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Spermatogenesis and sperm ultrastructure in the polychaete genus *Ophryotrocha* (Dorvilleidae)*

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ABSTRACT: The details of spermatogenesis and spermiogenesis are described for *Ophryotrocha puerilis*. The ultrastructure of mature sperm is shown for *O. puerilis, O. hartmanni, O. gracilis, O. diadema, O. labronica,* and *O. notoglandulata*. Clusters of sixteen cells each are proliferated by two stem cells in each setigerous segment of *O. puerilis* representing the very early stages of both oogenesis and spermatogenesis. In each spermatocyte-I cluster, the cells are interconnected by cytoplasmic bridges. Early clusters are enveloped by peritoneal sheath cells. These transient gonad walls break down prior to meiosis. The meiotic processes may start in the clusters, with the cells still interconnected, or during breakdown of the original cluster, giving rise to smaller subclusters of both spermatocytes I and spermatocytes II with various numbers of cells. Finally, spermatid tetrads are present. As spermiogenesis progresses, the tetrads disintegrate. Golgi vesicles in both spermatocytes and spermatids contain electron-dense material, presumably preacrosomal. The acrosome is formed by such vesicles. In the six species studied here, the acrosomes appear to be of a similar overall structure but are of different shape. Centrioles are usually located beneath the acrosome. The distal centriole forms the basal body of a flagellum-like cytoplasmic process. The microtubules of these flagellar equivalents do not show a normal ciliar arrangement. The flagellar equivalent appears to be non-motile. In *O. hartmanni* and in *O. notoglandulata*, a flagellar equivalent is missing. Microtubules originating from the proximal end of the distal centriole stretch to the nuclear envelope. This feature appears to be especially conspicuous in *O. puerilis* and in *O. labronica*. In *O. labronica* and in *O. notoglandulata*, bundles of microtubules paralleling the cell perimeter appear to stabilise the sperm. Various numbers of mitochondria are either randomly distributed around the nucleus or accumulate on one side, often directly under the acrosome.

INTRODUCTION

In polychaetes, as in other marine invertebrates, sperm morphology is thought to be of adaptive significance. Usually, external fertilisation is accomplished by so-called

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primitive sperm. These types of sperm consist of a more or less spherical nucleus, a relatively short middle piece, and a long tail. Aberrant sperm (Franzén, 1956, 1977) are thought to be accompanied by modified fertilisation (for review see Sawada, 1984). In *Ophryotrocha*, male and female gametes are shed during pseudocopulation. Males and females appear to cooperate in building mucous tubes inside which the young develop. Although the eggs are externally fertilised, sperm in this genus show a highly derived morphology (Berruti et al., 1978; Troyer & Schwager, 1979). At present, little is known about cellular processes and the ultrastructure of spermatogenesis. Also, nothing is known about species-specific differences in the process of fertilisation and about possibly corresponding sperm morphology.

In *Ophryotrocha*, chromosomal events during spermatogenesis have been studied at the light microscope level by Grégoire & Deton (1906). These authors, however, do not report on cellular events or on the localization of spermatocytes or spermatids within the worms. One of the interesting features of gametogenesis in the protandric hermaphrodite *O. puerilis* is that both oogonia and spermatogonia are proliferated by the same stem cells. The proliferation of the stem cells and the early stages of oogenesis have been described earlier (Pfannenstiel & Grünig, 1982). The present paper deals with cellular events of spermatogenesis, the different stages of spermiogenesis, and the morphology of sperm in six *Ophryotrocha* species.

MATERIAL AND METHODS

Animals

Specimens of *Ophryotrocha puerilis, O. hartmanni, O. diadema, O. labronica,* and *O. notoglandulata* used in this study have been bred in our laboratory. Culture methods have been published in a previous paper (Pfannenstiel, 1973b). Specimens of *O. gracilis* were collected at Helgoland in the summer of 1988.

Young worms with 6–9 setigerous segments were collected from mass cultures and examined with Nomarsky optics. Only those without freely floating sperm were taken for the EM study of spermiogenesis *(O. puerilis).* Adults with ripe sperm were taken for the documentation of sperm morphology.

Electron microscopy

For electron microscopy, specimens were fixed for 1 h in an ice-cold 1 : 1 mixture (pH 7.4, 1000–1100 mosmol) of (i) 2.5 % glutaraldehyde in 0.1 mol/l phosphate buffer and (ii) 4 % osmium tetroxide. They were rinsed in phosphate buffer (pH 7.4) and dehydrated in ethanol. During dehydration, specimens were stained for 10–15 min in a saturated uranylacetate solution in 70 % ethanol. Specimens were then transferred to propylene oxide and embedded in Araldite. Sections cut on a Reichert OmU 3 were stained with lead citrate and examined with a Zeiss 9-S 2 or a Zeiss 10 C transmission electron microscope.

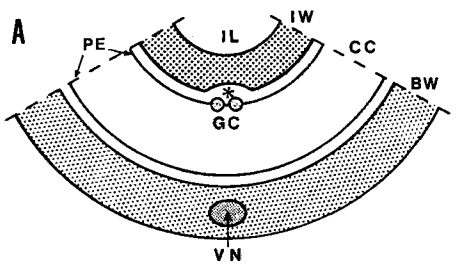
RESULTS

Spermatogenesis in *Ophryotrocha puerilis*

Two primordial germ cells are found in each setigerous segment. They are located in the coelomic lining of the ventral gut wall (Pfannenstiel & Grünig, 1982) (Fig. 1A). At the beginning of spermatogenesis the primordial germ cells undergo rapid mitotic proliferation producing clusters of 16 cells each (Figs 1B, 2). The cells of the 16-cell clusters are interconnected by cytoplasmic bridges. They represent the gametocytes I (Figs 2, 3). The gametocyte clusters are covered by peritoneal sheath cells which form a transient gonad wall (Fig. 3). It appears that up to this stage the fate of these cells is not fixed. In young specimens they will undergo meiosis and differentiate into sperm (Figs 1C, 2) whereas in older worms half of the cells will become oocytes I and half will become nurse cells. Spermatocytes I do not differ markedly in size from the stem cells as they appear to grow rapidly during the series of mitotic divisions by which they are produced. Profiles of the rather big nuclei of spermatocytes I (Fig. 3) are not round but form lobes and protrusions. Golgi stacks are found synthesizing preacrosomal vesicles. The more or less spherical mitochondria are numerous. Only few cristae are present in each mitochondrion. Mitochondria of this type are found throughout spermatogenesis (Fig. 12). Later on, the spermatocyte I clusters disintegrate and at about the same time the meiotic divisions begin. Meiosis and the breakdown of spermatocyte I clusters do not occur in a strict temporal order. The first meiotic division may occur with cells of the early cluster still in cytoplasmic continuity or after detachment of groups or single spermatocytes I from the original cluster. Thus, clusters with cell numbers varying within a broad range are observed in males engaged in the processes of spermatogenesis (Fig. 2). These clusters accordingly contain both spermatocytes I and spermatocytes II. Usually, these clusters still adhere to the peritoneal lining of the gut although the gonad wall itself has broken down. As the two meiotic divisions follow each other immediately, spermatocytes II (Fig. 4) are rarely seen. The nuclei of spermatocytes II tend to round up (Fig. 4). Finally, the spermatocytes completely detach from each other and after the second meiotic division spermatid tetrads (Figs 2, 5) are found adhering to the coelomic epithelium of the gut.

Spermiogenesis and sperm morphology in *Ophryotrocha puerilis*

Spermiogenesis takes place in spermatid tetrads still attached to the coelomic lining. Mature sperm are found floating freely in the coelomic cavity. The four spermatids of a tetrad are in cytoplasmic continuity. A peculiar type of cell bridge (Fig. 7) interconnects the 4 cells in the middle of a tetrad. Preacrosomal material of Golgi origin which can be found accumulating at the anterior pole of spermatocytes II (Fig. 5) forms the acrosome in spermatids (Fig. 6). In spermatids, Golgi stacks are still producing vesicles which are thought to contribute to acrosome formation. Normally, the acrosome forms as a cap at one cell pole.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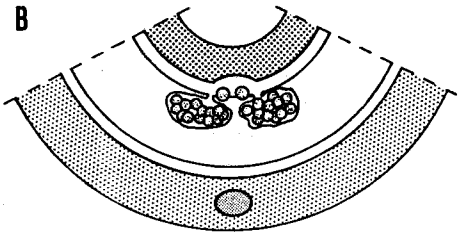
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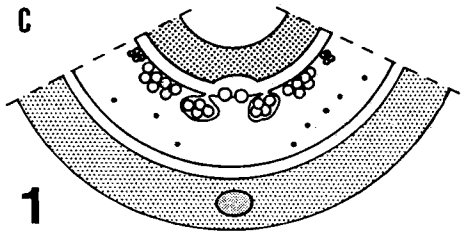
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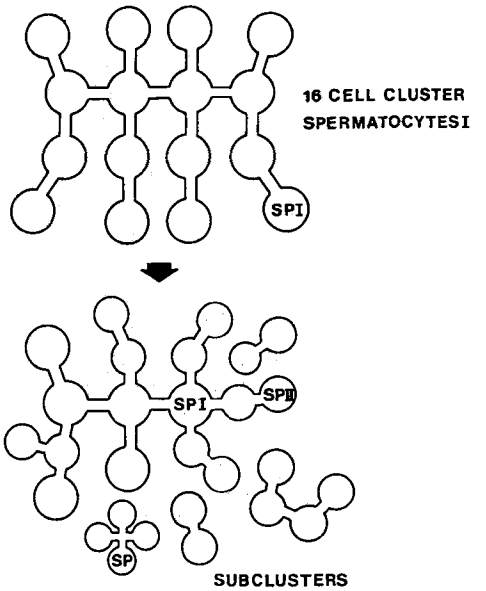
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SPI
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SP
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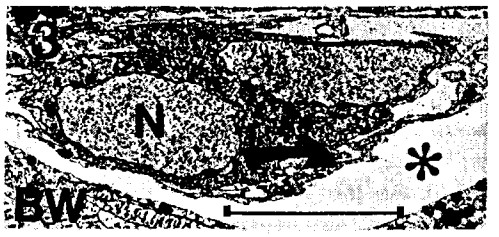
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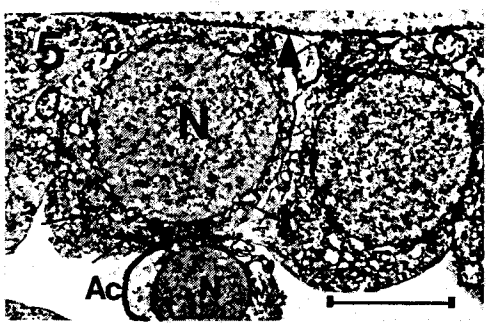
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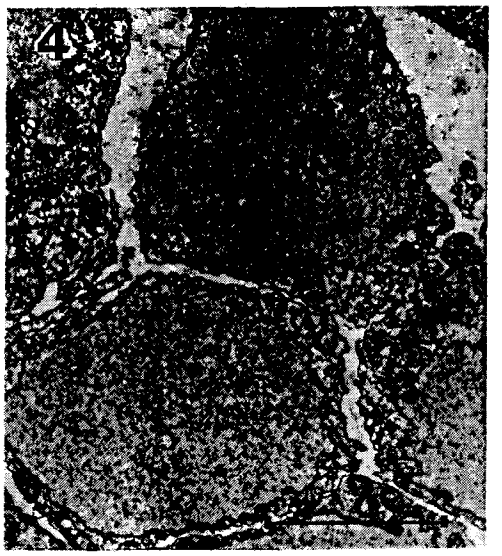
4


6
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Spermatid nuclei are easily recognized by their homogeneous chromatin (Fig. 6). Nuclear condensation does not lead to very dense chromatin as is normal for other sperm. The homogeneously looking fine granular nuclear material is found in spermatocytes II (Fig. 5), and in both early and late spermatids (Fig. 6). In almost mature sperm (Figs 8, 10), the fine granular nuclear material forms slightly larger aggregates giving a flocculent impression. At that stage, centrioles are usually found just beneath the acrosome where they are also found in mature sperm. The two centrioles are interconnected by microtubule bundles (Fig. 9). Bundles of microtubules also stretch from the distal and from the proximal centriole to the nuclear envelope (Figs 8, 9). These microtubule bundles sometimes resemble rootlet structures (*O. notoglandulata*, Fig. 15; *O. labronica*, Fig. 20). Some variation appears to exist as to the relative position of the two centrioles. In some cases they are positioned at right angles to each other while in other sperm they appear to form a tandem (*O. puerilis*, Fig. 9; *O. diadema*, Fig. 23). In *O. notoglandulata* in one exceptional case two centrioles have been found at opposite cell poles (Fig. 16). The distal centriole forms a basal body giving rise to 10–15 microtubules which form a so-called flagellar equivalent (Troyer & Schwager, 1979). The microtubules of this non-motile flagellar equivalent do not show the 9+2 arrangement which is normal for motile cilia. Normally, the flagellar equivalent evaginates from the lower rim of the acrosome. The number and location of spermatid and sperm mitochondria obviously is not fixed. Sometimes, mitochondria are situated between nucleus and acrosome (*O. hartmanni*, Fig. 12). Consequently, a middle piece of the spermatozoon is missing in the present case. Sperm mitochondria sometimes appear oblong rather than spherical (*O. labronica*, Fig. 21). It is not known whether these mitochondria are formed by fusion of smaller spherical spermatid mitochondria (Fig. 4). The morphology of a ripe spermatozoon of *O. puerilis* is schematically depicted in Figure 24.

Figs 1–6. *Ophryotrocha puerilis*

Fig. 1. Schematic presentation of gonad development. A: ventral sector of sexually immature segment. IL – lumen of intestine, IW – intestine wall, PE – peritoneum, CC – coelomic cavity, GC – gonocytes, asterisk – genital blood vessel, BW – body wall, VN – ventral nerve cord; B: cluster of 16 gametocytes I proliferated by gonocytes within outpocketing of peritoneum; C: gonad disintegrates, subclusters of spermatocytes attached to peritoneum, ripe sperm freely floating in coelomic cavity, gonocytes start new proliferation cycle

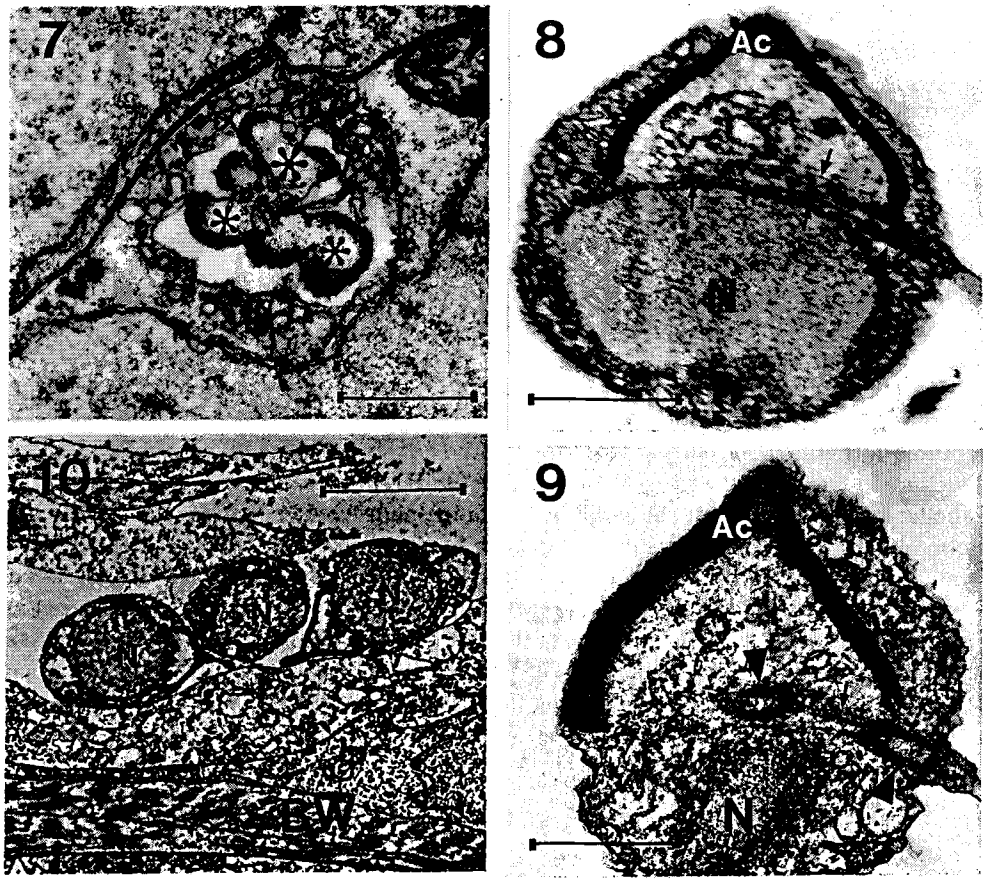
Fig. 2. Schematic presentation of spermatocyte I cluster and of subclusters produced during breakdown of gonad. SPI – spermatocytes I, SPII – spermatocytes II, SP – spermatids

Fig. 3. Gametocytes I located in coelomic cavity between intestine and body wall (BW). Scale bar – 10 μm

Fig. 4. Spermatocytes II. Note spherical mitochondria and large nuclei (N). Scale bar – 5 μm

Fig. 5. Young spermatids attached to basal lamina (arrowhead) of genital blood vessel. Note spherical nuclei (N). Arrows – accumulating preacrosomal material, Ac – acrosome of almost mature sperm. Note condensed nuclear material in sperm nucleus (N). Scale bar – 5 μm

Fig. 6. Advanced spermatid. Ac – forming acrosome, arrowheads – vesicles of Golgi origin contribute to acrosome formation, Ce – centriole, N – nucleus with advanced condensation. Scale bar – 1 μm



Figs 7–10. *Ophryotrocha puerilis*

Fig. 7. Cross sections of cytoplasmic bridge of spermatid tetrad. Note lumina (asterisks) of bridge. Scale bar – 1 μm

Fig. 8. Almost mature sperm. Note conical acrosome (Ac). Basal body (arrowhead) gives rise to microtubules (arrows) stretching parallel to nuclear envelope: Scale bar – 1 μm

Fig. 9. Almost mature sperm. Ac – acrosome, arrowheads – tandem centrioles connected by microtubules (arrows), N – nucleus. Scale bar – 1 μm

Fig. 10. Nearly mature sperm in coelomic cavity. N – nuclei, BW – body wall. Note elongate tip of acrosome. Scale bar – 5 μm

Comparative sperm morphology

The most striking structural differences in sperm of the six species studied here concern the acrosomes and the flagellar equivalents. In *O. hartmanni* and in *O. notoglandulata*, flagellar equivalents are completely missing, although centrioles are present at

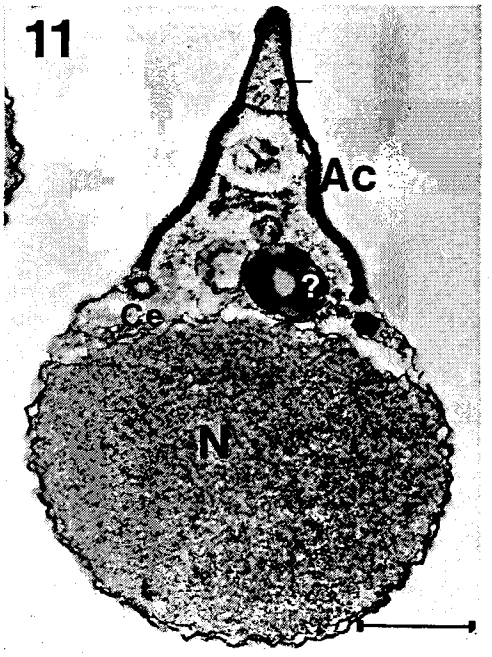
the location where they are usually found, i.e. beneath the acrosome (*O. hartmanni*, Figs 11, 12, 24; *O. notoglandulata*, Figs 13, 15, 16, 24). Whereas acrosomes in *O. puerilis* (Figs 10, 24), *O. gracilis* (Figs 17, 24), and *O. hartmanni* (Figs 11, 24) are conical or cap-shaped with elongated tapering tips, acrosomes in *O. diadema* (Figs 22–24), *O. labronica* (Figs 18–20, 24), and *O. notoglandulata* (Figs 13, 15, 16, 24) are rather flat with only slightly protruding tips *(O. labronica, O. notoglandulata).* The space of the *O. hartmanni* acrosome tip is filled with flocculent material (Fig. 11). In *O. hartmanni* and in *O. gracilis*, vesicles with electron-dense contents are usually found beneath the acrosomes (Figs 11, 17, 24) the origin of which is unknown. In *O. diadema*, the acrosome shows a concave surface which is covered by numerous indentations. In *O. gracilis* and in *O. hartmanni*, mitochondria are usually found accumulating beneath the acrosomes (Figs 11, 17), while in the other species mitochondria are scattered around the nuclei.

In *O. labronica* (Figs 18, 19, 24) and in *O. notoglandulata* (Figs 13–15, 24), bundles of microtobules paralleling the cell perimeter form a ring just beneath the cell membrane which appears to stabilise the discoidal shape of the cells. Cytoplasmic membranes (Figs 13, 18, 19) appear to subtend or encircle the nuclei in *O. labronica* and in *O. notoglandulata*. These membranes sometimes look like derivatives of the nuclear envelope (Fig. 19). In Figure 18, the peripheral bundle of microtubules appears to be encircled by such a membrane.

DISCUSSION

The early stages of gametogenesis up to oocytes I or spermatocytes I appear to be identical in male- or female-phase individuals of the protandric *Ophryotrocha puerilis*. These early stages are represented by clusters of 16 cells, each proliferated by a single stem cell. Oocytes I and spermatocytes I cannot be told apart before oocytes and nurse cells start to differentiate. Oocytes enter vitellogenesis and nurse cells become polyploid. The first visible sign of spermatocyte differentiation is the appearance of preacrosomal vesicles. Male-female differentiation is controlled by an ootropic hormone in the presence of which gametocytes differentiate into oocytes and nurse cells (Pfannenstiel, 1973a; for review see Pfannenstiel, 1984). The fact that early stages of gametogenesis are very similar if not identical in both males and females of *O. puerilis* suggests that the ootropic hormone triggers a switch from male to female differentiation at the level of gametocytes (Franke & Pfannenstiel, 1984). A general bisexual potency appears to be inherent in a variety of organisms where it is expressed by the development of male and female sex characters from identical anlagen in both sexes. In *O. puerilis*, even the early stages of gametogenesis in both males and females are the same and may be regarded as an expression of that general bisexual potency.

The structure of mature sperm in the genus *Ophryotrocha* deviates from what is thought to be normal in two different ways: (i) the general organisation of sperm shows adaptations to an almost complete loss of motility and (ii) the localisation and ultrastructure of cell organelles display a considerable degree of both inter- and intraspecific variation. The adaptations concerning the loss of motility appear to reflect the mode of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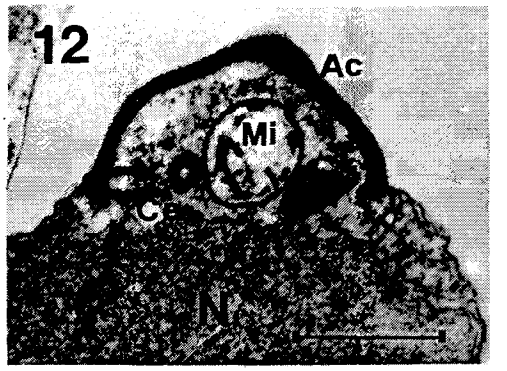
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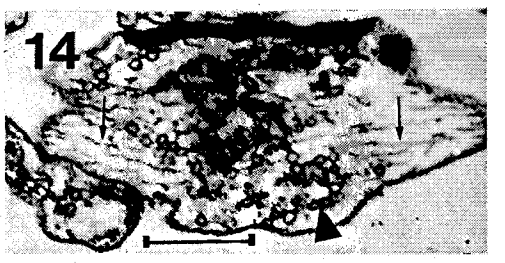
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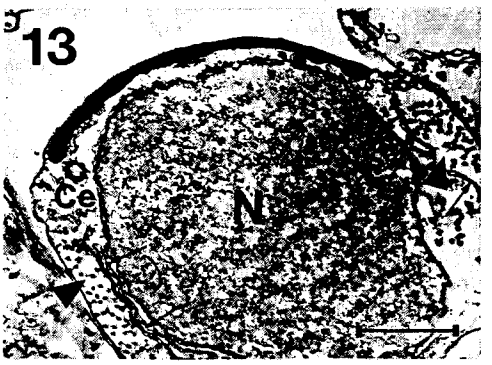
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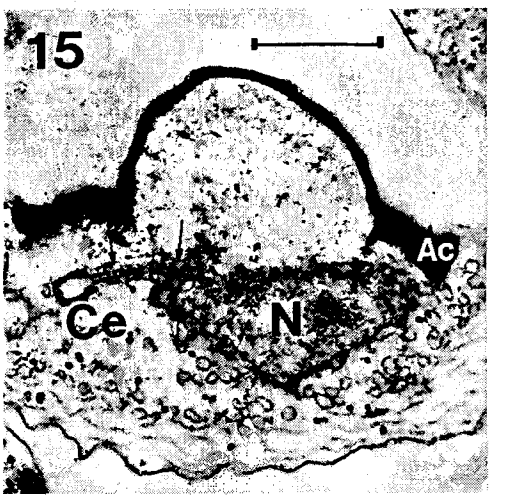
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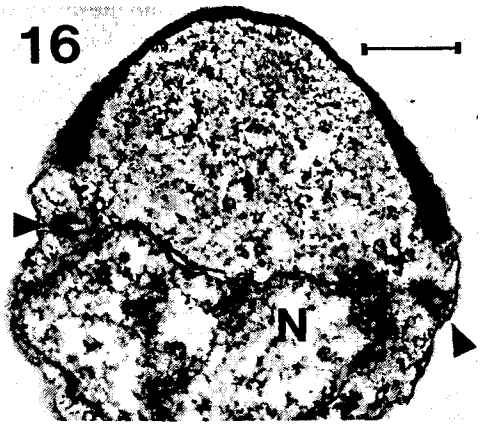
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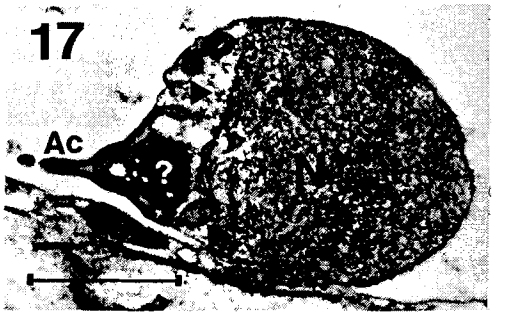
17
Ac
?
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Table 1. Comparison of sperm morphology, reproductive characters, and chromosome numbers in six *Ophryotrocha* species

	Sperm morphology			Reproductive characters		Chromosome number (2n =)
	Shape of sperm	Flagellar equivalent	Acrosome	Shape of eggmass	Mucus covering eggmass	
Protandric hermaphrodite						
O. puerilis	spherical	present	conical	irregular	soft	8
Contemporary hermaphrodites						
O. hartmanni	spherical	absent	conical	cylindric	soft	10
O. gracilis	spherical	present	conical	fusiform	hard	10
O. diadema	flat	present	flat	fusiform	hard	8
Gonochorers						
O. labronica	flat	present	flat	tube	hard	6
O. notoglandulata	flat	absent	flat	tube	hard	6

sperm transfer which is accomplished during pseudocopulation (Åkesson, 1973). The most striking interspecific variations concern the shape of the acrosome and the presence or absence of a flagellar equivalent. A middle piece is completely lacking in all sperm studied here. Table 1 summarizes some features of sexual differentiation, sperm ultrastructure, reproductive characters, and chromosome numbers in the six *Ophryotrocha*-species studied. In the schematic representation of sperm ultrastructure (Fig. 24), species are ranked according to Table 1. Up to now, there is no clue to the actual adaptation of sperm to some known reproductive feature in the species investigated here. Neither the shape nor the consistency of the material covering eggmasses allows any correlations to

Figs 11, 12. *Ophryotrocha hartmanni*

Fig. 11. Mature sperm. Ac – acrosome. Note flocculent material in acrosome tip (arrow). Ce – centriole in cross section, N – nucleus in final stage of condensation, ? – vesicle with electron-dense material of unknown origin. Scale bar – 1 µm

Fig. 12. Detail of acrosome (Ac) with the two centrioles (Ce) and a mitochondrion (Mi) with few cristae and electron-lucent matrix. Scale bar – 1 µm

Figs 13–16. *Ophryotrocha notoglandulata*

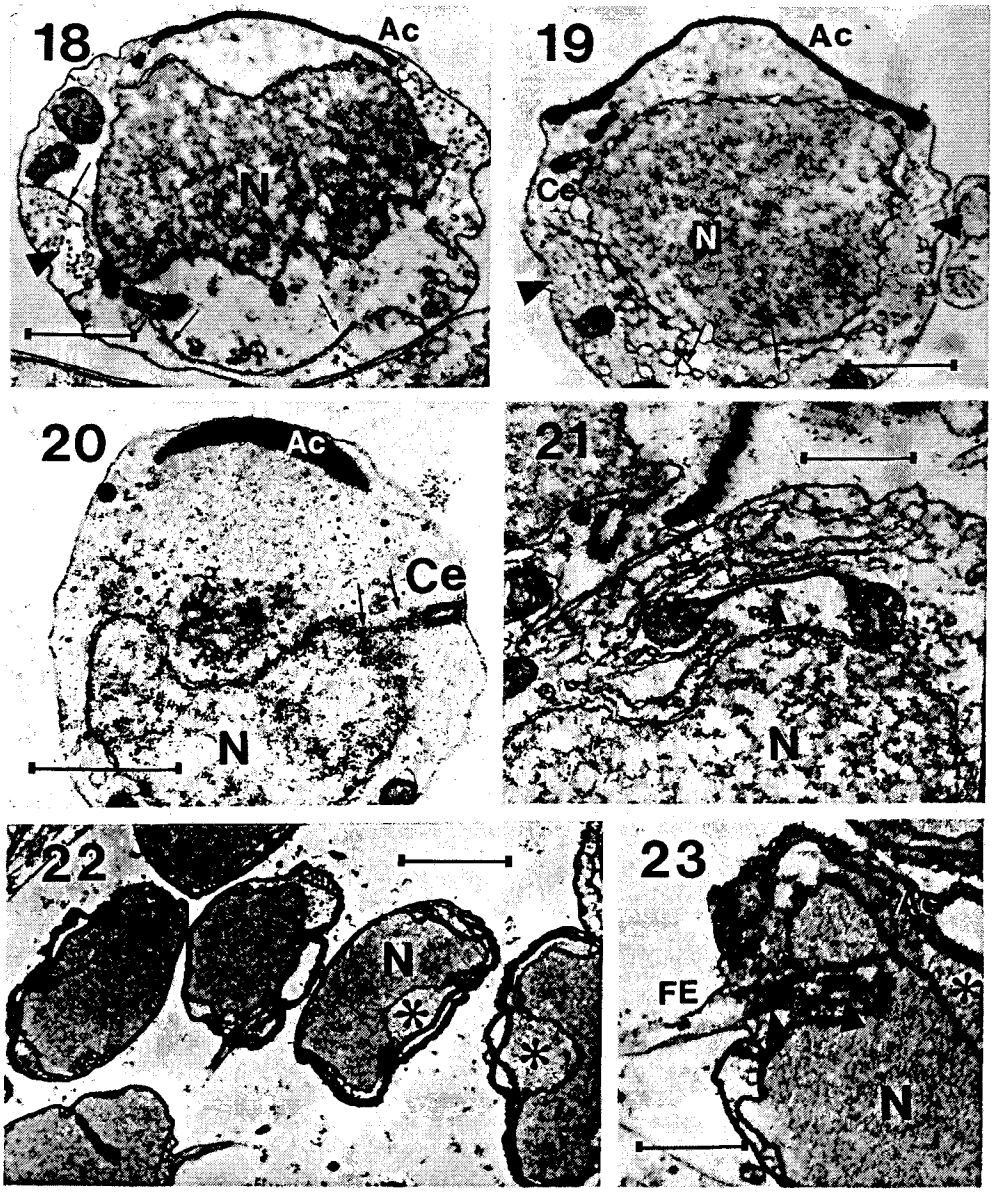
Fig. 13. Mature sperm. Ce – centriole beneath acrosome, N – nucleus, microtubule bundles in cross section (arrowheads) encircled by cytoplasmic membranes (arrows). Scale bar – 1 µm

Fig. 14. Microtubule bundles (arrows) in tangentially sectioned mature sperm. Note cytoplasmic membranes forming chains of vesicles (arrowhead). Scale bar – 1 µm

Fig. 15. Distal centriole of mature sperm (Ce) connected to nuclear envelope by rootlet-like structure (arrows). Scale bar – 1 µm

Fig. 16. Centrioles (arrowheads) located at opposite ends of acrosome in mature sperm. Scale bar – 1 µm

Fig. 17. *Ophryotrocha gracilis.* Mature sperm. Acrosome (Ac) with prominent tip. Note indented basal rim of acrosome (arrowheads). N – nucleus, ? – vesicle with electron-dense contents of unknown origin. Scale bar – 2 µm


18
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acrosome shape or to presence or absence of a flagellar equivalent. It should be noted that sperm of *O. hartmanni* and *O. gracilis* show a lot of striking similarities in the overall shape of the sperm and of the acrosome. However, even sperm of these two species are easily distinguished. The lower rim of the acrosome is indented in *O. gracilis* sperm, and a flagellar equivalent is missing in *O. hartmanni* sperm.

Within the genus *Ophryotrocha*, sperm of all species studied are of the aberrant type. We therefore have to take into account that the interspecific modifications found may as well reflect the phylogenetic relations of species within the genus (see also Westheide, 1984). However, when both aberrant and primitive sperm are found in closely related species (for example: *Platynereis dumerilii* – primitive sperm; Hanske, 1989; *Platynereis massiliensis* – aberrant sperm; Lücht & Pfannenstiel, 1989) these differences must be of adaptive significance. It remains to demonstrate to what features of reproduction or fertilisation sperm are adapted in the respective cases.

Acknowledgements. This work was supported by the Deutsche Forschungsgemeinschaft (Pf 116/4-4, Pf 116/5-1).

Figs 18–21. *Ophryotrocha labronica*

Fig. 18. Almost mature sperm. Ac – acrosome, N – nucleus. Note cross sections of microtubule bundles (arrowheads). Note also cytoplasmic membranes (arrows) subtending nucleus and encircling microtubule bundles. Scale bar – 2 µm

Fig. 19. Mature sperm. Ac – acrosome, Ce – centriole, N – nucleus, arrowheads – microtubule bundles. Note that cytoplasmic membrane (arrows) subtending nucleus (N) resembles nuclear envelope. Scale bar – 2 µm

Fig. 20. Microtubules (arrows) stretching from centriole (Ce) to nuclear envelope. Ac – acrosome. Scale bar – 2 µm

Fig. 21. Elongate mitochondrion (arrow) in almost mature sperm. N – nucleus. Scale bar – 1 µm

Figs 22, 23. *Ophryotrocha diadema*

Fig. 22. Mature sperm in coelomic cavity. N – nucleus, asterisk – subacrosomal space filled with flocculent material. Scale bar – 3 µm

Fig. 23. Tandem centrioles (arrowheads) in deep indentation of nucleus (N). FE – flagellar equivalent, Ac – acrosome, asterisk – subacrosomal space. Scale bar – 1 µm

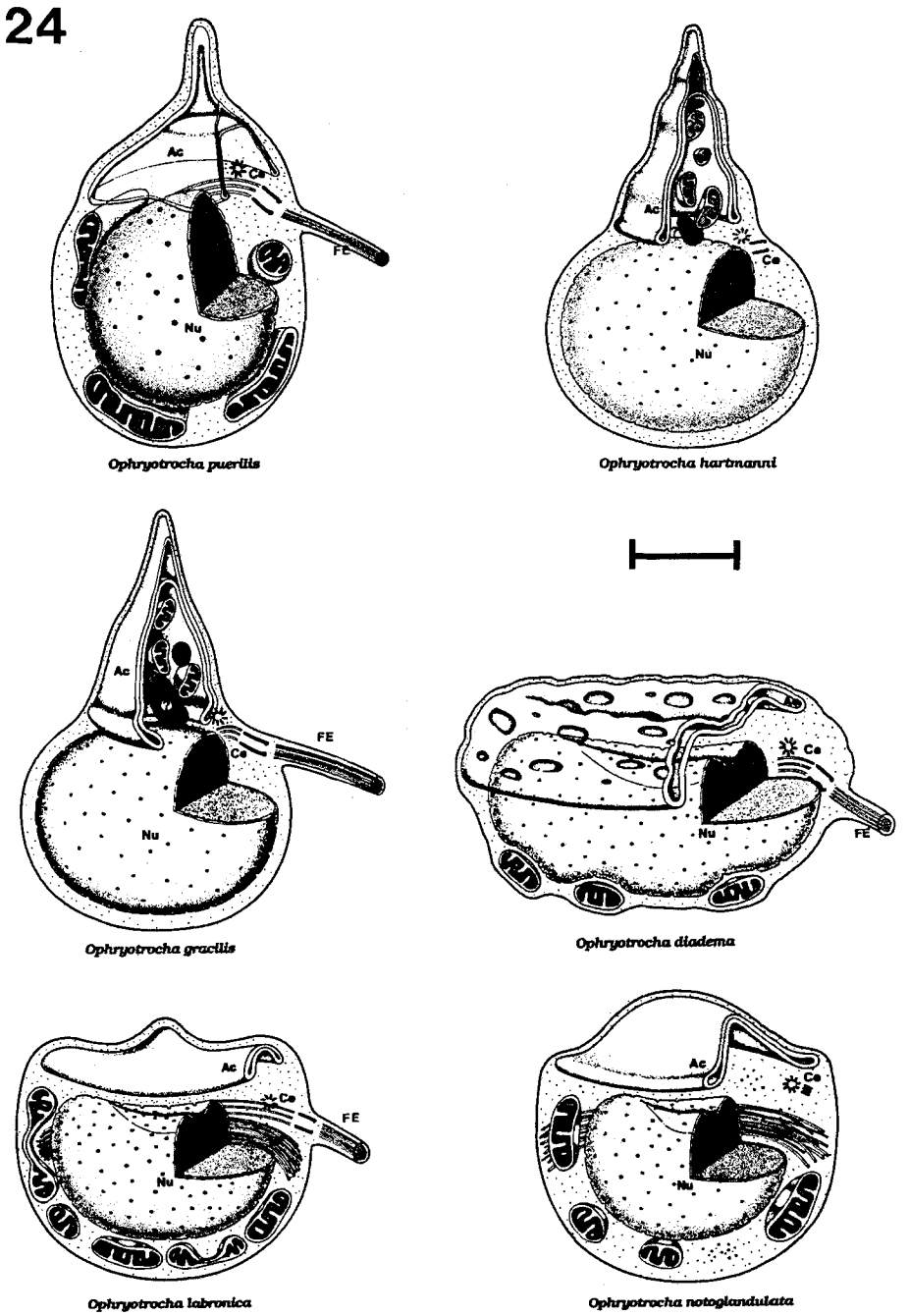

Fig. 24. Schematic reconstruction of sperm ultrastructure in six species of *Ophryotrocha*. Scale bar – 2 μm

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