

# THE SPERMATOOZOA OF THE POLYCHAETA (ANNELIDA): AN ULTRASTRUCTURAL REVIEW

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## I. INTRODUCTION

'Primitive' sperm (*sensu* Retzius, 1904, 1905, 1909; Franzén, 1956, 1977*a*), seen in polychaetes, alone of the annelids and in several other phyla, have been termed 'aquasperm' by Jamieson (1986*a, b*), a term in accordance with the equally appropriate 'aquatic sperm' of Baccetti (e.g. 1979). Recently Rouse & Jamieson (1987) proposed a system of nomenclature, based on function, for the various manifestations of the spermatozoon. Two chief types of aquasperm are recognized: ect-aquasperm and ent-aquasperm. These contrast with sperm which do not enter the water, for which we have proposed the term introsperm. All three types are seen in the Annelida. These terms

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Table 1. *Ultrastructural studies on polychaete sperm*

Polychaete orders yet to be examined for sperm ultrastructure		
Magelonida, Psammodrilida, Cossurida, Spintherida, Sternapsida, Flabelligerida, Fauvelopsida		
Polychaete families for which only aquasperm have been demonstrated ultrastructurally		
Ect-aquasperm only		
AMPHINOMIDA:	Amphinomidae	<i>Eurythoe complanata</i>
EUNICIDA:	Eunicidae	<i>Marphysa sanguinea</i>
	Onuphidae	<i>Hyalinocia tubicola</i> , <i>Diopatra</i> sp. (both possibly ent-aquasperm), <i>Onuphis mariahirsuta</i>
	Lumbrineridae	<i>Lumbrineris</i> sp.
TEREBELLIDA:	Sabelliariidae	<i>Sabellaria alveolata</i> , <i>Phragmatopoma lapidosa</i> , <i>P. californica</i> , <i>Idanthyrus pennatus</i>
	Pectinariidae	<i>Cistenides okudai</i>
CIRRATULIDA:	Cirratulidae	<i>Cirriformia tentaculata</i> , <i>Cirriformia</i> sp.
OWENIIDA:	Oweniidae	<i>Owenia fusiformis</i>
CAPITELLIDA:	Arenicolidae	<i>Arenicola brasiliensis</i> , <i>A. marina</i> (both possibly ent-aquasperm)
SPIONIDA:	Chaetopteridae	<i>Chaetopterus pergamentaceus</i> , <i>C. variopedatus</i> , <i>Mesochaetopterus</i>
	Acrocirridae	<i>Acrocirrus validus</i>
OPHELIIDA:	Opheliidae	<i>Armandia</i> sp., <i>Travisia japonica</i>
PHYLLODOCIDA:	Sigalionidae	<i>Sigalion</i> sp.
	Polynoidae	<i>Lepidonotus</i> cf. <i>carinulatus</i> , L. sp.
	Phyllodocidae	<i>Eulalia</i> sp.
POLYGORDIIDA:	Polygordidae	<i>Polygordius lacteus</i>
Ent-aquasperm only		
SABELLIDA:	Spirorbidae	<i>Spirorbis morchi</i> , <i>S. spirorbis</i>
CAPITELLIDA:	Maldanidae	<i>Clymenella</i> sp., <i>Micromaldane</i> sp.
PHYLLODOCIDA:	Tomopteridae	<i>Tomopteris helgolandica</i>
Ect*- and ent-aquasperm		
TEREBELLIDA:	Terebellidae	<i>Streblosoma acymatum</i> *, <i>Nicolea zostericola</i>
SABELLIDA:	Sabellidae	<i>Sabella penicillum</i> *, <i>Fabricia sabella</i> , <i>Oriopsis</i> sp., <i>Pseudopotamilla reniformis</i> *, <i>Perkinsiana rubra</i> *
	Serpulidae	<i>Hydroides hexagonus</i> *, <i>Galeolaria caespitosa</i> *, <i>Pomatoleios krausii</i> *, <i>Serpula</i> sp.*, <i>Chitinopoma serrula</i>

define sperm with regard to fertilization biology and complement but do not replace terms based on spermatozoal morphology such as 'modified' and 'aberrant' sperm of Franzén (1977*a*) or 'eupyrene', 'oligopyrene' and 'apyrene' sperm of Meves (1903). A recent classification of polychaete sperm types in a brief but valuable, functionally orientated review by Olive (1983) also remains useful. Olive recognized five types of polychaete sperm: I, primitive; II, with a complex acrosome; III a, with a mitochondrial sheath or collar; III b, with a mitochondrial sheath which has fused mitochondria; IV, filiform sperm; V, other (aflagellate and biflagellate). Nevertheless, the term 'primitive' sperm (Retzius, 1904; Franzén, 1956, 1977*a*) is no longer considered valid as, although it is considered to refer both to sperm which are genuinely plesiomorphic, some aquasperm (notably in teleosts) may have attained a similar 'primitive' morphology secondarily. The new terms represent concepts already recognized by Afzelius (1972).

In polychaetes, ect-aquasperm usually have a morphology close to the idealized 'primitive' type, termed the 'plesiosperm' by Jamieson (1987*a, b*), though some modification, such as fusion of mitochondria in some nereids may occur. In a few groups (e.g. urochordates, agnathans, a few teleosts) more complex ect-aquasperm may occur (see below). Ent-aquasperm may be only slightly modified, as shown by some

Table 1. (cont.)

Polychaete families for which ent-aquasperm and introsperm only, have been demonstrated ultrastructurally  
None

Polychaete families for which ect-aquasperm\* and introsperm only, have been demonstrated ultrastructurally

SPIONIDA:	Spionidae	<i>Prionospio</i> cf. <i>queenslandica</i> *, <i>Polydora ciliata</i> , <i>P. ligni</i> , <i>P. socialis</i> , <i>P. websteri</i> , <i>Streblospio benedicti</i> , <i>Tripolydora</i> sp.
	Nereidae	<i>Nereis irrorata</i> , <i>N. diversicolor</i> , <i>N. limbata</i> , <i>N. pelagica</i> , <i>Nereis virens</i> , <i>Neanthes japonica</i> , <i>Perinereis brevicirrus</i> , <i>Platynereis dumerilii</i> , <i>P. massiliensis</i> (pseudintrosperm or ent-aquasperm?), <i>Tylorrynchus heterochaetus</i>
Polychaete families with ect <sup>-1</sup> , ent <sup>-2</sup> aquasperm and introsperm <sup>3</sup>		
PHYLLODOCIDA:	Syllidae	<i>Typosyllis pulchra</i> , <i>Typosyllis</i> sp. <sup>1</sup> , <i>Grubea clavata</i> <sup>2</sup> , <i>Autolytus</i> sp. <sup>1,2</sup> <i>Calamyzas</i> sp. <sup>3</sup> ?
Polychaete families for which only introsperm have been demonstrated ultrastructurally		
EUNICIDA:	Histriobdellidae	<i>Histriobdella homari</i> , <i>Stratiodrillus novaehollandiae</i>
	Dorvilleidae	<i>Ophryotrocha puerilis</i> , <i>O. labronica</i> , <i>Ikosipodus carolensis</i>
	Dinophilidae	<i>Dinophilus</i> , sp. <i>Trilobodrillus axi</i> , <i>T. heideri</i>
ORBINIDA:	Questidae	<i>Questa ersei</i>
CAPITELLIDA:	Capitellidae	<i>Capitella capitata</i> , <i>Capitella</i> , 5 sibling spp., <i>Capitomastus</i> 3 spp., <i>Capitellides</i> 1 sp.
PHYLLODOCIDA:	Hesionidae	<i>Hesionides arenaria</i> , <i>Microphthalmus listensis</i> , <i>Microphthalmus</i> sp.
DIURODRILIDA:	Diurodrilidae	<i>Diurodrilidus westheidei</i>
NERILLIDA:	Nerillidae	<i>Nerilla antennata</i>
PROTODRILIDA:	Protodrilidae	<i>Protodrilus adhaerens</i> , <i>P. ciliatus</i> , <i>P. gracilis</i> , <i>P. haurakiensis</i> , <i>P. helgolandicus</i> , <i>P. hypoleucus</i> , <i>P. jaegersteni</i> , <i>P. jouinae</i> , <i>P. litoralis</i> , <i>P. oculifer</i> , <i>P. purpureus</i> , <i>P. rubropharyngeus</i> , <i>P. submersus</i> , <i>Protodriloides symbioticus</i>
	Saccocirridae	<i>Saccocirrus</i>
	Pisionidae	<i>Pisionia</i>

elongation of the nucleus in *Clymenella*, to highly modified, as in *Micromaldane*. Introsperm in polychaetes are always modified or aberrant sperm; in contrast some teleosts (goodeid cyprinodontiformes, Grier *et al.*, 1978) which have apparently only recently developed internal fertilization have retained the simple sperm characteristic of externally fertilizing teleosts.

#### (1) Definition of the ect-aquasperm

The ect-aquasperm is an aquasperm which is liberated freely into the ambient water in which it (potentially at least) fertilizes the similarly free egg. Ect-aquasperm usually correspond with the basic primitive sperm of Retzius (1904), Franzén (1956, 1970) and Afzelius (1972). This type is characterized, in 'anterior-posterior' sequence, by a caplike acrosome (sometimes absent, as in Cnidaria and the possibly secondary aquasperm of teleosts), a subspherical nucleus, a small number of rounded, cristate mitochondria and a free axoneme with the 9+2 arrangement of microtubules. In its most basic form two centrioles are retained, the distal (posterior) of which forms the basal body of the axoneme, and a satellite- or anchoring-apparatus arises from this centriole. In polychaetes, ect-aquasperm are the most common type of sperm. All known annelid examples of ect-aquasperm are restricted to the polychaetes and those examined ultrastructurally are listed in Table 1. The hypothetical, idealized form, or ground plan, often closely approached in reality, is the plesiosperm. The term

plesiosperm recognizes that, whether or not aquasperm have evolved in some sections of the Metazoa from more complex sperm, the 'primitive' sperm facies may be genuinely plesiomorphic in many metazoan groups. This is not to exclude the possibility (a) that the earliest metazoans may have been free-spawning with sperm of an even simpler form than the plesiosperm or (b) that they were internally fertilizing with more or less complex sperm. In the present work the term plesiosperm will be used to denote spermatozoal morphology agreeing with that of the 'primitive' sperm with no necessary connotation of its being primitive.

Ect-aquasperm of more complex structure occur in urochordates in which, like their ent-aquasperm, the nucleus is elongate and mitochondria are located laterally to it; these modifications appear to be related to complication of the egg envelopes. Highly 'modified' or complex externally fertilizing sperm occur in the Agnatha (e.g. *Eptatretus*, Myxinidae, Jespersen, 1975, and *Lampetra*, Petromyzontidae, Nicander & Sjöden, 1971) and in a few teleosts (e.g. the elopomorph, *Albula vulpes*, Mattei & Mattei, 1973; the lophiiform *Neoceratias*, Jespersen, 1984) in all of which it appears possible that external fertilization has been secondarily acquired.

### (2) Taxonomic occurrence of ect-aquasperm

The following is a list of ultrastructural descriptions of ect-aquasperm available to date. Illustrations in the present work are indicated. For brevity, in the subsequent account references are usually not repeated where they conform exactly with this list.

AMPHINOMIDA: Amphinomidae – *Eurythoe complanata*, Rouse & Jamieson (1987) (Figs 1 A, 14 A).

EUNICIDA: Eunicidae – *Marphysa sanguinea*, Harley & Jamieson, unpublished (Fig. 1 B). Onuphidae – *Hyalinoecia tubicola*, Cotelli & Lora Lamia Donin (1975) (Fig. 1 C); *Diopatra* sp. Rouse (1986) (Figs 1 E, 14 I) (both possibly ent-aquasperm); *Onuphis mariahirsuta*, Harley & Jamieson, unpublished (Fig. 1 D). Lumbrineridae – *Lumbrineris* sp., Rouse (1988 a) (Fig. 1 F).

TEREBELLIDA: Terebellidae – *Streblosoma acymatum*, Harley & Jamieson, unpublished (Fig. 4 A).

SABELLIDA: Sabellidae – *Sabella penicillum*, Graebner & Kryvi (1973 a, b), Kryvi & Graebner (1975) (Fig. 4 B); *Pseudopotamilla reniformis*, *Perkinsiana rubra* (Fig. 4 E), Chughtai (1986). Serpulidae – *Hydroides hexagonus*, Colwin & Colwin (1961 a) (Fig. 4 C); *Galeolaria caespitosa*, by SEM, Grant (1981), by TEM, Jamieson & Harley, unpublished (Figs 4 G, 14 D); *Pomatoleios krausii*, Sawada (1984) (Fig. 4 F); *Serpula* sp., Rouse (1986) (Figs 4 D, 14 B, C). Sabellariidae – *Sabellaria alveolata*, Pasteels (1965); *Phragmatopoma lapidosa*, Eckelbarger (1984) (Fig. 1 H); *P. californica*, Kopp

Fig. 1. Longitudinal sections of polychaete ect-aquasperm or, C and E, putative ent-aquasperm. Only the base of the flagellum is included. (A) *Eurythoe complanata* (Amphinomidae) (from Rouse & Jamieson, 1987). (B) *Marphysa sanguinea* (Eunicidae) (after Harley, 1982)\*. (C) *Hyalinoecia tubicola* (Onuphidae) (after Cotelli & Lora Lamia Donin, 1975). (D) *Onuphis mariahirsuta* (Onuphidae) (after Harley, 1982)\* (Acrosome and tip of nucleus only). (E) *Diopatra* sp. (Onuphidae) (from Rouse, 1986). (F) *Lumbrineris* sp. (Lumbrineridae) (from Rouse, 1986). (G) *Phragmatopoma californica* (Sabellariidae) (after Kopp, 1985)\*. (H) *Phragmatopoma lapidosa* (Sabellariidae) (after Eckelbarger, 1984)\*. (I) *Idanthyrsus pennatus* (Sabellariidae) (from Rouse, Harley & Jamieson, unpublished) (acrosome and tip of nucleus only).

\*Drawn from micrographs. Except where indicated the bar line on each illustration in Figs. 1–15 is 1  $\mu$ m.

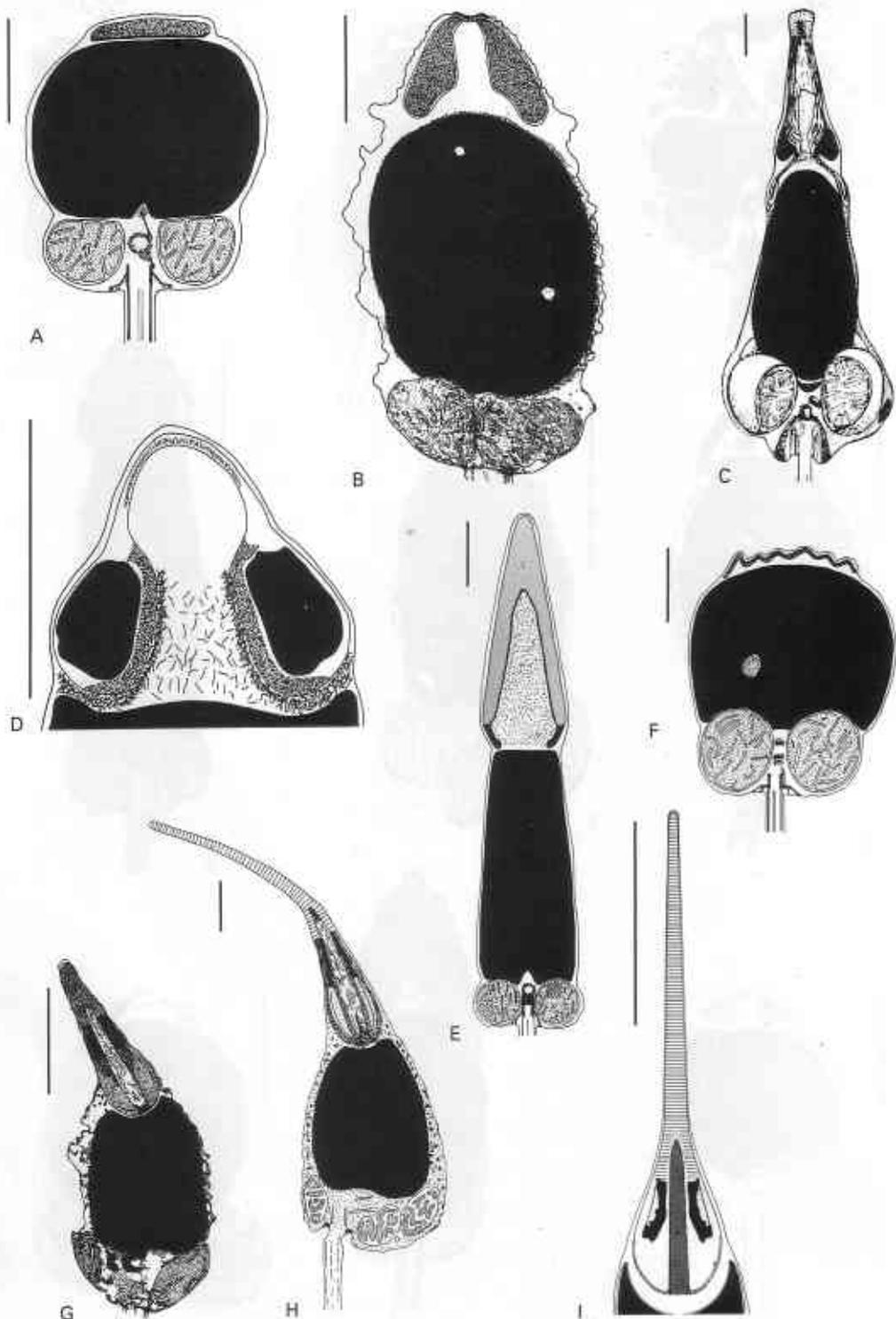


Figure 1. For legend see opposite.

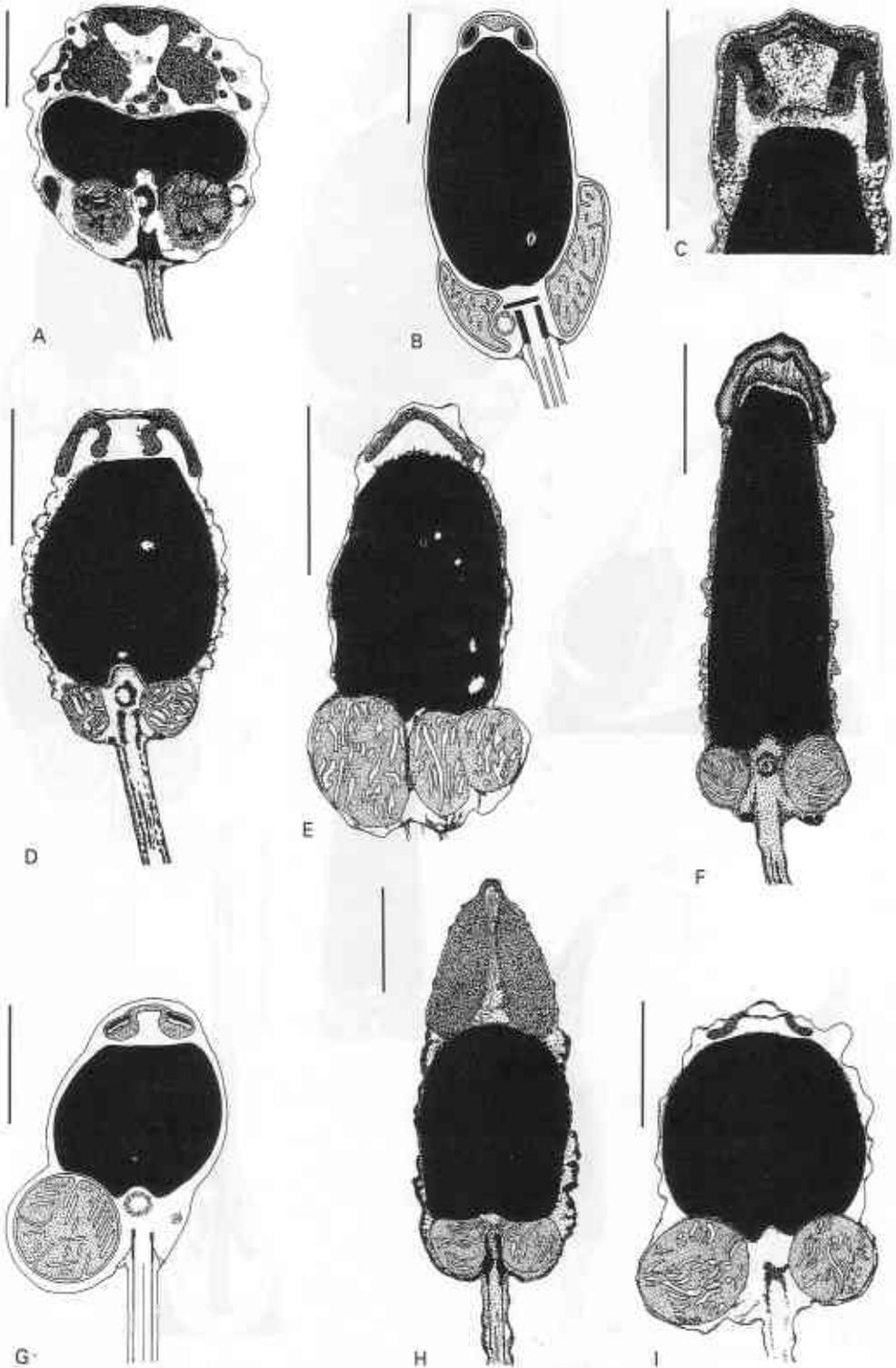


Figure 2. For legend see opposite.

(1985) (Fig. 1 G); *Idanthysus pennatus*, Rouse, Harley & Jamieson, unpublished (Fig. 1 I). Pectinariidae – *Cistenides okudai*, Sawada (1984).

CIRRATULIDA: Cirratulidae – *Cirriformia tentaculata*, Sawada (1984) (Fig. 2 A); *Cirriformia* sp., Harley & Jamieson, unpublished.

OWENIIDA: Oweniidae – *Owenia fusiformis*, Rouse (1988 a) (Fig. 2 B).

CAPITELLIDA: Arenicolidae – *Arenicola brasiliensis*, Sawada & Ochi (1973), Sawada (1975, 1984) (possibly an ent-aquasperm) (Fig. 2 C); *A. marina*, Meijer (1979) (Fig. 2 D).

SPIONIDA: Spionidae – *Prionospio* cf. *queenslandica*, Rouse (1988 b) (Fig. 4 H). Chaetopteridae – *Chaetopterus pergamentaceus*, Anderson & Eckberg (1983) (Fig. 2 F), Eckberg & Anderson (1985); *C. variopedatus*, Harley & Jamieson, unpublished (Figs 2 E, 14 E); *Mesochaetopterus*, Kubo, unpublished *fide* Sawada (1984). Acrocirridae – *Acrocirrus validus*, Sawada (1984).

OPHELIIDA: Opheliidae – *Armandia* sp., Rouse (1986) (Figs 2 G, 14 H); *Travisia japonica*, Ochi *et al.* (1977), Sawada (1984) (Fig. 2 H).

PHYLLODOCIDA: Phyllodocidae – *Eulalia* sp., Rouse (1988 a) (Fig. 3 B). Polynoidae – *Lepidonotus* cf. *carinulatus*, Rouse (1986) (Fig. 3 A); *L.* sp., Rouse (1988 a) (Figs 14 F, G). Nereidae – *Nereis irrorata* (Fig. 3 D); *N. pelagica*, Defretin & Wissocq (1974); *N. diversicolor*, Bertout (1976) (Fig. 3 E); *N. limbata*, Fallon & Austin (1967), Austin (1963) (Fig. 3 G); *Nereis virens*, Bass & Brafield (1972); *Neanthes japonica*, Takashima & Takashima (1963) (as *Nereis japonica*), Sato & Osanai (1986) (Fig. 3 F); *Perinereis brevicirrus*, Kubo & Sawada (1977), Sawada, (1984) (Fig. 3 H); *Platynereis dumerilii*, Pfannenstiel *et al.* (1987) (Fig. 4 I); *Tylorrhynchus heterochaetus*, Sato & Osanai (1981, 1983), Osanai (1983) (Fig. 3 I). Syllidae – *Typosyllis pulchra*, Heacox & Schroeder (1982); *Typosyllis* sp., Harley & Jamieson, unpublished (Fig. 4 J); *Autolytus* sp. group, Franzén (1982 b) (Fig. 4 K). Sigalionidae, *Sigalion* sp., Harley & Jamieson, unpublished (Fig. 2 I).

GORDIIDA: Polygordidae *Polygordius lacteus*, Franzén (1977 b) (Fig. 3 C).

### (3) Ultrastructural variation in ect-aquasperm

#### (a) The acrosome

The acrosome of most polychaete ect-aquasperm show some modification from the simple rounded cap-like vesicle with uniform contents, proposed as primitive by Franzén (1977 a). Sawada (1984) classified acrosome vesicles of polychaete 'primitive' sperm into four types; conical, beret-like, cup-like and pouch-like. These terms have descriptive but little taxonomic value. Despite its variation in form, the acrosome of ect-aquasperm is radially symmetrical with the exception of *Prionospio* cf. *queenslandica*

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Fig. 2. Longitudinal sections of polychaete ect-aquasperm or, (C) putative ent-aquasperm. Only the base of the flagellum is included. (A) *Cirriformia tentaculata* (Cirratulidae) (after Sawada, 1984)\*. (B) *Owenia fusiformis* (Oweniidae) (from Rouse, 1986). (C) *Arenicola brasiliensis* (Arenicolidae) (after Sawada, 1984)\* (acrosome and tip of nucleus only). (D) *Arenicola marina* (Arenicolidae) (after Meijer, 1979)\*. (E) *Chaetopterus variopedatus* (Chaetopteridae) (after Harley, 1982)\*. (F) *Chaetopterus pergamentaceus* (Chaetopteridae) (after Anderson & Eckberg, 1983)\*. (G) *Armandia* sp. (Opheliidae) (from Rouse, 1986). (H) *Travisia japonica* (Opheliidae) (after Ochi *et al.*, 1977)\*. (I) *Sigalion bandaensis* (Sigalionidae) (after Harley, 1982)\*. \* Drawn from micrographs.

(Fig. 4H) in which symmetry is bilateral, *Autolytus* sp. (Fig. 4K), in which it is disc-shaped, on one side of the nucleus and *Phragmatopoma* (Fig. 1H) in which it is curved.

Variation in the form of the acrosome is detailed in the systematic section below but a representative sample of variation, seen in Figs 1-4, ranges from the simple disc of *Eurythoe complanata* (Fig. 1A) to more modified forms (for references see figure legend). In *Lumbrineris* (Fig. 1F) the vesicle has the shape of a crenulated dish; in *Eulalia* sp. (Fig. 3B) of an inverted dish; it has the form, very approximately, of an inverted bowl with thickened rim in *Onuphis mariahirsuta* (Fig. 1D), *Owenia fusiformis* (Fig. 2B), *Armandia* sp. (Figs 2G, 14H), *Sigalion* sp. (Fig. 2I), *Lepidonotus* cf. *carinulatus* (Fig. 3A), *L.* sp. (Fig. 14F), *Galeolaria caespitosa* (Figs 4G, 14D), and *Tylorrhynchus heterochaetus* (Fig. 3I); it is a low cone in *Polygordius lacteus* (Fig. 3C) and, although somewhat inflated, in *Marphysa sanguinea* (Fig. 1B); it has the form of two inverted cups, one inside the other, in *Arenicola brasiliensis* (Fig. 2C) (possibly an ent-aquasperm); in nereids (Figs 3D-H, 4I, 8B) it is typically a more-or-less inflated cone penetrated to near its anterior limit by a well-developed perforatorium, *Tylorrhynchus heterochaetus* (Fig. 3I) being a notable exception; in *Prionospio* cf. *queenslandica* (Fig. 4H) it is a bilaterally compressed dome with deep, narrow subacrosomal invagination; in the sabellariids *Phragmatopoma californica* (Fig. 1G), *P. lapidosa* (Fig. 1H) and *Idanthyrus pennatus* (Fig. 1I), it forms a complexly structured vesicle which surrounds an ill-defined fibrous perforatorium and has an apical cross striated extension which is particularly long in the latter two species; and it is a long cone with a deep conical subacrosomal invagination in *Diopatra* sp. (Figs 1E, 14I) (possibly an ent-aquasperm, however) and, with considerable complexity, in *Hyalinoecia tubicola* (Fig. 1C).

Commonly modification of acrosome takes the form of minor to extreme basal invagination of the vesicle. The resultant subacrosomal space is not infrequently filled with material which is flocculent in *Diopatra* sp., in which it also contains occasional membranous bodies, *Lumbrineris* sp., and *Prionospio* cf. *queenslandica*, granular in *Marphysa sanguinea*, *Onuphis mariahirsuta*, *Galeolaria caespitosa*, *Serpula* sp. and *Chaetopterus pergamentaceus* or fibrous, consisting in *Phragmatopoma lapidosa* of longitudinal fibrils. The subacrosomal material presumably has a perforatorial role and may show transitions to a definite axial rod or perforatorium. Thus in *Cistenides okudai* subacrosomal material consists of coarse materials around a dense core in a deep, round indentation of the vesicle. In the syllids *Typosyllis pulchra* and *T.* sp. (Fig. 4J), the posterior rim of the acrosome vesicle leaves only a small aperture between the enclosed subacrosomal material and the underlying nucleus. The subacrosomal material within the enclosed cavity is differentiated respectively in the two species as an ellipsoidal dense body or as a number of thick oblique and possibly helical filaments, each structure virtually constituting an acrosomal rod. In the opheliid and in the nereids

Fig. 3. Longitudinal sections of polychaete ect-aquasperm. Only the base of the flagellum is included. (A) *Lepidonotus* sp. (Polynoidae) (from Rouse, 1986). (B) *Eulalia* sp. (Phyllodocidae) (from Rouse, 1986). (C) *Polygordius lacteus* (Polygordidae) (after Franzén, 1977)\*. (D) *Nereis irrorata* (Nereidae) (from Defretin & Wissocq, 1974). (E) *Nereis diversicolor* (Nereidae) (after Bertout, 1976)\*. (F) *Neanthes japonica* (Nereidae) (after Takashima & Takashima, 1963). (G) *Nereis limbata* (Nereidae) (from Fallon & Austin, 1967). (H) *Perinereis brevicirrus* (Nereidae) (after Sawada, 1984)\*. (I) *Tylorrhynchus heterochaetus* (Nereidae) (after Sato & Osanai, 1983)\*. \* Drawn from micrographs.

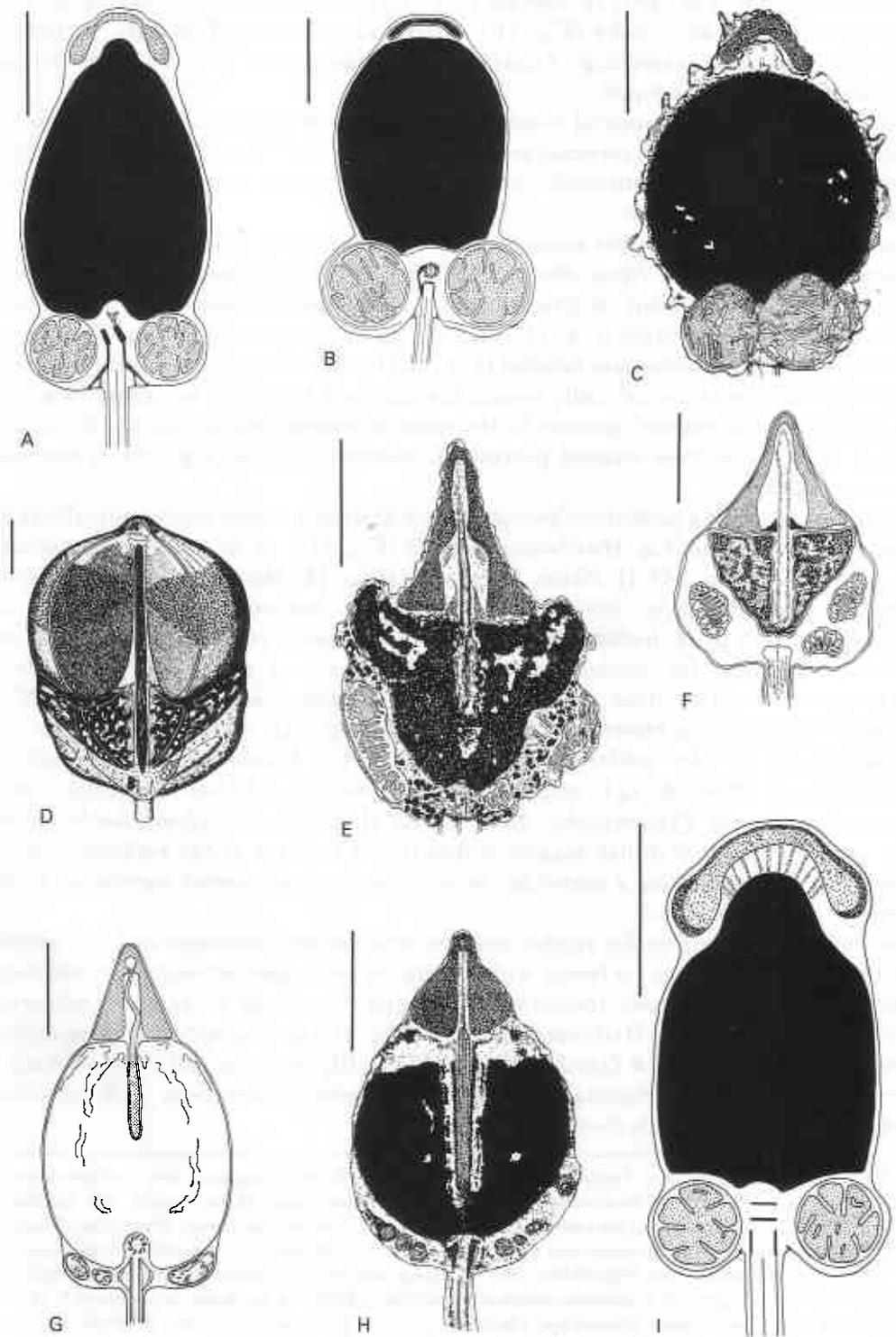


Figure 3. For legend see opposite.

*Neanthes japonica* (Fig. 3F); *N. limbata* (Fig. 3G); *N. virens*; *N. irrorata* (Fig. 3D); *Nereis pelagica*; *N. diversicolor* (Fig. 3E); *Perinereis brevicirrus* (Fig. 3H), *Platynereis dumerilii* (Fig. 4I), excepting *Tylorrhynchus heterochaetus* (Fig. 3I), a definite perforatorial rod is developed.

In some species subacrosomal material is negligible in amount as in *Eulalia* sp., or absent as in *Lepidonotus* cf. *carinulatus* (Fig. 3A) and *L.* sp. (Fig. 14F), though in these species some flocculent material lies between the outer acrosomal and plasma membranes.

Sawada (1984) observes that acrosomes often have peculiar structures in their apical region. The acrosomes of *Nereis diversicolor*, *Perinereis brevicirrus* and *Travisia japonica* have a protuberant tip but in *Nereis irrorata* and *Arenicola brasiliensis* this is more differentiated. In *N. irrorata* (Fig. 3D) it is termed the 'acrosomal button' (Defretin & Wissocq, 1974). In *Hyalinoecia tubicola* (Fig. 1C) the apical region of the very elongate acrosome contains a concentrically wound filament and *Hydroides hexagonus* (Fig. 4C) has a small 'apical vesicle' anterior to the main acrosome vesicle (Colwin & Colwin, 1961a). The apical cross striated process of sabellariid sperm (Fig. 1G-I) has been mentioned above.

Spermatozoa with a prominent perforatorium or subacrosomal space generally show a flattened nuclear tip, e.g. *Hyalinoecia tubicola* (Fig. 1C), or an invaginated nucleus, e.g. sabellariids (Fig. 1G-I). *Nereis diversicolor* (Fig. 3E) shows further modification with an invagination in the nucleus also being formed to accommodate the perforatorium, as in *N. limbata* (Fig. 3G), and with more posterior extension of the perforatorium into the nucleus, in *Perinereis brevicirrus* (Fig. 3H) and, almost reaching the posterior limit of the nucleus, in *Neanthes japonica* (Fig. 3F). This tendency is taken to its extreme in *Nereis irrorata* (Fig. 3D) where the entire nucleus is penetrated by the perforatorium. Alternatively, *Lepidonotus* cf. *carinulatus*, *Lepidonotus* sp. (Figs 3A, 14F) and *Owenia fusiformis* (Fig. 2B) have a nuclear cone, a feature also seen in *Tylorrhynchus heterochaetus* (Fig. 3I), and *Hydroides hexagonus* (Fig. 4C). Rouse (1988a) has suggested that it acts in place of the perforatorium in supporting and presenting a particular area of the inner acrosomal membrane to the oolemma on fertilization.

Ect-aquasperm fertilization studies indicate that the perforatorium acts to present a specific area of membrane for fusion with the egg. In some cases no single perforatorium is formed, rather the inner (posterior) acrosomal membrane invaginates numerous times. This is a feature of *Hydroides hexagonus* (Fig. 4C) and, as apparent homologues, *Sabella penicillum* (Kryvi & Graebner, 1975) (Fig. 4B), the latter authors (Graebner & Kryvi, 1973a) originally maintaining that the acrosome invaginations of *S. penicillum* were not homologous with those of *H. hexagonus*.

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Fig. 4. Longitudinal sections of polychaete ect-aquasperm and, (K) ent-aquasperm. Only the base of the flagellum is included. (A) *Streblosoma acymatum* (Terebellidae) (after Harley, 1982). (B) *Sabella penicillum* (Sabellidae) (from Graebner & Kryvi, 1973a). (C) *Hydroides hexagonus* (Sabellidae) (from Colwin & Colwin, 1961a) (acrosome and tip of nucleus only). (D) *Serpula* sp. (Serpulidae) (from Rouse, 1986). (E) *Perkinsiana rubra* (Sabellidae) (after Chughtai, 1986)\*. (F) *Pomatoleios krausii* (Sabellidae) (after Sawada, 1984)\*. (G) *Galeolaria caespitosa* (Serpulidae) (Harley & Jamieson, unpublished)\*. (H) *Prionospio* cf. *queenslandica* (Spionidae) (Rouse, 1986). (I) *Platynereis dumerilii* (Nereidae) (from Pfannenstiel *et al.*, 1987). (J) *Typosyllis* sp. (Syllidae) (Harley & Jamieson, unpublished)\*. (K) *Autolytus* sp. (Syllidae) (from Franzén, 1982). \* Drawn from micrographs.

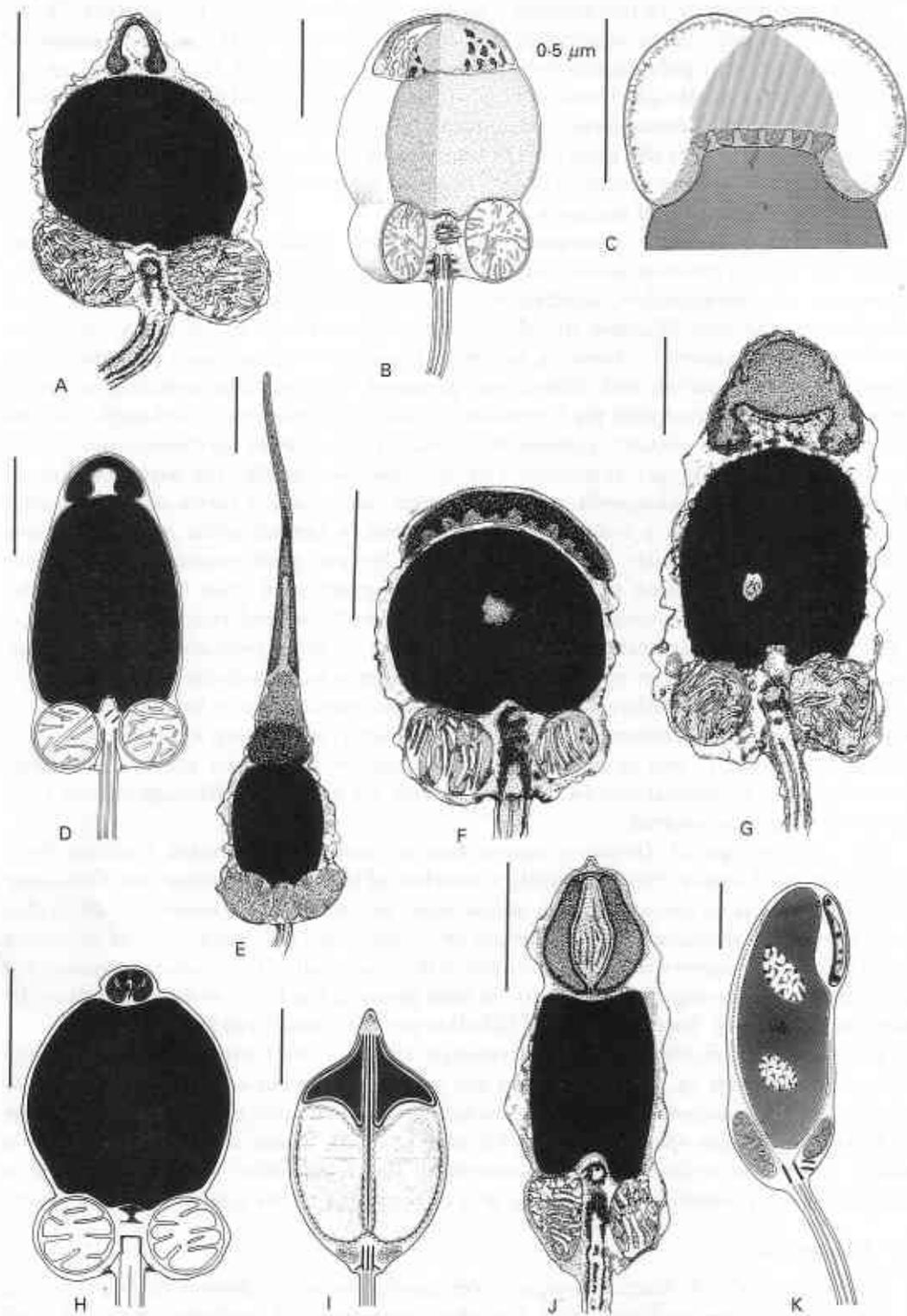


Figure 4. For legend see opposite.

Other modifications to the acrosome involve substructuring of the contents of the vesicle. Some polychaete ect-aquasperm have acrosomes with vesicle contents of uniform density: the polygordiid *Polygordius lacteus*, the opheliid *Travisia japonica*, the serpulid *Serpula* sp., the phyllocid *Eulalia* sp., the amphinomid *Eurythoe complanata*, the chaetopteriid *Chaetopterus variopedatus*, *Typosyllis* sp., and the terebellid *Streblosoma acymatum*; the putative ent-aquasperm of the maldanid *Clymenella* sp. is similar. The fertilization reactions of these species are possibly less complex than those with structured acrosomal contents.

Most other polychaete ect-aquasperm examined ultrastructurally to date show regions of varying electron density within the acrosome vesicle, these include, *inter alia*, *Prionospio* cf. *queenslandica*, *Lumbrineris* sp., *Owenia fusiformis*, *Armandia* sp., and *Onuphis mariaehirsuta*, *Diopatra* sp., *Arenicola brasiliensis* (Sawada, 1975) (the latter two possibly ent-aquasperm), *Arenicola marina*, *Hyalinoecia tubicola* and the sabellariids *Phragmatopoma lapidosa* and *Idanthyrus pennatus*. Where substructuring occurs it appears to be associated with the formation of a subacrosomal space, although *Travisia japonica*, without vesicular substructure, has a prominent perforatorium. Such heterogeneity is strikingly exemplified by the two sabellariids: the acrosome has an unusual, exceedingly elongated anterior portion containing a series of very regular transverse striations in a scalariform arrangement, a central collar and a posterior bulbous region, the latter regions penetrated by the perforatorium. Dan (1970) postulated that, for fusion to the egg, membrane-precursors must be present in the acrosome. She also proposed that the precursors would be lipid-rich and osmiophilic. Fertilization studies indicate, from the occurrence of these presumably lipid-based, osmiophilic densities, that such precursors are present in polychaete sperm.

*Diopatra* sp. and another onuphid *Hyalinoecia tubicola* have exceptionally large aquasperm with the acrosome (4  $\mu\text{m}$  long in *Diopatra*) making up approximately half of the head length; this is possibly a family tendency, but the sperm of *Onuphis mariaehirsuta* is of normal size (with a head about 2.5  $\mu\text{m}$  long) although almost 1  $\mu\text{m}$  of this is, again, acrosomal.

The mature egg of *Diopatra cuprea* has an extremely complex vitelline layer (Anderson & Huebner, 1968), possibly a correlate of the large acrosome; the possibility that the sperm is an ent-aquasperm is discussed in Section IV. Jamieson *et al.* (1983) have shown that in oligochaetes the length of the acrosome (specifically of the acrosome tube) is precisely correlated with the length of the microvilli of the vitelline envelope but in *Capitella* a high negative correlation is seen between the latter and the length of the nucleus (see Fig. 5, based on data of Eckelbarger & Grassle, 1983, 1987 *a, b*).

The functional significance of the crenulate shape of the flattened acrosome of the related *Lumbrineris* sp., a modification not seen in other ect-aquasperm, is possibly similar to that proposed for the many invaginations of the inner acrosomal membrane of *Sabella penicillum* sperm (Kryvi & Graebner, 1975). These authors suggest that it allows a better distribution of lytic enzymes. The fertilization reaction involved in *Lumbrineris* in presenting such a large area of acrosome to the egg is worthy of study.

#### (b) *The nucleus*

The nucleus of polychaete ect-aquasperm usually shows condensed chromatin. This is uniformly dense in (*inter alia*) *Eurythoe complanata*, *Idanthyrus pennatus*, and

*Phragmatopoma lapidosa*. In several species spaces of electron transparency (lacunae) are present in small (*Diopatra* sp., *Lepidonotus carinulatus*, *L.* sp., *Autolytus* sp.; *Polygordius lacteus*) or large numbers (*Chaetopterus variopedatus*). In *Streblosoma acymatum* the nuclear chromatin is, however, poorly condensed and thus has a granular and sporadically lacunate appearance.

There is considerable variation in the nuclei in size and shape, though most showed a subspherical or ellipsoid shape with or without flattening at one or both poles. Such anterior and posterior flattening is seen in *Eurythoe complanata* (Fig. 1 A). Flattening is extreme in *Cirriformia tentaculata* (Fig. 2 A) in which the nucleus is several times wider than long. In most nereids (Fig. 3 D-H) the nucleus is variably depressed by the acrosome, depression, and enlargement of the acrosome, reaching an extreme in *Nereis irrorata* (Fig. 3 D).

Many polychaete species show a central fossa in the base of the nucleus, which functions as an implantation fossa for the proximal centriole (*Lumbrineris* sp., *Nereis limbata*, *Typosyllis pulchra* and *Typosyllis* sp.) or for processes from this (*Cirriformia tentaculata*; *Lepidonotus* spp. *Prionospio* cf. *queenslandica*, *Galeolaria caespitosa*), though this association is not always apparent. The fossa is shallow in *Eurythoe complanata*, *Streblosoma acymatum* and *Owenia fusiformis*, small but pronounced in *Diopatra* sp., *Cirriformia tentaculata* and *Galeolaria caespitosa*; semicylindrical in *Lumbrineris* sp. The nuclear membrane is thickened in the vicinity of the fossa in *Eurythoe complanata*.

In the ect-aquasperm the nucleus is slightly elongated and nearly oval in longitudinal section in *Marphysa sanguinea*, *Lepidonotus carinulatus*, *L.* sp. (Figs 3 A, 14 F) and *Chaetopterus variopedatus* (Figs 2 E, 14 E), *Owenia fusiformis* (Fig. 2 B) and *Eulalia* sp. (Fig. 3 B). In *Diopatra* sp. (Figs 1 E, 14 I), *Chaetopterus pergamentaceus* (Fig. 2 F), *Hyalinoecia tubicola* (Fig. 1 C), *Typosyllis pulchra* and *Typosyllis* sp., and *Arenicola brasiliensis* (Fig. 2 C) (Sawada, 1975) (but not *A. marina*, Fig. 2 D. in which it is ovoid), the nuclei are more elongate than normally seen in other ect-aquasperm, being shortly cylindrical. (The possibility that the sperm of *Hyalinoecia tubicola*, *Diopatra* sp. and some *Arenicola* species are in fact ent-aquasperm is discussed below). Franzén (1983) has demonstrated that certain bivalve species have also evolved ect-aquasperm with elongate nuclei. He suggests that this is correlated with increased egg size and lecithotrophic development (see Fig. 6).

### (c) The midpiece

In polychaete ect-aquasperm 4-6 spherical mitochondria with prominent cristae abut, and perhaps always scallop, the posterior end of the nucleus. The number of mitochondria is most commonly 4, as in *Eurythoe complanata*, *Diopatra* sp., *Lumbrineris* sp., *Pseudopotamilla reniformis*, *Perkinsiana rubra*, *Galeolaria caespitosa*, *Serpula* sp. (Fig. 14 C), *Travisia japonica* (Ochi *et al.*, 1977), *Arenicola brasiliensis* (twice as long as wide) (Sawada, 1975), *Prionospio* cf. *queenslandica*, *Eulalia* sp., and *Lepidonotus* cf. *carinulatus* but is 4 or 5 in *Streblosoma acymatum*, *Sabella penicillum* and *Polygordius lacteus*; 5 in *Lepidonotus* sp., *Idanthyrus pennatus* and *Phragmatopoma californica*; 5 or 6 in *Typosyllis pulchra*, *T.* sp. and *Phragmatopoma lapidosa* and 6 in *Marphysa sanguinea*. There are only three mitochondria in the opheliid *Armandia* in which the centriolar and anchoring apparatus lies between the 'base' pair of mitochondria, along

the central vertical axis of the sperm; the effect on motility would presumably be similar to those ect-aquasperm where the anchoring apparatus is off-centre (e.g. *Phragmatopoma lapidosa*).

The sperm of *Nereis virens*, *Chaetopterus pergamentaceus* and *Owenia fusiformis* are unusual in that they have a single ring-shaped mitochondrion (possibly C-shaped in *N. virens*) resulting from fusion of several smaller mitochondria. In *Owenia* the mitochondrion is circular in transverse section, shows prominent cristae and forms a ring around the centriolar apparatus and extends up one side of the nucleus approximately to its equator; there is no consistent orientation of this extension in relation to the position of the centrioles. In the syllid *Autolytus* there is a crescentic mitochondrion around the base of the nucleus, with or without a smaller mitochondrion. A C- or ring-shaped mitochondrion, encircling the basal body, is seen in the ect-aquasperm of several other invertebrate phyla, e.g. the anthozoan *Metridium* sp. (Afzelius, 1979), the echiurid *Ikedosoma gogoshimense* (Sawada *et al.*, 1975), the articulate brachiopod *Terebratulina caputserpentis* (Afzelius & Ferraguti, 1978) and is a feature of echinoderm sperm (See Jamieson, 1985*a* for review) and of the cephalochordate *Branchiostoma* (Jamieson, 1984). Little phylogenetic significance can be attached to this feature as it has evidently evolved several times and is, indeed, cited by Jamieson (1984) as a paramorphy. Within the Polychaeta this also seems to be the case. Thus the families where a single mitochondrion forms the midpiece, Chaetopteridae, Nereidae and Oweniidae, are only distantly related. The phylogenetic significance of this feature is further contraindicated by the presence of several midpiece mitochondria in other species of these families: the ect-aquasperm of *Chaetopterus variopedatus* Chaetopteridae and in several nereid species, e.g. *Perinereis brevicirrus* (Kubo & Sawada, 1977).

The nereids *Neanthes japonica*, *Nereis limbata*, *Nereis diversicolor*, *Nereis irrorata* and *Perinereis brevicirrus* also differ from the standard ect-aquasperm model (plesiosperm) in having several small mitochondria scattered around the base of the nucleus. Nevertheless, in all cases the mitochondrion or mitochondria form a ring around the centrioles, the distal centriole acting as a basal body for the flagellum.

The ect-aquasperm of the phyllodocid *Eulalia* sp., with one of the least modified acrosomes, is highly unusual in ensheathment of each pair of its four mitochondria by a discrete, extramitochondrial membrane of unknown spermiogenic origin. It is known that during spermiogenesis numerous smaller mitochondria fuse to form the final number (usually four) in mature aquasperm (Baccetti & Afzelius, 1976).

#### (d) *The centriolar and anchoring apparatus*

Polychaete ect-aquasperm retain the proximal and distal centrioles, unlike, for instance, the introsperm of oligochaetes which lose the proximal centriole during spermiogenesis. Most frequently the proximal centriole is perpendicular to the distal, the latter forming the basal body in the same longitudinal axis as the axoneme which arises from it. This condition, similar to the mutually perpendicular arrangement of centrioles in somatic cells, is rightly considered by Afzelius (1979) to be primitive for spermatozoa. It occurs in *Eurythoe complanata*; *Marphysa sanguinea* (approximately); *Streblosoma acymatum*; *Armandia* sp., *Diopatra* sp., *Lumbrineris* sp., *Prionospio* cf. *queenslandica*; *Cirriformia tentaculata*; *Sabella penicillum*; *Pseudopotamilla reniformis*;

*Idanthyrus pennatus*; *Arenicola brasiliensis*; *Arenicola marina*; *Typosyllis pulchra* and *Typosyllis* sp., *Tylorrhynchus heterochaetus* and *Polygordius lacteus*. This condition is also seen in the ent-aquasperm of *Clymenella* sp. and *Autolytus* sp.

*Nereis diversicolor* and *Perinereis brevicirrus* have the proximal centriole embedded in the side of the nucleus some distance from the distal centriole. Other polychaete ect-aquasperm are unusual in having the proximal centriole perpendicular to the distal centriole but lying to one side of it, e.g. *Arenicola brasiliensis* (Sawada, 1975), *Hyalinoecia tubicola*, *Phragmatopoma lapidosa* and *Owenia fusiformis*. However the presence of an anchoring plate, proximal to and crossing the two centrioles, appears unique to *O. fusiformis*.

In *Phragmatopoma* both centrioles are also displaced laterally to the sperm axis. Eckelbarger (1984) suggests that this is to offset the effect of a long curved acrosome. Such eccentricity of the entire centriolar complex is also a feature of the ent-aquasperm of *Fabricia sabella* and of other sabellids: *Fabricia* sp., and *Oriopsis* sp. The effect on motility of this eccentric tail complex would be marked and comparison of motility with that of ect-aquasperm with less modified centriolar complexes would be interesting.

In *Armandia* sp. and *Eulalia* sp. the proximal centriole is at right angles to the long axis of the sperm but the distal centriole is tilted at 30° to this axis.

In contrast, obliquity of the proximal centriole relative to the longitudinal distal centriole occurs in the ect-aquasperm of *Lepidonotus* sp. and *Lepidonotus* cf. *carinulatus*, and, at 45°, in the serpulids *Hydroides hexagonus*, *Galeolaria caespitosa* and *Serpula* sp. Obliquity is also seen in the introsperm of *Chitinopoma serrula* and of *Capitella capitata* (Franzén, 1982b).

Bridging material comparable with that in Anthozoan sperm (Schmidt & Zissler, 1979) connects the proximal and distal centrioles in a number of species, e.g. *Eurythoe complanata*, *Lumbrineris* sp., and *Diopatra* sp. In addition, links between the proximal centriole and the central fossa at the base of the nucleus occur in *Lepidonotus* sp., *Prionospio* cf. *queenslandica*, *Eurythoe complanata* and *Galeolaria caespitosa*, supporting the hypothesis of an anchoring role of the centrioles for the flagellum.

Nine rays (satellite rays) originate at the distal centriole and fuse with the plasma membrane. In some phyla these rays divide to give two or three branches and show constant cross-striations (see Afzelius, 1979). The existence of branching (bifurcation) of the satellite rays in the anchoring apparatus of polychaetes before they join the plasma membrane was shown for the first time in polychaetes in *Galeolaria caespitosa* (Harley & Jamieson, in preparation; cited by Ferraguti, 1983). This is also seen in *Eurythoe complanata*, *Eulalia* sp., *Owenia fusiformis*, *Lumbrineris* sp., and *Lepidonotus* sp. *Prionospio* cf. *queenslandica* and *Diopatra* sp., *Sigalion* sp., *Chaetopterus variopedatus*, in *Sabella penicillum*, in which there is an electron dense cushion at the end of each bifurcation and, with apparently more than one bifurcation, in *Serpula* sp. In *Marphysa sanguinea* the satellite rays are trifurcate at the tips. In *Galeolaria caespitosa*, *M. sanguinea*, and *Chaetopterus variopedatus*, and *Lepidonotus* cf. *carinulatus* and *L.* sp. (Fig. 14G) the rays and their branches are clearly cross-striated and (with the possible exception of *Chaetopterus variopedatus*) join a dense ring under the plasma membrane. The rays also join a ring in *Owenia fusiformis*. Satellite rays from the distal centriole to the plasma membrane are also known in *Typosyllis* sp. and *Polygordius lacteus*. In *Eurythoe complanata* each ray of the satellite complex has the two connections to the

plasma membrane distinct from terminal bifurcation of the ray. Branching, with cross-striation, is probably a widespread feature of polychaete ect-aquasperm but rarely preserves well for ultrastructural examination. In *Nereis irrorata* the normal satellite complex of other nereids is replaced with two longitudinally striated structures arranged like parentheses around the basal body as seen in longitudinal section.

*Eulalia* sp., *Owenia fusiformis* and *Phragmatopoma lapidosa*, with eccentric distal centriole, have a correspondingly displaced anchoring apparatus. In *Hyalinoecia tubicola* the sperm has a posterior collar-like extension, which contains continuations of the nine spokes of the satellite complex of the distal centriole, surrounding the initial portion of the flagellum. That this feature, with the complex acrosome, is related to aberrant fertilization biology is only conjectural and it is for the present regarded as an ect-aquasperm. A similar collar occurs in the putative introsperm of *Capitella*, *Capitomastos* and *Capitellides* (Franzén, 1982*b*; Eckelbarger & Grassle, 1987*a, b*).

The structure of the centrioles is rarely described. The distal centriole has the normal structure of 9 triplet microtubules in *C. variopedatus*, *Typosyllis* sp., *Lepidonotus* cf. *carinulatus*, *L.* sp. (Fig. 14F), *Lumbrineris* sp. and *Eulalia* sp. In *C. variopedatus* a granular matrix extends between adjacent triplets.

Ect-aquasperm of all species examined to date have a flagellum with a 9+2 organization of microtubules. However, some sperm of *Fabricia sabella* have 9+3 or even 9+4 axonemes (Franzén, 1975).

#### (4) *Functional aspects of the ect-aquasperm*

The structure of the externally fertilizing sperm (ect-aquasperm) in relation to function has been succinctly discussed by Afzelius (1972) and Baccetti & Afzelius (1976). It is placed in the wider context of egg sizes, life histories and phylogeny by Hermans (1979), Olive (1985) and Westheide (1984*b*, 1985).

As Afzelius (1972) and Baccetti & Afzelius (1976) argue, the likelihood of an individual sperm effecting fertilization is small because: (1) The sperm can only survive approximately 2–5 h and swim about 180 mm (Gemmill, 1900), largely because energy reserves (glycogen and mitochondrial phospholipids) (Anderson & Personne, 1970; Afzelius & Mohri, 1966) are limited. (2) Contact with the egg is random, as in sea urchins (Rothschild & Swann, 1951) or if chemotactic, as in *Tubularia* and other hydroids (Miller & O'Rand, 1974), the chemotactic substance acts over only a few mm. As an adaptation counteracting the difficulties of external fertilization, great numbers of gametes are typically produced:  $10^{11}$  spermatozoa and  $2 \times 10^7$  eggs in *Echinus esculentus* as a single ejaculation (Harvey, 1946). Accordingly sperm size is very small.

Afzelius (1972) rightly contests the view that 'primitive' sperm (ect-aquasperm of this account) are simple cells, and lists their several derived features (the acrosome; nucleus with evenly dispersed and moderately condensed chromatin but no nucleolus; nuclear membrane lacking pores; composite mitochondria; flagellar endpiece; absence of ribosomes, endoplasmic reticulum and Golgi apparatus; small size). That its general form is primarily an expression of the optimum design for swimming is by no means certain, however. It seems possible to the writers that the typical plesiosperm structure relates at least as much to economy of materials consummate with physiological demands and lack of necessity for complexity as to hydrodynamics. Thus, for modified sperm at least, as Baccetti & Afzelius (1976) point out, the shape of the head does not greatly influence the swimming properties of the sperm (Phillips & Olson, 1974), as can be

calculated from hydrodynamic data (Carlson, 1959). From the standpoint of fluid-dynamic efficiency it is also virtually immaterial whether the cross sectional shape of the sperm tail is circular or of another shape (Flower, 1967). This is not to suggest that many features of the ect-aquasperm are not adaptations for more efficient mechanical functioning, including swimming, and even small advantages in shape would be selected for. For instance, the satellite rays associated with the distal centriole presumably do have an important anchoring function for this basal body from which the vigorously beating flagellum arises, though physiological functions of this apparatus are also conceivable. It should not be difficult to test from theoretical and solid models whether the hydrodynamics of the ect-aquasperm are superior to those of a model, swimming in water, with modified features, such as an elongate nucleus or midpiece, features seen in some ent-aquasperm and even in urochordate ect-aquasperm. A very small size, typical of ect-aquasperm, clearly allows large numbers of sperm, a requisite of broadcast spawning, to be developed, in terms of resources, and stored in relation to available space in the body, prior to release. Given the generally assumed low probability of success for individual sperm and eggs of externally fertilizing organisms it is surprising that the complex vitelline layers of many polychaete eggs act to limit the amount of oolemma available for sperm to fuse with (Sato & Osanai, 1986). Possibly this minimizes deleterious polyspermy in species which swarm when spawning, as in many nereids.

External fertilization places a size constraint on the animal, imposing a lower limit. With a body length of 1 mm or less too few sperm are produced for effective external fertilization by broadcast spawning. Correspondingly, minute interstitial animals have internal fertilization by transfer of spermatophores, copulation and other means (Afzelius, 1972; Baccetti & Afzelius, 1976; Hermans, 1979; Olive, 1985; Swedmark, 1964; Westheide, 1984*b*, 1985).

#### (5) *Evolution of ect-aquasperm*

That the 'primitive' sperm (ect-aquasperm) is in fact the primitive type of sperm for the Metazoa, as advocated by Franzén (1956, 1977*a*) is not beyond question. Theories that the Metazoa derived from conjugating Protozoa would demand regarding external fertilization as secondary. Afzelius (1972) and Baccetti & Afzelius (1976) while considering and rejecting the view that the 'primitive' sperm has arisen independently on several occasions as the best technical solution for achieving external fertilization by gametes shed into water, have not addressed this problem beyond noting that the sperm of the most primitive multicellular phyla (red algae and dicyemid Mesozoa) do not have the 'primitive sperm' morphology but resemble somatic cells. Nevertheless, Baccetti (1985) and Jamieson (1987*a*) recognized the apparent re-evolution of 'primitive' sperm in teleosts. Evidence is presented elsewhere (Jamieson & Leung, in preparation) supporting the tentative proposition that teleosts have secondarily developed external fertilization and the 'primitive' sperm morphology (there lacking the acrosome). As evidence, the more primitive fish have 'modified', acrosomal sperm (Agnatha, Chondrichthyes, Cladistia, Actinistia, Chondrostei, Dipnoi) and either have internal fertilization (Chondrichthyes and coelacanth) or may show evidence of having recently lost this in favour of external fertilization (lampreys, in which a penis is retained).\* The

\* The evidence is equivocal. The proposition that the sperm of 'lower' fish are ect-aquasperm modified for features of the egg such as lecithotrophy and are not evidence for former internal fertilization is also discussed.

possibility that external fertilization has been re-expressed and is secondary to internal fertilization, and that there has been secondary development of the 'primitive' sperm facies, in other aquatic groups, if not in the Metazoa as a whole, deserves consideration. Furthermore, Afzelius (1972) has recognized that ect-aquasperm may have evolved twice, once in the Eumetazoa and independently in the Parazoa (Porifera), although in this case origin in each case from a simpler externally fertilizing sperm, rather than from an internally fertilizing sperm seems to have been envisaged. In view of the supposed phylogenetic discreteness of the Porifera this is a reasonable proposition despite recent demonstration of an acrosome in some poriferan sperm (e.g. Tripepi *et al.*, 1984).

Notwithstanding arguments above for some re-evolution of the 'primitive' sperm and for the possibility that the earliest Metazoa could conceivably have had internal fertilization, the 'primitive' sperm (ect-aquasperm) occurs in so many phyla (16 phyla of 20 listed by Afzelius, 1972) that it remains reasonable to regard it as plesiomorphic for the assemblage which they constitute, as Franzén (1956, 1977*a*) holds and parsimony demands. We have mentioned teleosts above as a possible exception, in which introsperm may have transformed to ect-aquasperm, but an aquasperm is, nevertheless, probably plesiomorphic for deuterostomes as a whole. We have long entertained the possibility of reversal to external fertilization from internal fertilization, or at least from the ent-aquasperm mode of fertilization, in some polychaetes but it would again, seem likely that the aquasperm is plesiomorphic for the Protostomia as a whole. As platyhelminthes have only internal fertilization, with introsperm, it nevertheless remains conceivable that internal fertilization is basic for the Metazoa.

Arguments for secondary development of 'primitive' sperm in sabellid polychaetes have been presented by Rouse (1986). Sabellids have varying reproductive biologies: the larger members of the Sabellinae, e.g. *Sabella penicillum*, generally exhibit external fertilization (Graebner & Kryvi, 1972*a, b*) but fabricines, small mobile worms, such as *Fabricia sabella* (Franzén, 1975), have ent-aquasperm and (Daly & Golding, 1977; Kahmann, 1984) spermathecae, like spirorbids. The fabricine sabellids are generally thought to be more primitive than other Sabellidae (Day, 1967). Knight-Jones (1981) envisages evolution from spirorbids to sabellids through early serpulids. Fabricines are not totally sessile and can move from their tubes, often aided by a pair of posterior eyes, and can make new tubes. Other sabellids, e.g. *Sabella* (Sabellinae) cannot leave their tubes, cannot reconstruct them if removed from them and are often blind (Knight-Jones, 1981). The possibility exists that the first sabellids were similar to the fabricine sabellids of today and, like them, had ent-aquasperm. With the evolution of larger forms, external fertilization and the production of many small planktotrophic larvae was possible, in response to the larger amount of energy, and larger number of cells, consequent on greater size, available for reproduction. The ect-aquasperm model may have redeveloped as most efficient for external fertilization. Chughtai (1986), has independently concluded that in the sperm of sabelliform polychaetes 'reversion to the primitive type could easily have happened through neoteny' of late spermatids, which have four spherical mitochondria. The spirorbid ancestors of sabelliforms (Knight-Jones, 1981), in Chughtai's view, would have been small brooders with modified sperm. Retention of a 'primitive' sperm stage in the spermiogenesis of modified sperm has been acknowledged by a number of workers (Franzén, 1956; Afzelius, 1972; Jamieson, 1987*a*) and has independently been envisaged by the present authors as at least a theoretical pathway to redevelopment of the 'primitive' sperm morphology.

Nevertheless, the alternative possibility of a moderate sized, mobile proto-sabellid with ect-aquasperm giving rise to the totally sessile large sabellids, (these species retaining ect-aquasperm) and the small mobile fabricines (these species having to develop ent-aquasperm) cannot be discounted and is in agreement with the orthodox view (e.g. Franzén, 1975), for the Metazoa as a whole, of the primitive nature of the ect-aquasperm.

## II. THE ENT-AQUASPERM

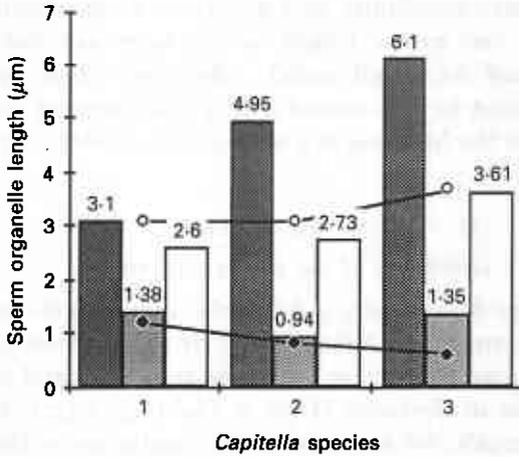
### (1) Definition of the ent-aquasperm

The ent-aquasperm (Rouse & Jamieson, 1987) is also shed into the ambient water but differs from the ect-aquasperm in that it is drawn in by the inhalent or feeding current of the female (sometime hermaphrodite), or otherwise reaches it, and may be stored, for instance in spermathecae, as in *Spirorbis* (Daly & Golding, 1977). Fertilization may occur in water within the female, for instance in the mantle cavity (bivalves) or in the tube (sedentary polychaetes). The ent-aquasperm mode of fertilization appears to be related chiefly to production by the female of large-yolked eggs, in smaller numbers than is effective for external fertilization, in animals which are not, or are poorly, motile. Ent-aquasperm may be significantly or profoundly modified from the basic aquasperm (plesiosperm) type. Modifications relate to features of the egg and to sperm storage. All known annelid examples are, again, restricted to the Polychaeta and those examined ultrastructurally are listed in Table 1 and Section II 2, below.

This subgroup of the 'primitive sperm' has already been recognized by Baccetti & Afzelius (1976). They point out that the difference from external fertilization is not great, since a great number of spermatozoa again have to be produced and released to ensure that the eggs will be fertilized. In contrast, a smaller number of eggs can be produced as the 'internal' fertilization ensures a safe environment for the eggs during fertilization and early development. The eggs tend to be larger than in species with external fertilization, with the notable exception of the bivalve *Teredo*. There Popham (1974) showed only small statistical differences, including shorter acrosomes, in internally fertilizing sperm (ent-aquasperm) compared with externally fertilizing sperm (ect-aquasperm). Correspondingly the eggs of these shipworms are small in both 'internally' and externally fertilizing species.

Baccetti & Afzelius (1976) claim that generally the midpiece of ent-aquasperm is relatively large and enlarges parallel to enlargement of the egg. However, in the putative introsperm of capetillids, mitochondrial dimensions show a high negative correlation with thickness of the egg envelope (Fig. 5). Elongation of the mitochondria does not show an obligate correlation with lecithotrophy for Franzén (1983) has drawn attention to the fact that 'deviated sperm' in bivalves, with elongate nuclei, have a mitochondrial morphology and centriolar apparatus agreeing with that of the 'primitive' spermatozoon even when the egg diameter is well into the range for lecithotrophy. Fig. 6 demonstrates that a bivalve sperm mitochondrial size shows insignificant correlation with egg-size.

Baccetti & Afzelius (1976) note loss of radial symmetry in some cases in what are here termed ect-aquasperm as the mitochondria have an asymmetrical location: tunicates, the mussel *Sphaerium*, the sea anemone *Tealia*, and, we may add, the maldanid polychaete *Micromaldane*. Whether these changes indicate a release from morphological constraints of external fertilization coincident with at least partially passive movement



	Nucleus	Acrosome	Mitochondria	Egg envelope	Yolk diameter
Nucleus	1				
Acrosome	-0.194	1			
Mitochondria	0.858	0.337	1		
Egg envelope	-0.998	0.249	-0.828	1	
Yolk diameter	0.792	0.446	0.993	-0.756	1

Fig. 5. *Capitella* spp. Correlation of various sperm and egg dimensions. Plotted from data of Eckelbarger & Grassle (1983, 1987a, b). ■, Nucleus; ▒, acrosome; □ mitochondria; ●, envelope; ○, yolk.

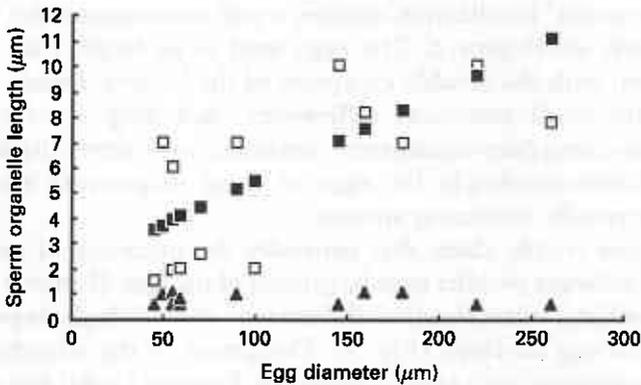


Fig. 6. Positive correlation (0.7) of length of the sperm nucleus with diameter of the egg in bivalves. Contrast the lack of significant correlation (-0.14) of length of the midpiece mitochondria with egg diameter. Plotted from data of Franzén (1983). □, Nucleus length (µm); ▲, midpiece length (µm); ■, Trend nucleus length/egg diameter.

of the sperm to the eggs or are positive adaptations to some features of the more lecithotrophic eggs (or both) is uncertain. Fukomoto (1981) considers that coiling of the mitochondria around the elongate nucleus of the ent-aquasperm of *Perophora formosana* facilitates locomotion of the sperm.

## (2) Taxonomic occurrence of ent-aquasperm

The following is a list of ultrastructural descriptions of ent-aquasperm available to date. Illustrations in the present work are indicated.

EUNICIDA: Onuphidae – *Hyalinoecia tubicola*, Cotelli & Lora Lamia Donin (1975) (Fig. 1C); *Diopatra* sp. Rouse (1986) (Figs 1E, 14I) (both putative ent-aquasperm, though possibly ect-aquasperm).

TEREBELLIDA: *Nicolea zostericola*, Eckelbarger (1975).

SABELLIDA: Sabellidae – *Fabricia sabella*, Franzén (1975); *Fabricia* sp., Rouse (1986) (Fig. 7A); *Oriopsis* sp., Rouse (1986) (Fig. 7B); possibly also *Perkinsiana rubra*, Chughtai (1986) (Fig. 4E). Serpulidae – *Chitinopoma serrula*, Franzén (1982b) (Fig. 7C). Spirorbidae – *Spirorbis morchi*, Potswald (1967); *S. spirorbis*, Daly & Golding (1977), Picard (1980).

CAPITELLIDA: Arenicolidae – *Arenicola brasiliensis* (putative ent-aquasperm) Sawada & Ochi (1973), Sawada (1975, 1984) (Fig. 2C). Maldanidae – *Clymenella* sp., (Fig. 7F) and *Micromaldane* sp. (Figs 7G, 15A, B), Rouse & Jamieson (1987).

PHYLLOCOCIDA: Tomopteridae – *Tomopteris helgolandica*, Franzén (1977a, 1982a) (Fig. 7E). Syllidae – *Grubea clavata*, Franzén (1974) (Fig. 7D); *Autolytus* sp., *prolifer* group (Franzén 1982b) (Fig. 4K).

## (3) Ultrastructural variation in ent-aquasperm

It is unfortunate that in most polychaete species to which ent-aquasperm are attributed and sperm ultrastructure is known this attribution is only tentative as the fertilization biology of the species concerned is not certainly known. In these cases, however, the mode of fertilization in closely related species is of the ent-aquasperm type, with free release of sperm into the ambient water but fertilization in the tube.

The ultrastructure of these putative and known ent-aquasperm varies over a spectrum from slight modification of the plesiosperm (chiefly in elongation of the head) to profound modification involving great elongation of the nucleus and displacement and alteration of the mitochondria; *Tomopteris helgolandica* constitutes a special case in which biflagellarity is developed. Details of the ultrastructure of ent-aquasperm are given in the systematic section (Section IV) but an outline is appropriate here.

Moderate but nevertheless significant elongation of the nucleus (not exceeding 8  $\mu\text{m}$ ), to give a bullet-shape or shortly cylindrical form, occurs in the terebellid *Nicolea zostericola* (Eckelbarger, 1975), the spirorbid *Spirorbis spirorbis* (Daly & Golding, 1977; Picard, 1980), and the maldanid *Clymenella* sp. (Rouse & Jamieson, 1987), as also in the questionable ect-aquasperm of both *Arenicola brasiliensis* (e.g. Sawada 1984) and the onuphid *Diopatra* sp.

Extreme elongation of the nucleus occurs in another maldanid, *Micromaldane* sp., in which, at 25  $\mu\text{m}$  long, it is exceeded only by the nucleus of the introsperm of *Streblospio benedicti* (Rice, 1981). In molluscs elongation of the nucleus has convincingly been correlated with increased diameter of the egg indicative of lecithotrophy (Franzén, 1983; see Section III, (2c), below, and Fig. 6). Indeed, modifications seen in the more derived ent-aquasperm are probably more related to features of the egg than to the occurrence of 'internal' fertilization *per se*. This is indicated by the fact that the type of modification seen in *Micromaldane*, elongation of the nucleus and of two mitochondria along it from its base, features also seen in ent-aquasperm of urochordates

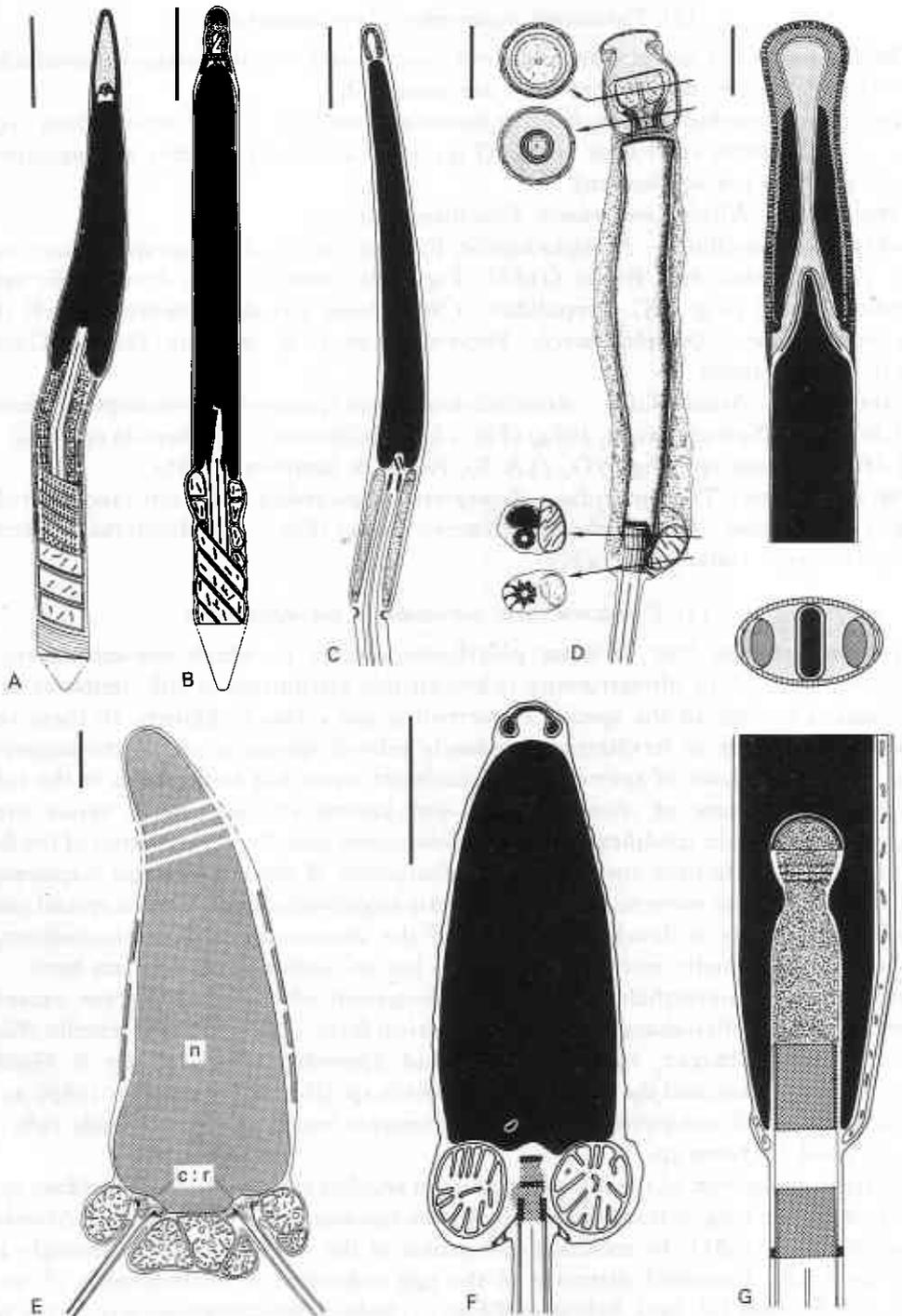


Fig. 7. Polychaete ent-aquasperm. (A) *Fabricia* sp. (Sabellidae) (from Rouse, 1986). (B) *Oriopsis* sp. (Sabellidae) (from Rouse, 1986). (C) *Chitinopoma serrula* (Sabellidae) (after Franzén, 1982b). (D) *Grubea clavata* (Syllidae) (from Franzén, 1974). (E) *Tomopteris helgolandica* (Tomopteridae) (from Franzén, 1982a). (F) *Clymenella* sp. (Maldanidae) (from Rouse & Jamieson, 1987). (G) *Micromaldane* sp. (Maldanidae). From top to bottom: longitudinal section of acrosomal region; cross section at tip of nucleus; and longitudinal section of centriolar region (after Rouse & Jamieson, 1987).

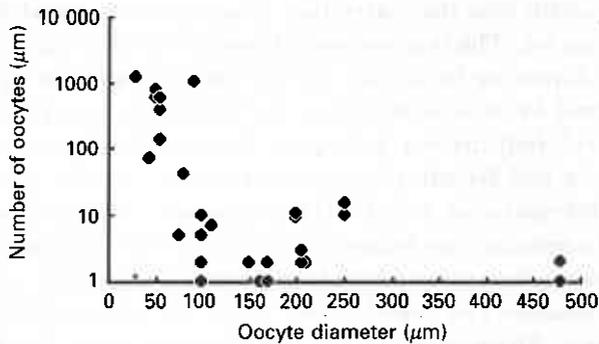


Fig. 8. Relationship between numbers of oocytes produced and their diameters in interstitial polychaetes. Plotted from data of Westheide (1984).

such as *Perophora formosana* (Fukamoto, 1981), also occur in externally fertilizing urochordates such as *Herdmania* (Jamieson, unpublished).

The nucleus is also strongly elongated, with elongation of the four mitochondria around the base of the axoneme, in the ent-aquasperm of the serpulid *Chitinopoma serrula*; with two mitochondria enclosing the base of the nucleus and the two centrioles, in the syllid *Grubea clavata*, in which sperm are deposited in a mass near the female (Franzén, 1974); and, with elongation of the mitochondria along the axoneme in a spiral or an obliquely stacked form, in species of *Fabricia* (Franzén, 1975; Rouse, 1986). In *Fabricia* spermathecae are associated with the tentacular crown (Daly & Golding, 1977; Kahmann, 1984).

In the indubitable ent-aquasperm of *Spirorbis spirorbis*, although elongation of the nucleus and of the mitochondria around the base of the axoneme is slight, profound modification is seen in development of processes of the acrosome which interdigitate with the wall of the spermatheca (Daly & Golding, 1977; Picard, 1980).

The mode of fertilization in *Tomopteris helgolandica* is not certainly known. Franzén (1982a) while classifying the sperm as a primitive sperm, noted three important aberrations: the presence of two flagella, the atypical 9+0 axonemal pattern, and the lack of a typical acrosome. *In vitro*, external fertilization can be achieved (Åkesson, 1962) but Franzén (1982a) considers that this does not disprove an earlier report of a kind of copulation in this species. Franzén (1982a) points out that in related alciopids some of the parapodial cirri are modified as seminal receptacles. If this were the case in *Tomopteris*, our tentative classification of the gamete as an ent-aquasperm would be justified. It may be, however, that as in the urochordate *Herdmania*, referred to above, the sperm are not taken up by the female and that modification of the sperm relates simply to features of the egg. The egg of *T. helgolandica* is exceptionally large for a polychaete, though exceeded by *Protodrilus albicans*, *Pisione remota*, and *Microphthalmus aberrans*, of some 20 species for which egg-size/number correlation has been listed by Westheide (1984b) (Fig. 8). Its diameter of 315 µm correlates with lecithotrophic development (Åkesson, 1962).

### III. THE INTROSPERM

#### (1) *Definition of the introsperm*

The introsperm (Rouse & Jamieson, 1987) never enters the water in aquatic species and is universal for terrestrial metazoans. (It is conceivable that some introsperm are

introduced very transiently into the water but, if so, it is envisaged that they do not swim freely or are immotile). The introsperm is transferred from the male to the female by some form of copulation or by uptake of free spermatophores by the female. In annelids it is transferred by true copulation, via female pores, as in some hesionid, pisionid and saccocirrid polychaetes and some leeches; by 'pseudocopulation', in which the apposed male and female shed gametes directly into a 'cocoon', as in the immotile sperm of *Ophryotrocha puerilis* (Dorvilleidae), or sperm received from a partner into seminal receptacles are subsequently shed onto the eggs within a cocoon as in questid polychaetes and most oligochaetes or, in a manner unknown, in the dorvilleid *Ikosipodus carolensis*; or transfer may be by transdermal injection, as in the histriobdellid polychaete *Stratiodrillus*, in which the male stabs the female with the penis and injects sperm into the female coelomic cavity. Spermatophores may be involved in all of the above modes of introsperm transfer; alternatively spermatophores may be transferred directly to the body surface of the partner, the sperm later penetrating the body wall to reach the eggs, as in some hesionids and leeches, or may be shed into the water and gathered by the female, as in some spionids.

In its typical and most common manifestation the introsperm participates in fertilization within the body of the female or hermaphrodite (i.e. internal fertilization), even where free spermatophores are shed into the water as in spionids. Where fertilization occurs outside the body, but with no motile aquatic phase, as in *Ophryotrocha*, or at least with no motile phase in the external water, as in *Platynereis massiliensis* (possibly, however, an ent-aquasperm), we might speak of a pseud-introsperm.

In an excellent discussion of modes of sperm transfer in polychaetes Westheide (1984*b*) has introduced the term 'hypodermic injection' in place of the imprecise 'hypodermic impregnation' but has used the former term not only for penetration by sperm or spermatophores but also for introduction of sperm through penetration of the integument by a penial apparatus. To the latter ambiguity is added imprecision due to a derived connotation of 'hypodermic' for a penetrative apparatus, a consideration which warrants replacement of 'hypodermic' with 'transdermal' (or, as a more general term to allow for rare absence of an epidermis as in neodermate platyhelminthes, 'transtegumental', although the tegument, as the neodermis, might be considered covered by the term dermis). It is here suggested that 'transdermal' or transtegumental injection' be limited to transdermal (hypodermic) introduction of sperm via a penial apparatus and that penetration of the integument by sperm or spermatophores, unaided by such an apparatus, may be termed 'transdermal spermio-penetration', a perhaps cumbersome term but a much-needed distinction. 'Transdermal insemination' might be employed to cover both terms.

A series in development of internal fertilization with true copulation is seen in eudrilid earthworms: sperm penetrate the wall of the spermatheca in *Stuhlmannia variabilis* to reach the eggs internally. In *S. asymmetrica* an open connection exists between the spermatheca (now effectively a vagina) and the ovarian apparatus (Jamieson, 1967). In *Pisione remota*, in an analogous system, sperm are transferred to the female by insertion of the copulatory organs of the male into genital pores of the female which lead to receptacular organs. These in turn are connected to segmentally arranged nephromixia which function as oviducts (Westheide, 1988).

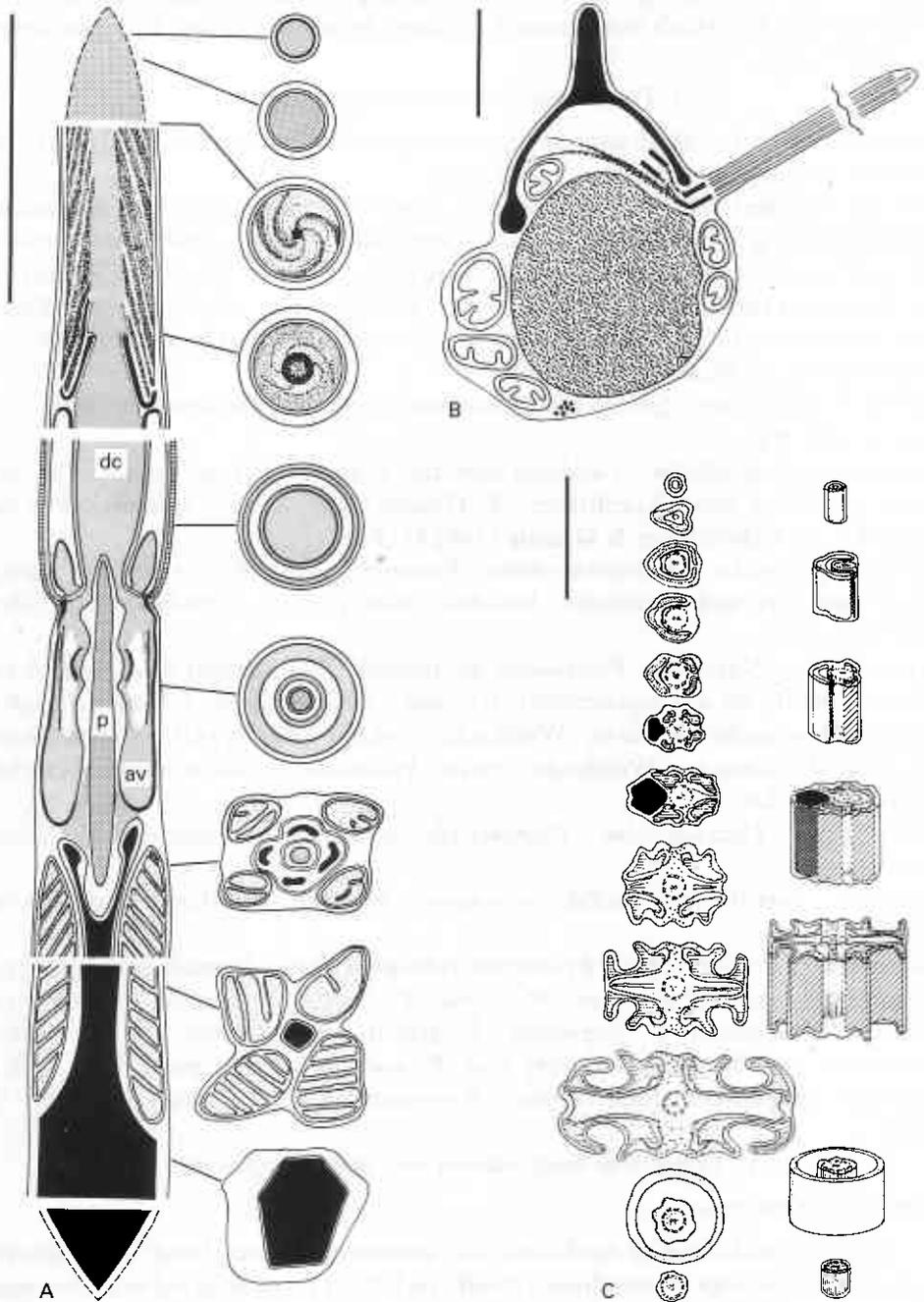


Fig. 9. Polychaete introsperm. (A) *Stratodrilus novaehollandiae* (Histriobdellidae) (from Jamieson *et al.*, 1985). (B) *Ophryotrocha puerilis* (Dorvilleidae) (Troyer & Schwager, unpublished). (C) *Trilobodrilus heideri* (Dinophilidae) (from Scharnfske, 1986).

Introsperm are the only sperm type in the non-polychaetous annelids. All known polychaete species for which introsperm have been demonstrated by TEM are listed in Table 1 and below.

### (2) *Taxonomic occurrence of introsperm*

The following is a list of ultrastructural descriptions of introsperm available to date. Illustrations in the present work are indicated.

EUNICIDA: Histriobdellidae – *Histriobdella homari* (Fig. 15 G, H) and *Stratiodrillus novaehollandiae* (Fig. 9 A), Jamieson *et al.* (1985). Dorvilleidae – *Ophryotrocha puerilis*, Berutti *et al.* (1978), Troyer & Schwager (1979) (Fig. 9 B); *O. labronica*, Berutti *et al.* (1978); *Ikosipodus carolensis*, Westheide (1982). Dinophilidae – *Dinophilus* sp., Franzén (1977 *b*), Westheide (1984 *b*); *Trilobodrillus axi*, *T. heideri* (Fig. 9 C), Scharnofske (1986, and unpublished, in Westheide (1984 *b*).

ORBINIIDA: Questidae – *Questa ersei*, Jamieson (1983 *a, b*), Jamieson & Webb (1984) (Figs 10 A, 15 C–F).

CAPITELLIDA: Capitellidae – *Capitella capitata*, Franzén (1977 *a*, 1982 *b*) (Fig. 10 B); *Capitella*, 5 sibling spp., Eckelbarger & Grassle (1987 *a, b*); *Capitomastus* 3 spp., *Capitellides* 1 sp. Eckelbarger & Grassle (1987 *b*) (Fig. 11).

SPIONIDA: Spionidae – *Polydora ciliata*, Franzén (1974) (Fig. 12 A); *P. ligni*, *P. socialis*, *P. websteri* and *Streblospio benedicti*, Rice (1981); *Tripolydora* sp., Rouse (1988 *b*) (Fig. 10 C).

PHYLLODOCIDA: Nereidae – *Platynereis massiliensis*, Pfannenstiel *et al.* (1987) (Fig. 12 B) (but possibly an ent-aquasperm). Syllidae – *Calamyzas* sp.? Franzén (1982 *a*). Hesionidae – *Hesionides arenaria*, Westheide (1984 *a*) (Fig. 12 C); *Microphthalmus listenis*, *Microphthalmus* sp., Westheide (1984 *b*). Pisionidae – *Pisione remota*, Westheide (1988) (Fig. 13 C, D).

DIURODRILIDA: Diurodrilidae – *Diurodrilidus westheidei*, Kristensen & Niilonen (1982).

NERILLIDA: Nerillidae – *Nerilla antennata*, Franzén & Sensenbaugh (1984) (Fig. 12 D, E).

PROTODRILIDA: Protodrillidae – *Protodrillus rubropharyngeus*, Franzén (1974, 1977 *a, b*), von Nordheim (1987), *P. purpureus*, *P. ciliatus*, *P. oculifer*, *P. jouinae*, *P. haurakiensis*, *P. litoralis*, *P. submersus*, *P. jaegersteni*, *P. gracilis*, *P. adhaerens*, *P. helgolandicus*, *P. hypoleucus*, von Nordheim (1987) (see *Protodrillus* ground-plan, Fig. 13 A, B); *Protodrilloides symbioticus*, Jouin (1979). Saccocirridae – *Saccocirrus*, Jamieson (unpublished).

### (3) *Introsperm modifications and their significance*

#### (a) *Chief introsperm types*

The adaptive significance of modifications, manifested in 'modified' and 'aberrant' sperm, from the plesiosperm condition, chiefly including those referred to as introsperm here, has been the subject of much debate. It would be consistent to classify introsperm according to fertilization biology, specifically the mode of transfer. This would, however, be artificial because of clearly convergent acquisition of modes of transfer, and we will here consider (and modify) the chiefly morphological classification of Westheide (1984 *b*). Space permits only a brief consideration of the main types of introsperm and of the probable adaptive value of the modifications seen.

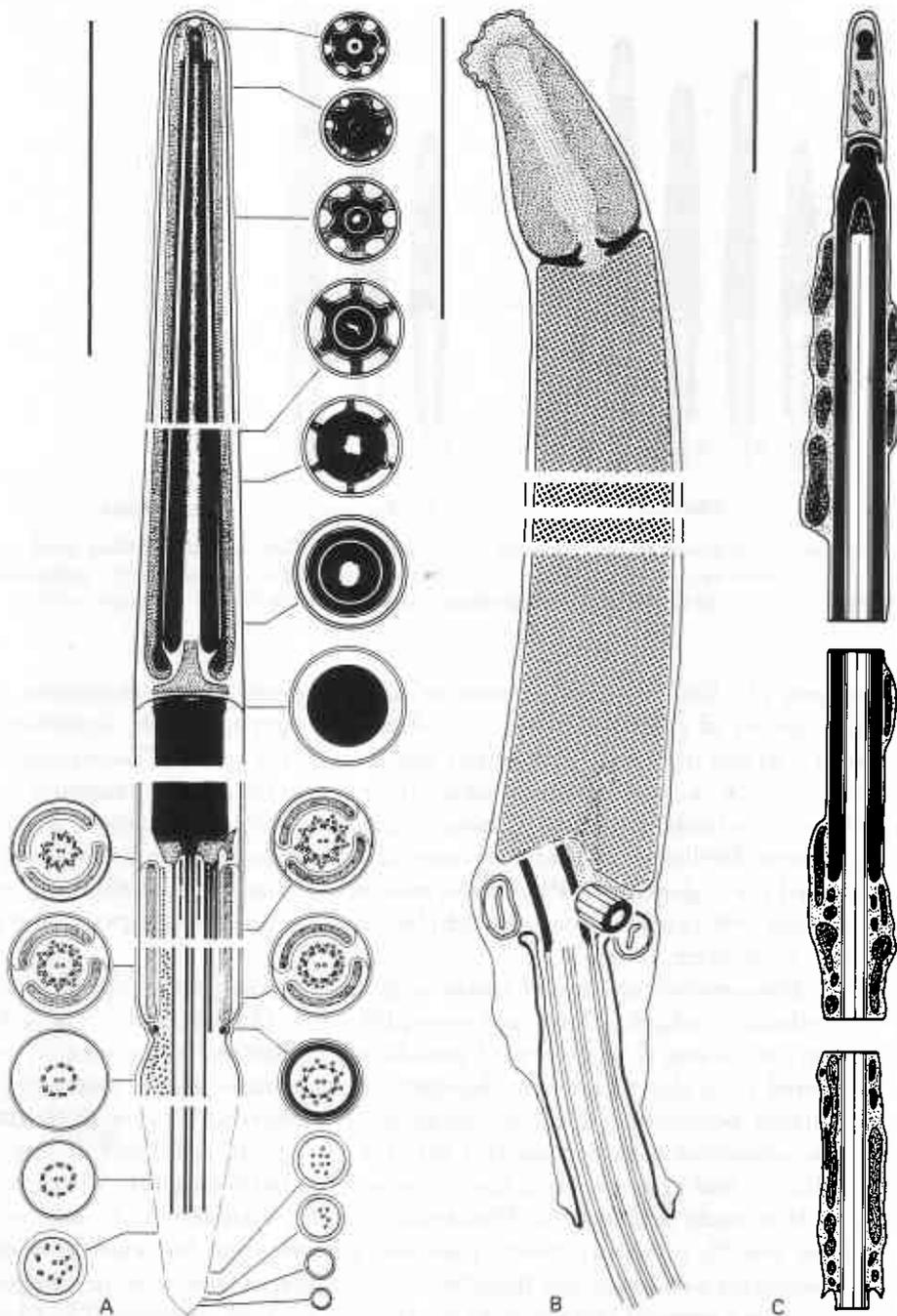


Fig. 10. Polychaete introsperm. (A) *Questa* sp. (Questidae) (from Jamieson, 1983). (B) *Capitella capitata* (Capitellidae; possibly a 'pseud-introsperm') (from Franzén, 1982b). (C) *Tripolydora* sp. (Spionidae) (from Rouse, 1986).

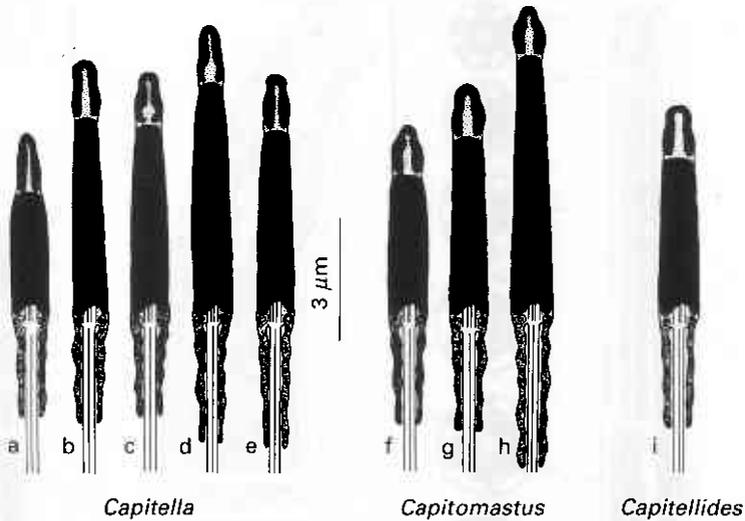


Fig. 11. Schematic diagrams of mature sperm of species of capetillids, including sibling species of *Capitella*. (a) *Capitella* sp. I; (b) *C. sp. Ia*; (c) *C. sp. II*; (d) *C. sp. IIIa*; (e) *C. ORL*; (f) *Capitomastus* MB/s; (g) *C. LYNN*; (h) *C. TRIN/1*; (i) *Capitellides* MB/sl (from Eckelbarger & Grassle, 1987b).

1. *Introsperm* (?) little modified relative to *ect-aquasperm* beyond elongation of the nucleus. The sperm of capetillids (Fig. 11) (Franzén, 1977a, 1982b; Eckelbarger & Grassle, 1987a, b) and the nereid *Platynereis massiliensis* (Fig. 12B) (Pfannenstiel *et al.*, 1987) (see Section IV, below) are morphologically of this type. These examples are not recognized by Westheide (1984b). Although questioned by Eckelbarger & Grassle (1987a, b), internal fertilization (or at least internal deposition of sperm) appears to have been established for *Capitella*. In *Platynereis massiliensis* (Fig. 12B) fertilization occurs in the brood-tube and its spermatozoon might be termed a 'pseudintrosperm' if it is not in fact an *ent-aquasperm*.

2. *Small, approximately spheroidal sperm with loss of a definite midpiece and non-motility* (Westheide, 1984b). These are exemplified by *Ophryotrocha* (Fig. 9B), in which the flagellum is said to be absent (*O. puerilis* and *O. labronica*, Berruti *et al.*, 1978) or is represented by a short immotile flagellum of inconstant length (mean 7.5  $\mu\text{m}$ ), curiously situated posterolateral to the acrosome (*O. puerilis*, Troyer & Schwager, 1979). Pseudocopulation in which contact between the sperm and the ambient water must be negligible and eggs are shed into a cocoon has been elegantly illustrated for *O. gracilis* by Westheide & Bürger (in Westheide, 1984b). Viviparity in *O. labronica* (as in *O. diadema* and *O. vivipara*) clearly indicates true internal fertilization. Another dorvilleid, *Ikosipodus carolensis*, has flagellate filiform sperm (referable to category 4a below) which enter a seminal receptacle in the female; internal, or external fertilization of an oligochaete type, are suspected (Westheide, 1982).

In *Protodriloides symbioticus* there is a complete reduction of the flagellum and the acrosome (Jouin, 1979). In the pseudocopulation seen in this species and *O. puerilis*, active movement of the sperm is purported to be disadvantageous, tending to remove the sperm from the vicinity of the eggs (Troyer & Schwager, 1979; Westheide, 1984b).

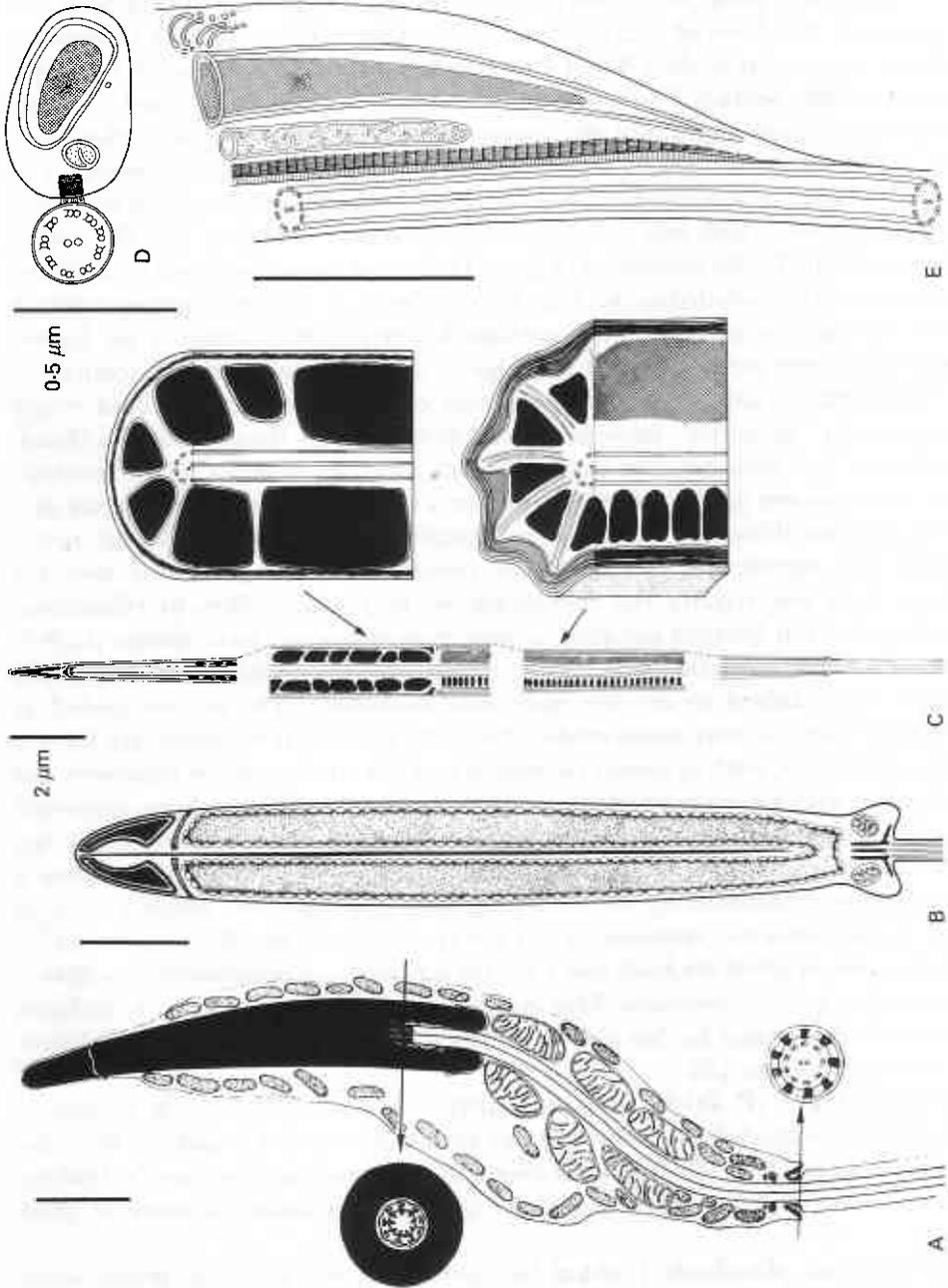


Fig. 12. Polychaete introsperm and, (B) ent-aquasperm. (A) *Polydora ciliata* (Spionidae) (from Franzén, 1974). (B) *Platynereis massiliensis* (Nereidae) (from Pfannenstiel et al., 1987). (C) *Hesionides arenaria* (Hesionidae) (from Westheide, 1984). (D, E) *Nerilla antennata* (Nerillidae) (from Franzén & Sensenbaugh, 1984).

The aflagellate, ellipsoid sperm of the syllid *Calamyzas*, described by Franzén, 1982a), may also be included in this category.

3. *Sperm superficially resembling 'primitive' sperm.* This category is recognized by Westheide (1984b) for sperm which have a compact spindle- or egg-shaped head and a clearly separated flagellum of varying length, e.g. Diurodrilidae. As he recognizes, their simplicity is spurious as their TEM structure is aberrant (Kristensen & Niilonen, 1982; see *Diurodrilus*, below). The mode of fertilization in *Diurodrilus* is not certainly known, as no males were found, but the sperm are probably introsperm as they have been found by the latter authors in the coelom near the oocytes. *Pisione remota* (Fig. 13 C, D), also included in this category by Westheide, is somewhat elongated and lacks a flagellum (Westheide, 1988) and would better be placed in category 4b. The sperm of nerillids, of which *Nerilla antennata* (Fig. 12 D, E), has been described by Franzén & Sensenbaugh (1984), included in this type by Westheide, is a filiform sperm in which the axoneme parallels the nucleus and is perhaps better placed in category 4a. In this species spermatophores are attached to the eggs or are deposited in their vicinity.

One is reminded, in category 3, of the sperm of schistosome trematodes which though superficially 'primitive' have peripheral microtubules throughout the 'head' and tail and have the mitochondria in an aberrant location, anterior to the nucleus. Supposedly, schistosome sperm have returned to a superficially primitive facies as a correlate of purported liberation into the gynaeocophoric groove (Kitajima *et al.* 1976).

Presumably the significance of superficial simplicity in category 3 is that the spermatozoon does not require the modifications, particularly filiform elongation, which we will discuss in the next category in more typical internal fertilization. As they nevertheless have to penetrate the eggs, which in diurodrilids are large and lecithotrophic, their failure to develop such modifications might be interpreted as indirect evidence that the more usual modifications for internal fertilization are for one or more other functions, such as sperm packaging and penetration of the tegument and of viscous media, with penetration of the egg less important than has been suggested elsewhere. A solution of this quandary might be found in investigation of the egg envelope which may well be the decisive feature, (with absolute egg-diameter a secondary correlate), determining, or covarying with, spermatozoal features such as nuclear and, in oligochaetes (Jamieson *et al.*, 1983), acrosomal length.

4. *Filiform sperm, in which the head, and often the acrosome and mitochondrial midpiece, is significantly, often greatly, elongated.* This is a very broad category and chiefly includes flagellate sperm (here termed 4a) but also rare aflagellate examples, as in histriobdellids (Fig. 9A) (here referred to 4b).

*Pisione remota* (Fig. 13 C, D) (Westheide, 1988) is here included in 4b. Sperm of category 1 could be included in category 4a as an initial step in elongation. We may attempt to relate some of the chief modifications to the particular fertilization biology of category 4 sperm and therefore to deduce the adaptive value of some of their modifications.

As a generalization, Westheide (1984a) has contended that elongate sperm reach their most complex and modified form in interstitial species with 'hypodermic injection' (transdermal injection), with minimization of diameter, formation of specific head region, and increase of the propulsory structures (e.g. *Protodrillus*, Fig. 13A, B, Franzén, 1977a, b, von Nordheim, 1987; *Microphthalmus listensis*, *M. sp.*, Westheide,

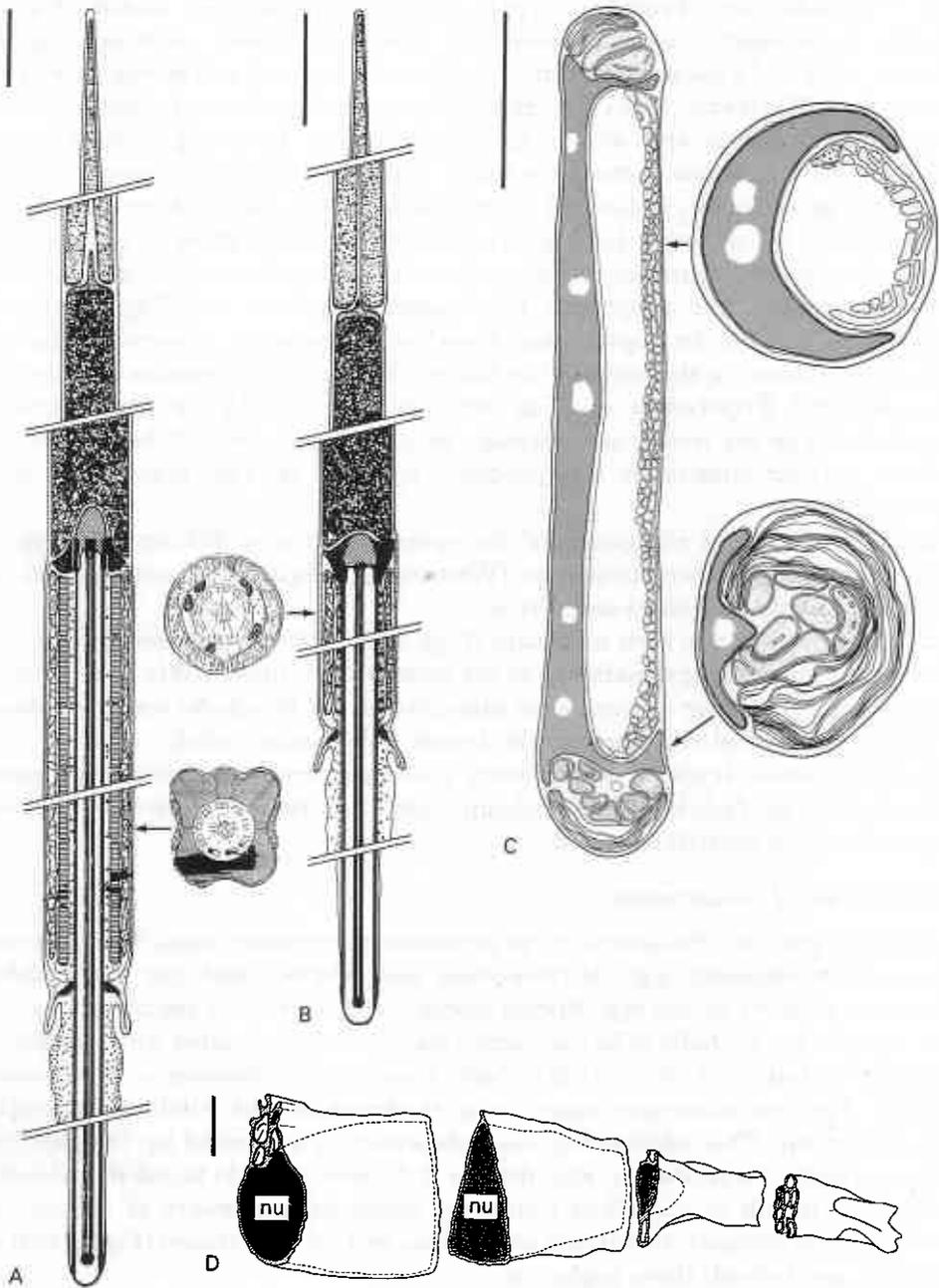


Fig. 13. Polychaete introsperm. (A, B) *Protodrilus* (Protodrilidae) ground-plan, (A) of eusperm, (B) of parasperm (from von Nordheim, 1987). (C, D) *Pisonie remota* (Pisonidae) in male and female respectively (from Westheide, 1988).

1984*b*; *Dinophilus* sp., Franzén, 1977*b*; *Trilobodrilus axi*, *T. heideri*, Fig. 9C, Westheide & Schmidt, 1974, Scharnoffske, 1986; *Hesionides arenaria*, Fig. 12C, Westheide, 1984*a*). In the last four forms the filamentous form and motility are perhaps augmented, as Westheide (1984*b*) suggests, by extension of the axoneme along the nucleus, to its anterior end within an extraordinarily long implantation fossa in *Hesionides*. The implication is that here much of the modification of the sperm, and of the flagellum in particular, is directed to penetration of the tissues of the partner rather than specifically of the egg. In support of this, in *Stratiodrillus* (Fig. 9A) where transdermal injection occurs but the sperm are injected into the coelom and do not have to penetrate tissues, the propulsive mechanism (centrioles and flagellum) is lost (Jamieson *et al.*, 1985). An implantation fossa for the axoneme, penetrating the whole length of the nucleus is also seen in the filiform sperm of the spionids *Polydora ligni* (Rice, 1981) and *Tripolydora* sp. (Fig. 10C) (see Section IV) in which uptake of spermatophores by the female is suspected; here the significance of the elongate fossa is unclear; spermatophores are also produced by other spionids which have shorter implantation fossae.

A further function of elongation of the spermatozoa to a filiform shape may be optimal packaging in spermatophores (Westheide, 1984*a*), seen not only in these spionids but also in *Hesionides* and *Nerilla*.

Filiform sperm are also seen in *Questa* (Figs 10A, 15C–F) (Jamieson, 1983*a, b*) in which they are stored in spermathecae of the concopulant, presumably to be shed from these onto the eggs in the cocoon, as in oligochaetes. In *Ikosipodus carolensis* they are also stored in a seminal receptacle in the female (Westheide, 1982).

Only in *Protodrillus* is sperm dimorphism, giving eusperm and parasperm, known in the Polychaeta (Fig. 13A, B) (von Nordheim, 1987); functional differences between the two types have not been determined.

#### (b) *Modification of the acrosome*

In category 4 sperm, elongation of the acrosome is frequently seen. This also occurs in some ent-aquasperm, e.g. *Micromaldane* (see above), and can reasonably be attributed to features of the egg. Sperm storage, either prior to ejaculation or in the female, appears too sporadic to be considered significantly correlated with elongation of the acrosome. Jamieson *et al.* (1983) have conclusively demonstrated a statistical correlation between acrosome length and thickness of the vitelline envelope in oligochaete sperm. This relationship was independently suggested by Franzén (1983) for bivalve sperm. Nevertheless, Eckelbarger & Grassle (1987*b*) found no evidence of correlation of length of the albeit minimally elongated acrosomes of the sperm of *Capitella* spp. (in category 1) with egg dimensions and we have shown (Fig. 5) that such correlations are, indeed, there negligible.

It is probably that the very complex substructure of the acrosome in, for instance, histriobdellids, relates to events in and after penetration of the egg envelopes, very probably denoting sequestering of enzyme systems.

Baccetti & Afzelius (1976) relate the absence of an acrosome in the ect-aquasperm of freshwater animals to the inability of this organelle, if it were present, to resist the stress of a hypo-osmotic medium. This may well be the reason in some cases for its absence but, if so, the sperm of freshwater bivalves and the lower fish which spawn in freshwater

(Agnatha, Cladistia, Holostei, Dipnoi) and which possess the acrosome, have overcome this problem (Franzén, 1983). Replacement of the acrosome in the biflagellate sperm, here considered an ent-aquasperm but possibly an introsperm (see Section II 3, above), of the marine polychaete *Tomopteris* with tegosome-like structures (Franzén, 1977a, 1982a) presumably relates to features of the egg. This is large, 280–315  $\mu\text{m}$  wide, at fertilization, but it is uncertain whether fertilization is by copulation or by free spawning in natural conditions (Franzén, 1982a). An acrosome otherwise appears to be present in polychaete introsperm.

(c) *Modification of the nucleus*

Elongation of the nucleus, a usual phenomenon of internally fertilizing sperm, is seen in some ect-aquasperm (e.g. urochordates) and ent-aquasperm (e.g. *Micromaldane*, urochordates). *Micromaldane* females store foreign sperm in spermathecae prior to fertilization in the tube (Rouse unpublished). Storage, particularly in spermathecae, appears to be strongly correlated with nuclear elongation, though this elongation has other correlates, such as transdermal injection and egg size. For bivalve ect- and ent-aquasperm Franzén (1983) has convincingly demonstrated, and indeed tabulated, the positive correlation between length of the nucleus and increasing diameter (and lecithotrophy) of the egg. His data are plotted in Fig. 6; there is a 0.7 positive correlation of the length of the nucleus with egg diameter and a clear trend is shown. Note that Franzén (1983) and Jamieson *et al.* (1983) in showing the correlation of acrosome length to thickness of the vitelline envelope in lumbricids independently used the midpiece as a 'control' organelle with no correlation with these features of the egg (see, however, Bacetti & Afzelius, 1976, below). We have shown (Fig. 5) a 0.99 negative correlation between nucleus length and thickness of the egg envelope for published data on three *Capitella* species, (Eckelbarger & Grassle, 1983, 1987a, b) but great caution must be attached to results from so small a sample.

Correlation of nuclear elongation with sperm storage is nowhere better seen than in oligochaetes. In these packing of sperm side to side in the spermathecae may be extremely close, sometimes resembling a crystalline array (Jamieson, unpublished). Equally close packing could be achieved by spheres but a spherical form would be incompatible with maintenance of sufficient surface area for presumed exchanges between the sperm and the bathing medium together with the necessary volume of modified spermatozoal organelles, including the acrosome, nucleus and mitochondria.

Development of a deep implantation fossa, so that the axoneme penetrates virtually the entire length of the nucleus, which is converted into a very elongate thin cap, for instance, in *Hesionides*, is seen by Westheide (1984a) as a device for increasing motility and penetrative ability of the sperm. As noted above, it also occurs in the spionids *Polydora ligni* (Rice, 1981) and *Tripolydora* sp., in which it is not correlated with transdermal impregnation. Unfortunately, the mode of fertilization is unknown in these worms beyond that it involves spermatophores. Elongation of the nucleus along but external to the anterior region of the axoneme in *Nerilla antennata* (Franzén & Sensenbaugh, 1984) and *Trilobodrilus* (Scharnoffske, 1986) presumably subserves a similar function. In *Trilobodrilus* although there is transdermal impregnation (with spermatophores) this is supposedly aided by histolysis (Westheide, 1984b).

*(d) Modification of the mitochondria*

Of the major modifications of the mitochondria which Baccetti & Afzelius (1976) recognize for modified sperm in general, the following can be said in relation to polychaetes.

1. The ratio of mitochondrial volume to cell volume is higher than in 'primitive' sperm (Favard & André, 1970).

2. The mitochondria form one or a few long strands which extend along or twist around the flagellum (or the nucleus in histriobdellids and *Micromaldane*). Baccetti & Afzelius (1976), with Davey (1964), reasonably relate the filiform condition of insect sperm, and of their mitochondria, to the existence of a narrow micropyle on the egg while recognizing that this is not a universal explanation of elongation of the mitochondria in introsperm. In fish, development of a single micropyle is a correlate of development of simple sperm (ect-aquasperm) lacking an acrosome, seen in teleosts; only the sturgeon (*Chondrostei*) combines occurrence of micropyles (there several) with retention of a 'modified' ect-aquasperm with an acrosome.

We have seen that ent-aquasperm are said to show a facultative positive correlation between mitochondrial length and increasing egg-size (Baccetti & Afzelius, 1976), a correlation which is lacking in bivalves (Franzén, 1983) and that Jamieson *et al.* (1983) found no correlation in mitochondrial length in the introsperm of oligochaetes and thickness of the vitelline envelope. Nevertheless, the overwhelming majority of, 'directly transferred' sperm, including those of 'internally fertilizing polychaetes and most oligochaetes, have elongate mitochondrial sheaths. Such elongation, with or without longitudinal and transverse fusion, is well exemplified by the introsperm of histriobdellids (Jamieson *et al.*, 1985), *Dinophilus* sp., *Trilobodrilus axi* and *T. heideri* (Scharnofske, 1986), *Questa ersei* (Jamieson, 1983 *a, b*), *Hesionides arenaria* (Westheide, 1984 *a*), *Microphthalmus* sp. (Westheide, 1984 *b*), *Nerilla antennata* (Franzén & Sensenbaugh, 1984, though not sheathlike in this species) and *Protodrilus* spp. (Franzén, 1974, 1977 *a, b*; von Nordheim, 1987) and in the spionids *Polydora*, *Streblospio* (Franzén, 1974; Rice, 1981) and *Tripolydora*. Mitochondrial elongation is not exclusive to introsperm, however, as it is seen in other 'modified' sperm: the ent-aquasperm of *Micromaldane* (though not in that species as a sheath; Jamieson & Rouse, 1987), and of the sabellids *Fabricia* (Franzén, 1975; Rouse, 1986) and *Oriopsis* (Rouse, 1986).

3. The inner organization of the mitochondria is more regular than is the case in primitive sperm or in somatic cells. This is not notably the case in polychaete sperm but is exemplified by histriobdellids (Fig. 9A) in which a regular, scalariform arrangement of transverse cristae is seen throughout the very elongate mitochondria (Jamieson *et al.*, 1985).

4. Enlargement of the intracrystal space relative to the matrix, frequent in introsperm (André, 1962), does not appear to be a feature of polychaete sperm.

5. Development of mitochondrial crystals, seen in insect sperm (references in Jamieson, 1987 *b*) and in pulmonates (Favard & André, 1970) has not been reported for annelids. Paracrystalline bodies in *Hesionides arenaria*, though remarkably reminiscent of those in insect sperm, do not appear to be mitochondrial derivatives (Westheide, 1984 *a*).

(e) *Modification of the axoneme*

The axoneme, of the 9+2 type in ect-aquasperm and ent-aquasperm, with the exception of an occasional 9+3 or 9+4 condition in *Fabricia sabella* (Franzén, 1975) and the biflagellate 9+0 condition in the putative ent-aquasperm of *Tomopteris* (Franzén, 1977a, 1982a), retains the 9+2 condition in most polychaete introsperm. We have seen, though, that the flagellum is reduced or absent in *Ophryotrocha* and totally absent in *Protodriloides symbioticus*, in histriobdellids and in the syllid *Calamyzas*.

Baccetti & Afzelius (1976) note that the flagella of modified sperm often have an extra set of nine accessory elements, peripheral to the 9+2 microtubules. They report this for oligochaetes, gastropods, cephalopods, onychophorans, insects, spiders, cyclostomes and higher vertebrates. Of these, only onychophorans and insects have true peripheral microtubules, as distinct from coarse fibres [in oligochaetes such fibres are in fact known only for *Cryptodrilus* sp. (Jamieson, 1978)]. Peripheral tubules occur in annelids only in the introsperm of the oligochaetoid polychaete *Questa*. There, anteriorly in the axoneme, there are three microtubules outside each of the nine doublets, progressively reducing more posteriorly to one and then none (Jamieson, 1983). The function of these peripheral microtubules is not known.

#### IV. SYSTEMATIC ACCOUNT OF POLYCHAETE AQUASPERM AND INTROSPERM

The classification of the Polychaeta followed here is that adopted by Fauchald (1977) but the arrangement of orders is in accordance with the evolutionary sequence, based on feeding mechanisms, argued by Dales (1962) notwithstanding some justifiable criticism of Dales' system by Orrhage (1973). The various families of the 'Archannelida' are assigned to polychaete orders in accordance with the suggestion of Westheide (1985).

Several polychaete orders have yet to be examined for sperm ultrastructure: Magelonida, Psammodrillida, Cossurida, Spintherida, Sternapsida, Flabelligerida, and Fauvelopsida. Of the orders studied, only ect-aquasperm are known for the largest assemblage of families (Table 1): Amphinomidae (Amphinomida), Eunicidae, Onuphidae and Lumbrineridae (Eunicida), Sabellariidae and Pectinariidae (Terebellida), Cirratulidae (Cirritulida), Oweniidae (Oweniida), Arenicolidae (Capitellida), Chaetopteridae (Spionida), Opheliidae (Opheliida), Sigalionidae, Polynoidae (however, *Harmothoe imbricata* broods larvae under elytra and light microscopy reveals elongate sperm, here considered ent-aquasperm, with a head 28  $\mu\text{m}$  long (Daly, 1972)), Phyllodocidae, Nereidae (Phyllodocida) and Polygordidae (Polygordiida). Only ent-aquasperm, considerably modified, are known in the Spirorbidae (Sabellida), Maldanidae (Capitellida) and Tomopteridae (Phyllodocida).

The influence of fertilization biology on sperm structure, even where phylogenetic relationship is close, is reflected by intrafamilial variation from the plesiosperm to the modified form of the sperm coincident with corresponding changes in fertilization biology from the ect-aquasperm type to the ent-aquasperm type or from the ect-aquasperm to the introsperm type or, at present known for the Syllidae only, variation from the ect-aquasperm to the ent-aquasperm and introsperm type. An exclusive combination of ent-aquasperm and introsperm in a single polychaete family is at present unknown. Knowledge of the sperm of polychaetes is, however, in its infancy.

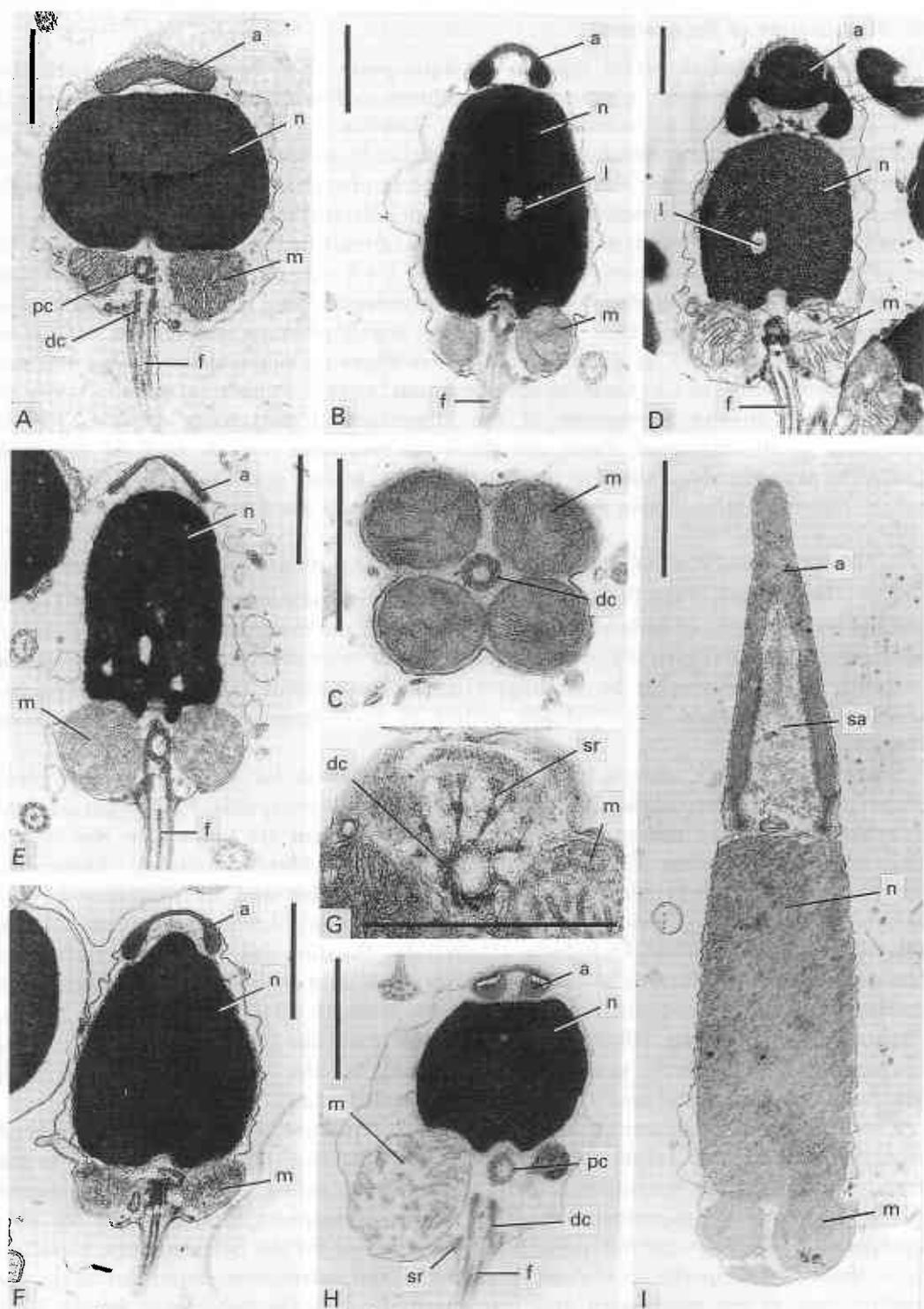


Figure 14. For legend see opposite.

In descriptions of ect-aquasperm in the following accounts, features already described in the overview in Section I will generally not be reiterated. Accounts will chiefly be limited to the acrosome. Ent-aquasperm and introsperm will be more fully described.

## AMPHINOMIDA

### Amphinomidae

The mature spermatozoon of *Eurothoe complanata* (Figs 1 A, 14 A) conforms closely to the basic 'primitive' type (plesiosperm). The vesicle is disc-shaped; the inner acrosomal membrane (closest to the nucleus) is distinctly thickened and electron dense (Rouse & Jamieson, 1987).

## EUNICIDA

### Eunicidae

The eunicid *Marphysa sanguinea* has an ect-aquasperm (Fig. 1 B). The acrosome vesicle is a truncated cone; the subacrosomal space extends anteriorly nearly to the tip of the acrosome and posteriorly becomes greatly widened (Harley & Jamieson, unpublished).

### Onuphidae

In the Onuphidae, although *Onuphis mariahirsuta* (acrosome, Fig. 1 D) has a near-plesiosperm, the ect-aquasperm of *Hyalinoecia tubicola* (Fig. 1 C) has a complex and elongate acrosome indicating that this species may brood larvae. The possible ent-aquasperm of *Diopatra* sp. (Rouse, 1986) has an acrosome that comprises half of the 8  $\mu\text{m}$  length of the head (Fig. 1 E, 14 I). Okuda (1946) reviewed breeding behaviour in onuphids and found that several species of *Diopatra* brood larvae in or attached to the female's tube.

### Lumbrineridae

In the ect-aquasperm of *Lumbrineris* sp. (Fig. 1 F) the acrosome vesicle has the shape of a crenulated dish to which the plasma membrane is closely applied. The acrosome contents are differentiated into an outer electron transparent layer and an inner electron dense layer (Rouse, 1988 a).

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Fig. 14. Transmission electron micrographs of polychaete ect-aquasperm (A-H) and ent-aquasperm (I). Longitudinal sections (LS) excepting (C) and (G) which are transverse sections (TS). (A) *Eurythoe complanata* (from Rouse & Jamieson, 1987). (B, C) *Serpula* sp. (B) LS, (C) TS, through mitochondria and distal centriole (from Rouse, 1986). (D) *Galeolaria caespitosa* (from Jamieson & Harley, unpublished). (E) *Chaetopterus variopedatus* (from Harley & Jamieson, unpublished). (F, G) *Lepidonotus* sp. (F) LS, (G) Oblique TS through mitochondria and distal centriole with branching satellites (from Rouse, 1986). (H) *Armandia* sp. (from Rouse, 1986). (I) *Diopatra* sp. Putative ent-aquasperm (from Rouse, unpublished). *Abbreviations*: a, acrosome vesicle; dc, distal centriole; f, flagellum; l, lacuna in acrosome; m, mitochondrion; n, nucleus; pc, proximal centriole; sa, subacrosomal material; sr, satellite ray of anchoring apparatus.

## Dorvilleidae

In the dorvilleid *Ophryotrocha puerilis* the axoneme of the short, immotile flagellum of the putative introsperm (Fig. 9B) has neither doublets nor dynein arms but merely some 8–16 microtubules (Troyer & Schwager, 1979). Berruti *et al.* (1978) found this species and *O. labronica* to lack axonemes but this conclusion must be questioned. A structure immediately posterolateral to the acrosome in their Figure 4 is probably the axoneme which Troyer & Schwager (1979), in ignorance of the previous work, showed to insert at this position, giving a lateral flagellum, in *O. puerilis* (Fig. 9B). The two centrioles retain the triplet structure and one, the basal body, lies in a notch in the underside of the rim of the acrosome and is anchored to the opposite side of the acrosome by bifurcating rootlets with a weak cross striation. The mitochondria are randomly arranged, their matrices are electron lucent, and they do not respond to Janus Green B or Nadi staining, indicating depressed respiratory capacity. The immotility of this spermatozoon is related to the fact that *Ophryotrocha* undergoes a type of pseudocopulation and the sperm and eggs are deposited in a common gelatinous envelope (Berruti *et al.*, 1978; Troyer & Schwager, 1979). Motility would tend to remove the sperm from the vicinity of the egg. Immotility is achieved by loss of the midpiece and random location of the mitochondria with consequent interruption of the direct path of transfer of ATP to the flagellum; depression of mitochondrial respiration; and modification of the flagellum in disruption of microtubules and loss of dynein arms. Absence of motility is here taken to indicate that the sperm do not enter the water and that they are therefore introsperm.

## Dinophilidae

The Dinophilidae is a family which, until recently, was included in the Archiannelida. It has now been transferred to the Eunicida, close to the dorvilleids (see Westheide, 1985). *Dinophilus* sp. and *Trilobodrilus axi* have a second mode of hypodermic (transdermal) injection in which the integument is opened histolytically during copulation (Westheide, 1984b). The mode of fertilization in *T. heideri* is said by Scharnofske (1986) to be similar. In both *Dinophilus* and *Trilobodrilus* the nucleus of the introsperm runs along the axoneme (Westheide, 1984b; Scharnofske, 1986).

In *Dinophilus* sp., there is an asymmetric, elongate, pointed acrosome with lateral flanges resting on top of the filiform nucleus. The nucleus is highly condensed; anteriorly it forms a cone before extending as a rod-like structure forming a ring with the four mitochondrial derivatives, in transverse section, around the 9+2 axoneme (Franzén, 1977b).

The sperm of *Trilobodrilus* is filiform, 200  $\mu\text{m}$  long with a maximum width, in the mitochondrial region, of 0.7  $\mu\text{m}$  (*T. axi*) or 2  $\mu\text{m}$  (*T. heideri*) (Fig. 9C). Acrosome, nucleus, mitochondria and axoneme partly run parallel with each other. The acrosome vesicle is bilaterally symmetrical, more complex in structure in *T. heideri* (Fig. 9C) than in *T. axi*, in each forming, in cross section, a V-shaped structure on the side of the anterior tip of the axoneme which is itself flanked by the beginning of the nucleus. In both species the vesicle surrounds a rod-like structure (putative perforatorium). At the posterior end of the elongate, condensed nucleus and surrounding the axoneme there

are four elongate mitochondrial derivatives, each formed by fusion of several spherical mitochondria during spermiogenesis. In cross section each mitochondrial derivative is dumbbell-shaped (*T. axi*) or more complex (*T. heideri*). In the major part of the mitochondrial region, two electron dense cytoplasmic supporting structures lie between the axoneme and cell membrane. The basal body of the axoneme is situated at the anterior tip of the nucleus; no centrioles have been observed. An annulus is present at the junction of the mitochondrial region and the tail, the latter containing only the axoneme which is of the 9+2 type (Scharnofske, 1986).

#### Histriobdellidae

The histriobdellid *Stratiodrillus* uses transdermal (hypodermic) injection to transfer sperm from the male to the female. Transfer in *Histriobdella* is probably by apposition of genital pores. Jamieson *et al.* (1985) have described the sperm of members from both genera in this family. The chief features of the filiform histriobdellid spermatozoon are a very long, complex acrosome, with preformed perforatorium; an elongate nucleus, a long anterior portion of which forms a narrow shaft surrounded by regularly cristate mitochondria (four in *Stratiodrillus*, Fig. 9A, eight or nine in *Histriobdella*); anterior invagination of the tip of the nucleus to form a beaker-shaped pedestal, fenestrated in *Histriobdella* (Fig. 15G), and subdivided into four prongs in *Stratiodrillus* or eight in *Histriobdella*, supporting the base of the acrosome; and posterior narrowing of the wide, elongate hind portion of the nucleus to form the posterior end of the spermatozoon. The anterior region of the acrosome is composed of spiral laminae (4 in *Stratiodrillus*, 6 in *Histriobdella*). A flagellum is totally absent at all stages of development, though centrioles are transiently present in the spermatid (*Stratiodrillus*). Remarkably, in *Stratiodrillus* mitochondrial cristae of sperm which do not penetrate the egg give rise to a reticular structure (egg envelope?). As a result of polyspermy, it appears that non-fertilizing sperm contribute materials to the egg (Jamieson *et al.*, 1985).

#### TEREBELLIDA

The writers agree with Fauchald (1977) and differ from Dales (1961) in including the Sabellariidae in the Terebellida. Striking somatic similarities between sabellariids and pectinariids are here taken to indicate real relationship though Dales' well argued claims for a relationship between sabellariids and the spionid-chaetopterid assemblage require further consideration.

The sperm of the Ampharetidae, Pectinariidae, Trichobranchidae, and Bogueidae (now a subfamily in the Maldanidae) have not been examined ultrastructurally.

#### Terebellidae

*Ect-aquasperm.* An ect-aquasperm is seen in the terebellid *Streblosoma acymatum* (Fig. 4A). The small, simple acrosome is nearly conical in shape. The posterior margins of the vesicle are thickened, thus forming a ring around the base of the acrosome.

*Ent-aquasperm.* *Nicola zostericola* has an ent-aquasperm. Males leave their tubes and join tentacles with a female before emitting sperm which must penetrate through mucus to reach the eggs (Thorson, 1946). The total length of a spermatozoon is about

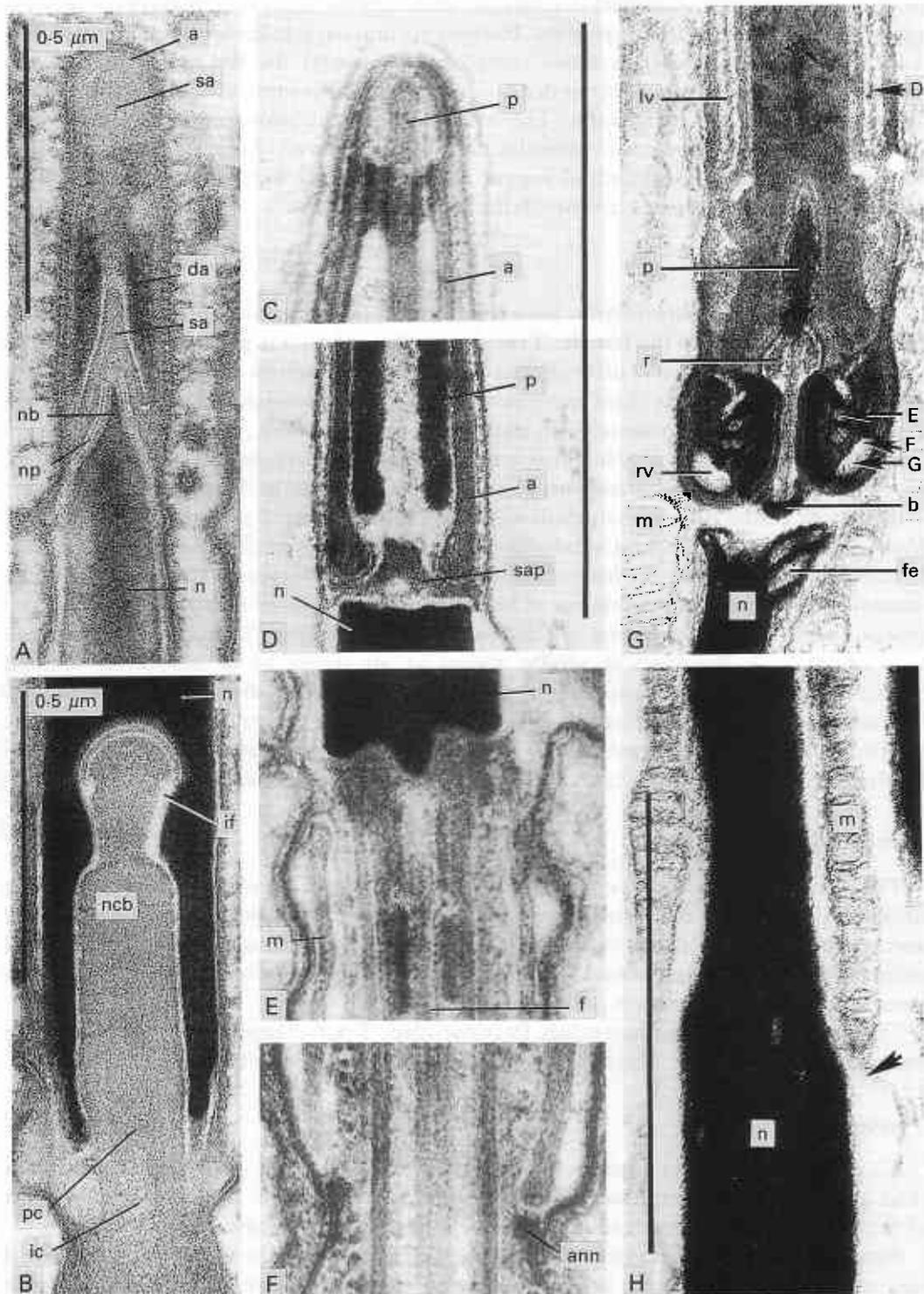


Figure 15. For legend see opposite.

56  $\mu\text{m}$  of which 14  $\mu\text{m}$  is the head. The nucleus is bullet-shaped, 8.5  $\mu\text{m}$  long, with a bulb-shaped apical acrosome; it has four deep longitudinal grooves in its posterior two thirds within which elongate mitochondria are situated. The concave base of the nucleus encloses the proximal centriole; the distal centriole is supposedly positioned just below this (though the structure micrographed resembles an immature or lateral acrosome) (Eckelbarger, 1975).

### Sabellariidae

Some polychaete sperm while biologically ect-aquasperm have distinct modifications. Thus in the sabellariids *Idanthysus pennatus* (Harley, 1982, honours thesis; Rouse, Harley & Jamieson, unpublished) (Fig. 1 I) *Phragmatopoma lapidosa* (Fig. 1 H) (described in an exemplary paper by Eckelbarger, 1984), and *P. californica* (Fig. 1 G) the acrosome develops a long apical process with a remarkable cross striation. Otherwise these spermatozoa are typical plesiosperm. A similar cross striated anterior extension of the unreacted acrosome is seen in the sperm of *Sabellaria* sp. (Rouse, unpublished). Failure to observe it in *S. alveolata* examined by Pasteels (1965), may have been because of obliquity of the section. Nevertheless, in *P. californica*, the anterior extension of the vesicle, though conspicuous, is relatively short and appears straight, the acrosome being 2  $\mu\text{m}$  long and 0.6  $\mu\text{m}$  wide. In *P. lapidosa* the striated portion is strongly curved.

The acrosome vesicle, 2.5  $\mu\text{m}$  long in *I. pennatus* (Fig. 1 I) but 6.5  $\mu\text{m}$  long in *P. lapidosa* (Fig. 1 H), resting in an anterior concavity of the nucleus, has three main components in anteroposterior sequence: (1) a narrow spire with cross-striations formed by horizontal, regularly spaced electron-dense layers, alternating with electron transparent layers; (2) behind the striated portion, a posteriorly widening electron dense collar or (Rouse *et al.*, unpublished) osmiophilic cylinder, open at both ends and with irregular inner and outer borders; (3) a central subacrosomal space. The anterior rim of the cylinder lies in the basal electron transparent matrix of the striated structure. The base of the acrosome vesicle is invaginated to a point reaching into the striated structure (Fig. 1 I). In *I. pennatus*, the subacrosomal space, within the cylinder, is filled with a fibrous material. In *P. lapidosa* the material consists of longitudinal parallel fibrils which are most clearly defined in the anterior region where they have a diameter of about 15–18 nm. In *P. lapidosa*, correlated with curvature of the acrosome, the proximal and distal centrioles, and associated flagellum, are laterally displaced, and the centriolar fossa of the nucleus is lateroposterior, giving the flagellum a rotational

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Fig. 15. Transmission electron micrographs of polychaete ent-aquasperm (A, B) and introsperm (C–H) in longitudinal section. (A, B) *Micromaldane* sp. ent-aquasperm. (A) Acrosome and anterior nucleus. (B) Posterior end of nucleus, and centriolar region (from Rouse & Jamieson, 1987). (C–F) *Questa ersei*. Introsperm. Successive regions from acrosome tip to posterior end of midpiece (from Jamieson, 1983b). (G, H) *Histriobdella homari*. Introsperm. (G) Base of acrosome, and anterior nucleus. (H) Nucleus showing posterior end of surrounding mitochondria (from Jamieson *et al.* 1985). *Abbreviations*: a, acrosome vesicle; ann, annulus; b, buttonlike end of perforatorium; da, dense contents of acrosome vesicle; f, flagellum; fe, fenestrations of nucleus; ic, intercentriolar link; if, implantation fossa; lv, lateral vesicle of acrosome; m, mitochondrion; n, nucleus; nb, nuclear blade; ncb, nuclear-centriolar bridge; np, nuclear plate; p, perforatorium; pc, proximal centriole; r, ringlike appendage of perforatorium; rv, ring vacuole; sa, subacrosomal material; sap, subacrosomal plate.

asymmetry. The flagellum is about  $36\ \mu\text{m}$  long, with 2 dynein arms on each doublet (Eckelbarger, 1984).

Fertilization in *Idanthysus* and the two *Phragmatopoma* species occurs freely in the water and, as Eckelbarger (1984) notes, the mode of sperm transfer cannot therefore be related to observed alteration in acrosome morphology. His suggestion that structural alterations in the acrosome are related to modifications of the egg envelope has been verified independently by Rouse (unpublished), who has shown, in *Idanthysus*, a close correlation between alteration of the various acrosomal components on fertilization and specific stages of penetration.

#### Pectinariidae

An ect-aquasperm occurs in *Cistenides okudai* which has a spherical nucleus and a small acrosome (Sawada, 1984).

#### CIRRATULIDA

##### Cirratulidae

An ect-aquasperm occurs in *Cirriformia tentaculata* (Fig. 2A), and in *C.* sp. (Jamieson & Harley, unpublished).

#### OWENIIDA

##### Oweniidae

In the ect-aquasperm of *Owenia fusiformis* (Fig. 2B) the acrosome vesicle has the shape of an inverted bowl with a thickened rim. Its contents show two distinct regions of different electron density of which one, which is dense, occupies the rim. The boundary between the two regions shows undulations which in favourable sections show a distinct spiral arrangement. Some scattered material occupies the sub-acrosomal space and appears to extend down the side of the nucleus to the midpiece. The single asymmetric ring-shaped mitochondrion in the midpiece is a rare condition shared with some nereids (Rouse, 1988a).

#### SABELLIDA

##### Sabellidae

*Ect-aquasperm.* Sufficient ect-aquasperm have been described in sabellids to merit a brief comparative treatment. The sperm of *Sabella penicillum* (Graebner & Kryvi, 1973a, b; Kryvi & Graebner, 1975) (Fig. 4B) and *Pseudopotamilla reniformis* (Chughtai, 1986), both with broadcast spawning, well exemplify the ect-aquasperm as manifested by the Polychaeta. The acrosome is about  $1.5\ \mu\text{m}$  (*Sabella*) or  $2\ \mu\text{m}$  wide (*Pseudopotamilla*). In *S. penicillum* it has the form of a hemisphere consisting of an outer cap, a central core and intermediate tubules, in continuity with the core, which branch to form a network corresponding with invaginations of the basal acrosome membrane (Graebner & Kryvi, 1973a, b; Kryvi & Graebner, 1975) which are considered homologous with the tubules of the acrosome of *Hydroides hexagonus* described by Colwin & Colwin (1961a).

In *Pseudopotamilla reniformis* the acrosome is transversely ellipsoid and its base indents the nucleus; its apical region contains a laminated body with two electron densities, the laminae crossed by occasional lines of dislocation, as in a crystal. A peripherally situated lucent zone contains tubules which are very like those of the *Sabella* sperm and are similarly homologized with those of *Hydroides*, though, at maturity at least, lacking connections with the acrosome base. Further detail is given by Chughtai (1986). The basal body has been shown in *Sabella* to have nine triplets. The flagellum, has a length of 40  $\mu\text{m}$  in *Sabella* and 64  $\mu\text{m}$  in *Pseudopotamilla*.

The sperm of *Perkinsiana rubra* (Fig. 4E) resembles that of *Pseudopotamilla reniformis* in features of the nuclei, mitochondria, centrioles and flagella but exceeds even the sabellariid *Phragmatopoma* in elongation of the acrosome, which is 8  $\mu\text{m}$  long. The acrosome consists of an apical, easily dihisced, filament and a basal tapering region. Both of these are uniformly electron dense. Further back, in the middle of the tapering region, there is a more electron lucent posteriorly widening core. Anteriorly, where the core is narrow, it is surrounded by two rings which are made up of longitudinal units which are larger than microtubules and are composed of concentric densities. As the acrosome and its core widen posteriorly the two rings widen and merge, with their units distributed in a single ring. The basal region of the acrosome is very electron dense though separated from the one-ring region by a lesser, though still high density. Females of another species of *Perkinsiana*, *P. antarctica*, are reported to brood embryos (Knight-Jones & Bowden, 1984).

*Ent-aquasperm*. It was formerly postulated that the male of *Fabricia sabella* leaves its tube and deposits its sperm in direct contact with the female (Franzén, 1975) but Kahmann (1984) has demonstrated that contact between sexes is not necessary for sperm transfer in this species. Spermathecae are associated with the tentacle crown, (Kahmann, 1984), and it is likely that the sperm are collected in these from the feeding current as Daly & Golding (1977) suggest for *Spirorbis spirorbis*. *Fabricia* sperm have several aberrant features relative to ect-aquasperm; the acrosome, nucleus and midpiece are elongate; the acrosome is penetrated posteriorly by a diverticulum of the nucleus; the nucleus has a deep, eccentric posterior fossa for a dense structure which resembles a basal body or proximal centriole but for which only doublets have been demonstrated; a second centriole is lacking; the midpiece mitochondria form a continuous sheath would helically around the axoneme; internal to this sheath is an helical sheath of dense material which appears to originate at the posterior aspect of the centriole and is linked throughout its length with those mitochondrial cristae which remain; posteriorly to the midpiece, the axonemal doublets are surrounded by nine coarse fibres each of which appears more or less stellate in cross section (Franzén, 1975).

In the generally similar sperm of *Fabricia* sp. (Fig. 7A), the mitochondria of the midpiece appear as cylinders split from a single large mitochondrion. This feature is apparently unique in spermatozoa. Its function is probably to facilitate efficient motility as well as allow sperm storage. The putative glycogen around the axoneme of *Fabricia* sp. spermatozoa is unique to sabellid sperm studied to date. Glycogen has widespread occurrence in introsperm but examples within the Polychaeta are few; they include *Questa* sp. (Jamieson, 1983) and *Capitella capitata*; in ent-aquasperm it is seen in *Micromaldane* sp. (Rouse & Jamieson, 1987).

The difference in sperm structure between genera of the sabellid subfamily

Fabricinae is pronounced, as indicated by distinctive morphology of that of *Oriopsis* sp. When considering that the worms share a very similar morphology and habitat it must be presumed that their reproductive biologies are markedly different.

The midpiece of *Oriopsis* sp. is distinctly different from that of *Fabricia* sp. and *Fabricia sabella*. It is formed by the elongation and spiralling of five separate mitochondria and has no helical sheath between the axoneme and the mitochondria (Rouse, 1986). In the early spermatid these mitochondria have an arrangement similar to that of ect-aquasperm (e.g. *Sabella penicillum*, Kryvi & Graebner, 1975). The sperm of *Spirorbis morchi* (Potswald, 1967) and *Questa ersei* Jamieson, 1983 *a*) show a similar midpiece development to *Oriopsis* sp. The four mitochondria of *S. morchi* and *Q. ersei* initially appear as spheres distal to the nucleus. They then elongate down the axoneme. They do not, however, spiral around it and form a helix as in *Oriopsis* sp. There is also a significant difference in spermiogenesis between *Oriopsis* sp. and *Fabricia* spp. The sperm of the former develop as tetrads of spermatids. *Fabricia* sp. sperm result from large masses of spermatids connected to a cytophore (Rouse, 1986). The reason for this difference is obscure and renders suspect any taxonomic or evolutionary hypotheses based on types of spermiogenesis, as proposed by Sawada (1984).

#### Serpulidae

*Ect-aquasperm.* Ect-aquasperm comparable to that in *Sabella penicillum* are seen in the serpulids *Hydroides hexagonus* (Fig. 4C) (Colwin & Colwin, 1961 *a*), *Galeolaria caespitosa* (Grant, 1981, SEM; Harley & Jamieson, unpublished, transmission electron microscopy) (Fig. 4G), *Serpula* sp. (Figs 4D, 14B, C) (Rouse, 1986) and *Pomatoleios krausii* (Fig. 4F) (Sawada, 1984). Note, for *Galeolaria* and *Serpula*, the inverted bowl-shaped acrosome with broad posterior rim, rounded, electron dense nucleus, four rounded mitochondria, and two centrioles of which the proximal is here at approximately at 45° to the distal. In the subacrosomal space there is granular material surrounded by the posterior rim. In *Galeolaria* (Figs 4G, 14D) the proximal centriole, lying posterior to but not enclosed in the fossa, is connected to the walls of the fossa by an apparatus which in longitudinal section has the appearance of a Y-shaped filament. There is a small electron dense body at the branching of the Y and at the end of each branch where this makes contact with the wall of the fossa. The centrioles are surrounded by granular material which extends between them and possibly has a supporting function. In *Serpula* the proximal centriole lies partly in a small nuclear fossa and to the axial, longitudinal distal centriole. The centrioles are very closely applied, if not fused to one another.

In *H. hexagonus* (Fig. 4C) the thickly cap-shaped acrosome has several zones around a central 'acrosomal granule' and is notable for numerous tubular invaginations of the basal surface which on reaction become multiple equivalents of the acrosomal filaments of other sperm. The ultrastructure of the acrosome, its reaction, and penetration of the vitelline membrane are illustrated by Colwin & Colwin (1961 *b*) and incorporation in the egg by Colwin & Colwin (1961 *c*). These accounts are difficult to reconcile with an earlier account of Colwin *et al.* (1957) reporting a single acrosomal filament in the form of a stout rod with terminal knob in this species.

*Ent-aquasperm.* In contrast with the virtual plesiosperm of the serpulid *Galeolaria*,

the ent-aquasperm of *Chitinopoma serrula* (Fig. 7C) (Franzén, 1982b) is modified with regard to nuclear elongation and modification of the mitochondria. The acrosome is not complex, being a thimble-shaped structure consisting of an acrosome vesicle, with homogeneously electron dense contents, and ill-defined subacrosomal material. The nucleus is an anteriorly somewhat narrowing slightly bent cylinder. A basal nuclear fossa is partly occupied by the proximal centriole. The distal centriole (basal body) is almost parallel to the longitudinal axis of the sperm. The midpiece contains four rod-shaped mitochondria; at its junction with the tail there is an annulus. The axoneme is of the 9+2 type (Franzén, 1982b).

### Spirorbidae

With its ent-aquasperm biology, the sperm of *Spirorbis spirorbis* is, again, considerably modified (Daly & Golding, 1977; Picard, 1980); the nucleus is somewhat elongated, with a deep basal fossa which surrounds the basal region of the axoneme, the mitochondria are elongate and surround a considerable length of the axoneme to form the midpiece and the most striking feature is the presence of processes on the acrosome which interdigitate with a spermathecal cell; these processes apparently develop only when the sperm is in the spermatheca. The acrosome and much of the nucleus are embedded in the spermathecal cell and the two cells are linked by scalariform junctions (Day & Golding, 1977). The statement that the mature sperm lack centrioles (Picard, 1980) requires confirmation. Picard states that spermathecae occur in all spirorbids so far investigated and lists nine species from five genera. In an account chiefly limited to spermiogenesis Potswald (1967) indicates the existence of four very long mitochondrial derivatives and of a basal nuclear fossa for *Spirorbis (Laospira) morchii*.

## CAPITELLIDA

### CAPITELLIDAE

*Capitella capitata* shares with *Questa* and histriobdellids, among polychaetes, the possession of prominent external sexual characters distinguishing the sexes. In *Capitella* special clasping organs ('genital tubes') of the male are said to be used in ejaculation and copulation; spermatophores are supposedly produced and transferred to the female; and there is brood care (Eisig, 1887, Franzén, 1956). Again this unusual fertilization biology is reflected in modification of the sperm (Fig. 10B) (Franzén, 1956, 1982b).

In male *Capitella* the notopodial hooks of two setigers (8 and 9) in the vicinity of the gonadial segments, are replaced with specialized genital spines. These spines have been demonstrated in an elegant study by Eckelbarger & Grassle (1987a) for five unnamed sibling species of the genus in which they have investigated spermatozoal ultrastructure but these workers question the occurrence of spermatophore production and, indeed, of copulation and internal fertilization in *Capitella*. In a further work on these species, on three species in the related genus *Capitomastus*, and on one species of *Capitellides* they again found no evidence for the production of spermatophores and stated that 'the method of sperm transfer is not understood' (Eckelbarger & Grassle, 1987b).

There is no doubt that some capitellids (*Notomastus latericeus* and *Heteromastus*

*filiformis*) have 'primitive' sperm (Franzén, 1956) and presumably broadcast spawning but we are inclined to view the genital spines of *Capitella* which are so like the genital setae of obligatory copulants, the oligochaetes, as evidence of copulation. It is possible, nevertheless, that as in most oligochaetes fertilization occurs outside the body. The fact that sperm production is low in all species studied and that 100% of eggs from a given laying are fertilized (Eckelbarger & Grassle, 1987*b*) supports the existence of internal fertilization (or that of the pseudo-introsperm type by exudation of stored foreign sperm over the eggs outside the body). Nevertheless, Eckelbarger & Grassle (1987*a, b*) may be correct in suggesting that modifications of the sperm of *Capitella*, *Capitomastus* and *Capitellides* relative to the 'primitive plan' correlate primarily with known sperm storage in inflated male ducts rather than with the method of sperm transfer. They are unable (Eckelbarger & Grassle, 1987*b*) to relate small interspecific differences in sperm morphology to previously reported variation in egg envelope morphology.

In *Capitella capitata* as identified by Franzén (1956, 1982*b*), the large thimble-shaped acrosome is somewhat asymmetrically disposed on the elongate nucleus; the midpiece consists of a cytoplasmic sleeve or collar which begins at the level of the centrioles and extends about 3  $\mu\text{m}$  along the proximal part of the flagellum; around the centrioles it is stated to contain one or two small mitochondria (but see below); granules in the collar are probably mainly glycogen. The proximal centriole is oblique to the sperm axis and has 9 triplets. The distal centriole (basal body) has its long axis oblique to the nuclear axis. The flagellum is about 23  $\mu\text{m}$  long and has the 9+2 pattern (Franzén, 1977*a*; 1982*b*).

Great morphological similarity exists among the mature sperm of the nine capitellid species examined by Eckelbarger & Grassle (1987*a, b*) (Fig. 11). The species were designated *Capitella* sp. I and II, *Capitella* ORL, *Capitomastus* LYNN, *Capistomastus* TRIN/1, and *Capitellides* MB/sl, all of which have relatively large, yolky eggs and lecithotrophic or direct development; and the planktotrophic *Capitella* sp. Ia and *Capitomastus* MB/s. All have a conical acrosome, an elongate, tapering nucleus, and long midpiece composed of a cytoplasmic sleeve or collar which extends posteriorly along the proximal portion of the flagellum, as described by Franzén for the included *C. capitata*. A single ring-shaped mitochondrion encircles the centriolar region (an important difference from Franzén's account) as a result of fusion of smaller mitochondria; and large amounts of glycogen are located in the cytoplasm of the midpiece. Although morphologically similar, the sperm of all nine capitellid species differ significantly from each other in the lengths of their acrosomes, nuclei and midpieces. Subtle differences also occur in acrosome morphology, including the shape and volume of the vesicle and the subacrosomal space. Intraspecific variation is minor and does not extend to acrosome morphology (Eckelbarger & Grassle, 1987*b*).

#### Maldanidae

The mature ent-aquasperm of *Clymenella* sp. (Fig. 7F) has a head length of 4  $\mu\text{m}$ . The acrosome is cap-shaped and the nucleus (2.7  $\mu\text{m}$  long) is elongate-bullet shaped, tapering anteriorly, and uniformly electron dense. Posteriorly it has a small central fossa and five marginal depressions for the mitochondria. The nuclear envelope is closely applied to the nucleus and shows distinct thickening in the nuclear foss (Rouse & Jamieson, 1987).

The mature ent-aquasperm of *Micromaldane* sp. is 50  $\mu\text{m}$  long, the head being 26  $\mu\text{m}$  long (Figs 7G, 15A, B). The acrosome is an approximately bilaterally symmetrical, apically somewhat spatulate cone 0.9  $\mu\text{m}$  long. In a longitudinal section through the broad axis of 'the nuclear blade' the acrosome vesicle appears short and symmetrical. However in sections at 90° to this, in the narrow axis of the nuclear blade, the acrosome extends down each side of the blade. The acrosome vesicle has two distinct components. The nucleus, subelliptical in cross section, is electron dense and approximately 25  $\mu\text{m}$  long. At the extreme anterior end the nucleus alters from a cylindrical ellipse to become a thin blade (nuclear blade), rounded anteriorly. Posteriorly the nucleus has a deep nuclear fossa (implantation fossa) occupied by the centriolar complex.

A pair of mitochondria runs from the inter-centriolar region along the nucleus anteriorly for approximately 9  $\mu\text{m}$ . A ringlike structure within the 9+2 axoneme, immediately behind the distal centriole may be a basal cylinder (Rouse & Jamieson, 1987).

#### Arenicolidae

Okuda (1946) observed spermatophore release in *Abarenicola pacifica* (as *Arenicola claparedii*). Spermatophores were shed freely into the water and some were fortuitously incident in or near a female's tube. The spermatophores were composed of a simple thin membrane around the sperm, which had spherical nuclei a triangular acrosome and short midpiece. The spermatophore membrane broke easily allowing the sperm to swim freely through the water of the tube to the eggs attached, in jelly, around the female's body. Brooding of larvae has been recorded in several other arenicolids (see Schroeder & Hermans, 1975) though not in *Arenicola marina*. In the putative ent-aquasperm of *Arenicola brasiliensis* (Fig. 2C) (Sawada & Ochi, 1973; Sawada, 1975, 1984) the nucleus is considerably longer than wide (3 × 1  $\mu\text{m}$ ). In *A. marina* (Fig. 2D) it is ovoid with a broad flattened anterior end. In both species the acrosome has the structure of an inverted cylindrical cup in which a smaller cup is suspended, the two forming a continuous acrosome vesicle. Fine particulate subacrosomal material lies between the convolutions of the acrosome vesicle and the nuclear envelope (Sawada, 1975, 1984). In both species the basal nuclear fossa contains the proximal centriole.

#### SPIONIDA

##### Spionidae

*Ect-aquasperm.* The sperm of *Prionospio* cf. *queenslandica* (Fig. 4H) is unique among polychaete ect-aquasperm in possessing an acrosome with bilateral symmetry (Rouse, 1988b). Other species of *Prionospio* have been reported to swarm and purportedly release their gametes into the water (Schroeder & Hermans, 1975). This hypothesis is supported by the anatomy of the sperm.

The low but transversely elongate acrosome is penetrated almost to its anterior limit by the narrow sub-acrosomal space which is filled with a flocculent material that extends around the base of the acrosome. The contents of the vesicle are divided into three layers interspersed between which is a matrix of moderate electron density. The proximal centriole is linked to the small nuclear fossa by a thin arm that expands in the fossa and fills it (Rouse, 1988b).

*Introsperm.* In *Polydora* and, it is suspected *Tripolydora*, spermatophores are gathered by the female while in *Streblospio* copulation is suspected (Franzén, 1974; Rice, 1978, 1979, 1981; Rouse, 1988b). As is common in introsperm there is conspicuous elongation of the nucleus but only in *Polydora* and *Tripolydora* is the midpiece elongated.

The advanced spermatid of *Polydora ciliata* (Fig. 12A) has been described ultrastructurally by Franzén (1974) and the mature sperm of *P. ligni*, *P. socialis* and *P. websteri* described by Rice (1981) and of *Tripolydora* sp. (Fig. 10C) by Rouse (1988b). The basic similarity of structure, its peculiarities, and interspecific differences in spionid introsperm exemplify the taxonomic value of spermatozoal ultrastructure, here at generic and specific levels, Rice (1981) lists quantitative interspecific differences in *Polydora* for several dimensions of the sperm. Further differences within the basically similar structure include the exceptionally long basal nuclear (implantation) fossa in *P. ligni* which extends almost the entire length of the nucleus, as in *Tripolydora* (an exceptional condition in the Polychaeta) compared with well developed but relatively short fossae in *P. socialis*, *P. websteri* and also *P. ciliata*. The acrosome is complex but similar between species. It is conical and is divisible into three regions. In all four species the nucleus and midpiece is ensheathed by platelet-shaped electron dense bodies. The mitochondria of the midpiece ensheath a considerable length of the axoneme behind the nucleus. They are described as a number scattered in the cytoplasm for *P. ciliata* (Franzén, 1974) but in the other three species it is stated the mitochondria each appear to form a long sinuous tube extending from the base of the nucleus to the end of the midpiece (Rice, 1981). At the posterior end of the midpiece there is a cytoplasmic indentation accompanied by an annulus which has a nine-rayed symmetry. In an interesting paper Rice (1980) describes ultrastructurally the role of the male nephridium in spermatophore formation in *Polydora ligni* and *P. websteri*.

In *Streblospio benedicti* sperm are elongate with an average head length of 55  $\mu\text{m}$  and a tail reaching 50  $\mu\text{m}$ . The acrosome is about 7  $\mu\text{m}$  long but the midpiece is short, about 0.6  $\mu\text{m}$ . Mitochondria and other inclusions are distributed along the nuclear region of the sperm: mitochondria in the basal third, slightly elongated but unmodified, platelets throughout the length of the nucleus. The implantation fossa penetrates about 1.22  $\mu\text{m}$  into the base of the nucleus (Rice, 1981).

The spermatozoon of *Tripolydora* sp. (Fig. 10C) is 90  $\mu\text{m}$  long, with a head length of 40  $\mu\text{m}$ . The acrosome consists of a single bullet shaped vesicle, occupied by an electron dense spheroidal structure. The elongate nucleus, is almost completely penetrated by the axoneme, only its anterior 0.3  $\mu\text{m}$  being solid. Between the plasma membrane and nucleus are numerous moderately electron dense, irregularly spaced bodies (platelets of Rice, 1981); they also extend into the midpiece. The midpiece shows three elements; the axoneme emergent from the base of the nucleus; numerous platelets; and many elongate cylindrical sparsely cristate mitochondria. The midpiece terminates with an annulus (Rouse, 1988b).

#### Chaetopteridae

Ect-aquasperm also occur in the chaetopterids *Chaetopterus variopedatus* (Harley & Jamieson, unpublished) and *C. pergamentaceus* (Anderson & Eckberg, 1983) (Fig. 2F).

In *C. variopedatus* (Figs 2E, 14E), the acrosome has the form of a hollow, flattened cone.

In *C. pergamentaceus* (Fig. 2F), the dimensions of the slightly elongated head, with the midpiece, are  $4\ \mu\text{m}$  long by  $1\ \mu\text{m}$  wide. The acrosome vesicle, which is inverted-cup-like and contains fibrous material associated with its membranes, embraces the tip of the nucleus (Anderson & Eckberg, 1983).

## OPHELIIDA

### Opheliidae

The ect-aquasperm of *Armandia* sp. (Figs 2G, 14H) is remarkable for asymmetry imposed by the presence of only three mitochondria (Rouse, 1986), as in the cnidarian *Hydra viridis* (Burnett *et al.*, 1966). In *Travisia japonica* (Fig. 2H) a definite perforatorial rod is said to be developed (Ochi *et al.*, 1977) but this does not appear as well defined as that of a typical nereid sperm.

## PHYLLODOCIDA

Approximately 22 phyllodocid families remain to be investigated for sperm ultrastructure. The following have been briefly investigated.

### Phyllodocidae

In the mature spermatozoon of *Eulalia* sp. (Fig. 3B) the acrosome has the shape of an inverted dish, flattened anteriorly. The nucleus is spheroidal and four mitochondria abut its posterior end. The midpiece is unusual in that the mitochondria are ensheathed in an extra-mitochondrial membrane (Rouse, 1988a).

### Sigalionidae

The ect-aquasperm of *Sigalion* sp. (Fig. 2I), has a simple acrosome, though with apical protuberance and some internal zonation. The subspherical nucleus has a well developed posterior fossa. The long axis of the proximal centriole is slightly tilted relative to that of the longitudinal distal centriole (Harley & Jamieson, unpublished).

### Hesionidae

The small interstitial polychaete *Hesionides arenaria* has an extremely modified introsperm (Westheide, 1984a) (Fig. 12C). The filiform,  $110\ \mu\text{m}$  long, cell has a maximum diameter of about  $1.1\ \mu\text{m}$ . The acrosome is a tapering conical structure, at least  $1.2\ \mu\text{m}$  long, with transparent inner core and is followed basally by a very short, broadly conical subacrosomal structure which is possibly the homologue of a euclitellate acrosome tube. It indicates how the acrosome tube might have evolved from subacrosomal material in the annelidan line leading to euclitellates though it is not here suggested that hesionids have any direct affinity with the latter. The nucleus forms a long, thin-walled tube, with closed anterior end, which encloses a long proximal region of the axoneme. Directly under the tip of the nucleus lies the basal body of the axoneme. In the headpiece the axoneme is surrounded by numerous large electron dense

membrane-bound bodies. The boundary of the midpiece is a small ring-shaped invagination of the plasma membrane. From here posteriorly, different and smaller electron dense bodies and nine conspicuous double radial supporting walls with a 'cross-hatched' substructure surround the axoneme. In the periphery of the midpiece a mitochondrial derivative forms a continuous circumferential sheath. The tail region consists only of the axoneme and surrounding plasma membrane. The striking morphology of this sperm is correlated with transmission in spermatophores and with transdermal injection (hypodermic impregnation).

In several *Microphthalmus* species the nucleus is screw-shaped. In *M. listensis* it abuts on the anterior end of a midpiece which, from a micrograph, is about  $3.5 \mu\text{m}$  long and consists of a mitochondrial sheath around the anterior end of the axoneme. In *Microphthalmus* sp., in contrast, the nucleus is coiled around the axoneme (Westheide, 1984b).

### Tomopteridae

The ent-aquasperm of *Tomopteris helgolandica*, (Fig. 7E), which is motile, is unique in investigated polychaetes in having two flagella. The two basal bodies, separated by  $2 \mu\text{m}$ , are connected with the posterior part of the nuclear envelope by granular dense material. Distally each centriole carries a satellite apparatus of 9 spoke-like arms which contact the plasma membrane. The flagella have an aberrant 9+0 pattern, though it is possible that the 9+2 arrangement occurs distally. The short midpiece, consisting of about 10 cristate mitochondria is deeply invaginated at the insertion of each flagellum. The sperm is further aberrant in having groups of vesicles (tegosomes) peripheral to the middle region of the broad anteriorly tapering nucleus in place of the acrosome (Franzén, 1982a).

### Polynoidae

The spermatozoon of *Lepidonotus* sp. (Figs 3A, 14F) (Rouse, 1988a) and *Lepidonotus carinulatus* are very similar. In both species the acrosome, semi-spherical, flattened anteriorly and posteriorly, has a distinct rim. The vesicle has uniformly electron dense contents. A prominent conical projection of the ellipsoidal nucleus occupies the space created by the acrosome invagination. The mitochondria, four in *L. carinulatus* and five in *Lepidonotus* sp. are spherical with prominent cristae and enclose the centriolar complex. The proximal centriole lies on the central axis at approximately  $30^\circ$  to vertical. A satellite complex is present (Fig. 14G). The 9+2 axoneme arises from the distal centriole (Rouse, 1986, 1988a).

### Syllidae

*Ect-aquasperm*. A common modification of the ect-aquasperm in various phyla consists of organization of subacrosomal material to form an acrosome rod. An approach to this is seen in the syllids, *Typosyllis pulchra* and *Typosyllis* sp. (Fig. 4J). In both species the posterior rim of the acrosome vesicle leaves only a small aperture between the enclosed subacrosomal material and the underlying nucleus. The subacrosomal material within the enclosed cavity is differentiated respectively in two

species as in an ellipsoidal dense body or as a number of thick oblique and possibly helical filaments, each structure virtually constituting an acrosomal rod. The nucleus in both species has the form, approximately of a short, stout cylinder almost twice as long as wide; the transverse proximal centriole is partly contained in a moderate posterior fossa. The distal centriole (basal body) in *Typosyllis* sp. has the usual nine triplets.

*Ent-aquasperm.* Functionally, the sperm of *Autolytus* sp. (*prolifer* group) (Fig. 4 K) appears to be an ent-aquasperm, as indicated by the only description of mating behaviour in this group, for *Autolytus edwardsii* (Gidholm, 1965). Spermatozoa are released by males in a nuptial dance and eventually attach to the ventral surface of the female some time before oviposition. The acrosome of *Autolytus* sp. (*prolifer* group) spermatozoa is situated along one side of the anterior part of the nucleus. It consists of a flattened acrosome vesicle and subacrosomal substance. The ovoid highly condensed nucleus has some scattered lacunae. There are one or two mitochondria, one always bigger than the other and forming a crescent-shaped structure around the base of the nucleus. There are two mutually perpendicular centrioles of the triplet type. Cross striated flagellar rootlets, generally three in number, extend from the anterior part of the distal centriole (basal body) along the nucleus to the anterior pole. A flagellar rootlet known elsewhere in annelid sperm in *Eurythoe complanata* (Fig. 1 A, 14 A) (Rouse & Jamieson, 1987), and apparently in *Galeolaria caespitosa* (Fig. 4 G), in both of which it links the centriole with the nucleus within the basal fossa is doubtfully homologous with those of *Autolytus*. The long bifurcated cross striated rootlets of *Ophryotrocha puerilis* spermatozoa (Troyer & Schwager, 1979) are possibly homogenous with those of *Autolytus* and may be a modified anchoring apparatus. An elongate cross striated rootlet-like ribbon structure paralleling the axoneme and arising from the centriolar region in *Nerilla antennata* is thought to correspond with that of *Autolytus* but to have originated independently (Franzén & Sensenbaugh, 1984). Cross striated flagellar rootlets of the *Autolytus* type occur, *inter alia*, in the sperm of the siphonophore *Muggiaea* (Carré, 1979), the cephalochordate *Branchiostoma* and holothurians, such parallel development being termed paramorphy by Jamieson (1984c, 1985a). The 9 + 2 flagellum of the *Autolytus* sperm is about 70  $\mu\text{m}$  long (Franzén, 1982b).

In the syllid *Grubea clavata* (Fig. 7 D) the male discharges its sperm as a large mass near the female. The acrosome vesicle has the form of an inverted wine-glass (Fig. 2.2 I, J) containing subacrosomal material and is separated from the nucleus by a post-acrosomal plate. The acrosome is disposed slightly asymmetrically on the nucleus. The nucleus is 5  $\mu\text{m}$  long and 0.8  $\mu\text{m}$  wide anteriorly. At the posterior end of the nucleus there are two centrioles, of which the proximal is somewhat oblique to the long axis and overlaps the end of the nucleus; alongside both is an oval, unilaterally situated mitochondrion (Franzén, 1974).

*Introsperm.* The aberrant syllid *Calamyzas amphictenicola* is ectoparasitic on the polychaete *Amphicteis gunneri*. Both sexes occur in close contact, suggesting that the sperm do not have to swim to the eggs. The sperm are approximately spherical cells, 4  $\mu\text{m}$  in diameter, with no flagellum but (Franzén, pers. comm.) possessing centrioles. The nucleus, disc-shaped with concave posterior face, 3.5  $\mu\text{m}$  wide, and about 1  $\mu\text{m}$  thick, is dislocated to one pole, leaving space for cytoplasm with a larger spherical vacuole and a varying number of mitochondria at the opposite pole. Its convex surface is separated from the plasma membrane by a thin layer of cytoplasm. The otherwise

thin limiting membrane of the vacuole is thick and electron dense in the part nearest the nucleus (Franzén, 1982a).

### *Nereidae*

The sperm, ect-aquasperm and rarely (*Platynereis massiliensis*) ent-aquasperm or possibly introsperm, of the Nereidae deserve separate mention as the considerable number investigated affords one of the few opportunities for comparative ultrastructure within a family. Nereids have taken the trend towards specialization of the subacrosomal material further in the development of a definite acrosome rod and this development coincides with a number of modifications which include the centriolar system and mitochondria. The acrosome rod (perforatorium) lies in a deep fossa in the acrosome vesicle and extends from near the tip of the acrosome, through the vesicle to deeply or completely penetrate the nucleus. The sperm of *Platynereis massiliensis*, alone in the studied nereids, is not an ect-aquasperm but is, debatably, an ent-aquasperm, an introsperm, or a pseudintrosperm (Fig. 12B), and correspondingly has a strongly elongated nucleus about  $6.4 \mu\text{m}$  long, although otherwise with the nereid features mentioned above. This modification is correlated with fertilization (self-fertilization has been reported) of the  $280\text{--}300 \mu\text{m}$  eggs in a thick-walled brood tube and absence of a trochophore. In contrast, its somatically indistinguishable sibling species, *P. dumerilii*, spawns freely, has  $160\text{--}180 \mu\text{m}$  eggs,  $2 \mu\text{m}$  long sperm nuclei (Fig. 4I), and has a trochophore (Pfannenstiel *et al.*, 1987).

In *Nereis limbata* (Fig. 3G) the conical acrosome vesicle encloses the rod, the posterior half of which extends almost to the equator of the ovoid nucleus; a posterior fossa of the nucleus contains the proximal centriole which is perpendicular to the distal centriole; relatively small mitochondria surround the distal centriole (Fallon & Austin, 1967). The sperm of *Perinereis brevicirrus* (Fig. 3H) is very similar but in it the rod extends almost to the base of the nucleus which is perforated by an axial orifice. The portion of the rod within the nucleus is surrounded by a dense sheath which, were it outside the nucleus, would closely resemble the acrosome tube of euclitellate sperm. In *Nereis diversicolor* (Fig. 3E), *Platynereis dumerilii* (Fig. 4I) and *P. massiliensis* (Fig. 12B) the rod is tubular and penetrates the nucleus posteriorly almost as far as the nuclear membrane. In *N. diversicolor* (Fig. 3E) it contains acid phosphatase which is envisaged as being involved in reorganization of the tube to form the acrosome filament on reaction (Bertout, 1976). That of *Neanthes japonica* (Fig. 3F), for which the acrosome reaction is well-documented, is very similar, including the tubular nature of the perforatorium.

The fullest development of distinctive characters of the nereid sperm is seen in *Nereis irrorata* (Fig. 3D). Here the acrosome vesicle is very large; the acrosome rod penetrates from near the acrosome tip to the anterior part of the basal body; the nucleus, penetrated by the rod, is reduced in size; the mitochondria take on elongate, oblong shapes; and the fairly normal satellite complex of other nereids is replaced with two longitudinally striated structures arranged like parentheses around the basal body as seen in longitudinal section. However, the sperm of *Nereis pelagica*, though again basically primitive, is in other respects as modified as that of *N. irrorata*. The acrosome is almost as long, at  $1.4 \mu\text{m}$ , as the well developed ovoid nucleus and the acrosome rod,

which extends for an unspecified distance into the nucleus, ends near the apex of the acrosome with a swelling similar to the capitulum of some oligochaete sperm. The appearance of the acrosome is remarkably oligochaetoid except for the important absence of an acrosome tube external to the acrosome vesicle. Lengths and basal widths respectively of nereid acrosomes are (in  $\mu\text{m}$ ): *N. irrorata* 3.4, 4.1; *N. limbata* 1, 0.5; *N. virens* 0.8, 0.6; *N. pelagica* 1.4, 0.7; *Neanthes japonica* 1.2, 1.2 (References in lists Section I 12); and *Platinereis dumerilii* 0.5, 1.5  $\mu\text{m}$  (Pfannenstiel *et al.*, 1987). In *Nereis virens* the one or two large mitochondria are wrapped around the posterior part of the nucleus and the satellite complex. The mitochondria in *Neanthes japonica*, *Nereis limbata* and *Perinereis brevicirrus* do not appear to be regularly arranged as spheres around the centriole. The general form of these sperm still allows them to be classified as 'primitive' sperm; all are ect-aquasperm. *Nereis limbata* sperm were the subject of classical studies of the acrosome reaction (Metz & Morrill, 1955; Fallon & Austin, 1967; Austin, 1968).

The egg of *Tylorrhynchus heterochaetus* has complex vitelline layers very similar to those of other nereids. However the sperm acrosome (Fig. 3I) is much simpler in construction than that of any other nereid examined to date (Sato & Osanai, 1981, 1983; Osanai, 1983). There is no perforatorium, rather the tip of the nucleus forms a cone upon which the acrosome rests. The acrosome vesicle contains electron dense matter at the periphery and there is a small amount of subacrosomal material. Fusion, as seen in *Chaetopterus pergamentaceus* (Anderson & Eckberg, 1983), occurs between the inner acrosomal membrane and the tip of an egg microvillus projecting through the vitelline layers. From micrographs, the nucleus is almost twice as long as wide. The two centrioles are fused, with the proximal perpendicular to the distal and lying in a shallow posterior nuclear fossa.

#### Pisionidae

The ultrastructure of the spermatozoon of *Pisione remota* and its transformation in the female are unique in the Polychaeta. In the male, the most prominent organelles of the rod-shaped, 10–12  $\mu\text{m}$  long and 0.9  $\mu\text{m}$  wide, aflagellate sperm (Fig. 13C) are an elongate, canoe-shaped nucleus, a longitudinal vacuole-like structure, contained in its concavity, with a peripheral network of cisternae, and, at both ends of the cell, mitochondria and lamellar bodies. In the female (Fig. 13D) the vacuole-like structure detaches and is phagocytosed by a receptacle cell, the nucleus expands and the cytoplasmic sheath of the sperm differentiates to form a system of pseudopodium-like projections which envelop the apical projections of the receptacle cell (Westheide, 1988). We note a remarkably similar transformation in the female in nematode (e.g. ascaridoid) sperm, which similarly have peripheral cisternae and pseudopodia, lack an evident acrosome and are aflagellate.

#### ORBINIIDA

##### Questidae

The Orbiniidae and Paraonidae have yet to be examined but the Questidae have been recently studied.

Questids are clitellate annelidas with oligochaetoid features but examination of spermiogenesis and spermatozoal ultrastructure of *Questa ersei* (Figs 10A, 15C-F) (Jamieson, 1983*a, b*; Jamieson & Webb, 1984) has confirmed the conclusion of Giere & Riser (1981) that they are not oligochaetes. The sperm are deposited in spermathecae of the partner and correspondingly are highly modified. The very elongate acrosome vesicle and contained tubular perforatorium, somewhat exceeding 5  $\mu\text{m}$ , are followed by a highly condensed elongate nucleus. Between acrosome and nucleus a sub-acrosomal- and a narrow nuclear-plate intervene. Behind the nucleus a complex centriolar apparatus with evidence of two centrioles, of which the distal has triplets and accessory microtubules, give rise to a long glycogen-containing flagellum. At least 12  $\mu\text{m}$  of the axoneme, constituting the midpiece, is invested by two mitochondrial derivatives, each with a narrow crescentic transverse profile, posteriorly limited by an annulus. The axoneme is of the 9+2 type but in the mitochondrial region as many as 27 accessory peripheral microtubules, in 9 triads, encircle it. Presence of accessory microtubules is unique in the Polychaeta. Elongation of the primary vesicle and of the mitochondrial derivatives, and presence of an extensive axial rod, are seen in sperm of protodrilids with which, as with dinophilids, questids have somatic similarities suggestive of a relationship closer than with other annelids.

#### POLYGORDIIDA

##### Polygordidae

The sperm of *Polygordius lacteus* (Fig. 3C), described ultrastructurally by Franzén (1977*b*) is an ect-aquasperm with classical plesiosperm morphology. This is appropriate to the fact that polygordiids, unlike other 'archannelid' families do not occupy an interstitial niche. The acrosome vesicle is cone-shaped, with an electron density which is nearly as great as that of the nucleus. It is underlain by diffuse subacrosomal material of lesser density. The gametes are discharged into the water by bursting of the body wall or, occasionally dropping of the posterior end of the body (Franzén, 1977*b*).

#### DIURODRILIDA

##### Diurodrilidae

By light microscopy the putative introsperm of *Diurodrilus westheidei* appear plesiosperm-like but TEM investigations show that the round head does not contain the nucleus, but a giant acrosome. The nucleus is situated in the so-called midpiece between the 7-8 mitochondria. The plasma membrane in this region is modified and covered with mushroom-shaped protuberances. The flagellum is only slightly modified (Kristensen & Niilonen, 1982).

#### NERILLIDA

##### Nerillidae

The mature introsperm of *Nerilla antennata* (Fig. 12D, E) is filiform and about 47  $\mu\text{m}$  long. It has an anterior elongated flattened region and a posterior cylindrical tail region. Through both of these regions runs the 9+2 axoneme. The anterior region,

which corresponds with the head and midpiece of the 'primitive' spermatozoon, contains a nucleus, a mitochondrion and a striated ribbon-like structure (flagellar rootlet) parallel with the axoneme. In this region, the flagellum is connected by junctions with the rest of the cell by only a very small part of its circumference, a junctional complex composed of electron dense fibres. The posterior region, consisting only of the axoneme, is 13  $\mu\text{m}$  long. The nucleus, axoneme and striated rootlet are in approximately the same plane of symmetry but the single mitochondrion is located to one side of this plane (Franzén & Sensenbaugh, 1984).

## PROTODRILIDA

### Protodrilidae

Introsperm have been described ultrastructurally for the genera *Protodrilus* (Franzén, 1974, 1977*a, b*; von Nordheim, 1987) and *Protodriloides* (Jouin, 1979). Only von Nordheim has recognized the occurrence of eusperm and parasperm in *Protodrilus*. From 19 'potential' species (see Table 1) he describes schematic ground plans for the two sperm types (Fig. 13 A, B). Because of the constant arrangement of the peripheral microtubules in the axoneme it is possible to designate X (dorsal and ventral) and Y (lateral) planes in cross sectional views of the axoneme. In all examined *Protodrilus* species the sperm is filiform (Fig. 13 A, B). Eusperm are 80–255  $\mu\text{m}$  long, with widths according to the region of the sperm and the species of 0.03 to a maximum of 2.5  $\mu\text{m}$ . Parasperm are as a rule thinner, 0.03  $\mu\text{m}$  to a maximum of 0.7  $\mu\text{m}$  wide, and shorter, 50–130  $\mu\text{m}$  long. In most species the sperm is clearly divided into acrosomal, nuclear, midpiece and flagellar regions but in some species these regions overlap. In parasperm the overlapping is never pronounced.

Each sperm type is surrounded by a trilaminar plasma membrane, about 8 nm thick, and, because of the structure of the axoneme, is bilaterally symmetrical. In the segments containing sperm ducts, about 5–20% of sperm are parasperm. In both sperm types the axoneme undulates whereas the nucleus and acrosome are stiff, but the eusperm are the more motile (von Nordheim, 1987).

The acrosome vesicle of the eusperm is an elongate cone, 7–51  $\mu\text{m}$  long, with a circular, elliptical or rounded triangular cross section, 60–750 nm in diameter. In some species the acrosome vesicle extends far distally into the nuclear region, in an extreme case, the eusperm of *P. jaegersteni*, as a thin rod spiralling around the nucleus to the beginning of the midpiece.

The parasperm differ with regard to the subacrosomal space. This is as a rule only a thin tubular passage which extends eccentrically through about two thirds of the acrosome. The elongate eusperm nucleus, 4–13  $\mu\text{m}$  long, narrows apically and has a circular to bean-shaped cross section, 0.12–1.0  $\mu\text{m}$  in diameter.

In most of the investigated species, the nucleus has a basal, implantation fossa, of varying depths, which contains the basal body of the axoneme. In *P. litoralis* and *P. submersus*, the axoneme runs parallel to the nucleus as far as the acrosome region and no implantation fossa is present.

In paraspermatozoa the nucleus is usually thinner and longer, 7.3–25  $\mu\text{m}$  long, 0.17–0.57  $\mu\text{m}$  in diameter, than in euspermatozoa and there is always a basal implantation fossa.

Dimensions of the midpiece of the eusperm are very variable, with lengths of 18–195  $\mu\text{m}$  and widths of 0.75–2.4  $\mu\text{m}$ . Its form is very complex, containing three distinctly demarcated components: the axoneme with accessory structures, the various riblike elements, and the mitochondrial derivatives. An annulus lies at the posterior end of the midpiece. The midpiece of the parasperm is clearly distinguishable from that of the eusperm and is of a much less complex construction.

The introsperm of *Protodrilus*, transferred via spermatophores to fertilize in the coelom of the female, is thus highly modified (Franzén, 1974, 1977*a, b*). Another protodrilid, *Protodriloides symbioticus*, has strikingly different sperm, aflagellate and nonmotile with no acrosome (Jouin, 1979). The reproductive biologies of the respective species are correspondingly dissimilar. Instead of utilizing spermatophores *Protodriloides symbioticus* spermatozoa are shed into a cocoon where they fertilize the eggs (Jouin, 1979).

#### V. CONCLUSIONS

The spermatozoa of the Polychaeta are divisible into externally fertilizing sperm (ect-aquasperm), sperm which enter the water but are drawn into the female (ent-aquasperm), and sperm which do not enter the water and which fertilize internally (introsperm). Sperm which are received into receptacula directly from the male (e.g. via apposed male pores, penis or spermatophore) but are exuded to fertilize the eggs in a cocoon or in the tube are here included as introsperm but might be termed pseudintrosperm.

Ect-aquasperm are the commonest type of polychaete sperm and are considered plesiomorphic for the Polychaeta. Re-evolution of ect-aquasperm (as neo-aquasperm) is, nevertheless, tentatively hypothesized for some Sabellida.

Ent-aquasperm are rare in the Polychaeta whereas introsperm occur in many families and are typical of interstitial forms.

In terms of ultrastructural studies of sperm in the investigated polychaete families, only ect-aquasperm have been demonstrated for 16 families (Amphinomidae, Eunicidae, Onuphidae, Lumbrineridae, Sabellariidae, Pectinariidae, Cirratulidae, Oweniidae, Arenicolidae, Chaetopteridae, Acrocirridae, Opheliidae, Sigalionidae, Polynoidae, Phyllodocidae, Polygordidae); only ent-aquasperm for 3 families (Spirorbidae, Maldanidae, Tomopteridae); ect- and ent-aquasperm for 3 (Terebellidae, Sabellidae, Sepulidae); ect- and intro-sperm for 2 (Spionidae, Nereidae); ect-, ent- and intro-sperm for 1 family (Syllidae); and only introsperm for 11 families (Histriobdellidae, Dorvilleidae, Dinophilidae, Questidae; Capitellidae\*; Hesionidae, Diurodrilidae, Nerillidae, Protodrilidae, Saccocirridae, Pisionidae) but investigations can only be regarded as preliminary. To date no family is known to have ent- and intro-sperm only. Sperm ultrastructure has yet to be examined in the orders Magelonida, Psammodrilida, Cossurida, Spintherida, Sternapsida, Flabelligerida and Fauvelopsida.

Ect-aquasperm show gross approximation to a hypothetical, but often approximately realized, morphology termed the plesiosperm, corresponding with the 'primitive sperm' *sensu* Franzén (1956, 1977*a*). Nevertheless, much variation occurs in gross morphology, ultrastructure and configuration of the several components: acrosome,

\* Some capitellids are known optically to have ect-aquasperm.

nucleus, mitochondria, and centrioles and associated anchoring apparatus. A 9+2 axoneme is constant for polychaete ect-aquasperm.

In most families insufficient numbers of species have been investigated to establish whether the ect-aquasperm of each supraspecific taxon has group-specific characters. However, examination of considerable numbers of nereid species has established the occurrence of a nereid-specific ect-aquasperm type, although exceptions exist. Such specificity has been demonstrated for introsperm in at least the families Histriobdellidae, Questidae; Capitellidae, Spionidae and Protodrilidae. Species-specificity of all classes of spermatozoa is well established.

The very small size of ect-aquasperm is correlated with production of large numbers of sperm as an adaptation to the hazards of broadcast spawning. Simplicity of structure possibly relates more to conservation of materials than to hydrodynamics. Broadcast spawning imposes a size limit on the spermatozoon if sufficient numbers of sperm are to be produced to ensure fecundity.

Fertilization by ent-aquasperm similarly requires large numbers of sperm but fewer eggs than in external fertilization and is accompanied by a tendency to lecithotrophy. Ent-aquasperm are negligibly to greatly modified relative to ect-aquasperm. Whether these changes are related to transfer or to other features, such as lecithotrophy, is uncertain. Elongation of the nucleus and development of asymmetry are seen in several of the few known examples of ent-aquasperm.

Evidence that introsperm have evolved independently many times in the class is seen in the co-occurrence of ect-aquasperm (apparently plesiomorphically) and introsperm as an intraspecific variation in the Spionidae, Nereidae and Syllidae. The independent development of ent-aquasperm is possibly seen in their coexistence with ect-aquasperm in Terebellidae and Sabellida (Sabellidae and Serpulidae) but the issue is here clouded by the possibility that it is ect-aquasperm which are secondary in the Sabellida. Putative pseudintrosperm (possibly an introsperm or ent-aquasperm) in the nereid *P. massiliensis* are clearly an independent development as indicated by the occurrence of ect-aquasperm in its sibling species *Platynereis dumerilii* and other nereids. The multiple origins of introsperm contraindicate their value in establishing relationship between families in which they have separately developed, in contrast with groups such as decapod crustacea where sperm structure defines groups such as the Brachyura and their respective families. (For references to works which demonstrate and independently attest the value of spermatozoal ultrastructure for reconstructing phylogeny see Wirth, 1984, and Jamieson, 1987*b*.) There is, nevertheless, the possibility that related polychaete families will have developed similar modifications of sperm structure, even if only by parallelism as a result of relationship, which will afford some indication of affinity. Some similarities of the sperm of protodrilids and dinophilids may be of this type. At the intrafamilial level introsperm have taxonomic and phylogenetic value. Thus spionids have familial spermatozoal characteristics, including the usual distribution of mitochondria and platelets along the nucleus. Whereas several investigated species of *Polydora* confirm their intrageneric relationship and relationship to *Tripolydora* in closely similar though, again, species-specific spermatozoal structure, the genus *Streblospio* has its own variant on the spionid pattern. The sperm of the several sibling species once referable to *Capitella capitata* have similar but morphometrically distinct sperm. At higher taxonomic levels, the ultrastructure of questid

sperm has confirmed that the family, though oligochaetoid, has no close relationship with or at least cannot be included in, the euclitellates, each class of which has its own distinctive subtype of the euclitellate introsperm.

A number of categories of introsperm may somewhat arbitrarily be recognized: those which differ from ect-aquasperm chiefly in elongation of the nucleus (capitellids, possibly *Platynereis massiliensis*, though this may be a pseudintrosperm or even ent-aquasperm); approximately spheroidal, non-motile sperm (*Ophyrotrocha*); sperm with superficially 'primitive' facies but in fact modified (*Diurodrilus*); and filiform flagellate sperm (*Protodrilus*, *Microphthalmus*, *Dinophilus*, *Trilobodrilus*, *Hesionides*, *Ikosipodus*, *Questa*) or filiform aflagellate sperm (*Pisione*, histriobdellids). In all but the last two flagellate examples filiform development and extreme modification appear to be related to transdermal insemination but may also be adaptive to packaging in spermatophores and/or seminal receptacles. Spermatophores and filiform flagellate introsperm occur in spionids in the absence of transdermal insemination.

We concur with Westheide (1982) that in polychaetes there has been multiple parallel evolution of both reproductive behaviour and sperm structure; that the capacity for modification of sperm and reproductive biology is high in the class, whenever adaptive needs make it necessary; and that this may have been one of the prerequisites of the evolutionary success of the Polychaeta.

#### VI. SUMMARY

1. Polychaete sperm are divisible into ect-aquasperm, ent-aquasperm, and introsperm.
2. Ect-aquasperm are the commonest type of polychaete sperm and are considered plesiomorphic for the Polychaeta. Re-evolution of ect-aquasperm (as neo-aquasperm) is, nevertheless, tentatively hypothesized for some Sabellida.
3. In terms of ultrastructural studies of sperm in the investigated polychaete families, only ect-aquasperm have been demonstrated for 16 families; only ent-aquasperm for 3 families; ect- and ent-aquasperm for 3; ect- and intro-sperm for 2; ect-, ent- and intro-sperm for 1 family; and only introsperm for 11 families but investigations can only be regarded as preliminary. To date no family is known to have ent- and intro-sperm only. Sperm ultrastructure has yet to be examined in the orders Magelonida, Psammodrilida, Cossurida, Spintherida, Sternapsida, Flabelligerida and Fauvelopsida.
4. Much variation occurs in gross morphology, ultrastructure and configuration of the several components of ect-aquasperm: acrosome, nucleus, mitochondria, and centrioles and associated anchoring apparatus. A 9+2 axoneme is constant.
5. Group-specific sperm structure has been demonstrated for the Nereidae (chiefly ect-aquasperm), and for introsperm of the families Histriobdellidae, Questidae; Capitellidae, Spionidae and Protodrilidae. Species-specificity of all classes of spermatozoa is well established.
6. The very small size of ect-aquasperm is correlated with production of large numbers of sperm as an adaptation to broadcast spawning. Simplicity of structure may relate more to conservation of materials than to hydrodynamics.
7. Fertilization by ent-aquasperm requires fewer eggs than in external fertilization and is accompanied by a tendency to lecithotrophy. Elongation of the nucleus and

development of asymmetry are seen in several of the few known examples of ent-aquasperm. Whether modifications are related to transfer or to other features, such as lecithotrophy, is uncertain.

8. Evident multiple origins of polychaete introsperm contraindicate their value in establishing relationship between families, in contrast with their utility in groups such as decapod crustacea.

9. At the intrafamilial level polychaete introsperm have taxonomic and phylogenetic value, as seen in the Spionidae, Capitellidae, and Histriobdellidae, and are distinctive of each of these and other families.

10. At higher taxonomic levels, the ultrastructure of the sperm of the oligochaetoid Questidae distinguishes this family from euclitellates, each class of which has its own distinctive subtype of the euclitellate introsperm.

11. A number of categories of polychaete introsperm, with examples, are recognized: those which differ from ect-aquasperm chiefly in elongation of the nucleus; approximately spheroidal, non-motile sperm; sperm with superficially 'primitive' facies but in fact modified; and filiform flagellate or aflagellate sperm.

12. Filiform development and extreme modification appear often to be related to transdermal insemination but may also be adaptive to packaging in spermatophores and seminal receptacles.

13. Polychaetes exemplify multiple and pronounced parallel evolutionary modification of both reproductive biology and sperm structure which must have contributed substantially to the evolutionary success of the Polychaeta.

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## IX. ADDENDUM

In a recent review of spermatogenesis, male gametes and gamete interaction in polychaetes Franzén & Rice (1988) illustrated and described the structure of sperm from several polychaete species not included in the present review.

The spermatozoon of *Prionospio fallax* (Spionidae) is strikingly similar to that of *P. cf. queenslandica* (Rouse 1988*b*) but the bilateral symmetry of the acrosome seen in *P. cf. queenslandica* was not demonstrated.

The spermatozoa of *Alentia gelatinosa* (Polynoidea) and *Branchiomma bombyx* (Sabellidae), called primitive sperm by Franzén & Rice (1988), are here tentatively categorized as ect-aquasperm. *B. bombyx* has a rounded nucleus, a large triangular acrosome of uniform electron density and spherical mitochondria at the base of the nucleus. *A. gelatinosa* has a slightly elongate nucleus with a simple acrosome and four spherical mitochondria.

The sperm of *Sabellaria cementarium* (Sabellariidae) is similar to the other sabellariids previously described. The acrosome has three distinct regions typical of this family and is similar in overall structure to *S. alveolata* Pasteels (1965) in having a short striated structure.

The sperm of several species of Alciopidae are also illustrated. For two of these, *Naiades cantranii* and *Torrea candida* the sperm were taken from seminal receptacles in the female. They are thus classifiable as ent-aquasperm or introsperm but interestingly have a morphology typically seen in ect-aquasperm. *Vanadis formosana* has an elongate nucleus and sperm storage. *Krohnia lepidota* has a simple acrosome, and an elongate cylindrical nucleus with small spherical mitochondria abutting its base.

FRANZÉN, Å. & RICE, S. A. (1988). Spermatogenesis, male gametes and gamete interactions. In *The Ultrastructure of the Polychaeta* (ed. W. Westheide and C. O. Hermans), *Microfauna Marina* vol. 4, pp. 309-333. Gustav Fischer Verlag, Stuttgart.

nucleus, mitochondria, and centrioles and associated anchoring apparatus. A  $9+2$  axoneme is constant for polychaete ect-aquasperm.

In most families insufficient numbers of species have been investigated to establish whether the ect-aquasperm of each supraspecific taxon has group-specific characters. However, examination of considerable numbers of nereid species has established the occurrence of a nereid-specific ect-aquasperm type, although exceptions exist. Such specificity has been demonstrated for introsperm in at least the families Histriobdellidae, Questidae; Capitellidae, Spionidae and Protodrilidae. Species-specificity of all classes of spermatozoa is well established.

The very small size of ect-aquasperm is correlated with production of large numbers of sperm as an adaptation to the hazards of broadcast spawning. Simplicity of structure possibly relates more to conservation of materials than to hydrodynamics. Broadcast spawning imposes a size limit on the spermatozoon if sufficient numbers of sperm are to be produced to ensure fecundity.

Fertilization by ent-aquasperm similarly requires large numbers of sperm but fewer eggs than in external fertilization and is accompanied by a tendency to lecithotrophy. Ent-aquasperm are negligibly to greatly modified relative to ect-aquasperm. Whether these changes are related to transfer or to other features, such as lecithotrophy, is uncertain. Elongation of the nucleus and development of asymmetry are seen in several of the few known examples of ent-aquasperm.

Evidence that introsperm have evolved independently many times in the class is seen in the co-occurrence of ect-aquasperm (apparently plesiomorphically) and introsperm as an intraspecific variation in the Spionidae, Nereidae and Syllidae. The independent development of ent-aquasperm is possibly seen in their coexistence with ect-aquasperm in Terebellidae and Sabellida (Sabellidae and Serpulidae) but the issue is here clouded by the possibility that it is ect-aquasperm which are secondary in the Sabellida. Putative pseudintrosperm (possibly an introsperm or ent-aquasperm) in the nereid *P. massiliensis* are clearly an independent development as indicated by the occurrence of ect-aquasperm in its sibling species *Platynereis dumerilii* and other nereids. The multiple origins of introsperm contraindicate their value in establishing relationship between families in which they have separately developed, in contrast with groups such as decapod crustacea where sperm structure defines groups such as the Brachyura and their respective families. (For references to works which demonstrate and independently attest the value of spermatozoal ultrastructure for reconstructing phylogeny see Wirth, 1984, and Jamieson, 1987b.) There is, nevertheless, the possibility that related polychaete families will have developed similar modifications of sperm structure, even if only by parallelism as a result of relationship, which will afford some indication of affinity. Some similarities of the sperm of protodrilids and dinophilids may be of this type. At the intrafamilial level introsperm have taxonomic and phylogenetic value. Thus spionids have familial spermatozoal characteristics, including the usual distribution of mitochondria and platelets along the nucleus. Whereas several investigated species of *Polydora* confirm their intrageneric relationship and relationship to *Tripolydora* in closely similar though, again, species-specific spermatozoal structure, the genus *Streblospio* has its own variant on the spionid pattern. The sperm of the several sibling species once referable to *Capitella capitata* have similar but morphometrically distinct sperm. At higher taxonomic levels, the ultrastructure of questid

sperm has confirmed that the family, though oligochaetoid, has no close relationship with or at least cannot be included in, the euclitellates, each class of which has its own distinctive subtype of the euclitellate introsperm.

A number of categories of introsperm may somewhat arbitrarily be recognized: those which differ from ect-aquasperm chiefly in elongation of the nucleus (capitellids, possibly *Platynereis massiliensis*, though this may be a pseudintrosperm or even ent-aquasperm); approximately spheroidal, non-motile sperm (*Ophyrotrocha*); sperm with superficially 'primitive' facies but in fact modified (*Diurodrilus*); and filiform flagellate sperm (*Protodrilus*, *Microphthalmus*, *Dinophilus*, *Trilobodrilus*, *Hesionides*, *Ikosipodus*, *Questa*) or filiform aflagellate sperm (*Pisione*, histriobdellids). In all but the last two flagellate examples filiform development and extreme modification appear to be related to transdermal insemination but may also be adaptive to packaging in spermatophores and/or seminal receptacles. Spermatophores and filiform flagellate introsperm occur in spionids in the absence of transdermal insemination.

We concur with Westheide (1982) that in polychaetes there has been multiple parallel evolution of both reproductive behaviour and sperm structure; that the capacity for modification of sperm and reproductive biology is high in the class, whenever adaptive needs make it necessary; and that this may have been one of the prerequisites of the evolutionary success of the Polychaeta.

#### VI. SUMMARY

1. Polychaete sperm are divisible into ect-aquasperm, ent-aquasperm, and introsperm.
2. Ect-aquasperm are the commonest type of polychaete sperm and are considered plesiomorphic for the Polychaeta. Re-evolution of ect-aquasperm (as neo-aquasperm) is, nevertheless, tentatively hypothesized for some Sabellida.
3. In terms of ultrastructural studies of sperm in the investigated polychaete families, only ect-aquasperm have been demonstrated for 16 families; only ent-aquasperm for 3 families; ect- and ent-aquasperm for 3; ect- and intro-sperm for 2; ect-, ent- and intro-sperm for 1 family; and only introsperm for 11 families but investigations can only be regarded as preliminary. To date no family is known to have ent- and intro-sperm only. Sperm ultrastructure has yet to be examined in the orders Magelonida, Psammodrillida, Cossurida, Spintherida, Sternapsida, Flabelligerida and Fauvelopsida.
4. Much variation occurs in gross morphology, ultrastructure and configuration of the several components of ect-aquasperm: acrosome, nucleus, mitochondria, and centrioles and associated anchoring apparatus. A 9+2 axoneme is constant.
5. Group-specific sperm structure has been demonstrated for the Nereidae (chiefly ect-aquasperm), and for introsperm of the families Histriobdellidae, Questidae; Capitellidae, Spionidae and Protodrilidae. Species-specificity of all classes of spermatozoa is well established.
6. The very small size of ect-aquasperm is correlated with production of large numbers of sperm as an adaptation to broadcast spawning. Simplicity of structure may relate more to conservation of materials than to hydrodynamics.
7. Fertilization by ent-aquasperm requires fewer eggs than in external fertilization and is accompanied by a tendency to lecithotrophy. Elongation of the nucleus and