Spermiogenesis in three species of cicadas (Hemiptera: Cicadidae)

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Abstract

Spermiogenesis in three species of cicadas representing one cicadettine (*Monomatapa matoposa* Boulard) and two cicadines (*Diceroprocta biconica* [Walker] and *Kongota punctigera* [Walker]) was investigated by light and electron microscopy. Although spermiogenesis was occurring in the testis of adult males of all species, earlier spermiogenic stages were observed in *D. biconica* only. While spermiogenesis was similar to that described for other insects, some differences were noted. For example granular material did not assemble around the centriole to form a centriolar adjunct but did accumulate in the cytoplasm of early spermatids adjacent to a region of the nuclear membrane where nuclear pores were aggregated. In late spermatids this material accumulated anterior to the mitochondrial derivatives in a developing postero-lateral nuclear groove. While this material has been named the 'centriolar adjunct' by previous authors, its formation away from the centriole raises questions about its true identity. Second, during acrosome maturation an ante-acrosomal region of cytoplasm develops. Although present in later spermatids, this region is lost in spermatozoa. Interspecific variations in chromatin condensation patterns and the number of microtubule layers encircling the spermatid nucleus during spermiogenesis were noted.

Introduction

In the majority of insects that are short-lived as adults, e.g. mayflies and caddis flies, the adult testis contains only mature spermatozoa because spermatogenesis has ceased (Phillips 1971). Myers (1929) suggested that this would also be expected in cicadas because they have a short adult life. While spermatogenesis has been well studied in insects (e.g. Phillips 1970, 1974; Friedländer and Meisel 1977; Fuller 1993; Friedländer 1997; Hess 1999; Jamieson et al. 1999), there is little information on cicadas. Contrary to the prediction by Myers (1929), Folliot and Maillet (1970) and Kubo-Irie et al. (2003) found that spermiogenesis was still underway in the testis of the last larval instars and some adults of the cicadine cicadas Cicada orni L., Lyristes plebejus (Scopoli) and Graptosaltria nigrofuscata (de Motschulsky). This indicates that in cicadas spermatogenesis commences in one of the earlier nymphal stages. Unfortunately, studies of early spermatogenesis are a challenge because cicada nymphs are extremely difficult to locate in their subterranean habitats.

Cicadas (Hemiptera: Cicadomorpha) are a diverse group of insects with over 2000 species catalogued worldwide (Metcalf 1963a,b,c; Duffels and van der Laan 1985; Villet 1999a,b). Currently they are divided into three subfamilies, Cicadinae, Cicadettinae and Tibicininae (Moulds 2005). Some variations in the sperm morphology of cicadine and cicadettine cicadas have since been noted (Chawanji et al. 2005, 2006) and therefore there might be variations during spermatogenesis in these cicada subfamilies. A notable feature of cicada spermatozoa is the granular material located in a postero-lateral nuclear groove, anterior to the mitochondrial derivatives (Folliot and Maillet 1970; Kubo-Irie et al. 2003; Chawanji et al. 2005, 2006). Even though this material has no obvious link to the centriole, Folliot and Maillet (1970) identified this structure as the centriolar adjunct. The position of this so-called centriolar adjunct suggests that it is misnamed. The aims of this study were therefore to describe and compare some stages of spermiogenesis in the subfamilies Cicadinae and Cicadettinae, and thus provide further clarification on the formation of the so-called centriolar adjunct.

Materials and methods

Male cicadas of three species, representing three tribes, were collected in the Eastern Cape, South Africa, south-eastern Zimbabwe and Florida, United States of America. *Kongota punctigera* (Walker 1850) (Cicadinae: Platypleurini) was collected near East London, South Africa (33°1′ S, 27°55′ E) in December 2002; *Monomatapa matoposa* Boulard 1980 (Cicadettinae: Taphurini) was collected in Malilangwe Private Game Reserve, Zimbabwe (20°58′ S, 31°47′ E) in December 2003; and *Diceroprocta biconica* (Walker 1850) (Cicadinae: Cryptotympanini) was collected in Key Largo (25°6′ N, 80°23′ W, and 25°5′ N, 80°27′ W), Upper Matecumbe Key (24°55′ N, 80°38′ W), Lower Matecumbe Key (24°51′ N, 80°44′ W) and Conch Key (24°47′ N, 80°53′ W), Monroe County, Florida, United States in August 2005.

Testes and seminal vesicles were dissected in 2% saline solution before fixation overnight at 4 °C in 2.5% glutaraldehyde in 0.1 m phosphate buffer (pH 7.2). After washing in 0.1 m phosphate buffer, tissues were post-fixed in 1% osmium tetroxide in 0.1 m phosphate buffer for 90 min at room temperature, dehydrated in ethanol and embedded in Epon 812 resin. Some semi-thin sections were stained with toluidine blue and examined and photographed with an Olympus BX-60 microscope. Ultrathin sections (silver-gold) were cut using a diamond knife and collected on 300-mesh copper grids before staining with uranyl acetate and lead citrate. Sections were examined and photographed with a JEOL 1210 transmission electron microscope at 120 kV.

Results

As spermiogenesis was similar in all three species, only a single description is given, with any species differences noted.

Light microscopy

Each testis comprises a series of follicles that are connected to the vas deferens. Numerous spermatids aligned parallel to one another are visible within the individual follicles (Fig. 1A–C). The interstitial tissue is dominated by large irregularly shaped cyst cells that often contain large spherical granules that stain densely in toluidine blue (Fig. 1B,C). No early spermatogenic stages (spermatogonia or spermatocytes) were observed in any of the species. Spermiogenesis is synchronous within a follicle but not between follicles (Fig. 1A).

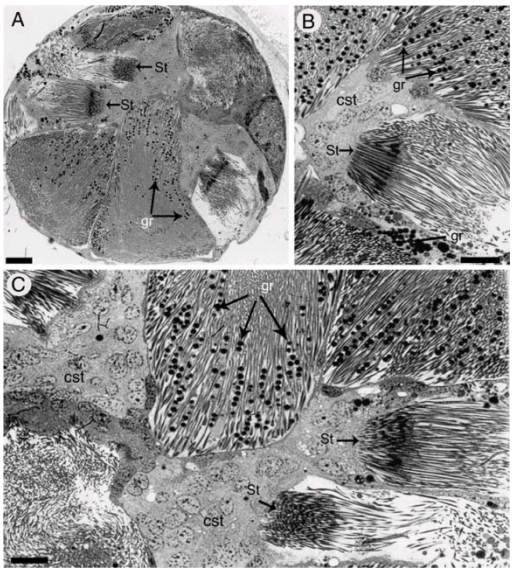

Figure 1—. A. A light micrograph of some follicles in the testis of *Kongota punctigera*. Numerous spermatids (St) and granules (gr) can be seen in each follicle. Scale bar = 10 µm. —B. A higher magnification light micrograph of some follicles in the testis of *K. punctigera*. Spermatids (St) are already aligned in a parallel

fashion within each follicle and are associated with the large spherical granular bodies (gr). Several large cyst cells (cst) can be seen within the interstitial tissue. Scale bar = 10 µm. —C. A light micrograph of some follicles in the testis of *K. punctigera*. Large cyst cells (cst) line the interstitial spaces. Note the elongated profiles of the spermatids (St) and the large spherical granules (gr). Scale bar = 10 µm.

Electron microscopy

Early stages of spermiogenesis were present in the testis of D. biconica, but not in K. punctigera or M. matoposa. Nevertheless, spermiogenesis was occurring in the adults of all species. Spermatids have a close association with cyst cells whose cytoplasmic projections enclose the anterior regions of spermatids (Figs 2F and 3E). In addition, the cytoplasm of the cyst cells contains large spherical (0.8–3 µm diameter) and homogeneously electron-dense granules (Fig. 3E).

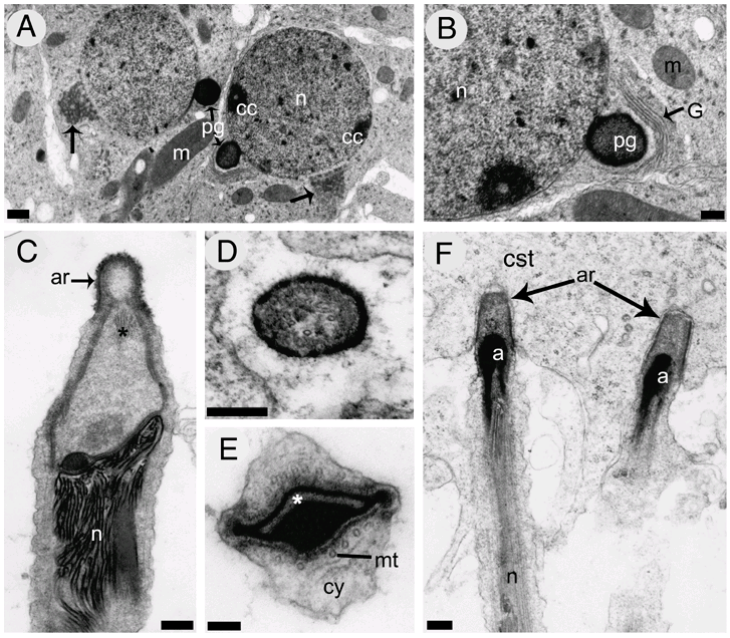

Figure 2—. A. *Diceroprocta biconica*. Sections of early spermatids in which some coarse granular material (arrows) is accumulating in a region adjacent to the nucleus (n) of each spermatid. cc, early chromatin condensation; pg, proacrosomal granule; m, mitochondrion. Scale bar = 500 nm. —B. *D. biconica*. The proacrosomal granule (pg) in an early spermatid lying adjacent between the nucleus (n) and the concave side of the Golgi complex (G). m, mitochondrion. Scale bar = 200 nm. —C. *Kongota punctigera*. Longitudinal section of the anterior region of a developing sperm head. At this stage the largely undifferentiated acrosome has an ante-acrosomal region (ar); in addition microtubule-like elements (asterisk) are beginning to form. n, nucleus. Scale bar = 200 nm. —D. *K. punctigera*. Transverse section through the ante-acrosomal region of a spermatid. Note the microtubules within the interior. Scale bar = 200 nm. —E. *Monomatapa matoposa*. Transverse section through the developing acrosome. Note the subacrosomal space (asterisk) and the microtubules (mt). cy, cytoplasm. Scale bar = 100 nm. —F. *M. matoposa*. Longitudinal sections of the heads of mid-spermatids. At this stage the ante-acrosomal region (ar) has extended along the acrosome. a, acrosome; cst, cyst cell; n, nucleus. Scale bar = 200 nm.

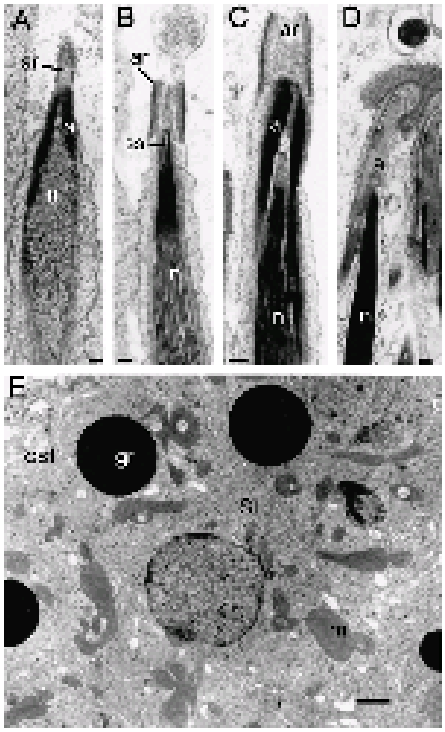

Figure 3—. A. *Kongota punctigera*. Longitudinal section of a mid-spermatid head. The developing acrosome (a) extends as two processes on either side of the condensing nucleus (n). ar, ante-acrosomal region. Scale bar = 200 nm. —B. *K. punctigera*. Longitudinal section through a later stage spermatid head. The developing acrosome has developed a subacrosomal space (ss). The plasma membrane of the ante-acrosomal region (ar) has become elongated. n, nucleus. Scale bar = 200 nm. —C. *K. punctigera*. Longitudinal section of a late spermatid head. The acrosome (a) now has a well-developed subacrosomal space; chromatin condensation in the nucleus (n) has produced thick lamellae. ar, ante-acrosomal region. Scale bar = 200 nm. —D. *K. punctigera*. Longitudinal section of the anterior head of a very late spermatid. The nucleus (n) and acrosome (a) are surrounded by cytoplasmic extensions of cyst cells. Scale bar = 1 µm. —E. *Diceroprocta biconica.* cst, cyst cell; early spermatids (St). gr, granule. M, mitochondrion. Scale bar = 1 µm.

Development of the acrosome

Early spermatids possess a spherical proacrosomal granule (about 500 nm diameter) that is located between the concave face of the Golgi complex and the spermatid nucleus (Fig. 2A,B). In mid- to late spermatids the developing acrosome is positioned at the presumptive anterior of the elongating nucleus and microtubular-like elements start appearing in the developing acrosome (Fig. 2C). Both the anterior nucleus and acrosome are embedded in cyst cells (Fig. 2F). As the spermatid matures the acrosome elongates, invaginates and develops a posterior lateral subacrosomal space (Figs 2C,E,F and 3A–D). In mid-spermatids there is a small region of cytoplasm anterior to the acrosome (Figs 2C,F and 3A–D), hereafter referred to as the ante-acrosomal region. This region is circular in cross-section (Fig. 2D) and has a thickened plasma membrane that is in close contact with the membrane of the cyst cell (Figs 2D,F and 3C,D). It is absent in mature spermatozoa (see Chawanji et al. 2005, 2006). The developing acrosome is surrounded by some microtubules that continue into the ante-acrosomal region (Fig. 2D,E). Elongation of the acrosome anteriorly and posteriorly as two lateral processes either side of the nucleus, is accompanied by differentiation of the acrosomal contents (Fig. 4F,G).

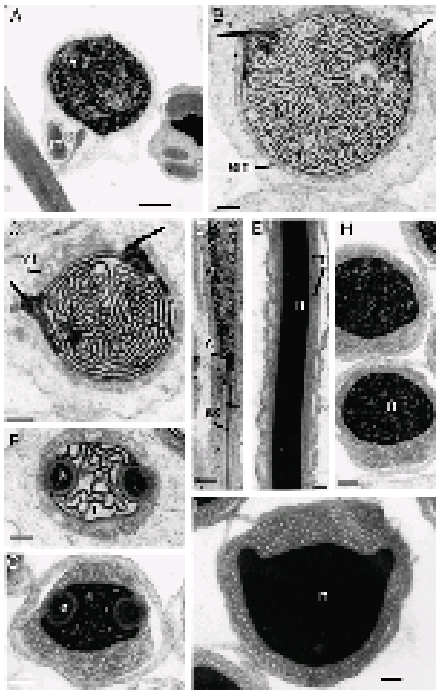

Figure 4—. A. *Monomatapa matoposa.* Early spermatid in which chromatin condensation in the nucleus (n) has begun and some small mitochondria (m) are aggregating around the developing axoneme. Scale bar = 1 µm. —B. *M. matoposa*. Transverse section of a spermatid nucleus in which the chromatin is in the form of lamellae and condensation is more concentrated at two lateral regions of the nucleus (arrows). The nucleus is surrounded by a manchette of microtubules that are in turn surrounded by an endomembrane (em). Scale bar = 200 nm. —C. *Kongota punctigera*. Transverse section of a spermatid nucleus in which the condensing chromatin appears as thicker dense lamellae. However, in the region where condensation commences (arrows), the chromatin has formed thick granules. Note also the developing nuclear invagination, which contains four discontinuous rows of microtubules (mt). Scale bar = 200 nm.—D. *Diceroprocta biconica*. Longitudinal section of the spermatid nucleus (n) and mid-piece. ax, axoneme; c, centriole. Scale bar = 1 µm. —E. *K. punctigera*. Longitudinal section of the elongated nucleus (n) of a late spermatid in which the chromatin has completely condensed. Note the microtubules (mt) aligned parallel to the long axis of the nucleus. Scale bar = 200 nm. —F. *K. punctigera.* Transverse section through the anterior nuclear region (n) of a mid-spermatid showing the two acrosomal processes (a). Note the ring of microtubules surrounding the spermatid. Scale bar = 100 nm. —G. *D. biconica.* Late spermatid cross-sectioned at the same level as in (F). Condensation of the nucleus (n) is almost complete. Note the abundant microtubules. a, acrosome. Scale bar = 100 nm. —H. *D. biconica.* Transverse sections of late spermatids showing two condensing nuclei (n). Note the manchette surrounding each spermatid. Scale bar = 200 nm. —I. *D. biconica.* A late spermatid showing the nucleus (n), which is no longer speckled, and chromatin condensation has been completed. The manchette is still present. Scale bar = 100 nm.

Development of the nucleus

The early spermatid nucleus (Figs 2A,B, 3E and 4A), is ovoid (about 3–3.5 µm in diameter) and contains heterogeneous chromatin with a granular appearance. In D. biconica the nucleus also contains small, dense aggregations of heterochromatin close to the nuclear envelope (Fig. 2A,B). These aggregations are the first indications of chromatin condensation. During spermiogenesis the spermatid nucleus changes shape, elongates, and develops a posterior lateral invagination (Fig. 4C).

As the nucleus changes shape, chromatin condensation begins at two lateral regions of the spermatid nucleus (Figs 2A and 4B,C). Within an individual spermatid the degree of chromatin condensation is not uniform within the nucleus (Fig. 4C,D). The elongation of the spermatid nucleus is accompanied by the chromatin becoming fibrous (Fig. 3A) and then lamellate in appearance (Fig. 4B). As the chromatin condenses, the lamellae increase

in electron density (Figs 3C and 4C) until it becomes compact, electron opaque and devoid of visible substructures (Figs 3D and 4E,I). The pattern of chromatin condensation in K. punctigera and M. matoposa is very similar. In D. biconica, however, the condensing nucleus of the late spermatid develops a freckled appearance (Fig. 4G,H) caused by isolated nucleoplasm forming numerous small light regions in the dark chromatin.

During elongation of the nucleus, it becomes surrounded by microtubules that form a manchette (Fig. 4B–I). In all species the microtubules are in turn surrounded by an endomembrane (Fig. 4B,C). In K. punctigera neighbouring microtubules are very close to each other and to the nuclear envelope (Fig. 4C), but the microtubules in M. matoposa are arranged sparsely and seem to be unconnected to the nuclear envelope (Fig. 4B). In M. matoposa a single layer of microtubules surrounds all stages of spermatids (Fig. 4B), while in K. punctigera and D. biconica one side of the nucleus is surrounded by either a single or double layer of microtubules and the opposite side is encircled by three to six layers of tightly packed microtubules (Fig. 4H,I). Up to 220 microtubules are visible in late D. biconica spermatids. The spermatid nucleus becomes laterally invaginated posteriorly in a region sandwiched between the two zones where chromatin condensation commences (Fig. 4C). Within this region a body of granular amorphous material becomes closely associated with the nucleus by adhering to it laterally (Fig. 5B).

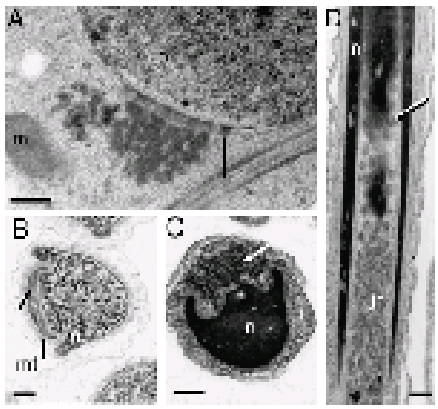

Figure 5—. A. *Diceroprocta biconica.* Early spermatid in which granular material has begun to accumulate in the cytoplasm adjacent to nuclear pores (arrowed) of the nuclear membrane. m, mitochondrion. Scale bar = 0.5 µm. —B. *Monomatapa matoposa.* Mid-spermatid cross-sectioned at the level of the nuclear invagination. The granular material (arrowhead) is now positioned in the invagination of the nucleus (n). mt, microtubules. Scale bar = 300 nm. —C. *M. matoposa.* Transverse section of the nucleus (n) of a late spermatid showing the lateral nuclear invagination with the granular material (arrowed). Note that the number of microtubules in the manchette has decreased. Scale bar = 100 nm. —D. *M. matoposa*. Longitudinal section of the lateral nuclear invagination of a late spermatid showing the juxta-nuclear material (jn) and putative nuclear pores (one arrowed). n, nucleus. Scale bar = 200 nm.

Development of the so-called centriolar adjunct

The cytoplasm of early spermatids contains a conglomerate of electron-dense granules (about 50 nm diameter) that lie close to the nuclear membrane (Figs 2A and 5A). This region of the nuclear membrane is characterized by an accumulation of pore-like complexes, each about 90 nm in diameter (Fig. 5A). In later stages, cross-sections of the spermatid nucleus show the granular material accumulating within the developing posterior lateral invagination of the nucleus (Fig. 5B,C). The nucleus and accumulating material, which gradually become more electron-dense, is surrounded by a microtubular manchette. In longitudinal sections of very late spermatids, structures that are of a similar diameter to nuclear pores, namely 90 nm in diameter, are associated with the granular material and nuclear invagination (Fig. 5D).

Development of the sperm mid-piece, mitochondrial derivatives and axoneme

Early spermatids possess a single centriole, surrounded by a number of small mitochondria, which is located in a shallow nuclear fossa (Figs 4A and 6A). As spermatids mature and elongate, the mitochondria also begin to elongate and amalgamate to form two mitochondrial derivatives (Fig. 6B). In mid-spermatids the mitochondrial derivatives have an almost circular cross-sectional profile and homogeneous content (Fig. 6C). The early mitochondrial derivatives are also surrounded by cytoplasm and some microtubules; the latter are not surrounded initially by an endomembrane (Fig. 6C). During further development, the derivatives elongate and an endomembrane develops around them (Fig. 6D,E). Some electron-dense crystallized regions start to form in the core of each derivative until they almost fill up the spaces within the mitochondrial matrix. The peripheral cristae become regularly aligned and perpendicular to the longitudinal axis of each derivative to form striations of constant periodicity (Fig. 6G).

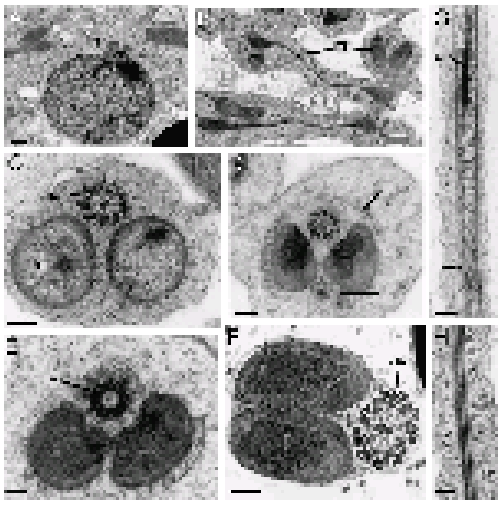

Figure 6—. A. *Diceroprocta biconica.* An early spermatid with the centriole sitting within a shallow fossa of the nucleus and surrounded by small mitochondria (m). Scale bar = 200 nm. —B. *D. biconica.* Mid-spermatids in which the mitochondrial derivatives (m) are elongating. Scale bar = 500 nm. —C. *Monomatapa matoposa*. Transverse section through the developing mitochondrial derivatives (m) and axoneme (ax) of a mid-spermatid. Scale bar = 100 nm. —D. *M. matoposa.* Transverse section of a later spermatid in which most of the space in each mitochondrial derivative is occupied by crystalline material. A few microtubules (mt) and an endomembrane (arrowed) surround the axoneme. Scale bar = 100 nm. —E. *Kongota punctigera.* A late spermatid cross-sectioned at the level of the centriole (c). Scale bar = 200 nm. —F. *K. punctigera*. Transverse section of the mature sperm tail. The manchette microtubules and cytoplasm have been lost. ax, axoneme. Scale bar = 100 nm. —G. *K. punctigera*. Longitudinal section through a mitochondrial derivative of a mid-spermatid. Here the mitochondrial cristae (arrow) are arranged into regularly spaced lamellae, which only extend part way across the mitochondrion, leaving most of the space for crystalline material (cr). Scale bar = 200 nm. —H. *K. punctigera*. Longitudinal section of the tail of a later spermatid showing the axoneme and the surrounding cytoplasm. Scale bar = 200 nm.

During all these stages the elongating spermatid tail is surrounded by a manchette of microtubules but their numbers are fewer compared to those found surrounding the spermatid nucleus. Between 30 and 60 microtubules surround the tail; some lie between the mitochondrial derivatives (Fig. 6D). Throughout spermiogenesis the developing mid-piece and tail contain excess cytoplasm (Fig. 6C–E,G,H) which is lost in mature spermatozoa (Fig. 6F).

Discussion

Spermiogenesis in D. biconica, K. punctigera and M. matoposa is similar to that described in other cicadas (Folliot and Maillet 1970; Kubo-Irie et al. 2003), hemipterans generally (Lee 1985; Fernandes et al. 2001) and other insect orders (Ndiaye et al. 1996, 2005; Baccetti 1998; Jamieson et al. 1999; Dallai et al. 2001, 2004). In early spermatids the Golgi complex lies in close proximity to a proacrosomal granule suggesting that it is involved in the formation of the acrosome. The role of the Golgi complex in the formation of the proacrosomal granule and its differentiation into a proper acrosome has been reported in several insects, e.g. spitbugs (Folliot and Maillet 1970), butterflies (Phillips 1970; Mancini and Dolder 2004), beetles (Baccetti 1975), honeybees (Peng et al. 1993) and silverfish (Dallai et al. 2001). As the acrosome matures an ante-acrosomal region of cytoplasm develops. This region persists in late spermatids but is absent in mature spermatozoa (Chawanji et al. 2005, 2006), unlike in lacewings, antlions and beetles where there is an extra-acrosomal layer in mature spermatozoa (Baccetti et al. 1973a,b; Baccetti 1998). According to Baccetti (1998) this layer, in which granular cytoplasmic material is aggregated, originates from the spermatid proacrosomal granule.

The ante-acrosomal bleb described in the spermatozoa of the cicadas, Lyristes plebejus, Cicada orni (Folliot and Maillet 1970) and Graptosaltria nigrofuscata (Kubo-Irie et al. 2003) could be an ante-acrosomal region because it is so similar in appearance to the ante-acrosomal region observed in this study. In addition, a layer of cytoplasm is evident in some of the electron micrographs used by Folliot and Maillet (1970). The current study has shown that spermatids possess a conspicuous amount of peripheral cytoplasm. This indicates that the other authors could have examined late spermatids instead of mature spermatozoa and suggests that mature cicada spermatozoa lack ante-acrosomal blebs.

The maturation of the nucleus, characterized by elongation and a reduction in diameter with a simultaneous condensation of chromatin, is typical of most insects (Phillips 1970, 1974; Baccetti 1998; Jamieson et al. 1999; Dallai et al. 2004). In D. biconica, K. punctigera and M. matoposa chromatin condensation commenced at two lateral regions of the spermatid nucleus. Studies of other animals have shown that during spermiogenesis, the arrangement of chromatin within the nucleus is mediated by two specific areas, each of which is known as the polar nuclear matrix (Ribes et al. 2004). Such a polar nuclear matrix has been described from the spermatids of mammals, fish and a cephalopod (Ribes et al. 2004). From the present study the zones from which chromatin condensation commenced were not at the poles, but because these zones were in similar positions in all three cicadas examined, this suggests a homologous role in the orientation of chromatin fibres during spermiogenesis.

The packaging of DNA and the architecture of the chromosomes in mature sperm nuclei are the result of a series of structural and chemical modifications including an organized substitution of structural proteins (mainly histones) in the heads of early spermatids by sperm nuclear basic proteins (SNBPs/protamines) in mature sperm nuclei, which are rich in arginine and/or lysine residues (Friedländer and Hauschteck-Jungen 1982; Hauschteck-Jungen and Rutz 1982; Ausio 1995; Hammadeh et al. 1999; Gimenez-Bonafe et al. 2002; Ribes et al. 2004). During spermiogenesis, the types of condensation patterns shown by chromatin are influenced by the interaction of nuclear sperm-specific proteins with DNA (Ribes et al. 2004). For example, interaction of DNA with proteins of the histone type (H-type) promotes a granular condensation, while other basic proteins condense chromatin in fibres or lamellae (Saperas et al. 1993; Ausio 1995; Càceres et al. 1999; Ribes et al. 2004). The evolution of SNBPs appears to be saltatory rather than continuous because these proteins can differ markedly between related taxa (Kasinsky 1995). The variation in the characteristic chromatin condensation patterns in D. biconica, K. punctigera and M. matoposa could be the result of differences in the protamines and DNA of each species. Protamines show very little evidence of conservation, and have an elevated degree of heterogeneity, both at the protein and gene levels (Kasinsky 1989; Ausio 1995; Lewis et al. 2003). Condensation by these basic amino acids can thus be considered as a first level of genome organization of the sperm nucleus (Ribes et al. 2004).

A characteristic feature of spermiogenesis in the cicadas studied is the presence of a transient microtubular manchette. This structure, which surrounds the acrosome, nucleus and mitochondria, has been observed in a wide range of invertebrates (including insects, e.g. Phillips 1970; Afzelius 1988; Ndiaye et al. 1997; Baccetti 1998; Jamieson et al. 1999) and some vertebrates (Russell et al. 1991; Soley 1997; Vieira et al. 2001; Al-Dhoki 2004). Some authors (Kessel 1966; Tandler and Moriber 1966; Lee 1985) are of the opinion that the manchette may be involved in the establishment and maintenance of cell shape, mechanical support and in intracellular transport, in addition to inducing elongation of the nucleus and the mitochondrial derivatives. The presence of both nucleocytoplasmic transport elements and microtubule motor elements such as kinesins and dyneins has been demonstrated in the manchette in mice, suggesting its role in protein or organelle transport in a process termed intramanchette transport (Tovich et al. 2004). The manchette microtubules may possibly have similar functions in cicada spermatids. The microtubules may also be essential for the redistribution of cytoplasm that takes place during spermatid elongation.

The variation in the number and arrangement of the manchette microtubules that encircle the spermatid nucleus in the three cicada species studied might have some phylogenetic value. The number and arrangement of microtubules in the manchette of several insects tends to vary between taxa (Jamieson et al. 1999). For example, the microtubules in true bugs (Hemiptera) cluster together in groups of three and five (Danilova et al. 1984), while in stick insects they fill up the entire cytoplasm (Afzelius 1988). Studies on spermiogenesis of more cicada taxa are required to evaluate whether microtubular arrangements could be used as a phylogenetic character.

During spermiogenesis in many insects, granular material accumulates around the centriole adjacent to the nuclear envelope to form the centriolar adjunct (Yasuzumi et al. 1970; Taffarel and Esponda 1980; Dallai et al. 2001, 2004). Kubo-Irie et al. (2003) reported that this also occurs in the cicada Graptosaltria nigrofuscata. In contrast to most insects where the centriolar adjunct of mature spermatozoa remains close to the centriole (and often other mid-piece elements) at the base of the nucleus (Wheeler et al. 1990; Jamieson et al. 1999; Fausto et al. 2001), Kubo-Irie et al. (2003) observed that in G. nigrofuscata the material that develops around the centriole becomes positioned anterior to the mitochondrial derivatives in the posterior lateral nuclear groove. While the results from our study also show that granular material accumulates in the posterior lateral nuclear groove of elongating cicada spermatids, we found that this material did not have any direct association with the centriole at any stage of spermiogenesis. In light of the final position of this structure and its formation away from the centriole, we suggest that this organelle be referred to as juxta-nuclear material until its composition and function are determined.

The material of the centriolar adjunct of insects is composed of ribonucleoproteins and proteins (Yasuzumi et al. 1970; Taffarel and Esponda 1980), and some authors suggest that it originates within the nucleus and moves through the nuclear pores (e.g. Cruz-Landim 1979; Quagro-Grassiotto and De Lello 1995). Troyer and Schwager (1982) showed that during spermiogenesis in Locusta migratoria, nuclear pores migrated and aggregated next to the site of formation of the centriolar adjunct material. The granular material that eventually occupies the postero-lateral nuclear canal in cicada spermatozoa also first appears next to a region of the nuclear envelope that has prominent pores. This suggests that this material may have a nuclear origin and that nuclear pores migrate posteriorly during spermiogenesis. In very late spermatids accumulations of pore-like structures were observed in the posterior nuclear groove in close association with the nucleus and juxta-nuclear material. Thus the vesicle-like elements described by Chawanji et al. (2005, 2006) in the mature spermatozoa of cicadas are probably nuclear pores.

Transmission electron microscopy revealed numerous large, electron-dense granules that were present in the cytoplasm of cyst cells. They are not present in mature spermatozoa. Presumably these granules are some kind of storage products and could have a role in spermiogenesis.

Varying stages of spermiogenesis were observed in the testes of three cicada species, thus spermiogenesis probably continues after cicada adults eclose. This suggests that cicada males are capable of mating more than

once and can replace the mature spermatozoa stored in their seminal vesicles. However, spermatogonia and spermatocytes were not apparent in the adults examined, which suggests that these species will have a finite ability to produce spermatozoa and fertilize eggs during the four or so weeks of their short adult lives.

Acknowledgements: The authors acknowledge Shirley Pinchuck and Marvin Randall (Electron Microscopy Unit, Rhodes University) for technical assistance; Derek de la Harpe, Pandeni Chitimela, Bruce Clegg, Sarah Clegg and Nicky Lunt (Malilangwe Private Game Reserve, Zimbabwe) for access to the estate, accommodation and support; and the National Research Foundation and Rhodes University for funding.

References

Afzelius, B. A. 1988. Microtubules in the spermatids of stick insects.– Journal of Ultrastructure and Molecular Research 98: 94–102.

Al-Dhoki, O. A. 2004. Electron microscopic study of sperm head differentiation in the Arabian horned viper Cerastes cerastes (Squamata, Reptilia). – Journal of Biology Sciences 4: 111–116.

Ausio, J. 1995. Histone H1 and the evolution of the nuclear spermspecific proteins. – Mémoires Du Muséum National D'histoire Naturelle 166: 447–462.

Baccetti, B. 1975. The role of the Golgi complex during spermiogenesis. – Current Topics in Developmental Biology 10: 103–122.

Baccetti, B. 1998. Spermatozoa. In Harrison, F. W. and Locke, M. (Eds): Microscopic Anatomy of Invertebrates 11C, Insecta, pp. 843–894. Wiley-Liss, New York.

Baccetti, B., Burrini, A. G., Dallai, R., Giusti, F., Mazzini, M., Reineiri, T., Rosati, F. and Selmi, G. 1973a. The spermatozoon of Arthropoda. − XX. − Structure and function in the spermatozoon of Tenebrio molitor. – Journal of Mechanochemistry and Cell Motility 2: 149–161.

Baccetti, B., Burrini, A. G., Dallai, R., Pallini, V., Piantelli, F., Rosati, F. and Selmi, G. 1973b. Structure and function in the spermatozoon of Bacillus rossius. − The spermatozoon of Arthropoda. − XIX. – Journal of Ultrastructure Research 44: 5–73.

Càceres, C., Gimenez-Bonafe, P., Ribes, E., Wouters-Tyrou, D., Martinage, A., Kouach, M., Sautière, P., Muller, S., Palau, J., Subirana, J. A., Cornudella, L. and Chiva, M. 1999. DNAinteracting proteins in the spermiogenesis of the mollusk Murex brandaris. – The Journal of Biological Chemistry 274: 649–656.

Chawanji, A. S., Hodgson, A. N. and Villet, M. H. 2005. Sperm morphology in four species of African platypleurine cicadas (Hemiptera: Cicadomorpha: Cicadidae). – Tissue and Cell 37: 257–267.

Chawanji, A. S., Hodgson, A. N. and Villet, M. H. 2006. Sperm morphology in five species of cicadettine cicadas (Hemiptera: Cicadomorpha: Cicadidae). – Tissue and Cell 38: 373–388.

Cruz-Landim, C. 1979. Ultrastructural characterization of nuage in spermatocytes of Myogryllus sp. (Orthoptera). – The Nucleus 22: 67–74.

Dallai, R., Carapelli, A., Nardi, F., Fanciulli, P. P., Lupetti, P., Afzelius, B. A. and Frati, F. 2004. Sperm structure and spermiogenesis in Coletinia sp. (Nicoletiidae, Zygentoma, Insecta) with a comparative analysis of sperm structure in Zygentoma. – Tissue and Cell 36: 233–244.

Dallai, R., Lupetti, P., Frati, F., Nardi, F. and Afzelius, B. A. 2001. Sperm ultrastructure and spermiogenesis in the relic species Tricholepidion gertschi Wygodzinsky (Insecta, Zygentoma). – Tissue and Cell 33: 596–605.

Danilova, L. V., Gaber, E. S. and Shinyaeva, L. I. 1984. Spermiogenesis and the ultrastructure of mature sperm in Eurygaster intergriceps. – Gamete Research 9: 75–86.

Duffels, J. P. and van der Laan, P. A. 1985. Catalogue of the Cicadoidea (Homoptera, Auchenorrhyncha). 1956–80. Series Entomologica 34. Dr Junk W. Publishers, The Hague.

Fausto, A. M., Belardinelli, M., Fochetti, R. and Mazzini, M. 2001. Comparative spermatology in Plecoptera (Insecta): an ultrastructural investigation on four species. – Arthropod Structural Development 30: 55–62.

Fernandes, A. P., Curi, G., Franca, F. G. R. and Báo, S. N. 2001. Nuclear changes and acrosome formation during spermiogenesis in Euchistus heros (Hemiptera: Pentatomidae). – Tissue and Cell 33: 286–293.

Folliot, R. and Maillet, P. L. 1970. Ultrastructure de la spermiogenèse et du spermatozoïde de divers insects Homoptères. In Baccetti, B. (Ed.): Comparative Spermatology, pp. 289–300. Academic Press, New York.

Friedländer, M. 1997. Control of eupyrene-apyrene sperm dimorphism in Lepidoptera. – Journal of Insect Physiology 43: 1085–1092.

Friedländer, M. and Hauschteck-Jungen, E. 1982. Differential basic nucleoprotein kinetics in the two kinds of Lepidoptera spermatids: nucleate (eupyrene) and anucleate (apyrene). – Chromosoma 85: 387–398.

Friedländer, M. and Meisel, S. 1977. Spermatid anucleation during the normal atypical spermatogenesis of the warehouse moth, Ephestia cautella. – Journal of Submicroscopic Cytology 9: 173–185.

Fuller, M. T. 1993. Spermatogenesis. In Bate, M. and Arias, A. M. (Eds): The Development of Drosophila melanogaster, pp. 71–148. Cold Spring Harbour Laboratory Press, Plainview, USA.

Gimenez-Bonafe, P., Ribes, E., Sautière, P., Gonzalez, A., Kasinsky, H. E., Kouach, M., Sautière, P. E., Ausio, J. and Chiva, M. 2002. Chromatin condensation, cysteine-rich protamine, and establishment of disulphide interprotamine bonds during spermiogenesis of Eledone cirrhosa (Cephalopoda). – European Journal of Cell Biology 81: 341–349.

Hammadeh, M. E., Al-Hasani, S., Doerr, S., Stieper, M., Rosenbaum, P., Schmidt, W. and Diedrich, K. 1999. Comparison between chromatin condensation and morphology from testis biopsy extracted and ejaculated spermatozoa and their relationship to ICSI outcome. – Human Reproduction 14: 363–367.

Hauschteck-Jungen, E. and Rutz, G. 1982. Arginine-rich nucleoprotein transition occurs in the two size classes of spermatozoa of Drosophila subobscura males. – Genetica 62: 25–32.

Hess, R. A. 1999. Spermatogenesis, Overview. – Encyclopedia of Reproduction 4: 539–545.

Jamieson, B. G. M., Dallai, R. and Afzelius, B. A. 1999. Insects: Their Spermatozoa and Phylogeny. Science Publishers, Enfield, NH.

Kasinsky, H. E. 1989. Specificity and distribution of sperm basic proteins. In Hnilica, H. S., Stein, G. S. and Stein, J. L. (Eds): Histones and Other Basic Nuclear Proteins, pp. 73–163. CRC Press, Boca Raton, FL.

Kasinsky, H. E. 1995. Evolution and origin of sperm nuclear basic proteins. – Mémoires Du Muséum National d'Histoire Naturelle 166: 463–473.

Kessel, R. G. 1966. The association between microtubules and nuclei during spermiogenesis in the dragonfly. – Journal of Ultrastructure Research 16: 293–304.

Kubo-Irie, M., Irie, M., Nakazawa, T. and Mohri, H. 2003. Ultrastructure and function of long and short sperm in Cicadidae (Hemiptera). – Journal of Insect Physiology 49: 983–991.

Lee, Y. H. 1985. Spermatogenesis of the water strider, Gerris paludum (Heteroptera, Gerridae). – Journal of Ultrastructure Research 90: 235–250.

Lewis, J. D., Song, Y., De Jong, M. E., Bagha, S. M. and Ausio, J. 2003. A walk through vertebrate and invertebrate protamines. – Chromosoma 111: 473–482.

Mancini, K. and Dolder, H. 2004. Dichotomic spermiogenesis in Euptoieta hegesia (Lepidoptera: Nymphalidae). – Brazilian Journal of Morphological Science 21: 13–23.

Metcalf, Z. P. 1963a. General catalogue of the Homoptera, Fascicle VIII. Part 1. Cicadidae. Section 1. Tibiceninae. – North Carolina State College Contribution 1502: 1–585.

Metcalf, Z. P. 1963b. General catalogue of the Homoptera, Fascicle VIII. Part II. Gaeaninae and Cicadinae. – North Carolina State College Contribution 1564: 587–919.

Metcalf, Z. P. 1963c. General catalogue of the Homoptera, Fascicle VIII. Cicadoidea. Part 2. Tibicinidae. – North Carolina State College Contribution 1564: 1–492.

Moulds, M. 2005. An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. – Records of the Australian Museum 57: 375–446.

Myers, J. G. 1929. Insect Singers – a Natural History of the Cicadas. Mayflower Press, Plymouth.

Ndiaye, M. and Mattei, X. 2005. Process of nuclear envelope reduction in spermiogenesis of a mosquito Culex tigripes. – Molecular Reproduction and Development 34: 416–419.

Ndiaye, M., Mattei, X. and Thiaw, O. T. 1996. Spermatogenesis in mosquitoes (Diptera: Culicidae). – Journal of Submicroscopy Cytology and Pathology 28: 375–384.

Ndiaye, M., Mattei, X. and Thiaw, O. T. 1997. Ultrastructural diversity of the manchette in spermatids of mosquitoes (Diptera: Culicidae). – Acta Zoologica 78: 47–49.

Peng, C. Y., Yin, S. and Yin, L. R. S. 1993. Ultrastructure of honey bee, Apis mellifera, sperm with special emphasis on the acrosomal complex following high-pressure fixation. – Physiological Entomology 18: 93–101.

Phillips, D. M. 1970. Insect sperm: their structure and morphogenesis. – Journal of Cell Biology 44: 243–277.

Phillips, D. M. 1971. Morphogenesis of the lacinate appendages of lepidopteran spermatozoa. – Journal of Ultrastructure Research 34: 567–585.

Phillips, D. M. 1974. Spermiogenesis, pp. 1–68. Academic Press, New York and London.

Quagio-Grassiotto, I. and De Lello, E. 1995. Occurrence of ‘nuages’ and ‘lamellae anulata’ during spermiogenesis in Dermatobia hominis (Diptera: Cuterebridae). – Memürias Do Instituto Oswaldo Cruz 90: 537–545.

Ribes, E., Gimenez-Bonafe, P., Martinez-Soler, F., Gonzalez, A.,

Saperas, N., Kasinsky, H. E. and Chiva, M. 2004. Chromatin organization during spermiogenesis in Octopus vulgaris. 1: Morphological structures. – Molecular Reproduction and Development 68: 223–231.

Russell, L. D., Russell, J. A., MacGregor, G. R. and Meistrich, M. L. 1991. Linkage of manchette microtubules to the nuclear envelope and observations of the role of the manchette in nuclear shaping during spermiogenesis in rodents. – American Journal of Anatomy 192: 97–120.

Saperas, N., Ribes, E., Buesa, C., Garcia-Hegart, F. and Chiva, M. 1993. Differences in chromatin condensation during spermiogenesis in two species of fish with distinct protamines. – Journal of Experimental Zoology 265: 185–194.

Soley, J. T. 1997. Nuclear morphogenesis and the role of the manchette during spermiogenesis in the ostrich (Struthio camelus). – Journal of Anatomy 190: 563–576.

Taffarel, M. and Esponda, P. 1980. Fine-structural cytochemistry of the centriolar adjunct in grasshopper spermatids. – Mikroskopie 36: 35–42.

Tandler, B. and Moriber, L. G. 1966. Microtubular structures associated with the acrosome during spermiogenesis in the water strider, Gerris remigis. – Journal of Ultrastructure Research 14: 391– 404.

Tovich, P. R., Sutovsky, P. and Oko, R. J. 2004. Novel aspect of perinuclear theca assembly revealed by immunolocalization of non-nuclear somatic histones during bovine spermiogenesis. – Biology of Reproduction 71: 1182–1194.

Troyer, D. and Schwager, P. 1982. Evidence for nuclear membrane fluidity: proacrosome migrations and nuclear pore redistribution during grasshopper spermiogenesis. – Cell Motility and the Cytoskeleton 2: 355–367.

Vieira, G. H. C., Wiederhecker, H. C., Colli, C. R. and Báo, S. N. 2001. Spermiogenesis and testicular cycle of the lizard Tropidurus torquatus (Squamata, Tropiduridae) in the Cerrado of Central Brazil. – Amphibia-Reptilia 22: 217–233.

Villet, M. H. 1999a. On two species of Paectira Karsch (Hemiptera: Cicadidae) from Kenya. − -African Entomology 7: 151–153.

Villet, M. H. 1999b. A new cicada of the genus Severiana Boulard 1972 (Homoptera: Cicadidae) from northern Namibia. – Cimbebasia 15: 149–153.

Wheeler, D. E., Crichton, E. G. and Krutzsch, P. H. 1990. Comparative ultrastructure of ant spermatozoa (Formicidae: Hymenoptera). – Journal of Morphology 206: 343–350.

Yasuzumi, G., Sugioka, T., Tsubo, I., Yasuzumi, F. and Matano, Y. 1970. Spermatogenesis in animals as revealed by electron microscopy. XX. Relationship between chromatoid bodies and centriole adjunct in spermatids of grasshopper, Acrida lata. – Zeitschrift für Zellforschung und Mikroskopische Anatomie 110: 231–242.