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27. CHAETOGNATHA

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I. INTRODUCTION

The Chaetognatha, generally called arrow-worms, are hermaphrodites; the testes are in the tail, totally independent of the ovaries. The male reproductive system—which comprises the testes, vasa deferentia, and seminal vesicles—occupies completely the coelom of the caudal segment, except in *Sagitta enflata*. In this species, the testes occupy the posterior half of the tail segment. The tail segment is divided into a right and a left chamber by the dorsoventral mesentery. At the centre of each chamber is a median longitudinal wall (secondary mesentery) extending along each half of the tail segment, and held ventrally and dorsally. This secondary mesentery does not divide completely the right or the left chamber; and the material contained in each left and right part of the tail segment can circulate freely and independently. The right and left chambers do not communicate with each other; each chamber is occupied by one testis.

II. TESTIS

The testis (Fig. 1) is covered by a thin layer of endothelium continuous with the body wall along the line of attachment. In young animals approaching sexual maturity, testes are full of sperm-mother cells which grow and develop floating in the fluid filling the tail cavity. Testes in adult animals are filled with masses of spermatozoa at various stages of development. Groups of spermatogonia develop from the germinal layer in testes, and give rise to spermatocytes, spermatids, and finally spermatozoa which gradually fill the testes in the tail cavity.

In the testes are masses of sexual elements, all at the same or nearly the same stage of development (Bordas, 1914; Ghirardelli and Arnaud, 1966). However, different stages of spermatogenesis can also coexist in the testes. The masses of sperm formations are continuously on the move in the testes. The speed of movement varies with species and with the degree of fullness of the testes, and is enhanced by temperature. In *Spadella*, circulation is slower than in *Sagitta bipunctata* and related species (Ghirardelli; 1968).



Fig. 1. Cross-section through the testes of *Sagitta euneritica*. (Courtesy: Dr. Bernhard E. F. Reimann). ($\times 410$)

As can be seen from Fig. 2, in each chamber of the tail cavity, spermatogonial masses and spermatozoa move forward along the outer side, pass around the anterior end of the secondary mesentery, and then move towards the tip of the tail alongside the median longitudinal mesentery of the tail. Interestingly, small isolated masses of spermatocytes move very rapidly when close to the median longitudinal mesentery, which indicates this mesentery might be ciliated. Spermatogonia and developing spermatozoa seem to be kept in continuous movement in the testis cavity by ciliated cells of the lateral line and median mesentery. Spermatocytes, arranged in groups ('polyplasts' of Bordas, 1912, 1914), are separated from each other by the trabeculae in testicular space. The movement is circulatory and can be easily observed; it is not straight, as schematically illustrated in Fig. 2, but tortuous, following the irregularities of the paths interlocked among the trabeculae of spongy mesh structure. Burfield (1927) indicates that the movement of 'polyplasts' is due to the activity of the ciliated cells covering the median mesentery, whereas Bordas (1912, 1914) considers it is due to the tail action of mature spermatozoa. Interestingly, spermatocytes circulate even when the spermatozoa are absent. It is suggested that circulation could be also helped by ciliation at the opening of the vas deferens. With approaching maturity this meshwork, with its numerous groups of spermatocytes, fills up the whole space of the tail cavities.

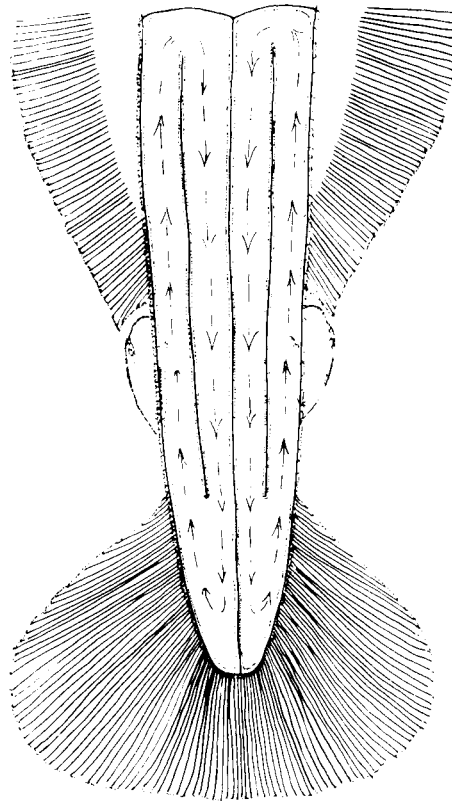


Fig. 2. General circulation of spermatozoa in testes of chaetognaths

The progressive development of the spermatocytes is almost synchronous; they all divide together into spermatids and these differentiate into spermatozoa. As the testis matures, groups of spermatozoa are distinctly visible among the spermatocytes. The formation of spermatozoa (Alvariño, 1965) starts at the anterior part of the testis, but a number of ripe spermatozoa may be found in the posterior part of the testis even while groups of spermatocytes are present anteriorly. Kramp (1917) considered an animal to be ripe, 'when ripe polyplasts are found through the whole length of the testes'. Spermatozoa are orientated in the testis with their head towards the anterior part of the animal; that is, facing the transverse wall.

The vas deferens communicates each testis with the seminal vesicle (Section V) of that side. These ducts are relatively long and may be shorter, longer, or about the length of the seminal vesicle. The sperm duct is lined with a simple epithelium (Burfield, 1927) and opens anteriorly into the tail coelom by an oval opening with slightly thickened lips; it proceeds down partially embedded in the body wall of the lateral line and opens by a ciliated opening into the seminal vesicle.

III. SPERMATOGENESIS

Spermatogenesis in the Chaetognatha has been studied mainly by Hertwig (1880), Bolles-Lee (1888), Stevens (1903, 1910), Bordas (1912, 1914), Tuzet (1931), and Ghirardelli (1954). The species studied is *Sagitta friderici*, and not *Sagitta bipunctata* as stated by these authors. *Sagitta friderici* is abundant at locations sampled by them, while *Sagitta bipunctata* is an oceanic species of scanty populations, not found at locations where the samples were obtained.

Chromosomes are 3 μm long, but in secondary spermatocytes they are only slightly over 1 μm long. Eighteen chromosomes (diploid number), about 3 μm long, were observed in *Sagitta friderici* (Boveri, 1890), *Sagitta enflata* (Buchner, 1910; Ghirardelli, 1954), *Sagitta minima* (Ghirardelli, 1954), *Sagitta elegans* (Stevens, 1910), and *Spadella cephaloptera* (Ghirardelli, 1959). Some differences may appear in the karyogram of various species. For instance, during spermatogonial metaphase of *Sagitta enflata*, five pairs of metacentric chromosomes were observed (Ghirardelli, 1954), while in *Sagitta setosa* there were only four pairs. The squash technique confirms that, apart from small differences in detail, meiosis in *Sagitta setosa* follows the classic scheme (Ghirardelli and Arnaud, 1966). The X pair of chromosomes (Stevens, 1910) are named m chromosomes by Ghirardelli and Arnaud (1966) to avoid any confusion with the sex chromosomes. At the first metaphase, a pair of chromosomes moves more precociously than the others towards the opposite extremities of the thread spindle much like the sex chromosomes. Although these chromosomes cannot be considered strictly as sex chromosomes, the animal being hermaphroditic, this phenomenon was observed only during spermatogenesis and not in oogenesis.

Spermatogonia have a clear reniform nucleus (Tuzet, 1931) with one or two nucleoli. Chromatin appears in small roundish packs. The young spermatids have horseshoe-shaped dictyosomes and one or many small, granular chondriokonts, two of which are sometimes attached to form a large ball (mitochondrial balls).

During the so-called 'resting stage', in the nucleus of spermatids, the chromatin is irregularly netted, but suddenly a crown of condensed chromatin is formed along the periphery of the nucleus, until the whole nucleus is a sphere of chromatin of pycnotic characteristics. The elongated nucleus reaches close to the proximal and distal centrioles, which are connected. The proximal centriole is associated with the acrosome; the distal one gives rise to the caudal flagellum.

IV. THE SPERMATOZOA

Spermatozoa of chaetognaths are elongated thin filaments (Tuzet, 1931; Jägersten, 1940) with a long and fine acrosome at the anterior end. The proximal centriole is at the base of the acrosome, followed by chromatin

filament; next is the short middle piece; and the caudal flagellum is short and placed towards one side. The spermatozoa of *Spadella* (Fig. 3) and *Sagitta* (Figs. 4 and 5) share some peculiar characteristics (Ghirardelli, 1968). They are both elongated with a centriole at the base of the long and thick acrosome, and are provided with an external chromatin thread placed at the side of the head, connected to the proximal centriole. The middle piece and the caudal flagellum are short in relation to the size of the head. Retzius (1909) gave a different description of the spermatozoa, in which the anterior part was reported as posterior and *vice versa*, and this research has now only historical value. Jägersten (1940), who studied chaetognath spermatozoa, indicated that isolated spermatozoa in water are unable to move. This is not true: I have observed such movement of spermatozoa in living *Sagitta enflata* and *Sagitta euneritica* studied under the microscope; they moved rapidly in water propelled by undulation of their tails. In these experiments, the spermatozoa flowed out in a current from the bursting seminal vesicles and spread like the flow of water from a hose; no spermatophore was formed.

Van Deurs (1972) studied mature spermatozoa of *Spadella cephaloptera*, and indicated that they corresponded to the filiform type, common to cirripedes, ostracods (see Pochon-Masson, this volume), insects, and other arthropods and also to gastropods (see Maxwell, this volume). However, the fine structure of the spermatozoa of the Chaetognatha (Fig. 5) does not agree with the ultrastructure described for any other filiform spermatozoon.

Most of the spermatozoa in the seminal vesicles of *Spadella cephaloptera* and also *Sagitta euneritica* are orientated in a direction parallel to the longitudinal axis of the seminal vesicle and the whole animal. Mature spermatozoon is approximately 290 μm long and 0.7 μm wide. Usually, the spermatozoon is orientated for definition laying ventrally; the median plane is vertical to the axoneme. At the anterior end of the spermatozoon are the membrane cap, acrosomal structure, and beaded vesicles; at the top is the centriole-like structure from which extends the axoneme running along the spermatozoon (Fig. 3). A few microns behind the centriolar region begins the long mitochondrion (about 250 μm) which extends along the spermatozoon till almost the end of the nucleus. Next to the beaded structure are the longitudinal sacs, at the central half of the spermatozoon between the mitochondrion and axoneme and continuing with the nuclear region and extending farther back than the mitochondrion. At the other end of the spermatozoon is a free flagellum, about 20 μm long.

The membrane cap is a complicated structure, 60 μm long at the anterior end of the sperm. It may be divided into four regions: (1) terminal region (1–2 μm), (2) centriolar region, (3) the axoneme, (4) axoneme and mitochondrion. The membrane cap is formed of two membranes in a double ring. The beaded structure, which extends for 50 μm , starting 8 μm off the top front, appears associated with the cap. The terminal region is in front of the centriolar region,

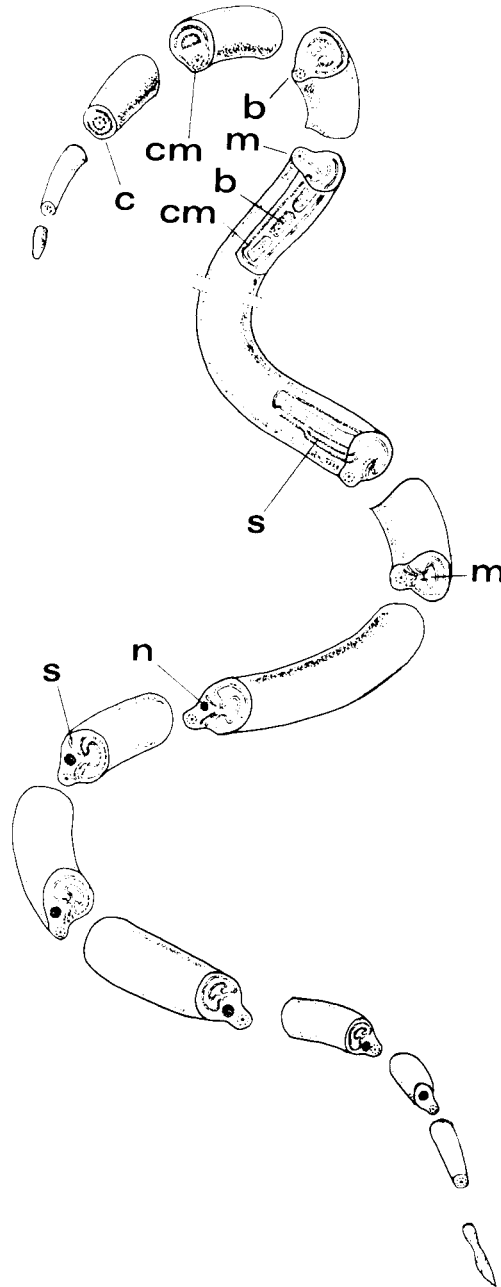


Fig. 3. Structure of mature spermatozoon of *Spadella* (after van Deurs, 1972). b, Beaded structure; c, centriolar structure; cm, membrane cap; m, mitochondrion; n, nucleus; s, sacs. At the lowest end is the free flagellum.



Fig. 4. A section through testis and neighbouring tissues of *S. euneritica*. Note the arrangement of the axoneme of the spermatozoa ($\times 24,800$). (Courtesy: Dr. Bernhard E. F. Reimann)

and here the complete double membrane ring of the cap is present. In the rest of the cap, the membrane has an opening where the outer and inner cap membranes are connected. The beaded structure in the cap region is flat with outer and inner membranes, and lies dorsally displaced to one side of the



Fig. 5. A section through testis and neighbouring tissues of *S. euneritica*. The spermatozoa have been sectioned through nucleus, membrane cap, mitochondrion, etc. ($\times 19,200$). (Courtesy: Dr. Bernhard E. F. Reimann)

spermatozoon. The beaded structure at the dorsal part of the spermatozoon consists of a series of vesicles or sacs of rectangular or elliptical shape. Each sac is 0.8–1.4 μm long, 0.4 μm wide, and 0.2 μm high. Distance between sacs is small, and sometimes several sacs may merge together. Both cap membranes may be continuous with the sac membrane, although this is not very clear in mature spermatozoa. The nucleus of mature spermatozoon is at the posterior

dorsal end of the axoneme. The nucleus appears as a dense rod, 60 μm long and 0.25 μm in diameter at the centre, tapering at both ends. The nuclear region overlaps in part the longitudinal sac region.

The mitochondrion is bilaterally symmetrical in structure, but not all along the spermatozoon, as it turns around the longitudinal axis with some lateral displacement. The two longitudinal sacs are $0.03 \times 0.15 \times 1.5 \mu\text{m}$, situated between the mitochondrion and axoneme; or there may be two sacs on one side and one on the other. The transition from beaded sacs to longitudinal sacs is probably a gradual one. Axoneme (Fig. 4) extends from the front end of the spermatozoon, parallel to the mitochondrion surrounded by the membrane cap, and ends in a free flagellum (Fig. 3). Similarities are found between the spermatozoa of *Spadella* and *Sagitta*, and van Deurs (1972) indicates that a distinct acrosomal structure is not present in the spermatozoon of *Spadella*, but the membrane cap at the anterior end of the spermatozoon could be considered an acrosome. The mitochondrion and axoneme run parallel through almost the complete length of the spermatozoon (van Deurs, 1972). The ripe filiform spermatozoa move along the vas deferens from the testes, to be stored in the seminal vesicles.

V. SPERM STORAGE: SEMINAL VESICLES

Spectacular taxonomic differences exist in morphology of the seminal vesicles of the Chaetognatha (Tokioka, 1939; Alvariño, 1963, 1965, 1967, 1969). This may be because seminal vesicles need to be different in each species to avoid interspecific cross fertilization (Table 1). A detailed analysis of the structure of the opening of the oviducts would show that for each species oviducal openings and the devices of the seminal vesicles adapt and adjust conveniently. Also, the distance of the opening of the oviduct to the seminal vesicles is particular to each species, and this meristic factor is specific, and of primary importance to ensure copulation. The seminal vesicles are located laterally in the caudal portion of the body, just anterior to the caudal fin (Fig. 6). They are clothed by a layer of epidermis and lined with a layer of small gland cells, which probably produce a mucous substance.

The seminal vesicles of mature arrow-worms are tightly packed with filiform spermatozoa. The method of sperm emission is by rupture of the seminal vesicles by a slit or pore. Spermatophore formation has been mentioned by Dallot (1967), Ghirardelli (1968), and Nagasawa and Marumo (1978). I have observed extrusion of spermatozoa in *Sagitta enflata*, *Sagitta euneritica*, and other species; no formation of spermatophore was accomplished. Also, I have seen many hundreds of thousands of specimens of chaetognaths alive and preserved, from all the world oceans, but I have never found spermatophores. Although the chaetognaths are hermaphrodites, cross-fertilization is usual and obligatory. The semen is introduced by copulation into the receptaculum

Table 1
Chaetognatha: seminal vesicles (most common pelagic species)

Species	Seminal vesicles
<i>Eukrohnia bathyantarctica</i>	ovoid, opening at lateral dorsal side
<i>E. bathypelagica</i>	ovoid, opening at lateral dorsal side
<i>E. fowleri</i>	ovoid, opening at dorsal edge of the anterior part
<i>E. hamata</i>	ovoid, elongated, opening at dorsal edge of the anterior part
<i>E. minuta</i>	ovoid, elongated, opening at dorsal edge of the anterior part
<i>Krohnitta mutabbii</i>	oval, longer than in <i>K. pacifica</i> , touching both lateral and tail fins
<i>K. pacifica</i>	pear-shaped, oval, opening at top and dorsolaterally; fins cover them at the ventral side
<i>K. subtilis</i>	elongated oval; open at the anterolateral edge of dorsal side
<i>Pterosagitta draco</i>	elongated, oval, anterior part rounded; open dorsally at the anterolateral region
<i>Sagitta gazellae</i>	roundish, oval, opening at midlength of dorsal edge
<i>S. lyra</i>	oval, breaking at midlength of dorsolateral side
<i>S. scrippsae</i>	roundish, oval; open at midlength of dorsolateral side
<i>S. maxima</i>	oval, opening at midlength of dorsal side
<i>S. hexaptera</i>	small, spherical; rupture takes place at midlength over dorsolateral border
<i>S. enflata</i>	spherical, small, bursting by a fissure at the anterolateral edge along the dorsal side
<i>S. pulchra</i>	include two parts, an oval part and a spherical top, and open at the anterolateral dorsal side
<i>S. bierii</i>	pear-shaped, one prominence at top of anterior end, bending towards dorsal side; breaking by a pore at the base of the prominence.
<i>S. pacifica</i>	conspicuous with chitinous teeth at the anterolateral ventral side; a shield covers the anterior part, a device to ensure copulation. Bursting occurs at the anterodorsal side; the plate with the teeth opens like a lid leaving exposed a hole in the vesicle.
<i>S. pseudoserratodentata</i>	triangular, with prominence at lateral anterior corner, and small teeth or chitinous indentations at anterior end; open at anterior end of dorsal side
<i>S. serratodentata</i>	two prominences or horns at anterior lateral corner, opening at anterodorsal side
<i>S. tasmanica</i>	oval, with top formed by soft abundant papillae, a cap or lid covering the opening; the cap adapts to female openings, if evident
<i>S. bombayensis</i>	pear-shaped, anterior part large, opening at anterolateral dorsal edge
<i>S. bruuni</i>	oval, anterior part enlarged, opening out by anterodorsal fissure.

- S. crassa*
S. euneritica
S. euxina
S. friderici
S. peruviana
S. popovicii
S. setosa
S. tenuis

S. bipunctata
S. macrocephala
S. helenae
S. marri
S. planctonis
S. zetestos
S. bedoti

S. nagae

S. elegans
S. minima

S. decipiens

S. ferox

S. hispida

S. robusta
S. tokiokai

S. neglecta
- oval, with a roundish swelling at anterolateral end
 oval, with a roundish body at anterior part
 oval, with roundish body at anterior part
 oval, with a large posterior receptacle and a roundish anterior part
 oval, with posterior receptacle and roundish anterior part, opening at anterodorsal side
 oval, with a prominence at anterolateral side
 oval, inconspicuous
 oval and elongated, with a swelling at anterior end; open by a latero-anterior fissure at dorsal side
 pear-shaped, anterior part globular, opening at the anterodorsal side
 oval, opening along a fissure extending at the dorsal outer edge
 oval, with enlarged anterior part, opening at anterodorsal end
 conical, bursting at midlength of the dorsal edge
 oval, breaking at edge of dorsal side
 oval, open by a ridge along the dorsal side edge
 ovoid, with small prominence at anterolateral region; burst along the lateral edge by a fission line which extends from anterior end to the posterior part of the vesicle
 oval with small prominence at the anterior part; open by a slit along the dorsolateral side; on the ventral side of the vesicle appears a triangular thin shield partially covering the vesicle.
 conical; open at laterodorsal side
 oval, elongated, anterior part larger than the posterior; opening extends along dorsolateral side
 pear-shaped with the widest part at anterior end; a prominence or small shield at the ventral side
 oval with a cup-like extension at the anterior end; they have two parts, an oval posterior section and an anterior section, covered by a shield like the shell of a bivalve mollusc; shield is of glandular and protective function
 oval with hemispherical protuberance at anterior end; opening by a fissure extending along the dorsal edge
 pear-shaped, anterior part roundish; opening at anterodorsal side
 large oval with strong walls; opening is covered by a shield like the bivalve shell; the concave part of the shield lies against the opening of the spermatid sac and is lined with papillae
 oval, elongated, anterior part larger than the posterior; open along edge of dorsal side

Table 1 (continued)

Species	Seminal vesicles
<i>S. regularis</i>	elongated, oval; anterior part larger than the posterior; open along edge of dorsal side
<i>S. bedfordii</i>	pear-shaped; opening at anterolateral end of the dorsal side
<i>S. johorensis</i>	oval, almost spherical with a rounded prominence like a shield at anterolateral end, protecting the opening of the vesicle. At the anterior ventral side, a hard structure extends from the tail to the vesicle
<i>S. oceania</i>	pear-shaped with opening at the anterolateral part of dorsal side; a flap protects the opening ensuring copulation
<i>S. septata</i>	roundish oval, with a roundish extension at the anterior end; they open at the edge of dorsal side, and the line of rupture is well marked on the vesicle; a fine membrane extends from posterior end of the vesicle to base of the tail, holding the vesicles.

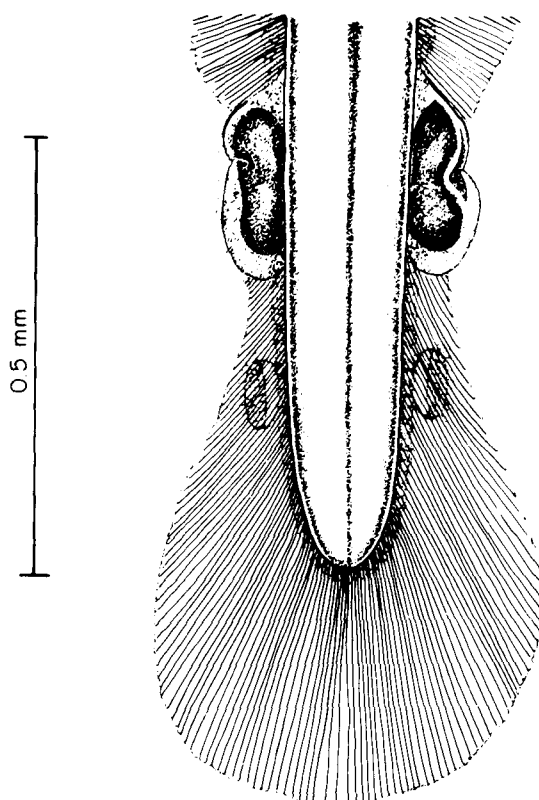


Fig. 6. Seminal vesicles of *Spadella gaetanoi* (a benthic chaetognath), dorsal view
 seminis (annex to the ovary), each mating partner acting as a functional male
 and female.

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