Meiosis in *Sciara coprophila*: Structure of the Spindle and Chromosome Behavior during the First Meiotic Division

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ABSTRACT Light microscope descriptions of meiosis I in males of the fungus gnat *Sciara coprophila* suggested the presence of a monopolar spindle in which maternal and limited chromosomes move poleward while paternal chromosomes "back away" from the pole. The ultrastructural analysis reported here, based upon serial sections of cells in different stages of meiosis I, shows that the spindle is indeed monopolar with a distinctive differentiation, the polar complex, at one pole. This complex is the focus of a conical radiation of spindle microtubules. Kinetochores of paternal chromosomes face the complex and microtubules associated with these kinetochores run toward the complex. No kinetochore microtubules were discovered on maternal or limited chromosomes. When the position of paternal, maternal, and limited chromosomes is compared at various stages, it is found that limited chromosomes always remain near the polar complex, paternal chromosomes remain far from it and only maternal chromosomes move closer to the pole. Apparently, chromosome segregation does not depend on paternal chromosomes "backing away" from the pole, and the required movement of maternal chromosomes takes place in the absence of kinetochore microtubules. In the prophase nucleus, limited and maternal chromosomes are already spatially separate from paternal chromosomes before the spindle forms. Thus, the monopolar spindle functions only to increase the distance between already segregated sets of chromosomes. An extensive system of microtubule-associated membranes outlines the spindle; the possibility that maternal chromosome movement is somehow related to the presence of this membrane is discussed.

The precise chromosome behavior characteristic of mitosis and meiosis is understood, at least in purely mechanical terms, as resulting from the interaction of chromosomes with a spindle of bipolar symmetry (e.g. see reference 12). In typical meiosis I, for example, homologous maternal and paternal chromosomes pair during prophase and these pairs congress midway between the two spindle poles. At this stage, metaphase, each pair of chromosomes is linked with both spindle poles via chromosomal spindle fibers, the fiber associated with one chromosome running toward one pole, the fiber of the other running toward the opposite pole (bipolar orientation). In anaphase, the two chromosomes of each pair separate, and each moves toward the pole to which it is linked by a spindle fiber. In other words, the twofold symmetry of the spindle and the symmetrical manner in which chromosomes engage on the spindle is fundamental for the separation of equivalent sets of chromosomes.

Descriptions of monopolar spindles in several insects (for references, see 25, p. 22) challenge the generalization that the spindle is "essentially always a bipolar structure" (12). Perhaps most widely-known is the spindle of meiosis I in males of the lower dipteran *Sciara*. According to Metz and his co-workers (16, 17), this spindle is unusual because of its asymmetry or monopolarity, i.e., it is a conical structure with one acuminate pole and an opposite broad base. This monopolar spindle regularly segregates two sets of chromosomes and is, therefore, no less effective than a conventional bipolar spindle. However, the precise behavior of chromosomes throughout meiosis in *Sciara* and the chromosome composition of the segregated chromosome sets suggests important differences between the function of the monopolar spindle and more ordinary bipolar meiotic spindles.

The following is a summary of the light microscope descriptions of chromosome behavior in *Sciara coprophila*, the best studied species (review: reference 16). Primary spermatocytes usually contain ten chromosomes, four paternal, four maternals and two limiteds (limiteds are chromosomes present only in the germ line and they may be of either maternal or paternal origin; they vary in number both between individuals and in cells of one individual [23]). In prophase I, maternal and
paternal chromosomes do not pair. When the spindle forms, the ten unpaired chromosomes remain scattered approximately as they were in the prophase nucleus and never become aligned in a metaphase-like array. Each chromosome is associated with a spindle fiber and all of these chromosomal fibers run toward the acuminate spindle pole. Despite this identical orientation of all chromosomes, some move toward the pole while others move away from it, striking contrast to chromosome behavior in typical spindles where chromosomes always move progressively closer to a spindle pole. The fact that S. coprophila males do not transmit their paternally derived genetic traits (26) points to another functional oddity of this monopolar spindle. Chromosomes which back away from the spindle pole eventually degenerate; thus, these chromosomes are obviously identifiable as of paternal origin. Chromosomes which move toward the pole include only maternals and the genetically inert limited chromosomes. This nonrandom chromosome segregation distinguishes the monopolar meiosis of Sciara from more conventional bipolar spindles where each of the segregated chromosome sets includes a random assortment of maternal and paternal chromosomes.

If the light-microscopic observations of Sciara meiosis are accepted at face value, they present “difficulties for all mitotic hypotheses thus far devised” (25). It has been suggested that the spindle is in reality bipolar, the “monopolar” appearance resulting because one pole is acuminate while the other is diffuse (25). Even if this is so, the perfectly nonrandom segregation effected by such a bipolar spindle requires further explanation. Also, the unusual orientation of a V-shaped metacentric paternal chromosome which moves with its arms, not the kinetochore, leading the way to the diffuse pole remains unexplained. The suggestion that neocentric activity at the chromosome ends is responsible for the peculiar configuration of this particular chromosome (13) has been negated by recent experiments reported by Abbott, et al. (1).

The ultrastructural evidence presented here confirms some of the light microscope observations, in particular, the meiosis I spindle in S. coprophila is obviously monopolar, and paternal chromosomes are indeed oriented toward the one pole. In addition, the new evidence in this report explains nonrandom segregation as the result of differences between the kinetochore activity of paternal chromosomes and maternals and limited chromosomes, as was suspected by Luykx (11) and by Bajer and Molé-Bajer (3).

MATERIALS AND METHODS

Cultures containing fourth instar male larvae of Sciara coprophila derived from male-producing females of the stock 6980 (1) were generously provided by Dr. Susan A. Gerbi (Division of Biology and Medicine, Brown University, Providence, RI). These were maintained at 16–18°C.

As prepupae and pupae appeared in the cultures, they were transferred to solid 2% agar in petri dishes where subsequent development could be observed with ease. When the pupal eyes were ½ to ¾ pigmented, the stage at which meiotic divisions are most numerous (Dr. Helen V. Crouse, personal communication), testes were dissected into Shen’s Ringer solution (0.9 g NaCl, 0.42 g KCl, 0.25 g CaCl₂, per liter), freed of fat, and transferred to freshly prepared modified Karnovsky fixative (24) (pH 6.8, 1 h at room temperature). All further preparations for electron microscopy, including serial sectioning procedures, were as described previously (24).

Although pupal eye pigmentation aids in selecting testes certain to contain cells in spermatogenesis, the stages during which the meiotic spindle is present are apparently transitory. Usually, a testis will contain none, or only few cysts, of cells at these important stages. Unfortunately, it is not possible to preselect individual cells in desired stages by light microscopy before electron microscopy has been done for other spermatocytes (e.g., see reference 20). This is because the large refractile mass of mitochondria always present in spermatocytes hides nuclei and spindles from view (7). Therefore, to find the relatively rare meiotic stages which might be present in a given testis, serial sections through ~½ of the testes were prepared (~500 sections). The approximate stage of each cyst within the section series could then be judged by electron microscopic examination at low magnification using criteria defined by Metz, et al.'s light microscope studies (17). In all testes I examined, meiotic spindles were rare. Fortunately, one testis proved to contain cells in a variety of stages ranging from prophase of meiosis I through metaphase of meiosis II. The observations reported here are based on the analysis of six cells from this testis.

Micrographs were taken with a Siemens 101 electron microscope or a Zeiss 104a electron microscope using 70 mm roll film (Kodakith LR 2372). Magnifications ranged between 1,600 and 4,600 times. When the lowest magnifications were used, a number of cells in one cyst were included in each micrograph. These negatives were used to assess the relative synchrony between cells of a cyst and to identify cells sectioned in an orientation appropriate for further analysis. Serial micrographs of the cells chosen by this means were then prepared at increased magnification.

Two- or three-dimensional reconstructions were made to clarify the relationships between chromosomes, spindle fibers, polar structures, membranes, etc. They were prepared in accordance with the general procedures already described (10).

RESULTS

General

The peculiarity of meiosis I in S. coprophila males precludes use of conventional terms to identify stages of meiosis. Because homologous chromosomes do not pair in prophase (17), there are no stages corresponding to leptotene through diakinesis; and, because chromosomes do not align at the spindle equator before poleward movement is initiated (17), the terms prometaphase, metaphase, and anaphase are meaningless. In describing this meiotic division, then, various stages will be designated simply early or late prophase followed by mid or late meiosis I. The criteria for the stage designations will become apparent as the description is given.

Cell Selection: For an account of testis structure and meiosis I in Sciara based upon light microscopy, consult Metz et al. (17) and Metz (16). In summary, each spherical testis is composed of a number of cysts. In each cyst (Fig. 1), spermatogenic cells are arrayed at the periphery so that a cyst sectioned near the center appears as a rosette of cells surrounding a central noncellular area. At meiosis I, the “monopolar” spindle forms with its long axis radial to the cyst center and chromosomes thus pass toward or away from the center; only those chromosomes which move toward the periphery of the cyst (limited and maternals) are eventually included in sperm while the ones that move toward the interior of the cyst (paternals) are enclosed in a small bud destined to degenerate. Because buds accumulate within the cyst as a result of the first meiotic division, scanning of serial sections of the testis allows rough classification of cysts as follows: prophase (no buds in cyst, chromosomes more or less condensed, nuclear envelope intact and cytoplasmic microtubules present in most cells); mid-to-late meiosis I (no buds, spindle present in majority of cells); post-meiosis I (buds but no spindles present); meiosis II (buds and spindles present). One testis was found to contain cysts in all of these categories, and the cells described in this paper were selected from one prophase cyst and one mid-to-late meiosis I cyst of that testis. Some asynchrony is evident in each cyst. In the prophase cyst, the degree of chromosome condensation was an immediate clue to early vs. late stages; from this cyst an early prophase cell (slight chromosome condensation) and a meiosis I cell (spindle present) were chosen for analysis. Similarly, asynchrony within the mid-to-late meiosis I cyst permitted choice of cells in various stages. However, an estimate of the relative stage of each cell was
meiosis I chromosomes are scattered throughout the cell while a similar stage were examined in serial low magnification traces meiosis I in reverse order. Late meiosis I is consid-erred first because questions raised by older descriptions of meiosis in *S. coprophila* can be answered unequivocally on the basis of that description. Next, cells in intermediate stages of meiosis I chromosomes are scattered throughout the cell while paternal chromosomes \( (P) \) are nearer the interior of the cyst.

**SEQUENCE OF DESCRIPTION:** For clarity, this description traces meiosis I in reverse order. Late meiosis I is considered first because questions raised by older descriptions of meiosis in *S. coprophila* can be answered unequivocally on the basis of that description. Next, cells in intermediate stages of meiosis I (mid meiosis I) are considered; in these, features are found which were not anticipated by light microscopy. The newly discovered characteristics of meiosis I are, in turn, understandable only with reference to the unusual structure of prophase nuclei. Therefore, prophase cells are described last.

**Late Meiosis I**

This description is based upon complete three-dimensional reconstruction of a single cell. However, six additional cells of a similar stage were examined in serial low magnification micrographs and found to have comparable characteristics.

**POLAR COMPLEX:** A distinctive ringlike structure, the polar organelle (Fig. 2), appears at one end of the meiotic spindle, at the pole nearest the exterior of the cyst. This structure is perhaps related to but not identical with the "giant centrioles" described by Phillips (21). There is only one polar organelle per cell, and its location is invariant. A flocculent mass of intermediate electron density encircles the polar organelle. Together, the organelle plus the concentric ring of material surrounding it will be referred to as the polar complex. Microtubules composing the meiosis I spindle radiate from the vicinity of the polar complex (Fig. 2).

**SPINDLE STRUCTURE:** Spindle microtubules diverge from the region of the polar complex and run toward the end of the cell closest to the interior of the cyst. Thus, the spindle is a roughly conical structure with the acuminate pole (vertex) always positioned near the exterior of the cyst and the base near the interior. Spindle dimensions are given in Table 1. For a more precise appreciation of spindle shape, see the description of mid meiosis I and Fig. 7.

Spindle microtubules are unevenly distributed so that in reconstructions one finds small bundles of microtubules which course over all or part of the spindle length. In addition, some of the microtubules are associated with a system of membranes outlining the spindle area. This membrane system is not so prominent at late meiosis I as it is in earlier stages (see the description of mid meiosis I), but the juxtaposition of mem-brane and microtubules at the spindle boundaries is discernible upon close inspection (Fig. 2). Some microtubule bundles are associated with chromosomes while others simply terminate at various positions along the length of the spindle or impinge on membrane and become indistinguishable from the membrane-associated microtubule complex.

In contrast with the acuminate spindle pole where the polar complex lies at the focus of spindle microtubules, the opposite broad end of the spindle exhibits no polelike focus of micro-tubule termination. Rather, the membrane system continues around the broad end of the spindle and microtubules associated with this membrane system end at indeterminate positions on the membrane (observed in three-dimensional reconstruc-tions but not illustrated).

**CHROMOSOME DISTRIBUTION:** On the late meiosis I spindle, the segregation of chromosomes is virtually complete in that two sets of chromosomes are spatially separated (Fig. 3). According to the cytological and genetic evidence of Metz and his collaborators (for review, see reference 16), chromo-somes which collect near the acuminate spindle pole (near the polar complex) include maternals plus a variable number of limited chromosomes while the set at the opposite broad end of the spindle includes only the four paternal chromosomes (Fig. 1).

In the cell under consideration, maternal and limited chro-mosomes form a compact, roughly prolate mass near the polar complex (Fig. 3a). They are gathered so closely that it is not possible to verify that the group contains an appropriate num-ber of chromosomes. No kinetochores were found within this group of chromosomes; i.e., although the chromosomes are in an area of relatively high microtubule density and microtubules touch or even penetrate chromatin, no specific sites of termina-tion of microtubule bundles were found on chromosomes near the acuminate spindle pole.

The four paternal chromosomes appear at the broad end of the spindle, about six micrometers from the maternal chro-mosome group (Fig. 3b). These are not closely grouped but are well-separated from each other within the broadest spindle region, exactly as described by Metz et al. (17). Each paternal chromosome bears a kinetochore (Fig. 4) most readily recog-nized by its association with a bundle of microtubules. These kinetochores are minimally differentiated and appear simply

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**Figure 1** Diagram of the meiosis I cyst as seen in a section near the middle of the cyst. In each cell, the spindle is oriented radial to the cyst center so that maternal and limited chromosomes \( (M & L) \) accumulate at the end of the cell nearest the periphery of the cyst while paternal chromosomes \( (P) \) are nearer the interior of the cyst.
TABLE I
Dimensions of Spindles during Meiosis in S. coprophila

<table>
<thead>
<tr>
<th>Meiotic stage and cell identification</th>
<th>Spindle length* μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid meiosis I, cell a</td>
<td>14</td>
</tr>
<tr>
<td>Mid meiosis I, cell b</td>
<td>14</td>
</tr>
<tr>
<td>Mid meiosis I, cell c</td>
<td>12</td>
</tr>
<tr>
<td>Late meiosis I</td>
<td>13</td>
</tr>
</tbody>
</table>

* Distance between the polar organelle and the chromosome end farthest from it; calculated from 
\[ a = \sqrt{b^2 + c^2} \]
where \( a \) is spindle length, \( b \) is the distance between the polar organelle and the farthest chromosome end in the two-dimensional reconstruction divided by the magnification of the reconstruction, and \( c \) is the number of sections between polar organelle and chromosome end multiplied by the average section thickness.

as a mass of fibrils of finer texture and lesser electron density than the remainder of the chromosome. On three of the chromosomes, the kinetochore is at the end of the chromosome and on the fourth it is approximately at the middle (e.g. see Fig. 3 b). In other words, the chromosomes farthest from the acuminate spindle pole include three acrocentric chromosomes and one metacentric. This is precisely the number and form of chromosomes expected for the paternal set (17).

The orientation of paternal chromosomes is unambiguous: the kinetochore of each chromosome faces the polar complex and all kinetochore microtubules thus run toward the acuminate spindle pole (Figs. 3 b and 4). Moreover, chromosome shapes suggest that the kinetochore microtubules are exerting at least some tension toward the spindle pole. This is most

Figure 3 Reconstruction of parts of the late meiosis I cell showing chromosome segregation. (a) Maternal and limited chromosomes (M & L) are grouped near the polar organelle (po), (b) Paternal chromosomes lie far from the polar organelle. In this portion of the reconstruction, only two of the four paternals are included, the metacentric (m) and an acrocentric (a); arrows represent their kinetochore fibers (cf. Fig. 4).

Figure 2 (a) Late meiosis I, portion of the spindle nearest the exterior of the cyst. The polar complex contains a polar organelle (po) in the form of an electron-dense ring ~1 μm in diameter (see Fig. 5 for demonstration of the overall form of the organelle). The organelle lies at the spindle pole with its diameter perpendicular to the spindle long axis; therefore, in sections near the center of the spindle and parallel to the spindle long axis as illustrated here, the organelle appears as two electron-dense segments of the ring. Flocculent, intermediately electron-dense material (f) forms a concentric ring around the organelle. Some of the chromosomes (c) are grouped near the polar complex and spindle microtubules (mt) are relatively sparse at this stage (cf. mid meiosis, Fig. 6). A system of membranes is found at the margins of the spindle (arrowheads) and the close apposition of these membranes and microtubules is often recognizable. This membrane-microtubule relationship appears as either the parallel alignment of microtubules and membrane profiles (1) or the association of microtubules and tangentially sectioned membrane (2). Bar, 1 μm × 28,000.
(b) Association of membranes and microtubules. The majority of microtubules (mt) associated with the membrane (m) surrounding the spindle occur on the spindle face of the membrane, i.e., on the side of the membrane facing the chromosome (c), but some are found on the cytoplasmic face of the membrane, i.e., on the side facing a mitochondrion (mt). Bar, 1 μm × 34,100.
particularly evident in the case of the metacentric chromosome which is V-shaped with the kinetochore at the V apex and facing toward the polar complex; precisely such a chromosome configuration is seen for metacentric chromosomes in conventional bipolar spindles while they are moving poleward under tension exerted by kinetochore fibers. Similarly, the acrocentric chromosomes are extended along the length of the spindle so that they appear as rods aligned along radii drawn from the polar complex to the base of the spindle.

**CONCLUSIONS:** In summary, Metz and co-workers' description of the meiosis I spindle of *S. coprophila* spermatoocytes as a structurally and functionally monopolar structure is verified. The presence of the polar complex, a differentiation undetectable by light microscopy and so not described by Metz, and the absence of any comparable polar differentiation at the opposite broad end of the spindle certainly demonstrates the structural asymmetry of this spindle. Functional monopolarity is decisively established by the fact that the position of all chromosomes, maternal as well as paternal, is determined with reference to the polar complex—the acuminate end of the spindle is not only the focus of maternal chromosome accumulation, it is also the focus toward which the more distant paternal chromosomes orient via kinetochore microtubules.

**Mid Meiosis I**

Three cells in mid meiosis I, cells *a*, *b*, and *c*, were examined in detail. All three are considered to be in relatively earlier stages than the cell already described because chromosomes are scattered throughout the length of the spindle. Cell *a* was found in the prophase cyst, the remaining two in the mid-to-late meiosis I cyst.

In mid meiosis I, some aspects of spindle structure are as described for late meiosis I: an identical polar complex lies at the acuminate spindle pole (Figs. 5 and 6), the distribution of microtubules within the spindle is similar and spindle shape and dimensions (Table 1) are approximately the same. Despite these general similarities, it soon becomes apparent that cells in early stages of meiosis I are identifiable in single sections without resorting to reconstructions. This is possible because the entire spindle area has a distinctive appearance (Fig. 6). Three factors contribute: (a) the system of membranes outlining the spindle is decidedly more prominent than in late meiosis I; (b) microtubules are numerous and, in particular, the microtubules associated with membrane are more closely spaced (Fig. 6 b); and (c) the spindle matrix has a recognizable character—small electron dense patches within the spindle area clearly differentiate the spindle from surrounding cytoplasm. Together, these characteristics render mid meiosis I cells immediately identifiable even when the distribution of chromosomes within the spindle is not known.

Reconstructions which include chromosomes as well as membrane profiles at the spindle margins were prepared (e.g. see Fig. 7). With regard to the membrane system, such reconstructions are imperfect due to the difficulty of recognizing membranes sectioned tangentially. Nevertheless, the general outline of the spindle area as defined by the membrane system is well-represented by such reconstruction, as is the manner in which membranes loosely follow the chromosome contours.

The relative positions of chromosomes within each mid meiosis I spindle are summarized in Fig. 8. On many chromosomes, there is one discrete mass of fine fibrils having a finer texture and lesser electron density than the chromosome...
itself (Fig. 9). Such regions are presumed to represent kinetochores; and in Fig. 8 arrowheads denote their position. Certain of the presumed kinetochores are associated with microtubules (Fig. 9b), a fact not always easily ascertained in single sections due to the few microtubules involved. However, reconstructions prepared at high magnification which include chromosomes and all nearby microtubules verify that the finely fibrillar region of the chromosome is an exclusive site of microtubule termination. All microtubule-associated kinetochores are oriented toward the polar organelle (Fig. 8); note, however, that presumed kinetochores that are not associated with microtubules are not necessarily similarly oriented (Figs. 8b and 9; the kinetochores on chromosomes identified by arrows in Fig. 8b are illustrated in Fig. 9).

When the distribution of chromosomes in the spindle and, in particular, the positions of chromosomes bearing microtubule-associated kinetochores is considered, four chromosomes lying farthest from the acuminate spindle pole are almost certainly identifiable as the paternal set of chromosomes. The several observations which, taken together, lead to this conclusion are illustrated in Fig. 8. These are: (a) In each cell, four chromosomes of the complement lie in the one-third of the spindle farthest from the polar complex. (b) Where it is possible to recognize relative kinetochore position on all four of these chromosomes (cells b and c), the chromosomes include three acrocentrics and one metacentric. (c) The only microtubule-associated kinetochores found in these cells occur on chromosomes in the one-third of the spindle farthest from the polar complex. (d) The greatest number of microtubule-associated kinetochores in any cell is four (cell c). Since only the four paternal chromosomes are associated with microtubules in late meiosis I, and here again no more than four chromosomes show such microtubule association, it is logical to conclude that paternals are the chromosomes shown in grey in Fig. 8.

If, as is indicated by the mid meiosis I cells selected for detailed analysis, paternal chromosomes are always positioned farther from the acuminate spindle pole than maternals and limiteds, we must consider the possibility that Metz and his co-workers were incorrect in concluding that chromosome segregation is accomplished because paternal chromosomes move away from the acuminate spindle pole. Additional evidence against that conclusion is the fact that spindle length in the three mid meiosis I cells does not differ from the length in late meiosis I (Table 1, Fig. 8) as would be expected if the distance between the pole and paternals increases during the period of chromosome segregation. In fact, there are suggestions that paternal chromosomes may move slightly toward the polar complex. This impression is gained from examination of three-dimensional reconstructions which demonstrate the following: (a) When the fewest kinetochore microtubules are present (cell a), all four putative paternal chromosomes are rather compactly coiled, indicating that the chromosomes are subject to little or no tension. (b) When a reasonable number of kinetochore

**FIGURE 5** Polar complex of mid meiosis I, cell b. As in late meiosis I, the polar complex consists of an electron-dense polar organelle (po) within a concentric mass of flocculent material (f); microtubules (mt) radiate from the vicinity of this complex. The oblique sections shown (four nonadjacent sections of a series of 13) clearly indicate the ringlike form of the complex. A membrane profile (m) appears in the lumen of the polar organelle, but its relationship with membranes surrounding the spindle could not be determined due to the difficulty of reconstructing membrane. Bar, 1 μm. × 29,200.
Microtubules are present on two of the four chromosomes (cell b), only the two paternals with no kinetochore microtubules, those farthest from the spindle pole, are coiled; in contrast, the two chromosomes associated with a reasonably well-developed bundle of kinetochore microtubules are relatively extended as if subject to tension and the kinetochores are closer to the polar complex. (c) When all four paternals are associated with microtubules (cell c), two chromosomes are rather coiled and two are extended as is reasonably clear even in the two-dimensional view shown in Fig. 8; kinetochores of the extended chromosomes lie closest to the spindle pole. Combined with evidence from the late meiosis I cell (well-developed kinetochore fibers on each paternal, and all paternals extended along the spindle long axis), these observations suggest that kinetochore microtubules exert a force on paternal chromosomes which tends to (a) extend the chromosome along the spindle axis and (b) move the kinetochore relatively closer to the pole.

In addition to the four chromosomes just discussed, each spindle includes six chromosomes which occupy the two-thirds of the spindle closer to the acuminate pole. Two of these six are appreciably larger than any other chromosomes and are identified as the large limited chromosomes (16, 23). The remaining four are, presumably, the maternal chromosomes.

In each cell, the two limited chromosomes are found to one side of the spindle near the pole, ~1.3 micrometers from the polar complex. In every case, the limiteds are so closely associated that the two cannot be distinguished when serial sections are examined. However, in three-dimensional reconstructions, the overall size and shape of the limited-chromatin mass discloses the presence of two closely apposed chromosomes. This relationship is shown in the two-dimensional reconstructions (Fig. 8). Neither microtubule-associated kinetochores nor presumptive kinetochore-like regions were found on any limited chromosomes of the mid meiosis I cells.

While paternal chromosomes were found only far from the spindle pole and limited chromosomes only close to it, the spindle position of putative maternal chromosomes is variable. For the three cells, the differences in number of chromosomes found within the middle one-third of the spindle versus the one-third closest to the pole (Fig. 8) indicates that the spatial segregation of maternal and paternal chromosome sets is effected because maternal chromosomes move progressively poleward.

As indicated in Fig. 8, one region on the majority of maternal chromosomes is distinctly kinetochore-like insofar as it appears as a mass of fine fibrils of intermediate electron density (Fig. 9). Surprisingly, however, in reconstructions of the chromosomes and all nearby microtubules, neither the presumed kinetochores nor any other region of the chromosome shows a specific association with microtubules. Presumed kinetochores are usually near or in contact with the membrane surrounding the spindle (Fig. 9a); this is true for all maternal kinetochores of cells b and c. When we recall that large areas of these membranes are closely associated with microtubules, it seems likely that kinetochores of maternal chromosomes could interact specifically with microtubules in such regions. Yet, no evidence for this was found.

Conclusions: Kinetochores of paternal chromosomes associate with microtubules during mid meiosis I so that by late meiosis I each paternal exhibits a well-developed kinetochore fiber. However, the presence of paternal kinetochore fibers is correlated with no appreciable chromosome displacement. A slight poleward movement of kinetochores may be responsible for the extension (uncouling) of the chromosome so that the chromosome arms align with the spindle long axis; but, throughout meiosis, paternal chromosomes maintain approximately the same relative position within the spindle, i.e., at the broad end, far from the polar complex.
Figure 8 Chromosome positions in mid meiosis spindle, cells a, b, and c. These drawings, traced from two-dimensional reconstructions, indicate the general disposition of the ten chromosomes in each cell relative to the polar complex (*). The locations of microtubule-associated kinetochores (●) and of kinetochore-like regions free of microtubules (▲) are shown. Criteria for identifying particular chromosomes as limited (black), maternal (white), or paternal (grey) are given in the text. Arrows identify chromosomes illustrated in Figs. 9 a and b. Metacentric chromosomes (m) are the only chromosomes with median kinetochores.

Figure 9 Kinetochore in mid meiosis I, cell b. (a) The maternal chromosome indicated by an arrow in Fig. 8 b shows a region of fine fibrils that is less electron-dense than the remainder of the chromosome; this is presumed to represent the kinetochore (kc). Because no microtubules were found associated with this differentiation, its kinetochoric nature is, of course, uncertain. Note the proximity of this presumed kinetochore and the membrane (m) surround the spindle. (b) The paternal chromosome indicated by an arrow in Fig. 8. The kinetochore (kc) is a mass of fine fibrils associated with several microtubules (mt). The chromosomes in Figs. 8 and 9 are shown in the same view; that is, the polar complex lies toward the top of the page. With this in mind, note that the microtubule-associated paternal kinetochore is oriented toward the polar complex while the microtubule-free kinetochore-like region on the maternal chromosome is turned away from the complex. Bar, 1 μm. × 37,400.

The remaining chromosomes, maternals and limiteds, never form kinetochore fibers. Judging from the relative position of chromosomes in the spindle in different cells, limiteds are always close to the spindle pole and only maternals undergo significant poleward movement. The structural basis for this movement is unclear.
Prophase of Meiosis I

The evidence already presented suggests that paternal chromosomes always lie far from the acuminate spindle pole in meiosis I, effectively segregated from maternal and limited chromosomes from mid meiosis onward. This may mean either (a) paternal chromosomes are already spatially segregated from the remaining chromosomes in prophase, before the spindle forms, or (b) segregation is accomplished quite early in meiosis I and the cells chosen for examination included none in which the segregation is in progress. In an attempt to resolve this ambiguity, the structure of two prophase nuclei was examined.

The cells to be discussed include one early prophase cell selected from the prophase cyst and one late prophase cell from the mid-to-late meiosis I cyst. Both are judged to be in prophase based upon the presence of cytoplasmic microtubules in the vicinity of the polar organelle, the absence of intranuclear microtubules, and the presence of more- or less-condensed chromosomes within an intact nuclear envelope. A slight difference of stage is apparent, the early prophase cell having only loosely condensed chromosomes and compact spherical nucleoli (Fig. 10), the late prophase cell having more highly condensed chromosomes and nucleoli that have begun to disperse.

In the early prophase cell, a polar organelle similar to that of meiosis I cells is present (Fig. 10, inset), and the preponderance of cytoplasmic microtubules occur in the vicinity of this structure. The polar organelle of the late prophase cells was, unfortunately, not observed due to the loss of some sections. However, a cytoplasmic region of high microtubule density toward one pole of the nucleus is recognizable and thus allows a rough approximation of the position of the polar organelle relative to the nucleus.

Prophase nuclei are not spherical. Rather, the nuclear surface is greatly deformed, as is immediately apparent in individual sections (Fig. 10) or solid reconstructions (Fig. 11). Chromosomes are arrayed around the periphery of the nucleus and each protrusion of the nucleus contains a chromosome or chromosomes so that there is extensive contact between chromosomes and the nuclear envelope. Just what is responsible

![Figure 10](image-url)
for the irregular contours of the prophase nucleus is unclear. No intranuclear microtubules are present and the only appreciable concentration of cytoplasmic microtubules occurs near the pole of the nucleus closest to the polar organelle. Perhaps nuclear shape is simply related to the degree of chromosome condensation, a speculation suggested by the large areas of contact between chromosomes and nuclear envelope and by the more extensive deformations of the late prophase nucleus.

In transparent three-dimensional reconstructions, ten chromosomes in each prophase nucleus are counted with no difficulty. However, only certain of the ten are identifiable, two large heterochromatic limiteds and two euchromatic, nucleolus-associated X chromosomes (Fig. 10). The rules of chromosome segregation for S. coprophila require that at the end of meiosis I three of the four chromosomes, the two limiteds and a maternal X chromosome, lie close to the polar organelle. Only one of the four, the paternal X chromosome, is to be found at the opposite end of the spindle, far from the polar organelle. This is precisely the arrangement of limiteds and X chromosomes found within both prophase nuclei before spindle formation. As indicated in Fig. 11, limiteds are close to each other and at the end of the nucleus closest to the polar organelle; the two X chromosomes, on the other hand, are far separated within the nucleus, one just as close to the polar organelle as the limiteds, the other at the extreme opposite end of the nucleus. Plainly, in the case of limited and X chromosomes, spatial segregation of chromosomes within the prophase nucleus foreshadows the genetic segregation to be accomplished by meiosis I.

It is not possible to determine if there is a similar segregation of the remaining euchromatic chromosomes in the prophase nuclei. Since the three euchromatic maternal chromosomes are indistinguishable from the three euchromatic paternal chromosomes, all that can be discovered is that these are equally distributed throughout the nucleus. However, when the overall distribution of chromosomes in prophase nuclei is examined, it is found that a greater number of chromosomes is concentrated in approximately one half of the nucleus, the half closest to the polar organelle. In late prophase, for example, six chromosomes (two limiteds, an X, and three euchromatic chromosomes) lie within the half nucleus closest to the polar organelle (38 sections) while only four chromosomes (an X and three euchromatic chromosomes) appear within the remainder of the nucleus (49 sections). The same was found to be true of the chromosome distribution in the early prophase nucleus. Numerically, at least, this is consistent with the idea that groups of chromosomes destined to segregate from each other on the meiosis I spindle (six chromosomes including maternals plus limiteds versus four paternal chromosomes) are already effectively segregated in two halves of prophase nuclei.

**General**

In each cell studied, one or two distinctive agglomeration(s) of membrane are found (Fig. 12). In the early prophase cell, one such agglomeration is present and it is in the cytoplasm at some distance from the nucleus. In later stages, no cytoplasmic masses of membrane of similar appearance are present, but instead the agglomeration(s) are found only in close association with the nuclear envelope (late prophase) or with the membrane system at the periphery of the spindle as in Fig. 12. In favorable sections, it is possible to trace a direct continuity between membranes of the agglomeration and membranes surrounding the spindle (observed but not illustrated).

There are differences in the number and disposition of the agglomeration(s) during meiosis. In early stages, the one accumulation of membrane in each cell is located far from the polar organelle (in late prophase, at the end of the nucleus close to the polar organelle; in mid meiosis cells a and b, at the broad end of the spindle). In later meiotic stages, when kinetochore fibers are present on all paternal chromosomes, two agglomerations are present in each cell, a relatively small mass close to the polar organelle plus a larger one farther away (in mid meiosis cell c, at the broad end of the spindle, in late meiosis, approximately at the middle of the spindle). Still later, in meiosis II, a single membranous mass is found in each cell, always quite close to the polar complex (unpublished observations).

The most obvious interpretation of these observations is that the agglomerations of membrane represent an excess over the amount of membrane required to enclose the prophase nucleus or meiotic spindle; if this is true, the changes in number and position of the membrane masses suggest some dynamic change in membrane distribution over the course of meiosis.

**DISCUSSION**

The meiosis I spindle in male Sciara was considered an exception to the general rule (12, 18) that chromosomal spindle fibers are responsible for poleward movement of chromosomes. According to interpretations based on light microscopy all chromosomes orient identically at meiosis I in Sciara (chromosomal fibers all associated with the one pole of a monopolar spindle); and yet chromosomes move in opposite directions (some move toward the pole while others retreat from it). In this context, the most basic result of the ultrastructural examination of meiosis I in S. coprophila is disproof of the claim that all chromosomes engage on the spindle in the same fashion. Consequently, a complete reevaluation of chromosome behavior in this monopolar spindle is required.

**FIGURE 11** Solid reconstruction of the early prophase nucleus, viewed at right angles to the plane of section of Fig. 10. The irregular surface of the nucleus shows a number of elongate protrusions. Chromosomes lie within these protrusions (cf. Fig. 10). The position of all ten chromosomes within the nucleus was determined in a transparent three-dimensional reconstruction, and the approximate positions of limited chromosomes (L), X chromosomes (X), and the polar organelle (*) are indicated in this figure.
FIGURE 12. Membrane agglomeration in mid meiosis I, cell b. A distinctive mass of membrane (m) is closely associated with the membrane enclosing the spindle (arrowheads). One chromosome (c) is visible. Bar, 1 μm. × 29,200.

No evidence for interaction of spindle microtubules and kinetochores on either limited or maternal chromosomes was ever found. My failure to discover kinetochore microtubules (chromosomal fibers) on these chromosomes was, of course, totally unexpected in light of previous assertions that they show "ordinary mitotic behavior...as expected" (14, 15). This discrepancy is resolved when the original detailed description is consulted (17). There (p. 247), Metz et al. reported that spindle fibers on the six chromosomes that collect near the spindle pole were observed only in exceptional cases. In subsequent discussions by Metz and by others, this qualification was forgotten, and the presence of chromosomal fibers on all chromosomes was taken as given. Apparently, in light microscopic preparations, nonchromosomal spindle fibers near limiteds and maternals were mistaken for chromosomal fibers.

In S. coprophila, the only specific association of chromosomes and microtubules is found on paternal chromosomes. In late meiosis, the kinetochore of each of the four paternal chromosomes faces the polar complex (spindle pole), and kinetochore microtubules run from the kinetochore toward the polar complex. Earlier, in mid-meiosis, only four or fewer chromosomes interact similarly with microtubules; by inference, these are identified as paternals.

The differential capacity of maternal and paternal chromosomes to form spindle fibers has all the hallmarks of "imprinting", a chromosome conditioning which "determines that a chromosome will behave differently from a homologous chromosome in the same nucleus" (4). Crouse (5) introduced the concept of imprinting to explain various anomalous chromosome behaviors in Sciara, all of which depend on the parental history of a chromosome. According to Crouse, a chromosome that passes through the female germ line acquires an imprint different from the imprint the same chromosome would acquire upon passage through the male germ line. The reversible nature of imprinting is crucial (6), as is even more clear now that we have seen precisely how maternal and paternal imprints affect chromosome-microtubule interactions. At meiosis I, the kinetochores of maternally derived chromosomes are somehow prevented from interacting with microtubules. But, no permanent chromosomal change can be involved since this anomaly is restricted to meiosis I and normal chromosomal spindle fibers (kinetochore microtubules) soon appear on maternal chromosomes during meiosis II (17, my unpublished observations). Moreover, in the succeeding generation of males, these very chromosomes have become paternals and their ability to interact with kinetochore microtubules even in meiosis I must be restored. Paradoxically, the chromosomes that have reacquired an ability to form spindle fibers at meiosis I of the second male generation are destined to be lost, the result of paternal chromosomes being included in a degenerating bud. Somehow, imprinting and the unusual meiosis of male Sciara combine in a mechanism causing loss of any chromosome (other than a limited) transmitted from father to son.

The presence of spindle fibers on only a selected few chromosomes during meiosis I in S. coprophila males is a sufficient basis for nonrandom chromosome segregation. However, the segregation is not effected, as one might reasonably expect, because a set of chromosomes associated with kinetochore microtubules actively moves away from a set of chromosomes...
lacking such spindle connection. Instead, the converse is true: conspicuous movement of chromosomes not directly associated with microtubules (maternals) is countered by inactivity of chromosomes with kinetochore microtubules (paternals). The poleward movement of maternals is unmistakable. Chromosomes which were scattered at various distances from the polar complex in mid-meiosis collect in a compact group close to the complex by late meiosis. In contrast, the absence of paternal chromosome movement is evident because there is no significant change in spindle length or the distance between paternal and the polar complex from mid- to late meiosis. One conclusion seems inescapable, that is, the chromosome behavior involved in nonrandom segregation in S. coprophila is almost totally different from the chromosome behavior leading to random segregation in typical spindles.

Interpretations which dismissed movement of paternal chromosomes as an instance of "ordinary mitotic behavior" (15; see also 14) are invalidated by the ultrastructural evidence. Only the poleward directionality of the movement is conventional. Because maternal chromosome movement in S. coprophila does not require direct kinetochore-microtubule connection, we are forced to conclude that this movement has essentially nothing in common with the microtubule-mediated chromosome movements of conventional mitosis or meiosis. Unfortunately, there is little if any ultrastructural indication of a basis for the unorthodox movement. Only the tendency for presumptive maternal kinetochores to lie near or in contact with the system of membrane surrounding the spindle is suggestive. Conceivably, if this membrane moves in a poleward direction, it could drag maternals poleward. Chromosome movements mediated by membrane but not microtubules are not unheard of (see reference 22 and references therein) and I suggest that changes in position of the peculiar membrane agglomerations which are part of the membrane system outlining the spindle may reflect exactly the kind of membrane activity that would produce chromosome movement. However, the suggestion is tentative and all that can be said with certainty is that maternals do move and the cause of the movement is unknown.

Misconceptions regarding paternal chromosome behavior also can be corrected on the basis of ultrastructural information. Metz (14, 15) was convinced that chromosome segregation was the result of paternal chromosomes "backing away" or "retreating" from maternal chromosomes and the spindle pole. In his view, chromosomal fibers orienting paternals toward the pole do exert a poleward force, but this force is overwhelmed by a counterbalancing force to produce net movement away from the pole. Because the ultrastructural study was limited to stages involving chromosome segregation per se (mid to late meiosis), it is not determined unequivocally that paternal chromosomes never retreat from the pole; perhaps such movement is observable when paternals are extruded into the bud that forms at cytokinesis. However, it is certain that paternals undergo no net displacement up to the stage when segregation is virtually completed. This is made plain by comparison of mid and late meiosis cells where both spindle length and pole-to-paternal distance remain approximately constant.

There is some indication that kinetochore microtubules exert poleward forces on paternal chromosomes since the presence of kinetochore microtubules on those chromosomes is correlated with a slight decrease in chromosome-to-pole distance and with extension of chromosome arms along the spindle axis. But, this force is simply not sufficient to carry the whole chromosome poleward. A reasonable interpretation of the facts is that the kinetochore microtubules of paternal chromosomes serve as anchors that impede poleward progress, preventing paternals from approaching the pole as rapidly as maternals. In other words, in the monopolar meiosis of S. coprophila, the role of microtubules as governors (9, 19) of paternal chromosome velocity predominates over their force producing or transmitting functions.

In principle, a spindle that permits (or promotes) movement of only certain chromosomes could separate two sets of chromosomes regardless of the specific arrangement of chromosomes in the prophase nucleus. Grouping of maternal and limited chromosomes in one half of the nucleus and paternals in the other half is certainly not a necessary precondition for the later segregation on the spindle. Yet, chromosomes in the two prophase nuclei analyzed for this study are apparently grouped just so. There are several independent indications that this is not fortuitous and may indeed by common to all premeiotic nuclei in the male germ line: (a) Although the maternal and paternal chromosome sets are intermingled in somatic cells, as indicated by their conspicuous somatic pairing (specific association of homologous chromosomes, a characteristic of Diptera; 16), somatic pairing is absent in germ line nuclei (16, 17, 23). That is, maternal and paternal chromosomes are not obviously intermingled in germ line nuclei as they are in somatic nuclei. (b) At a stage in early development of the germ line when maternals and paternals stain differentially, there is a degree of chromosome "clustering"; maternal chromosomes tend to lie close together (23). (c) Limited chromosomes usually lie close to each other within a nucleus (17, 23); and they tend to be close to the position where the acuminate spindle pole will form at meiosis I (17). All of this suggests that spatial separation of maternals and limiteds from paternals persists through all stages of germ line development and does not result from rearrangement of chromosomes within the nucleus in preparation for meiosis I. The disposition of maternals and paternals in germ line nuclei likely results because sets of chromosomes contributed by egg and sperm never commingle. Limiteds on the other hand, may be of either maternal or paternal origin (6, 23) and their grouping together with maternals is not so simply explained. Regardless of how the orderly arrangement of chromosomes in prophase nuclei arises, it is advantageous because it permits clear-cut separation of sets of chromosomes with relatively little chromosome movement, i.e., a shorter spindle is required.

Metz, et al. (17) based their description of meiosis I in Sciara on examination of two species, S. similans and S. coprophila. The description given here represents the first complete reexamination of events in one of those species, S. coprophila. Although the interpretation suggested by my ultrastructural data is fundamentally different from that of Metz and his colleagues, there is agreement about the basic monopolar nature of the spindle and the orientation of paternal chromosomes. Since Metz (16) reported that the same pattern of spermatogenesis is found in 14 species of Sciara, reasonably detailed light microscopic descriptions of meiosis I in S. paulicosta (27), Plastosciara pectiniventris (8) and Trichosia pubescens (2) have been published. The last of these is the most clearly documented. In addition, there is a particularly striking micrograph of meiosis I in S. impatiens (6). Most noteworthy in each case is the information regarding chromosome orientation. Chromosomes destined for the bud are in haphazard array and have no common focus of orientation while the chromosomes collected near the spindle pole are plainly oriented to that pole. This is quite the opposite of the situation in S. coprophila where the configuration of chromosomes to be included in the bud is

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an immediate clue to their poleward orientation (e.g. Figs. 1b and 10b in reference 1) and where chromosomes moving poleward have no recognizable orientation (Fig. 8). Nevertheless, general conclusions regarding spindle functions are quite similar for S. coprophila and for the other species; the same mechanism cannot account for behavior of the two sets of chromosomes on the meiosis I spindle and chromosomal fibers on one of the sets appear to act more as anchors than as agents of chromosome movement (2). Taken together, the studies of meiosis in Sciaridae indicate that only the monopolar structure of the meiosis I spindle is common to all and it is inconsequential which set of chromosomes orients to the pole via spindle fibers. It will be interesting to have ultrastructural information about meiosis in species other than S. coprophila. What seems most curious when available information about the different species is compared is the following: in some (species other than S. coprophila), the chromosomes which do not form chromosomal fibers are included in the bud destined for degeneration and so are eliminated immediately as a result of meiosis I. In S. coprophila, on the other hand, the chromosomes which do not form chromosomal fibers (maternals) are retained and transmitted to sperm; they will be lost only if transmitted to another male individual (at which time they have regained the capacity to form chromosomal fibers).

The extraordinary first meiotic division in male Sciara is interesting as a puzzle in its own right. Beyond this, it is instructive in some matters of general importance. It demonstrates that spindle structure and chromosome behavior can be very different in different cells of the same organism—monopolar division occurs at only one stage in the life cycle; quite conventional bipolar spindles are involved in somatic and germ line mitoses and in the second meiotic division (16). The orderly chromosome distribution that takes place on the monopolar spindle is evidence that bipolar spindle organization is not an absolute requirement for appropriate segregation of chromosome sets. And, finally, the static position of paternal chromosomes in the monopolar spindle shows that the simple association of chromosomes and kinetochore microtubules does not assure poleward chromosome movement; mechanisms that generate chromosome movement in conventional bipolar spindles are obviously altered or inoperative in meiosis I of S. coprophila males.

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