Spermatogenesis and sperm structure in some *Meloidogyne* species (Heteroderoidea, Meloidogynidae) and a comparison with those in some cyst nematodes (Heteroderoidea, Heteroderidae)

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Summary

Sperm structure and development was examined in the mitotic (obligatory) parthenogens *Meloidogyne incognita incognita, M. incognita wartellei* and *M. arenaria*, in the meiotic (facultative) parthenogens *M. hapla* (race A) and *M. graminicola*, in *M. oryzae* (reproductive status unknown), and in the amphimictic species *M. acronea*. Spermatogenesis in *Meloidogyne* differs from that in the cyst nematodes, in which the maturation divisions occur only in the testis of the pre-adult ; cells in all phases of spermatogenesis were present in adult *Meloidogyne* males. The structure of the spermatid in *M. hapla* is discussed. In *Meloidogyne*, differences in sperm structure between species concerned mainly : the polarisation of the spermatozoa in some into a main « body » and a pseudopodium ; persistence or otherwise of the fibrillar bodies ; abundance of filopodia, particularly at earlier stages ; state of the nuclear material and size of mature sperm. Sperm of four *Meloidogyne* species were, like cyst-nematode testicular sperm, unpolarised. The form of the condensed chromatin in the sperm nucleus was similar in most *Meloidogyne* to that in *Heterodera*. In *Globodera* and in *M. oryzae* the form was different. So-called nucleolar bodies, present in the *Meloidogyne* species, were absent from sperm in the testis of cyst nematodes. Cortical microtubules lining the plasma membrane of the sperm, and the absence of membranous organelles, were constant features throughout the two families.

Résumé

Spermatogénèse et structure des spermatozoïdes chez quelques Meloidogyne *(Heteroderoidea, Meloidogynidae) comparées avec certaines espèces de nématodes à kystes (Heteroderoidea, Heteroderidae)*

La structure et le développement des spermatozoïdes ont été observés chez les espèces à parthénogénèse mitotique (obligatoire) *Meloidogyne incognita incognita, M. incognita wartellei* et *M. arenaria*, chez les espèces à parthénogénèse méiotique (facultative) *M. hapa* (race A) et *M. graminicola*, ainsi que chez l'espèce *M. oryzae*, dont les modalités de reproduction sont inconnues, et chez l'espèce amphimictique *M. acronea*. La spermatogénèse chez *Meloidogyne* est différente de celle des nématodes à kystes où les divisions de maturation ont lieu dans le testicule du pré-adulte ; chez les mâles de *Meloidogyne*, des cellules à toutes les phases de la spermatogénèse étaient présentes. La structure de la spermatide chez *M. hapla* est discutée. Chez *Meloidogyne* les différences entre espèces dans la structure des spermatozoïdes concernent principalement : la « polarisation » entre corps principal du spermatozoïde et pseudopode ; la persistance ou la disparition des corps fibrillaires ; l'abondance des filopodes, particulièrement aux stades les plus jeunes ; l'état du matériel nucléaire et la taille du spermatozoïde mûr. Chez quatre espèces de *Meloidogyne* les spermatozoïdes ne sont pas polarisés, de même que les spermatozoïdes testiculaires des nématodes à kystes. Chez de nombreux *Meloidogyne* l'aspect de la chromatine condensée dans le noyau du spermatozoïde est identique à celui des *Heterodera*. Chez *Globodera* et *M. oryzae* cet aspect est différent. Les corps nucléolaires présents chez *Meloidogyne*, manquent dans les spermatozoïdes testiculaires des nématodes à kystes. La présence de microtubules corticaux bordant la membrane plasmatique du spermatozoïde, ainsi que l'absence d'organites membraneuses, représentent des caractères constants chez l'une et l'autre familles.

Differences between the structure of sperm of cyst nematodes *(Heterodera* and *Globodera)* (Shepherd, Clark & Kempton, 1973) in the Tylenchida and of *Aphelenchoides* (Shepherd & Clark, 1976) in the Aphelenchida (Siddiqi, 1980) are fundamental. However, more examples from each group are needed to confirm that these basic structural differences are characteristic of the respective groups as a whole. For comparison with cyst-nematode sperm we selected *Meloidogyne*, representing a family (Meloidogynidae) close to the Heteroderidae, and we examined sperm structure and development in several species to see if they varied. This selection included meiotic (facultative) and mitotic (obligatory) parthenogens, and one species *(M. acronea)* thought to be entirely amphimictic (Page, pers. comm.).

The main object of this study was to compare sperm morphology; we have not attempted to interpret genetical differences. The ultrastructure of cytological differences between the meiotic and mitotic races of *M. hapla* during spermatogenesis was described by Goldstein and Triantaphyllou (1978*b*), and sperm development in *M. hapla* by Goldstein and Triantaphyllou (1980). We briefly described sperm development in *M. incognita incognita* previously (Shepherd & Clark, 1979); here we describe it fully, and comment on the other species where differences exist. The males of all the species examined are telogonic and usually monorchic (although some individuals may have two testes), and their gonads are usually straight but may be reflexed.

Materials and methods

Adult males of the various *Meloidogyne* spp. were obtained from the sources and populations expressed in Table 1, kept in a greenhouse at Rothamsted.

The males were fixed in 6% glutaraldehyde in cacodylate buffer at pH 7.2 for 22 h in an ice bath. The posterior two thirds or so was then excised and cut into at least two pieces; each piece was embedded in 1% agar and returned to the cold fixative for an additional 2 h. They were then thoroughly rinsed in buffer, post-fixed in 1% OsO_4 in veronal acetate buffer for 3 h, dehydrated in an alcohol series and infiltrated and embedded in Spurr low viscosity resin. Longitudinal and transverse serial sections (60 mm) were cut and mounted on large-slot grids coated with a necoloidine film. Sections were stained on the grids using lead citrate, either alone or after uranyl acetate. Some were also pretreated with potassium permanganate (Soloff, 1973).

Observations

Mitotic (obligatory) parthenogens

M. incognita incognita.

All stages of sperm development are found in the adult male gonad, the tip of which is usually about midway along the body.

Spermatogonia. Two or three intermingled rows of spermatogonial cells (sg) (Fig. 1a) extend from the testis tip for about 150 μm. The cells are packed closely together and we could not distinguish a definite rachis. The testis wall (tw) is very thin in this region. Each spermatogonium has a large, amoeboid, membrane-bound nucleus (nu) occupying much of the cell and containing a prominent, electron-dense, spherical nucleolus (nl). The spermatogonia, which usually number about 50-70, are about 6 μm in diameter and are rich in ribosomes (ri) and mitochondria (m); Golgi bodies (gb) are also plentiful.

Table 1

| Species	Source	Cultured on	No. specimens cut
M. acronea Coetzee	Ngabu, Malawi	sorghum	7
M. arenaria (Neal)	origin unknown	coleus	7
M. graminicola Golden & Birchfield	Bangladesh	rice	9
M. hapla (race A) Chitwood	Norfolk, England	tomato	4
M. incognita incognita (Kofoid & White)	origin unknown	tomato	11
M. incognita wartellei Golden & Birchfield	N.C. State Univ., U.S.A.	soybean	7
M. oryzae Maas, Sanders & Dede	Surinam	rice	2
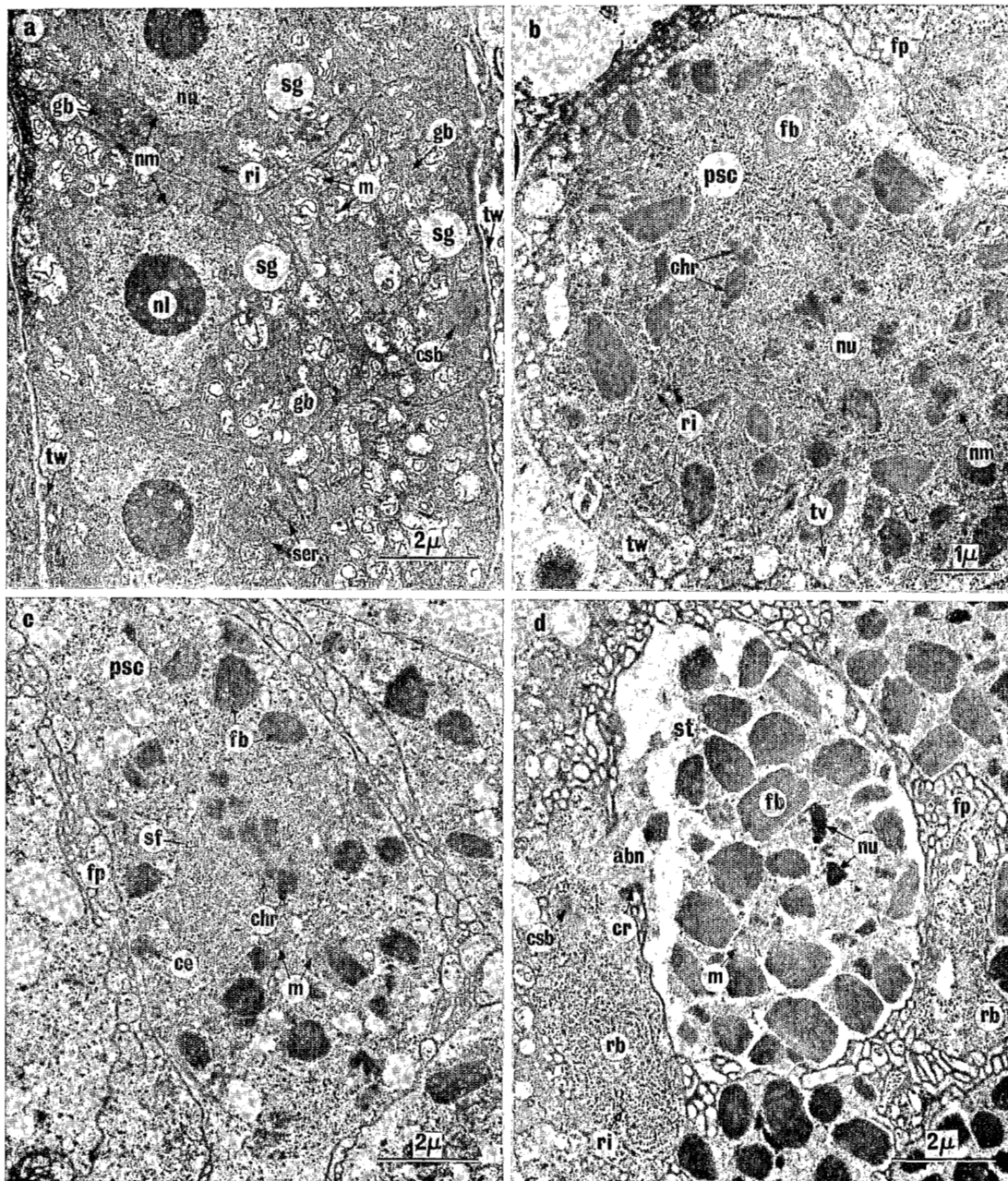
Fig. 1. *M. incognita incognita* adult male. a : L.S. testis tip showing spermatogonia (sg) with large amoeboid nucleus (nu), spherical nucleolus (nl), nuclear membrane (nm), ribosomes (ri), Golgi bodies (gb), mitochondria (m), smooth endoplasmic recticulum (ser) and cytoplasmic spherical bodies (csb). × 7 300 ; b : T.S. testis showing a primary spermatocyte (psc) at prophase, with chromosomes (chr) condensing and nuclear membrane (nm) almost dispersed. Cytoplasm contains fibrillar bodies (fb) and many ribosomes (ri). × 9 200 ; c : L.S. testis showing primary spermatocyte (psc) at metaphase with chromosomes (chr) aligned along the metaphase plate ; also spindle fibres (sf) and centriolar complex (ce). × 7 300 ; d : T.S. testis showing spermatid (st) with homogeneous nucleus (nu), fibrillar bodies (fb) and mitochondria (m) and a residual body (rb) with many ribosomes (ri), joined by abscission neck (abn) containing contractile ring of fibrils (cr). × 7 500.

Abbréviations : abn : abscission neck ; bmu : body wall muscle ; ce : centriole ; chn : chromatin ; chr : chromosome ; cmt : cortical microtubules ; cr : contractile ring ; csb : cytoplasmic spherical body ; cu : cuticle ; cyt : cytoplasmic tubules ; esc : early spermatocyte ; fb : fibrillar body ; fp : filopodium ; gb : Golgi body ; int : intestine ; m : mitochondrion ; mt : microtubules ; mtc : compressed microtubules ; nl : nucleolus ; nlb : nucleolar body ; nm : nuclear membrane ; nu : nucleus ; osm : outer sperm membrane ; pm : plasma membrane ; ps : pseudopodium ; psc : primary spermatocyte ; rb : residual body ; ri : ribosomes ; sc : spermatocyte ; ser : smooth endoplasmic recticulum ; sf : spindle fibres ; sg : spermatogonium ; sp : spermatozoon ; spb : sperm body ; st : spermatid ; svw : seminal vesicle wall ; tn : telophase neck ; tv : electron-translucent vesicle ; tw : testis wall.

Patches of moderately dense granular material in the cytoplasm (csb) are presumably profiles of spherical bodies of some kind, and there is some smooth endoplasmic reticulum (ser) (Fig. 1a).

Spermatocytes. With the nuclear changes that mark the inception of the spermatocyte stage, the first signs appear of the fibrillar material (fb) characteristic of most nematode sperm at some stage (as illustrated for *M. hapla*, Fig. 4a). The roughly cuboidal fibrillar bodies (Fig. 1b, c) grow to about 0.5-0.6 μm in diameter. They are not associated with any specialised membrane system nor are they even contained within a vesicle but lie free in the cytoplasm from their inception. There are usually from 12 to 20 cells in various phases of the maturation division at any one time, occupying 35-40 μm of the length of the testis, whose wall (tw) is thicker in this region and contains ribosomes and vesicles. Each spermatocyte is slightly elongated, about 15 μm by 10 μm, with many ribosomes (ri) and fibrillar bodies, some mitochondria (m) and some electron-translucent vesicles (tv), whose contents may have been leached out during preparation. The outline of the spermatocyte becomes complex with the first infoldings of the boundary membrane that eventually become filopodia (fp) (Fig. 1b, c). The cell is enclosed only by a plasma membrane. The earliest indication of the start of the maturation division is the condensation of the chromatin (chr) within the nuclear membrane (nm) at early prophase (Fig. 1b), followed by dissolution of the nuclear membrane. Fig. 1c shows metaphase, with fully condensed chromosomes aligned along the equator of the spindle.

Spermatids. After the maturation division the chromatic material of the nucleus re-organises into a homogeneous, electron-dense mass (nu) with no reconstitution of the nuclear membrane (Fig. 1d). The ribosomes, Golgi bodies and cytoplasmic spherical bodies, with some cytoplasm, are segregated from the nuclear region of the spermatid into the residual body (rb) (Fig. 1d). A cleavage furrow develops between the two portions so that they are joined only by an abscission neck (abn) (Fig. 1d). This has similar characteristics to the telophase neck between daughter cells following cell division (as seen in *M. hapla* (tn in Fig. 4d)), with a contractile ring of fibrils (cr) seen as a dark zone just inside the cell membrane and many microtubules passing through the neck (mt in Fig. 5c, d). These often become highly compressed (mtc) at the centre (see Dyson, 1978). The residual body is eventually discarded and engulfed by the testis wall as previously described for *Globodera* (Shepherd, Clark & Kempton, 1973). In the spermatid the fibrillar bodies (fb) remain in the nuclear portion of the cell, where they occupy a large proportion of its volume, together with the mitochondria (which are poorly preserved in Fig. 1d). The cell outline becomes increasingly complex around this region but not around the residual body.

Throughout this stage the cell is still bounded only by the plasma membrane but in very late spermatids the wall shows signs of thickening. In the specimens we sectioned there were about a dozen cells at the spermatid stage, occupying 35-40 μm of the testis. The spermatid, including its attached residual body, is about 20 μm by 8 μm.

Spermatozoa. In our interpretation, the transition from spermatid to spermatozoon comes with the sloughing off of the residual body (Shepherd, 1981). The early spermatozoa contain a central nucleus of homogeneous, very electron-dense chromatin (nu in Fig. 2b), irregular in shape but with smooth outlines and cavities. There are also several spherical satellite bodies, probably nucleolar material (nlb) (Goldstein & Triantaphyllou, 1980), scattered throughout the cytoplasm. These, too, are homogeneous but less electron-dense and more granular than the chromatin. The contours of these various bodies were determined by reconstruction from serial sections. The mitochondria (m) and fibrillar bodies (fb) are evenly distributed throughout the cytoplasm and the many filopodia (fp) are free-standing, no longer compacted or possibly even adhering together as earlier. The outer coat over the whole sperm surface, including the filopodia, is complex at this stage (Fig. 3f), with an inner plasma membrane (pm), and an outer membrane (osm) that has two dense layers separated by an electron-translucent zone, with an additional dense, fuzzy layer on the outside. Microtubule-like structures (cmt) (about 20 nm diam.) in parallel arrays line the inner surface of the plasma membrane (Figs 2b ; 3b, f). These look finer and less obviously attached to the plasma membrane than those in cyst-nematode sperm (Shepherd, Clark & Kempton, 1973), and the fingerprint-like patterns often noted in tangential sections of *Globodera* sperm were not seen in *M. incognita incognita* sperm. The only indication of the parallel arrangement was seen in small areas such as that depicted in Fig. 3e.

Very soon after maturation, all mitochondria and fibrillar bodies become concentrated in one region surrounding the nucleus, and most of the cytoplasm forms a large pseudopodium (ps) (Figs 2a ; 3a). The fine structure of these two parts is shown in Figs 2b and 3b respectively. Scattered throughout the cytoplasm of both portions are numerous microtubule-like structures (cyt) similar to those in *Aphelenchoides* sperm (Shepherd & Clark, 1976). In *M. incognita incognita* sperm this irregularly-organised system of tubules appears continuous
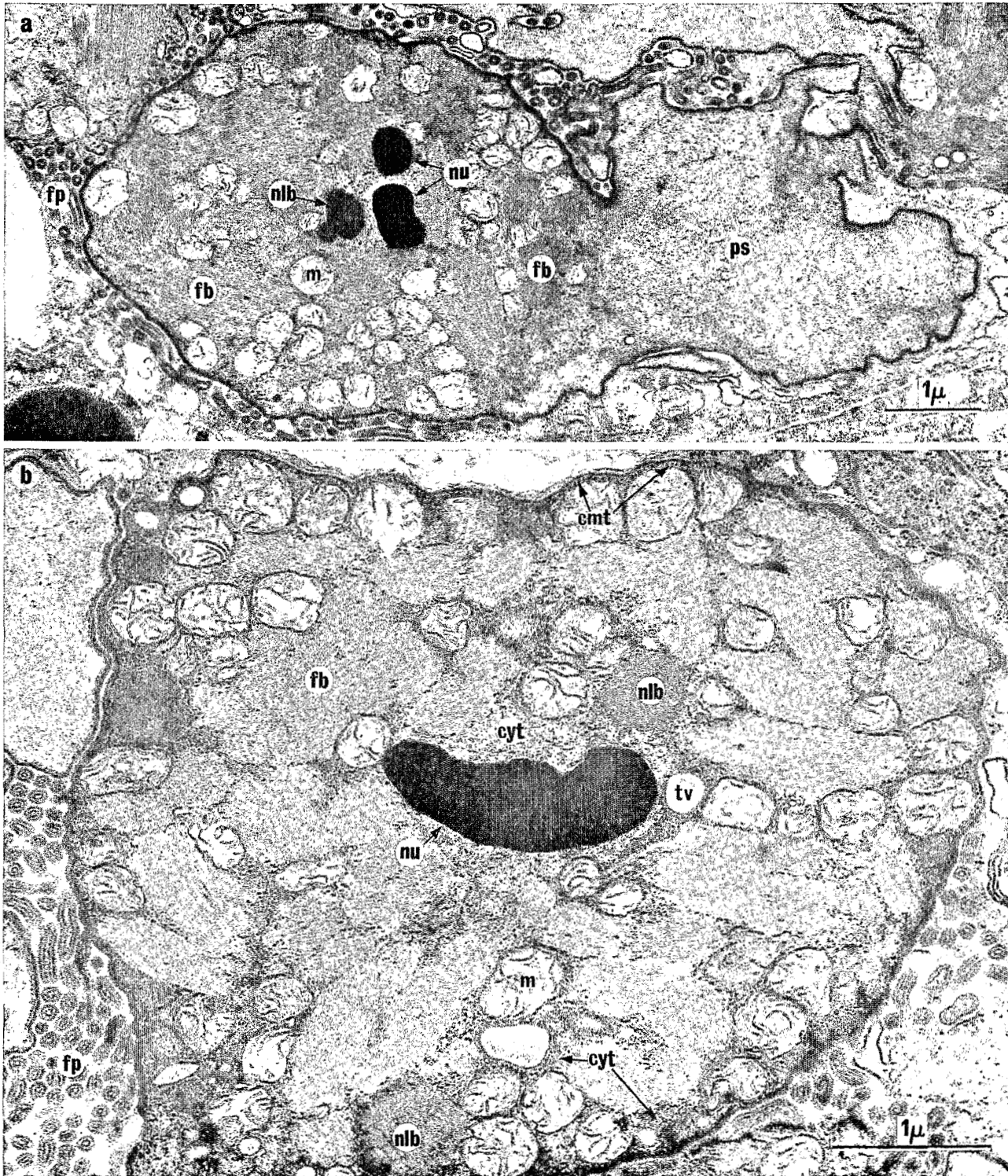
Fig. 2. *M. incognita incognita* adult male. a : T.S. seminal vesicle showing spermatozoon with nuclear region with dense nucleus (nu), nucleolar bodies (nlb), fibrillar bodies (fb), mitochondria (m) and filopodia (fp), and pseudopodial region (ps). × 16 000 ; b : Nuclear region of testicular spermatozoon, showing nucleus (nu), nucleolar bodies (nlb), and other organelles, also cortical microtubules (cmt) lining peripheral membrane, and tubular elements (cyt) throughout cytoplasm. × 26 000.

Abbreviations : see under Fig. 1.
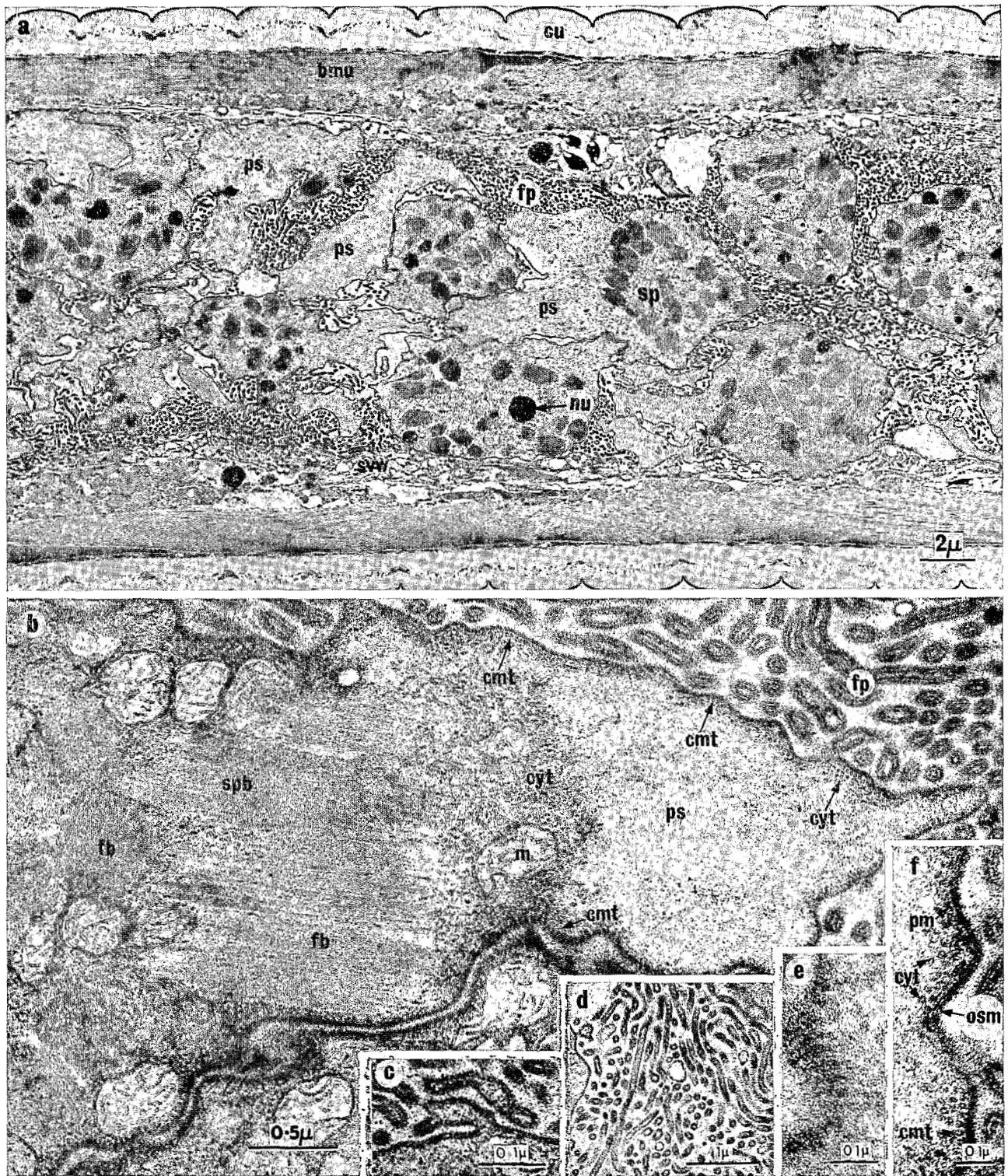
Fig. 3. *M. incognita incognita* adult male. a : L.S. showing seminal vesicle filled with mature spermatozoa (sp) with large pseudopodia (ps). × 4 400 ; b : Pseudopodial region (ps) of spermatozoon extending from main sperm body (spb), showing cortical microtubules (cmt) and cytoplasmic tubular elements (cyt). × 30 000 ; c : Branched filopodium. × 109 000 ; d : Long portions of filopodia. × 12 400 ; e : Slightly tangential section through sperm coat, showing parallel cortical microtubules. × 76 000 ; f : Section through periphery of spermatozoon showing plasma membrane (pm), triple-layered outer membrane (osm) with external fuzzy layer, cortical microtubules (cmt) and cytoplasmic tubular elements (cyt). × 58 000.

Abbreviations : see under Fig. 1.

with the regularly arranged array lining the plasma membrane. The two distinct types of (presumed) nuclear material in the spermatozoon are seen in Fig. 2a and b. Most of the very electron-dense chromatin (nu) is in the centre of the cell. The less dense, spherical 'nucleolar' bodies (nlb), which look granular, are dispersed throughout the cell, often around the periphery, and are rarely attached to the chromatin. They look somewhat similar in texture and electron density to the spherical bodies seen in the spermatogonial cytoplasm, although perhaps slightly more coarse-grained.

The fibrillar bodies are present in even the most advanced sperm in the seminal vesicle. Their structure is somewhat less compact in spermatozoa (Figs. 2b ; 3b) than in the earlier stages and eventually becomes rather wispy but they disappear almost completely only in defunct sperm.

The very numerous filopodia (fp in Fig. 3a) are mostly confined to the main body of the sperm. The several layers of their boundary membrane are clearly seen in Fig. 3b and they are often very long (Fig. 3d) (portions up to 4 μm long have been measured), and sometimes branched (Fig. 3c).

The mature sperm are about 12 μm long including the pseudopodium, the main body being about 6-7 μm in diameter. They are stored, packed quite tightly together (Fig. 3a), in the seminal vesicle before being passed out at copulation via the glandular vas deferens, the lumen of which is normally occluded.

M. incognita wartellei

While spermatogenesis was the same as in *M. incognita incognita*, the spermatozoa of *M. incognita wartellei* (Fig. 8a) were different. With the light microscope, sperm in fixed specimens appear spherical, about 7-8 μm in diameter, and less granular than *M. incognita incognita* sperm. In sections they appeared slightly elongated, about 8-9 μm × 4-5 μm. They showed no clear polarisation, the organelles being distributed throughout most of the cell, whose whole outline was amoeboid. Some fibrillar material persisted into maturity, although in a fairly dispersed form, but it sometimes disappeared entirely. The microtubular elements, both cortical and cytoplasmic, were well-defined in this material. The character of the nuclear material was like that of *M. incognita incognita*.

M. arenaria

Early stages in spermatogenesis of *M. arenaria* had fewer incipient filopodia than those of the other species examined. The spermatozoa (Fig. 7c) were about 10 μm × 8 μm, and were like those of *M. incognita incognita*.

MEIOTIC (FACULTATIVE) PARTHENOGENS

M. hapla (race A).

As with *M. incognita incognita*, all stages of sperm development were present in the adult males of *M. hapla* (race A). The spermatogonia (Fig. 4a), which at 16 μm × 8-10 μm were larger than those of *M. incognita incognita*, contained a large, amoeboid, membrane-bound nucleus (nu) ; moderately dense, cytoplasmic spherical bodies and smooth ER were again present. Some phases of the meiotic sequence are shown in Fig. 4b-e, progressing to the spermatid with a highly condensed nucleus lacking an envelope (Fig. 5a, c). There was no reconstitution of the nuclear membrane during spermatid development in the adult male specimens we examined. Fibrillar bodies (fb) were present throughout all stages after first appearing in the primary spermatocyte. Fig. 4e shows late telophase and cytokinesis in the secondary spermatocyte. In the telophase neck (tn) there are many compressed microtubules (mtc) of the spindle (sf) within the contractile ring (cr) cleaving the dividing cell. As mentioned for *M. incognita incognita*, it appears that a contractile ring also contributes to the severing of the residual body from the spermatid (Fig. 5c, d).

The mature sperm were almost indistinguishable from those of *M. incognita incognita*, having a pseudopodium (ps), although a somewhat smaller one, distinct from the main body of the sperm (Fig. 5e). The overall length of the mature sperm was about 9 μm, with the main body about 6 μm in diameter. The only other difference we could detect between the structure in the two species was a tendency for *M. hapla* to develop considerably more filopodia, particularly in the earlier stages, as seen in the spermatocytes in Fig. 4b, compared with *M. incognita incognita* at the same stage (Fig. 1c).

M. graminicola

Like *M. hapla*, *M. graminicola* has very abundant filopodia in both early and late stages (fp in Fig. 6a-c). The spermatozoa, which were about 9 μm × 5 μm, showed no clear polarity into 'body' and 'pseudopodial' regions. In this they were like *M. incognita wartellei*. As with *Globodera* sperm, they also lost their fibrillar material immediately on becoming spermatozoa. In this species the chromatin appeared as smaller, sometimes rather diffuse clumps (chn in Fig. 6c). The less dense 'nucleolar' (nlb) material was mostly gathered into a smooth-contoured central mass, but smaller spherical bodies were also scattered throughout the cytoplasm. The testis was proportionally longer, often occupying three quarters of the body length.
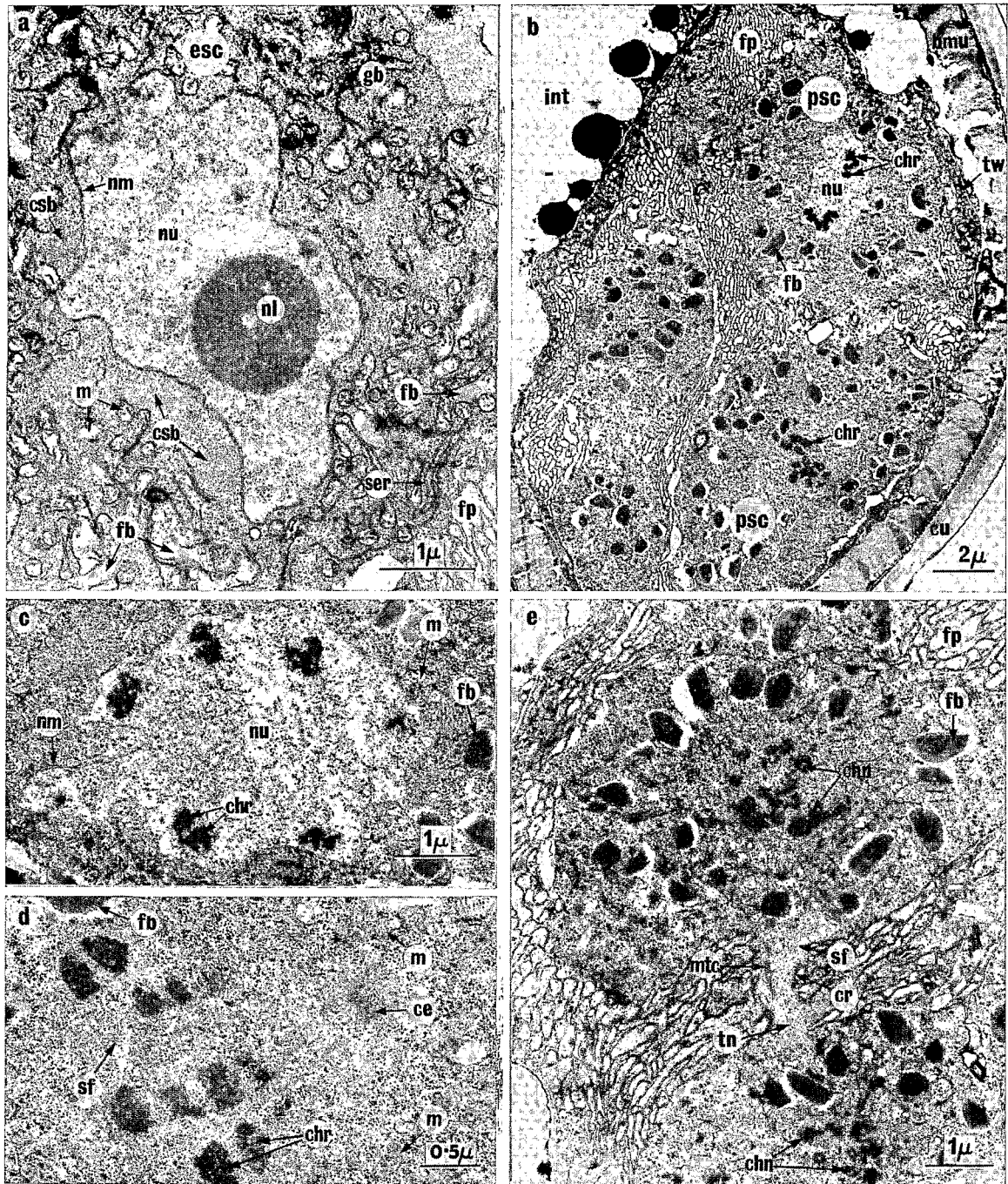
Fig. 4. *M. hapla* adult male. a : T.S. testis showing very early primary spermatocyte (esc) with large amoeboid nucleus (nu), nuclear membrane (nm), spherical nucleolus (nl), mitochondria (m), incipient fibrillar bodies (fb), Golgi bodies (gb), cytoplasmic spherical bodies (csb), smooth endoplasmic reticulum (ser) and incipient filopodia (fp). × 14 500 ; b : Testis showing primary spermatocytes (psc) at various phases of meiosis. × 4 300 ; c : Early prophase I nucleus (nu) of primary spermatocyte, with nuclear membrane (nm) still intact and homologous pairs of chromosomes (chr) condensing around periphery of nucleus. × 13 000 ; d : Chromosomes (chr) at metaphase I in nucleus of primary spermatocyte ; homologous pairs lined up along metaphase plate. Showing also centriole (ce) and microtubules of spindle (sf). × 17 600 ; e : The end of telophase II in secondary spermatocyte, with chromatin (chn) recondensing. Telophase neck (tn) contains compressed spindle fibres (sf). × 9 500.
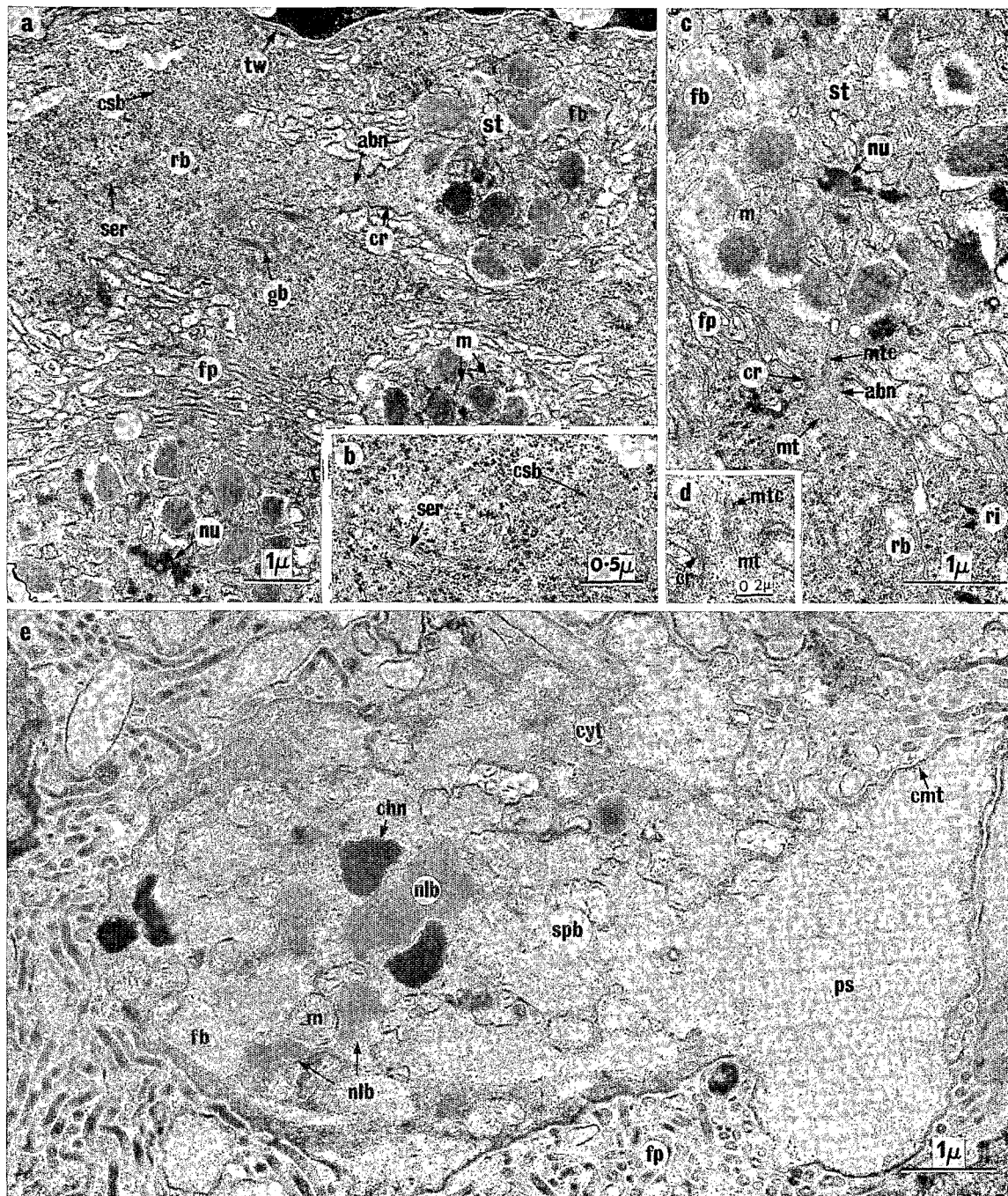
Abbreviations : see under Fig. 1.

Fig. 5. *M. hapla* adult male. a : T.S. testis showing spermatids (st) with large residual bodies (rb). Upper spermatid shows abscission neck (abn) with contractile ring of fibrils (cr). × 9 800 ; b : Enlargement of residual body showing smooth ER (ser) and cytoplasmic spherical bodies (csb). × 18 300 ; c : Spermatid (st) showing abscission neck (abn) between main cell body and residual body (rb), similar to telophase neck in fig. 4d, with contractile ring (cr) and compressed microtubules (mtc). × 14 800 ; d : Enlargement of abscission neck. × 27 000 ; e : Spermatozoon, showing the two regions, one containing organelles (spb), the other a pseudopodium (ps) with cortical microtubules (cmt) and cytoplasmic tubular elements (cyt). × 14 000.
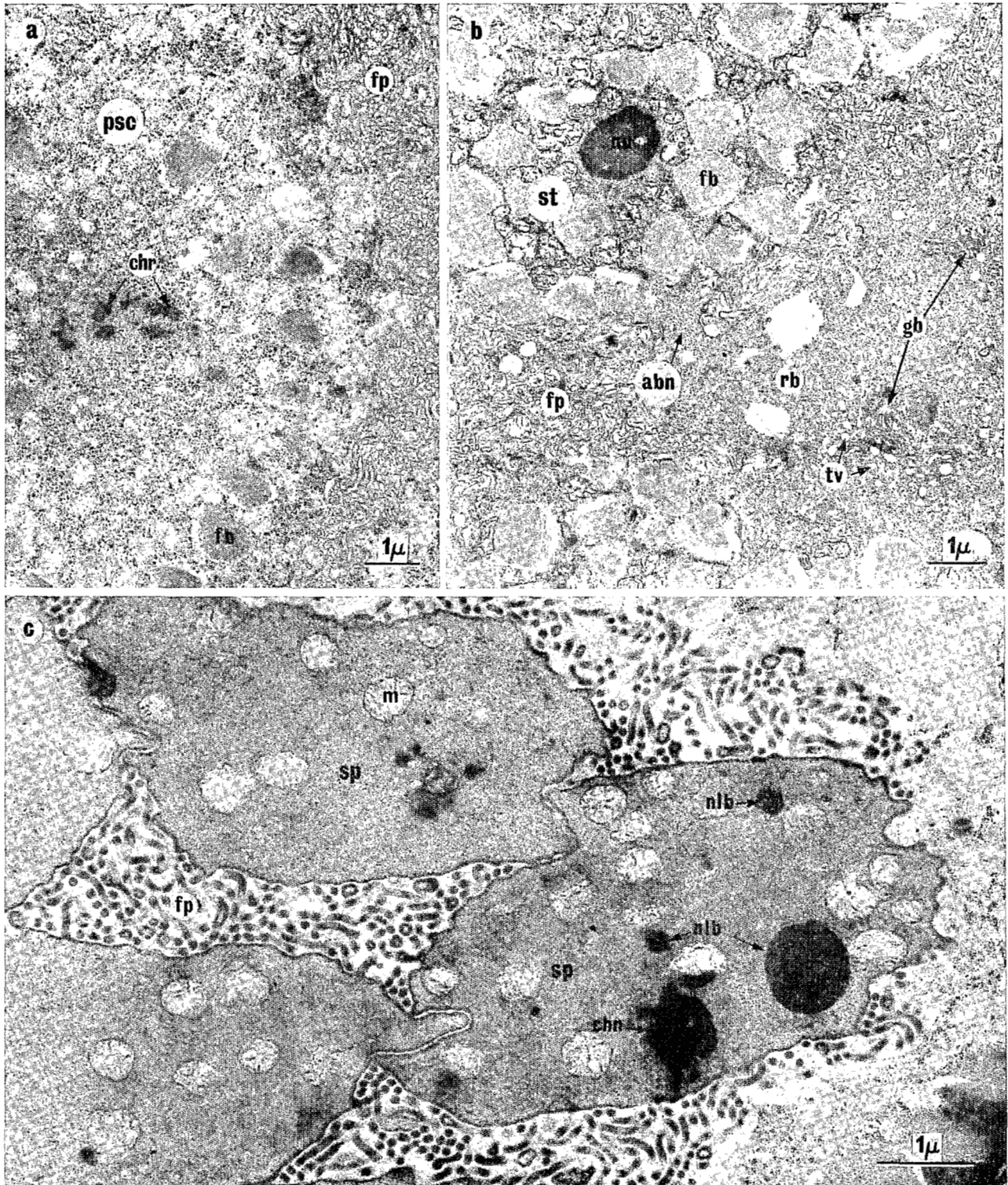
Abbreviations : see under Fig. 1.

Fig. 6. *M. graminicola* adult male. a : Primary spermatocyte (psc), with nucleus in metaphase I, also showing many incipient filopodia (fp). × 9 000 ; b : Spermatid (st) with large residual body (rb) showing abscission neck (abn). Residual body contains many ribosomes, large Golgi complexes (gb) and electron-translucent vesicles (tv). × 8 600 ; c : Spermatozoa, one showing large and small nucleolar bodies (nlb) and rather diffuse chromatin material (chn). (Slight stain precipitation in nucleolar material.) × 16 100.

Abbreviations : see under Fig. 1.

REPRODUCTIVE STATUS UNDETERMINED

M. oryzae

M. oryzae is considered by Maas, Sanders and Dede (1978) to be closely related to *M. graminicola*, but its reproductive status is not yet determined. Its sperm (Fig. 7a) somewhat resembled those of *M. graminicola* but were larger (about 15-20 μm × 8-12 μm). As with *M. graminicola*, the chromatin was less compacted than in the other species ; in *M. oryzae* it was mostly dispersed as fine strands or granules, or as small clumps around a central, large, smooth-contoured 'nucleolar' body (Fig. 7a, b). Smaller 'nucleolar' bodies throughout the cytoplasm were also associated with clumps of chromatin (Fig. 7b). The cytoplasm was frequently vacuolated. Although there was no clear polarity, the nuclear material and mitochondria were often concentrated towards the centre, leaving large areas of cytoplasm free of organelles.

AMPHIMICTIC SPECIES (see Introduction)

M. acronea.

The spermatogonia were smaller (4.5-5.5 μm) in this species and more numerous in a cross section of the testis. Their nuclei were less amoeboid, and more like the ovoid nuclei of the cyst-nematode spermatogonia.

Late spermatids were sometimes seen joined together (Fig. 8b). Filopodia were abundant in this species and some fibrillar material was retained in the sperm which, at about 4.5 μm × 3.5 μm, were smallest by far of all the species examined. Late sperm (Fig. 8d) tended to be more generally amoeboid rather than obviously polarised, although early sperm showed some larger pseudopodia (Fig. 8c).

Discussion

A characteristic of cyst-nematode spermatogenesis is the termination of cell division at the last moult of the male, so that shortly after the moult the testis of the adult contains only spermatids and spermatozoa. Shepherd, Clark and Kempton (1973) suggested that this might be associated with the apparent inability of the males to feed, a condition shared, as far as is known, by *Meloidogyne* males. However, all stages of sperm development were found in the testes of adult males of all the *Meloidogyne* species examined, so conservation of energy resources may not be the major factor we previously thought.

In *Meloidogyne* the gonad occupies in general the same proportion of the body length as in the cyst nematodes, averaging 400-500 μm from tip to cloaca, about half to two thirds the nematode's length. There are several thousand fully developed sperm in the gonoduct of a young adult *Globodera* male. Most of the relatively long vas deferens acts also as a seminal vesicle, housing the full complement of sperm that results from most of the germ cells maturing before mating occurs. Only the posterior c. 50 μm of the duct acts as a valve. In *Meloidogyne* the whole of the much shorter vas deferens is closed except to allow the passage of sperm (Shepherd & & Clark, 1982). The space occupied by the immature stages leaves room for relatively few fully developed sperm (only several hundred) in the gonoduct.

Although a rachis has been clearly demonstrated in the ovary of *Meloidogyne* (McClure & Bird, 1976 ; Goldstein & Triantaphyllou, 1978a), and one is seen in the testis of *Globodera* pre-adult males, we could not recognise one in the testis of the *Meloidogyne* males.

The structure of the early stages of the germ cells does not differ greatly between the two families. In most *Meloidogyne* species the nucleus of the spermatogonium is very amoeboid in shape (*M. acronea* is an exception) whereas in the cyst nematodes it is oval. Golgi bodies were either rare or else poorly preserved in the cyst-nematode material but were abundant in the spermatogonia and spermatocytes of *Meloidogyne*. In *Meloidogyne* the incipient fibrillar material appeared first in the primary spermatocytes and was well developed in all subsequent stages with the exception of spermatozoa of three of the species. In *Globodera* it first appeared in the spermatid and had disappeared in the testicular spermatozoa, whereas in *Heterodera* some remained briefly after loss of the residual body. Spermatids were essentially similar in the three genera.

There are greater differences between the spermatozoa in the two families, although some similarities exist. From early maturity the sperm of some *Meloidogyne* species *(M. arenaria, M. hapla* and *M. incognita incognita)* are distinctly polarised into a nuclear region and a large pseudopodium ; others *(M. graminicola, M. incognita wartellei* and *M. oryzae)*, like the cyst-nematode sperm, are not. *M. acronea* is intermediate, with some indication of polarity in early sperm but the mature sperm are more generally amoeboid. In unpolarised sperm, in both families, fibrillar material either becomes rather diffuse or disperses entirely as the sperm matures. Polarisation occurs only after insemination of the female in some nematode species, e.g. *Aphelenchoides blastophthorus* (Shepherd & Clark, 1976) and *Caenorhabditis elegans* (Ward & Carrel, 1979).
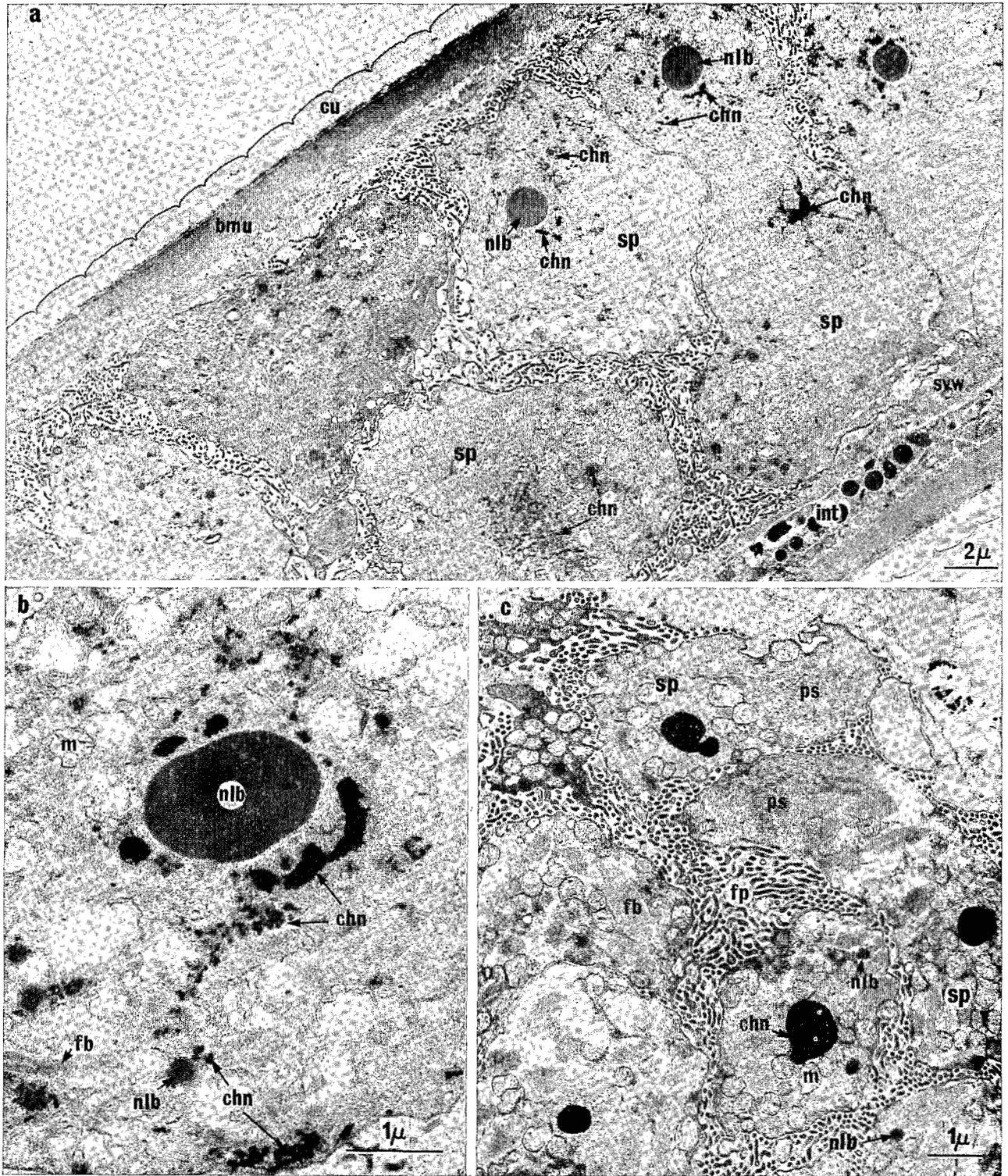
Fig. 7. a-b *M. oryzae* adult male. a : L.S. through seminal vesicle showing very large spermatozoa (sp) lacking fibrillar material ; also large spherical nucleolar bodies (nlb) and dispersed chromatin (chn). × 4 400 ; b : Spermatozoon, showing large and small nucleolar bodies (nlb), and diffuse clumps and small granules of chromatin material (chn) ; also remnants of fibrillar material (fb). × 15 400 ; c : *M. arenaria* adult male. Spermatozoa, showing fibrillar bodies (fb) and pseudopodia (ps). × 8 600.
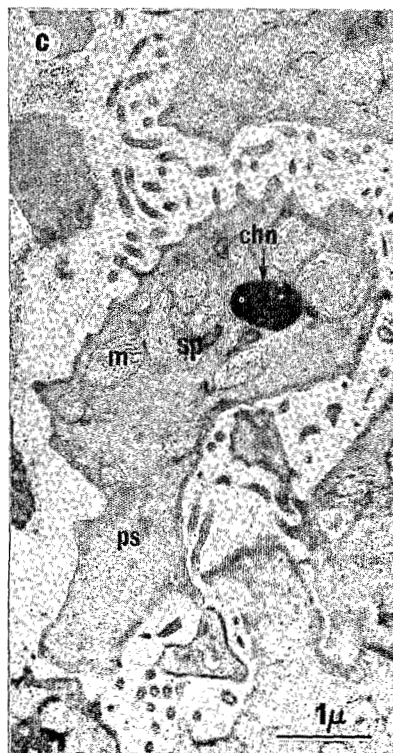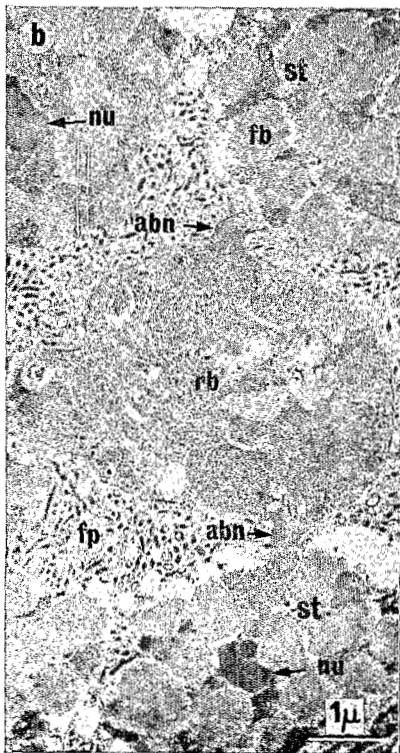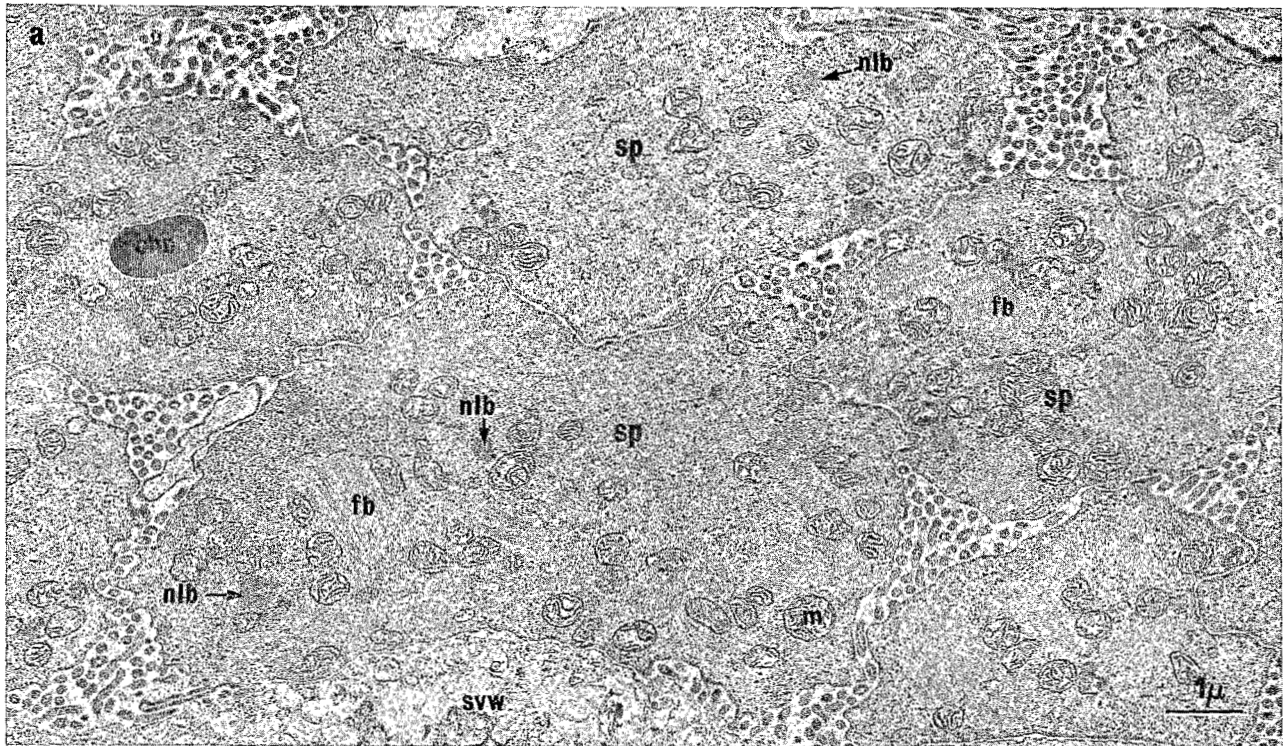
Abbreviations : see under Fig. 1.

Fig. 8. a : *M. incognita wartellei* adult male. L.S. seminal vesicle with non-polarised spermatozoa. × 10 400. b-d *M. acronea* adult male ; b : L.S. testis showing two spermatids (st) with a common residual body (rb). × 8 600 ; c : Early spermatozoa, showing pseudopodia (ps) and some fibrillar material (fb). × 13 000 ; d : Late spermatozoa near vas deferens. × 9 000.

Abbreviations : see under Fig. 1.

Nelson and Ward (1980) were able to induce polarisation in *C. elegans* testicular sperm using the ionophore monensin.

The state of condensation of the chromatin in the testicular sperm is similar in *Meloidogyne* and *Heterodera*, where, in most of the species we examined, it is a solid, homogeneous mass ; in *M. oryzae*, however, it is partially dispersed in granular form. In *Globodera* it passes through a distinctive, beaded-filamentous phase before consolidating after passage of the sperm through the vas deferens.

The spherical bodies, considered by Goldstein and Triantaphyllou (1980) to be nucleolar bodies, are found in *Meloidogyne* species but not in testicular sperm of the cyst nematodes. In *Globodera* they appear first in sperm in the spermatheca of the female ; in that instance they are attached to the coarse threads of chromatin. Goldstein and Triantaphyllou considered them to be RNA on the grounds of the response of the material to Bernhard's (1969) procedure. This seems a plausible assumption although the method used does not differentiate unequivocally between ribonucleoprotein and pure protein (Franke & Falk, 1970). When the material being characterised is dispersed in the cytoplasm, as in these nematode sperm, rather than within a nuclear membrane, there is less certainty of its being RNA when shown not to be DNA. Our designation of structures as 'chromatin' or 'nucleolar material' is based only on electron-density following staining with lead citrate and uranyl acetate, unconfirmed by cytochemical tests.

The 'cytoplasmic spherical bodies' seen in spermatocytes and spermatids of *Meloidogyne* species (e.g. *M. hapla*, csb in Figs 4a ; 5a, b) resemble those described by Kessel (1981) in *Drosophila* spermatocytes, where they were associated with smooth ER and annulate lamellae, and were thought to contain both RNA and protein. Kessel suggested that, like nuclear membrane, the system might assemble polyribosomes. Annulate lamellae, resembling portions of nuclear membrane, were present in *G. rostochiensis* spermatids (Fig. 2c of Shepherd, Clark and Kempton (1973) but not named) but were not seen in *Meloidogyne*.

Sperm of all three genera had cortical microtubules, although they seemed to differ slightly in the two families. Intracytoplasmic tubules were not seen in the cyst-nematode sperm, apart from the more typical microtubules closely associated with the nuclear material.

Within the genus *Meloidogyne*, then, the differences between species concerned mainly : the polarisation of the spermatozoa in some ; the persistence or otherwise of the fibrillar material ; the abundance of filopodia, particularly at the earlier stages ; the

state of the nuclear material ; the size of the mature sperm. The smallest sperm *(M. acronea)* were only about one twentieth the volume of the largest *(M. oryzae)*. Some of these characteristics are summarised in Table 2. The difference between the sperm in the two subspecies of *M. incognita* is noteworthy. Also there was a clear distinction between the condition of the nuclear material in *M. graminicola* and *M. oryzae*, where the less electron-dense 'nucleolar' material was compacted into the larger mass, compared with the other species where the denser chromatin predominated.

With our material of *M. hapla*, we found no evidence in the adult male testis of a brief reconstitution of the nuclear membrane in the early spermatid, which Goldstein and Triantaphyllou (1980) described from the pre-adult testis, nor of the transitory nature of the fibrillar material during spermateleosis. Development proceeded as in other nematode sperm and culminated in polarised spermatozoa, and not in the unpolarised sperm reported by these authors.

Although most of the *Meloidogyne* species studied were parthenogenetic, we found no morphological abnormalities in any of the stages during sperm development, nor any strictly morphological differences distinguishing mitotic from meiotic types. This is in agreement with the findings of Goldstein and Triantaphyllou (1980) for the two races of *M. hapla*. Even though, unlike in cyst-nematode males, the facility for continued sperm development exists in *Meloidogyne* males, individuals in which the spermatozoa were degenerating in the seminal vesicle were often encountered, and so evidently no great advantage accrues in terms of the eventual number of available sperm. In fact, it seems possible that the system evolved in the cyst nematodes is the more efficient, since the males are at least guaranteed a relatively large number of fully developed sperm, whereas in *Meloidogyne* males sperm production might become impaired if energy resources were depleted by activity.

It is clear that although there are some differences in sperm structure and development between these two families in the Heteroderoidea, their basic sperm structure is fundamentally similar. Subject to confirmation, it seems the Aphelenchida may be the only group among the plant-parasitic nematodes in which the sperm are of the type with membranous organelles, as in most animal-parasitic orders and in the rhabditids and enoplids (see Bird, 1971 ; Shepherd Clark & Kempton, 1973 ; Shepherd & Clark, 1976 ; Shepherd, 1981). These organelles do not occur in any of the Tylenchida sperm so far studied, including *Dilylenchus dipsaci* and *Pralylenchus penetrans*, which were cursorily examined

Table 2

Some characteristics of testicular spermatozoa in the genera *Globodera, Heterodera* and *Meloidogyne*

Species and reproductive status	sperm polarised	fibrillar material	no. filopodia	nucleolar bodies	cortical microtubules	cytoplasmic microtubules
Globodera spp. (am)	−	−	**	−	+	−
Heterodera spp. (am)	−	*	**	−	+	−
M acronea (am)	+ → −	*	***	+	+	+
M. arenaria (mi)	+	**	*	+	+	+
M. graminicola (me)	−	−	***	+	+	+
M. hapla (me)	+	**	***	+	+	+
M. incognita incognita (mi)	+	**	**	+	+	+
M. incognita wartellei (mi)	−	*	**	+	+	+
M. oryzae (?)	−	−	***	+	+	+

− absent ; + present ; *5, 55, 555* few-many ; am, amphimixis ; mi, mitotic (obligatory) parthenogenesis ; me, meiotic (facultative) parthenogenesis

by Shepherd and Clark (1975), nor in the sperm of the dorylaims *Longidorus macrosoma* and *Xiphinema index* (Shepherd & Clark, 1980).

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