Ultrastructure of spermatozoa in four species of Alvinellidae
(Annelida: Polychaeta)

Claude JOUIN-TOULMOND1, Masina MOZZO and Stéphane HOUDEZ2
(1) Station Biologique UPMC-CNRS-INSU, BP 74, F 29682 Roscoff Cedex France
E-mail : jouin@sb-roscoff.fr
(2) 208 Mueller Laboratory, The Pennsylvania State University,
University Park, PA 16802, USA
E-mail : shousdez@bio.psu.edu

Introduction

Polychaete species from the family Alvinellidae are exclusively found at deep-sea hydrothermal vents in the Pacific Ocean. Twelve species have been described so far, occurring in different microhabitats of the vent ecosystem. Comparative studies of the genital apparatus and ultrastructure of spermatozoa in these species can be used to better understand the reproductive biology of these species and their phylogenetic relationships within the order Terebellida.

The single pair of gonoducts in alvinellids, and the presence of spermathecae in females and seminal vesicles in males were first described in Paralvinella grasslei Desbruyères & Laubier, 1982 (Zal et al., 1994). Investigations of Alvinella pompejana Desbruyères & Laubier, 1980 revealed a similar organization in the genus Alvinella (Jouin-Toulmond et al., 1997; Desbruyères et al., 1998; Zhadan et al., 2000). In P. grasslei and A. pompejana, spermatozoa transferred from males to females during a probable pseudocopulatory process are stored in the spermathecae located anteriorly, just below the genital pore opening, at the base of the gills.

We conducted similar anatomical and ultrastructural investigations in Paralvinella palmiformis Desbruyères & Laubier, 1986, P. pandorae irlandei Desbruyères & Laubier, 1986 and Alvinella caudata Desbruyères & Laubier, 1986. The aim of this study was to examine specific characters of the spermathecae and spermatozoa, and eventual ultrastructural similarities between closely related species and sub-species.

Material and methods

Animal collection
Specimens of Paralvinella palmiformis were collected from the Endeavour Segment, Juan de Fuca Ridge (JDF) (47°57'N, 129°05'W; 2195 m depth) and provided by Dr. V. Tunnicliiffe; four females were studied. Specimens of P. pandorae irlandei were collected by Dr. D. Desbruyères from the East Pacific Rise (EPR) (12°48.8'N, 103°56.5'W; 2635 m depth): six specimens were studied. Specimens of Alvinella caudata were collected by C. J-T. during the HERO 91 cruise, from the EPR, (12°48.66'N, 103°56.43'W): four specimens were examined. All material was initially fixed in a solution of 10% neutral formalin.

Tissue preparation
Tissues were prepared as described in Zal et al. (1994). Ultrathin sections were examined with a Jeol JEM 1200 EX.

Results

Spermathecae and spermatozoa of Paralvinella palmiformis
As in Paralvinella grasslei, two spermathecae, located in the dorsal part of segment 4, form the terminal part of two oviducts. They communicate with the exterior by a short common canal, located medially at the base of the most posterior gills, that opens at the genital pore. The spermathecae have a muscular peripheral layer and a luminal epithelium forming cristae (Fig. 1A-C). The spermatozoa, clustered together on the cristae or in the lumen, are just adjacent to the cristae and do not penetrate the spermathecal epithelium (Fig. 1C).

The spermatozoon of P. palmiformis (Fig. 1D) is very similar to that of P. grasslei: it has no acrosome, no
Figure 1. A–D: *Paralvinella palmiformis*. A. Histological section through a spermatheca, showing cristae of the luminal epithelium and spermatozoa clustered in the cavity. B. Scanning electron microscopy, SEM, view of a crista covered with aflagellate spermatozoa. C. Transmission electron microscopy, TEM, micrograph showing spermatozoa not attached to the spermathecal epithelium. D. A spermatozoon showing a convex cortical layer of electron-dense grains, an opposite vesicular side, part of the central nucleus, few mitochondria and granules. Inset: plasma membrane protrusions between the spermatozoa. E–J: *Paralvinella pandorae irlandei*. E–F Histological sections through the spermathecae, showing a smooth luminal epithelium, abundant spermatozoa attached to the spermathecal wall and long flagella in the spermathecal lumen. G. SEM view of morulae of spermatids from the coelomic fluid of a male; long flagella are at the periphery of the morulae. H. Middle part of a spermatozoon with part of the nucleus and convoluted structure. I. Posterior part of spermatozoa in the spermduct of a male. The flagella are retro-oriented (toward anterior end); basal body (arrow). J. Spermatozoa attached to the spermathecal wall by their posterior ends; basal bodies (arrows). K. *Alvinella pompejana*: spermatozoon from a spermatheca. L. *Alvinella caudata*: spermatozoon from a spermduct. Note the proximal centriole adjacent to the distal one.  

*Abbreviations*: (c) cristae; (cs) convoluted structure; (ep) spermathecal epithelium; (f) flagella; (n) nucleus; (pc) paracrystalline body; (sp) spermatids; (spz) spermatozoa; (v) vesicles with an electron lucent core.

midpiece, no flagellum and it comprises an oval “head”, (ca.
10 x 4 µm) containing the central nucleus and a few
mitochondria. It has a convex-shaped cortical layer of
electron-dense granules, very similar to that of P. grasslei.
On the opposite vesicular side, the plasma membrane can
form elongate extensions between the spermatozoa (Fig. D,
inset) which could be adhesive material favouring the
clustering and storage of the male gametes in the
spermatotheca. This spermatozoon possess a short process
different from the long “caudal” process present in
P. grasslei (Fig. 2).

Spermathecae and spermatozoa of Paralvinella pandorae
irlandei
The anatomy of the genital apparatus is similar to that of
P. grasslei and P. palmiformis: one pair of spermathecae is
present in the females, located in the dorsal part of
setigerous segment 4 and connected laterally to one pair of
oviducts (Fig. 1 E).
The flagellate spermatozoa are quite different from that of
P. grasslei and P. palmiformis, and very similar to that of
P. pandorae pandorae described by McHugh (1995).
Groups of late spermatids in the coelom of males show long
flagella at the periphery of the morulae (Fig. 1G). Each
spermatozoon is an elongate cell (about 19 µm in length)
comprising several parts which are, following the
nomenclature introduced by McHugh (1995): an apical
elongation, an electron-dense nucleus, a midpiece
comprising two mitochondrial areas anterior and posterior
to a convoluted structure (Fig. 1 H, J), then a short area
bearing the basal body of a long flagellum forming an angle
with the cell and directed anteriorly (Fig. 1, I, J ; Fig. 2).

In all the females examined, the spermathecae harboured
bundles of spermatozoa attached to the spermathecal wall
by their posterior (flagellate) end (Fig. 1 J), of which about
1 µm penetrates the epithelial surface; the basal bodies are
superficially inserted in the epithelium. The flagella, along
with the rest of the sperm cell, are free, but all aligned in the
spermathecal lumen (Fig. 1 E, F, I, J).

Spermathecae of Alvinella pompejana and A. caudata
The anatomy of the gonoducts, and the ultrastructural
features of the spermatozoa have already been described for
A. pompejana (see Introduction). The arrangement of the
genital apparatus is similar in A. caudata. In both species,
the spermathecae are located in the dorsal part of setigerous
segment 1.

Numerous spermatozoa are present in the spermathecae
of A. pompejana, free in the spermathecal lumen. They have
a small size, a conical shape, and a rather flat (ca. 4 µm
diameter), vesicular surface, in the middle of which is
inserted a short (ca. 1 µm) flagellum (Fig. 1K). The central
nucleus is round (ca. 2 µm in diameter) and surrounded by
few mitochondria; adjacent to the nucleus there is a
paracrystalline body, made of tubules and lying always
against the nucleus, but with a variable position (near the
flagellum or opposite to it) (Fig. 1K). The distal centriole
(basal body) is clearly visible, but the proximal centriole is
lacking.

In A. caudata, spermatozoa have only been examined in
the sperm ducts of a male specimen, the spermathecae
examined being empty. The spermatozoa are similar to
those of A. pompejana: a cell comparatively more flattened
than that of A. pompejana, a slightly elongate nucleus (Fig.
1 L), a short flagellum (ca. 1 µm in length) in the middle of
the flat vesicular surface (about 4.5 µm in diameter).
A proximal centriole is present next to the distal centriole
in A. caudata, but the paracrystalline body is lacking.

Discussion
The six alvinellid species investigated so far possess
spermathecae which harbour spermatozoa transferred from
males to females, by the way of spermatzeugmata, during
a probable pseudocopulatory process. The ultrastructural
similarities of spermatozoa of the two sub-species
Paralvinella pandorae pandorae and P. p. irlandei strongly
suggest that, although not seen by McHugh (1995), the
spermathecae are also present in P. p. pandorae. The
gonoducts and spermathecae of this species have been
studied by Zhadan et al. (2000), who also described a
similar anatomy for the genital organs in Paralvinella dela.
The Alvinellidae is, at present, the only taxon in the order
Terebellida known to possess spermathecae, a feature which
may represent an apomorphy for this family. The luminal
wall of the spermathecae have either internal cristae (e.g.
P. palmiformis, P. grasslei and the two species of Alvinella)
or a smooth surface (e.g. P. pandorae irlandei) and the

Figure 2. Schematic drawings of spermatozoa of six species of
Alvinellidae highlighting the ultrastructural diversity of spermato-
zoa in the genus Paralvinella and the morphological similarities of
sperms of two closely related species, of two sub-species and of
the two species of Alvinella. All species at the same scale: 2 µm.
Abbreviations as in Figure 1.
storage of spermatozoa differs according to the sperm morphology of the species. In Alvinella pompejana, A. caudata, Paralvinella grasslei and P. palmiformis, the sperm cells, almost immobile, with flagella reduced or absent, are stored without any special attachment at the surface of cristae in the spermathecae. In contrast, in P. pandorae irlandei, the elongate spermatozoa displaying long flagella are firmly implanted in the smooth spermathecal epithelium by their “posterior” flagellate end. In the Alvinellidae, it is still unknown whether fertilization occurs in the spermathecae or at spawning, when oocytes and spermatozoa, both mature, are emitted through the female genital pore.

There are three types of spermatozoa in the species investigated (Fig. 2). All belong to the introsperrn category and display modified structures (see Jamieson & Rouse, 1989) being flagellated or not, having no acrosome and an atypical or absent midpiece. In P. p. pandorae and P. p. irlandei, the elongate spermatozoa exhibit an atypical midpiece and a long flagellum, with a typical pattern, but a reverse orientation, producing a movement of the sperm cells with the flagellar part ahead, since this part is implanted in the spermathecal wall after the sperm transfer.

The aflagellate and oval shaped spermatozoa of the two closely related species P. grasslei and P. palmiformis represent another type of modified sperm having no acrosome, no midpiece, and no flagellum. The role of the cortical layer of dense granules remains unknown, but in P. palmiformis the cell membrane of the vesicular side produces some material that could be adhesive, and thus favour clustering and storage in the spermathecae.

Alvinella pompejana and A. caudata also have modified spermatozoa, conical in shape and very small sized. They have no acrosome, no midpiece and a short flagellum with a typical pattern. Their vesicular surface could be also involved in adhesiveness in the spermathecae.

In a study of phylogenetic relationships of eleven species of Alvinellidae using allozymes, Jollivet et al. (1995) showed that paralvinellid species are separated into three major clades that reflect an old habitat radiation. This radiation seems to have occurred before the ridge major clades that reflect an old habitat radiation. This study, species from the family Alvinellidae using allozymes, Jollivet et al. (1995) shows a typical pattern. Their vesicular surface could be also involved in adhesiveness in the spermathecae.

In the Alvinellidae, it is still unknown whether fertilization occurs in the spermathecae or at spawning, when oocytes and spermatozoa, both mature, are emitted through the female genital pore.

Acknowledgements

We are most grateful to Dr. D. Desbruyères and Dr. V. Tunnicliffe who generously provided specimens of Paralvinella pandorae irlandei and Paralvinella palmiformis and to Didier Jollivet for fruitful discussions. We thank J. Sourimant (Electron Microscopy Service, S.B. Roscoff) for his assistance.

References


Desbruyères D. & Laubier L. 1993. New species of Alvinellidae (Polychaeta) from the Pacific hydrothermal vents of the Pacific Ocean and for those whose reproductive structures were studied, specific features are notable: the single pair of gonoducts and the regular presence of spermathecae and seminal vesicles in six of twelve species suggest that these structures were present in the common ancestor that colonized the vent environment.
