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COMPARATIVE SPERMATOLOGY OF THREE SPECIES OF *DONAX* (BIVALVIA) FROM SOUTH AFRICA

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ABSTRACT

The fine structure of the sperm and spermatogenesis in three species of *Donax* (*D. madagascariensis, D. sordidus* and *D. serra*) are described. Although the morphology of the sperm of all species is very similar, each has unique features. *Donax madagascariensis* and *D. sordidus* reportedly hybridize in regions of sympatry and their spermatozoa are morphologically closer to one another than to *D. serra*. All sperm are of the primitive type with a head (about 2 μm long), mid-piece of four mitochondria and tail. The head comprises a barrel-shaped nucleus which is capped by a small, complex acrosome. The structure of the acrosome is typical of heterodont bivalves. During spermatogenesis the pattern of nuclear chromatin condensation is granular. Glycogen first appears in the cytoplasm of spermatids, and in the mature sperm is sited in the mid-piece and base of the acrosome.

INTRODUCTION

Bivalves of the genus *Donax* are common inhabitants of marine sandy sediments throughout the tropical and temperate regions of the world (Ansell, 1983). In South Africa three species, *D. serra* Dillwyn, 1817, *D. sordidus* Hanley, 1845 and *D. madagascariensis* Wood, 1828, are found intertidally. *Donax serra* is found along the west and south coast, *D. sordidus* the south, and *D. madagascariensis* extends from the east coast northwards to East Africa (Kilburn & Rippey, 1982).

Donax serra and *D. sordidus* are the dominant members of the macrofauna in South African sandy beaches and have been the subjects of considerable research (for reviews see Ansell, 1983; McLachlan, 1983). Despite the importance of these animals in sandy beach ecosystems very little is known about their reproduction with only a few published studies on reproductive cycles of *D. serra* and *D. sordidus* (De Villiers, 1975; McLachlan & Hanekom, 1979; McLachlan, 1979; McLachlan & Van der Horst, 1979) and no such published studies on *D. madagascariensis*.

It has been proposed that, when evaluated correctly, a knowledge of bivalve sperm structure could be used for taxonomic purposes (Franzén, 1970, 1977, 1983; Popham, 1979; Hodgson & Bernard, 1986a, b), and since a revision of the genus *Donax* is warranted (R. Balley, pers. comm.), a study of spermatozoon morphology could be of value. Furthermore an examination of the sperm structure of *D. sordidus* and *D. madagascariensis* may be particularly valuable as Kilburn and Rippey (1982) suggest that where they overlap in their distribution they interbreed. To date there is no information on the sperm structure of the family Donacidae. In this paper we give the first description and compare the sperm structure and spermatogenesis of the three South African intertidal species of *Donax*.

METHODS

Animals were collected from sandy beaches of Natal (c. 30°S, 31°E; *D. madagascariensis*), eastern Cape (c. 33°S, 27°E; *D. serra* and *D. sordidus*) and western Cape (c. 33°S, 18°E; *D. serra*) of South Africa in December 1986 and 1988. After collection, the animals were immediately transported back to a laboratory where portions of the testis were dissected from the gonad and prepared for electron microscopy.

The portions of testis were fixed by one of two methods. The first involved fixing for 12 hours at room temperature in 2.5% glutaraldehyde in filtered sea water (35‰), the fixative having an osmolarity of 1200 mOsmols (sea water = 1059 mOsmols). The second method followed Hackney, McCrohan & Hawkins (1983), the tissue being fixed for 12 hours in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer and sea water (pH 7.4 and isosmotic with sea water). The

Table 1. Dimensions of the spermatozoa (mean ± S.D.; n = 20). All measurements (in micrometers) were taken from electron micrographs.

	Nucleus		Acrosome	
	length	mid-diameter	length	basal diameter
D. serra	1.33 ± 0.02	1.5 ± 0.01	0.9 ± 0.01	1.0 ± 0.02
D. sordidus	1.50 ± 0.03	1.4 ± 0.02	1.0 ± 0.02	0.8 ± 0.01
D. madagascariensis	1.56 ± 0.03	1.4 ± 0.02	1.07 ± 0.01	0.98 ± 0.01

quality of fixation by both these methods is equally good (Cross, Hodgson & Dower, 1986). All tissues were postfixed in 1% osmium tetroxide in 0.1M sodium cacodylate and sea water (after Hackney *et al.*, 1983). Specimens were then dehydrated and embedded in an Araldite CY212/Taab resin mixture (Cross, 1989). Thin sections (silver/gold) were mounted on 300 mesh copper grids and stained in 5% aqueous uranyl acetate (30 minutes) and lead citrate (3 minutes). Some sections were stained for glycogen using the periodic acid-sodium chlorite-uranyl acetate method (Vye & Fischman, 1971). All sections were examined on a JEOL 100CXII electron microscope.

RESULTS

Although the spermatozoa of all three species are very similar in appearance (Fig. 1), each has distinguishing features. All have a head comprising a barrel-shaped nucleus, of slightly differing dimensions (Table 1), and an anterior acrosome which sits in a nuclear fossa (Figs 1 & 2).

The acrosomes of *D. sordidus* and *D. madagascariensis* are cone-shaped (Figs. 1 & 2A, C), that of the former being slightly smaller (Table 1). In these two species the acrosome comprises two regions of differing electron densities (Fig. 2A, B, C). The anterior and central portions are electron-lucent, this material comprising about 50% of the total acrosomal volume. The outer basal area is more electron-opaque and contains regularly spaced lamellae which run parallel to the outer acrosomal membrane (Fig. 2A, B, C). By contrast the acrosome of *D. serra* (Figs 1, 2D) is almost spherical (0.9 μm long × 1.0 μm mid-acrosomal diameter; Table 1). Internally three regions can be recognized. There is an electron-lucent region which comprises about 16% of the acrosomal volume and which is restricted to the anterior of the organelle. The majority of the acrosomal material (c. 84%) is more electron-dense and is divided into two morphologically distinct areas. The central portion of material is homogeneous and is surrounded by a region containing lamellae (Fig. 2). Finally all the acrosomes are deeply invaginated and have an axial rod (about 0.12 μm diameter) which projects from the subacrosomal space through the centre of the acrosome (Figs 1 & 2). In the mature sperm, glycogen granules are present at the base of the acrosome (Fig. 3B).

The mid-piece of all species comprises a ring of four spherical mitochondria (about 0.5 μm diameter) with numerous well developed cristae (Fig. 3A). In the centre of the mitochondrial ring are the proximal and distal centrioles, and scattered throughout the mid-piece are numerous glycogen granules (Fig. 3A, B). The tail (which has the normal 9 + 2 arrangement of microtubules) emerges from the distal centriole (Figs 1B, 3B), and in some spermatozoa of *D. madagascariensis* (<10%) the distal centriole is rotated through 90° and the tail displaced laterally (Fig. 3C).

Spermatogenesis

The process of spermatogenesis in all species is similar and therefore a single description is given. Spermatogonia lie to the outside of the lobes of the testis and as the sperm cells mature they are displaced towards the centre.

Early spermatogonia are characterized by a round (5 μm diameter) nucleus with a prominent electron-dense nucleolus (1 μm diameter). The nucleus contains small clumps of electron-dense chromatin which are often associated with the inner nuclear membrane. In late spermatogonia the nucleus becomes more oval in shape (about 4 μm × 3 μm) (Fig. 4A). The cytoplasm of all spermatogonia contains numerous mitochondria, free ribosomes and small amounts of rough endoplasmic reticulum (Fig. 4A).

The spermatocytes have a nucleus that is similar in size and shape to that of the late spermatogonia. The nucleolus, however, is no longer present, and the chromatin is more evenly dispersed within the nucleus (Fig. 4B). In addition synaptonemal complexes can be seen

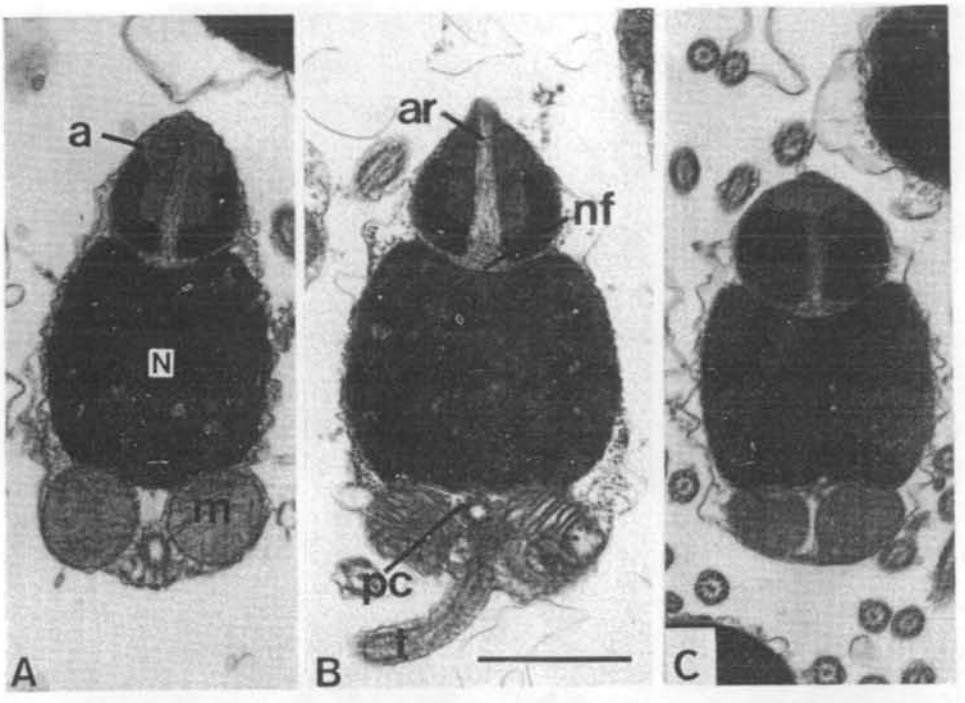

Fig. 1. Mid-longitudinal sections through the spermatozoa of A. *Donax sordidus*, B. *D. madagascariensis*, C. *D. serra*. a, acrosome; ar, axial rod; m, mitochondrion; n, nucleus; nf, nuclear fossa; pc, proximal centriole; t, tail. Scale bar = 1.0 μm.

within the nucleus (Fig. 4B). The cytoplasm of the cell now contains numerous electron-dense, membrane-bound proacrosomal vesicles (about 0.2 μm diameter) which appear to be formed by a Golgi complex (Fig. 4B & inset).

Early spermatids have a round nucelus (about 3 μm diameter), the chromatin of which is in the form of a patchwork (Fig. 5A). As the spermatid matures the nucleus decreases in size and becomes more oval in shape with a distinct anterior fossa (Fig. 5B). Chromatin condensation commences with the formation of spherical chromatin granules, each about 45 nm diameter (Fig. 5C). By the late spermatid stage, chromatin condensation is almost complete and the nucleus has begun to assume its mature shape (Fig. 5E).

In early spermatids (Fig. 5A) there are numerous proacrosomal vesicles. By the mid-spermatid stage these appear to have coalesced to form a single, spherical proacrosomal vesicle about 0.8 μm in diameter (Fig. 5B). As the spermatid matures the contents of the vesicle begin to differentiate (Fig. 5C-E) and at the same time the vesicle migrates towards the presumptive anterior end of the cell where it occupies the nuclear fossa. The centre of the acrosome then begins to invaginate posteriorly, thus forming a central canal in which the axial rod develops (Fig. 5D, E). At the same time the mitochondria, which are ellipsoidal in early stages and distributed throughout the cytoplasm, decrease in number and increase in size. By the mid-spermatid stage they are spherical and have migrated to the posterior of the nucleus (Fig. 5C). The tail first appears in early spermatids (Fig. 5A). Cytochemical staining reveals that glycogen granules first appear in the cytoplasm of mid-spermatids. It initially accumulates around the developing mid-piece and later at the base of the acrosome. No glycogen has been found in earlier stages.

Intercellular bridges connect developing spermatocytes and spermatids (Fig. 5B) and remain until spermatozoa are nearly mature. The bridges are short, cylindrical and the cytoplasmic side of the plasma membrane of each bridge thickened.

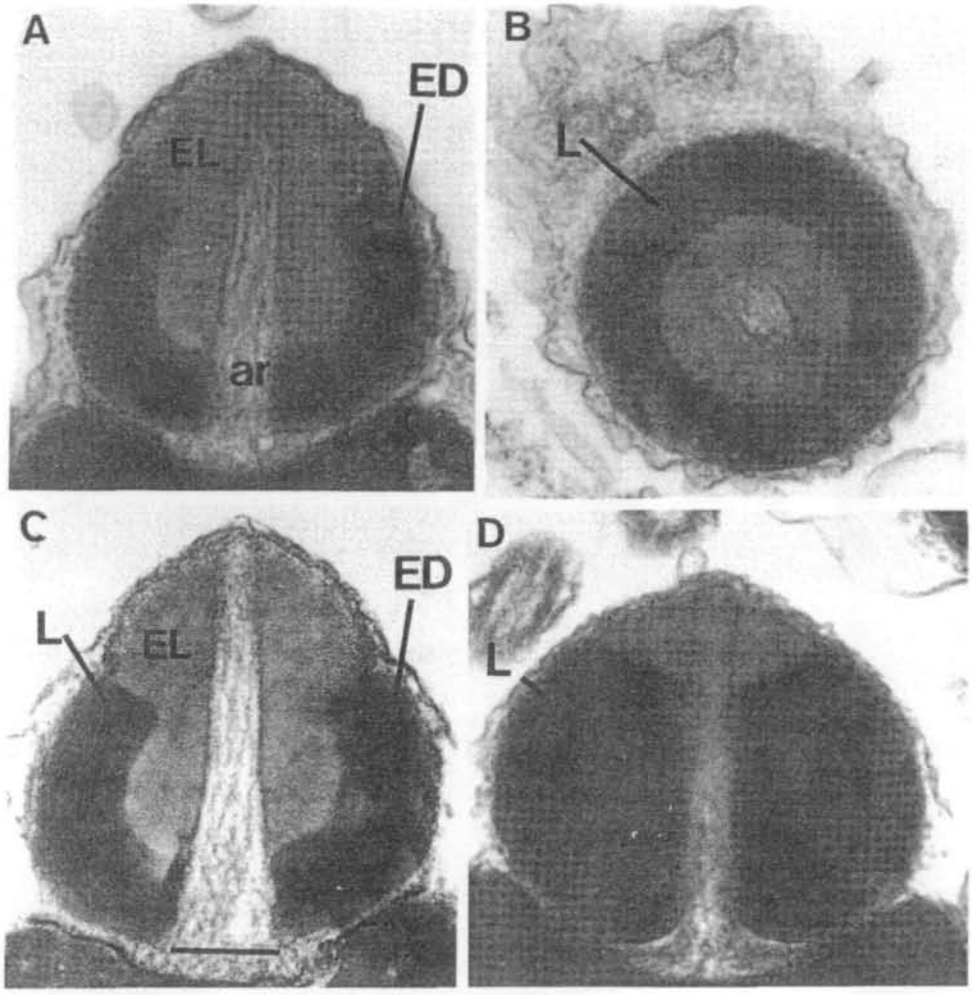

Fig. 2. Longitudinal and transverse sections through the acrosomes showing electron-lucent (EL) and electron-dense (ED) regions. In addition lamellae (L) and the axial rod (ar) can be seen. A and B = *D. sordidus;* C = *D. madagascariensis*; D = *D. serra.* Scale bar = 0.25 μm.

DISCUSSION

The spermatozoa of all three species of *Donax* examined are of the primitive type (Franzén, 1955). More recently Rouse & Jamieson (1987) renamed this type of sperm ect-aquasperm in order to remove any phylogenetic implications of the term 'primitive'. Invertebrates which produce such sperm fertilize the eggs externally (Franzén, 1956; Rouse & Jamieson, 1987). Field observations of spawning in the genus *Donax* have been unsuccessful (Ansell, 1983) however recent laboratory experiments with *D. serra* and *D. sordidus* (van der Horst unpublished observations) have resulted in successful spawning and fertilization. It appears therefore that in the genus *Donax*, sperm morphology and mode of fertilization fit the pattern proposed by Rouse and Jamieson (1987).

The comparison of sperm structure of the three species of *Donax* shows that they closely resemble one another. The sperm of *D. sordidus* and *D. madagascariensis* are notably similar, differing only in overall dimensions, suggesting that these two species are closely related. In view of the reported hybridization between *D.*

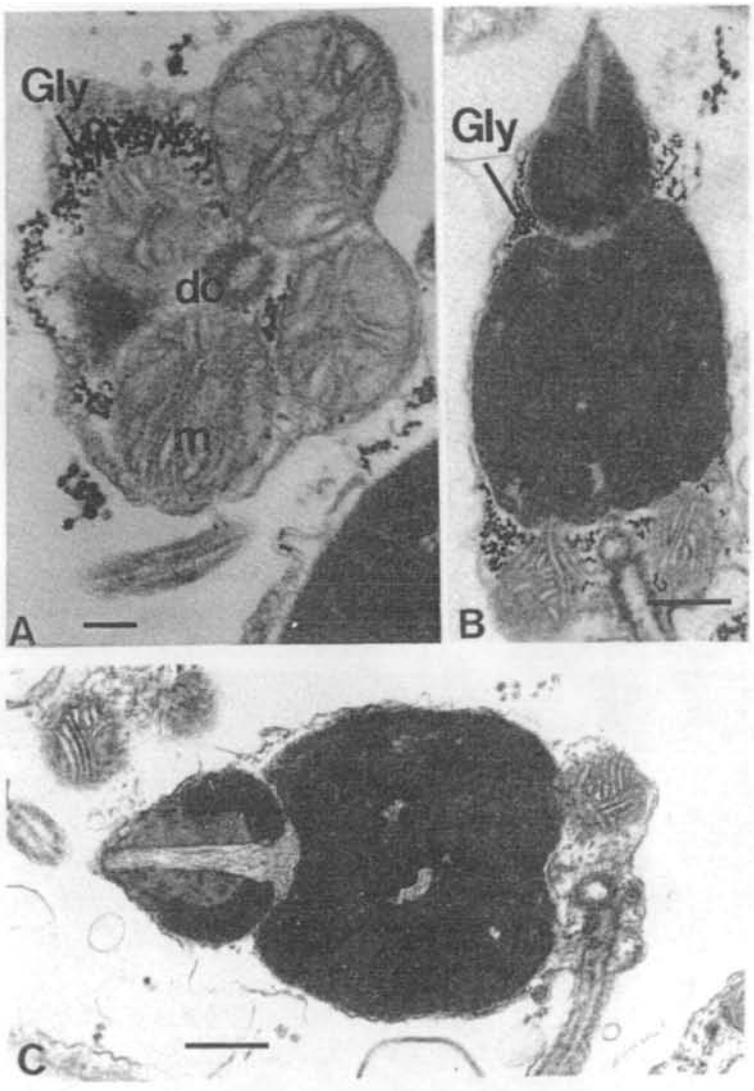

Fig. 3. A. Transverse section of the mid-piece of *D. madagascariensis* showing ring of four mitochondria (m) and distal centriole (dc). The section is stained to demonstrate the presence of glycogen (Gly). Scale bar = 0.25 μm. B. Spermatozoon of *D. madagascariensis* stained to show distribution of glycogen (Gly) in mid-piece and base of acrosome. Scale bar = 0.5 μm. C. Defective sperm of *D. madagascariensis* in which the distal centriole and tail are displaced Scale bar = 0.5 μm.

sordidus and *D. madagascariensis* (Kilburn & Rippey, 1982) this finding is perhaps not surprising.

Members of the genus *Donax* belong to the subclass Heterodonta and it is not surprising to find that their sperm resemble those described for other heterodonts (Popham 1974a, b; Popham, Dickson & Goddard, 1974; Popham, 1979; Franzén, 1983; Maxwell, 1983). Our findings therefore support the view that sperm structure of bivalves can be a valuable character for taxonomic studies at both the species and higher levels (Franzén, 1970, 1977, 1983; Popham, 1979; Hodgson & Bernard, 1986a, b).

Although the nucleus and mid-piece of the sperm of *Donax* are unremarkable being typical

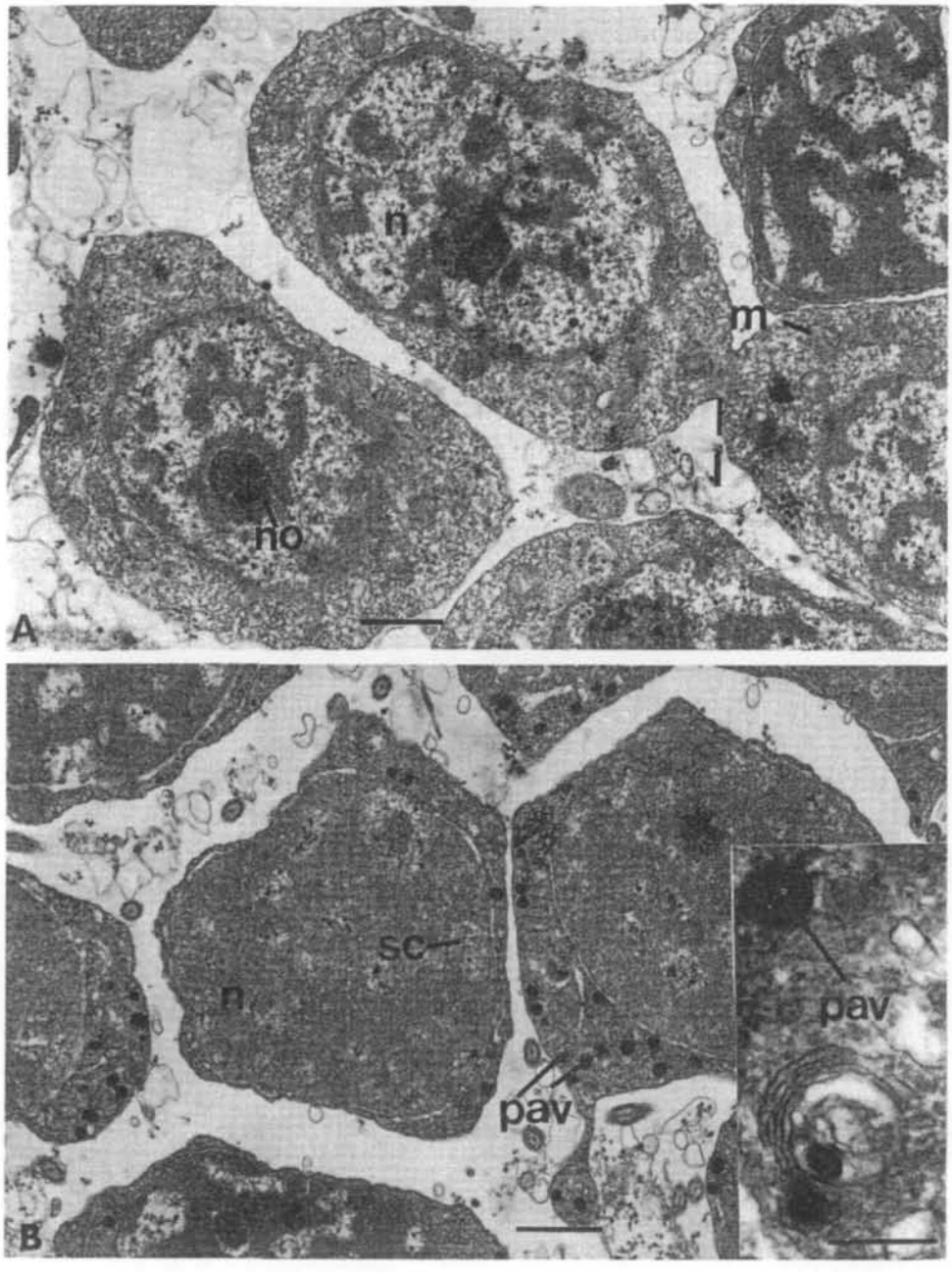

Fig. 4. Stages in spermatogenesis from the testis of *D. madagascariensis*. A. Late spermatogonia. Scale bar = 1.0 μm. B. Spermatocytes with synaptonemal complexes (sc) in the nucleus (n). Scale bar = 1.0 μm. Inset, Golgi body. Scale bar = 0.25 μm; I, intercellular bridge; m, mitochondrion; n, nucleus; no, nucleolus; pav, proacrosomal vesicles.

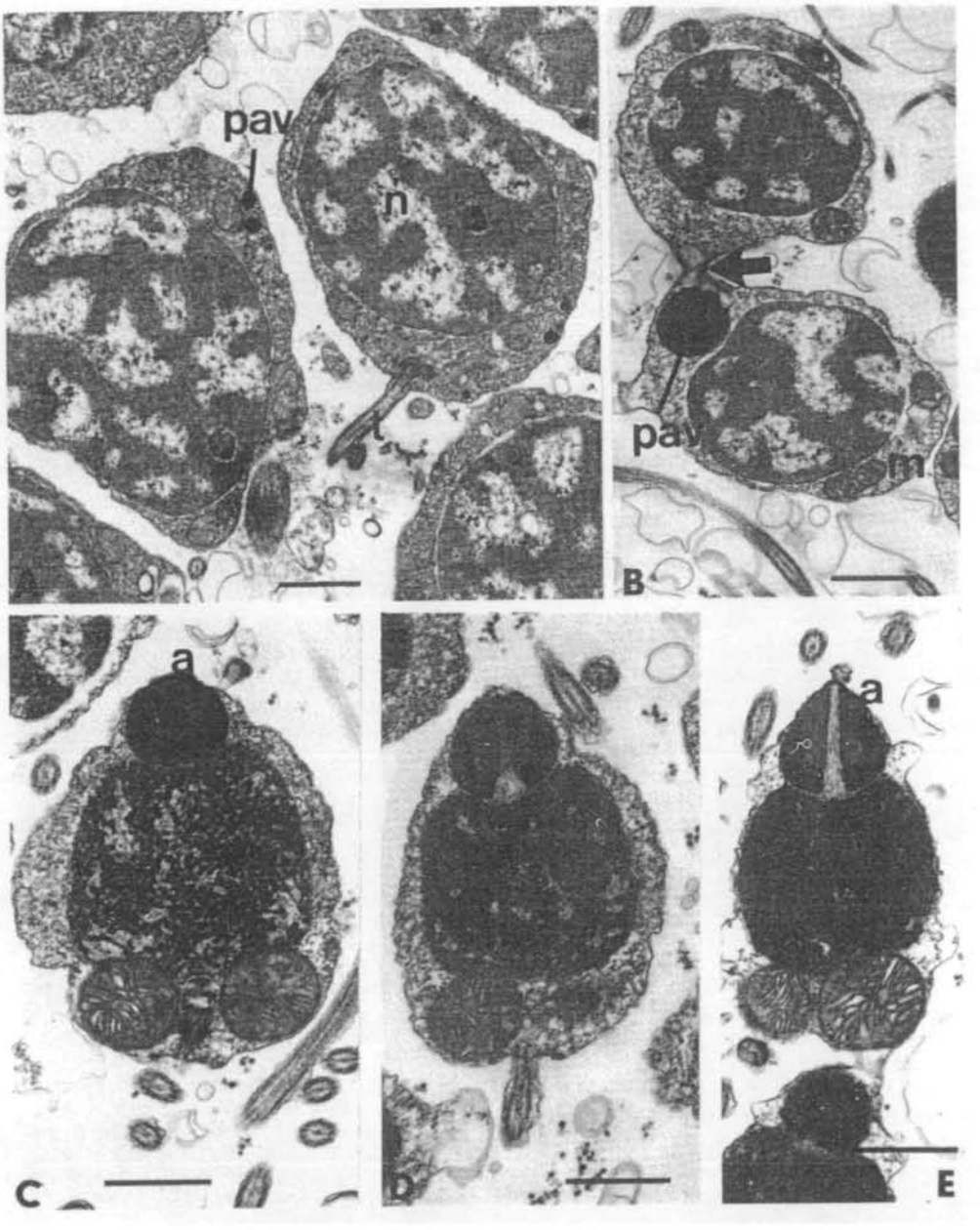

Fig. 5. Stages in spermiogenesis of *D. madagascariensis*. A. Early spermatids with round, central nucleus (n), proacrosomal vesicles (pav) and developing tail (t). Scale bar = 1.0 μm. B. Two spermatids joined by cytoplasmic bridge (arrow). Note the single large proacrosomal vesicle (pav) at the presumptive anterior of the cell, and the mitochondria (m) at the opposite end of the cell. Scale bar = 1.0 μm. C,D,E. Mid to late spermatids showing stages of chromatin condensation, acrosome (a) development and reduction of excess cytoplasm. Scale bar = 1.0 μm.

of ect-aquasperm, the structure of the acrosome is more interesting. The acrosome is differentiated into electron-dense and electron-lucent regions, a feature recorded from other bivalves (Popham, 1974a, b; Hylander & Summers, 1977; Franzén, 1983; Maxwell, 1983; Bernard & Hodgson, 1985; Hodgson & Bernard, 1986a, b). These regions of differing electron density probably reflect the differing functions of the acrosome during fertilization (Popham, 1974b). Hylander & Summers (1977) have shown that in the bivalves *Chama macerophylla* and *Spisula solidissima* the outer electron-dense region of the acrosome is the region that binds the sperm to fibrillar tufts of the microvilli of the egg surface. The results of Popham (1974b) on *Bankia australis* would suggest that in this species it is the outer electron-lucent region which has a role in binding. Presumably, the equivalent region of the acrosomes of *Donax* performs a similar function. However in addition the electron-dense portion of the acrosome has a well ordered substructure similar to that reported by Popham (1974b) for *B. australis* and Franzén (1983) for *Dreissena polymorpha*. Periodic ordering of acrosomal contents has been described for other molluscs, in cephalopods (Galangau & Tuzet, 1968; Longo & Anderson, 1970) and the patellid limpet *Cellana capensis* (Hodgson & Bernard, 1988). Although the functional significance of such ordering is still to be determined, it has been proposed that periodicity of acrosomal contents represents a crystalline alignment of enzymes within the acrosome (Friend & Fawcett, 1974).

The structural changes that occur during spermatogenesis are similar to those described for many other invertebrates (see Roosen-Runge, 1977; Adiyodi & Adiyodi, 1983; Hodgson, 1986 for reviews) with the greatest morphological changes occurring during spermatogenesis. Chromatin condensation within the nucleus is of the granular pattern which is typical in sperm heads that are short and squat (Maxwell, 1983). Acrosome formation occurs early in spermiogenesis, with several small vesicles produced by a Golgi body coalescing to form a single larger vesicle. During spermiogenesis it was noted that glycogen particles accumulated in the cytoplasm of spermatids and that in the mature sperm these were sited around the mid-piece and base of the acrosome. Whilst the presence of glycogen in the mid-piece of molluscan sperm is well documented (Anderson & Personne, 1970; Maxwell, 1983) its presence in the head region has only been previously recorded in the bivalve *Bankia australis* (Popham & Dickson, 1975; see also Maxwell, 1983; Table 2). The actual source of the glycogen is not known but it was observed that the lumen of the testis contained large quantities of free glycogen. Accumulation of glycogen in the developing spermatids contrasts with euthyneuran gastropods in which glycogen only appears after the sperm leave the testis (Maxwell, 1983).

Finally some of the spermatozoa (<10%) of *D. madagascariensis* were found to have a sperm defect whereby the tail emerged at right angles to the long axis of the sperm. Such a defect has only been reported in one other mollusc, the limpet *Nacella delesserti* (Hodgson & Bernard, 1989). As *Donax* produce some 10^{10} sperm/ml. (van der Horst, unpublished data) it is unlikely that the few defective sperm will affect the fecundity of the animals.

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