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TESTIS MORPHOLOGY AND SPERMATOGENESIS IN TWO SPECIES OF *ELENCHUS* (STREPSIPTERA: ELENCHIDAE)

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Abstract—Testes ultrastructure and spermatogenesis were studied in two species of Strepsiptera (Insecta), namely, *Elenchus tenuicornis* and *E. japonicus*, using light and electron microscopy. In both species, the testis is paired and consists of several large irregularly shaped follicles. Each follicle consists of a single clone of germ cells surrounded by a thin epithelium. During the larval and pupal stages, all the germ cells of each testis develop synchronously, and at eclosion, the gonads contain solely mature sperm. One of the most interesting findings is the morphogenesis of a large nuclear vesicle bounded by the fenestrate part of the nuclear envelope. This vesicle contains an electron-dense spherical structure, the chromatoid body. At the end of spermiogenesis, both the nuclear vesicle and its chromatoid body are eliminated with the excess cytoplasm. Large drops of residual cytoplasm containing several nuclear vesicles are present in the lumen of the testis and inside the cytoplasm of phagocytic cells. © 1998 Elsevier Science Ltd. All rights reserved

Index descriptors (in addition to those in the title): Reproduction, insect spermatozoa, nuclear transformation, nuclear vesicle.

INTRODUCTION

Strepsiptera have an unusual parasitic life cycle and their hosts include seven orders of Insecta. The two free-living stages are the first-instar larva and the adult male. The other stages are totally endoparasitic in the host, except in the family Mengenillidae, where both the male and female emerge to pupate externally (Kathirithamby, 1989). At the last-instar larva, the head and prothorax of the endoparasitic male extrude through the cuticle of the fifth-instar nymph, or that of an adult host. The anterior region (cephalotheca) forms the cap of the puparium, which seals and sclerotises with the host cuticle. On emergence of the free-living male, a gaping hole is left in the host. Stylopisation extends the life cycle of the host, and death occurs when the empty puparium becomes fungal-infected (Kathirithamby, 1978). Strepsiptera are parasites of important pests of crops such as the rice plant in South East Asia and the oil palm in New Guinea and the Pacific islands and may act as natural control agents. Despite their potential ecological importance, very little is known about the reproductive biology of these insects.

The ultrastructure of the mature spermatozoa of Strepsiptera has only recently been studied (Baccetti, 1989; Mazzini, *et al.*, 1991; Kathirithamby, *et al.*, 1992, 1993; Carcupino *et al.*, 1993, 1995; Afzelius and Dallai, 1994). There has been one study on the spermatogenesis of Strepsiptera using light microscopy (Hughes-Schrader, 1924), and spermiogenesis has been briefly described by Kathirithamby *et al.* (1993) at the ultrastructural level.

In this paper, the organisation of the testes and the spermatogenetic process in two species of Elenchidae, namely, *Elenchus tenuicornis* (Kirby) and *E. japonicus* (Esaki and Hashimoto), is described.

E. tenuicornis is a Palaearctic species and parasitizes some 42 species of Delphacidae (Homoptera). The host collected for this study was *Javesella dubia* (Kirschbaum), which is mainly brachypterous and *E. tenuicornis* pupates mainly in the 5th-instar nymphal hosts. When the free-living male emerges, the long-living 5th-instar host dies without moulting to the adult stage.

E. japonicus is a parasitoid of the rice planthoppers *Nilaparvata lugens* (Stål) and *Sogatella furcifera* (Horváth) in south- and southeast Asia. It is mainly macropterous and the male pupates in the adult host.

Specimens of *J. dubia* were collected in north of Oxford, U.K. *S. furcifera* and *N. lugens* were collected in Matsue, Shimane Prefecture, Japan, by means of sweep netting in rice fields. Some hosts were reared in the laboratory to obtain various stages of the larvae and pupae.

MATERIALS AND METHODS

The larvae of Strepsiptera of different ages were dissected from the host in 0.1 M cacodylate buffer (pH 7.2), cut to enable penetration of the fixative (2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2 for 2 h), washed overnight in the same buffer, and postfixed for 1 h in 1% osmium tetroxide in 0.1 M cacodylate buffer. The specimens were dehydrated with a graded ethanol series and embedded in Epon-Araldite.

Both 1 μm- and thin sections were cut with LKB Nova and Reichert

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ultramicrotomes and routinely stained with toluidine blue (1 μm sections) and uranyl acetate and lead citrate (thin sections) and observed with a ZEISS Axiophot light microscope and a JEM 1200 JEOL EX II electron microscope, respectively.

RESULTS

The morphology of the testes and the spermatogenetic events were similar in *E. tenuicornis* and *E. japonicus*; the results described below refer to this process in both species and the figures to only *E. tenuicornis*.

The testes are paired and lie on either side of the gut (Fig. 1A–C and Fig. 2B). During the larval and pupal stages, each testis is bounded by a monolayered epithelium, and is divided into many follicles of irregular shape, containing a single clone of germ cells (Fig. 1C–F and Fig. 2D). At the end of the 4th larval instar and beginning of the pupal instar, the testes are small and elliptical in shape. They are located on the anterior side of the abdomen and contain only spermatogonia (Fig. 1A–C). During pupal development, the testes enlarge and occupy, as in the adult, most of the abdominal cavity (Fig. 2A–C). Thin somatic cells delimit the testis and

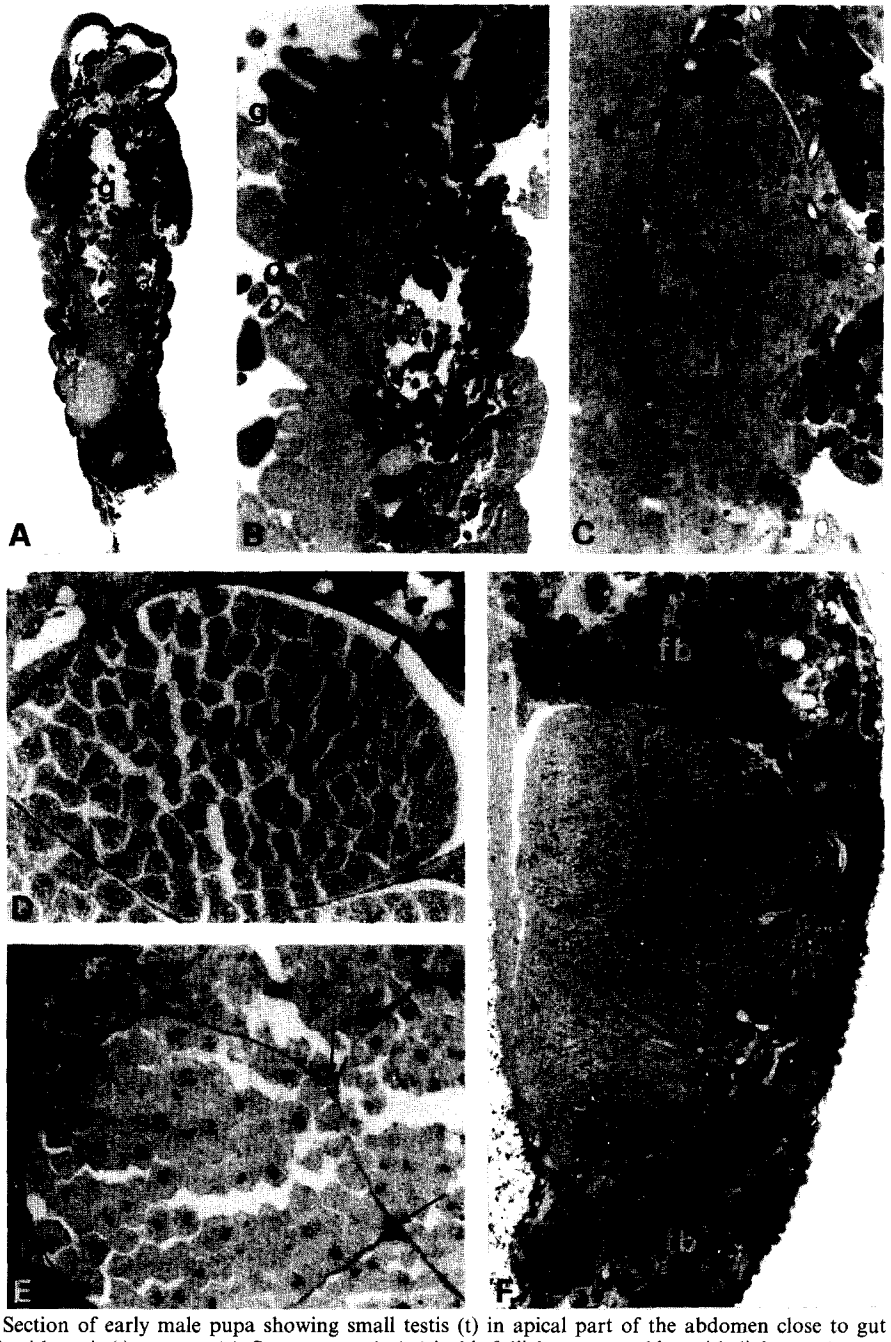

Fig. 1 (A–F). A. Section of early male pupa showing small testis (t) in apical part of the abdomen close to gut (g). B–C. Higher magnification of A with testis (t) near gut (g). Spermatogonia (sg) inside follicles separated by epithelial septa (arrows). D–E. Germinal follicles containing a single clone of prophase (D) and metaphase (E) spermatocytes (sc) surrounded by a monolayer of flat epithelial cells (arrows). F. Longitudinal section of late pupa abdomen. Testis containing mature spermatozoa (sp) is surrounded by fat body (fb). A × 100; B × 240; C–E × 700; F × 350.

Fig. 2 (A–E). A. Lateral view of abdomen of an adult male of *E. tenuicornis* showing internal genitalia: Testis (t), aedeagus (ad). B. Entire section of a late pupa prior to emergence showing large testes (t). Fat body (fb), gut (g). C. Section of adult abdomen with testis filled with mature spermatozoa (sp) surrounded by fat body (fb). D. Late pupa testis with developing spermatids (sd) in follicles separated by epithelial septa (ep); E. High magnification of testis wall with epithelial cells (ec) having flat elongated nuclei. Muscle (ms), external cuticle (cu). A–B × 140; C × 280; D × 900; E × 6000.

divide it into several follicles (Fig. 1D–F). The somatic cells of the follicular septa and testicular wall are both characterized by an elongate nucleus surrounded by a thin cytoplasmic layer (Fig. 2D, E).

The spermatogenetic process occurs synchronously in all germ cells of both testes (Fig. 1C–F), and at the end of the pupal development, just before eclosion, each testis contains only mature sperm (Fig. 2C).

At the beginning of the pupal instar, all germinal follicles contain only spermatocytes I (Fig. 1D and Fig. 3A). Prophase I spermatocytes have a large, ovoid, eccentrically located nucleus with a distinct granular nucleolus and several synaptonemal complexes (Fig. 3A, C, D). The nuclear envelope has many pores clustered in 2 or 3 distinct groups (Fig. 3A). In the cytoplasm around the nucleus, small mitochondria, long cisterns of endoplasmic reticulum and free ribosomes are present (Fig. 3 A). Each spermatocyte is connected to its sister-cells by

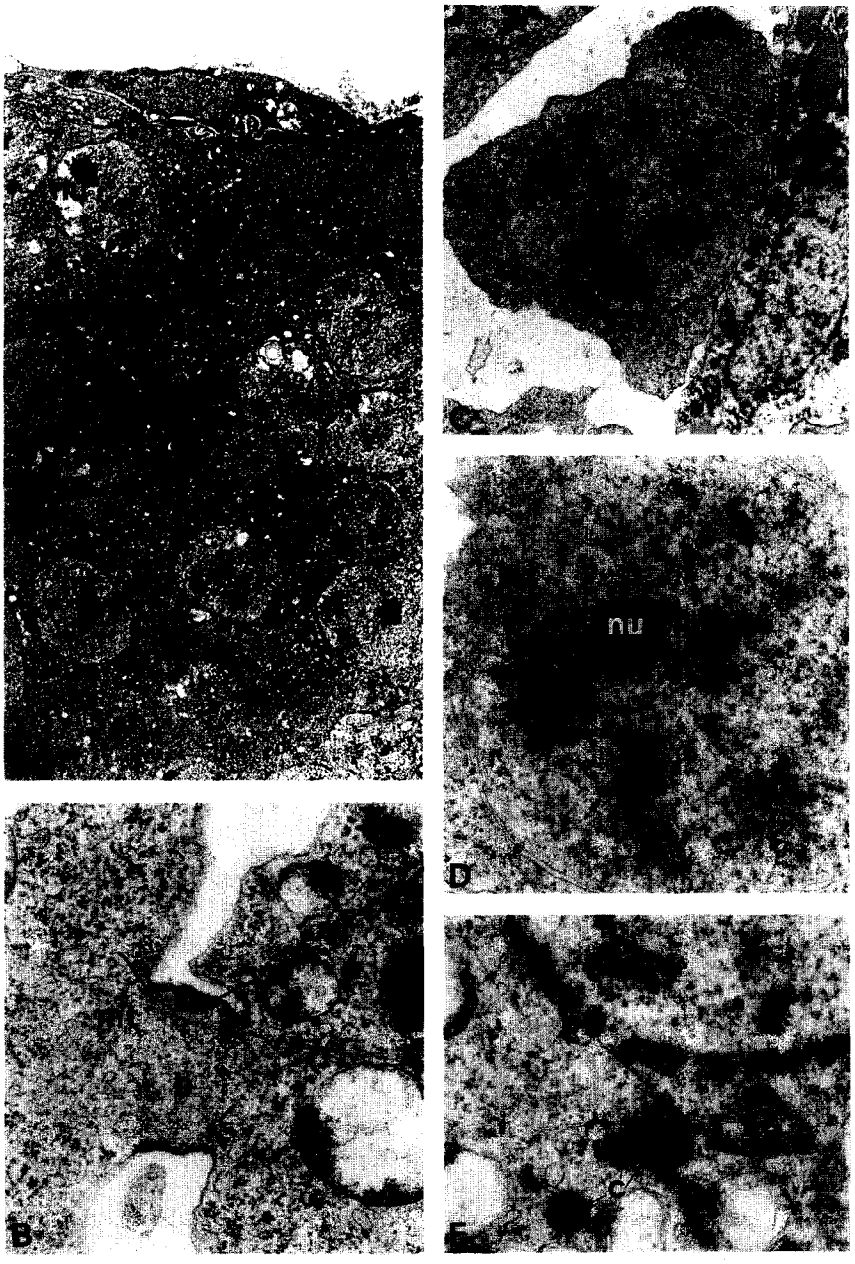

Fig. 3 (A–E). A. Transmission electron micrograph of germinal follicle with spermatocytes characterized by round nuclei (n) with distinct nucleolus (nu). Cytoplasmic bridges (b) between germ cells. B. High magnification of dense material beneath plasma membrane (arrows) of cytoplasmic bridges (b). Microtubule (mt). C–D. Spermatocyte I with nucleus characterized by several synaptonemal complexes (arrows) and nucleolus (nu) of granular appearance. E. Prophase spermatocyte I with diplosomes adjacent to nuclear envelope. Centrioles (c) with classical nine microtubular triplets are orthogonally arranged and embedded in dense pericentriolar material. Nuclear pores (p). A × 3500; B × 31,000; C × 7000, D × 15,500; E × 24,000.

large cytoplasmic "bridges", in which the plasma membrane is clearly visible (Fig. 3A, B). Narrow filaments arise from the plasma membrane of the bridges and contact the microtubules disposed across the bridges (Fig. 3B).

At the beginning of the spermatocytes I division, centrioles replicate as in mitosis. A diplosome is located beneath the fenestrated part of the nuclear envelope (Fig. 3E). Two typical centrioles (with nine microtubular triplets) orthogonally arranged are embedded in the dense pericentriolar material (Fig. 3E). During metaphase I and II, a normal bipolar microtubular spindle with two well-defined polar centrosomes are visible (Fig. 4). In the spermatocytes II the centrosomes have one centriole (Fig. 4C) so that only one centriole is included in each spermatid. Spindle-associated membranes are not visible (Fig. 4).

Before the chromatin begins to condense, the young

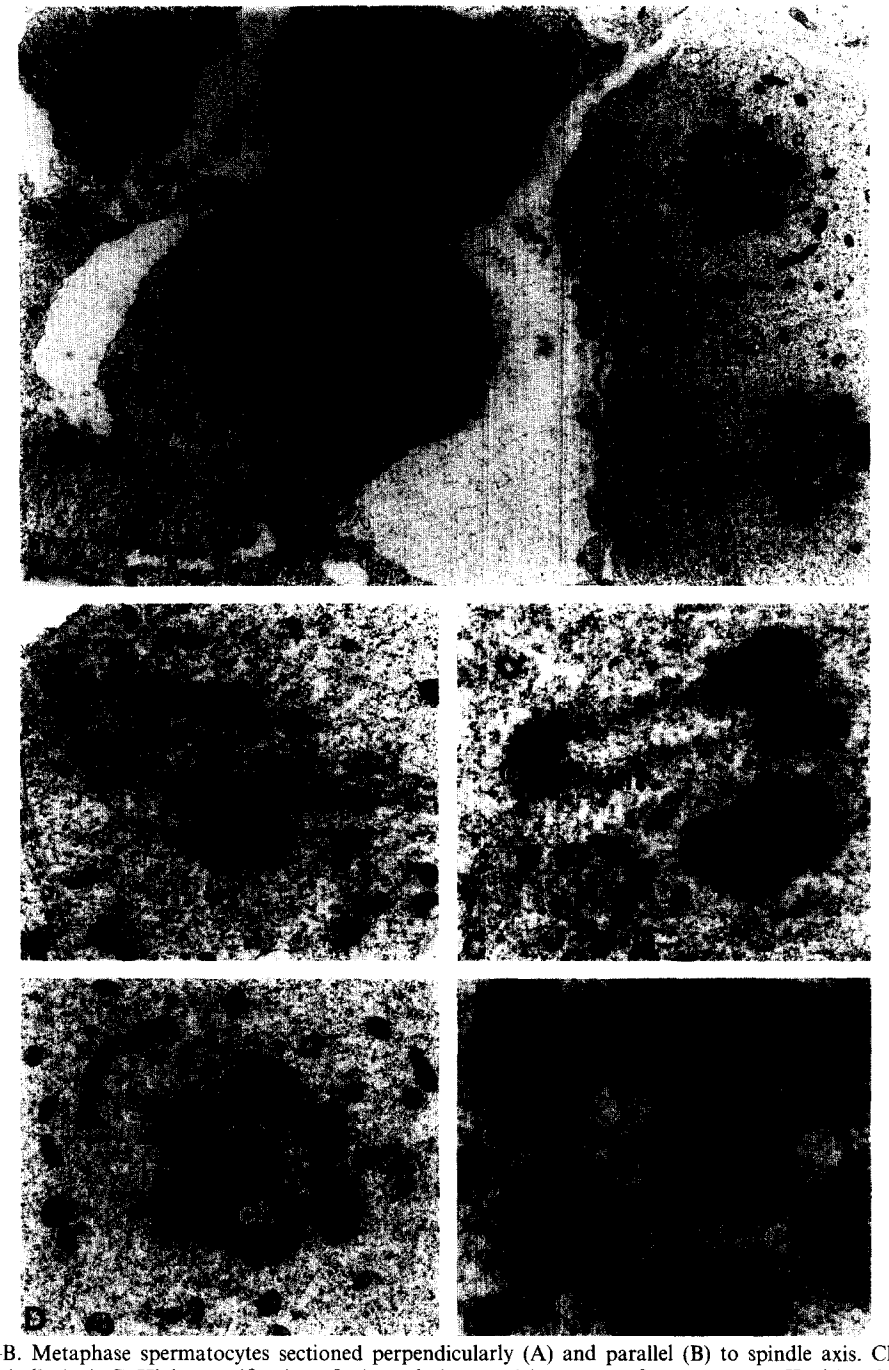

Fig. 4 (A–E). A–B. Metaphase spermatocytes sectioned perpendicularly (A) and parallel (B) to spindle axis. Chromosomes (ch), microtubules of spindle (mt). C. High magnification of microtubule-organizing center of spermatocytes II with only one centriole (c). Microtubules (mt), Chromosomes (ch). D–E. Metaphase chromosomes (ch) scattered among the microtubules (mt). A × 6000; B × 12,000; C × 15,500; D × 12,000, E × 26,000.

spermatids have a small acrosomal vesicle at the anterior part of the nucleus, and the centriole lodged in the posterior nuclear fossa (Fig. 5A). The axoneme and mitochondrial derivatives are also present (Fig. 5A). At this stage, the nuclear pores have an asymmetrical distribution, being grouped on one side of the nucleus, which has a fenestrated and a non-fenestrated side (Fig. 5A, D). On the non-fenestrated side, a narrow layer of heterochromatin is closely associated with the nuclear envelope (Fig. 5A, C, D), whereas on the other side there is a small group of electron-dense granules of nuclear material (Fig. 5A, C, D). At this stage, microtubules and most of the cytoplasm is on the non-fenestrated side of the nucleus (Fig. 5C, D).

As the stage advances, the heterochromatin layer becomes ticker and the small electron-dense granules fuse into a single, compact, spherical chromatoid body (Fig. 5E). The two sides of the nucleus become more evident and the fenestrated side project from the nucleus. The electron-dense body occupies an eccentric position (Fig. 5E). Later, the chromatin continues to condense so that the nuclear volume decreases. The microtubules move

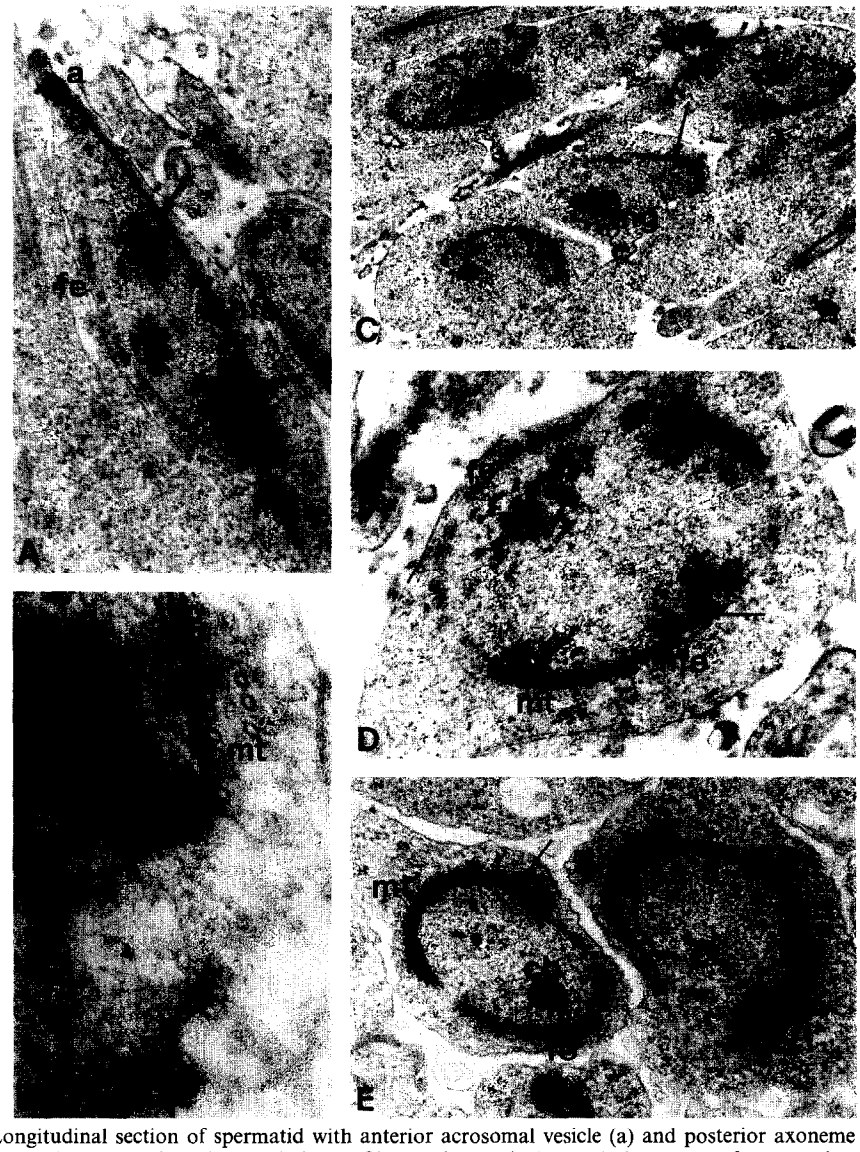

Fig. 5 (A–E). A. Longitudinal section of spermatid with anterior acrosomal vesicle (a) and posterior axoneme enveloped by mitochondrial derivatives (md). Oval nucleus shows thin layer of heterochromatin (arrows) close to non-fenestrated nuclear envelope (nfe), and small electron-dense nuclear granules (ng) near fenestrated nuclear envelope (fe). B. High magnification of microtubules (mt) closely associated with non-fenestrated nuclear membrane. C–D. Cross section of spermatids at same stage as A. Heterochromatin (arrows), fenestrated (fe) and non-fenestrated (nfe) nuclear envelope, nuclear electron-dense granules (ng), microtubules (mt). E. Cross-section of spermatids at later stage of development showing electron-dense nuclear granules fused into spherical chromatoid body (cb), and thick layer of heterochromatin (arrows). Fenestrate nuclear envelope (fe), microtubules (mt). A × 19,000; B × 84,000; C × 10,500; D × 26,000; E × 21,000.

progressively to form a tight sleeve around the nucleus pushing the fenestrated part outwards (Fig. 6A, B). When the condensation of the chromatin is in an advanced stage, the nucleus is completely enveloped by microtubules, and the fenestrated side of the nucleus resembles a large vesicle separated from the other side by a narrow nucleoplasmic bridge (Fig. 6A, B, E). The nucleoplasmic bridge arises from the point where the microtubules converge (Fig. 6A, B). At this stage, the nucleus is elongated and kidney-shaped in cross section (Fig. 6C, D) with the concavity facing the nuclear vesicle (Fig. 6A, B, E, F).

At the same stage, the other components of the mature sperm differentiate (Fig. 7). In the centriolar region, the "9+9+2" axoneme originates from the centriole (Fig. 7A). The centriole and the first portion of the axoneme are progressively surrounded by pericentriolar material (Fig. 7A, B, D). This material progressively occupies the pericentriolar region, in which the first part of axoneme is embedded (Fig. 7A–D). Two membranous systems (internal and external) surround the axoneme, which is also flanked by two mitochondrial derivatives. The internal membranous system separates the axoneme from the two mitochondrial derivatives, while the external one envelopes both the axoneme and the mitochondrial derivatives (Fig. 7C–E). Between the membranous systems, microtubules are scattered in two or three groups around the axoneme, while microtubules closely envelope the two mitochondrial derivatives (Fig. 7B–E). Few mic-

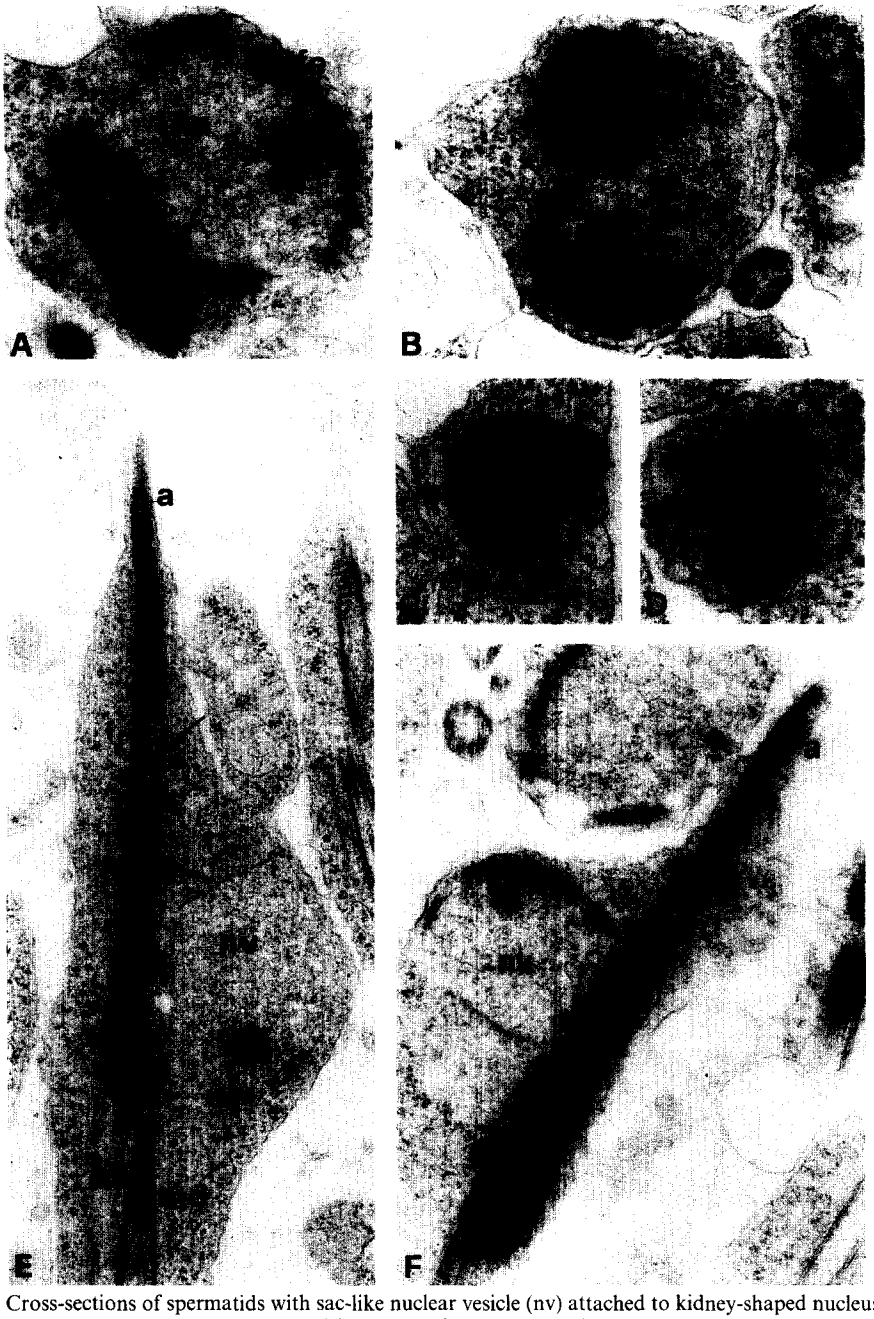

Fig. 6 (A–F). A–B. Cross-sections of spermatids with sac-like nuclear vesicle (nv) attached to kidney-shaped nucleus (n) by a bridge of nucleoplasm (arrows). Microtubules (mt), chromatoid body (cb), fenestrated nuclear envelope (fe). C–D. Cross-sections of kidney-shaped nucleus (n) completely surrounded by microtubules (mt). E–F. Longitudinal section of spermatids with nuclear vesicle (nv) moving posteriorly. Acrosome (a), axoneme (ax), nucleus (n) with internal channel of uncondensed chromatin (arrows), mitochondrial derivatives (md). A × 24,500; B × 29,500; C × 42,000; D × 49,000; E–F × 21,000.

rotubules are also associated with the external membranous system (Fig. 7C, E). Towards the posterior end of the tail, microtubules progressively decrease in number until they completely disappear. Mitochondrial derivatives reduce in size and the axoneme disorganises (Fig. 7F, G), so that the terminal part of the tail has a "9+0" axoneme embedded in the surrounding cytoplasm (Fig. 7G).

At the end of spermiogenesis, the nuclear vesicle moves posteriorly to the sperm body (Fig. 6C, F), and is eliminated with the excess cytoplasm. Large cytoplasmic drops containing unmodified nuclear vesicles mixed with mature spermatozoa appear in the lumen of the testicular follicles (Fig. 8A, B). Degenerating drops of cytoplasm are also visible inside phagocytic cells (Fig. 8B).

DISCUSSION

During its short life span of a few hours, the adult male of Strepsiptera has to seek and inseminate the neotenic female, which is simply a cigar-shaped sac filled with eggs (Kathirithamby *et al.*, 1990).

As in other short-lived insects, spermatogenesis in Strepsiptera occurs during the larval and pupal stages

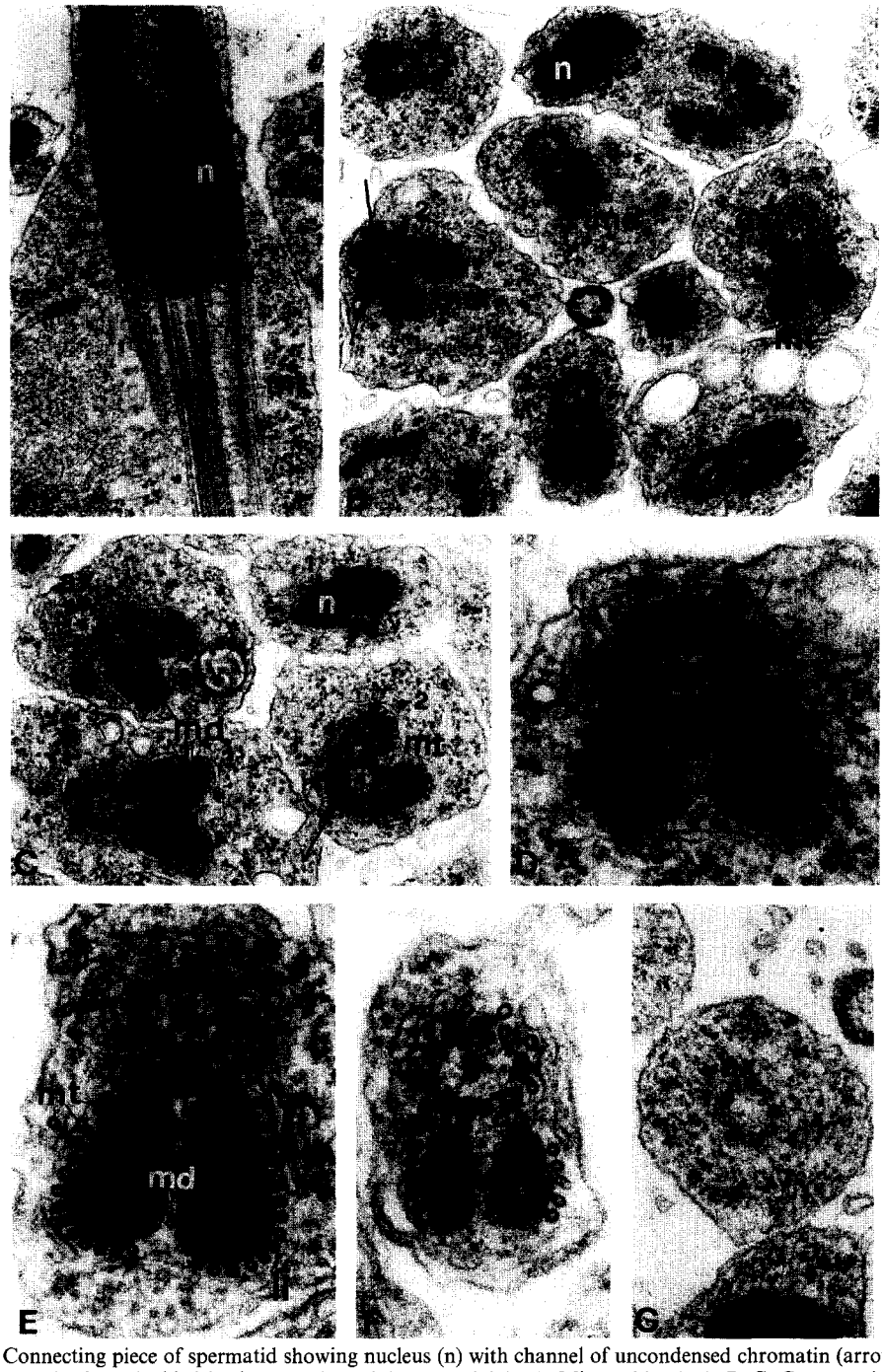

Fig. 7 (A–G). A. Connecting piece of spermatid showing nucleus (n) with channel of uncondensed chromatin (arrows) and beginning of axoneme (ax) completely embedded in dense pericentriolar material (pm). Microtubles (mt). B–C. Cross-sections of spermatids. Head (1) with condensed nucleus (n), connecting piece (2) with beginning of axoneme embedded in developing pericentriolar material (arrows), principal piece of tail (3) with "9+9+2" axoneme and the two mitochondrial derivatives (md). Microtubules (mt). D. High magnification of beginning of axoneme (ax) surrounded by developing pericentriolar material (arrows). E. Principal piece of tail with "9+9+2" axoneme (ax) flanked by two mitochondrial derivatives (md). One of two membranous systems (I) surround the axoneme and the other one (II) surrounds both axoneme and mitochondrial derivatives. Microtubules (mt). F–G. Caudal portion of tail showing reduced mitochondrial derivatives (md) and disorganized axoneme (ax). A × 31,000; B × 23,000; C × 26,000; D × 57,000; E–G × 60,000.

(Phillips, 1970; Jamieson, 1987). Mitotic divisions of the spermatogonia occur in larval instars, whereas meiotic divisions and sperm differentiation take place during the pupal stage. During this period, no seriation of development is observed, and all the germ cells mature just before the emergence of the adult male from the puparium.

As in many other animal groups (from Cnidaria to mammals), the germ cells are divided into several clones. In Strepsiptera, each germinal clone occupies an entire follicle in which germinal cysts are not recognizable. The germ cells of each clone are connected by cytoplasmic "bridges" in which the plasma membrane is strengthened by dense material. Insect ovarioles stained with Rh-phal-

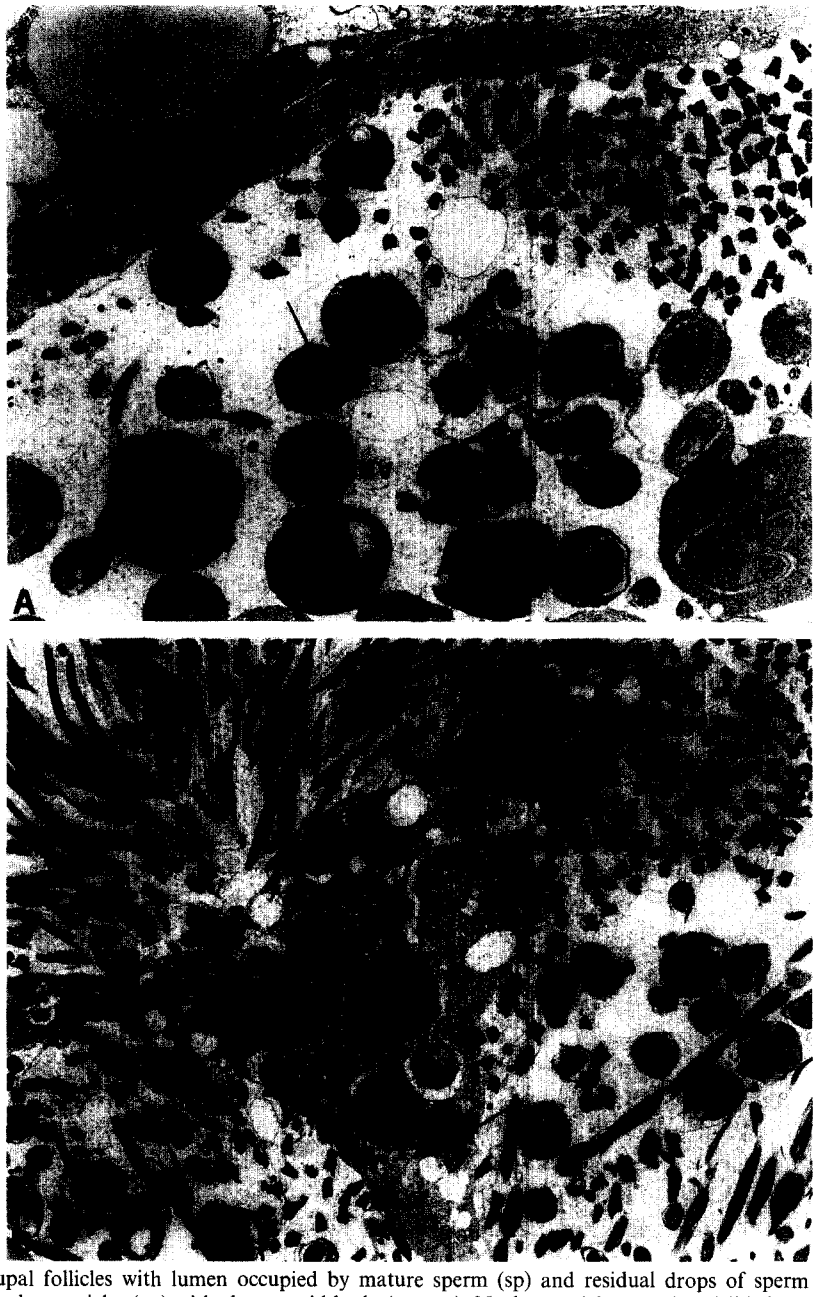

Fig. 8 (A–B) Late pupal follicles with lumen occupied by mature sperm (sp) and residual drops of sperm cytoplasm containing unmodified sac-like nuclear vesicles (nv) with chromatoid body (arrows). Nuclear vesicles are also visible inside phagocytic cells (pc). A × 13,500; B × 10,500.

loidin have shown that F-actin microfilaments, which have probably lost their contractile properties, are located beneath the membrane of the cytoplasmic bridges the so-called "ring canals" of the female germ cells (Warn *et al.*, 1985; Carcupino *et al.*, 1992). By virtue of the cytoplasmic canals, each germinal clone is a functional syncytium, which permits synchrony in cell differentiation, so that different clones have different degrees of development (King and Büning, 1985). On the contrary, in Strepsiptera each testis is a unique physiological compartment, in which the germ cells develop synchronously.

Spermatogenesis in the two species of Strepsiptera examined is similar to that described for other insect orders (Phillips, 1970; Baccetti, 1972; Yasuzumi, 1974; Jamieson 1987; Henning and Kremer, 1990). Division of the spermatocytes occurs with the disorganization of the nuclear membrane, which is replaced by a normal bipolar microtubular spindle. The spindle poles are marked by the presence of centrosomes consisting of centrioles embedded in dense pericentriolar material. As shown in Fig. 3E, centrioles in first spermatocytes replicate as in mitosis (due to an incomplete section, only three centrioles are visible in the diplosome shown in the figure) so that two centrioles must be present at each pole of first meiotic spindle. Only one centriole is present in the centrosomes of Strepsiptera spermatocytes II. According to Friedlander and Wahrman (1971), in second sper-

matocytes of Strepsiptera as in all other insects, no centriole replications takes place and only one centriole is present in each spermatids.

Spindle-associated membranes, common to many invertebrate germ cells, including Strepsiptera oocytes (Wolf, 1995), are not recognizable. Substantial differences in spindle organization exist between female and male germ cells in Strepsiptera. Strepsipteran oocytes have spindles containing an abundance of membranes and diffuse poles that lack distinct polar microtubule-organizing centers (Hughes-Schrader, 1924; Rieder and Nowogrodzki, 1983). From the first optical observations by Hughes-Schrader (1924), spindle formation in the strepsipteran oocyte has been cited as evidence to support the hypothesis that kinetochores play a pivotal role in the organization of a functional bipolar spindle in the absence of a MTs organizing center (for a review see Rieder and Nowogrodzki, 1983). Rieder and Nowogrodzki (1983) studied spindle formation in the oocyte of *Xenos peckii* at ultrastructural level. Their data suggested that membranes associated with the spindle determine the orientation of the spindle microtubules and also partially regulate their formation. The reason for this marked difference in spindle organization in Strepsiptera germ cells is not clear.

During spermiogenesis, the nucleus elongates and becomes surrounded by a microtubular sleeve. The "9+9+2" axoneme develops from the distal centriole (basal body), while the proximal one disappears. The mitochondria transform into two long mitochondrial derivatives flanking the axoneme. At the end of the spermiogenetic process, the excess cytoplasm is extruded, and, according to Baccetti (1975), the plasma membrane of the spermatid is replaced by a membrane derived from Golgi vesicles surrounding the axoneme.

As indicated above, the spermatids undergo several modifications, the most interesting of which is the transformation of the nucleus. Nuclear elongation coincides with the development of MTs around the nucleus. They first appear on the non-fenestrated side of the nucleus, later embracing the entire nuclear surface. The microtubular sleeve is involved in guiding the elongation of the nucleus, as it is claimed to do in other insects (Tokuyasu, 1974). In Strepsiptera, however, the microtubular movement could also be responsible for the formation of the nuclear vesicle, which is discarded from the nucleus. This vesicle is then ejected into the testicular lumen and phagocyted by somatic cells of the follicles.

The elimination of the nuclear vesicle may be a way of eliminating the nuclear pores. In many other insect groups (Baccetti, 1972; Tokuyasu, 1974), the nucleus of the mature sperm is devoid of nuclear pores, as in Strepsiptera. On the other hand, a single large vesicle of nuclear membrane, which is discharged in a drop of cytoplasm in the lumen of the testis, has not been observed in previous studies. A spherical electron-dense body is also eliminated from the fenestrated part of the nucleus, so that the nuclear vesicle might also be involved in the disposal of nuclear material, which is no longer useful. A similar vesicle, which contains electron-dense material, has also been reported in the spermatids of Coleoptera such as *Cicindela campestris* and *Carabus catenulatus* and Hemiptera such as *Nepa rubra* (Werner, 1965, 1966). In these species, the vesicle remains attached to the nucleus during the process of sperm differentiation, and the electron-dense material has been interpreted as material dispersed through the nuclear pores to form the centriolar adjunct, after which, the empty vesicle disappears (Werner, 1965, 1966). In Strepsiptera, elimination of the nuclear vesicle with its electron-dense body may be related to secondary loss of the centriolar adjunct.

The electron-dense body eliminated from the nucleus in *E. tenuicornis* and *E. japonicus* could be related to a different process, such as the secondary loss of the centriolar adjunct, as mentioned above, or elimination of some other nuclear material, such as the basic protein involved in the condensation of chromatin. In fact, the nucleus of the mature sperm of the five strepsipteran specie studied so far (Mazzini *et al.*, 1991; Kathirithamby, *et al.*, 1992, 1993; Carcupino *et al.*, 1993, 1995) is characterized by a portion of uncondensed chromatin.

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REFERENCES

Afzelius, B. A. and Dallai, R. (1994) Characteristics of the flagellar axoneme in Neuroptera, Coleoptera and Strepsiptera. *J. Morphol.* **219,** 15–20.

Baccetti, B. (1972) Insect sperm cells. *Adv. Insect Physiol.* **9,** 315–397.

Baccetti, B. (1975) The role of Golgi complex during spermiogenesis. *Curr. Top. Dev. Biol.* **10,** 103–122.

Baccetti, B. (1989) The spermatozoon of Strepsiptera (Insecta) and its value in the systematic position of the group. *J. Submicrosc. Cytol. Pathol.* **21,** 397–398.

Carcupino, M., Yin, C.-M., Stoffolano, J. G. Jr., Scapigliati, G. and Mazzini, M. (1992) F-actin distribution in the ovaries of pre-vitellogenic and vitellogenic black blowflies, *Phormia regina* (Meigen) (Diptera: Calliphoridae). *Int. J. Insect. Morphol. Embryol.* **21,** 77–86.

Carcupino, M., Mazzini, M., Olmi, M. and Kathirithamby, J. (1993) The spermatozoon of *Halictophagus chilensis* Hofmann (Strepsiptera, Halictophagidae). *Boll. Zool.* **60,** 361–365.

Carcupino, M. Profili, G., Kathirithamby, J. and Mazzini, M. (1995) Sperm ultrastructure of *Xenos vesparum* (Rossi) and its significance in the taxonomy and phylogeny of Strepsiptera (Insecta). In *Advances in Spermatozoal Phylogeny and Taxonomy*, ed. B. G. M. Jamieson, J. Ausio and J.-L. Justine *Mém. Mus. nat. Hist. nat.* **166,** 291–296, Universal Book Services, Leiden.

Friedlander, M. and Wahrman, J. (1971) The number of centrioles in insect sperm: a study in two kinds of differentiating silkworm spermatids. *J. Morphol.* **134,** 383–398.

Henning, W. and Kremer, H. (1990) Spermatogenesis of *Drosophila hydei*. *Int. Rev. Cytol.* **123,** 129–175.

Hughes-Schrader, S. (1924) Reproduction in *Acroschismus wheeleri* Pierce. *J. Morphol. Physiol.* **39,** 157–204.

Jamieson, B. G. M. (1987) The *Ultrastructure and Phylogeny of Insect Spermatozoa*. Cambridge University Press, Cambridge.

Kathirithamby, J. (1978) The effects of stylopisation on the sexual

development of *Javesella dubia* (Kirschbaum) (Homoptera: Delphacidae). *Biol. J. Linn. Soc.* **10,** 163–179.

Kathirithamby, J. (1989) A review of the order Strepsiptera. *Syst. Entomol.* **14,** 41–92.

Kathirithamby, J., Carcupino, M. and Mazzini, M. (1990) Ovarian structure in the order Strepsiptera. *Frustula Entomol.* **13,** 1–8.

Kathirithamby, J., Carcupino, M. and Mazzini, M. (1992) Ultrastructure of the spermatozoon of *Elenchus japonicus* and its bearing on the phylogeny of Strepsiptera. *Tissue Cell* **24**(3), 437–442.

Kathirithamby, J., Carcupino, M. and Mazzini, M. (1993) Comparative spermatology of four species of Strepsiptera and comparison with a species of primitive Coleoptera. *Int. J. Insect Morphol. Embryol.* **22,** 459–470.

King, R. C. and Büning, J. (1985) The origin and function of insect oocyte and nurse cells. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, ed. G. A. Kerkut and L. I. Gilbert, pp. 83–111. Pergamon Press, Oxford.

Mazzini, M., Carcupino, M. and Kathirithamby, J. (1991) Fine structure of the spermatozoon of the strepsipteran *Xenos moutoni*. *Tissue Cell* **23**(2), 199–207.

Phillips, D. M. (1970) Insect sperm: their structure and morphogenesis. *J. Cell. Biol.* **44,** 243–277.

Rieder, C. L. and Nowogrodzki, R. (1983) Intranuclear membranes and the formation of the first meiotic spindle in *Xenos peckii* (*Acroschismus wheeleri*) oocytes. *J. Cell Biol.* **97,** 1144–1155.

Tokuyasu, K. T. (1974) Dynamics of spermiogenesis in *Drosophila melanogaster*. IV. Nuclear transformation. *J. Ultrastruct. Res.* **48,** 284–303.

Warn, R. M., Gutzeit, H. O., Smith, L. and Warn, A. (1985) F-actin rings are associated with the ring canals of the *Drosophila* egg chamber. *Exp. Cell Res.* **157,** 355–363.

Werner, G. (1965) Untersuchungen über die Spermiogenese beim Sandläufer, *Cicindela campestris* L. *Z. Zellforsh.* **66,** 255–275.

Werner, G. (1966) Untersuchungen über die Spermiogenese bei einem Laufkäfer *Carabus catenulatus* Scop., und der Skorpion-wasserwanze, *Nepa rubra* L. *Z. Zellforsch.* **73,** 576–599.

Wolf, K. W. (1995) Spindle membranes and spindle architecture in invertebrates. *Micron* **26,** 69–98.

Yasuzumi, G. (1974) Electron microscope studies on spermiogenesis in various animal species. *Int. Rev. Cytol.* **37,** 53–119.