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Centrosome hyperamplification with the formation of multiple asters and programmed chromosome inactivation in aberrant spermatocytes during male meiosis in *Acricotopus*

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Abstract In the germ line of the midge *Acricotopus lucidus* an unequal chromosome segregation occurs in the last gonial mitosis prior to meiosis. This results in one daughter cell receiving only somatic chromosomes (=Ss), whereas the other cell is given all the so-called germ line limited chromosomes (=Ks) in addition to the Ss. The cytokinesis following this differential mitosis is incomplete and the daughter cells remain connected by a permanent cytoplasmic bridge. The cell with the Ss and Ks develops into a primary oocyte or spermatocyte, whereas the cell containing only Ss differentiates as a nurse cell in the female and as an aberrant spermatocyte in the male. When the primary spermatocyte enters meiosis, the Ss in the connected aberrant spermatocyte undergo chromosome condensation but the aberrant spermatocyte remains undivided, with the condensed metaphase status and inactivation of the Ss persisting during both meiotic divisions. These events indicate a programmed inactivation of all chromosomes in the aberrant spermatocyte at the beginning of meiosis. The alterations in the microtubule arrangements and of the distribution of mitochondria in the spermatocytes during meiosis have been were followed via live-cell fluorescence labelling using TubulinTracker and MitoTracker reagents and by transmission electron microscopy. The observations revealed a hyperamplification of the centrosomes and the formation of tetrapolar asters in the non-dividing aberrant spermatocytes containing the condensed Ss. The programmed inactivation of the Ss in the aberrant spermatocyte is suggested to have developed during evolution to inhibit the entry of the aberrant spermatocytes into meiosis, thereby preventing the formation of sperms containing only Ss but no Ks.

Key words Aberrant spermatocyte · centrosome hyperamplification · germ line limited chromosomes · spermatogenesis · tetrapolar asters · Midge (*Acricotopus lucidus*) (Insecta, Diptera, Chironomidae)

Introduction

In the last gonial mitosis prior to meiosis, an unequal segregation of chromosomes takes place in the chironomid *Acricotopus lucidus*, producing daughter cells with two different sets of chromosomes. This differential mitoses represents one kind of unusual mitosis occurring in the complex chromosome cycle in the subfamily Orthocladiinae of the Chironomidae, during which the germ line limited chromosomes (Ks) pass together with the regular somatic chromosomes (Ss) into the primary spermatocyte or primary oocyte (Bauer and Beermann 1952; Redi et al. 2001; Staiber 2006). In this particular mitosis, all Ks move undivided to only one pole of the cell, whereas the Ss separate and segregate equally. However, cytokinesis is incomplete and the two resulting cells remain interconnected by a permanent cytoplasmic bridge (Staiber 2007). The cell that receives only Ss differentiates into a polyploid nurse cell in the female or into an aberrant spermatocyte in the male, whereas the cell receiving Ss and all Ks develops into the oocyte or the primary spermatocyte, which then undergoes meiosis (White 1973).

An asymmetric distribution of the mitochondria, accompanied by a directed and opposite asymmetric arrangement of spindle microtubules (MTs), has been found in cells passing through the differential mitosis and this resulted in an unequal partitioning of mitochondria to the daughter cells in both sexes (Staiber 2007). In the male, a compensation then occurs between the daughter cells by the active transport of mitochondria through the cytoplasmic bridge from the aberrant spermatocyte, which has received most of the mitochondria, to the primary spermatocyte (Staiber 2007). The occurrence of such stable cytoplasmic bridges, or canals, between germ cells, allowing the exchange of cytoplasmic components, is a widespread phenomenon of gametogenesis from insects to mammals (Hime et al. 1996; Ventelä et al. 2003; Greenbaum et al. 2006).

Little is known about interactions and changes between and within living aberrant and regular spermatocytes during meiosis, which, because of the connecting canal, share a syncytium-like common cytoplasm. The present investigation into the MT arrangements, by using live-cell tubulin fluorescence labelling, and of chromosome behaviour during male meiosis in *Acricotopus* has revealed a hyperamplification of the centrosomes, the formation of tetrapolar asters and a programmed inactivation of all the chromosomes in aberrant spermatocytes.

Materials and methods

Cell preparation and fluorescence staining

Testes and spermatocytes from 4th instar larvae and prepupae of a laboratory stock of *A. lucidus* (Diptera, Chironomidae) were prepared according to the protocol described in Staiber 2007. MTs and mitochondria were visualised via direct fluorescence labelling, by incubating living cells for about 10 min in a 1:1 mixture of Ringer's solution (100 mM NaCl, 3 mM KCl, 3 mM $CaCl_2$, pH 7.2), and Shields and Sang M3 insect medium (Sigma) supplemented with 1-4 μM

TubulinTracker Green reagent (Molecular Probes) and 0.2–0.5 μM MitoTracker Red CMXRos (Molecular Probes).

Chromosome banding and fluorescence in situ hybridisation

Giemsa banding and chromosome painting were carried out as described previously (Staiber 1988; Staiber and Schiffkowski 2000).

Light microscopy

Phase contrast and fluorescence images were captured with a Canon A 80 camera on a Zeiss epifluorescence microscope equipped with a Neofluar 100×/1.30 oil objective. Digital images were further processed using a Corel Draw software package.

Electron microscopy

Testes were prepared in MT-stabilising buffer (100 mM PIPES, pH 6.8, 1 mM $MgSO_4$, 1 mM EGTA; Wolf and Bastmeyer 1991) and then fixed with 2.5% glutaraldehyde in cacodylate buffer, pH 7.3, for 1 hour at 4°C. After postfixation with 1% osmium tetroxide for 1 hour at 4°C and dehydration with ethanol, the testes were embedded in ERL 4206. Ultra-thin sections (50 nm) were cut with a diamond knife on an Ultratom IV (LKB). The sections were stained with uranyl acetate and lead citrate solutions, and examined and photographed using a Zeiss electron microscope EM 10 A.

Results and discussion

A characteristic feature of the last gonial mitosis before meiosis in *Acricotopus lucidus* is the migration of all Ks as unseparated sister chromatids to only one pole of the cell, as shown in the differential mitoses in Figs. 1a and Fig. 5a. In contrast, the regular Ss (2n=6) first remain at the metaphase plate as three groups of paired homologues (Fig. 1a). Clear tension forces mediated by attached MTs pull the trilaminar kinetochores of the S sister chromatids towards the opposite poles (Fig. 5a-d). Then, just before the Ks arrive at one spindle pole, the chromatids separate and are partitioned equally to the daughter cells (Staiber 2007). In the male, the cell receiving Ss and all the Ks develops into a primary spermatocyte, which undergoes meiosis (see the metaphase I primary spermatocyte in Fig. 1b,c), whereas the other cell with only Ss follows the developmental pathway of an aberrant spermatocyte (Fig. 1b,c). The cytokinesis after this differential mitosis is incomplete, and the primary and the aberrant spermatocyte remain connected by a permanent canal-like cytoplasmic bridge (Fig. 2a, arrowhead). Chromosome painting, using probes specific for the three Ss (Fig. 1c), clearly demonstrates the distinctly different chromosome constitutions of the primary and the aberrant spermatocytes.

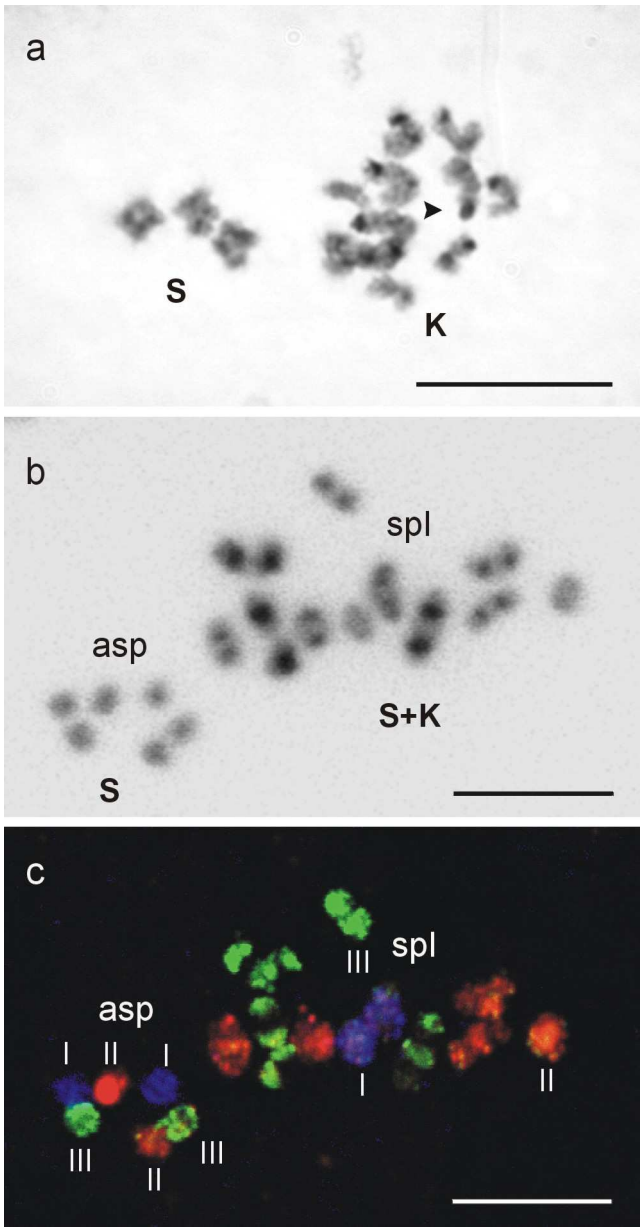

Fig. 1 a G-banded male differential gonial mitosis of *Acricotopus*. All Ks (*K*) move as unseparated sister chromatids to the right spindle pole (*arrowhead*), whereas the six Ss (*S*) remain in the equatorial plane in three groups of paired homologues. **b** Meiotic metaphase I plate of a primary spermatocyte (*spI*, *right*) showing chiasmate S bivalents and K homologues. The connected aberrant spermatocyte (*asp*, *left*) exhibits six unpaired Ss. DAPI (4,6-diamidino-2-phenylindole) C-banding image. **c** Chromosome painting of the stages in **b** with probes for the three Ss: I (*blue*), II (*red*) and III (*green*). The Ks in the metaphase I are stained in their S-homologous euchromatic sections. *Bars* 10 µm.

In the interphase following the differential mitosis, one prominent nucleolus is formed within the nucleus of the aberrant spermatocyte and is clearly demarcated from the homogeneous chromatin (Fig. 2a). In contrast, the connected primary spermatocyte exhibits extensive masses of highly condensed chromatin within the karyoplasm (Fig. 2a). At this stage, in vivo tubulin staining shows that dense bundles of MTs extend from the aberrant spermatocyte through the cytoplasmic bridge to the primary spermatocyte (Fig. 2a',a''). Most probably, the vast majority of the mitochondria of the aberrant spermatocyte are transported along these MT fibres to the primary spermatocyte (Fig. 2a''') (Staiber 2007).

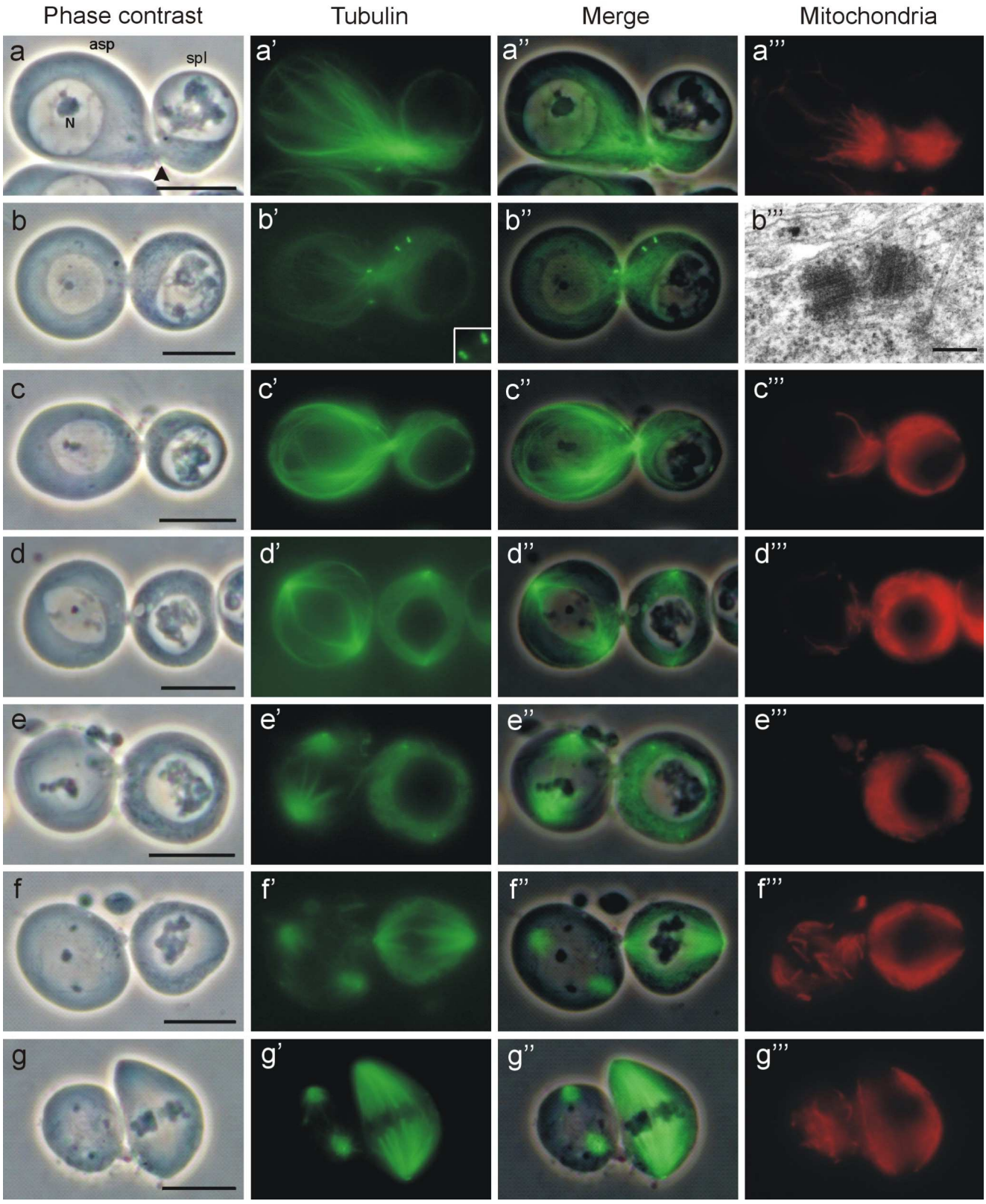

Fig. 2a-g''' Male meiosis in *Acricotopus* shown in phase-contrast, TubulinTracker fluorescent staining of polymerised tubulin, merged phase-contrast and tubulin fluorescence images and MitoTracker fluorescent staining of mitochondria. **a-a'''** Interphase before meiosis. **a** Primary spermatocyte (*spI*) and aberrant spermatocyte (*asp*) are connected by a permanent cytoplasmic canal (*arrowhead*). **b-b''', c-c'''** Prophase I, pachytene. Duplication and polar movement of centrosomes and centrioles. **b'** *Insert*: Separating centrosomes of the primary spermatocyte each containing a pair of centrioles. **b'''** Electron microscope image of a centriole pair from a spermatocyte. *Bar* 0.2 µm. **d-d'''** Prophase I, polar positioning of

centrosomes and aster formation. **e-e'''** Prophase I. Nuclear membrane breakdown in the aberrant spermatocyte. Most of the mitochondria are concentrated in the primary spermatocyte. **f-f'''** Pro-metaphase I. Spindle formation in the primary spermatocyte. **g-g'''** Metaphase I of the primary spermatocyte with a prominent bipolar spindle and equatorially arranged K and S bivalents attached to many bundles of kinetochore microtubules (MTs). The six Ss in the aberrant spermatocyte are separated and distributed in the cell. *Bars* 10 µm (for all except **b''')**.

The onset of meiosis in a primary spermatocyte is clearly visible at pachytene of prophase I, when thick threads and loops of paired homologues extend from the condensed chromatin masses into the karyoplasm (Figs. 2b, 4e, 5e and insert in Fig. 5e). Synaptonemal complexes exhibiting the typical tripartite structure with two lateral elements and a central element have been verified between these paired homologues by electron microscopy of ultra-thin sections of pachytene primary spermatocytes (Fig. 5e,f). *Drosophila* males in contrast, in which no crossing over takes place, lack such a synaptonemal complex (Vazquez et al. 2002). During pachytene the pair of centrioles in the single centrosome of the primary spermatocytes duplicates synchronously with the pair in the centrosome of the aberrant spermatocytes (Fig. 2b-b'') and can be clearly seen as fluorescent twin dots (the insert of the tubulin image in Fig. 2b'). The mother and the daughter centrosomes migrate around the periphery of the cell to opposite poles (Fig. 2c-c'''), thereby initiating the nucleation of MTs and the formation of prominent asters, which begin to organise the spindle (Fig. 2d-d''). Following the duplication of the centrosomes in *Drosophila*, in contrast, the two centriole pairs first move to the cell surface and remain there until shortly before meiosis. Then, they migrate back to the nucleus and shift along the nuclear membrane to opposite positions in the cell. This was clearly shown by time-lapse images of centriole migration in primary spermatocytes expressing a centriole specific protein tagged with green fluorescent protein (Rebollo et al. 2004). The presence of a pair of short centrioles in the centrosome in spermatocytes of *Acricotopus* is shown in the electron microscope image in Fig. 2b'''.

In the growing asters of the primary and the aberrant spermatocyte, fibres of emanating MTs run around the ovoid or round nuclei in the direction of the opposite poles (Fig. 2d',d'') and, by this time, the vast majority of the mitochondria of the aberrant spermatocyte has been transported to the primary spermatocyte (Fig. 2d'''). During regression of the nucleolus, the chromosomes in the aberrant spermatocyte begin to condense. Then, with the breakdown of the nuclear membrane at prophase, an unstructured mass of condensing chromatin can be seen (Fig. 2e-e''') and three clumps, each containing a couple of paired S homologues, become clearly visible (Fig. 2f). Whether this pairwise arrangement of the Ss results from somatic or from meiotic pairing of the homologues is unclear. In the aberrant spermatocyte, the prophase breakdown of the nuclear membrane takes place somewhat earlier (Fig. 2e) than in the primary spermatocyte (Fig. 2f).

Following breakdown of the nuclear membrane in the primary spermatocyte, a new spindle is formed by an increasing number of MT fibres originating from the polar centrosomes and extending to the condensing bivalents, which congregate in the equatorial zone of the cell (Fig. 2f',f''). The mitochondria then arrange themselves in the periphery of the cell outside this growing spindle (Figs. 2f''', 5h). In the connected aberrant spermatocyte, no MTs extend from

the polar asters to the kinetochores of the paired S homologues. The Ss show no directed movements to congregate in the equatorial plane and seem to be randomly distributed between the polar asters. In metaphase I spermatocytes, a prominent spindle is formed (Fig. 2g-g’’’) and many dense bundles of MTs extend from the polar centrosomes to the kinetochores of the equatorially arranged S and K bivalents. Clear tension forces acting from the MTs draw the centromeres attached to the kinetochores draw the centromeres of the chiasmate S and K homologues in the direction to the opposite poles (Fig. 5g). By this time, the paired Ss in the aberrant spermatocyte have separated (Figs. 1b, 2g) and six condensed Ss each composed of two sister chromatids can be observed at stochastically distributed positions within the cell, but not attached to MTs. The polar centrosomes of the cell are surrounded by only small asters (Fig. 2g’-g’’). Chiasma formation between S homologues and K homologues has only been observed in the primary spermatocytes and not between the S homologues in the aberrant spermatocytes.

In the primary spermatocyte, the chiasmata between the homologues of the S and K bivalents dissolve at the metaphase I/anaphase I transition (Fig. 5i) and the homologues move to the opposite poles through their attachment to the shortening kinetochore MTs (Fig. 3a-a’’). The Ss in the connected aberrant spermatocyte remain scattered within the cell and show no directed chromosome movements (Fig. 3a). Mitochondria aggregate around the spindle, at the periphery of the anaphase primary spermatocyte (Fig. 3a’’’). During the formation of the cleavage furrow between the telophase I nuclei, dense bundles of MTs concentrate in the mid-body that forms (Fig. 3b-b’’). In the course of the spindle elongation and cytokinesis, the mitochondria partition equally to the developing secondary spermatocytes (Fig. 3b’’’).

In late telophase I to early interphase II, the single centrosome in each of the two secondary spermatocytes and each of the two centrosomes in the aberrant spermatocytes reduplicate. Whether the centrosome duplication in both cell types is accompanied with a duplication of the centriole pair or only with a separation and a partitioning of the two parent centrioles, so that each of the daughter centrosomes contains only a single centriole, is not known. The latter case of a reductional segregation of the centrioles before meiosis II (Gonzales et al., 1998), resulting in unicentriolar centrosomes that then form the spindles in the second meiotic division, occurs in *Drosophila* males, as established by electron microscopy (Tates 1971 cited in Fuller 1993; Cenci et al 1994). There are indications that this situation may also apply in *Acricotopus* males. In all centrosomes analysed at present, only one fluorescent dot per centrosome have been seen after the meiosis II centrosome duplication. Furthermore, in a longitudinal section of a metaphase II secondary spermatocyte only one centriole has been detected in the polar centrosome (Fig. 5j), but this observations need to be verified.

The cytokinesis following meiosis I is incomplete, as for the cytokinesis after differential gonial mitosis, and thus the two secondary spermatocytes remain connected through a cytoplasmic bridge (Figs. 3c-c’’’, 4f); and the primary cytoplasmic bridge to the aberrant spermatocyte also remains. This bridge first connects the aberrant spermatocyte with only one of the arising secondary spermatocytes (as shown in Fig. 3b) but it then shifts in direction to the

bridge joining the secondary spermatocytes and fuses to form a new Y-shaped cytoplasmic canal complex connecting the three cells (Fig. 3d-d’’’).

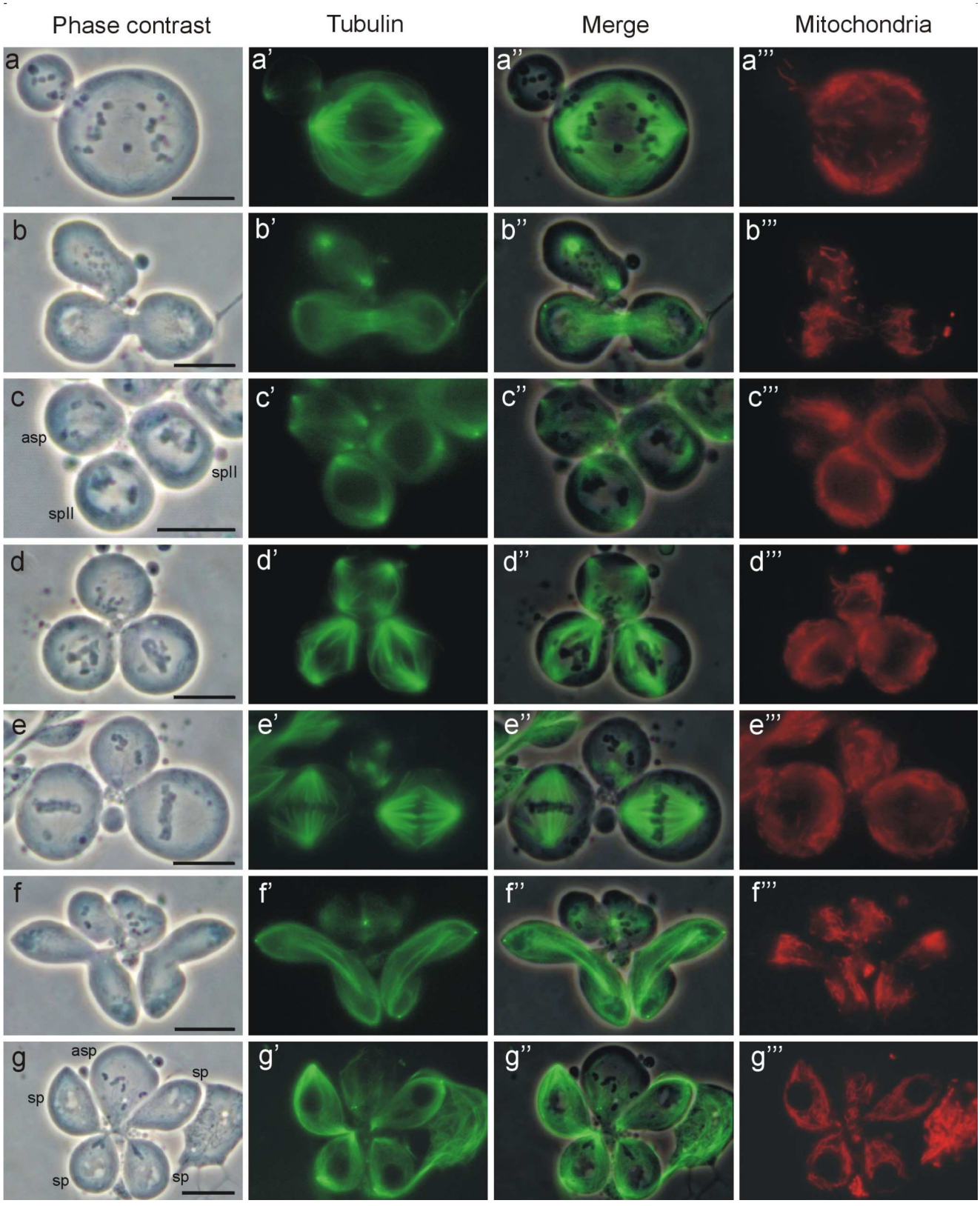

Fig. 3a-g’’’ Male meiosis in *Acricotopus*. **a-a’’’** Mid anaphase I. Polar movement of the S and K homologues. **b-b’’’** Telophase I with cleavage furrow. Formation of the secondary spermatocytes. **c-c’’’** Interphase II. Polar positioning of the duplicated centrosomes in the secondary spermatocytes (*spII*). The Ss in the aberrant spermatocyte remain condensed. Formation of four asters in the aberrant spermatocyte

(*asp*). **d-d'''** Late prophase II. Nuclear membrane breakdown and spindle formation in secondary spermatocytes. Tetrapolar arrangement of centrosomes and asters in the aberrant spermatocyte. **e-e'''** Metaphase II with prominent bipolar spindles. **f-f'''** Telophase II. Beginning of cytokinesis and formation of spermatids. The Ss in the aberrant spermatocyte remain condensed. **g-g'''** The four early spermatids (*sp*) and the aberrant spermatocyte (*asp*) are connected with each other through cytoplasmic bridges. *Bars* 10 µm.

During interphase II to prophase II the centrosomes in secondary spermatocytes move to opposite poles and organise bipolar spindles (Fig. 3c',c'',d',d''), while in the aberrant spermatocyte, the centrosomes with their surrounding asters arrange a tetrapolar spindle within the cell (Fig. 3d',d''). In the aberrant spermatocytes (Fig. 4a,c), the MTs of the four prominent asters can be clearly seen but no kinetochore MTs extend from the asters to the six metaphase condensed Ss (Fig. 4b). Some MTs emanating from the centrosomes overlap as do polar MTs in a normal spindle at the mid-zones to each of the neighbouring centrosomes (Fig. 4c).

The tetrapolar positioning of the asters and the condensed status of the Ss in the non-dividing aberrant spermatocyte persist during metaphase II, with prominent spindles formed in the secondary spermatocytes (Figs. 3e-e'', 4d) up until telophase II (Fig. 3f-f''). The mitochondria in metaphase II cells are clustered equally in the cell periphery around the spindle (Fig. 3e'''). They are then evenly distributed during telophase II (Fig. 3f''') and partition equally to the developing early spermatids (Fig. 3g'''). Cytokinesis following the second meiotic division is also incomplete and the aberrant spermatocyte remains connected with the four early spermatids by small cytoplasmic bridges (Figs. 3g-g'', 4g). The centrosomes of the aberrant spermatocytes lack most of the emanating MTs and occupy variable positions within the cell. At the beginning of spermiogenesis, the early spermatids begin to elongate (Fig. 4h). The aberrant spermatocytes also undergo a partial cell elongation and adopt a spindle-like shape (Fig. 4h-j). The six Ss of the aberrant spermatocyte condense further and become heteropycnotic (Fig. 4h-j).

The scheme in Fig. 6 summarizes the behaviour of Ss and Ks and of centrosomes, and all relevant changes that occur in mitochondria distribution, chromosome condensation and formation of spindle MTs and cytoplasmic bridges during differential gonial mitosis and male meiosis in *Acricotopus.*

Male meiosis proceeds asynchronously in the various cells of the *Acricotopus* testes: it begins in some cells at the late 4th larval stage and in others at the early prepupal stage, and is completed in all cells before pupation. The images of male meiosis I and meiosis II shown in Figs. 2a-g''', 3a-g''' clearly demonstrate that the aberrant spermatocytes undergo no cell divisions. This is in contrast to observations of Bauer and Beermann (1952), who reported that, in the orthocladiid species that they investigated, the aberrant spermatocytes carry out two divisions in synchrony with the meiotic divisions of the regular spermatocytes.

In many cases, cytoplasmic bridges connecting two aberrant spermatocytes have been observed, e.g. before meiotic divisions (Fig. 4e, arrowhead), and after the first meiotic division (Fig. 4f, arrowhead) and also after the second division (Fig. 4g, arrowhead). These bridges result from incomplete mitotic divisions of spermatogonial cells, which take place before differential

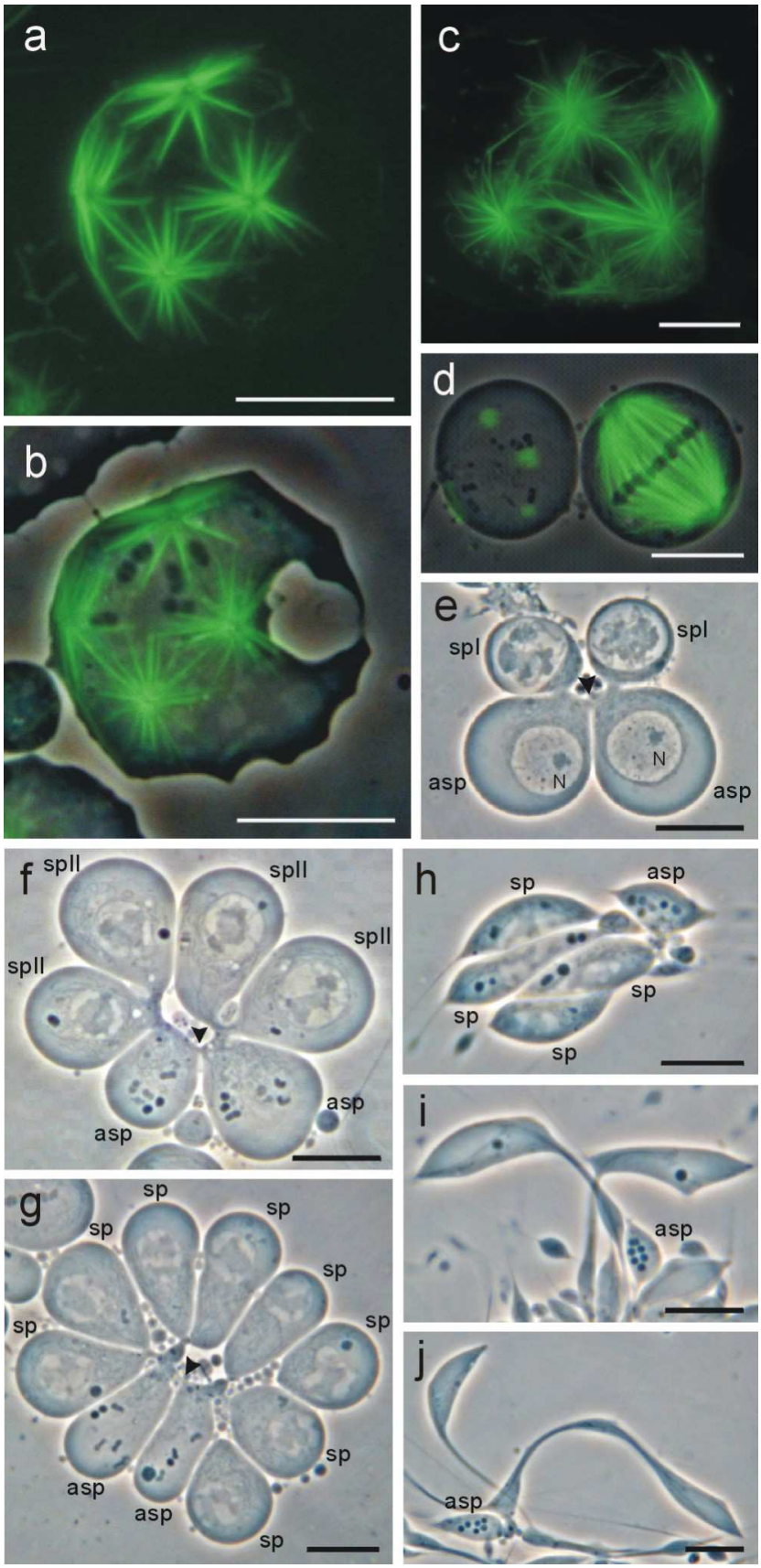

Fig. 4 a-c Aberrant spermatocytes of *Acricotopus* with tetrapolar arranged asters. **a**, **c** TubulinTracker fluorescent staining of polymerised tubulin. **b** Merged fluorescence and phase-contrast images. No MTs attached at the condensed Ss. **d** Metaphase II secondary spermatocyte showing dense bundles of MTs attached at the kinetochores of the equatorially congregated Ks and Ss. The connected aberrant spermatocyte exhibits only small asters. **e** Group of four cells consisting of a pair of primary

spermatocytes (*spI*) in pachytene and a pair of aberrant spermatocyte (*asp*) in interphase connected by cytoplasmic bridges (*arrowhead* intercellular bridge between the aberrant spermatocytes). **f** Group of six cells composed of two sets of two secondary spermatocytes (*spII*) in interphase and one pair of aberrant spermatocytes (*asp*) with condensed Ss. All cells are connected through cytoplasmic bridges (*arrowhead* intercellular bridge between the aberrant spermatocytes). **g** Group of ten cells representing two sets of four early spermatids (*sp*) and one pair of aberrant spermatocytes, the latter with permanently condensed Ss. Most of the cells are still connected by intercellular bridges (*arrowhead* cytoplasmic bridge joining the aberrant spermatocytes). **h-j** Early spermatid differentiation with progressive elongation of the spermatids. Each of the associated aberrant spermatocytes (*asp*) adopts a spindle-like shape and exhibits six rounded strongly condensed Ss. *Bars* 10 µm.

gonial mitosis. The intercellular cytoplasmic bridges also remain after differential mitosis and, in all cases, connect the aberrant spermatocytes with each other (Fig. 4e-g). The occurrence of cytoplasmic bridges interconnecting gametogenic cells is a widespread phenomenon established from insects through to mammals (Moens and Go 1972; Hime et al. 1996; Greenbaum et al. 2006, 2007). In *Acricotopus*, the cytoplasmic bridge between aberrant and primary spermatocytes has been suggested to be needed to transport mitochondria and other cytoplasmic components to the primary spermatocyte in order to support the growing germ cell until the beginning of meiosis (Staiber 2007). Intercellular bridges between germ cells may also serve for the interchange of signals coordinating meiotic divisions and for the sharing of gene products in haploid gametocytes (Guo and Zheng 2004; Greenbaum et al. 2006).

The hyperamplification of centrosomes and the resulting formation of tetrapolar asters before the second meiotic division are remarkable events in the non-dividing aberrant spermatocyte. Recent findings implicate the centrosomes, or more generally the MT organising centres (MTOCs), as having a role as cellular control centres coordinating cell cycle progression, MT organisation and MT turnover (Cuschieri et al. 2007). In addition, centrosomes and centrosomal proteins also seem to have important functions in cytokinesis (Fabbro et al. 2005; Azimzadeh and Bornens 2007). Thus, the duplication of the centrosomes in cells must also be closely coordinated with other processes during the cell cycle. Studies that have identified the cell cycle kinase CDK2-cyclin A and CDK2-Cyclin E complexes as essential factors necessary for centrosome reproduction support this idea (Hinchcliffe et al. 1999; Meraldi et al. 1999). In *Acricotopus*, such cell cycle regulators present in the secondary spermatocytes may act via the cytoplasmic bridge to the non-dividing aberrant spermatocyte and may induce the extra-duplication of the two centrosomes leading to the formation of tetra-asters. Thus, in the aberrant spermatocyte, the centrosome cycle and the cell cycle seem to be decoupled.

The programmed inactivation of all Ss by permanent condensation in the aberrant spermatocyte during male meiosis of *Acricotopus* might be regarded as a special form of chromatin silencing of the whole genome of a cell, although the mechanism involved is unknown. Histone modifications or DNA methylation might play a role in this inactivation process. A well-known example of chromosome inactivation is the X chromosome condensation that takes place as a specific form of dosage compensation in somatic cells during female mammalian development.

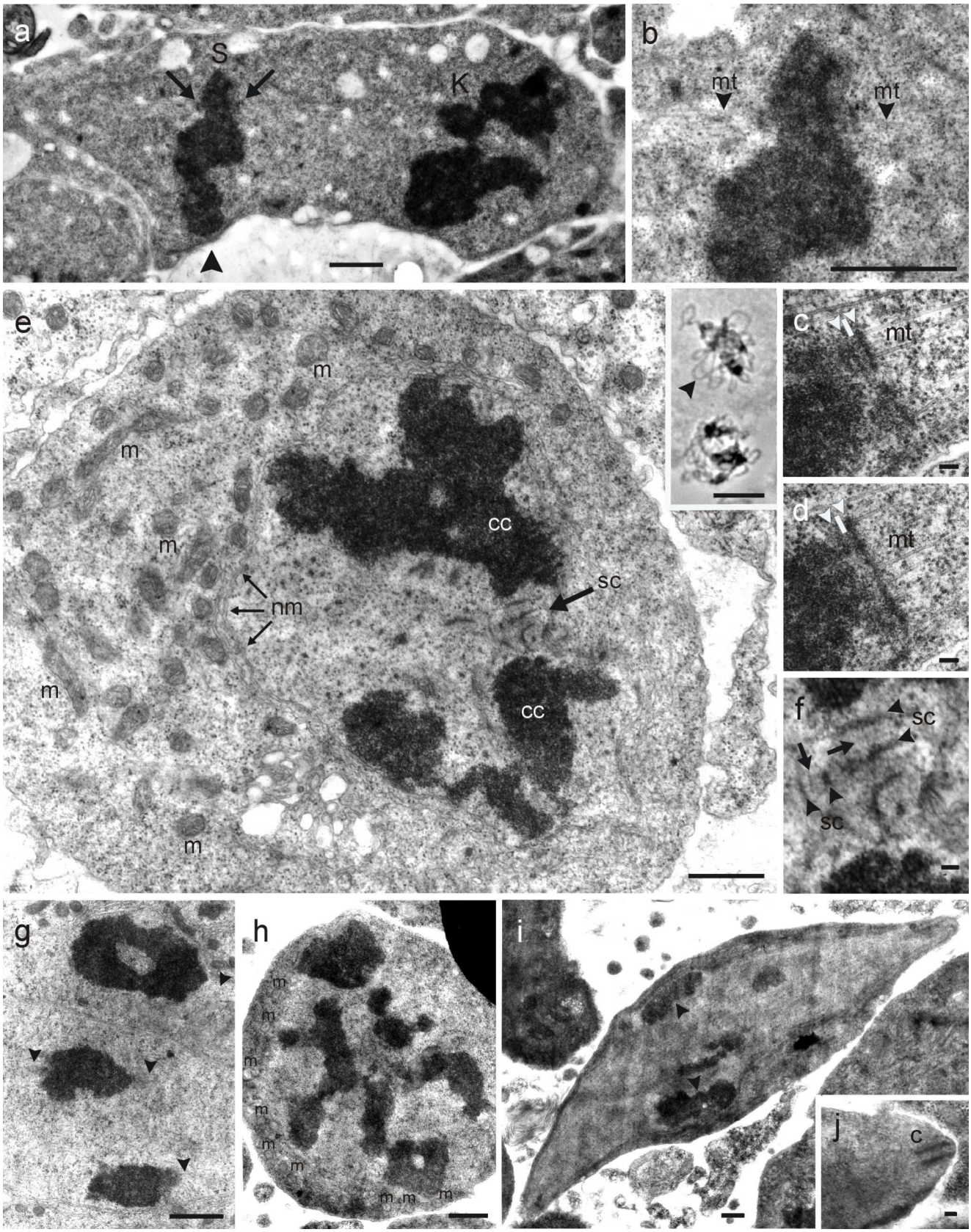

Fig. 5 a Longitudinal section through a spermatogonial cell in differential mitosis. All Ks move unseparated to the right spindle pole, whereas the Ss remain in the equatorial plane (*arrowhead*; cf. the differential mitosis in Fig. 1a). In one of the Ss (*S*), the kinetochores of the two sister chromatids are peripherically sectioned (*arrows*). The kinetochores are slightly pulled out of the chromatids and are oriented to the opposite poles. **b** The sectioned S sister kinetochores of **a** at a higher magnification. The right kinetochore shows a faintly triple-layered internal structure. Both kinetochores are attached to

microtubules (*mt*, *arrowheads*). **c, d** Consecutive sections through a kinetochore of a pole moving anaphase chromosome from a spermatogonial mitosis showing more clearly the three distinct layers (*arrowheads* outer layers, *bar* inner layer) of a trilaminar kinetochore. MTs (*mt*) are attached to the outer layer. **e** Median section through a pachytene primary spermatocyte. The majority of the mitochondria (*m*) are accumulated in the left half of the cell in direction to the aberrant spermatocyte. The same unilateral accumulation of mitochondria is shown in the pachytene primary spermatocyte in Fig. 2c''' visualised by MitoTracker fluorescent staining. *Insert* in **e**: Phase-contrast image of two carmine-stained pachytene nuclei showing large loops of paired homologous chromosomes (*arrowhead*) extending from blocks of condensed chromatin. Some short longitudinal sections of such loops can be seen in **e** in the region between the two condensed chromatin (*cc*) blocks (*arrow*), indicating the presence of a synaptonemal complex (*sc*). **f** Bivalent loops of **e** at a higher magnification. The typical tripartite structure of a synaptonemal complex (*sc*) reflecting the pairing of the homologous chromosomes is clearly visible: two dark stained lateral elements (*arrowhead*s) and the central element (*arrow*). **g** Longitudinal section through three metaphase I bivalents. Bundles of MTs pull the homologues in opposite directions (*arrowheads*). **h** Cross section through the congregated bivalents of a metaphase I plate. The mitochondria (*m*) are arranged in the periphery of the cell outside the spindle. The same peripherical arrangement of the mitochondria can be viewed in the metaphase I cell in Fig. 2g'''. **i** Longitudinal section of an early anaphase I primary spermatocyte. The chiasmata are dissolving in two of the bivalents (*arrowheads*). **j** Longitudinal section through a polar centriole (*c*) of a metaphase II secondary spermatocyte. *Bars* 10 µm (*insert* in **e**), 1 µm (**a**, **b**, **e**, **g**, **h**, **i**), 0.1 µm (**c**, **d, f, j**)

Another form of X chromosome inactivation or silencing, called meiotic sex chromosome inactivation (MSCI), occurs in the male germ line of mammals in meiotic prophase I at pachytene (Schimenti 2005; Turner 2007). MSCI seems to be one aspect of a more general meiotic-silencing mechanism, called meiotic silencing of unsynapsed chromatin (MSUC), whereby chromosomes that do not pair with their homologues are silenced, leading to the failure of gametogenesis. MSUC has also been established for autosomal regions in male and female meiosis in mice (Baarends et al. 2005; Turner et al. 2005); and may be a part of a general surveillance mechanism to protect against the formation of chromosomally imbalanced gametes (Turner 2007).

Another programmed inactivation, but in this case of entire paternally derived haploid chromosome sets by heterochromatisation, occurs in early embryogenesis in male mealybugs (Nur 1990; Khosla et al. 2006) and an inactivation of the whole nucleus of a spermatocyte has been reported by Dallai et al. (2000) for two collembolan species. In these springtails, the secondary spermatocyte, which received no sex chromosomes in meiosis I, forms "a smaller cell with very reduced cytoplasm, two centrioles, and a condensed nucleus" and does not carry out the second meiotic division and degenerates (Dallai et al. 2000).

A mechanism like MSUC might explain the inactivation Ss, which are probably unsynapsed, in the aberrant spermatocyte of *Acricotopus.* Factors inducing MSUC may act through the cytoplasmic bridge from the pachytene in primary spermatocytes, containing completely synapsed S and K homologues, to the aberrant spermatocyte and there induce the permanent inactivation and silencing of the Ss. The purpose of a programmed inactivation of the Ss in the aberrant spermatocyte of *Acricotopus* might be to inhibit the entry of the aberrant spermatocytes into meiosis and thereby prevent the generation of sperms that contain only Ss, but no Ks. This mechanism would perpetuate and guarantee the presence of the Ks in the germ line.

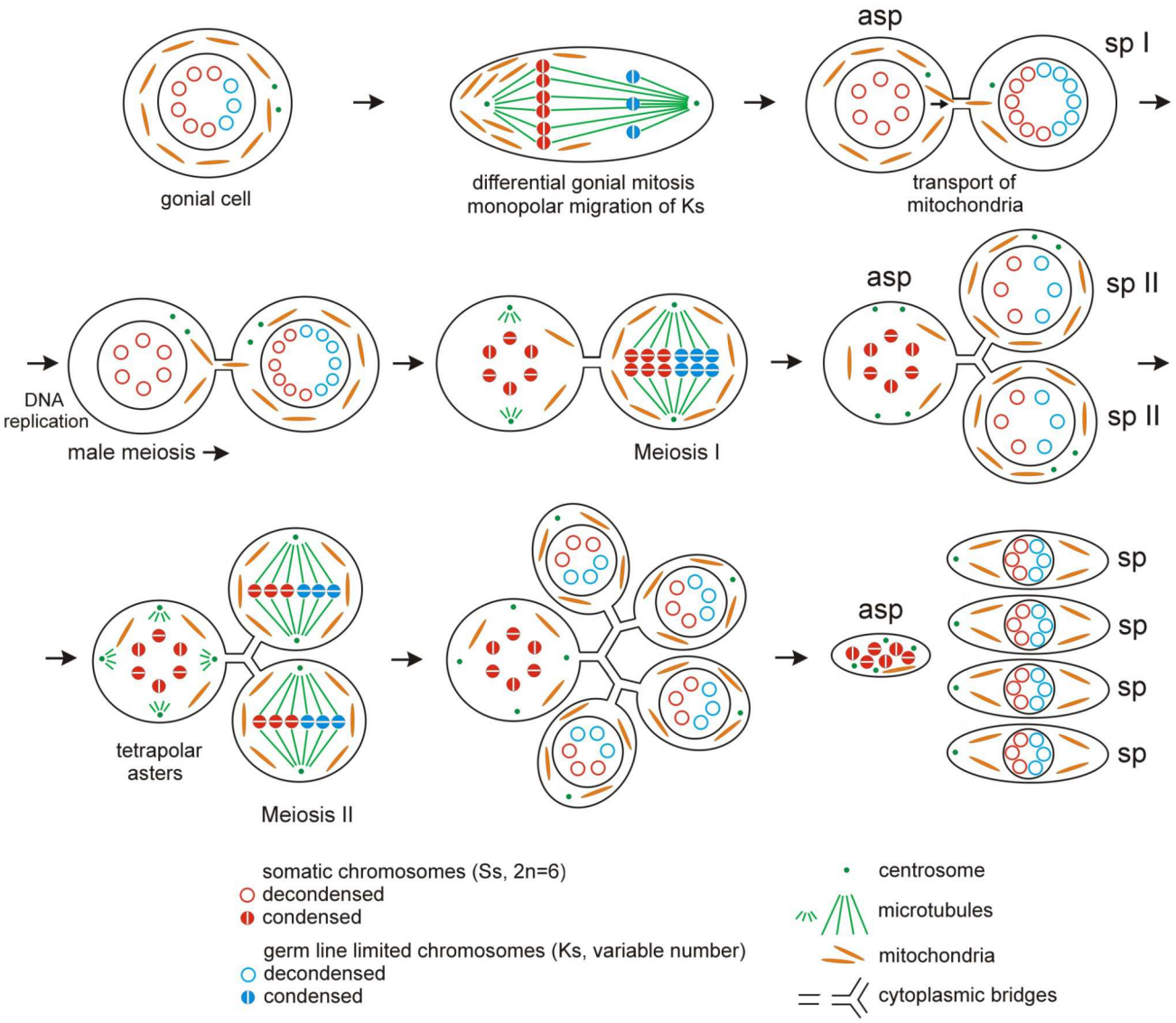

Fig. 6 Representation summarising the relevant changes in chromosome and mitochondria distribution, chromosome condensation, behavior of centrosomes, and formation of spindle microtubules and cytoplasmic bridges during differential gonial mitosis and male meiosis in *Acricotopus*. To clarify interpretation, both chromatids are represented in the condensed chromosomes. *asp* aberrant spermatocyte, *spI* primary spermatocyte, *spII* secondary spermatocyte, *sp* spermatid.

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