Journal of Cell Science 107, 299-312 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994
JCS8407

Unorthodox male meiosis in *Trichosia pubescens* (Sciaridae)

Chromosome elimination involves polar organelle degeneration and monocentric spindles in first and second division

Harald Fuge

Department of Biology, University of Kaiserslautern, PO Box 3049, D-67653 Kaiserslautern, Federal Republic of Germany

SUMMARY

Male meiosis in *Trichosia pubescens* (Sciaridae) was investigated by means of serial section electron microscopy and immunofluorescence light microscopy. From earlier studies of another sciarid fly, *Sciara coprophila* (Phillips (1967) *J. Cell. Biol.* 33, 73-92), it is known that the spindle poles in sciarid spermatogonia are characterized by pairs of 'giant centrioles', ring-shaped organelles composed of large numbers of singlet microtubules. In the present study spermatocytes in early prophase of *Trichosia* were found to possess single giant centrioles at opposite sides of the nucleus. The obvious reduction in centriole number from the spermatogonial to the spermatocyte stage is suggested to be the result of a suppression of daughter centriole formation. In late prophase, a large aster is developed around the centriole at one pole. At the opposite pole no comparable aster is formed. Instead, a number of irregular centriolar components appear in this region, a process that is understood to be a degeneration of the polar organelle. The components of the degenerate pole migrate into a cytoplasmic protrusion ('bud'), which later is also utilized for the elimination of paternal chromosomes. The existence of only one functional polar centre is the reason for the formation of a monopolar monocentric spindle in first meiotic division, which in turn is one of the prerequisites for the elimination of paternal chromosomes. While the set of maternal and L chromosomes orientates and probably moves towards the pole, paternal chromosomes seem to be unable to contact the pole, possibly due to an inactivation of their kinetochores. Retrograde ('away from the pole') chromosome motion not involving kinetochores is assumed. Eventually, paternal chromosomes move into the pole-distal bud and are eliminated by casting off, together with the components of the degenerate polar organelle. Chromosome elimination can be delayed until the second meiotic division. The spindle of the second meiotic division is bipolar and monocentric. One spindle pole is marked by the polar centre of first division. The opposite spindle apex is devoid of a polar centre. It is assumed that spindle bipolarity in the second division is induced by the amphi-orientated chromosomes themselves. The maternal and L chromosome set (except the non-disjunctional X chromosome, which is found near the polar centre) congress in a metaphase plate, divide and segregate. Of the two daughter nuclei resulting from the second meiotic division, the one containing the X chromatids is retained as the nucleus of the future spermatozoon. The other nucleus becomes again eliminated within a second cytoplasmic bud.

Key words: giant centriole, spindle formation, kinetochore orientation, monopolar monocentric division, bipolar monocentric division, serial section electron microscopy, immunofluorescence

INTRODUCTION

Since Metz' pioneering work (Metz, 1927, 1931, 1933; Metz et al., 1926) male meiosis of sciarid flies has been repeatedly investigated (e.g. see Rieffel and Crouse, 1966; Phillips, 1966, 1967; Amabis et al., 1979; Abbott and Gerbi, 1981; Abbott et al., 1981; Kubai, 1982, 1987). It is especially interesting with respect to unusual orientation and movement of chromosomes during non-random segregation and elimination in the first meiotic division. A comprehensive review was presented by Gerbi (1986). Apart from Amabis et al. (1979), who investigated meiosis in *Trichosia pubescens* using standard light microscopical techniques, most other workers studied members of the genus *Sciara*, particularly *Sciara coprophila*.

In the following, a synopsis of only those events that were the subject of the present investigation is given. The spermatocyte enters the first meiotic division with a diploid set of univalent, i.e. unpaired chromosomes consisting of ordinary autosomes, germline limited chromosomes (L chromosomes), and two X chromosomes. The first meiotic division is characterized by the formation of a monopolar spindle. Chromosomes inherited from the mother (maternal chromosomes) together with L chromosomes and those inherited from the father (paternal chromosomes) are non-randomly distributed. Maternal chromosomes (autosomes and one X chromosome) and L chromosomes are displaced to the pole of the monopolar spindle, whereas paternal chromosomes (autosomes and one X chromosome) become eliminated in a cytoplasmic protrusion

('bud'). With respect to autosomes and the X this directed segregation is reductional. Kinetochores of paternal chromosomes of *Sciara* were found to be connected with the pole via kinetochore microtubules (Kubai, 1982), although the chromosomes become eliminated. Whether or not the paternal haploid set is definitely 'backing away' from the spindle pole is a matter of controversy (Gerbi, 1986). The second meiotic division in male sciarids is bipolar (Gerbi, 1986). Maternal autosomes and L chromosomes congress in a metaphase plate and their chromatids are conventionally distributed to opposite poles in anaphase (equational division). The maternal X chromosome does not divide but is found at one pole already at metaphase ('precocious' X chromosome; Abbott and Gerbi, 1981). Two daughter nuclei are formed and, while the nucleus containing both chromatids of the X is retained as the future spermatid nucleus, the other nucleus is again eliminated in a cytoplasmic bud. Hence, male meiosis results in a single spermatozoon containing only chromosomes inherited from the mother and the L chromosomes. According to Amabis et al. (1979) male meiosis in *Trichosia pubescens* is similar to that in *Sciara*, with the exception that paternal chromosomes obviously are not orientated polewards during the first meiotic division. In a preliminary study using indirect immunofluorescence staining of tubulin Bastmeyer (1989) observed that one spindle pole in the second meiotic division in *Trichosia* lacked aster microtubules.

In conclusion, male sciarid meiosis does not seem to be less enigmatic today than it was in the time of Metz. Regarding the general problem of spindle function in mitosis and meiosis the study of such 'exceptional' cases is a meaningful attempt, to cite a statement of the late Max Hartmann: '...Ihre (der Sciaridenmeiose) Analyse könnte deshalb für das Mitoseproblem aufschlußreicher sein, als wiederholte Untersuchungen an der 'normalen' Mitose. Ehe es nicht gelingt, alle verschiedenen Mitoseabläufe einer einheitlichen Vorstellung einzuordnen, kann das Rätsel vom Mechanismus der Kernteilung nicht als gelöst angesehen werden.' (Hartmann, 1953).

The data of the present study of spermatogenesis in *Trichosia* are based on serial section electron microscopy and immunofluorescence light microscopy. The observations suggest that a modification of the centriole cycle causes the generation of a monopolar monocentric spindle in the first meiotic division and, in succession, of a bipolar monocentric spindle in the second meiotic division. Both seem to be essential prerequisites for chromosome eliminations and the final outcome of only a single spermatozoon containing the maternal and L chromosome set.

MATERIALS AND METHODS

IVth instar larvae of *Trichosia pubescens* (Nematocera, Sciaridae), a species from Brazil, were obtained from a laboratory stock of the Max-Planck-Institut für Biologie, Tübingen (Laboratory of Dr Claus Pelling). To avoid drying, animals (prepupae and early pupal stages) were dissected in a moisture chamber at 95% relative humidity. For a preliminary study of meiosis with the light microscope, squash preparations of ethanol/acetic acid (3:1 v/v)-fixed testes were stained with Darlington's orcein (1% orcein in 45% acetic acid). For electron microscopy and immunoflorescence light microscopy testes were opened in a 40 µl drop of fixative on a microscope slide previously coated with poly-L-lysine or collagen to improve adhesion of the spermatocytes. The suspended spermatocytes were slightly separated with a fine needle, an 18 mm × 18 mm coverslip was applied, and fixation was carried out under the coverslip.

Electron microscopy

The first fixation was carried out for 30 minutes at room temperature in 0.03 M Pipes buffer (pH 7) containing 2% glutaraldehyde. After fixation the coverslip was removed by dipping the slide in buffer. The selection of appropriate cell stages in the embedded material with the phase-contrast microscope is prevented by a dense layer of mitochondria masking the nuclei and division spindles of *Trichosia* spermatocytes. For this reason, chromosomes were stained with DAPI (4′,6-diamidino-2-phenylindole-dihydrochloride, 2×10^{-5}% in a 1:1, glycerol/phosphate-buffered saline (PBS) medium) prior to second fixation and resin embedding, and examined with the fluorescence microscope. The positions of appropriate stages were recorded and the chromosomes photographed. Then the cells were washed in buffer, fixed for 30 minutes in 0.03 M Pipes containing 1% OsO_4, dehydrated in an acetone series, and flat-embedded on the slide in a drop of Vestopal W polyester resin. After removal from the slide the selected cells (prophase stages, 9 cells from 7 animals; first meiotic division, 8 cells from 4 animals; interkinesis, one cell; second meiotic division including telophase stages, 15 cells from 7 animals) were sectioned with a LKB Ultrotome III (section thickness 50 nm). Sections were double-stained with uranyl acetate and lead citrate, and examined with a Zeiss EM10A electron microsocope working at 80 kV.

Immunofluorescence

The cells were fixed in 0.03 M Pipes (pH 7) containing 2.5% formaldehyde (freshly prepared from paraformaldehyde), 0.5% glutaraldehyde, 0.2% Triton X-100, 1 mM EGTA, and 1 mM $MgCl_2$ for 10 minutes. After fixation slides were washed 3 times for 5 minutes in PBS (pH 7), or stored for longer at 4°C in the same buffer. Tubulin staining: cells were incubated with anti-α-tubulin (mouse monoclonal antibody, IgG1, Serva, Heidelberg, 1:2000 dilution in PBS) for 1 hour at room temperature. Three 5-minute rinses in PBS were followed by incubation in fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (affinity-isolated antibody, Sigma Chemical Co., St Louis; 1:100 dilution in PBS) for 30 minutes at room temperature. After rinsing three times for 5 minutes in PBS (with the last step containing 2×10^{-5}% DAPI for chromosome staining) the slides were embedded in a 9:1, glycerol/Tris-HCl medium containing 2.3% 1,4-diazabicyclo-[2.2.2]octane (DABCO, Sigma) as an antifade agent. The cells were examined with a Zeiss Axioskop using filter sets H485SB (blue excitation) for FITC and G365 (UV excitation) for DAPI. Photos were taken on Kodak Technical Pan 2415 using a ×40 Plan-Neofluar oil objective.

RESULTS

General

Primary spermatocytes of *Trichosia pubescens* have a chromosome set of $2n$=11 (12). This comprises three (occasionally 4; see Amabis et al., 1979) L chromosomes, six ordinary autosomes, and two X chromosomes. Ordinary and L chromosomes could not unequivocally be distinguished during first and second division, with either the light or the electron microscope. In the group of seven chromosomes retained in the first meiotic division, three large metacentrics, one small metacentric and three medium-sized acrocentrics could be distinguished in orcein squash preparations.

Early prophase (stage I)

The earliest cell stage studied is round or elliptical. After DAPI

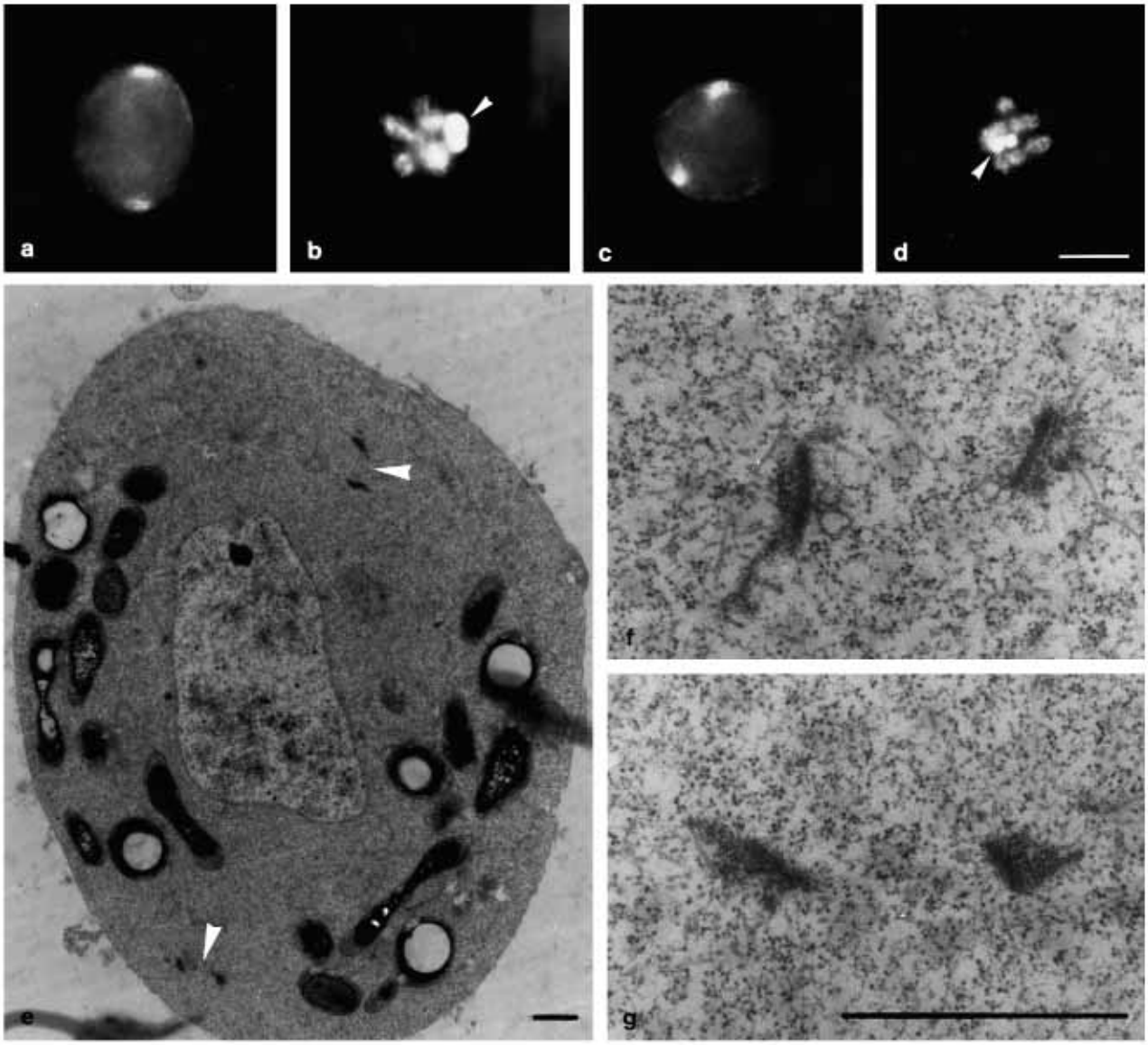


Fig. 1. Early prophase (stage I). (a,b and c,d) Two cells labeled with anti-tubulin-FITC (a,c) to show distribution of giant centrioles and associated microtubules. Chromosomes stained with DAPI (b,d). Arrowheads point to condensed L chromosomes. Bar in d, 10 μm. (e) Ring-shaped giant centrioles (arrowheads at opposite sides of the nucleus). The numerous mitochondria of *Trichosia* spermatocytes appear black or 'empty' in this and the following micrographs. (f,g) Giant centrioles at higher magnification surrounded by a few aster microtubules, same cell as in (e), neighbouring section. Bars, 1 μm.

staining chromosomes can be distinguished as individuals but most of them are still relatively decondensed. Three (exceptionally four) chromosomes are heavily stained with both orcein and DAPI (Fig. 1b,d). These are the condensed (heterochromatic) L chromosomes (Rieffel and Crouse, 1966; Amabis et al., 1979). This stage can be regarded as very early prophase of the primary spermatocyte (Rieffel and Crouse, 1966) and is called here 'Stage I'. After anti-tubulin labeling the bulk of stage I cells shows two signals, generally lying opposite to one another at the cell periphery (Fig. 1a). In some cells the two signals were found not exactly opposite to each other (Fig. 1c), and occasionally cells with four signals were observed (not shown). The tubulin signals can be similar in size and staining intensity or one signal can be more pronounced. Fig. 1e shows an electron micrograph of a stage I cell. It becomes evident that the tubulin staining pattern is due to two 'giant centrioles' with associated aster microtubules lying at opposite sides of the nucleus. From studies of *Sciara coprophila* it is known that giant centrioles composed of 60-90 singlet microtubules are typical of sciarid germ line cells (Phillips, 1967). Since the centriole is a ring-shaped organelle, it generally appears as two darkly staining profiles when viewed from the side in ultrathin sections (Figs 1f,g and 6k-m). Serial section analysis revealed a maximum centriole diameter of 2 μm. It can be assumed that asymmetry of centres in the anti-tubulin staining pattern is caused by different aster microtubule amounts.

Early prophase (stage II)

Later in prophase the cell becomes pear-shaped. This is due to

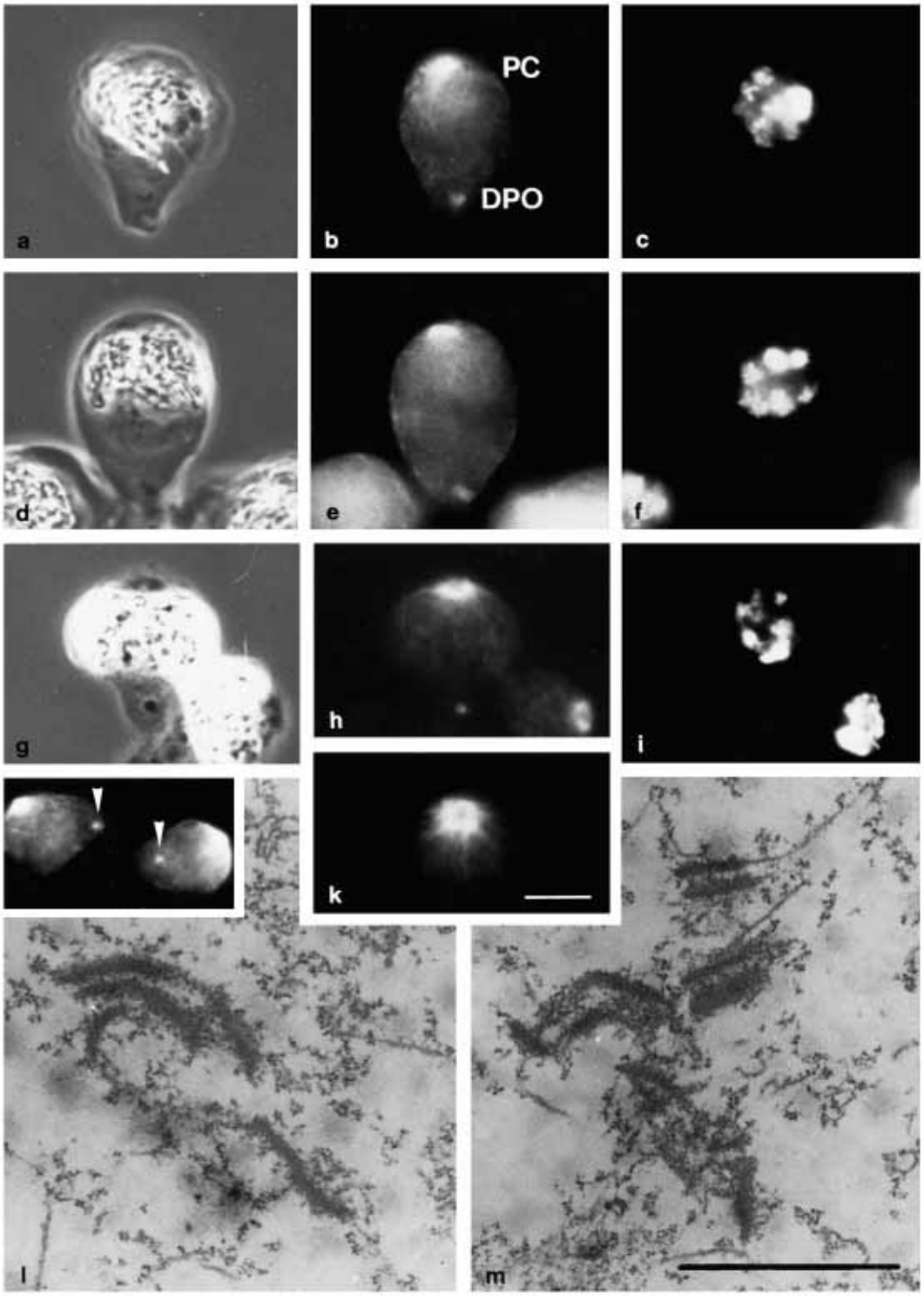


Fig. 2. Early prophase (stage II). (a-c, d-f and g-i) Three cells in phase-contrast (a,d,g), and anti-tubulin-FITC (b,e,h) to show polar centres (PC) and degenerate polar organelles (DPO). Chromosomes stained with DAPI (c,f,i). DPO regions, and partly the PC (g), appear as dark zones in phase-contrast. k, PC viewed from above, anti-tubulin-FITC. Bar in k, 10 μm. (l,m) Ultrathin sections of two DPO regions previously labeled with anti-tubulin (inset, arrowheads). The bad preservation of the cytoplasm is due to formaldehyde fixation used for immunofluorescence. Bar, 1 μm.

the development of a cytoplasmic protrusion ('bud') at one cell pole. As Amabis et al. (1979) have shown, up to 15 of these pear-shaped spermatocytes in *Trichosia* form globular cysts with the buds pointing towards the centre of the cyst. This arrangement of cells is also typical of *Sciara* (Metz et al., 1926; Kubai, 1982). In the present study these cysts were mostly disrupted during preparation. However, if several cells happened to stay together on the slide, the distal ends of the buds were constantly observed in ultrathin sections to be connected by thin cytoplasmic channels. Hence, spermatocytes of a cyst form a syncytium. Chromosome condensation of stage II is fairly similar to that of stage I. The anti-tubulin staining pattern is characterized by an intense and broad signal at the cell pole opposite to the bud (Fig. 2b,e,h). When viewed from above this signal has a star-like appearance (Fig. 2k). Studies of later division stages (see below) clearly indicate that we are dealing here with the polar centre (PC) of the future monopolar spindle of the first meiotic division, composed of

one giant centriole surrounded by large numbers of aster microtubules. The other tubulin signal of lesser intensity is generally located near or inside the cytoplasmic bud (Fig. 2b,e,h). In phase-contrast this fluorescence signal is a dark body of irregular shape (Fig. 2a,d,g). To study this structure at higher resolution, some cells previously examined with the fluorescence microscope were post-fixed with OsO_4 and re-embedded for ultrathin sectioning and electron microscopy. It turns out that the tubulin signals are caused by a number of differently shaped centriole-like components, suggesting irregular generation of giant centriole material in this region (Fig. 2l,m). This is understood to be a process of degeneration, and for this reason the entirety of the components was named 'degenerate polar organelle' (DPO).

Different morphological aspects of DPOs and their fate during meiotic divisions will be discussed below in more detail.

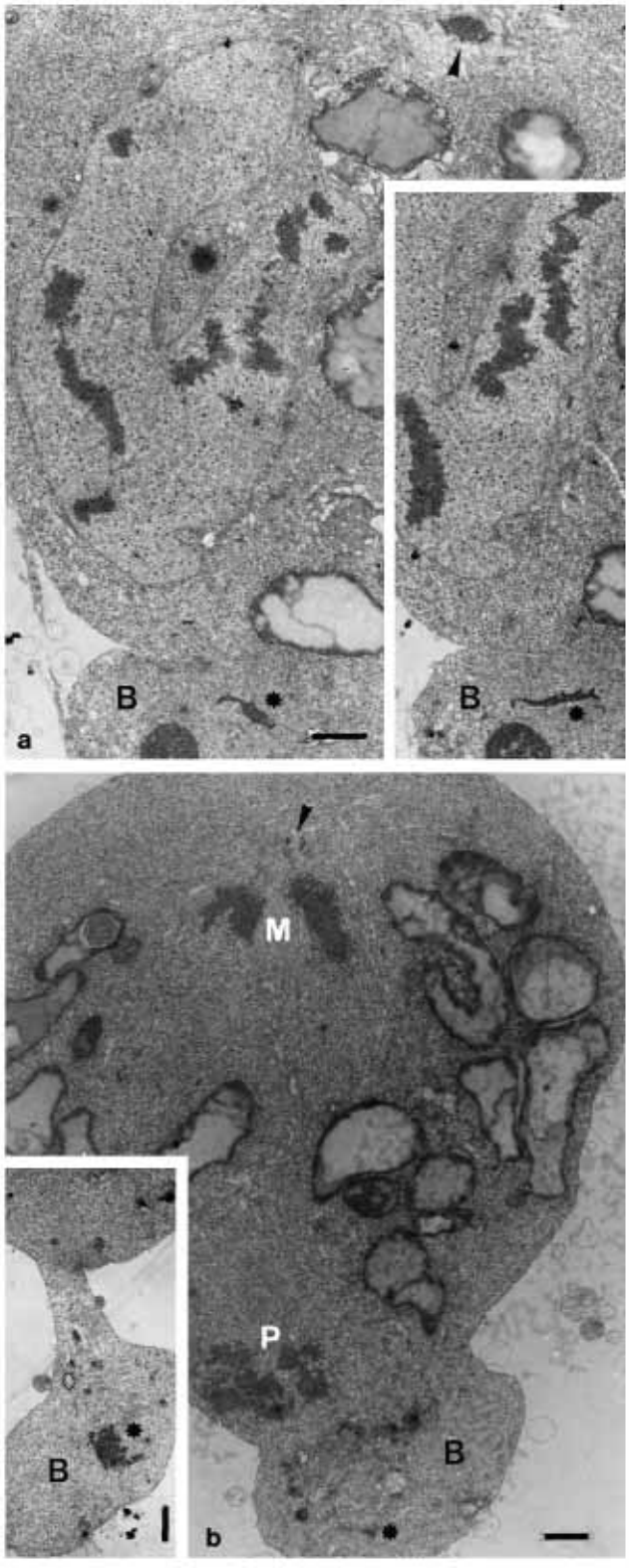


Fig. 3. (a) Late prophase. Adjacent to the deeply indented nucleus lies a giant centriole with darkly staining appendage (arrowhead). A DPO (asterisk) is seen in the cytoplasmic bud (B). Chromosomes can be recognized as individuals. Inset: neighbouring section showing a different profile of the DPO. (b) First meiotic division. The set of maternal and L chromosomes (M) lies close to the PC (giant centriole, arrowhead). The paternal chromosomes (P) are located in the vicinity of the cytoplasmic bud (B). The DPO is caught just at its periphery (asterisk). A complete DPO region in the bud of another cell of the same stage is shown in the inset. Bars, 1 μm.

Late prophase

Fig. 3 shows a spermatocyte in late prophase. Adjacent to the PC the nucleus is deeply indented. All 11 chromosomes are condensed. They are recognized as individuals in the section series, clearly indicating that meiotic synapsis does not take place in male sciarids (Metz et al., 1926). From this stage on, a round to ellipsoidal appendage of densely staining material is found intimately associated with the giant centriole of the PC. The appendage is located inside the centriolar ring, mostly sticking to the inner wall. It is present throughout the first meiotic division (see below), but is no longer found in the second division. A DPO is located in the cytoplasmic bud (Fig. 3a,b, asterisk in inset).

First meiotic division

Without exception two well-separated groups of chromosomes were found in the cells studied, with both light and electron microscopes. One group comprises the maternal and L chromosomes, the other the paternal chromosomes (Fig. 3b). The set of maternal and L chromosomes lies within a cone-shaped monopolar spindle (Fig. 4a,b), always close to the PC (Fig. 4c-e). Pole-proximal parts of the chromosomes are characterized by regions of fibrous material, presumably representing kinetochore regions. This is suggested by microtubules inserting into or traversing these regions. Spindle microtubules are also laterally associated with the chromatin (Fig. 4e). A centriolar appendage was observed in all eight first division cells studied by serial sectioning. Different section planes of appendages are shown in Fig. 4c and d.

Paternal chromosomes were found at distances of 11.4-15.2 μm from the giant centriole (measured from the middle of the chromosome group to the centre of the centriole; mean = 13.1 μm, s.d. = 1.5 μm, *N*=8 cells from 4 animals) in the vicinity of the cytoplasmic bud (Fig. 3b). Poleward orientation of paternal chromosomes was not observed either in the eight cells studied with the electron microscope or in orcein-stained squash preparations. This is consistent with the light microscope data of Amabis et al. (1979). No structures reminiscent

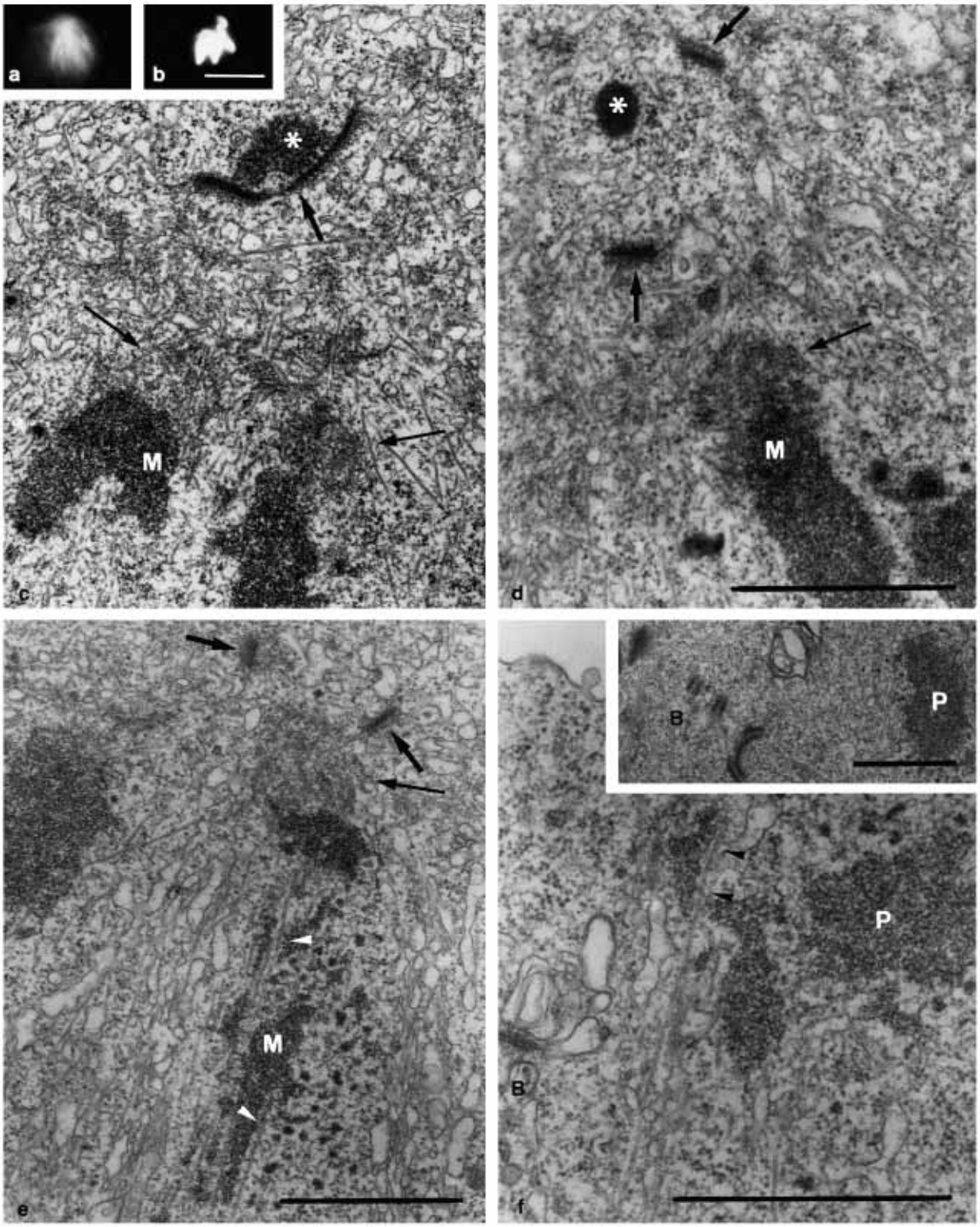


Fig. 4. First meiotic division. (a) Monopolar monocentric spindle labeled with anti-tubulin-FITC. The set of maternal and L chromosomes stained with DAPI (b). Bar in b, 10 μm. (c-e) Maternal chromosomes (M) facing the PC. Giant centriole profiles (short arrows) with appendages caught in (c) and (d) (asterisks). Long arrows point to regions of fibrous kinetochore material with associated microtubules. Peripheral section through a chromosome in (e) shows non-kinetochore lateral association (arrowheads). (f) Paternal chromosomes (P) near the cytoplasmic bud (B) with associated microtubules (arrowheads). Inset: survey from a neighbouring section showing a DPO region inside the bud (B). Bars, 1 μm.

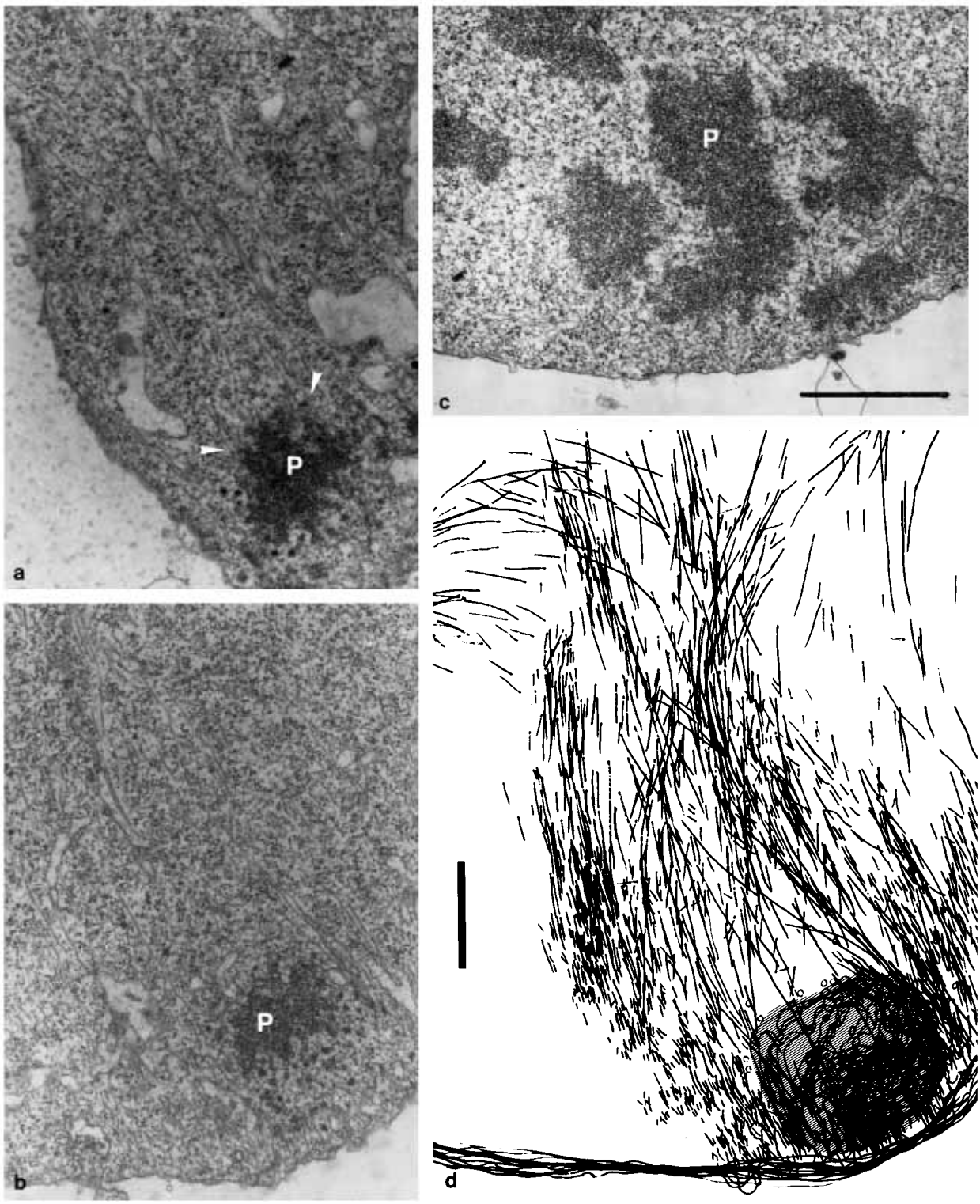


Fig. 5. First meiotic division. (a,b,d) Paternal chromosomes (P) distant from the pole, and their relation to spindle microtubules. Arrowheads in (a) point to microtubules inserting at the poleward side. In (d) microtubule profiles of 15 consecutive sections are shown on overlaid transparent sheets. The area occupied by the chromosomes is hatched. The pole is situated at a distance of approx. 15 μm above. (b) One of the sections used for reconstruction. (c) Paternal chromosomes surrounded by negligible numbers of microtubules (for chromosome position cf. Fig. 3b). Bars, 1 μm.

of kinetochores were detected. In only two out of eight cells were paternal chromosomes observed to have any connection with pole-oriented spindle microtubules. In Fig. 5d the tightly packed group of paternal chromosomes lies some 15 μm distant from the pole, and the accumulation of microtubules around them is not continuous with the cone-shaped monopolar spindle. It is, however, reasonable to assume that these microtubules originated from the monopolar spindle and have been separated in the course of division. Some microtubules insert into the chromatin at the poleward side of chromosomes (Fig. 5a). In five cells microtubules were scarce or absent in the neighbourhood of paternal chromosomes (Fig. 5c). These cells are supposed to be in a still later stage of division. In one cell paternal chromosomes were found to be associated with several microtubules facing the bud (Fig. 4d).

Second meiotic division

On superficial inspection the bipolar spindle of the second meiotic division in male sciarids seems to be conventional and the division orthodox (Amabis et al., 1979; Gerbi, 1986). Except for the precocious X, the chromosomes assemble in the 'sloppy' equatorial metaphase plate of a bipolar spindle (Fig. 6a,d), and they are divided into their chromatids in anaphase. On further consideration the metaphase spindle is recognized to be slightly asymmetrical (Metz et al., 1926), which can also be seen in Amabis' micrographs (Amabis et al., 1979). The conus of the half-spindle with the X chromatids is slightly longer and more pointed than the conus of the other half-spindle (Fig. 6a). After anti-tubulin labeling the pole with the X chromosome shows indications of astral rays, whereas the opposite (bud-proximal) spindle apex appears roundish (Fig. 6b,c). This has already been observed in preliminary studies carried out by Bastmeyer (1989, and unpublished observations). Electron microscope analysis reveals that the asymmetry is due to the presence or absence of a polar centre. In 11 out of 12 meta- and anaphase cells (from 5 animals), a PC was found only at the pole with the X chromosome (Fig. 6d,e). The bud-proximal spindle apex was devoid of any polar organelles. Hence, the second meiotic division spindle can be regarded as bipolar and monocentric. In only one cell was a giant centriole also found at the bud-proximal spindle apex. Since the axes of both meiotic spindles in the sciarid spermatocyte are oriented towards the cytoplasmic bud, i.e. towards the centre of the spermatocyte cyst (Metz et al., 1926), the PC of the second division spindle is obviously identical to that of the first division. In contrast to first division, however, the PC has no appendage (Fig. 6k-m). Maternal autosomes and L chromosomes could not be differentiated. Kinetochores are composed of fibrous material without any layered arrangement. Fig. 6f shows an acrocentric metaphase chromosome with the two chromatid kinetochores orientated to different poles (amphitelic orientation; Bauer et al., 1961). Kinetochores are even less conspicuous in anaphase (Fig. 6h,i). Additionally, lateral contact with kinetochores and chromatin is frequently observed in anaphase. The precocious X chromosome near the pole is generally split into two chromatids (Fig. 6a,c,d). A zone of fibrous material with inserting microtubules shown in Fig. 6g presumably represents the kinetochore region of an X chromatid. This indicates that the X chromosome has some physical connection with the PC. It is not known whether the position of the X represents the final state of chromosomal approach to the pole, or whether the X chromatids occupy a polar position already in the interkinesis nucleus, as suggested by Abbott and Gerbi (1981) for *Sciara.*

In three cells (anaphases) the DPO region was found inside the cytoplasmic bud. In one cell (metaphase) a bud containing both DPO and paternal chromosomes was already cast off but still lying alongside the cell. In three cells (anaphases) paternal chromosomes were not yet displaced into the bud, and in two cells (meta- and anaphase) paternal chromosomes were located inside the bud, yet a DPO was not found. In five cells (anaphases) neither paternal chromosomes nor a DPO were found. Since these cells were also devoid of buds, it is assumed that both components had already been cast off and removed. This indicates that during the second division the DPO is either located within the bud or already cast off. The different positions that paternal chromosomes can occupy during the second division indicate that the final elimination in a cytoplasmic bud is not correlated with the segregation of maternal and L chromatids.

In telophase of the second division two conventional daughter nuclei are formed (Fig. 7a). The PC-distal nucleus migrates into a second cytoplasmic protrusion and will later be eliminated. It is found to be surrounded by cytoplasmic microtubules (Fig. 7b,c), suggesting that nuclear migration is a microtubule-mediated process. The retained nucleus will be the nucleus of the future spermatozoon.

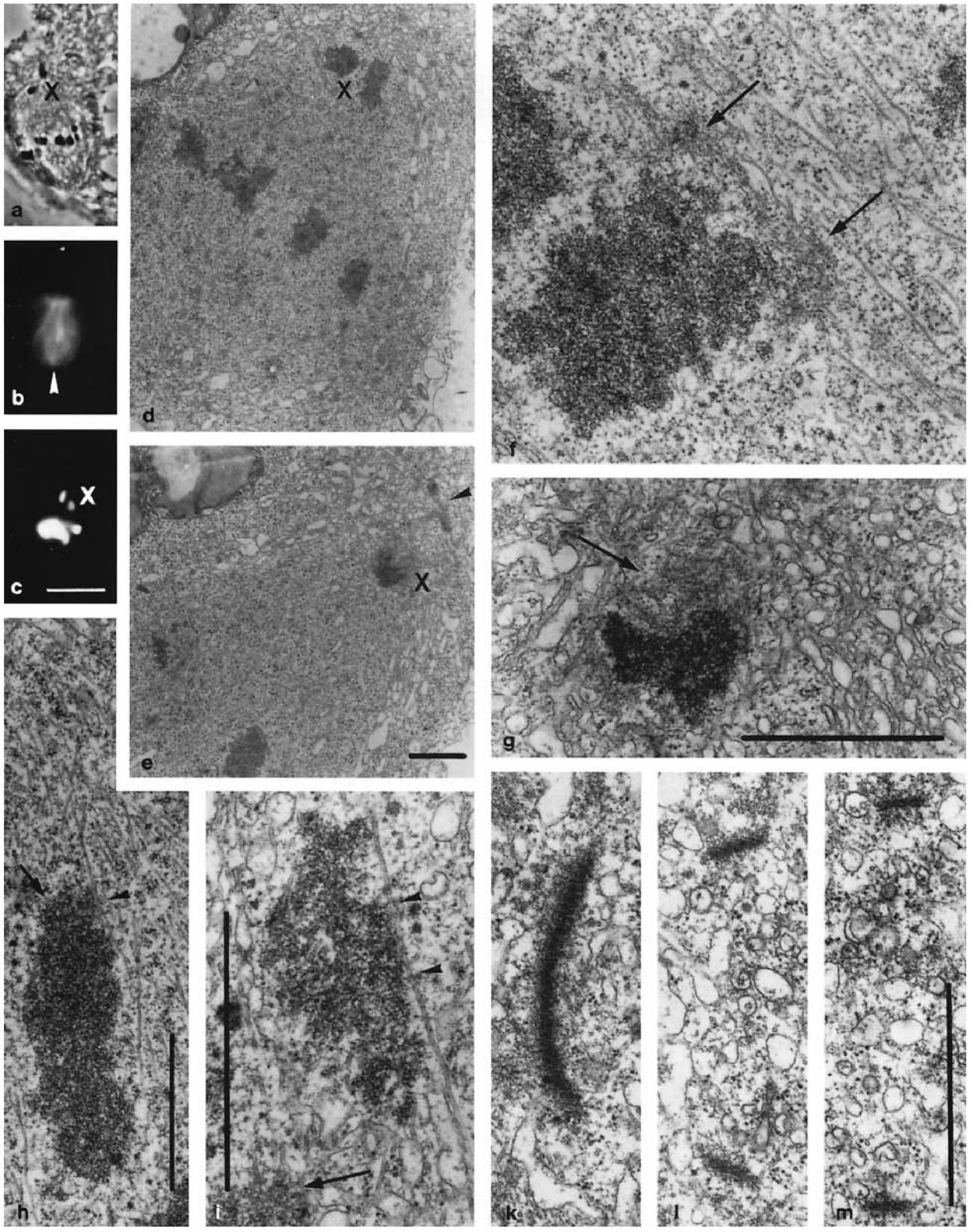
Morphological and dynamic aspects of degenerate polar organelles

As described above, DPOs are first observed in prophase cells (stage II) distally from the PC, located inside or in the vicinity of the cytoplasmic bud. Both positions could also be recorded throughout the first meiotic division (compare Figs 3b and 8a). During the second meiotic division DPOs were either found inside the bud or already cast off. From the relative positions of the DPO and paternal chromosomes it can be concluded that the DPO components migrate into the bud before the chromosomes. The DPO seems to be cast off in a late stage of the second division. Fig. 8b-e shows different sections through DPO components. Some constantly observed components may be complete rings (equivalent to normal giant centrioles),

Fig. 6. Second meiotic division. (a) Metaphase, orcein staining. (b,c) Metaphase, anti-tubulin immunofluorescence (b) and DAPI (c). Arrowhead in (b) points to the bud-proximal spindle apex lacking a polar organelle (bipolar monocentric spindle). Chromatids of the precocious X chromosome are seen in (a) and (c). Bar in c, 10 μm. (d,e) Different section planes through the bipolar monocentric spindle at metaphase, showing the equatorial plate, the precocious X, and the giant centriole of the PC at one pole (arrowhead). (f) Metaphase chromosome with the two amphitelically oriented chromatid kinetochores (arrows). The spindle poles are at the upper left and at the lower right-hand corner. (g) X chromatid facing the PC. Arrow points to a region with inserting microtubules, presumably a kinetochore. (h,i) Anaphase chromosomes. Kinetochores (arrows) are less conspicuous than in metaphase. Microtubule lateral contact with kinetochore and chromatin is marked by arrowheads. (k-m) Three different section profiles of a ring-shaped giant centriole. Bars, 1 μm.

incomplete rings (Fig. 8b), or concentric double rings (Fig. 8c,d). Hook-like components (Fig. 8e) are section profiles of the same structures.

Microtubules are often seen to grow out from centriolar units (Figs 2a and 8c,d). Some of the degenerate centriolar components are associated with a mass of dense, electron-scattering material (Fig. 8b,c,e). This material is not yet present in early prophase. It is constantly observed in late prophase and throughout first division. A three-dimensional reconstruction (Fig. 8f) reveals a most complex arrangement of centriolar components in association with the dense material. The nature of this material, as well as of the PC appendage that appears



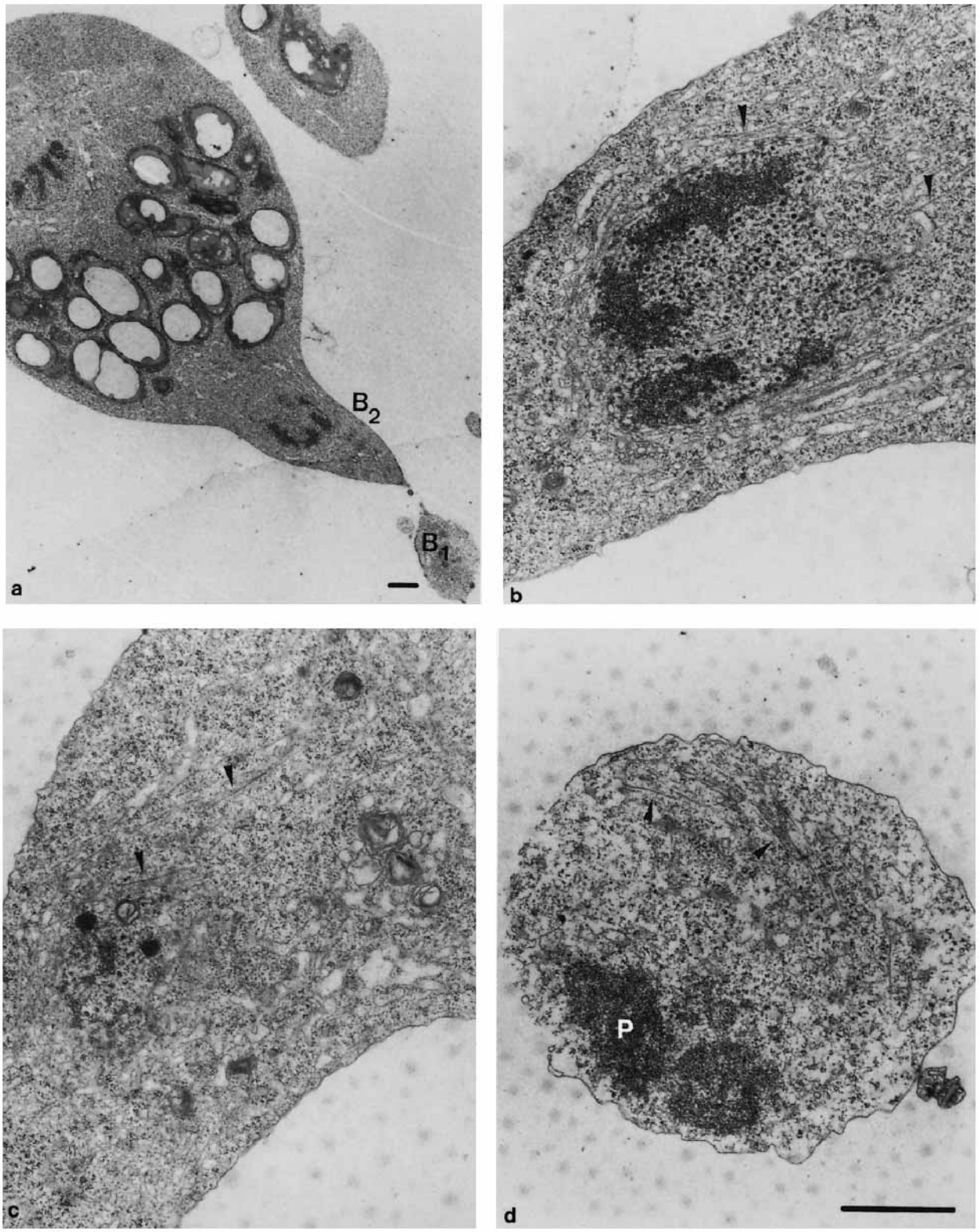


Fig. 7. Telophase of second meiotic division. (a) The retained nucleus is to the left, the nucleus to be eliminated has migrated into a second cytoplasmic bud (B_2). The first bud (B_1) has been cast off. (b,c) Different section profiles of the nucleus in B_2. The nucleus is surrounded by microtubules (arrowheads). (d) Eliminated paternal chromosomes (P) inside B_1 with microtubules (arrowheads) in the neighbourhood. Bars, 1 μm.

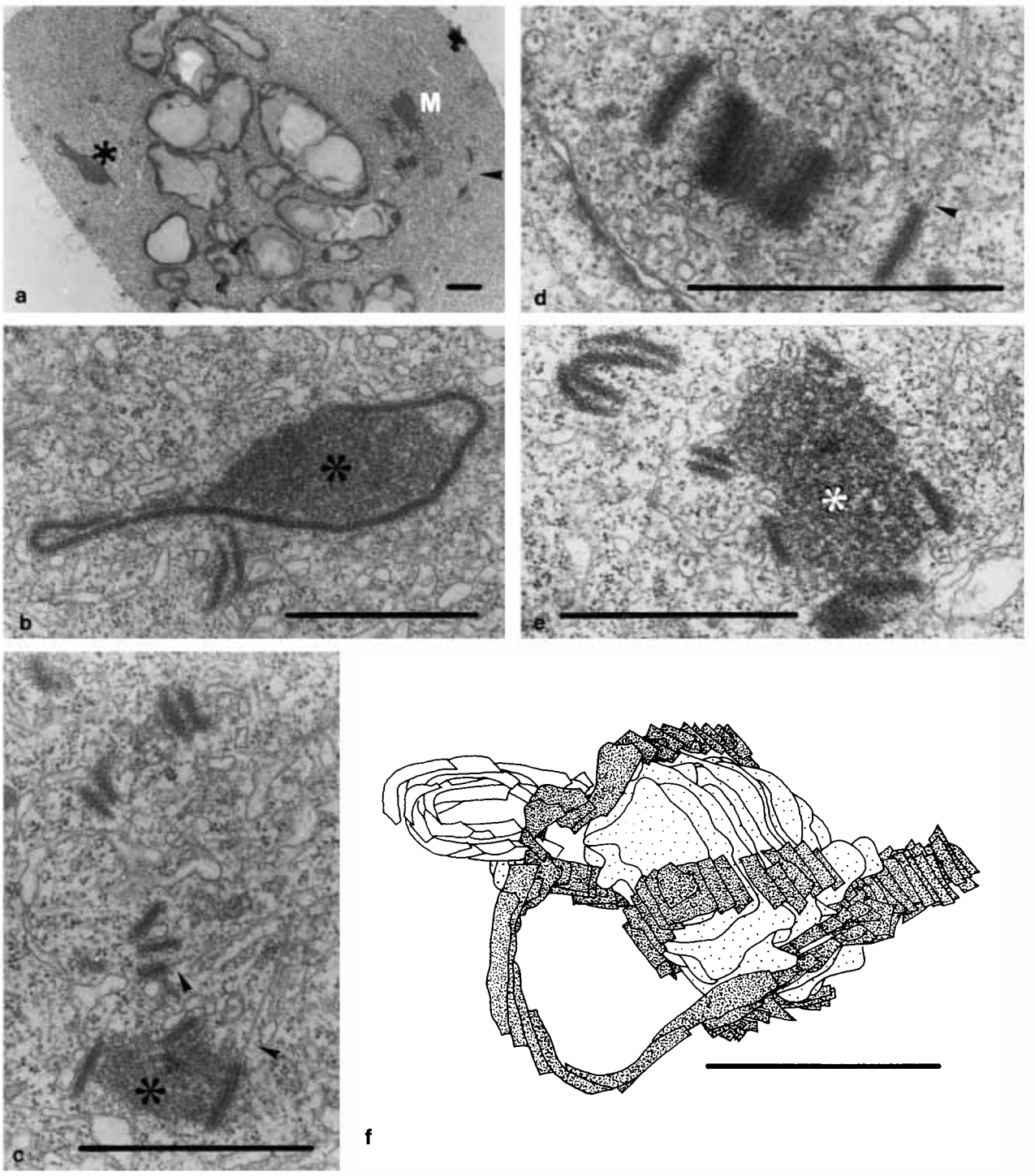


Fig. 8. Morphological aspects of the degenerate polar organelle (DPO) in first meiotic division. (a) The DPO (asterisk) lies opposite to the PC (arrowhead), but not yet inside the cytoplasmic bud. M, maternal chromosomes. (b-e) Different sections through DPO regions: incomplete rings (b), apparent double rings (c-e), and associated electron-scattering material (asterisks). Arrowheads point to elongated microtubules of the centriolar unit. (f) 3-D reconstruction of a DPO from 32 consecutive sections (e is one section of the series; survey in 3b, inset). Centriolar components (densely dotted) envelope a body of electron-scattering material (less densely dotted). Bars, 1 μm.

at the same time (see above), is obscure. Phillips (1967) mentioned dense 'fibrous material' associated with giant centrioles in spermatogonia of *Sciara*, but he did not discuss his observation. There are no other reports of similar structures that are known to the author.

DISCUSSION

Polar organelles in prophase and the first meiotic division

According to Phillips (1967) the spindle poles in spermatogo-

nial divisions of *Sciara* are characterized by pairs of giant centrioles. In consequence, each spermatocyte can be expected to receive a pair as a result of the last spermatogonial division. In normal cell division the two centrioles of a pair would separate and duplicate to form *two pairs* as the presumptive centres of the two spindle poles (e.g. see Vorobjev and Nadezhdina, 1987; Kochanski and Borisy, 1990). Kubai (1982) recognized the pole of the monopolar spindle of the first meiotic division in *Sciara* to be characterized by only a single giant centriole. This observation is confirmed in the present study of *Trichosia* spermatogenesis. How is this reduction achieved? As shown in Fig. 1e, prophase cells already have single centrioles at opposite sides of the nucleus. This suggests that the centrioles of the pair inherited from the last spermatogonial stage disorientate and separate, but do not induce the formation of daughter centrioles. Migration of one centriole around the nucleus would lead to the situation shown in Fig. 1e. Cells in which the two tubulin signals were found not exactly opposite to one another (Fig. 1c) may be intermediate stages of centriole migration. Exceptional cells with four centres may indicate that parent centrioles had nucleated daughter centrioles and lost their orthogonal relationship afterwards.

Different activities of the two centrioles in the pear-shaped prophase cell (stage II) finally lead to the formation of a monopolar monocentric spindle. While microtubules grow around one centriole to form a PC, the other centriole lying inside or near the cytoplasmic bud degenerates to form a DPO. The particulars of degeneration are not clear. The observations suggest an uncontrolled nucleation of new centriolar components. These are obviously unable to function as a polar centre.

Chromosome orientation and segregation during the first meiotic division

The degeneration of one polar organelle explains why the first meiotic division in sciarid males is monopolar and monocentric. It does not answer the question of what causes the nonrandom selection of chromosomes. The differential behaviour of maternal and paternal chromosomes is attributed to genetical 'imprinting', i.e. the predetermination of one of the homologues to behave differently from its counterpart at some stage of development. It is still unknown how and when this imprinting occurs in sciarids (Gerbi, 1986). As to *Trichosia,* paternal chromosomes do not show poleward orientation (Amabis et al., 1979). Since functional kinetochores can generally be regarded as prerequisites for proper chromosome orientation, the failure of paternals to orientate could indicate inactivation of their kinetochores. On the other hand, the presence of functional kinetochores in maternal and L chromosomes would enable these chromosomes to adopt syntelic orientation, i.e. orientation of both chromatid kinetochores to the same pole (Bauer et al., 1961). Hence, a reasonable working hypothesis would be that the imprint mainly affects paternal chromosome kinetochores. Inactivation could make paternal kinetochores too inconspicuous to be detected in ultrathin sections. Preliminary studies using a human anti-centromere serum to identify these regions were not successful, since the serum failed to detect any centromere regions in *Trichosia.* The observations in *Trichosia* stand in variance with those in *Sciara.* It has repeatedly been documented that paternal chromosomes of *Sciara* are pole-oriented, and that they possess distinct kinetochores connected with pole-orientated kinetochore microtubules (Metz et al., 1926; Abbott et al., 1981; Kubai, 1982). Since it is hardly imaginable that fundamental physiological differences exist between two species of the same family, it may be concluded that poleward orientation of kinetochores alone cannot be the crucial point. *Trichosia* and *Sciara* may demonstrate two different levels of paternal kinetochore dysfunction; a plausible explanation would be that *Sciara* kinetochores are still able to capture polar microtubules and, thus, orientate polewards, but that their kinetochore motor for poleward movement (Gorbsky et al., 1987) is defective. In contrast, *Trichosia* kinetochores may even be unable to bind microtubules. This point certainly needs further clarification.

In all first divisions so far examined, with both light and electron microscopes, the set of maternal and L chromosomes of *Trichosia* was found *very close* to the pole and the set of paternal chromosomes far away from the pole. This is in accordance with Metz' (Metz et al., 1926) and Kubai's (1982, 1987) observations in *Sciara*. Metz assumed fast poleward movement of the maternal and slow retrograde motion of the paternal set. Since the *Trichosia* chromosomes facing the pole possess poleward-orientated kinetochore regions with associated microtubules, it is suggested, in accordance with Metz, that they have moved polewards, at least for a short distance. If this polar approach is fast, as suggested by Metz, the probability of finding chromosomes more distant from the pole will be low.

The group of paternal chromosomes in the first division was regularly found at distances of more than 10 μm from the pole at the cell periphery. It is difficult to imagine that such a great distance can simply be the result of paternals remaining in the place they already occupied in the prophase nucleus, as suggested by Kubai (1982, 1987) for *Sciara.* As shown in Fig. 5d paternal chromosomes have contact with spindle microtubules during the first division at some time or other. This could be taken as an indication of microtubule-mediated chromosome transport away from the pole, not involving kinetochores (reviewed by Fuge, 1990), but more data are certainly necessary to confirm this.

Furthermore, paternal chromosomes must finally be transported into the bud at the end of the first or during the course of the second meiotic division. It would be reasonable to assume that this is also a microtubule-mediated process. The single observation of some bud-orientated microtubules in association with paternal chromatin (Fig. 4d) and microtubules found in the vicinity of chromatin after elimination (Fig. 7d) may be taken as clues.

Polar organelles and spindle formation in the second meiotic division

Amabis et al. (1979) described the spindle of the second meiotic division as being bipolar. The present study shows that one of the poles of the bipolar spindle is defined by the PC of the first meiotic division. Amabis et al. (1979) suggested that another polar centre is located inside the cytoplasmic bud. This is not confirmed by the present observations. The bud-proximal spindle apex does not reach into the bud and generally lacks a polar organelle in metaphase. Paternal chromosomes that were not yet eliminated may even be located between the spindle apex and the bud during the second division. Thus, the second meiotic division must be regarded as bipolar and monocentric. The single giant centriole in interkinesis may be unable to induce the formation of a second centriole. The centriolar

component found at the bud-proximal spindle apex in one out of 12 cells could have accidently been separated from the DPO region during its migration into the bud.

The question arises of how a bipolar spindle can be formed in the presence of only one polar centre. A widely accepted hypothesis of spindle formation suggests growth of microtubules from the two centrosomes. According to this hypothesis, capturing of polar microtubules by kinetochores finally leads to bipolar orientation of chromosomes (e.g. see Rieder, 1990). However, bipolar spindles can certainly also be generated *in the absence* of one (or both) centrosome(s) in certain systems. This is well documented in crane fly spermatocytes (Dietz, 1959, 1963, 1966; Steffen et al., 1986; Bastmeyer et al., 1986) and in *Drosophila* spermatocytes where micromanipulated bivalents can generate their own 'minispindles' without the involvement of centrosomes (Church et al., 1986). These and other observations (Nicklas and Gordon, 1985; Karsenti and Maro, 1986) indicate that chromosomes themselves can have the capacity to promote spindle microtubule assembly and/or orientation under certain conditions. The same principle may be realized in the second male meiotic division of *Trichosia*. During the first division the maternal and L chromosome set is obviously orientated syntelically. Stained squash preparations show that the chromosomes are again orientated syntelically towards the PC just after breakdown of the interkinesis nuclear envelope, in both *Sciara* and *Trichosia* (unpublished observations). This initial 'polarization' of chromosomes was also recognized by Metz (1926, p. 248). However, chromosome-induced formation of a bipolar monocentric spindle is imaginable only when the chromatid kinetochores point in opposite directions. Hence bipolarity of the spindle and metaphase congression of chromosomes in *Trichosia* may be assumed to be triggered by an early change from *syn*- to *amphi*-orientation. This change would involve the separation of chromatid centromeres, a process that begins in late anaphase I in orthodox bipolar meiosis (Rufas et al., 1989, and earlier investigators cited ibid.); and secondly, the re-orientation of one of the two kinetochore fibres. A transition from *syn*- to *amphi*-orientation at an early stage of spindle formation would be in accordance with observations made by Dietz (1959, 1963, 1966, and personal communication) on the behaviour of univalents in experimentally induced bipolar monocentric divisions of tipulid spermatocytes.

Conclusions

If the observations made here are correctly interpreted, there would be at least two prerequisites for the elimination of paternal chromosomes in the first meiotic division: (1) a modification of the centriole cycle in the young spermatocyte leading to the development of a monopolar monocentric spindle; and (2) the inability of the paternal chromosomes to achieve functional contact with the polar centre, probably due to a dysfunction of their kinetochores. In succession, (1) would explain why the bipolar spindle of the second division is monocentric, which in turn may partly or exclusively be the cause of the elimination of one of the daughter nuclei and the formation of a single spermatozoon. Inactive paternal kinetochores could be the manifestation of chromosome imprinting. How imprinting could be related to the disturbance of the centriole cycle remains obscure.

I thank Dr Claus Pelling (MPI für Biologie, Tübingen), who made this study possible by providing me with male *Trichosia* larvae. I am also most grateful to him and Dr Roland Dietz (Tübingen) for valuable suggestions during preparation of the manuscript.

REFERENCES

Abbott, A. G. and Gerbi, S. A. (1981). Spermatogenesis in Sciara coprophila. II. Precocious chromosome orientation in meiosis II. *Chromosoma* **83**, 19-27.

Abbott, A. G., Hess J. E. and Gerbi, S. A. (1981). Spermatogenesis in Sciara coprophila. I. Chromosome orientation on the monopolar spindle of meiosis I. *Chromosoma* **83**, 1-18.

Amabis, J. M., Reinach, F. C. and Andrews, N. (1979). Spermatogenesis in *Trichosia pubescens* (Diptera: Sciaridae). *J. Cell Sci.* **63**, 199-213.

Bastmeyer, M. (1989). Untersuchungen zur Entstehung des meiotischen Kernteilungsapparates bei Pales ferruginea: Die Bedeutungslosigkeit der Centrosomen und die Dynamik der Spindel hinsichtlich der Polarität ihrer Mikrotubuli. Dissertation, University of Kaiserslautern.

Bastmeyer, M., Steffen, W. and Fuge, H. (1986). Immunostaining of spindle components in tipulid spermatocytes using a serum against pericentriolar material. *Eur. J. Cell Biol.* **42**, 305-310.

Bauer, H., Dietz, R. and Röbbelen, C. (1961). Die Spermatocytenteilungen der Tipuliden. III. Mitteilung. *Chromosoma* **12**, 116-189.

Church, K., Nicklas, R. B. and Lin, H.-P. P. (1986). Micromanipulated bivalents can trigger mini-spindle formation in Drosophila melanogaster spermatocyte cytoplasm. *J. Cell Biol.* **103**, 2765-2773.

Dietz, R. (1959). Centrosomenfreie Spindelpole in Tipuliden-Spermatocyten. *Z. Naturforsch. Mikrosk. Anat.* **14b**, 749-752.

Dietz, R. (1963). Polarisationsmikroskopische Befunde zur chromosomeninduzierten Spindelbildung bei der Tipulide Pales crocata (Nematocera). *Zool. Anz. Suppl.* **23**, 131-138.

Dietz, R. (1966). The dispensability of the centrioles in the spermatocyte divisions of Pales ferruginea (Nematocera). *Heredity Suppl.* **19**, 161-166.

Fuge, H. (1990). Non-kinetochore transport phenomena, microtubule-chromosome associations, and force transmission in nuclear division. *Protoplasma* **158**, 1-9.

Gerbi, S. A. (1986). Unusual chromosome movements in sciarid flies. *Germ Line-Soma Differentiation*. In *Results and Problems of Cell Differentiation*, vol.13 (ed. W. Hennig), pp. 71-104. Springer Verlag: Berlin, Heidelberg, New York.

Gorbsky, G. J., Sammak, P. J. and Borisy, G. G. (1987). Chromosomes move poleward in anaphase along stationary microtubules that coordinately disassemble from their kinetochore ends. *J. Cell Biol.* **104**, 9-18.

Hartmann, M. (1953). *Allgemeine Biologie*, 4th edn. Stuttgart.

Karsenti, E. and Maro, B. (1986). Centrosomes and the spatial distribution of microtubules in animal cells. *Trends Biochem. Sci.* **11**, 460-463.

Kochanski, R. S. and Borisy, G. G. (1990). Mode of centriole duplication and distribution. *J. Cell Biol.* **110**, 1599-1605.

Kubai, D. F. (1982). Meiosis in Sciara coprophila: Structure of the spindle and chromosome behavior during the first meiotic division. *J. Cell Biol.* **93**, 655-669.

Kubai, D. F. (1987). Nonrandom chromosome arrangements in germ line nuclei of Sciara coprophila males: the basis for nonrandom chromosome segregation on the meiosis I spindle. *J. Cell Biol.* **105**, 2433-2446.

Metz, C. W., Moses, M. S. and Hoppe, E. N. (1926). Chromosome behavior and genetic behavior in Sciara (Diptera). I. Chromosome behavior in the spermatocyte divisions. Z. *indukt. Abstamm.-u. Vererbungsl.* **42**, 237-270.

Metz, C. W. (1927). Chromosome behavior in Sciara (Diptera). II. Genetic evidence of selective segregation in S. coprophila. III. Absence of parthenogenesis in S. coprophila. *Z. indukt. Abstamm.-u. Vererbungsl.* **45**, 184-201.

Metz, C. W. (1931). Chromosomal differences between germ cells and soma in Sciara. *Biol. Zentralbl.* **51**, 119-124.

Metz, C. W. (1933). Monocentric mitosis with segregation of chromosomes in Sciara and its bearing on the mechanism of mitosis. *Biol. Bull. Mar. Biol. Lab., Woods Hole* **64**, 333-347.

Nicklas, R. B. and Gordon, G. W. (1985). The total length of spindle microtubules depends on the number of chromosomes present. *J. Cell Biol.* **100**, 1-7.

Phillips, D. M. (1966). Fine structure of Sciara coprophila sperm. *J. Cell Biol.* **30**, 499-517.

Phillips, D. M. (1967). Giant centriole formation in Sciara. *J. Cell Biol.* **33**, 73-92.

Rieder, C. L. (1990). Formation of the astral mitotic spindle. Ultrastructural basis for the centrosome-kinetochore interaction. *Electron Microsc. Res.* **3**, 269-300.

Rieffel, S. M. and Crouse, H. V. (1966). The elimination and differentiation of chromosomes in the germ line of Sciara. *Chromosoma* **19**, 231-276.

Rufas, J. S., Mazella, C., Suja, J. A. and Garcia del la Vega, C. (1989). Kinetochore structures are duplicated prior to the first meiotic metaphase. A model of meiotic behavior of kinetochores in grasshoppers. *Eur. J. Cell Biol.* **48**, 220-226.

Steffen, W., Fuge, H., Dietz, R., Bastmeyer, M. and Müller, G. (1986). Aster-free spindle poles in insect spermatocytes: evidence for chromosome-induced spindle formation? *J. Cell Biol.* **102**, 1679-1687.

Vorobjev, I. A. and Nadezhdina, E. S. (1987). The centrosome and its role in the organization of microtubules. *Int. Rev. Cytol.* **106**, 227-293.

(Received 21 July 1993 - Accepted 30 September 1993)