

Intranuclear Membranes and the Formation of the First Meiotic Spindle in *Xenos peckii* (*Acroschismus wheeleri*) Oocytes

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ABSTRACT The ultrastructure of spindle formation during the first meiotic division in oocytes of the Strepsipteran insect *Xenos peckii* Kirby (*Acroschismus wheeleri* Pierce) was examined in serial thick (0.25- μ m) and thin sections. During late prophase the nuclear envelope became extremely convoluted and fenestrated. At this time vesicular and tubular membrane elements permeated the nucleoplasm and formed a thin fusiform sheath, 5–7 μ m in length, around each of the randomly oriented and condensing tetrads. These membrane elements appeared to arise from the nuclear envelope and/or in association with annulate lamellae in the nuclear region. All of the individual tetrads and their associated fusiform sheaths became aligned within the nucleus subsequent to the breakdown of the nuclear envelope. Microtubules (MTs) were found associated with membranes of the meiotic apparatus only after the nuclear envelope had broken down. Kinetochores, with associated MTs, were first recognizable as electron-opaque patches on the chromosomes at this time. The fully formed metaphase arrested *Xenos* oocyte meiotic apparatus contained an abundance of membranes and had diffuse poles that lacked distinct polar MT organizing centers.

From these observations we conclude that the apparent individual chromosomal spindles—seen in the light microscope to form around each *Xenos* tetrad during “intranuclear prometaphase” (Hughes-Schrader, S., 1924, *J. Morphol.* 39:157–197)—actually form during late prophase, lack MTs, and are therefore not complete miniature bipolar spindles, as had been commonly assumed. Thus, the unique mode of spindle formation in *Xenos* oocytes cannot be used to support the hypothesis that chromosomes (kinetochores) induce the polymerization of their associated MTs. Our observation that MTs appeared in association with and parallel to tubular membrane components of the *Xenos* meiotic apparatus after these membranes became oriented with respect to the tetrads, is consistent with the notion that membranes associated with the spindle determine the orientation of spindle MTs and also play a part in regulating their formation.

In oocytes of the insect *Xenos peckii* Kirby (*Acroschismus wheeleri* Pierce; 4) the first meiotic spindle, in specimens fixed and sectioned for light microscopy, appears to arise within the nucleus from the collocation of eight randomly oriented miniature spindles, each of which initially forms in association with a single tetrad (16). This unique manner of spindle formation has been cited, through the years, as evidence to support the argument that chromosomes themselves can organize a functional bipolar spindle in the absence of extra-

chromosomal organizers (32, 43, 45, 47), that spindle bipolarity is established by the kinetochores (43), and that chromosomal spindle fibers have a tendency to fuse (2). Indeed, more recently Hughes-Schrader's (16) observations on *Xenos* have even been extrapolated to indicate that kinetochores form their own fibers by nucleating microtubules (MTs¹; 7–

¹ Abbreviations used in this paper. MA, meiotic apparatus; MTs, microtubules; NE, nuclear envelope.

9, 28, 30, 32, 43, 47, 53).

Important suppositions from early light microscopic observations of fixed material should not be accepted at face value, a point that is amply illustrated in Kubai's (24) recent investigation of the monopolar spindle in *Sciara* spermatocytes. Her ultrastructural analysis of this unique system disproved the widely accepted light microscopic conclusion that anaphase chromosomes can move away from the pole to which they are connected. Her results forced "a complete reevaluation of chromosome behavior" in *Sciara* spermatocytes, and illustrate that much effort can be spent attempting to incorporate into a cohesive mitotic framework erroneous interpretations of exceptional light microscopic observations. In this respect the significant inferences drawn from Hughes-Schrader's (16) unique observations on *Xenos* also remain unproven (29, 32) since the first meiotic oocyte division in this elusive organism has yet to be examined at the ultrastructural level. Indeed, the most important conclusion drawn from her study, that the chromosomal spindle fiber may arise "entirely through the activity of the kinetochore alone" (45) remains controversial (34, 41, 49). These considerations prompted us to conduct a thorough ultrastructural examination of the first meiotic division in *Xenos* oocytes.

Our results demonstrate that the first meiotic oocyte division in *Xenos* is unique in that an elaborate intranuclear fusiform sheath, composed of tubular and vesicular membrane elements, formed in association with each individual tetrad during late prophase. These membranous sheaths formed and became aligned parallel within the nucleus, in the absence of MTs. Kinetochore and nonkinetochore spindle MTs were found associated with these existing parallel arrays of membrane elements only after the nuclear envelope (NE) had broken down. It can be concluded from these observations that chromosomal spindle fibers do not form in *Xenos* oocytes as commonly inferred from Hughes-Schrader's (16) light microscopic observations.

MATERIALS AND METHODS

Some notes on Xenos: The strepsipteran *Xenos peckii* Kirby (*Acroschismus wheeleri* Pierce; 4) is an obligate parasite of *Polistes fuscatus* Fabricius, the common paper wasp. Both the parasitic insect and its host are distributed throughout temperate North America (4, 5); the parasitized wasps (termed "styloized") have been recorded in most Northeastern states as well as Alabama, California, Indiana, Maryland, Michigan, Ohio, and Texas (16, 18, 23, 35, 36). Other *Xenos* species have been collected from various wasps in the family Vespidae in Europe, South America, Africa, and Asia (e.g. 3, 21, 22, 26).

Most of what is known of the biology of *X. peckii* has been described by Hughes-Schrader (16). As is generally true in the Strepsiptera, development takes place entirely within the abdominal cavity of the host (1). The minute male pupates in a cylindrical case that protrudes slightly through the wasp's sclerites; upon emergence the adult flies off to search for females. In contrast, the female does not pupate, and, as an adult, lacks wings, legs, and eyes. She remains within the wasp, eventually permanently exerting her white cephalothorax through the host's sclerites. Mating does not occur until 4 or 5 d after the first exertion, by which time the cephalothorax has become darkly pigmented. It is also at this time that the interphase eggs undergo meiotic maturation. Eggs that reach the first meiotic metaphase degenerate after 10–14 d if not fertilized.

Collection: Parasitized *P. fuscatus* were collected in early September on blooming goldenrod in Ithaca, New York, and at weekly intervals from early September to mid-October from nests in East Greenbush, New York. A styloized wasp can be identified with the naked eye by the fairly conspicuous asymmetric bulge in its abdomen, formed by either a male pupal case or an exerted female cephalothorax. Multiple *Xenos* in a single host are common.

The 37 styloized wasps collected yielded a total of 76 male pupal cases— from which most of the males had already departed—and 28 female *Xenos*, 21

of which had exerted the cephalothorax. The degree of pigmentation of the cephalothorax provided a rough estimate of the time since exertion.

Preparation and Fixation: Each gravid female parasite was found to contain 500–1,000 eggs (~80% of her body mass; 6), which could be easily liberated from the body cavity by simply teasing the parasite apart in fixative (or in insect culture medium). According to Hughes-Schrader (16) all of the eggs from any one female *Xenos* are in approximately the same stage of development. Unfortunately, the compound structure, large size, and amount of associated yolk of the *Xenos* egg, along with the diminutive size of the meiotic apparatus (MA) and chromosomes, prohibited any *in vivo* examination of the meiotic process. In fact, it was seldom possible to distinguish interphase from meiotic eggs prior to sectioning. Thus our approach was to collect, fix, and flat-embed on a single coverslip all the eggs from a particular female that had exerted her cephalothorax, and to make embeddings from as many such females as practical throughout the collection period. One or two eggs could later be cut from each of the embeddings to determine the approximate stage of the eggs from each female.

Gravid female parasites with lightly-to-heavily pigmented cephalothoraxes were teased apart directly into a 50:50 mixture of 4.5% glutaraldehyde and 2% OsO₄, each in 0.1 M PO₄ buffer (pH 7.1; 18°C) made up immediately prior to use (the final initial concentrations of glutaraldehyde and osmium were 2.25% and 1%, respectively). A glutaraldehyde/osmium fixation protocol (12, 38) was chosen because we found during the preliminary stages of this study that it preserved MTs and membranes within the MA of the mature metaphase-arrested *Xenos* egg. The liberated eggs, which are heavier than the remaining cellular debris, quickly sank to the bottom of the petri dish containing the fixative. After 90 min the eggs were transferred by pipetting into a 15-ml polystyrene centrifuge tube containing dH₂O and loosely pelleted by gentle centrifugation. They were then resuspended two more times in two changes of dH₂O, dehydrated in a graded series of ethanols, and flat-embedded in Polybed 812 (Polysciences, Warrington, PA) on 24 × 60-mm glass coverslips.

The glass coverslip embedding substrate was removed from the embedded eggs by a brief treatment with 4°C hydrofluoric acid (as in 40). The embedded eggs were then examined within the plastic by phase-contrast microscopy, and suitably oriented eggs were marked with a diamond objective scribe. Marked eggs were then excised from the embedment, mounted on EPON pegs, and serially thick- (0.25-μm) or thin-sectioned as described by Rieder (39). The 0.25-μm thick sections proved very practical for this study, since an egg could be completely sectioned in ~350 sections (vs. the 1,000 or more required for a conventional ultrathin section electron microscopic study). In addition, once mounted on slot grids, the ribbons of 0.25-μm thick sections could be quickly prescreened for the presence of the egg nucleus or MA with phase-contrast light microscopy before or after staining with uranyl acetate and lead citrate (see 42).

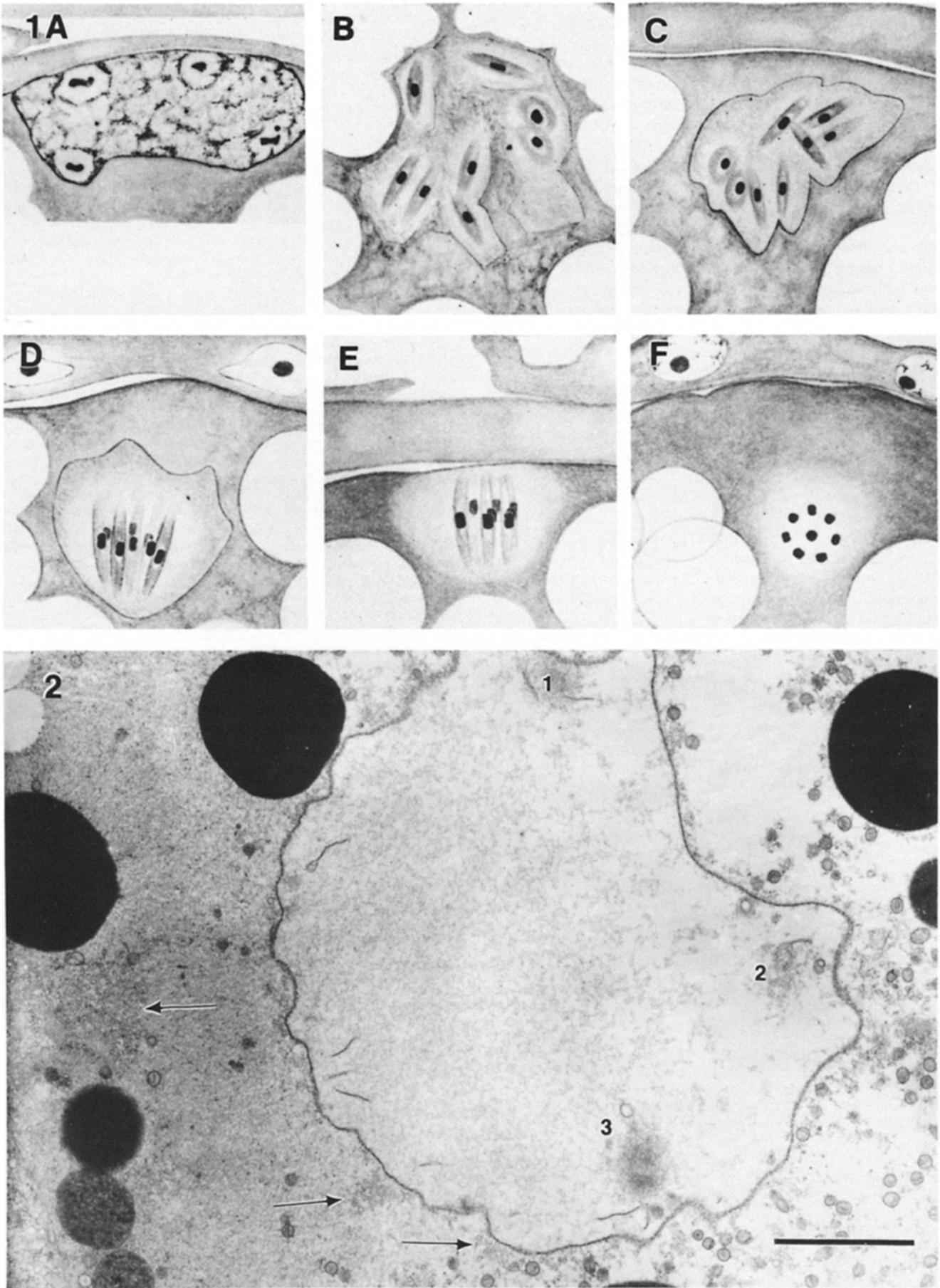
Since *Xenos* eggs have been reported to develop synchronously in any one female (see above), one or two initial eggs were cut from each of the 15 embeddings to determine the approximate stage of the eggs from each female. Additional eggs were sectioned from the four embeddings that contained eggs in various meiotic stages. A total of 19 eggs were examined, each in serial sections, from these four embeddings.

Electron Microscopy: Serial thin sections were examined at 80 kV with a Philips 300 electron microscope equipped with a goniometer stage and a 70-μm objective aperture. However, the great majority of data for this study was obtained by viewing serial 0.25-μm thick sections with the New York State Department of Health 1.2-MeV high voltage electron microscope, operated at 800–1,000 kV, and employing an objective aperture of 30-μm. All electron micrographs included herein are of 0.25-μm thick sections.

RESULTS

Salient Features of Hughes-Schrader's Observations on Spindle Formation in Xenos Oocytes

A brief description of Hughes-Schrader's (16) observations concerning spindle formation in *Xenos* oocytes is necessary, not only to understand our results, but also so that the reader may appreciate the correlation between her light microscopic observations and our ultrastructural data. Fig. 1 presents selected camera lucida drawings, reproduced from her original text, showing the unique method of spindle formation during the first meiotic (maturative) division of the egg. These drawings are of 5-μm-thick iron-haematoxylin-stained paraffin



sections cut through the nucleus from eggs preserved in Kahle's fixative (5% formic acid, 0.2% acetic acid, and 24% ethanol).

According to Hughes-Schrader (16), eight condensations of the chromatin appear within the nucleus during prophase (Fig. 1A). These condensing tetrads are positioned at the periphery of the nucleus and are surrounded by a clear zone. As "prometaphase" is initiated a fusiform body of nebulous material forms in association with the clear zone of each tetrad (Fig. 1B). At this time "the nuclear membrane collapses and becomes wrinkled and difficult to trace definitely" (16). During early "prometaphase" the eight tetrads and their fusiform sheaths may lie in any plane within the nucleus and show no common orientation. When the formation of the sheaths is well advanced the nuclear membrane reasserts itself, i.e., it appears to shrink and tighten up around the fusiform bodies (Fig. 1C). As this process continues the fusiform sheaths and their associated tetrads become aligned in a "metaphase" arrangement within the nucleus (Fig. 1D). The nuclear membrane then dissolves (Fig. 1E) and the fusiform bodies comprising the spindle "acquire a very constant and characteristic arrangement" (16; Fig. 1F). No further morphological changes occur in the metaphase-arrested MA until fertilization.

ULTRASTRUCTURAL ANALYSIS OF SPINDLE FORMATION IN *XENOS* OOCYTES

Late Interphase

Xenos eggs are broadly oval (~100 μm long; 60–70 μm wide) and slightly attenuated at one pole. Light and electron microscopy of thick and thin sections reveals that each definitive egg is encased in two distinct "envelopes." The inner envelope surrounds the yolk, egg nucleus, and cytoplasm and has been referred to as a chorion (6). The chorion is surrounded by numerous nurse cells that are in turn encased in a second, looser egg envelope formed by an epithelial cell layer which is thought to be derived from the follicular epithelium of the early egg tubules (16).

The late interphase *Xenos* egg nucleus is found at the periphery of the egg cytoplasm near the chorion, which is disrupted in the immediate vicinity of the egg nucleus. At this time the nucleus is roughly spherical and ~20–25- μm diam. Ultrastructural analysis reveals that it contains an extremely fine reticulum of chromatin threads enclosed in a porous NE which consists of the usual (inner and outer) nuclear membranes. Numerous slender membrane protrusions, which represent folds of the inner nuclear membrane, extend for varying distances into the nucleus (data not shown; see below). That part of the egg nucleus closest to the disrupted chorion is embedded in a fine but dense cytoplasmic ground substance that contains numerous mitochondria and annulate lamellae with associated membrane-bounded vesicles (data not shown).

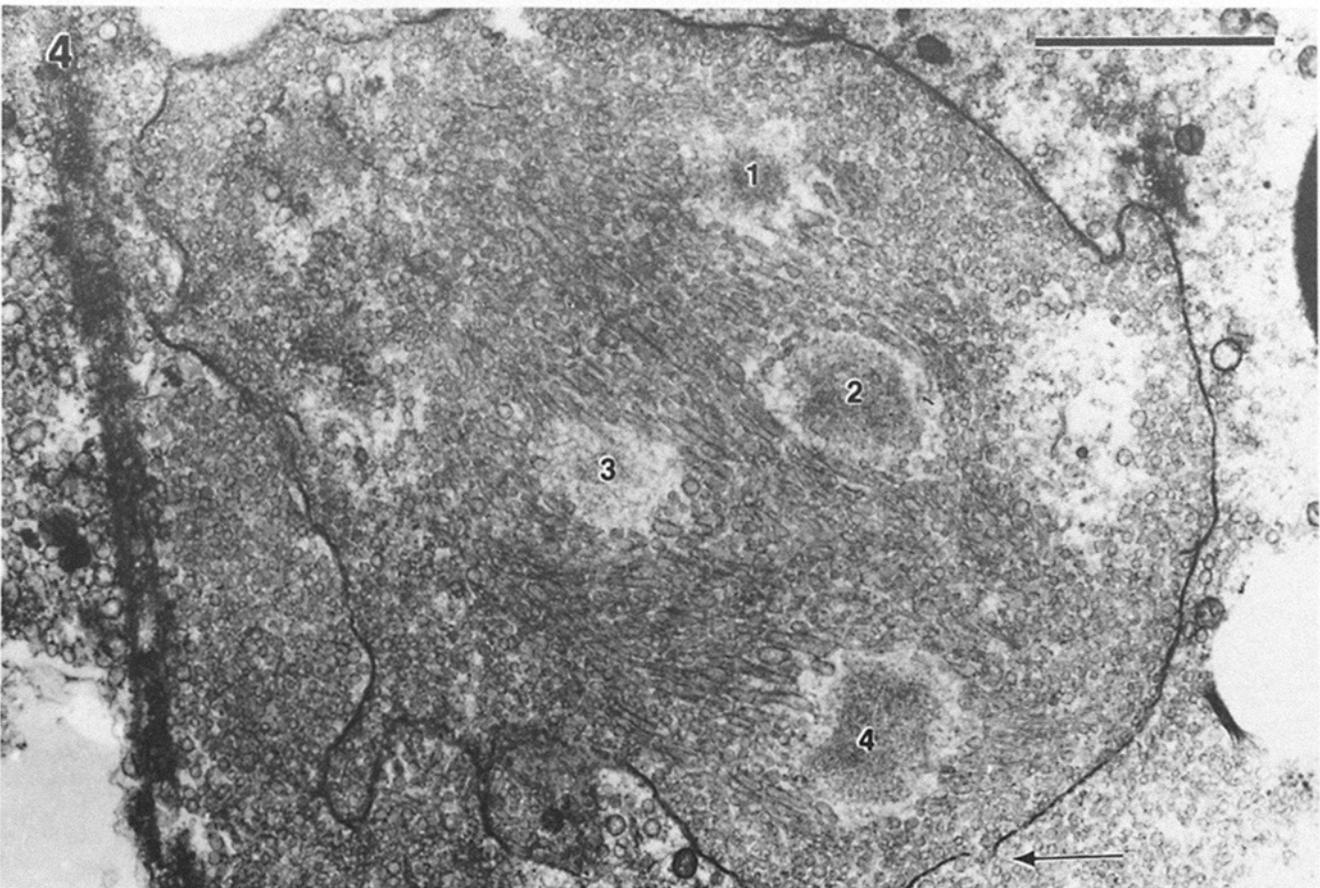
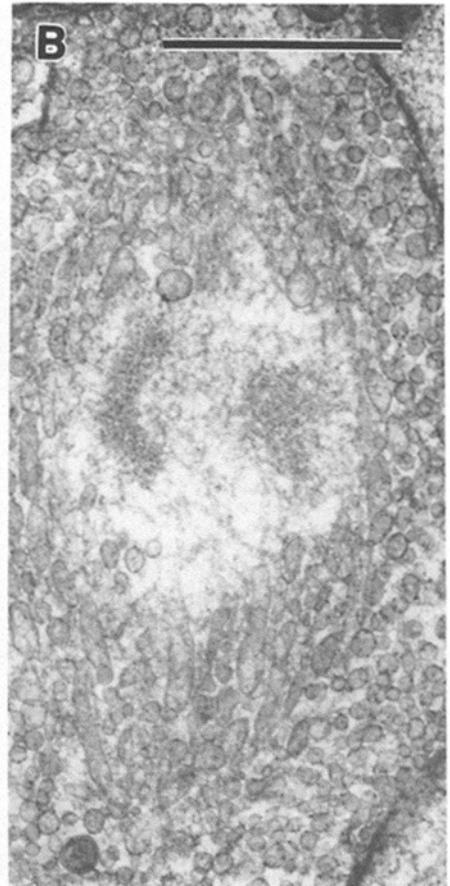
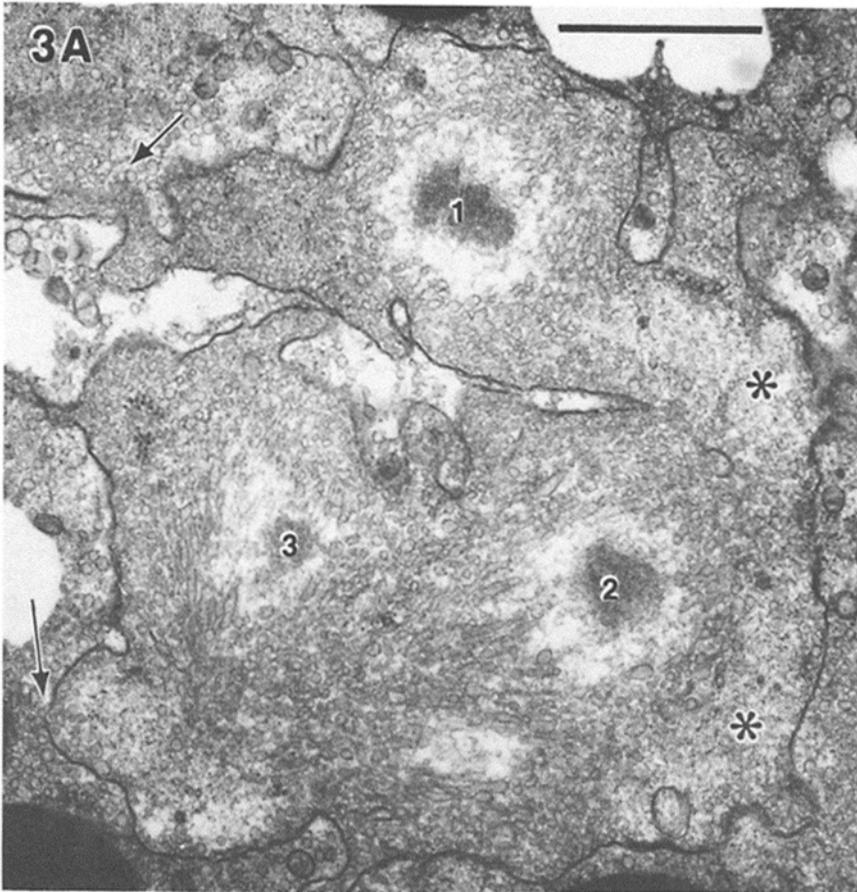
Prophase (10 Nuclei Reconstructed)

The early prophase egg nucleus resembles that of the late interphase egg nucleus, with the notable exception that the nucleus contains eight condensing tetrads (Fig. 2). Each of these tetrads is located at the periphery of the nucleus and is surrounded by a clear zone relatively free of chromatin (Fig. 2; also Fig. 1A). A reconstruction from serial sections revealed that each condensing tetrad is associated with one or more of the membrane projections that arise from the inner membrane of the NE (Fig. 2). Numerous aggregates of membrane-bounded vesicles, similar to those seen associated with annulate lamellae of late interphase oocytes, can be seen at or near the vicinity of the NE (Fig. 2, arrows). The annulate lamellae, characteristically seen in late interphase oocytes, were no longer found in the cytoplasm of eggs containing prophase nuclei.

Oocyte nuclei, classified as being in early (Fig. 1B), mid (Fig. 1C), or late (Fig. 1D) "prometaphase" by Hughes-Schrader (16), were, in reality, determined to be in mid-late prophase since the NE remained largely intact and no evidence of spindle formation could be found. These nuclei were invariably found near the chorion, which remained disrupted in the vicinity of the nucleus until metaphase (see below). Numerous small membrane-bounded vesicles were seen surrounding each mid-late prophase nucleus (Figs. 3–5), especially that part opposite the disrupted chorion (Fig. 4).

During mid-prophase (i.e., that stage corresponding to Fig. 1B) the NE appears extremely convoluted and contains numerous fenestrae, some as large as 1.0- μm diam (Fig. 3, arrows; also Figs. 4 and 5). Small membrane-bounded vesicles were frequently seen within these fenestrae. At this time the tetrads were nearly spherical and ~1–2- μm diam. Each is surrounded by an electron-translucent zone of variable width, which contains fine strands of chromatin (Fig. 3B). The electron-translucent zone surrounding each tetrad is, in turn, surrounded by a thin sheath of elongated tubular and spherical membrane elements, the latter of which also permeate the remainder of the nucleoplasm (Fig. 3). These fusiform membranous "chromosomal sheaths" were 5–7 μm long and 1.5–2.5 μm wide. The fusiform appearance of each sheath arose from numerous parallel-oriented tubular membrane elements, some as long as 1.0 μm , which curve around the clear zone associated with each tetrad and then taper toward focal points distal to the chromosome. These focal points lack any distinguishing ultrastructural features (e.g., Fig. 3B). During this stage the tetrads were scattered randomly within the bounds of the convoluted NE and the long axes of the individual chromosomal sheaths showed no common orientation. Kinetochores, if present, cannot be distinguished from the chromatin of the chromosome. No MTs are seen in serial thin or thick sections of these nuclei, regardless of the magnification (e.g., Fig. 3B).

FIGURES 1 and 2 Fig. 1: Camera lucida drawings of *Xenos* oocyte nuclei, reproduced from Hughes-Schrader (16), showing the various stages of spindle formation during the first meiotic division. (A) Prophase, (B) early-"prometaphase," (C) mid-"prometaphase," (D) late-"prometaphase," (E–F) metaphase. Reproduced by permission of Alan R. Liss, Inc. Fig. 2: Survey electron micrograph of a section through an early prophase *Xenos* oocyte nucleus. Three of the eight tetrads are caught in the plane of the section. The arrows denote aggregates of membrane-bounded cytoplasmic vesicles. Note the numerous intranuclear membrane projections of the NE and their association with the condensing tetrads. The disrupted chorion, not pictured, is at the left. Bar, 4.0 μm . $\times 6,500$.



Attempts to find nuclei in earlier stages of mid-prophase, during which some of the tetrads were not surrounded by membrane sheaths, were unsuccessful. Apparently, this situation was also encountered by Hughes-Schrader (16), since her earliest pictured "prometaphase" nucleus (Fig. 1B) shows sheath material associated with every chromosome. This would seem to indicate that the formation of the sheaths occurs very quickly, relative to the remainder of prophase. A careful inspection of serial sections through mid-prophase nuclei reveals areas free of chromosomes which are also relatively free of membrane elements (e.g., Fig. 3A, asterisks). This suggests that the membrane elements become localized first around the chromosomes and later fill the remaining nucleoplasm.

Oocyte nuclei judged to be in a later stage of mid-prophase (Fig. 4; corresponding to Fig. 1C) are similar to those described above, with the following exceptions: (a) Although the NE was still convoluted in certain areas, there exists an impression from serial sections that it is less convoluted than in earlier mid-prophase nuclei (data not shown). (b) The electron-translucent zone, which separates the tetrad from its associated membrane sheath, is diminished in size (cf., Figs. 3 and 4). (c) A reconstruction of these nuclei from serial sections indicates that the long axes of most of the chromosomal sheaths are oriented nearly parallel to one another (e.g., Fig. 4). As in earlier stages of mid-prophase, the NE was fenestrated, kinetochores could not be distinguished from the chromatin, and no MTs were present.

Late prophase nuclei (those corresponding to Fig. 1D) were 13–15- μm diam at their widest point. All of the chromosomal sheaths are near the middle of the nucleus, and all are oriented in the same direction. Sections through the middle of these nuclei, cut perpendicular to the long axis of the chromosomal sheaths, show most of the tetrads in the same section (Fig. 5). The NE remains somewhat convoluted but distinctly less so than in mid-prophase, and it is still fenestrated (Fig. 5, arrow). In cross-sections the tubular membrane components comprising the chromosomal sheaths appeared circular in profile and were difficult to differentiate from the vesicular membrane elements of the surrounding nucleoplasm. MTs and kinetochores are conspicuously absent from these nuclei (Fig. 6).

Metaphase (Five Nuclei Reconstructed)

As noted by Hughes-Schrader (16), the NE breaks down in *Xenos* oocytes sometime after the tetrads and their associated fusiform sheaths have become aligned within the nucleus (cf. Figs. 1, D and E; 5 and 8). The MA is then found attached to the chorion, which has reestablished itself (Figs. 7 and 8; [6,

16]). In longitudinal sections the MA appeared broadly fusiform and was 10 μm wide and 12 μm long. At this time individual chromosomal sheaths could not be differentiated from the additional membrane elements within the spindle. Kinetochores are now found to be associated with each tetrad, and these stain more electron-opaque than does the remainder of the chromosome (Figs. 7 and 8). Each tetrad is surrounded by a thin electron-translucent zone that is expanded in the kinetochore region (Fig. 7).

The fusiform appearance of the metaphase MA arose primarily from tubular membrane elements oriented roughly parallel to the long axis of the spindle. These elements appear to gently converge toward a diffusely defined polar area (cf. Figs. 1E and 7). An examination of serial sections through these metaphase spindles revealed 3–5 large membrane whorls (e.g., Fig. 7B asterisk and inset) which are usually, but not always, located in the vicinity of the polar areas. The polar areas were diffuse and contained no discrete polar organelles (centrioles, plaques, etc.) or electron-opaque aggregates of material, as found in the MA of mouse oocytes (48).

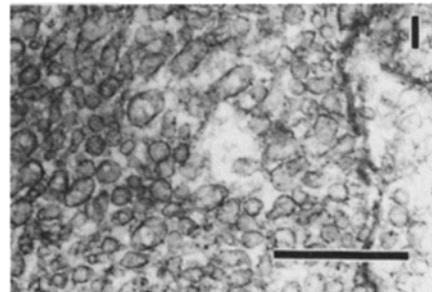
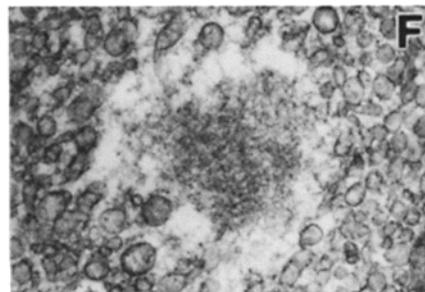
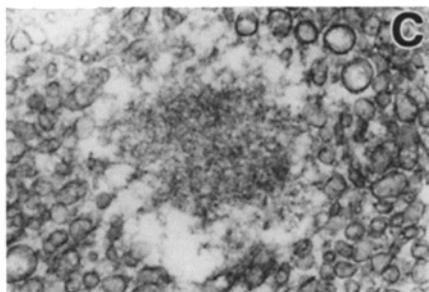
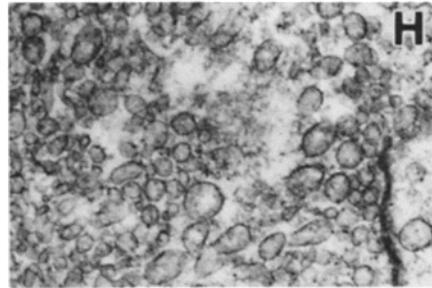
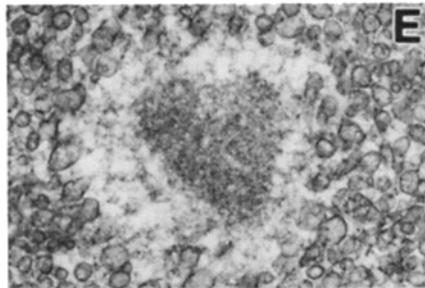
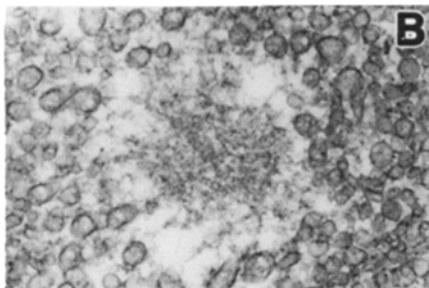
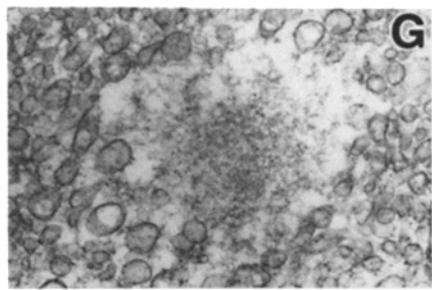
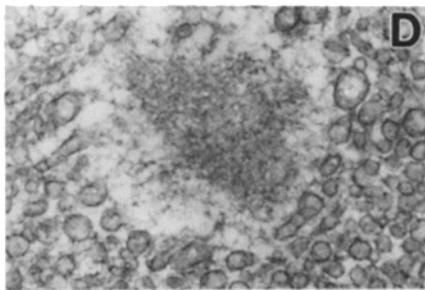
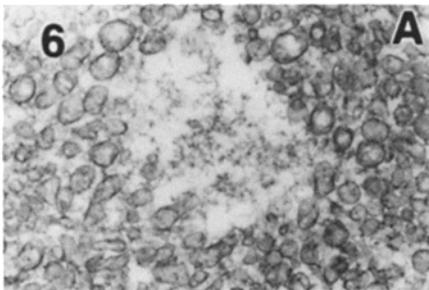
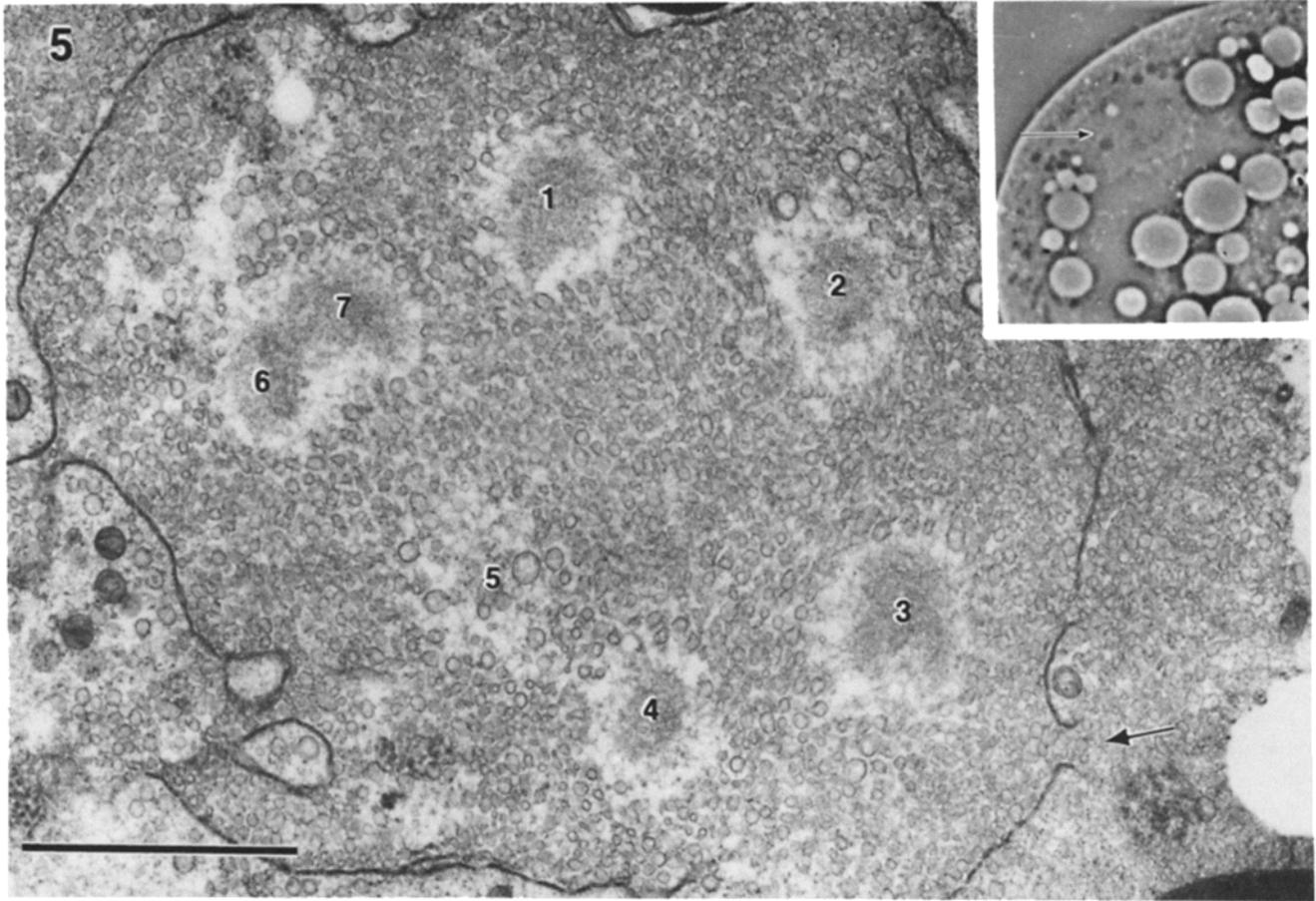
MTs could be seen in the thick longitudinal sections of *Xenos* metaphase spindles, but the overall density of the spindle caused by overlapping membranes makes it difficult to unequivocally identify them as MTs. They are, however, clearly visible in thinner longitudinal sections and in thick cross-sections of these spindles (Fig. 8). An examination of such sections revealed that these spindles contain both kinetochore and nonkinetochore MTs (Fig. 8, left inset). These MT appear scattered among, but not within, the membrane components of the spindle.

Sections cut perpendicular to the long axis of the metaphase MA revealed that the spindle boundary, as defined by the distribution of MTs (Fig. 8, large arrowheads), is surrounded by a thick layer of densely packed membrane elements from which MTs are largely excluded. Thus the spindle, containing the tetrads, membrane elements and MTs is ensheathed in an additional layer of membrane, and this spindle-membrane sheath boundary is very distinct (Fig. 8). Numerous 60–80-nm-diam electron-opaque deposits of an amorphous material are seen within the membrane region which ensheathes the spindle proper (Fig. 8, asterisks). This material, seen at high magnification (data not shown), was localized at the focal apex where radial arrays of elongated vesicular membrane elements converged.

DISCUSSION

On the whole, our ultrastructural observations correlate extremely well with Hughes-Schrader's (16) classic light micro-

FIGURES 3 and 4 Fig. 3: (A) Survey electron micrograph of a section through the mid-region of a mid-prophase *Xenos* oocyte nucleus. Three tetrads, each one surrounded by a clear zone and an associated membranous sheath, are pictured. Note that the long axis of each sheath differs in its orientation within the nucleus. The extremely convoluted NE, with fenestrae (e.g., arrows), is a characteristic feature of mid-prophase nuclei (cf. Fig. 1B). Areas that are relatively free of membrane elements are noted by the asterisks. (B) A mid-prophase tetrad and its associated membrane sheath. Note the absence of MTs. Bar, 3.0 μm . $\times 8,800$ (A); Bar, 2.0 μm . $\times 15,175$ (B). Fig. 4: Electron micrograph of a section through the middle of a mid-prophase *Xenos* oocyte nucleus which is at a later stage (cf. Fig. 1C) than that pictured in Fig. 3. The NE is less convoluted than that in Fig. 3, a smaller clear zone surrounds each tetrad, and the membrane sheaths of the four pictured tetrads are oriented along a similar axis. Bar, 3.0 μm . $\times 10,500$.



scopic observations concerning the unique method of spindle formation during the first meiotic division in *Xenos* oocytes. The intranuclear fusiform sheaths which she observed to form in association with each tetrad are clearly evident in our electron micrographs of 0.25- μm thick sections. The fact that they do not appear to be as well defined as those drawn by Hughes-Schrader is undoubtedly due, at least in part, to her choice of fixative, stain, and 5- μm section thickness. Kahle's fixative, containing a high percentage of ethanol, would be expected to artificially increase the definition of each sheath due to its coagulant action. Furthermore, iron haematoxylin (which was used by Hughes-Schrader because of its affinity for chromosomes) only lightly stained the sheath material against the background nucleoplasm—a fact that is mentioned in her text but not readily apparent from her drawings.

Our ultrastructural study has revealed additional important information concerning spindle formation in this organism. Specifically, we found that the intranuclear fusiform sheaths associated with each tetrad were devoid of MTs and consist primarily of short, parallel tubular membrane elements. The lack of MTs in these sheaths is, in fact, consistent with Hughes-Schrader's (16) observation that "from their earliest appearance the fusiform sheaths of the tetrads show no trace of fibrillar structure." It is also consistent with our observation that the tetrads in *Xenos* oocytes lacked well-defined kinetochores until after the NE broke down. Because our simultaneous glutaraldehyde-osmium fixation protocol preserved spindle MTs and kinetochores in fully formed metaphase-arrested *Xenos* oocytes, we are confident that it would also have preserved these structures, if present, in these early division oocytes. Therefore, an important conclusion of our study is that the long-standing assumption that the intranuclear fusiform sheaths associated with each *Xenos* tetrad contain MT and represent miniature bipolar spindles (32, 43, 45, 47) is erroneous. This assumption has been used to support the notion that chromosomes can form a spindle without reference to any extrachromosomal MT organizers (32, 45, 47)—i.e., that individual spindle fibers (MT) are formed from the kinetochore (8, 9, 28) and that these organelles contribute substantially to forming and organizing the spindle (27, 29, 30, 53). While there can be little doubt that kinetochores function to organize their associated fibers (regardless of the mechanism by which they acquire MTs), and while this contributes to the overall organization of the spindle, it remains to be clearly demonstrated that kinetochores themselves can form a functional bipolar spindle in the absence of extrachromosomal MT organizers or preformed MTs. Thus, Hughes-Schrader's (17) early observations on the meiotic prometaphase in Coccids, which have also been taken by

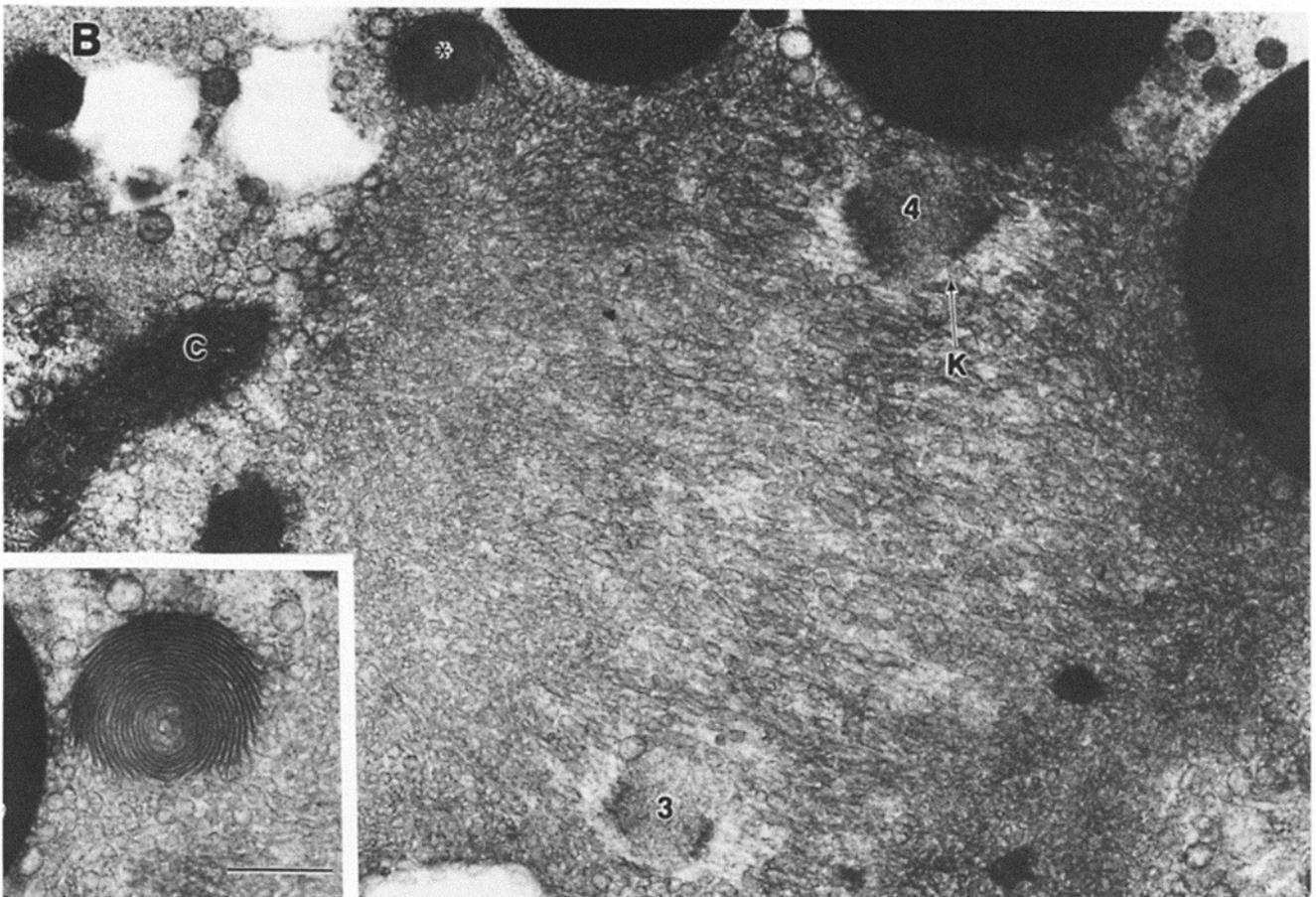
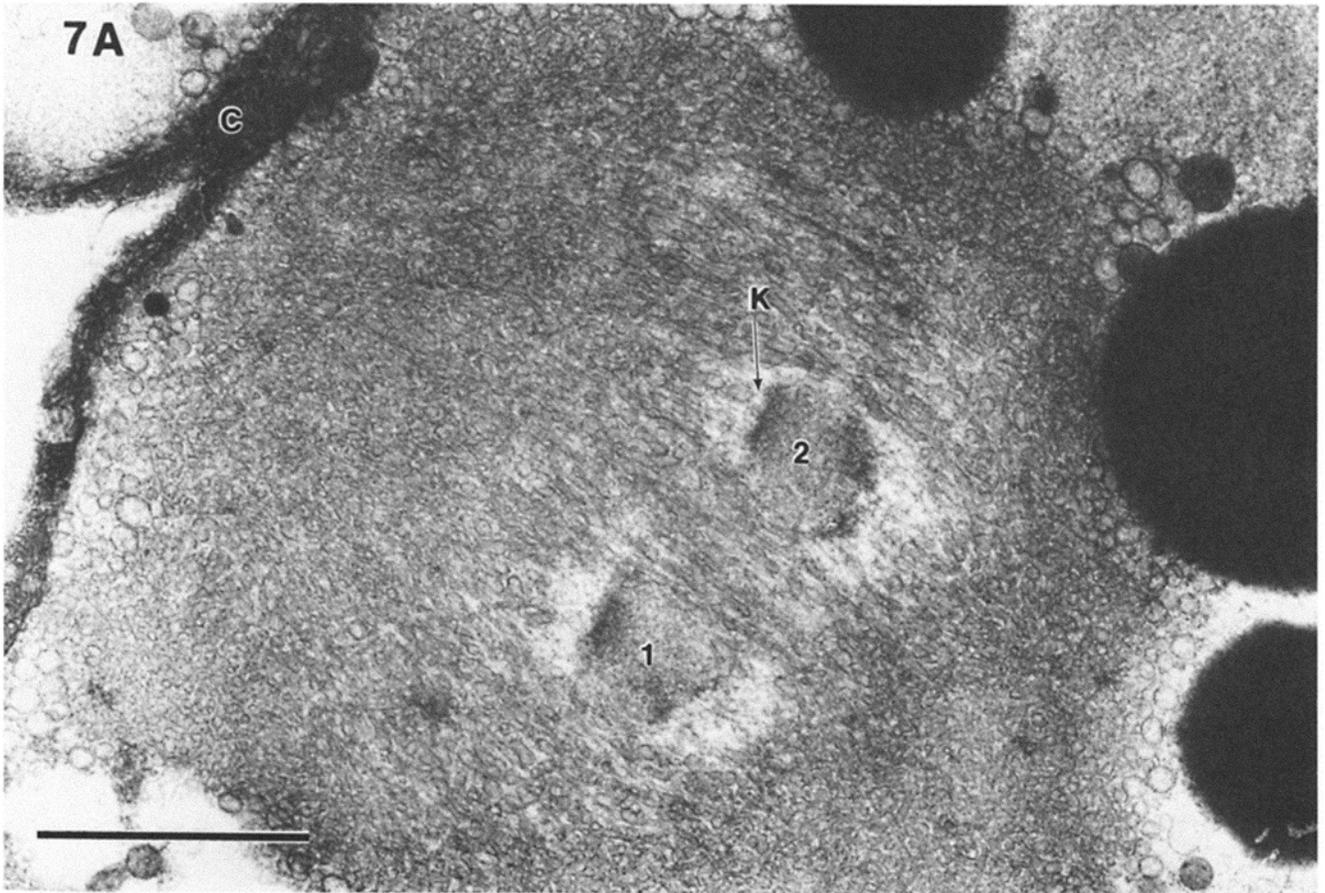
others (45) to indicate that kinetochores alone can independently organize a spindle, should be interpreted with care. Both the kinetochores and the polar areas in these organisms are diffuse and her original observations lack ultrastructural confirmation. While it is possible that the kinetochores on the *Xenos* tetrads nucleate their associated MTs, it is equally possible that they attach to MTs that form along existing parallel arrays of membrane sheath elements immediately after NE breakdown (see below). It should be noted that the latter possibility also offers a satisfactory explanation for the origin of the nonkinetochore spindle MTs in the absence of a well-defined polar microtubule organizing center.

Our ultrastructural observations suggest that the membrane elements that permeate the nucleus and form the fusiform sheath around each *Xenos* tetrad originate from the NE. However, the data are consistent with at least two possible alternative pathways: (a) These elements may arise from blebbing of the outer nuclear membrane as annulate lamellae form during late interphase (19) and then later invade the fenestrated nucleus as the annulate lamellae disperse during prophase, and/or (b) these membrane elements may arise inside the nucleus by blebbing from protrusions of the inner nuclear membrane which appear to be associated with the condensing tetrads. The key meiotic prophase event, which might resolve this question, appears to occur very quickly (see Results) and was not encountered in our study. However, either of the above mechanisms could ultimately produce the extremely convoluted and fenestrated NE characteristically seen in mid-late prophase *Xenos* oocyte nuclei.

Some of our observations may be relevant with respect to the mechanism by which the membrane components within the nucleus become aligned to form a distinct sheath around each tetrad. The short tubular membrane elements that form these sheaths appeared to arise near each tetrad from fusion of the more numerous and smaller vesicular components which permeated the nucleoplasm. This impression is based on the fact that these tubular elements were seen only in the vicinity of the chromosomes and that, as a rule, the volume of any individual tubular membrane component appeared to be greater than the volume of any individual vesicle within the nucleoplasm. The random orientation of the long axes of these sheaths during mid-prophase, and their intimate association with each tetrad, suggests that the tetrads themselves play a role in orienting these membrane elements. In this respect it is possible that this process was directed, in part, by membrane-chromatin interactions within the clear zone surrounding each tetrad.

We found that the long axes of the fusiform sheaths that form around each *Xenos* tetrad were initially oriented ran-

FIGURES 5 and 6 Fig. 5: Survey electron micrograph of a section through a late prophase *Xenos* oocyte nucleus at a stage similar to that pictured in Fig. 1D. The MA is cross-sectioned near its middle, and seven of the eight tetrads (as determined from serial sections) are caught in the plane of the section. Note that all of the membrane elements surrounding the tetrads appear circular in profile. Many of these are in reality cross-sections of tubular membrane elements (cf. Figs. 3 and 4) that form the sheath around each tetrad. There are no MTs within this nucleus (see Fig. 6: cf. Fig. 8). Arrows indicate obvious disruptions in the NE. Bar, 3.0 μm . $\times 11,500$. (Inset) A phase-contrast light micrograph of this nucleus (arrow) within an adjacent section. $\times 870$. Fig. 6: Sections 2, 4, 5, 7–10, 12, and 14, respectively, of a serial series through chromosome 1 in the late prophase nucleus pictured in Fig. 5. Note the absence of kinetochores and MT (cf. Fig. 8). Bar, 1.0 μm . $\times 17,500$.



domly with respect to each other, but that they became aligned parallel within the nucleus prior to the breakdown of the NE. This is consistent with Hughes-Schrader's (16) observations that suggest that a "metaphase-like" alignment of tetrads is reached in *Xenos* oocytes prior to NE breakdown. However, our results show that this intranuclear alignment of tetrads occurred during late prophase in the absence of spindle MTs. Therefore this process cannot be considered analogous to true prometaphase congression as commonly inferred from Hughes-Schrader's observations. Although the mechanism that generates this parallel alignment of chromosomal sheaths is unknown, there are several possibilities. It may be that one of the ends of each sheath is attached directly (or indirectly by additional membrane) to the inside of the NE and that forces generated at the surface of the NE lead to this alignment. This mechanism is suggested by Hughes-Schrader's (16) observation that the inner apices of the fusiform bodies approach a common point during this process, while at the same time their outer ends show varying degrees of divergence (Fig. 1D). Although speculative, this hypothesis draws support from those observations which indicate that pores within the NE can undergo an MT-independent "capping" (50) and that colcemid-insensitive prophase chromosome movements occur in crane fly spermatocytes in association with the NE (25). A possible alternative mechanism for this alignment, which also involves the NE, is suggested by the following observations of Hughes-Schrader (16): (a) "At all times each fusiform body maintains its form perfectly and seems quite rigid in its movements through the nucleoplasm," and (b) "After the formation and elongation of the fusiform bodies are well advanced, a change comes over the nucleus ... the nuclear membrane seems to shrink and tighten up around the fusiform tetrad sheaths ..." On the basis of her observations, and our observation that MTs were absent and therefore not involved in the alignment process, one can postulate that all of the sheaths and their associated tetrads became passively aligned within the nucleus from forces generated by a shrinking nuclear boundary. Such a mechanism, working alone or perhaps in combination with a tendency for chromosomal sheaths to interact laterally, would generate the metaphase-like alignment of tetrads characteristically seen in these late prophase oocytes.

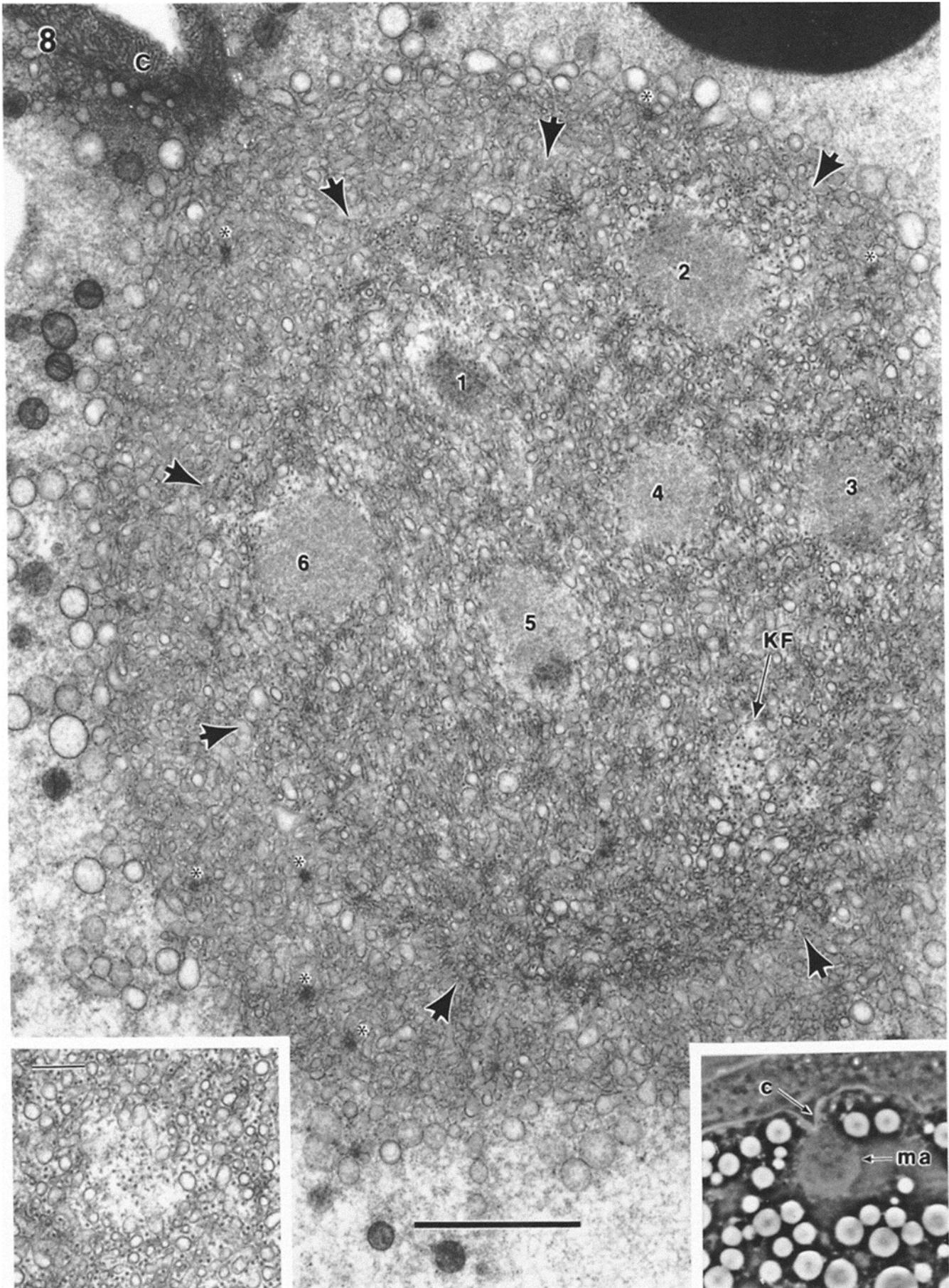
MTs first appear associated with the tetrads and membrane components of the *Xenos* MA after the NE has broken down and at this time they are oriented parallel to the tubular membrane components defining the long axis of the spindle (e.g., Fig. 7). The formation of spindle MTs in *Xenos* oocytes correlates with the formation of MT-containing cytasters in the cytoplasm of these eggs (C. Rieder, unpublished observations). Membrane-bounded vesicles indistinguishable from those in the spindle are associated with these cytasters. The

MT focal points of both the cytasters and the diffusely defined spindle poles lack a well-defined microtubule organizing center but contain an abundance of vesicles. There are a growing number of reports in the literature (reviewed in 14 and 33) showing that spindle (and other types of) MTs appear to be closely associated with and parallel the course of membranes. It has been suggested that membranes associated with the MA promote MT formation by functioning in a manner analogous to that of the Ca⁺⁺-sequestering sarcoplasmic reticulum of muscle (11, 13), and this concept has recently gained some experimental support (20, 44, 46, 51, 54). If the membranes associated with the metaphase *Xenos* MA do regulate MT assembly/disassembly, then the oriented tubular components of the chromosomal sheaths may act, after NE breakdown, as a scaffold not only for generating spindle bipolarity but also for establishing spatial relationships between the MT components of the spindle.

The metaphase-arrested *Xenos* oocyte spindle is surrounded by a thick layer of vesicular membrane elements from which MTs are largely excluded. The structural integrity of this membranous layer appears to be based in part on deposits of an electron-opaque material (glue?) which are localized at the focal apex where radial arrays of these membrane components converge. The membrane elements comprising this sheath are indistinguishable from and are intimately associated with the membranous components within the spindle proper. A similar but less elaborate membrane system, thought to be derived from the NE-endoplasmic reticulum complex, has been shown to surround and permeate the MA of many organisms (reviewed in 14 and 33) including sea urchin eggs (10), *Bombix mori* spermatocytes (15), spider spermatocytes (52), barley (13), onion root tip (37), and HeLa (31) cells. It has been suggested that these membranes play a role in regulating the local ionic environment (see above), and also that they act as a barrier that blocks yolk granules, mitochondria, and other cytoplasmic organelles from entering the spindle. The membrane sheath surrounding the metaphase *Xenos* MA may also have an additional function: Since the spindle MTs in the MA of this organism appear to lack well-defined (polar) anchor points, the membrane components within and surrounding the spindle may stabilize all of the tetrads in a bipolar metaphase arrangement until fertilization, which may occur up to 14 d (16) after the spindle has formed.

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FIGURE 7 Two sections from a *Xenos* oocyte MA in the same (metaphase) stage as the nucleus pictured in Fig. 1E. The NE has disappeared, the chorion (c) has reestablished itself, and the tetrads are aligned on a metaphase plate. Each tetrad is surrounded by a clear zone, and individual membranous chromosomal sheaths are impossible to delineate from the additional membrane elements within the spindle. MTs, which are more easily visualized at this magnification in cross-sections of metaphase nuclei (e.g., Fig. 8), are associated with the kinetochores (e.g., K). Each kinetochore stains more electron-opaque than the remainder of the chromosome. The asterisk in the upper left corner of B indicates a membrane whorl which is pictured, in the adjacent section, within the inset in B. Bar, 3.0 μ m. \times 12,000. (Inset) Bar, 1.0 μ m. \times 13,500.



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FIGURE 8 Electron micrograph of a section through a *Xenos* oocyte metaphase MA (cf. Fig. 1F). This MA has been cross-sectioned, and six tetrads plus one obvious kinetochore fiber (kf) are pictured within this section (cf. Fig. 5). Note that the kinetochores on chromosomes 1 and 5 are more electron-opaque than the remainder of the chromosome. Numerous spindle MTs can be seen in the region of the MA outlined by the large arrowheads. The asterisks near the spindle periphery denote areas where membranes appear attached to one another by an electron-opaque material. Bar, 2.0 μ m. \times 15,500. (lower left inset) Higher magnification micrograph of kinetochore and nonkinetochore MTs. Bar, 0.5 μ m. \times 18,750. (lower right inset) Phase-contrast light micrograph of a section through this nucleus. Note that the chorion (c) appears intact and contacts the MA via an invagination. \times 920.