Nonrandom Chromosome Arrangements in Germ Line Nuclei of Sciara coprophila Males: The Basis for Nonrandom Chromosome Segregation on the Meiosis I Spindle

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Abstract. Meiosis I in males of the Dipteran Sciara coprophila results in the nonrandom distribution of maternally and paternally derived chromosome sets to the two division products. Based on an earlier study (Kubai, D. E 1982. J. Cell Biol. 93:655-669), I suggested that the meiosis I spindle does not play a direct role in the nonrandom sorting of chromosomes but that, instead, haploid sets are already separated in prophase nuclei well before the onset of spindle formation. Here I report more direct evidence that this hypothesis is true; this evidence was gained from ultrastructural reconstruction analyses of the arrangement of chromosomes in germ line nuclei (prophase nuclei in spermatogonia and spermatocytes) of males heterozygous for an X-autosome chromosome translocation. Because of this translocation, the maternal and paternal chromosome sets are distinguishable, so it is possible to demonstrate that (a) the two haploid chromosome sets occupy distinct maternal and paternal nuclear compartments and that (b) nuclei are oriented so that the two haploid chromosome sets have consistent relationships to a well-defined cellular axis. The consequences of such nonrandom aspects of nuclear structure for chromosome behavior on premeiotic and meiotic spindles are discussed.

The idea that chromosomes are arranged nonrandomly in interphase or prophase nuclei is an old one, first suggested by the work of Rabl (20) and Boveri (7) and supported by diverse evidence (for review see reference 2). In the last few years, the credibility of the idea has increased appreciably, due mainly to studies such as those by Ashley (1, 2) and Bennett (see review in reference 3) and their collaborators. These investigators have clearly documented cases in which chromosomes assume specific arrangements in nuclei and on the mitotic spindle, and they have shown how those arrangements can have important implications for chromosome behavior.

Ashley and her associates, for example, found that the three chromosomes in the haploid complement of Ornithogalum virens usually exhibit a particular end-to-end order (1) and provided a model to show how the arrangement of two such haploid arrays in diploid nuclei could facilitate meiotic pairing (2). Bennett (3) and his co-workers discovered that in grasses the two haploid chromosome sets occupy separate domains on the mitotic spindle. In interspecies and intergeneric hybrids, one parental chromosome set is nearer the outside of the spindle, concentric with the second parent's chromosomes. Such hybrids tend to lose chromosomes derived from one parent, the very parent that contributes chromosomes nearer the outside of the spindle. Thus, Bennett's observations suggest that nonrandom chromosome positioning on the spindle is causally linked to nonrandom chromosome loss.

Another instance where a particular course of chromosome behavior and a particular arrangement of chromosomes within nuclei appear to be related occurs in the Dipteran Sciara coprophila. Here we are concerned with the perfectly nonrandom chromosome distribution that occurs during meiosis I in males. In this division, maternal chromosomes move poleward on a unique monopolar spindle, while paternal chromosomes remain in place, far from the spindle pole. As a result, all maternal chromosomes are transmitted to sperm and all paternal chromosomes are lost (for details see references 13 and 16). In my study of meiosis I in wild-type S. coprophila (13), I found that maternal and paternal chromosome sets are always in separate halves of the meiosis I spindle—maternals close to the pole and paternals far from it—even in the earliest stage examined. Moreover, I found indications that a similar segregation of maternal and paternal chromosomes exists in prophase nuclei, before the spindle forms. Consequently, I argued that the monopolar meiosis I spindle has no role in sorting chromosomes into maternal and paternal sets; it serves simply to increase the distance between chromosomes that were already nonrandomly arrayed in prophase nuclei.

In this paper, I present more direct evidence that the peculiar nonrandom meiotic chromosome distribution characteristic of S. coprophila males is most likely dependent upon the nonrandom structure of premeiotic nuclei. This evidence was obtained by exploiting a translocation that alters chromosome sizes (10) so that in males heterozygous for the
translocation, the parental chromosome sets have different size profiles. Moreover, in these heterozygotes, certain maternal and paternal chromosomes can be identified specifically on the basis of their size and their association with nucleoli. These characters allow me to use information gained from ultrastructural reconstruction of whole nuclei to demonstrate that parental chromosome sets are spatially separated in resting nuclei.

Materials and Methods

Cultures containing fourth instar male larvae of Sciara coprophila carrying the reciprocal chromosome translocation T23 (10) were generously provided by Dr. Susan A. Gerbi (Division of Biology and Medicine, Brown University, Providence, RI). The larvae were maintained at 16°-18°C until they developed into pupae, the stage when meiosis occurs (21).

Males heterozygous for chromosomes translocations are obtained by crossing wild-type males and heterozygous male-producing females (the translocations are homozygous lethal [9]). This means that translocation-bearing pupae must be selected from a mixture of wild-type and heterozygous progeny, a selection that requires examination of the chromosomes constitution of germ line nuclei (10). Therefore, the two testes from each pupa were treated differently, one being prepared for light microscopy (fixation with 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 6.8, for 30 min, followed by squashing and Feulgen staining [15]), and the other fixed for electron microscopy. Then, if the Feulgen-stained testes showed meiosis II division figures with the desired translocation chromosomes, the second testis of the pupa was a candidate for electron microscopy.

Because selection of heterozygotes was just the first of many tedious steps involved in serial section reconstruction analysis, I made a further selection before proceeding with electron microscopy. I discarded testes likely to have very few cells in the required stages—prophase I or earlier. (When meiosis II spindles are present, as required for diagnosis of chromosome constitution, most resting nuclei are in prophase II.) I also discarded testes from pupae with an abnormal chromosome number. As a result, very few testes proved suitable for my purposes, and the cells described in this report came from three pupae, progeny of different matings.

Methods used in preparing pupal testes for serial section electron microscopy and for recognizing meiotic stages in the electron microscope have been described (13). Micrographs of serial sections through resting nuclei, prophase I or earlier (× 3,300-4,300), and through meiosis II spindles (×3,300) were taken with a Zeiss 10A electron microscope operating at 80 kV, using 70-mm film (No. 613) (Chemco Photoproducts, Glen Cove, NY). For each micrograph series, the microscope was recalibrated using a grating-replica magnification standard.

Two- and three-dimensional reconstructions of nuclei or spindles, made by stacking serial section tracings as described earlier (14), were used to (a) assign the various chromatin masses seen in each section to particular chromosomes, (b) count chromosomes, (c) examine the shape of each chromosome, (d) establish each chromosome's position (recorded as the three-dimensional coordinates of a point approximately at the center of chromosome mass), and (e) examine the general spatial relationships between chromosomes and two structures peculiar to S. coprophila spermatocytes, the polar organelle, and the membrane agglomeration (13).

Computer-assisted reconstruction techniques were used to identify specific chromosomes and to analyze the arrangement of chromosomes on the meiosis II spindle or of parental chromosome sets within resting nuclei. This analysis was based upon programs which were written by Moens and Moens (17) for digitizing structural outlines and for manipulating and displaying digitized structural information. These programs were slightly modified for use with a model 2600 microcomputer (Vector Graphics, Thousands Oaks, CA), a model 220 digitizer (Numonics Corp., Lansdale, PA), and a model DMP-7 plotter (Houston Instrument, Austin, TX).

In addition, new programs were written specifically for this investigation. (a) A program to determine chromosome volumes: this program used data files containing the digitized outlines of all chromosomes or chromatin profiles in a nucleus; it calculated the areas within those outlines based on a formula for the area of polygons (6), summed the areas for all outlines assigned to a chromosome, corrected for final magnification of the reconstruction (division by magnification squared), and multiplied the total area by the estimated section thickness (80 nm) to give volume (cf. reference 5). (b) A set of related programs designed to test whether maternal and paternal chromosomes sets are in separate nuclear domains: these sorting programs are described in Results.

Results

Chromosomes in Males Bearing Translocation T23

The structure and behavior of chromosomes in Sciara coprophila males heterozygous for the X-autosome translocation T23 are well known as a result of light microscope studies by Crouse (9, 10). Since that knowledge is used to interpret the observations reported here, the pertinent facts are summarized in Table 1.

Identification of Chromosomes on the Meiosis II Spindle

Meiosis II spindles in T23 translocation males were used to

Table 1. Chromosomes in Sciara coprophila Males Heterozygous for Translocation T23

| Translocation set:* materials | Normal set: paternals | Limiteds† | Limiteds† |
|-------------------------------|-----------------------|-----------|-----------|
| X III | II | III | IV | X | II | III | IV | L₃ | L₄ |
| Relative size‡ | 0.40 | 0.21 | 0.07 | 0.32 | 0.21 | 0.21 | 0.26 | 0.32 | Very large | Very large |
| Type | M | A | A | M | A | A | A | M | M |
| Nuclear heterochromosomes | H1, H2, H3 | H1, H2, H3 | H1, H2, H3 | H1, H2, H3 | H1, H2, H3 | H1, H2, H3 | H1, H2, H3 |
| Position on meiosis II spindle** | Pole | Plate | Plate | Plate | Plate | Plate | Plate |

* The translocation is a reciprocal interchange involving chromosomes X and III; X III has the X centromere and a piece of chromosome III; III has the chromosome III centromere and a piece of the X chromosome (10).
† Limiteds (16, 21) are chromosomes found in the germ line but not in somatic cells (hence, they are "limited" to the germ line); they are of indeterminate parental origin; they are not eliminated at meiosis I and so are found as the largest chromosomes on the meiosis II spindle. Limiteds differ in size (see Table II), with one (L₃) being larger than the other (L₄).
‡ Derived from drawn-to-scale diagrams of salivary gland polytene chromosomes in reference 10, Fig. 1; calculated as (chromosome length)/(sum of the lengths of all chromosomes in a haploid set).
§ M, metacentric; A, acrocentric.
¶ Heterochromosomes H1, H2, and H3 compose the short arm of the normal X chromosome; in translocation T23, the exchange point lies between H2 and H3 so that H1 and H2 appear together on X III and H3 is translocated to III (10). Ribosomal RNA cistrons are distributed among these heterochromosomes: 10% in H1, 50% in H2, and 40% in H3 (11).
** As a result of the elimination of paternals in meiosis I (--, not present, eliminated at meiosis I), only maternals and limiteds participate in meiosis II; X III is the propcohesive chromosome (see text) and so is close to the spindle pole when the remaining chromosomes are on the metaphase plate (10).
test how well the small chromosomes of *S. coprophila* can be distinguished using volume reconstruction techniques. In principle, meiosis II is an ideal stage for this purpose. Because in *S. coprophila* males the paternal haploid set is eliminated at meiosis I, only six chromosomes participate in meiosis II: four maternal chromosomes (the translocation set with each chromosome having a distinctive size) and two limited chromosomes each larger than any maternal chromosome (Table I). Moreover, meiosis II in *S. coprophila* males always involves the unusual “precocious” behavior of one chromosome: that chromosome is found near the spindle pole while all others remain aligned on the metaphase plate of the bipolar spindle (16). In T23 males, it is usually translo-

![Diagram](image)

**Figure 1.** The arrangement of chromosomes on the meiosis II spindle in *S. coprophila* males heterozygous for translocation T23. (a) Cell A. (b) Cell B. These three-dimensional spindle representations (stereo pairs) are based on reconstructions from serial sections through whole cells. The position (center of mass; see Materials and Methods) of each chromosome (letters and roman numerals) is indicated. Dashed lines represent chromosome fibers (i.e., microtubules running from chromosomes to the polar organelle) except in the case of the precocious chromosome for which the connection of chromosome and pole via a chromosome fiber has not been verified; thus, for the precocious chromosome the dashed line is intended only to clarify the position of the chromosome relative to the pole. Solid lines connect the chromosomes on the metaphase plate. Only that half of the metaphase spindle having the polar organelle (r) at the spindle pole and the precocious chromosome (X m) nearby is shown. Computerized image rotation was used to show all spindles in the same orientation, with the precocious chromosome toward the right and the polar organelle at the top, regardless of original orientation relative to the section plane.

![Diagram](image)

**Figure 2.** The arrangement of chromosomes on the meiosis II spindle in *S. coprophila* males heterozygous for translocation T23. (a) Cell C. (b) Cell D. (c) Cell E. See Fig. 1 legend for general explanation. In these cells, either chromosome III' or IV is precocious, not X m as expected (cf. Fig. 1); this is explained by the presence of extra chromosomes in some cells of the testis (see reference 10); that is, chromosome II is duplicated in cells D and E.

Table I. Test how well the small chromosomes of *S. coprophila* can be distinguished using volume reconstruction techniques. In principle, meiosis II is an ideal stage for this purpose. Because in *S. coprophila* males the paternal haploid set is eliminated at meiosis I, only six chromosomes participate in meiosis II: four maternal chromosomes (the translocation set with each chromosome having a distinctive size) and two limited chromosomes each larger than any maternal chromosome (Table I). Moreover, meiosis II in *S. coprophila* males always involves the unusual “precocious” behavior of one chromosome: that chromosome is found near the spindle pole while all others remain aligned on the metaphase plate of the bipolar spindle (16). In T23 males, it is usually translo-
Table II. Volumes (μm³) of Chromosomes on the Meiosis II Spindle in Sciara coprophila Males Heterozygous for Translocation T23: Tentative Chromosome Identifications

| Translocation set: maternals | Limiteds |
|-----------------------------|----------|
| X\(\text{I}^{\text{m}}\) | II | III\(\text{I}^{\text{m}}\) | IV | L\(_5\) | L\(_4\) |
| Cell A | 1.52 | 1.21 | 0.16 | 1.39 | 1.95 | 2.20 |
| Cell B | 1.5 | 1.12 | 0.15 | 1.25\(^*\) | 1.69 | 2.50 |
| Cell C | 1.43 | 1.04 | 0.25 | 1.32 | 1.52 | 2.11 |
| Cell D | 1.29 | 0.76\(^*\) | 0.24 | 1.14 | 1.87 | 2.00 |
| Cell E | 1.31 | 0.96\(^*\) | 0.19 | 1.13 | 1.92 | 2.28 |

* Chromosomes are identified according to size; as expected from data in Table I, L\(_5\) > L\(_4\) > X\(\text{I}^{\text{m}}\) > IV > II > III\(\text{I}^{\text{m}}\).  
† The value was obtained by summing the volumes of two separated pieces of the chromosome; see text for explanation.  
‡ Two entries reflect the presence of an extra chromosome; see text.

2. Because some cells exhibited unusual chromosome numbers or behavior, the following paragraphs highlight the observed differences between the five cells.

**Cell A.** For this metaphase II cell, all of the chromosomes are tentatively identifiable as given in Table II. There are six chromosomes on the spindle, and the volume of each is different from the volume of any other chromosome. That the tentative identifications are correct is borne out by the following: the relative volume profile for the four chromosomes identified as maternals (Table III) is very similar to the relative length profile for the translocation set of polytene maternal chromosomes in salivary gland nuclei (Table I); further more, each chromosome is of the expected type (compare Table III with Table I) and in the expected spindle position with X\(\text{I}^{\text{m}}\) near the spindle pole and the remaining five chromosomes on the metaphase plate (Fig. 1 a; cf. Table I).

**Cell B.** This late metaphase/early anaphase cell appeared to carry seven chromosomes. However, two adjacent rod-shaped elements are not individual acrocentric chromosomes but are instead identifiable as the separated arms of chromosome IV. This is in accord with Crouse's report (8) that chromosome IV "tends to pull apart" at the centromere region (in smear preparations). The arms of chromosome IV measure 0.71 and 0.54 μm\(^3\), an arm ratio of 1.3, as compared to a ratio of 1.2 represented in Crouse's diagram of chromosome IV (Fig. 1 in reference 10). Once chromosome IV is recognized, tentative identifications can be based strictly on chromosome volume (Table II) and then verified by observing that the relative size and type of each chromosome (Table III) and its spindle position (X\(\text{I}^{\text{m}}\) near the pole, other chromosomes on the metaphase plate, see Fig. 1 b) are as expected (Table I).

**Cells C-E.** For each of these metaphase II cells, the tentative chromosome identifications are given in Table II; those identifications yield appropriate relative size profiles, as shown in Table III. In cells C and D (Fig. 2, a and b), chromosome III\(\text{I}^{\text{m}}\) is precocious (i.e., it is nearest the pole) while in cell E, chromosome IV is precocious. The precocious behavior of chromosomes other than X\(\text{I}^{\text{m}}\) is explained by the duplication of chromosome II in cells D and E (Fig. 2, b and c): as Crouse (10) has reported, the presence of an extra autosome in the germ line sometimes alters precocious chromosome behavior.

These results are a strong assurance that volume reconstruction is an effective means for identifying the chromo-

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Table III. Relative Sizes* and Types‡ of Chromosomes on the Meiosis II Spindle in Sciara coprophila Males Heterozygous for Translocation T23

| Maternals set | Limiteds |
|---------------|----------|
| X\(\text{I}^{\text{m}}\) | II | III\(\text{I}^{\text{m}}\) | IV | L\(_5\) | L\(_4\) |
| Cell A | 0.36 | 0.28 | 0.04 | 0.32 | 0.46 | 0.51 |
| Type | pc | A | A | M | M |
| Cell B | 0.37 | 0.28 | 0.04 | 0.31 | 0.42 | 0.62 |
| Type | pc | A | A | M | M |
| Cell C | 0.35 | 0.26 | 0.06 | 0.33 | 0.38 | 0.52 |
| Type | M | A | pc | M | M |
| Cell D | 0.38 | 0.21 | 0.07 | 0.34 | 0.55 | 0.59 |
| Type | M | A | pc | M | M |
| Cell E | 0.37 | 0.25 | 0.05 | 0.32 | 0.55 | 0.65 |
| Type | M | A | A | pc | M | M |
| Mean ± SEM | 0.366 ± 0.010 | 0.256 ± 0.014 | 0.052 ± 0.007 | 0.326 ± 0.006 | 0.470 ± 0.038 | 0.578 ± 0.031 |

* Based on chromosome volumes in Table II. For the maternal set, relative size = (chromosome volume/sum of the volumes of all chromosomes in the set); when two copies of a chromosome were present (cell D and E, chromosome II), the average volume was used in the calculation. For the limiteds, relative size = (volume of the limited/volume of the maternal set).

‡ Judged from the shape or position of the chromosomes as seen in the three-dimensional reconstruction; A, acrocentric (rod-shaped); M, metacentric (v-shaped); pc, precocious chromosome, near the spindle pole (shape uncertain).
some of *S. coprophila*: very similar relative chromosome volume profiles were obtained for five meiosis II cells (Table III), and these are not appreciably different from a relative chromosome length profile calculated for the translocation set of chromosomes in salivary gland nuclei (Table I).

The Order of Chromosomes on the Metaphase Plate in Meiosis II. Due to the unique structure of the meiosis II spindle in *S. coprophila* males, it is possible to determine how chromosomes are arranged on the metaphase plate with uncommon certainty. This is so because only one pole of the bipolar spindle is marked by the presence of a polar organelle and a nearby precociously chromosome (12, 13). The half-spindle including this differentiated pole and the ringlike array of chromosomes on the metaphase plate are represented in Figs. 1 and 2. With the two spindle poles so clearly differentiated, the order of chromosomes on the metaphase plate can be examined from a particular perspective (e.g., by looking toward the plate from the polar organelle) so as to determine chromosome order unambiguously. This contrasts with the case for ordinary spindles having undifferentiated poles where a chromosome arrangement viewed from one pole would appear in inverse order when viewed from the opposite pole.

For meiosis II cells A–E (Figs. 1 and 2), chromosome orders are listed in Table IV; these orders represent the clockwise sequence of chromosomes (starting with chromosome L₄) seen when the metaphase plate is viewed from the polar organelle. The listing shows the following. In the first two cells (cells A and C) four out of five positions are occupied by identical chromosomes, the exception being in position 3. Insofar as position 3 in each cell is occupied by a translocation chromosome, the two cells have virtually identical metaphase plate chromosome arrangements. Cell E resembles the first two cells in positions 1–4, cell B in positions 1–3, and cell D in positions 1 and 2.

The data in Table IV suggest that chromosomes tend to be arranged on the metaphase II plate in a specific order, rather than at random. To calculate the probability that the observed similarities in chromosome order resulted purely by chance, only cells A–C were considered, i.e., cells that have the standard number of five chromosomes on the metaphase plate. Two of these cells, cells A and C, exhibit identical chromosome order:

\[ L₄ - II - translocation chromosome - L₅ - IV. \]

The third, cell B, exhibits an identical order except for an inversion in positions 4 and 5:

\[ L₄ - II - translocation chromosome - IV - L₅. \]

If chromosomes assume random positions on the metaphase plate, the probability that chromosome order in a sample of three cells will match as well as or better than was observed for cells A–C is less than 0.03. This suggests that something other than chance determines the arrangement of chromosomes on the meiosis II metaphase plate in *S. coprophila*.

The Arrangement of Maternal and Paternal Chromosome Sets in Germ Line Nuclei and on the Meiosis I Spindle in *S. coprophila* Males

General Aspects of Cell Structure. In all of the prophase cells described here (spermatocytes, cells F–K; spermatogonia, cells M and N), euchromatic chromosomes are only loosely condensed (somewhat less condensed than in the prophase nucleus illustrated in Fig. 10 of reference 13) and only a small number of microtubules surround the polar organelle(s). Therefore, all of these cells are judged to be in a relatively early stage of prophase.

The Primary Axis. The arrangement of chromosomes in male germ line cells, either within nuclei or on the just-formed spindle, will always be described with reference to the cell's primary axis. In primary spermatocytes, this axis is defined by two differentiations that are located at opposite cell poles, the polar organelle and the membrane agglomeration (13). Because the monopolar meiosis I spindle always forms with the spindle pole focused on the polar organelle and the membrane agglomeration at the opposite, broad end of the spindle, the spindle long axis and the primary axis roughly coincide. And when the spindle is present, it is easy to recognize that all spermatocytes are identically oriented within spherical testicular cysts: the meiosis I spindle pole and the associated polar organelle are always near the exterior of the cyst (13).

The results of the present study show that the primary axis is well defined even before spindle formation, at prophase I. In six prophase I cells (cells F–K which are more fully described below), the polar organelle and the membrane agglomeration are positioned in the cytoplasm exactly as they would be if the spindle were present: the nucleus lies between the two structures, with the polar organelle near the nuclear surface closer to the exterior of the cyst and with the membrane agglomeration at the opposite end of the cell, nearer the interior of the cyst.

Criteria for Chromosome Identification Based on Ultrastructure and Chromosome Size. In *S. coprophila* spermatocytes at prophase I, chromosomes are ultrastructurally distinguishable (13) as being either heterochromatic limited chromosomes (highly condensed) or euchromatic chromosomes (relatively uncondensed); euchromatic chromosomes

1. Based on the sum of two probabilities, \( P_1 \), the probability of encountering three cells with identical chromosome arrangements (a match better than was observed), and \( P_2 \), the probability of encountering three cells among which two have identical chromosome orders while the third is the same except for two adjacent chromosomes that are in invented order with respect to their order in the other two cells (a match as good as was observed). For five chromosomes arranged in a ring, there are \((5 \times 1) = 24\) possible chromosome orders, and five potential sites for inverting the position of adjacent chromosomes so that

\[ P_1 = \frac{24}{24^2} = \frac{1}{24^2}; \]

\[ P_2 = 3 \times 5 \times \frac{1}{24^2} = \frac{15}{24^2}; \]

and

\[ P_1 + P_2 = \frac{16}{24^2} = \frac{1}{36}. \]
Table V. Absolute Sizes* of Chromosomes and Nucleoli in Sciara coprophila Males

Heterozygous for Translocation T23

| Translocation set: | Nontranslocation set: | Limiteds† |
|-------------------|----------------------|-----------|
| maternals         | paternals            | Ls        |
| X                 | II                   | III       | IV | X     | II       | III       | IV | Ls   | L    | Ls   |
| Cell F            | 1.73                 | 0.92      | 0.20 | 1.47  | 0.97     | 0.78      | 0.94 | 1.12 | 1.10 | 2.80 |
|                   | (1.14)               | (0.34)    | (0.00)| (1.77) | (0.78)   | (1.25)    | (1.46)| (1.72)|         |
| Cell G            | 1.54                 | 0.64      | 0.16 | 0.87  | 1.06     | 0.76      | 0.91 | 1.40 | 1.03 | 2.68 |
|                   | (0.26)               | (0.00)    | (0.35)| (0.53) | (0.95)   | (0.76)    | (1.40)| (1.72)|         |
| Cell H            | 4.46                 | 1.07      | 1.68 | 1.46  | 2.54     | 0.76      | 1.14 | 1.71 | 2.0  |       |
|                   | (0.51)               | (0.23)    | (0.95)| (0.76) | (0.76)   | (1.14)    | (1.71)| (2.0) |       |
| Cell I            | 3.05                 | 0.95      | 0.45 | 1.74  | 3.53     | 1.07      | 1.36 | 1.82 | 2.58 | 2.76 |
|                   | (1.09)               | (1.16)    | (0.70)| (0.35) | (1.07)   | (1.36)    | (1.82)| (2.58)| 3.97 |
| Cell J            | 4.79                 | 1.21      | 1.19 | 1.66  | 4.79     | 0.84      | 0.96 | 1.66 | 1.98 | 2.36 |
|                   | (0.66)               | (0.22)    | (0.00)| (1.01) | (0.84)   | (0.96)    | (1.66)| (1.98)|         |
| Cell K            | 3.47                 | 1.29      | 0.23 | 1.73  | 4.79     | 1.05      | 0.77 | 1.52 | 1.98 | 2.51 |
|                   | (0.82)               | (0.22)    | (0.00)| (0.18) | (1.05)   | (0.77)    | (1.52)| (1.98)|         |
| Cell L            | 1.88                 | 0.93      | 0.27 | 1.53  | 1.05     | 0.59      | 0.69 | 0.72 | 2.44 | 2.72 |
|                   | (0.00)               | (0.00)    | (0.00)| (1.33) | (0.59)   | (0.69)    | (0.72)| (2.44)|         |
| Cell M            | 3.94                 | 0.57      | 0.37 | 1.20  | 5.82     | 0.65      | 0.68 | 1.15 | 2.72 | 2.75 |
|                   | (0.58)               | (0.00)    | (0.04)| (2.16) | (0.65)   | (0.68)    | (1.15)| (2.72)|         |
| Cell N            | 3.27                 | 0.76      | 0.17 | 1.20  | 7.01     | 0.65      | 0.68 | 1.15 | 2.72 | 2.75 |
|                   | (0.66)               | (0.04)    | (0.00)| (2.16) | (0.65)   | (0.68)    | (1.15)| (2.72)|         |

* Volume in µm³; measured by the reconstruction method (see Materials and Methods). Numbers in parenthesis indicate the volume of the nucleolus associated with the chromosome.
† Chromosomes were arbitrarily identified while the analyses were performed; the final identifications as given here were assigned only after analyses were completed; see text.

are, in turn, of two types, associated with a nucleolus or not. The expectation is that each nucleus will carry ~10 chromosomes, 1–3 limiteds of unknown parental origin and 8 euchromatic chromosomes, 4 from each parent (16).

In the T23 translocation heterozygote males studied here, the haploid chromosome sets differ as to which chromosomes carry nucleoli and in the relative sizes of chromosomes in each set. The paternally derived set of four euchromatic chromosomes is wild type and includes one nucleolus-bearing chromosome, the X (II), plus chromosomes II, III, and IV; the relative size profile for the set is given in Table I. The maternally derived set of four euchromatics is the translocation set; it includes two nucleolus-bearing chromosomes, XHI and IIIx, the products of a reciprocal translocation between chromosomes X and III. Since the translocation caused a change in chromosome sizes as well as a redistribution of ribosomal RNA cistrons (Table I): (a) chromosome XHI is the largest maternal chromosome, it is larger than the paternal wild-type X chromosome, and its nucleolus should be smaller than a wild-type X nucleolus; and (b) chromosome IIIx is the smallest maternal chromosome, it is smaller than paternal chromosome III, and its nucleolus should be smaller than the nucleolus of either the maternal XHI or the paternal X chromosome. The maternal chromosome set that includes the two translocation chromosomes XHI and IIIx plus chromosomes II and IV thus has a relative size profile that is quite different from that of the paternal set, as shown in Table I.

From the foregoing, it is clear that 5 chromosomes of the 10 in a prophase I nucleus are distinctive: two heterochromatic limited chromosomes, and three nucleolus-bearing euchromatic chromosomes (the maternal XHI being the largest of the three, the paternal X the next largest, and the maternal IIIx the smallest). There are no criteria that would indicate the parental origin of the remaining five chromosomes, a pair each of chromosomes II and IV and the paternal chromosome III; but size differences (Table I) can be used to classify chromosomes as to type (e.g., II vs. IV).

Sorting Chromosomes into Maternal and Paternal Sets. Lacking the means to specify the parental origin of every chromosome in a nucleus, it is of course impossible to determine unequivocally if the chromosomes of one parent are spatially separate from those of the other parent. However, it is possible to discover if the arrangement of chromosomes in a nucleus is consistent with such a distinct grouping. For example, for nuclei of T23 translocation heterozygotes, one can ask if the two identifiable maternal nucleolar chromosomes, XHI and IIIx, are grouped with two other chromosomes whose sizes are appropriate for chromosomes II and IV while the single identifiable paternal nucleolar chromosome, the X, is grouped with three chromosomes having the sizes expected for chromosomes II, III, and IV.

To illustrate, the data for cell F (Table V) can be considered. The three nucleolus-bearing chromosomes in this meiosis I prophase cell measure 1.73, 0.20, and 0.97 µm³ and so are identifiable respectively as the maternals XHI and IIIx and the paternal X. Of the remaining five euchromatic chromosomes without nucleoli, the two largest (1.47 and 1.12 µm³) are taken to be homologous representatives of chromosome IV. The remaining three chromosomes, then, must include a pair of homologous chromosomes II and a paternal chromosome III. The similar size of two of these three (0.94 and 0.92 µm³) might suggest they are homologues; i.e., the chromosome II pair, with the remaining chromosome (0.78 µm³) being the paternal chromosome III. However, this cannot be true since it would mean that in the paternal set chromosome II is larger than III, the inverse of what is expected (Table I).
Table VI. Relative Sizes* of Chromosomes within Haploid Sets in Sciara coprophila Males Heterozygous for Translocation T23

|                  | Translocation set: maternals |                 | Nontranslocation set: paternals |                 |
|------------------|-----------------------------|----------------|-------------------------------|----------------|
|                  | X II III IV                 | X II III IV    |                               |                 |
| Expected*        | All chromosomes             | 0.40 0.21 0.07 0.32 | 0.21 0.21 0.26 0.32 |                 |
|                  | Nonnucleolar chromosomes   | 0.39 0.61      | 0.26 0.27 0.33 0.41          |                 |
| Cell F           | All chromosomes             | 0.40 0.21 0.05 0.34 | 0.26 0.20 0.25 0.29 |                 |
|                  | Nonnucleolar chromosomes   | 0.38 0.62      | 0.27 0.33 0.40              |                 |
| Cell G           | All chromosomes             | 0.48 0.20 0.05 0.27 | 0.23 0.17 0.28 0.32 |                 |
|                  | Nonnucleolar chromosomes   | 0.42 0.58      | 0.22 0.36 0.42              |                 |
| Cell H           | All chromosomes             | 0.51 0.12 0.19 0.17 | 0.53 0.12 0.14 0.21 |                 |
|                  | Nonnucleolar chromosomes   | 0.42 0.58      | 0.25 0.30 0.46              |                 |
| Cell I           | All chromosomes             | 0.50 0.15 0.07 0.28 | 0.38 0.13 0.19 0.30 |                 |
|                  | Nonnucleolar chromosomes   | 0.35 0.65      | 0.21 0.32 0.47              |                 |
| Cell J           | All chromosomes             | 0.54 0.14 0.13 0.19 | 0.45 0.14 0.17 0.23 |                 |
|                  | Nonnucleolar chromosomes   | 0.42 0.58      | 0.25 0.32 0.43              |                 |
| Cell K           | All chromosomes             | 0.52 0.19 0.03 0.26 | 0.58 0.10 0.12 0.20 |                 |
|                  | Nonnucleolar chromosomes   | 0.43 0.57      | 0.24 0.28 0.48              |                 |
| Cell L           | All chromosomes             | 0.41 0.20 0.06 0.33 | 0.25 0.18 0.21 0.36 |                 |
|                  | Nonnucleolar chromosomes   | 0.38 0.62      | 0.24 0.28 0.48              |                 |
| Cell M           | All chromosomes             | 0.65 0.09 0.06 0.20 | 0.74 0.08 0.09 0.09 |                 |
|                  | Nonnucleolar chromosomes   | 0.32 0.68      | 0.30 0.35 0.36              |                 |
| Cell N           | All chromosomes             | 0.61 0.14 0.03 0.22 | 0.74 0.07 0.07 0.12 |                 |
|                  | Nonnucleolar chromosomes   | 0.39 0.61      | 0.26 0.27 0.46              |                 |

* Relative size = (chromosome size)/(sum of the size of all chromosomes in the set); "size" refers to either length (salivary gland chromosomes; expected values) or volume (based on absolute volumes measured by the reconstruction method nuclei; see Table V).

Given the uncertainties that remain in identifying the chromosomes of cell F (Which of the IVth chromosomes is maternal? Which of the three smallest chromosomes is the paternal IIIrd? Which IInd chromosome is maternal?), there are six possible ways to sort chromosomes into a maternal set: three choices for chromosome II multiplied by two choices for chromosome IV (XII and IIIII are specified). The remaining chromosomes can be assigned to the paternal set in two ways—this because the two chromosomes which must be the paternal IInd and IIIrd chromosomes can be assigned alternative identifications (i.e., for two chromosomes, a and b, either a is chromosome II and b is III, or vice versa). Thus, 12 distinct maternal/paternal combinations are possible.

Using a computerized sorting routine designed specifically for this study, the 12 possible maternal/paternal combinations can be generated. Two sorting programs were used. One program gives the quantitative attributes all twelve possible combinations; that is, relative chromosome volume profiles for maternal and paternal chromosome sets are calculated for each of the twelve possible combinations (see Table VI for examples of such profiles). The other program yields spatial information on the 12 combinations by calculating for each combination the three-dimensional coordinates for two lines, one line which connects all chromosomes identified as maternals and a second line which connects all paternals (e.g., see Figs. 3 and 4). As a result of this computerized routine, each chromosome is given a specific identification indicating (a) whether it is assigned to the maternal or paternal set and (b) which of the four chromosomes of a set it represents. The objective was to discover if any of the possible maternal/paternal combinations satisfied two conditions by asking the following: (a) is there a clear-cut separation between putative maternal and paternal sets; and (b) are the relative sizes of chromosomes within each set as expected?

Among the 12 alternative combinations for cell F, all but one fail to satisfy condition 1, either because the line connecting maternal chromosomes intersects the line connecting paternals (that is maternals and paternals are intermingled; 10 cases) or because the lines connecting chromosomes of...
Figure 3. The arrangement of chromosomes in prophase I cells in S. coprophila males heterozygous for translocation T23. (a) Cell F. (b) Cell G. (c) Cell H. These simplified three-dimensional spindle representations (stereo pairs) were generated by a computerized sorting routine that assigned chromosomes to two haploid chromosome sets. The position (center of mass, see Materials and Methods) of each chromosome (letters and roman numerals) is indicated, and solid lines connect all chromosomes that are identified as maternally (shaded set) or paternally derived; limited chromosomes (Ls and L) are of unknown parental origin and so are not included in either haploid set. Dashed lines which connect each chromosome and the polar organelle do not represent real structures; they are intended to help delineate the spatial relationship between chromosomes and the polar organelle. The primary axis of the cell is indicated by a solid line joining the polar organelle (●) and the membrane agglomeration (●); this axis passes between the each set are very close (the separation between sets is not clear-cut; one case). Only one combination satisfied both conditions. In this case, the maternal and paternal chromosome sets each form a ringlike array (Fig. 3 a) with the two rings facing each other from opposite halves of the nucleus; specifically, the chromosome sets are situated on either side of the primary axis such that maternals would be cleanly separated from paternals if the nucleus were divided in two by a plane that contains the polar organelle at one pole of the nucleus and the membrane agglomeration at the opposite pole. For both sets, relative chromosome sizes agree very well with the expected values (Table VI).

Chromosome Arrangements in Meiotic Prophase Nuclei. Chromosome volumes for all cells considered in this section were computed as they were for chromosomes on the meiosis II spindle, but tracings were made from enlarged negatives (final magnifications: 19,000–47,000); enlargement was necessary because prophase chromosomes are not fully condensed so that chromatin profiles are very small and very numerous.

An analysis using the computerized chromosome-sorting scheme outlined in the preceding section was performed for a total of six cells in meiotic prophase I (cells F–K). For all of these cells, the results are comparable to those already described for cell F in that chromosomes are grouped in spatially separated sets (Figs. 3 and 4) that have appropriate chromosome volume profiles (Tables V and VI). However, the relationship between parental chromosome sets and the primary axis is not always as was found for cell F. In cells G and H (Fig. 3, b and c), ringlike arrays of maternal and paternal chromosomes face each other from opposite sides of the primary axis, just as in cell F (Fig. 3 a). In cells I–K (Fig. 4, a–c), although rings of maternal and paternal chromosomes are in opposing nuclear halves, facing one another, the rings are not aligned with the primary axis. Rather, both rings are tilted relative to the axis so that the maternal and paternal nuclear halves are defined by a line drawn at an angle to the primary axis. Thus, in cells I–K, the maternal chromosome set is nearer the polar organelle and the paternal set nearer the membrane agglomeration.

While the results for six prophase I cells are as a whole quite comparable, it is important to note how cell-to-cell variations complicated the analyses.

Differential Chromosome Condensation (Allocycle). For cell G, when the two largest nonnucleolar euchromatic chromosomes were identified as homologues of chromosome IV, as for all other prophase I cells, none of the maternal/paternal combinations given by the computerized sorting rou-
Figure 4. The arrangement of chromosomes in prophase I cells in *S. coprophila* males heterozygous for translocation T23. (a) Cell I. (b) Cell J. (c) Cell K. See Fig. 3 legend for general explanation. Haploid chromosome sets are not on either side of the primary axis and aligned with it as for cells F-G (Fig. 3); instead, the ringlike chromosome arrays are tilted relative to the primary axis. For both cells I and J, the sorting routine gave an alternative arrangement (not illustrated) in which haploid chromosome sets are clearly separated; the alternatives result from the exchange of homologues between maternal and paternal sets (cell I; IVth chromosome exchange; cell J: lind chromosome exchange). To examine the alternative arrangements, the reader can redraw Fig. 4, a and b, following in a general way instructions that are given in the legend for Fig. 3 to interchange the appropriate homologues between parental chromosome sets. For cell I, the alternative shows two chromosome rings that face directly and that are aligned with the primary axis (side-by-side arrangement, see text); thus, there is some resemblance to arrangements shown in Fig. 3. However, the alternative differs substantially from the examples in Fig. 3 in that the primary axis does not fall between chromosome rings; instead, the maternal chromosome ring is directly on the primary axis. For cell J, the alternative involves two chromosome rings that face directly, with both tilted relative to the primary axis; thus, in some respects, this alternative resembles the arrangement illustrated for this cell (Fig. 4 b) and those for cells I and K (Fig. 4, a and c). There is, however, a fundamental difference in that the nonillustrated alternative for cell J places the paternal chromosome set closest to the polar organelle while it is the maternals that are closer to the polar organelle in the other cases.
Chromosome Arrangements on the Meiosis I Spindle.

One cell, cell L, was judged to be in an early stage of meiosis I spindle formation. Although spindle microtubules are abundant, none of the chromosomes are associated with kinetochore microtubules; moreover, the presence of a nucleolus on one chromosome suggests that the cell had not progressed much beyond prophase, the stage during which nucleoli are expected to disassemble. Cell L is thus at a considerably earlier stage of meiosis I spindle function than any of the mid-meiosis cells previously studied. This permits a reexamination of the arrangement of haploid chromosome sets on the spindle, not only at an earlier spindle stage than was previously possible but also using the more direct means for differentiating between maternal and paternal chromosomes that was designed for this investigation.

Although in cell L chromosomes X, X m, and III X are not all tagged with nucleoli, it is nonetheless relatively easy to identify each of them. Assuming that the largest nucleolus would disappear last, the only chromosome still associated with a nucleolus in this cell can be identified as the X; and the largest and smallest of the remaining chromosomes are then taken to be X III and III X, respectively (see absolute chromosome sizes recorded in Table V).

Among the maternal/paternal combinations generated by the computerized sorting routine, one combination shows a clear-cut grouping of maternal vs. paternal chromosomes (Fig. 5) and excellent agreement between expected and measured chromosome size profiles (Table VI). In this combination, the maternal and paternal chromosome rings are in a mutually facing arrangement, a relationship between parental chromosome sets that was likewise indicated for each of the prophase cells already described. However, the relationship of chromosome sets to the primary axis of this cell (i.e., the long axis of the spindle) is distinctly different from what was indicated for any prophase cell; the parental chromosome rings are oriented almost perfectly perpendicular to the primary axis so that the entire set of maternal chromosomes is closer to the polar organelle than the paternal set, just as was concluded from earlier observations.

Chromosome Arrangements in Spermatogonial (Mitotic) Prophase Nuclei. A variety of observations suggested that the segregation of haploid chromosome sets in two halves of the nucleus is a characteristic of all cells of the germ line in S. coprophila males (summarized in reference 13). That is, a similar grouping of parental chromosomes might exist not only in meiotic prophase nuclei of spermatocytes such as those just described but also in prophase nuclei of spermatagonia, the mitotic precursors of meiotic nuclei.

Only two spermatogonia were encountered in the course of this study (spermatagonia are expected to occur infrequently, if at all, in prepupa; see reference 21). These cells, cells M and N, are indistinguishable from cells in meiotic prophase I in terms of nuclear structure (including chromosome ultrastructure and number). However, they are identifiable as spermatogonia owing to the presence of two polar organelles, one at each end of the cell, as described for the spermatogonia of second instar larvae (19). In cell M, for example, one polar organelle is located at the cell pole nearest the exterior of the cyst (i.e., in the same position as the single polar organelle of meiotic cells) and a second is at the opposite pole, in the vicinity of the membrane agglomeration. Cell N is similarly recognizable as a spermatogonium because the polar organelle distinctive of spermatogonia, the one near the membrane agglomeration, is present (the other polar organelle was not observed because the remainder of the cell was not included in the section series).

The identification of chromosomes X and X III for cells M and N was complicated by an anomalous nucleolus size/chromosome size relationship. The two largest chromosomes in each cell obviously represent X and X III; but, as in prophase cell K, the larger nucleolus is associated with the larger chromosome, not (as was expected) smaller nucleolus with larger chromosome (see Table I). Therefore, chromo-
Figure 6. The arrangement of chromosomes in spermatogonia at prophase in *S. coprophila* males heterozygous for translocation T23. (a) Cell M. (b) Cell N. See Fig. 3 legend for general explanations. In this case, two polar organelles (O) are present; the primary axis is thus redefined as the line joining these two structures. The membrane agglomeration, the structure at one end of the primary axis in other developmental stages, is present to one side of the polar organelle appearing at the bottom of the figures.

Chromosome Order in Haploid Sets. If chromosomes derived from different parents are indeed in different halves of prophase nuclei or the spindle, as illustrated in Figs. 4–6, with the chromosomes of each parent arrayed in a ring, it is possible that two levels of intranuclear order are maintained—

a segregation of the two haploid sets and a regular arrangement of the individual chromosomes within each set (ring). Therefore, the arrangement of chromosomes in the maternal chromosome ring was compared for several cells.

The comparison included six cells, those for which the analysis suggested just one way to arrange parental chromosomes into separate sets. When the maternal chromosome rings in these cells are viewed from outside the nucleus, three different chromosome orders are observed: *X*III - II - IIIx - IV (cells G, Fig. 3 b; cell L, Fig. 5); *X*III - IIIx - IV - II (cell K, Fig. 4 c; cells M and N, Fig. 6, a and b); and *X*III - IV - II - IIIx (cell F, Fig. 3 a). This indicates that chromosomes do not assume regular positions within a ring since the three observed orders correspond to the three2 (4-1)!/2 possible orders in which objects can be arranged in four-membered rings.

Discussion

Background

To discover if haploid chromosome sets occupy distinct nuclear domains in the male germ line of *S. coprophila*, a combination of spatial and quantitative information was obtained by serial section reconstruction. Although the labor involved is tedious and time consuming, reconstruction analysis circumvents certain errors that are unavoidable when unsectioned materials are examined (4). The technique is highly effective for examining the arrangement of chromosomes on metaphase spindles; based on this approach, Bennett and his co-workers have argued that chromosomes are positioned on the metaphase plate in an orderly way (for review see reference 3).

The reconstruction method has been used extensively to identify chromosomes at metaphase, a stage when chromosome volume accurately reflects chromosome DNA content (5). In the present study, the objective was to identify the chromosomes of *S. coprophila* at a different stage, prophase rather than metaphase. Nonetheless, an analysis of metaphase was undertaken, primarily to provide a foundation for the study of prophase nuclei. This analysis showed that there is a close correspondence between relative chromosome volumes of metaphase maternal chromosomes on the meiosis II spindle (Table III) and relative chromosome lengths of polytene maternal chromosomes in salivary gland nuclei (Table I). Since we can assume that metaphase and polytene chromosomes are roughly cylindrical so that volume is proportional to length in both cases, it is clear that chromosomes in very different states of condensation (polytene vs. metaphase) maintain the same relative sizes. And, it seems reasonable to think that this is so because in *Sciara* chromosome size (length or volume) is proportional to DNA content, just as was demonstrated more directly for other species. Thus, the results obtained by studying metaphase chromosomes bear on the problems connected with identify-

2. There are (4-1)! = 6 possible orders for four objects in a ring, but only half as many orders would be recognizable if orders that are inverse to one another cannot be distinguished. Though chromosome orders were determined by viewing rings from a fixed perspective, the outside of the nucleus, it would not be possible to distinguish one order from its inverse if a chromosome ring may be turned either way with respect to the outside of the nucleus.
ing prophase chromosomes in two respects. (a) The fact that the relative size profile for the maternal chromosomes is the same whether these chromosomes are found on the metaphase II spindle or in polytene salivary gland nuclei justifies using the sizes of salivary gland chromosomes as standards for comparison. This means that a relative size profile for each of the two parental chromosome sets is available from salivary gland data, as required for study of prophase nuclei. (b) The relative chromosome size profiles obtained for five metaphase cells are highly reproducible (Table III) even though the chromosomes were digitized at low magnification (×3,350). Because the many small chromatin masses composing each prophase chromosome were digitized at considerably higher magnifications (×19,000–47,000), prophase chromosome measurements should be at least as accurate as those for metaphase chromosomes.

Reconstruction data for prophase and metaphase are not precisely comparable because chromosomes at different stages of the cell cycle are very different structurally. For example, each metaphase chromosome can be classified as metacentric or acrocentric based on the position of the chromosome's kinetochore (centromere), and this information can be used together with size information to distinguish between chromosomes (see Table III and reference 3). In contrast, the kinetochores of prophase chromosomes in *S. coprophila* are not differentiated so these chromosomes cannot be classified as meta- or acrocentric. However, nucleoli are present at prophase (not metaphase), so prophase chromosomes can be classified as nucleolar vs. nonnucleolar; in addition, nucleolus size can be measured. Thus, chromosome type (nucleolar/nonnucleolar), nucleolus size, and chromosome size can be used together to identify prophase chromosomes (see Tables V and VI).

The fact that metaphase and prophase cells differ in chromosome condensation also affects the use of the reconstruction method for cells at different stages. With this method, only condensed chromatin can be measured; i.e., chromatin must be sufficiently condensed to appear as dense masses in electron micrographs. Obviously, then, fully condensed metaphase chromosomes are ideally suited for volume reconstruction measurements while prophase chromosomes are potentially difficult subjects because they are actually engaged in the gradual process of condensation. Of particular concern is the possibility that prophase chromosomes might condense in an allocyclic fashion; i.e., with some intra- or interchromosomal variation in the degree of condensation.

The indications that allocyly is a factor in *S. coprophila* prophase chromosomes were presented in Results. It is fortunate that allocyly seems to be most pronounced for nucleolar chromosomes: for these chromosomes, nucleolus size can, when necessary, serve as the sole basis for chromosome identification. For chromosomes less affected by allocyly, the nonnucleolar chromosomes, one cannot avoid the issue so directly. However, for these chromosomes, reconstruction-based identifications are possible if it is assumed that the relative chromosome size profile for the nonnucleolar chromosomes of either haploid set will be similar to the profiles for salivary gland chromosomes regardless of any allocyly. This assumption allows for the possibility that maternal and paternal nonnucleolar homologues may differ slightly in size due to allocyly but implies that allocyly is never so severe that, for example, a nonnucleolar chromosome that is relatively small in salivary gland nuclei becomes relatively large in prophase nuclei. Given that the identification of nonnucleolar chromosomes thus depends on an unverifiable assumption, the identifications are rather tentative and must be confirmed by further analysis (see next section).

In summary, reconstruction data can be used to identify prophase chromosomes in much the same way as they are used to identify metaphase chromosomes, but the allocyclic nature of prophase chromosomes introduces an element of uncertainty that is not a factor when metaphase chromosomes are under consideration.

The Three-dimensional Structure of Germ Line Nuclei in *S. coprophila* Males

The reconstruction-based investigation described in this report was undertaken to discover if chromosomes in prophase germ line nuclei of *S. coprophila* assume a particular arrangement, i.e., with haploid chromosome sets occupying separate intranuclear domains. Plainly, direct evidence for such order could not be obtained because some prophase chromosomes are only tentatively identifiable. However, when a computerized sorting routine was used to analyze the data from a number of cells, thus testing all of the possible ways that the chromosomes in each cell might be identified, there emerged strong evidence that parental chromosome sets do indeed occupy distinct compartments in prophase nuclei. Moreover, these nuclei appear to be orientated within the cell so that the haploid chromosome sets have a specific relationship to a well-defined cell axis.

Nine germ line cells were analyzed: six cells in prophase of meiosis I (cells F–K), one cell with a newly formed meiosis I spindle (cell L), and two cells in prophase of premeiotic mitosis (cells M and N). In every case, among the twelve alternative chromosome arrangements presented by the sorting routine, there were one or two showing a clear-cut separation between haploid chromosomes sets and appropriate chromosome volume profiles for both parental sets, as given in Figs. 3–6 and Tables V and VI. If chromosomes are correctly identified in each of the illustrated cases, as I am convinced they are, we can conclude that the haploid chromosome sets derived from two parents occupy separate compartments in all germ line nuclei of *S. coprophila* males.

My conviction that the chromosomes are accurately identified in Figs. 3–6 is based on some remarkably consistent aspects of the chromosome arrangements illustrated. (a) In every case, the chromosomes derived from each parent are in a compact ringlike array, the two parental chromosome rings are reasonably “flat” (i.e., the points indicating the position of each chromosome in a ring are not far from being co-planar), and the two rings face almost perfectly toward one another. (b) Parental chromosome rings are rather precisely oriented relative to intracellular landmarks. In premeiotic prophase (cells M and N) and some prophase I cells (cells F–H), the two chromosome rings are aligned on either side of the cell’s primary axis, parallel to it; otherwise (cells I–L), the rings are turned relative to the primary axis so that the maternal chromosome set is located nearer the polar organelle (i.e., if the cell were bisected so as to separate the two chromosome rings, the maternals and the polar organelle would both be found in the same half cell). In other words, the results indicate a striking regularity in the spatial rela-
relationship between parental chromosome sets and a limitation on the ways in which nuclei orient relative to the primary axis, a regularity that dictates my conclusion that the chromosome identifications have been made correctly.

This investigation of the S. coprophila male germ line was undertaken because the demonstration of an orderly segregation of haploid chromosome sets in resting nuclei would help define the functions of the unusual monopolar meiosis I spindle. The resulting evidence indicates that maternal/paternal chromosome segregation is well established in premeiotic nuclei (cells M and N) and persists in meiosis I prophase nuclei (cells F–K). This bears out my earlier suggestion that the meiosis I spindle has no active role in sorting maternal from paternal chromosomes (13); it simply maintains a pre-existing segregation of two chromosome sets while the distance between these sets increases. This is all that is required to ensure that upon cytokinesis only maternal chromosomes are included in one division product and only paternal chromosomes in the other, the perfectly nonrandom chromosome distribution peculiar to meiosis I in S. coprophila males.

As well as providing evidence for a highly ordered intranuclear chromosome arrangement, the present study gave some unanticipated indications that nuclei assume different orientations within cells at different stages of germ line development. A cursory examination of nuclei in late prophase I had suggested that maternal chromosomes occupy the nuclear hemisphere closest to the polar organelle (13), just as成熟 nuclei are closer to the organelle when on the meiosis I spindle. Consequently, I expected to find a similar relationship between chromosomes and cytoplasmic structures in all of the sets described in this report. In fact, these cells exhibited three distinct orientations of the parental chromosomes: side-by-side (maternals to one side of the primary axis, paternals to the other), tilted (with maternals closer to the polar organelle), and up/down (if the polar organelle is considered to be situated at upper pole of the cell, maternals are closer to it and therefore “up”). The side-by-side arrangement was found in premeiotic cells (cells M and N; Fig. 6) and in half of those in prophase I (cells F–H; Fig. 3); the tilted condition appeared in the remaining prophase I cells (cells I–K; Figs. 4); and the up/down arrangement was found in just one cell, a cell that had progressed beyond prophase and into the earliest stage of spindle formation (cell L; Fig. 5). Since the side-by-side orientation occurs early in germ line development (premeiotic prophase) while the up/down orientation appears late (in late prophase I cells with well-condensed chromosomes [see reference 13] and in early meiotic spindle formation, cell L), the tilted orientation found in some prophase I cells most likely represents a transition between the side-by-side and up/down states. Given that this transition appears to occur during prophase I, as a cell prepares for the first meiotic division, a change in nuclear orientation could well be connected with a germ cell’s switch from the mitotic (premeiotic) to the meiotic mode of reproduction.

A mitotic/meiotic transition from side-by-side to up/down nuclear orientation would require nuclear rotation (or a comparable coordinated intranuclear displacement of both parental chromosome sets); in order to bring a nucleus directly into the up/down (maternals up) orientation, a 90° rotation would suffice. Thus, it is significant that each of the presumed transition stages (cells I–K) shows a tilted chromosome arrangement with the maternal chromosomes nearer the polar organelle. This suggests that nuclear rotation is constrained to proceed in a particular direction, always moving maternals closer to the polar organelle; if this were not so, it is unlikely that all transition-stage cells would show such similar nuclear orientations.

Exactly how nuclear orientation is controlled is open to conjecture. Since microtubules were observed radiating from the polar organelle toward the nucleus in all meiotic prophase I cells, it is natural to suspect that meiotic nuclear rotation is a microtubule-related process. Moreover, a speculative explanation for differing nuclear behavior is suggested by the fact that there is only a single polar organelle and associated microtubule population in meiotic prophase while two such polar arrays, one at each pole of the nucleus, characterize mitotic prophase. If polar microtubule arrays promote nuclear rotation, a meiotic prophase nucleus would be subject to a single rotation promoter and should exhibit a net rotation; the direction and extent of the rotation could be controlled by an asymmetric development/functioning of the polar microtubule array. In contrast, a mitotic prophase nucleus would be affected by two rotation forces whose effects could counterbalance to maintain the nucleus in fixed orientation as the spindle forms.

One can easily imagine that different nuclear orientations might be required depending on whether a male germ line cell in S. coprophila is destined for mitosis or meiosis. It is conceivable, for example, that mitotic nuclei must maintain the orientation that puts chromosomes in the side-by-side arrangement in order to preserve a perfect intranuclear segregation of parental chromosome sets over the course of many cell generations. The reasoning is as follows: since chromosomes congress on the metaphase plate by moving on the spindle only in the pole-to-pole direction (18), the arrangement of chromosomes on the metaphase plate and in daughter nuclei will depend directly on how a nucleus is oriented when spindle formation begins. If a nucleus enters mitosis with parental chromosomes in a side-by-side arrangement, chromosomes will arrive at metaphase with all maternals to one side of the plate and all paternals to the other; then, in each daughter nucleus formed at telophase, haploid chromosome sets will remain perfectly segregated in different nuclear halves. In contrast, if a nucleus enters mitosis in any other orientation (i.e., with haploid chromosome rings in either the tilted or up/down arrangement) chromosomes from the two parents will intermingle on the metaphase plate; as a result, a complete randomization of maternal and paternal chromosomes within daughter nuclei will be effected over the course of several cell generations.

Just as the side-by-side chromosome arrangement might be required in S. coprophila while spermatogonia are undergoing mitotic reproduction, a different arrangement, up/down, could facilitate the unusual nonrandom segregation of maternal and paternal chromosome sets that is accomplished during the first meiotic division (13, 16). The monopolar meiosis I spindle always forms with its long axis coincident with the cell’s primary axis and with the polar organelle at the pole. Thus, a prophase nucleus in up/down orientation at the onset of spindle formation will position maternal chromosomes nearer the spindle pole and paternal chromosomes farther from it. This is exactly the maternal vs. paternal chromosome positioning that was observed on a just-formed spindle.
(cell L). And, it is precisely the chromosome arrangement maintained by spindles throughout meiosis I (13) in order to ensure that maternal and paternal chromosomes are nonrandomly distributed to the two division products. Therefore, the orientation of the late prophase I nucleus could well have a major influence on the arrangement of parental chromosome sets on the meiosis I spindle.

As discussed above, nuclei in the germ line of *S. coprophila* males are highly ordered structures with respect to both the intranuclear arrangement of the two haploid chromosome sets and the intracellular orientation of nuclei. As for the positioning of specific chromosomes within a haploid set, the evidence indicates randomness in prophase I nuclei but appreciable order in metaphase II spindles. Apparently, then, the ordered arrangement of chromosomes on the spindle does not depend on a comparable order in prophase nuclei. Instead, order must be imposed in the course of spindle formation, perhaps as a function of the physical constraints involved in accommodating chromosomes of varying size on the spindle. Therefore, the idea that "the arrangement of . . . chromosomes on the metaphase plate substantially reflects their interphase arrangement" (3) seems not to apply to *S. coprophila*.

Although the investigations described here were designed primarily to help us understand the peculiar first meiotic division in *S. coprophila* males, they are of broader significance. The results strengthen the general argument that the arrangement of chromosomes in resting nuclei can influence subsequent behavior of chromosomes on mitotic or meiotic spindles. Furthermore, this concept can now be extended to include the notion that not only the internal organization of a nucleus but also its specific orientation within the cell can profoundly affect chromosome–spindle interactions. This investigation also represents the first time that the spatial ordering of chromosomes within haploid sets in prophase nuclei and on metaphase spindles has been compared. The discovery that an orderly disposition of chromosomes on the metaphase plate does not stem from a similar arrangement in the prophase nucleus cautions against drawing conclusions about intranuclear structure from observations of metaphase spindles alone.

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