Comparative spermatology of two morphologically similar species of *Solen* (Mollusca : Bivalvia)

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The fine structure of the spermatozoa and spermatogenesis in *Solen cylindraceus* and *S. capensis* were studied by electron microscopy. The morphology of the sperm of both species is similar. Both are of the primitive type with a head about 1,5 μm long), mid-piece of five mitochondria, and tail. The head comprises a barrel-shaped electron-opaque nucleus (about 1,1 μm × 1,2 μm) which is capped by a small conical acrosome. The morphology of the acrosome of *Solen* is typical of heterodont bivalves, however, each species has an acrosome of differing dimensions which can thus be used to separate these two closely related bivalves. During spermatogenesis the pattern of nuclear chromatin condensation is granular. The acrosome is formed by a single Golgi body, and the five large mitochondria of the mid-piece are probably formed by fusion of several small mitochondria.

Die struktuur van die spermatozoa en spermatogenese in *Solen cylindraceus* en *Solen capensis* is met die electronmikroskoop bestudeer. Die morfologie van die sperma van albei spesies is soortgelyk. Albei is primitief met 'n kop (omtrent 1,5 μm lank), middelstuk van vyf mitokondria en stert. Die kop bestaan uit 'n vaatvormige, elektrondigte kern (omtrent 1,1 μm × 1,2 μm) wat bedek is deur 'n klein keëlvormige akrosoom. Die morfologie van die akrosoom van *Solen* is tipies van 'heterodont' tweekleppiges, nietemin het elke spesie 'n akrosoom van verskillende dimensies wat dus gebruik kan word om die twee naverwante tweekleppiges te skei. Gedurende spermatogenese is die patroon van die kernkromatinkondensasie korrelrig. Die akrosoom word gevorm deur 'n enkele Golgiliggaampie, en die vyf groot mitokondria van die middelstuk word waarskynlik gevorm deur die samesmelting van etlike klein mitokondria.

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Members of the bivalve family Solenidae are modified for burrowing rapidly into soft sediments. They are easily recognized as they have cylindrical shells which are elongated along the anterior/posterior axis. Two species are commonly found in the estuaries of southern Africa, *Solen cylindraceus* Hanley (Knysna to Natal) and *S. capensis* Fischer (Saldahna Bay to East London) (Day 1981; Kilburn & Rippey 1982). Within an estuary, *S. cylindraceus* prefers muddy substrates whereas *S. capensis* inhabits sand banks. Except for the work of McLachlan (1974), McLachlan & Erasmus (1974) and Hodgson (1984) very little is known about the biology of *Solen*. Indeed the family Solenidae are poorly researched bivalves. This is perhaps surprising as these animals can occur in very high densities. In South Africa, for example, *S. cylindraceus* reaches densities of 400 m^{-2} (Blaber, Kure, Jackson & Cyrus 1983; Hodgson 1987) and is thus an important component of the macrobenthos.

Although it is rare for the distribution of the two species to overlap within an estuary, small specimens are often difficult to identify by external morphological characteristics. The shell of large *S. capensis* (> 3cm) always bears a groove bordering the anterior margin but this feature is often lacking in smaller specimens (Kilburn & Rippey 1982). A number of workers have suggested that spermatozoon morphology could be used in the taxonomy of bivalves (Daniels, Longwell, McNiff & Wolfgang 1971; Gharagozlou-van Ginneken & Pochon-Masson 1971; Popham, Dickson & Goddard 1974; Franzén 1983; Hodgson & Bernard 1986a, b). Comparative studies by Hodgson & Bernard (1986 a, b) on closely related species of mytilid bivalves and of van der Horst, Bernard, Salie & Maasdorp (1986) on two species of *Donax*, have indeed shown that sperm structure can be used to distinguish between closely related species.

The aims of this study were to describe the structure of the sperm of two members of the Solenidae, to determine whether sperm morphology can be used to separate species of *Solen*, and to examine spermatogenesis.

Materials and Methods

Specimens (4–5 cm long) of *Solen cylindraceus* and *S. capensis* were collected during June 1986 from the Bushmans and Kariega estuaries of the eastern Cape Province, South Africa. The animals were transported back to the laboratory where portions of the testis were immediately prepared for electron microscopy.

Small portions of the testis were excised from animals, placed in 4% glutaraldehyde in cacodylate buffer (pH 7,4 at 4°C) and left in the fixative overnight. After buffer washing (cacodylate buffer, pH 7,4), the pieces of tissue were post-fixed in 1% osmium tetroxide for 90 min, dehydrated and embedded in an Araldite/Epon resin mixture via propylene oxide. Thin sections (about 65 nm) were cut, contrasted with uranyl acetate and lead citrate, and examined with a JEOL 100 CXII microscope.

Results

Spermatogenesis

Spermatogenesis was found to be very similar in *Solen*

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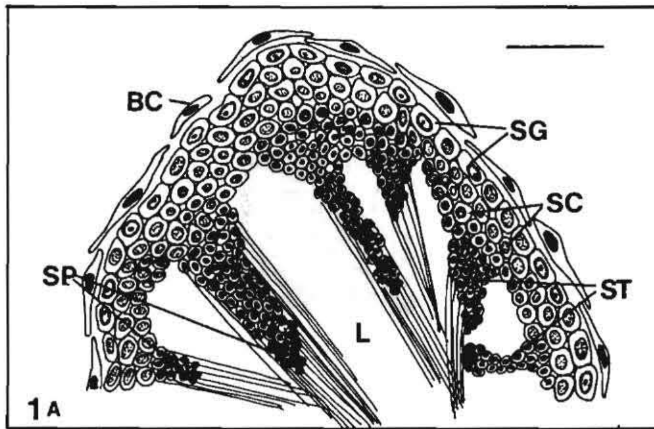


Figure 1A Diagrammatic representation of a light micrograph of a section through a germinal follicle in the testis of *S.cylindraceus*, showing the arrangement of spermatogonia (SG), spermatocytes (SC), spermatids (ST), and spermatozoa (SP). BC = basal cell; L = lumen. Scale bar = 30 μm.

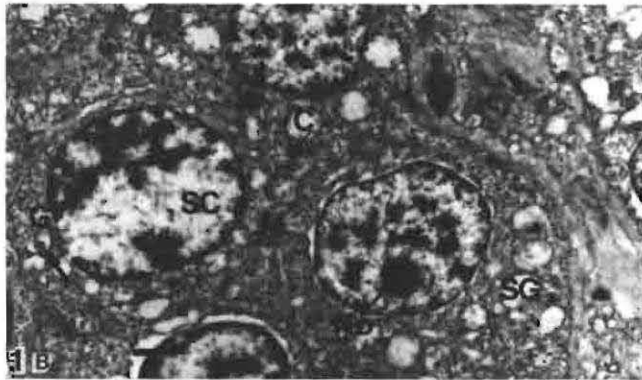
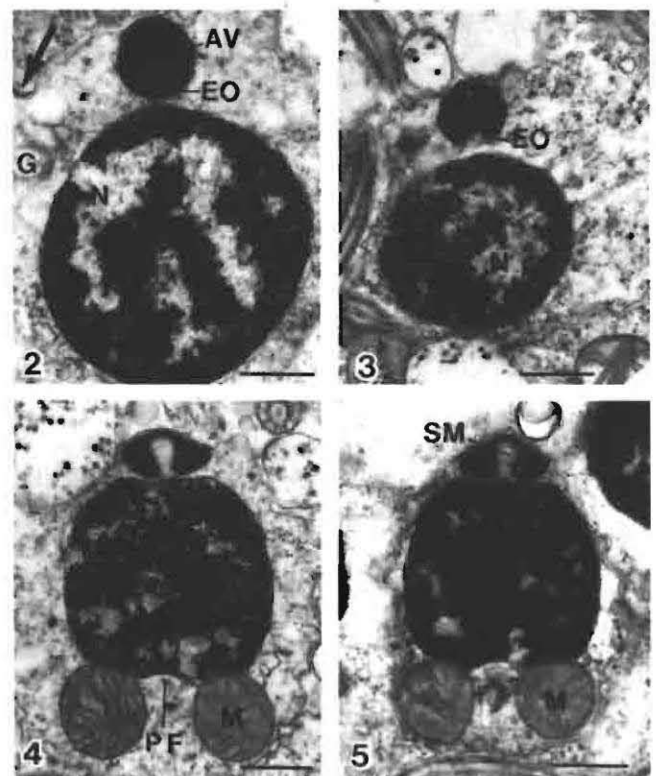


Figure 1B Section through the periphery of a germinal follicle of *S. cylindraceus*, showing a spermatogonium (SG) and spermatocyte (SC). C = centriole; G = Golgi body forming a proacrosomal vesicle (arrowed); M = mitochondrion; No = nucleolus. Scale bar = 1 μm.

cylindraceus and *S. capensis* and a single description will therefore be given. Most stages of spermatogenesis can be observed in the testis (Figure 1A) throughout the year. Spermatogonia lie close to the periphery of each germinal follicle (Figure 1A, B), and as the cells divide and mature they are displaced towards the centre (Figure 1A). Spermatogonia are characterized by an oval nucleus (3,5 × 4,5 μm) with a prominent nucleolus (0,7 μm diameter) (Figure 1B). The nucleus contains small clumps of electron-opaque chromatin which are often associated with the inner nuclear membrane. Cellular organelles are sparse, the cytoplasm containing a few mitochondria with poorly developed cristae and small amounts of rough endoplasmic reticulum. The spermatocytes are very similar in structure to the spermatogonia. However, the nucleolus is no longer prominent, and the chromatin is in the form of a patchwork (Figure 1B). The cytoplasm contains a similar complement of organelles to the spermatogonia, as well as a small Golgi body with three or four cisternae, and a few dense osmiophilic granules (Figure 1B). These granules appear to be formed by the Golgi body.

The early spermatid has a spherical nucleus (2 μm diameter) which occupies the centre of the cell. The cytoplasm contains numerous mitochondria, rough endoplasmic reticulum, a Golgi body and a few proacrosomal vesicles. Intercellular bridges, the plasma membrane of which is thickened, connect the spermatids (Figure 2) . As the spermatid matures and is displaced towards the centre of the germinal follicle, cytoplasm is lost by sloughing. The nuclear contents condense (Figures 2 – 5) and a single spherical proacrosomal vesicle, which is of uniform electron opacity (0,5 μm diameter) occupies the presumptive anterior end of the cell. The mitochondria become reduced in number, but increase in size and in addition, have well-developed cristae. As development progresses the mitochondria come to occupy a position in the cell opposite to that of the acrosome (Figure 4). The increase in size and decrease in mitochondrial number is believed to occur by mitochondrial fusion as illustrated by Figure 6. In conjunction with mitochondrial development, the two centrioles migrate to the posterior end of the cell where they lie between the mitochondria (Figure 5) thus beginning to form the sperm mid-piece. Chromatin condensation is of the granular pattern as described by Maxwell (1983). The homogeneous chromatin of the



Figures 2–5 Longitudinal sections through spermatids showing stages of chromatin condensation of the nucleus (N) and development of the acrosomal vesicle (AV). An electron-opaque (EO) region of the acrosome (Figures 2 & 3) lies close to the nuclear envelope. G = Golgi body; M = mitochondrion; PF = posterior fossa; SM = subacrosomal material. A cytoplasmic bridge is arrowed in Figure 2. Scale bar = 0,5 μm.

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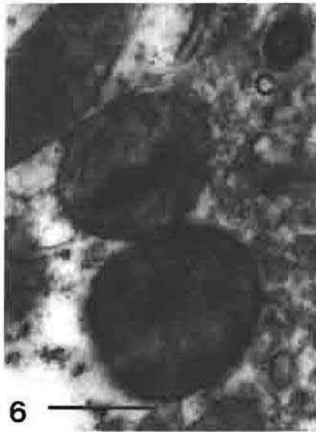


Figure 6 Transmission electron micrograph showing possible fusion of adjacent mitochondria in a spermatid. C = centriole. Scale bar = 0,5 μm.

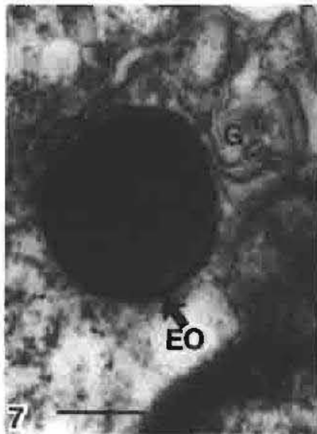
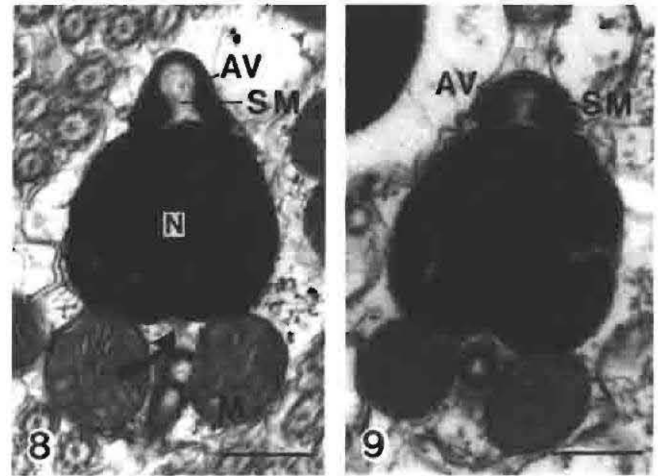


Figure 7 Early stage in acrosome development with the electron-opaque (EO) region lining the side furthest from the Golgi cisternae (G). Scale bar = 0,25 μm.

early spermatid develops a granular substructure (Figures 2 – 4) with a granular diameter of about 50 nm. Such a pattern of chromatin condensation has been described in other bivalves which have a sperm with an almost spherical nucleus (Maxwell 1983). As the chromatin of the nucleus condenses, the nucleus becomes more barrel-shaped with a flattened anterior end (Figures 4 & 5) and a small posterior fossa (Figure 4). Differentiation of the acrosomal vesicle begins with the development of an electron-opaque layer on the side of the acrosome furthest from the Golgi cisternae (Figure 7). This electron-opaque region eventually comes to face the nuclear envelope (Figures 2 & 3). The proacrosome then begins to invaginate on its adnuclear surface (Figure 3) and gradually assumes, during maturation, a conical form (Figures 4 & 5).

Structure of the spermatozoa

The spermatozoa of *S. cylindraceus* and *S. capensis* have a very similar morphology (Figures 8 & 9). Both are small being only 50 μm long. The sperm are of the



Figures 8 & 9 Transmission electron (mid-L.S. sections) micrographs of the spermatozoa of *S. cylindraceus* (Figure 8) and *S. capensis* (Figure 9). AV = acrosomal vesicle; M = mitochondrion; N = nucleus; SM = subacrosomal material. Posterior fossa is arrowed. Scale bars = 0,5 μm.

primitive type (Franzén 1955) and comprise three regions; a head, mid-piece and tail. The mid-piece of both species consists of a ring of spherical mitochondria (0,6 μm diameter) with large cristae, the mitochondria surrounding the proximal and distal centrioles (Figures 8 & 9). The tail (48 μm long), which has a 9 + 2 arrangement of microtubules originates from the distal centriole .

The head of the spermatozoon incorporates a barrel-shaped electron-opaque nucleus, (1,2 μm diameter) which is capped by a small acrosome. When seen in mid-longitudinal section the nucleus has a small but distinct posterior fossa (Figures 8 & 9). The acrosome is of differing dimensions in the two species. That of *S. cylindraceus* is longer and thinner (0,4 μm long × 0,5 μm basal diameter) as compared to *S. capensis* (0,3 μm long × 0,7 μm basal diameter) (Table 1, Figures 10 & 11). Although having different dimensions, the acrosomes have a similar shape when seen in mid-longitudinal section with the bulk of the acrosomal material confined to the base of the acrosome. In addition both acrosomes appear to be composed of two

Table 1 Dimensions of the head and mid-piece of the spermatozoa of *Solen.*

		Solen cylindraceus	*Solen capensis*
Acrosome	length (μm)	0,4 ± 0,01	0,3 ± 0,03
	width at base (μm)	0,5 ± 0,001	0,7 ± 0,002
Nucleus	length (μm)	1,08 ± 0,02	1,18 ± 0,06
	diameter (μm)	1,15 ± 0,06	1,25 ± 0,05
Mitochondria	number	5	5
	diameter (μm)	0,5–0,6	0,5–0,6
Overall head length (μm)		1,48 ± 0,07	1,5 ± 0,06

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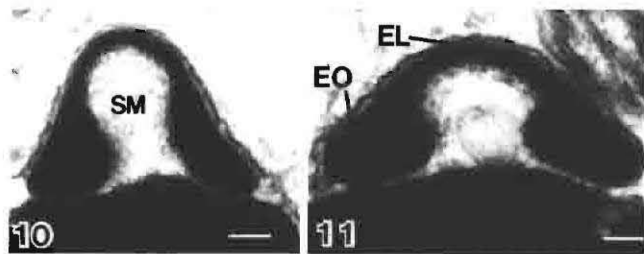

Figures 10 & 11 Longitudinal sections through the acrosomes of *S. cylindraceus* (Figure 10) and *S. capensis* (Figure 11). EO = electron-opaque region of acrosome; EL = electron-lucent region of the acrosome; SM = subacrosomal material. Scale bar = 0,1 μm.

materials, there being electron-opaque and electron-lucent regions. The electron-opaque areas are restricted to the base of the acrosome (Figures 10 & 11). Beneath the acrosome is diffuse subacrosomal material (Figures 10 & 11).

Discussion

The observations on the structure of the spermatozoon of *Solen cylindraceus* and *S. capensis* show that the two species differ only in the size of their acrosomes. This difference is consistent and can therefore be used to identify the two species. This finding adds further support to those workers who suggest that spermatozoon morphology could be of use in bivalve taxonomy (Daniels *et al.* 1971; Gharagozlou-van Ginneken & Pochon-Masson 1971; Popham *et al.* 1974; Franzén 1983; Hodgson & Bernard 1986a, b).

The structure of the acrosome of both species is typical of that of other members of the subclass Heterodonta (Hodgson & Bernard 1986b); the acrosomes having the majority of the acrosomal material confined to the base and sides of the vesicle. In addition the acrosome has an outer electron-opaque and an inner more electron-lucent region. Such differentiation has been recorded in the acrosomes of other bivalves (Popham 1974; Hylander & Summers 1977; Bernard & Hodgson 1985; Hodgson & Bernard 1986a, b; Bernard, Davies & Hodgson in press). These regions of differing electron opacity most probably reflect the differing functions of the acrosome during the process of fertilization. Hylander & Summers (1977) have shown that in the bivalves *Chama macerophylla* and *Spisula solidissima* the outer electron-opaque region of the acrosome is the region which binds the sperm to fibrillar tufts of the microvilli of the egg surface. It is, therefore, possible that the electron-opaque region of the acrosomes of *Solen* has a similar function.

The small difference in the structure of the sperm of the two species is probably not enough to prevent hybridization and this may account for those individuals which display external characteristics of both species. Although the binding agent of the acrosome is thought to play a role in gamete recognition, and in some invertebrates is species specific, (Summers & Hylander 1975, 1976; Hylander & Summers 1977) this does not prevent cross fertilization of some closely related bivalves. For example *Mytilus edulis* and *Mytilus galloprovincialis* have been shown to have acrosomes of differing morphologies (Hodgson & Bernard 1986a), yet hybridization between the two is possible (Lubet, Prunus, Masson & Bucaille 1984).

Finally spermatogenesis, as described here, is similar to that described for other bivalves with primitive spermatozoa (Longo & Dornfeld 1967; Longo & Anderson 1969; Maxwell 1983; Bernard & Hodgson 1985; Hodgson & Bernard 1986b; Bernard *et al.* in press). Early stages in the formation of the single acrosomal vesicle of the early spermatid have not yet been observed. However it is probable that the proacrosomal vesicles, produced by the Golgi body, fuse as has been shown in other invertebrates (Dan 1970; Bernard & Hodgson 1985; Hodgson & Bernard 1986b). Later stages in acrosome formation mirror the findings of Dan (1970). Our observations also add further support to the theory that, in bivalves, the large mitochondria of the sperm mid-piece are formed by fusion of several small mitochondria (Longo & Dornfeld 1967; Bernard & Hodgson 1985; Hodgson & Bernard 1986b).

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