inst. Monogr.* **18**:133.

15. WOLSTENHOLME, D. R., and G. F. MEYER. 1966. Some facts concerning the nature and formation of axial core structure in spermatids of *Gryllus domesticus*. *Chromosoma.* **18**:272.