AN ULTRASTRUCTURAL STUDY OF SPERMATOOZOA OF *HELIX ASPERSA* AND *HELIX POMATIA* (GASTROPODA, PULMONATA)

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(Received 23 May 1988, accepted 17 August 1988)

ABSTRACT

Spermatozoa of the pulmonates *Helix aspersa* Müller and *H. pomatia* Linnaeus are examined in detail using transmission electron microscopy (TEM). Important features such as the acrosome, perinuclear sheath, nucleus and terminal region of the midpiece are described for the first time. Also presented are the first ultrastructural observations on spermatozoa from spermatophores in any pulmonate gastropod (*H. aspersa*). No morphological differences could be found between sperm taken from spermatophores and those within the hermaphrodite duct in *H. aspersa*. Spermatozoa of *H. aspersa* and *H. pomatia* show all the characteristics of euthyneuran spermatozoa, namely: a helically-keeled nucleus; distinctive arrangement of acrosomal components (apical vesicle, acrosomal pedestal), and extremely elongate midpiece (axoneme and glycogen helix enclosed by matrix and paracrystalline layers). The sperm nucleus of both species is short, and the midpiece also forms the terminal portion of the spermatozoon (glycogen piece absent). The extraordinary positioning of the acrosome in *H. aspersa*—reflected backwards from the nuclear apex—is not observed in *H. pomatia*, though a perinuclear sheath (possibly another acrosomal component) is present in sperm of both species. *Helix* spermatozoa are compared with other euthyneuran sperm and briefly discussed from the systematic viewpoint.

INTRODUCTION

Previous studies of *Helix aspersa* Müller spermatozoa have demonstrated the internal structure and cytochemistry of the centriolar derivative ('basal body derivative') and various regions of the midpiece (Anderson & Personne, 1967, 1969a,b, see also 1970, 1976; Anderson *et al.*, 1968; Personne & Anderson, 1970; Ritter & André, 1975). These reports have dealt exclusively with sperm taken from the hermaphrodite duct: no ultrastructural observa-

tions have yet been published on spermatophore sperm in *H. aspersa* or any other euthyneuran gastropod (with the exception of two micrographs of *Runcina* sperm tails: Kress, 1985). Spermatozoa of *H. aspersa* (and other pulmonates) were examined by Maxwell (1975) using scanning electron microscopy (SEM) and Anderson & Personne (1976) have presented a small SEM micrograph of the spermatozoon of an unspecified *Helix* species (?*H. aspersa*). Some aspects of Golgi activity (Meek & Bradbury, 1963; Dauwalder & Whaley, 1975), nuclear maturation (Bloch & Hew, 1960) and midpiece development (Tahmisian, 1964; see also Anderson *et al.*, 1968) have been investigated in *H. aspersa* but the necessity for a full study of spermiogenesis in this important species is still apparent. It should also be noted that, at present, knowledge of sperm development in both euthyneuran groups (Pulmonata and Opisthobranchia) is limited to a few species, of which only two or three are known in any detail. The purpose of this paper is to demonstrate for the first time the morphology of the entire spermatozoon of *H. aspersa* (taken from the hermaphrodite duct and spermatophores after copulation) with particular emphasis being placed on the acrosome and the terminal region of the spermatozoon. The unusual arrangement of acrosomal components in *H. aspersa* prompted us to study spermatozoa of another *Helix* species (*H. pomatia* Linnaeus). These results, though limited to observations on sperm from the hermaphrodite duct of *H. pomatia*, are presented herein.

MATERIALS AND METHODS

Helix aspersa were collected from gardens in Brisbane (southern Queensland, Australia) and Sydney (New South Wales, Australia) between September and November (spring months). Animals were seen paired in November (Sydney) and from these individuals were obtained freshly deposited spermatophores. *H. poma-*

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Fig. 1. *Helix aspersa*: hermaphrodite duct spermatozoa. A. Longitudinal section (L.S.) of spermatozoan head showing poorly developed helical keels, portion of acrosomal pedestal, and perinuclear sheath (near nuclear apex). B. L.S. complete acrosome and anterior region of nucleus. Note: post-

eriorly-directed posture of apical vesicle and acrosomal pedestal. C. Transverse section (T.S.) through tip of nucleus and acrosomal pedestal. D. Oblique T.S. of acrosomal pedestal, nuclear tip, and perinuclear sheath, showing true profile of the acrosomal pedestal. E. T.S. through apical vesicle, nucleus

tia from the United Kingdom were obtained through the courtesy of Professor R. Chase.

Spermatophores (only *H. aspersa*) and hermaphrodite ducts (both *H. aspersa* and *H. pomatia*) were diced and fixed in 3% glutaraldehyde (at 0–4°C, in 0.1 M phosphate or cacodylate buffer, pH 7.2) for approximately two hours. Tissue pieces were then rinsed in buffer, osmicated for 80 minutes (1% osmium tetroxide in 0.1 M phosphate or cacodylate buffer at 0–4°C), rinsed again, dehydrated through a series of ethanols (20%–60% at 0–4°C, 70%–100% at room temperature) and gradually embedded in Spurr's epoxy resin. Thin sections were cut using LKB 2128 UM IV Ultratome and Huxley ultramicrotomes collected on coated or uncoated 200 mesh copper grids and stained with uranyl acetate and lead citrate. Specimen grids were examined with AEI Corinth 500, Hitachi 300, Siemens Elmiskop I and Philips 300 transmission electron microscopes.

RESULTS

Helix aspersa (hermaphrodite duct spermatozoa) Acrosome and nucleus.

The nucleus is approximately 8.1 µm in length, basally invaginated and exhibits one, or possibly as many as three, poorly developed helical keels (Fig. 1A). An acrosome is present and is composed of: (1) an apical vesicle (spherical, 0.2 µm in diameter) situated beside the tapered nuclear apex, and (2) a less electron-dense acrosomal pedestal (length 0.33 µm, circular in true transverse section) which connects the tip of the nucleus to the apical vesicle (Figs. 1B–E; Fig. 2A). A third, possibly acrosomal component (composed of a very electron-dense layer embedded in fine granular material) is intimately associated with the anterior half of the nucleus and supports the base of the apical vesicle and acrosomal pedestal (see Figs. 1A–G; Fig. 2A). This component is here termed the perinuclear sheath. The degree to which this sheath encloses the nucleus varies according to the level of section—the most complete enclosure of nucleus occurring near the nuclear apex

(below level of acrosomal pedestal) and perinuclear sheath. The electron-dense component of the sheath surrounds the nucleus while the less-electron-dense component abuts the apical vesicle. F. T.S. through nucleus and perinuclear sheath below level of apical vesicle and acrosomal pedestal—bilateral symmetry of the sheath is obvious. G. T.S. of nucleus and perinuclear sheath below level shown in Fig. 1F—note that the perinuclear sheath only surrounds approximately half of the nuclear membrane at this level. All scales = 0.2 µm except: 1A (scale = 1 µm), 1B (scale = 0.5 µm).

as shown in Figs. 1D–F. Transverse sections taken at lower levels of the nucleus (for example Fig. 1G; for orientation see also Fig. 1B), show the perinuclear sheath lining one half or less than one half of the nuclear membrane. Both transverse and longitudinal sections of the perinuclear sheath (and nucleus) reveal the bilateral symmetry of this component and of the associated apical vesicle and acrosomal pedestal (see Figs. 1B–G; Fig. 2A).

Coarse fibres, 'neck' region

Nine coarse fibres accompany the axonemal doublets throughout most of the midpiece (Figs. 2C–F, see also H) and together with the two central microtubules of the axoneme, attach to the centriolar derivative within the basal invagination of the nucleus (Fig. 2C). Figure 2C shows that the coarse fibres are thick (maximum width 0.13 µm) and periodically banded within the sperm 'neck' region (periodicity ranging from 400–500 Å). A well developed distal accessory sheath (length approximately 0.37 µm, width approximately 0.16 µm) surrounds the central pair of axonemal microtubules (Figs. 2C, D). At the base of the distal accessory sheath, an electron-dense granule is often observed (Fig. 2C arrow) and is also apparent in micrographs of *Euhadra hickonis* spermatozoa presented by Takaichi (1975) and Takaichi & Sawada (1973). The purpose or composition of this granule in *Helix* and *Euhadra* is unknown, but possibly it may act as a connecting link between the axonemal doublets (emergent from the coarse fibres) and the distal accessory sheath. From the base of the distal accessory sheath to the nuclear invagination, the axonemal doublets are individually enveloped (and therefore greatly obscured) by their accompanying coarse fibres (see Fig. 2C (longitudinal section), Figs. 2B, D (transverse section)). The composite coarse fibres and enclosed doublets are continuous with the cortical region of the centriolar derivative (see Fig. 2C). In contrast the central pair of axonemal microtubules (which in fact appear solid and not tubular in transverse section) attach directly to the core of the centriolar derivative (see Fig. 2C).

Midpiece

Some, though very minor, overlap of the nuclear base and midpiece is visible in longitudinal and transverse sections of the 'neck' region (see Figs. 2C, D). Initially the coarse fibres (with enclosed axonemal doublets) are surrounded only by

Fig. 2. *Helix aspersa*: hermaphrodite duct spermatozoa. A. Longitudinal section (L.S.) acrosomal pedestal and nuclear tip (detail of Fig. 1A)—note perinuclear sheath (this section taken at approximately 90° to the plane of section shown in Fig. 1B). B. Transverse section (T.S.) through basal invagination of nucleus showing central axonemal microtubules, and (arrows) axonemal doublets (faintly

visible) embedded in coarse fibres. C. L.S. sperm 'neck' region showing basal invagination of nucleus, centriolar derivative, periodically banded coarse fibres (continuous with cortical portion of centriolar derivative), distal accessory sheath, and also matrix and paracrystalline components of mitochondrial derivative. Note at base of distal accessory sheath, emergence of axonemal doublets from coarse fibres and

paracrystalline material (Figs. 2C, D). However, further posteriorly, the coarse fibres and emergent axonemal doublets are surrounded by both paracrystalline and matrix components of the mitochondrial derivative (see Figs. 2C, E, F). A single, glycogen helix is also enclosed by the mitochondrial derivative, but, in the immediate post-nuclear region of the midpiece, is occupied by tightly-packed membranous deposits. These deposits gradually become supplanted by glycogen granules in succeeding levels taken through the midpiece (see Figs. 2E, F, H). The shape of the midpiece changes considerably throughout its length, such that the glycogen helix gradually becomes reduced in extent (compare Figs. 2F, H with Figs. 3A, B) and eventually disappears altogether (Figs. 3C, D). The terminal region of the midpiece (the final 2.2 μm of the spermatozoon) commences with the loss of the two, central microtubules of the axoneme and is followed sequentially by: (1) the loss of all nine axonemal doublets (occurring simultaneously), (2) a plug of electron-dense material, (3) a partially occupied vesicle, (4) membrane deposits (see Figs. 3F, E for successive transverse sections through this region) and finally (5) a second, apparently empty vesicle. Figure 3G shows in full longitudinal section, the complete series of these structural changes within the midpiece. The mitochondrial derivative, therefore, forms the posterior extremity of the spermatozoon in *Helix aspersa*—there being no vestige of either a glycogen piece or dense ring structure (both features often occurring in sperm of opisthobranchs, basommatophoran pulmonates, and in euspermatozoa of all meso- and neogastropod prosobranchs).

(arrow) presence of a small, electron dense granule (? function). D. T.S. through sperm 'neck' region. Portions of nucleus and mitochondrial derivative (chiefly paracrystalline material) are visible as are coarse fibres (with enclosed axonemal doublets obscured in this section) and distal accessory sheath. E, F. T.S. through midpiece: E. immediately below nucleus (—note membranous deposits partly occupying glycogen helix; double matrix layer); F. posterior to 2E (—note absence of membranous deposits; single matrix layer; also intra-axonemal (glycogen) granules). G. Paracrystalline and matrix components of mitochondrial derivative as seen in oblique section. H. L.S. of midpiece at the same level as 2F. Scales. A, C, D, H (scale = 0.5 μm); B, E, F, G (scale = 0.2 μm).

Helix aspersa (spermatophore spermatozoa)

Spermatozoa from spermatophores (freshly exchanged during copulation) do not differ morphologically from those stored within the hermaphrodite duct. Figs. 4A-C show that the apical vesical and acrosomal pedestal are angularly positioned relative to the sperm longitudinal axis. The perinuclear sheath is fully intact and still retains its layered appearance (Figs. 4C-F). Other components of the spermatozoon, including the nucleus (Figs. 4A-F), midpiece (Figs. 4G, H) and terminal region of the cell (Fig. 4H) are as described for hermaphrodite duct spermatozoa.

Helix pomatia

Spermatozoa from only the hermaphrodite duct of this species were examined. The structure of the midpiece, nucleus and neck region (Figs. 5A-E) is essentially as described for *H. aspersa*. An important difference lies in the arrangement of the acrosomal complex. In *H. pomatia*, the apical vesicle (diameter 0.18 μm) and acrosomal pedestal (length, including pad over nuclear tip, approximately 0.25 μm , diameter 0.18 μm) lie on the nuclear apex (Figs. 5A, B) at only a slight angle to the sperm longitudinal axis (as opposed to the acute angle between nucleus and vesicle-pedestal complex in *H. aspersa*). The perinuclear sheath is present in *H. pomatia* spermatozoa (Figs. 5A, B) but lacks the layered organization seen in *H. aspersa* (compare with Figs. 1B-G, Figs. 4C-F). The length of the nucleus is approximately 10 μm .

DISCUSSION

Comparison with other euthyneuran spermatozoa

General features of euthyneuran spermatozoa. Euthyneuran spermatozoa—including those of *Helix aspersa* and *H. pomatia*—possess the following general characters: (1) an acrosome composed of an apical vesicle (usually round) and an acrosomal pedestal (variously shaped depending on taxon, often columnar), (2) helically-keeled nucleus (either distinct from or partially intertwined with the axoneme and mitochondrial derivative), (3) midpiece composed of a complex mitochondrial derivative—with one or more incorporated glycogen helices—which surrounds the axoneme and associated coarse fibres, and (4) ordered (usually 9 + 1) rows of intraaxonemal glycogen granules

throughout midpiece and, if present (absent in *Helix*), the glycogen piece. While exceptional character states occur in spermatozoa of some undisputed euthyneurans (for example, the absence of coarse fibres in spermatozoa of the opisthobranch *Tornatina* sp.—see Healy, 1982), the above listed features readily distinguish opisthobranch and pulmonate spermatozoa from the euspermatozoa of meso- and neogastropod prosobranchs.

'Neck' region and midpiece

The midpiece region of *Helix aspersa* spermatozoa has been the subject of previous ultrastructural studies (see Anderson & Personne, 1967, 1969a, b, see also 1970, 1976; Anderson *et al.*, 1968; Personne & Anderson, 1970; Ritter & André, 1975). These papers, in conjunction with much work on other pulmonate species, have dealt with the mitochondrial derivative and have helped to establish the presence of a glycogen-filled helix ('helice principale' of André, 1962) within the derivative. They have also explored the morphology and biochemistry of the two constituents of the derivative—the matrix material ('helice secondaire' of André, 1962) and the paracrystalline material. Anderson & Personne (1976) have presented a comprehensive review of the complex enzymatic and energy pathways occurring within the mitochondrial derivative of pulmonate spermatozoa and detailed the probable functional relation-

ships between the two components of the derivative, the incorporated glycogen helix (or helices) and the coarse fibres. They propose that the following metabolic function(s) can be ascribed to specific midpiece components: matrix material—Krebs tricarboxylic acid cycle; glycogen helix (or helices)—phosphorylase, dehydrogenase activity; axoneme, coarse fibre-axonemal doublet interface, surface of centriolar derivative—sites of Mg^{++} activated ATPase; paracrystalline material—cytochrome oxidase activity. Workers who have examined in detail the architecture of paracrystalline material in pulmonate spermatozoa generally agree that it is a continuous, three dimensional latticework composed of proteinaceous, hollow rodlets or granules (80–90 Å in diameter) (André, 1962; Ritter & André, 1975; Anderson & Personne, 1976; Reger & Fitzgerald, 1982; Reger *et al.*, 1982). Such an interpretation applies equally well to paracrystalline material of opisthobranch spermatozoa (for example see Healy, 1982; Healy & Willan, 1984) since it appears, morphologically at least, indistinguishable from that occurring in pulmonate sperm. Paracrystalline material has also been demonstrated within the midpieces of certain prosobranch euspermatozoa (*Cochlostoma montanum*—Selmi & Giusti, 1980; *Stenothyra* sp.—Healy, 1983a; *Cypraea errones*—Healy, 1986a but in all prosobranch cases the arrangement and form of this material differs substantially from that observed in sperm of euthyneurans. Ritter & André (1975) found that the 'crystal' (=paracrystalline material) of *Helix aspersa* spermatozoa, together with its contained cytochromes, resists even prolonged trypsin treatment, but is readily digested by pepsin. Their observation that cytochrome content of the paracrystalline material increases during the period of trypsin treatment (10 min to 12 h) seems to correlate well with the fact that trypsin can induce motility in otherwise immotile hermaphrodite duct spermatozoa of *Helix aspersa* (see Anderson & Personne, 1967). Anderson & Personne (1976) have even suggested that trypsin may directly stimulate ATPase activity within the axoneme/coarse fibre complex. However trypsin treatment does not apparently elicit movement in hermaphrodite duct spermatozoa of another stylommatophoran *Anguispira alternata* (Atkinson, 1982). It is likely that other chemical factors—intrinsic or extrinsic—may govern sperm motility, or at least, determine the degree of response of pulmonate sperm to stimulants such as trypsin.

While corroborating the work of other

Fig. 3. *Helix aspersa*: hermaphrodite duct spermatozoa. A. Transverse section (T.S.) of midpiece below level shown in Figs. 2G,H. The glycogen helix and components of the mitochondrial derivative have been reduced in extent. B. Longitudinal section (L.S.) through midpiece region shown in 3A. Intraaxonemal granules are clearly visible. C. T.S. through midpiece following termination of glycogen helix (axoneme/coarse fibre complex surrounded only by mitochondrial derivative). D. L.S. through midpiece region shown in 3C. G,E,F. L.S. and T.S. through terminal region of midpiece. Sequence of structural changes is best shown in 3G—note: (1) loss of central microtubules of axoneme, followed soon after by loss of doublets, (2) an electron-dense plug of material replaces axoneme, (3) a vesicle, partly occupied by dense material, (4) membranous deposits, (5) a second, more extensive vesicle. Note how mitochondrial derivative continues to the very tip of the spermatozoon. E,F are T.S. through upper (F) and lower (E) levels of the membranous deposit (area 4) shown in 3G. Scales. A,C,E,F (scale = 0.2 µm); B,D,G (scale = 0.5 µm).

Fig. 4. *Helix aspersa*: spermatophore spermatozoa. A. Longitudinal section (L.S. slightly oblique) showing angularly tilted apical vesicle and acrosomal pedestal near nuclear apex. B. L.S. of acrosome. C. Transverse section (T.S.) near nuclear apex showing nucleus, perinuclear sheath and laterally positioned apical vesicle. D, E. T.S. through anterior portion of nucleus and (layered) perinuclear sheath. F. T.S. through nucleus and base of perinuclear sheath. G. T.S. through anterior portion of midpiece. H. T.S. through posterior (left) and near-terminal (right) regions of the midpiece. Scales. A-H (scale = 0.2 μ m).

Fig. 5. *Helix pomatia*: hermaphrodite duct spermatozoa. A. Longitudinal section (L.S.) acrosomal complex (apical vesicle, acrosomal pedestal, perinuclear sheath) and anterior portion of nucleus. B. L.S. apical vesicle and acrosomal pedestal positioned at apex of nucleus. C. L.S. neck region of spermatozoon showing distal accessory sheath, centriolar derivative, coarse fibres (banded), nucleus and components of the mitochondrial derivative. D. Detail of paracrystalline component of mitochondrial derivative. E. Transverse sections through anterior region of midpiece and posterior region of midpiece. Scales. A, C, E (scale = 0.5 µm); B, D (scale = 0.2 µm).

authors who have studied the midpiece of *Helix aspersa* spermatozoa, our results have in addition shown that the axoneme terminates abruptly, and symmetrically, within the midpiece (2.2 μm from the posterior extremity of the spermatozoon), and is replaced by electron-dense material, two chamber-like vesicles and closely packed membranes. The same appears to be true of *H. pomatia* though we have not observed longitudinal sections of the terminal region in that species. The terminal region of *Euhadra hickonis* spermatozoa (see Takaichi, 1975, Figs. 12-15) is similar to that of *H. aspersa*, as in fact, are other features of the midpiece and neck region (including the presence of an electron-dense granule near the distal accessory sheath—see Takaichi, 1975; Takaichi & Sawada, 1973; Dan & Takaichi, 1979). The glycogen piece observed in spermatozoa of many opisthobranchs and basommatophoran (s.l.) pulmonates (Anderson & Personne, 1970; Ohsako, 1971; Thompson, 1973; Kitajima & Paraense, 1976; Ackerson & Koehler, 1977; Maxwell, 1980; Healy, 1982, 1983b; Healy & Willan, 1984) and in all investigated meso- and neogastropod euspermatozoa (see Healy, 1983c for references), does not occur in spermatozoa of *Helix aspersa*, *H. pomatia*, *Euhadra hickonis*, or, as far as is known, in any stylommatophoran pulmonate. In some opisthobranchs, the glycogen piece is short and may show partial degeneration or (rarely) total absence of the axoneme (Healy & Willan, 1984; Healy 1984). Such a trend towards regression of the glycogen piece in euthyneuran spermatozoa may be due to the fact that substantial glycogen deposits are enclosed by the mitochondrial derivative, thereby reducing the need perhaps for a glycogen piece (Healy & Willan, 1984).

Only a single glycogen helix occurs in spermatozoa of *Helix aspersa*, *H. pomatia* and other stylommatophorans (herein; Grasse *et al.*, 1956; André, 1962; Personne & André, 1964; Anderson & Personne, 1969a, b; Anderson *et al.*, 1968; Yasuzumi *et al.*, 1974; Ritter & André, 1975; Takaichi & Sawada, 1973; Takaichi, 1975; Dan & Takaichi, 1979; Shileiko & Danilova, 1979; Odiete, 1982; Atkinson, 1982; Reger & Fitzgerald, 1982) and this is also the case for most opisthobranchs (see Holman, 1972; Thompson & Bebbington, 1969; Healy, 1982; Healy & Willan, 1984; Healy, 1984) and at least some basommatophoran (s.l.) pulmonates (*Salinator*, *Siphonaria*—Healy, 1983b; *Onchidium*—Healy, 1986b). Multiple helices (ranging from two to three, possibly as many as four in some species) have been noted in sperm of some

basommatophorans (s.l.) (lymnaeids, planorbids, ellobiids—Anderson & Personne, 1970; Ohsako, 1971; Kitajima & Paraense, 1976; Healy, 1983b; see also Thompson, 1973) and certain cephalaspid opisthobranchs (Thompson, 1973—*Acteon*; Healy, 1984—*Bullina*). The possibility that some stylommatophoran spermatozoa may possess more than a single glycogen helix within the midpiece remains to be explored.

Finally, some comment should be made concerning the internal structure of the nine coarse fibres and their relationship with the axonemal doublets and centriolar derivative. In *Helix aspersa*, and *H. pomatia* (herein; see also Anderson & Personne, 1967): (1) the doublets are enveloped by the coarse fibres between the base of the distal accessory sheath and the centriolar derivative, and (2) the coarse fibres (with enclosed axonemal doublets) fuse to the cortex of the centriolar derivative. Precisely the same organization has been described in *Euhadra hickonis* (Takaichi, 1975; Dan & Takaichi, 1979) and other stylommatophorans (see Atkinson, 1982; micrographs of Maxwell, 1976; Yasuzumi *et al.*, 1974) some basommatophorans (s.l.) (Kitajima & Paraense, 1976; Healy, 1983b) and some opisthobranchs (nudibranchs—Healy, 1984). In some opisthobranchs however, the doublets either remain distinct from the coarse fibres or are only incompletely enclosed by them (Healy, 1984). Marked obscuring of the doublets by associated coarse fibres often makes it difficult to establish the exact spatial relationship between these two components in some species. Coarse fibres of all euthyneuran spermatozoa are periodically banded—the principal variables being the periodicity (ranging from 200 Å in *Anguispira alternata* (Atkinson, 1982), to 400–500 Å in *Helix aspersa* and *H. pomatia* (Anderson & Personne, 1967; herein) and as great as 750 Å in the lymnaeid *Radix japonica* (Ohsako, 1971)), width (variable depending on species and level of section), and persistence of periodicity into the midpiece region (according to Maxwell, 1976, banding in coarse fibres of *Discus rotundatus* extends from the neck region into the midpiece for a distance of up to 30 μm). The distal accessory sheath of *Helix aspersa* and *H. pomatia* spermatozoa is also observed in other stylommatophorans (*Euhadra hickonis*—Takaichi, 1975, Dan & Takaichi, 1973; Takaichi & Sawada, 1973; *Discus rotundatus*—Maxwell, 1976; *Limax flavus*—Yasuzumi *et al.*, 1974; *Bentosites bloomfieldi*—Healy, 1984) and in some basommatophoran (s.l.) species (*Biomphalaria glabrata*—Kitajima & Paraense, 1976;

Salinator fragilis, *S. solida*—Healy, 1983b). It is reminiscent of the inner dense cylinder described for the spermatozoal axoneme of the polychaete *Questa* by Jamieson (1983). No definite function for this structure has been determined, but it is likely that it performs some role in determining motility of the axial complex and does not, as suggested by Yasuzumi *et al.* (1974), represent the 'chromatoid body'.

Acrosome and nucleus

Perhaps the most interesting aspect of *Helix aspersa* and *H. pomatia* spermatozoa is the form of the acrosome and its positioning at the nuclear apex. Bloch & Hew (1960) recorded a marked difference in acid lability of nuclear histones between spermatozoa from the hermaphrodite duct and those from spermatophores in *H. aspersa*. We have observed no significant ultrastructural differences between spermatozoa taken from these two sources in *H. aspersa*—the apical vesicle and acrosomal pedestal continue to be angularly tilted, and associated with a perinuclear sheath within the spermatophore. The perinuclear sheath appears, therefore, to be a real feature of spermatozoa in *H. aspersa* and *H. pomatia*. Elsewhere within the Gastropoda, this structure has been recorded in the stylommatophoran *Bentosites bloomfieldi* (Healy, 1984; possibly also *Anguispira alternata*—Atkinson, 1982, and *Agriolimax reticulatus*—Bayne, 1970) and evidently in *Vaginulus borellianus* (see micrographs of Lanza & Quattrini, 1964).

The orientation of the acrosomal elements in *H. aspersa* may represent only an extreme situation within the Pulmonata, where 'grades' of tilting of the apical vesicle and acrosomal pedestal in relation to the longitudinal axis of the nucleus occur. A slightly angular orientation of the apical vesicle and acrosomal pedestal (relative to the nuclear tip) has been noted by Takaichi & Dan (1977) for spermatozoa of *Euhadra hickonis* and this is also the case in *Helix pomatia* (herein) and *Bentosites bloomfieldi* (Healy, 1984). In contrast, the acrosomal pedestal and apical vesicle of late spermatids of stylommatophoran species examined by Healy & Jamieson (unpublished data—*Pedinogyra hayii*, *Varohadra curtisiana*, *Xanthomelon pachystylum*), despite obvious signs of immaturity, are aligned in the longitudinal axis of the spermatid, and no equivalent of the perinuclear sheath can be identified.

Although we have studied spermatozoa from mature spermatophores, there is no guarantee

that the angular positioning of the *Helix aspersa* acrosome may not, in some way, alter prior to contact with the egg. Certainly an analysis of sperm-egg interaction would clarify this, and help determine what role the perinuclear sheath may play in fertilization.

The form of the sperm nucleus in *Helix aspersa* and *H. pomatia*—short, helically keeled, with a shallow basal invagination—occurs widely within the Euthyneura (? in all pulmonates) and almost certainly represents the primitive form for the ancestral euthyneuran spermatozoon. In contrast, sperm nuclei of some opisthobranchs (aplysiids—Thompson & Bebbington, 1969; Thompson, 1973; Kubo & Ishikawa, 1981; certain notaspids—Healy & Willan, 1984; the saccoglossan *Elysia australis*—Healy, 1984) take the form of an elongate cord which spirals around the proximal portion of the mitochondrial derivative and axial complex. Such a condition is here considered advanced and may have been independently acquired in these groups of opisthobranchs. The length of the spermatozoan head (that is, acrosome plus nucleus) of *Helix aspersa*, as determined in the present study is 8.1 μm —appreciably longer than the SEM results of Maxwell (1975) for this species (sperm head length $5.5 \pm 0.2 \mu\text{m}$), but reasonably close to the measurement of Anderson & Personne (1967) for typsin-treated spermatozoa (7.4 μm). Grasse *et al.*, (1956) give a length of 7.5 μm for the spermatozoan head of *H. pomatia*, while Thompson (1973) found this region of the spermatozoa of *H. pomatia* to measure 12 μm (including 2 μm acrosome). Our own observations on *H. pomatia* indicate a head length of approximately 10 μm . It seems possible that sperm head length may be variable in both *H. aspersa* and *H. pomatia* or that significant differences may exist between widespread populations. Further research on this aspect seems warranted. Table 1 summarizes comparative sperm morphology of *Helix* and other pulmonates.

Systematic significance of stylommatophoran spermatozoa

Apart from those studies dealing with the cytochemistry of pulmonate spermatozoa which have been briefly reviewed above, very little interest has been shown in comparative sperm morphology of the Stylommatophora. The order is large (over 50 families, approaching 15,000 species—see Boss, 1982) and diverse. Understandably, any comprehensive review of stylommatophoran spermatozoa would be onerous.

Table 1. Comparison of *Helix* and other Pulmonate Spermatozoa.

Taxon	Acrosome	Nucleus	Coarse fibre periodicity	Midpiece	Glycogen piece, annulus	Reference
ORDER BASOMMATOPHORA (S.L.)						
ONCHIDIOIDEA						
(<i>Onchidium damellii</i>)	0.87 µm long, pedestal tripartite and periodically banded	4 µm long	500 Å	1 glycogen helix	present (glycogen piece very short)	Healy, 1986b
SIPHONARIOIDEA						
(<i>Siphonaria funiculata</i>)	0.6 µm long, base of pedestal draped over nucleus	5 µm long	400 Å	1 glycogen helix	present	Healy, 1983b
AMPHIBOLOIDEA						
(<i>Salinator</i> spp.)	1.0 µm long, pedestal with helical perinuclear component	9-10 µm long	420 Å	1 glycogen helix	present, axoneme absent in glycogen piece	Healy, 1983b
ELLOBOIOIDEA						
(<i>Ophicardelus</i> spp.)	0.6 µm long	4.3 µm long	350-400 Å	3 glycogen helices	probably absent	Healy, 1983b
LYMNAEOIDEA						
(<i>Radix japonica</i>)	1.0 µm long, pedestal bilaterally symmetrical, basally draped over nucleus.	2.0 µm long	750 Å	2 glycogen helices	present	Ohsako, 1971
(<i>Lymnaea tessoni</i>)		2.0 µm long	630 Å	3 glycogen helices	present	Healy, 1983b
PLANORBOIDEA						
(<i>Biomphalaria glabrata</i>)	1.0 µm long, base of pedestal overlaps with nuclear apex	4.0 µm long	600 Å	2 glycogen helices	present	Kitajima & Paraense, 1976
ORDER STYLOMMATOPHORA						
SUCCINEOIDEA						
(<i>Succinea putris</i>)	0.6 µm long	4.9 µm long	7600 Å	1 glycogen helix	? absent	Shileiko & Danilova, 1979

ARIONOIDEA (<i>Anguispira alternata</i>)	0.45 µm long, pedestal overlaps with nucleus	13.0 µm long	200 Å	1 glycogen helix	? absent	Atkinson, 1982
RHYTIDOIDEA (<i>Pedinogyra hayii</i>)	0.22 µm long	8.0 µm long	400 Å	1 glycogen helix	? absent	Healy & Jamieson (Unpublished)
HELICOIDEA (BRADYBAENIDAE) (<i>Euhadra hickonis</i>)	0.3 µm long	6.0 µm long	420-460 Å	1 glycogen helix	absent	Takachi, Dan and colleagues
HELICOIDEA (CAMAENIDAE) (<i>Varohadra curtisiana</i>)	0.3 µm long	5.0 µm long	—	1 glycogen helix	absent	Healy & Jamieson (Unpublished)
(<i>Xanthomelon pachystylum</i>)	0.3 µm long	6.0 µm long	—	1 glycogen helix	absent	Healy & Jamieson (Unpublished)
(<i>Bentostites bloomfieldi</i>)	0.43 µm long, perinuclear sheath present	6.6 µm long	450 Å	1 glycogen helix	absent	Healy, 1984
HELICOIDEA (HELICIDAE) (<i>Helix aspersa</i>)	0.53 µm long, laterally positioned, perinuclear sheath present	8.1 µm long	400-500 Å	1 glycogen helix	absent	This paper (see also Anderson & Personne papers)
(<i>Helix pomatia</i>)	0.5 µm long, slight tilt, with perinuclear sheath	10.0 µm long	400-500 Å	1 glycogen helix	absent	This paper
(<i>Trichia hispida</i>)	0.3 µm long	6.0 µm long	?	1 glycogen helix	? absent	Shileiko & Danilova, 1979

It would, however, provide an opportunity to assess relationships within the order (from suborder to generic levels) and between these pulmonates, the Basommatophora (s.l.) and the Opisthobranchia.

In this paper we have presented structural information for the two most economically important species of *Helix* (*H. pomatia*—type species for the genus, prized as a delicacy, and *H. aspersa*—a widespread pest species). The results have shown numerous similarities between the two species (nucleus, midpiece) and one major difference (position of the acrosome—? possibly reflecting current subgeneric assignments of *H. pomatia* and *H. aspersa*). It is hoped that these data will provide the impetus for a wide-ranging survey of helioid spermatozoa.

On a more general level, spermatozoa of *H. aspersa* and *H. pomatia* (Helicidae (Helicinae), Helicoidea) are similar to those of other adequately studied stylommatophorans (*Euhadra hickonis*—Bradybaenidae, Helicoidea; *Anguispira alternata*—Punctidae, Arionoidea; *Trichia hispida*—Helicidae (Hygromiinae) Helicoidea; *Succinea putris*—Succineidae, Succinoidea). Varying amounts of data are available for other species though usually with no reference to acrosomal morphology. A perinuclear sheath similar to that of *Helix pomatia*, though less structured than that of *H. aspersa* is also observed in *Bentosites bloomfieldi* (Camaenidae, Helicoidea—Healy, 1984). *Bentosites bloomfieldi* and *Euhadra hickonis* (see—Takaichi & Dan, 1977) both have acrosomes only slightly tilted relative to the sperm longitudinal axis, as in *Helix pomatia*. The laterally-displaced acrosome of *Helix aspersa* has, as far as we are aware, no equivalent in any studied gastropod species. Interestingly, neither perinuclear sheath nor tilting of the acrosome are evident in late spermatids of the camaenids *Xanthomelon pachystylum* or *Varohadra curtisiana* (Healy & Jamieson, unpublished data) or the helioid *Trichia hispida* (Hygromiinae, Helicoidea—see Shileiko & Danilova, 1979). Clearly more information is required on spermatozoa of the Helicoidea before any useful discussion of systematics within the group can be attempted.

Absence of a glycogen piece in spermatozoa of all investigated stylommatophoran species suggests that this may even be an ordinal character (in all studied opisthobranchs, a glycogen piece, albeit sometimes very poorly developed, is still present—see Healy & Willan, 1984). Spermatozoa of basommatophoran (s.l.) pul-

monates *Salinator fragilis* and *S. solida* (see Healy, 1983b) approach the form of stylommatophoran spermatozoa (especially *Succinea putris* Succinoidea—see Shileiko & Danilova, 1979) more closely than do other basommatophorans, and it is pertinent that in *Salinator*, the axoneme terminates within the midpiece, leaving a glycogen piece composed solely of the plasma membrane and glycogen granules surrounding a central lumen. This arrangement, though neither proving nor refuting an Amphiboloidea-Stylommatophora link, provides a hypothetical intermediate towards the stylommatophoran condition (absence of glycogen piece).

ACKNOWLEDGEMENTS

The authors are grateful to Professor R. Chase (Department of Biology, McGill University) for providing material of *Helix pomatia*, and within the Zoology Department, University of Cambridge, to Professor G. Horn and Dr B. Gupta for facilities given to B.J. Thanks also are due to Mrs L. Daddow (Zoology Department, University of Queensland), Mr G. Rouse (now of the Zoology Department, University of Sydney) and the electron microscope units at the University of Cambridge, University of Sydney and University of Queensland for their combined technical assistance. Professor D. T. Anderson helped in collecting some of the spermatophores of *Helix aspersa* used in this study. Valuable criticisms of an earlier draft of this paper were provided by Drs M. Bishop (University of Cambridge) and R. C. Willan (Zoology Department, University of Queensland). Mrs H. Sanchez del Rio is thanked for her skilful typing of the manuscript. Financial support for this project was provided by a Commonwealth Postgraduate Award, Farrand Postdoctoral Research Fellowship and the Joyce W. Vickery Scientific Research Fund (J.H.) and Australian Research Grants Committee funding (B.J.).

ABBREVIATIONS USED IN FIGURES

ap	acrosomal pedestal
av	apical vesicle (of acrosome)
cd	centriolar derivative
cf	coarse fibres
das	distal accessory sheath
gh	glycogen helix (of midpiece)
ma	matrix material of mitochondrial derivative
n	nucleus

p paracrystalline material of mitochondrial derivative
 pns perinuclear sheath (? acrosomal component)

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