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Mating behavior of nemerteans: present knowledge and future directions

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Abstract

In most nemertean species, members of the two sexes aggregate before fertilization takes place. Few specific studies on the mating behaviour of nemerteans have been conducted but several observational reports indicate that important processes known from other organisms, such as sexual selection and sperm competition, may also be at work in nemerteans. Herein, we review some of these observations and discuss their possible implications. We produce a summary table and reproduce some important observations, placing them in an evolutionary context. Four types of gamete-transfer mechanisms are distinguished: (1) free-spawning, where members of both sexes release gametes freely into the water column; (2) mucus-spawning, where gametes are released within a mucus matrix; (3) internal fertilization, where spermatozoans are transferred to the immediate vicinity of oviducts, which they penetrate; and (4) gamete transfer aided by specific structures. While little is known about the last transfer mechanism, anecdotal observations are mainly available for the first three mechanisms. Mating interactions frequently involve more than two individuals. In several species, individuals only invest part of their gametes in a single mating interaction, apparently saving additional gametes for potential future mating events. These observations suggest that males of some species are exposed to mating competition. Males might counteract sperm competition by producing large numbers of sperm, as suggested by their high fertilization potential. Previous authors inferred that sperm morphology may be an adaptation to spawning behavior and possibly also to the risk of sperm competition. Based on the results of our analysis and the above observations, we suggest that sexual selection and sperm competition are not uncommon in nemerteans.

Keywords: *Nemertean, reproductive behavior, mating, fertilization, sperm competition*

Introduction

''The biology of fertilization within the Nemertini is very incompletely known…'' (Franzén 1956)

''Mating behaviour will help ensure fertilization of eggs in a situation in which few animals are found….'' (Roe 1988)

Sexual reproduction involves three major components: (1) production of gametes; (2) processes leading up to gamete transfer and successful fertilization; and (3) care of developing offspring. To achieve successful gamete transfer, many species show particular morphological and behavioural adaptations. In many free-spawning species, individuals aggregate before gamete release, which is interpreted as a mechanism to ensure high gamete concentrations in the surrounding water (Levitan 1995). In combination with this aggregation behaviour, the subsequent synchronization of gamete release leads to high fertilization success. In other species, gametes are released at least by one of the sexes, but then retained in particular structures that may be intracorporal (e.g. mantle cavity of some bivalves) or extracorporal (e.g. mucus masses of some polychaetes or nemerteans). These adaptations may aid in ensuring successful fertilization after gamete transfer. Finally, many species have evolved particular copulatory structures allowing direct insemination, thereby further reducing loss of gametes during transfer (e.g. crustaceans, gastropods). In these latter species, gamete transfer is typically accompanied by particular courtship behaviour, during which partners evaluate each other before proceeding with the mating interactions.

Most of these gamete-transfer mechanisms have been reported for nemerteans. However, very little is known about the numbers and behavior of the individuals participating in gamete transfer, since most descriptions of mating interactions in nemerteans are the result of casual observations (Friedrich 1979). Herein, we review a wide diversity of publications on nemerteans in a first attempt to summarize our present knowledge about the mating behavior of nemerteans and place it in an evolutionary context.

Most marine nemerteans are gonochoristic, but hermaphroditic species predominate among terrestrial and freshwater nemerteans (Turbeville 1999). The reproductive system of nemerteans is simple, consisting of multiple sac-like gonads, usually without particular organs for gamete storage or transfer (Turbeville 1991). Studies on the reproductive biology of nemerteans occasionally report important observations of their mating behavior (Coe 1943; Gontcharoff 1950; Bartolomaeus 1984; Roe 1988, 1993; Kruse and Buhs 2000). Some of these reports indicate that mating may include more than two individuals, suggesting that competition for fertilization may occur. Cantell (1989) remarked that many nemertean species become more gregarious with the beginning of the reproductive period, which could imply that gamete release is synchronized, thereby increasing the possibility of competition for fertilizations. Furthermore, there exists indication that individuals do not invest all gametes in a single-mating opportunity, but rather retain gametes, possibly to mate with multiple partners.

Materials and methods

We carefully reviewed studies in which observations on gamete transfer are described and categorized these according to the three main transfer mechanisms (free-spawning, mucus-spawning and internal fertilization). We briefly report the principal published descriptions on mating behavior in nemerteans to make these important observations easily accessible for a wider audience of scientists interested in mating behavior of marine invertebrates. From the available reports, we extracted information on the occurrence of: (1) partial spawnings; (2) multiple-mating partners involved in a single-mating interaction; and (3) spermatozoans in female reproductive tracts. We present a summary table on these gamete-transfer characteristics.

Main mechanisms of gamete transfer and examples

Free-spawning

Members of both sexes release gametes into the water column. Little is presently known of how gamete release is synchronized in free-spawning nemerteans. Chemical stimuli have been invoked to facilitate both spatial and temporal synchronization (Cantell 1989). Free-spawning nemerteans have also been reported to swim up the water column before gamete release (Berg 1972) and intertwine (Iwata 1960), which according to Friedrich (1979) ensures simultaneous release of oocytes and spermatozoans and, thereby, successful fertilization. Free-spawning species typically produce large quantities of gametes. Gonad counts range from several hundred to a few thousand pairs in free-spawning species, such as *Cerebratulus lacteus*, *Lineus longissimus*, *Micrura alaskensis*, *Pantinonemertes californiensis* (Bierne 1983; Roe 1993; Stricker et al. 2001). Individuals of these species release hundreds to thousands of oocytes in a single-spawning event (Roe 1993; Stricker et al. 2001). Depending on the number of individuals participating in these spawning events, sperm competition might be intense.

Cerebratulus lacteus. This species from the coasts of the northwest Atlantic apparently has a distinct reproductive period during the winter months (Coe 1895). This author also mentioned that not "all the products in a single individual are discharged at once, a specimen often showing a portion of its genital sacs to be empty, while other sacs are filled with mature ova or spermatozoa". Wilson (1900) observed that initiation of gamete release by females may induce release of spermatozoans in males. This author (cited in Friedrich 1979) saw the release of oocytes and spermatozoans in separate burrows from which they were extruded by the respiratory current into the open water column. During the reproductive period, Dean (1978) caught two specimens swimming at night in the water column, but whether this observation is related to spawning activities is not known.

Paranemertes peregrina. This is a free-spawning species. Roe (1976) remarked that only few individuals of a local population participate in a given spawning event, i.e. spawning does not appear to be synchronized in this species. Oocytes were freely shed into the water column (Roe 1976).

Riseriellus occultus. Rogers et al. (1993) observed spawning of a female in the laboratory: "The discharge of eggs is very similar to that described for *Lineus lacteus* by Goncharoff and Lechenault (1958); strings of eggs are released from the gonopores but quickly break up so that individual eggs are free". This description resembles other observations of free-spawning nemerteans, where authors also reported that, immediately after release, oocytes are surrounded by a gelatinous matrix, which quickly disintegrates (e.g. Friedrich 1979; Cantell 1989). Release of gametes may be enhanced by muscular contractions (Bierne 1983).

Mucus-spawning or burrow-spawning

Members of both sexes release gametes in a mucus mass or in a tube or burrow, which they inhabit. In some species, mucus envelopes are produced (primarily by the female) shortly before gamete release. This may facilitate successful fertilization. Given that often more than two sexual partners are found in the mucus masses or tubes/burrows, and that the sex ratio in these mating groups typically appears to be male-biased, sperm competition can be assumed to occur.

Lineus viridis. One of the most detailed reports on the mating process of a nemertean is from Bartolomaeus (1984) on the intertidal nemertean *L. viridis*, a very common species from the temperate regions of the northern hemisphere. The reproductive season of this species is during the spring months (March–May in Europe). A few days before spawning one to several males cling to the body of the larger female (Bartolomaeus 1984). Whether these males directly or indirectly compete for access to the female is presently unknown. However, Cantell (1975) reported that "a male pressed the ventral side of his head tight against the female and sinuously encircled her body. This continued for a rather long time and the worms were difficult to separate", which could suggest that males compete for the best position on a female. Shortly before spawning, the female stops active movements and releases a mucus cocoon around her body, which also includes the associated males. After completion of the cocoon, the female remains motionless, but the males actively crawl over her body. This phase lasts for 1–2 h, after which the males leave the cocoon – the testes of the males are only partially empty (Bartolomaeus 1984). When the males have left the cocoon, the female starts to press out the eggs, which are included in groups of up to 22 eggs in a pear-shaped capsule. The author emphasized that the content of each capsule represents the entire content of one female gonad. He also examined the egg capsules and found sperm therein. This led him to suggest that males release sperm into the mucus mass after formation of the cocoon and that sperm migrate into the female gonads before the egg capsule is formed. As soon as formation of egg capsules starts, the males leave the cocoon, but they may stay in the vicinity (Bierne 1983) (see Figure 1).

Lineus ruber. While observations on gamete transfer are not as detailed as for the previously mentioned species, they suggest that mating proceeds in the same manner and under similar conditions in *L. ruber* (Barrois 1876; Coe 1943; Gontcharoff 1951). Bierne (1975) reported that "the position of the spermioducts on the ventral surface of the body is an indication that internal fertilization takes place since such a position permits the male to press its body against the open oviducts located dorsally in the female". This conclusion is supported by other reports: "Upon reaching sexual maturity the male and female enclose themselves in a mucus envelope where the eggs are laid and fertilized. It is probable that the spermatozoa penetrate into the ovaries, which serve as receptacula seminis." (Gontcharoff 1951).

Carcinonemertes carcinophila. Humes (1942) recognized that spawning in a mucus mass or tube may favour successful fertilization: "…the two sexes must at times be together in the same sheath to bring about insemination. Otherwise the spermatozoa, which are limited in number as compared with the huge numbers present in some of the larger free-living nemerteans, would be so scattered that the majority of them would never come in contact with a female worm." Sometimes more than one pair of individuals are present in the mass (Riser 1974). Similar aggregations of several individuals are also reported for other species of the genus *Carcinonemertes* (Wickham 1980; Stricker 1986; Roe 1988; Kuris 1993; McDermott and Gibson 1993; McDermott 1998). From many of these reports it is not entirely clear whether both sexes release gametes simultaneously into the mucus envelope, or whether females retain gametes in their ovaries, into which spermatozoans then immigrate. However, Coe (1904) reported sperm in female ovaries and Stricker (1986, 1987) mentioned cleaving embryos in ovaries of female *C. epialti*. Internal fertilization may have evolved in species of *Carcinonemertes* because females lay embryo strings over several days (Roe 1988). Deposition of small numbers of embryos in a cocoon has been observed by Stricker et al. (2001). The male reproductive system of *Carcinonemertes* species shows
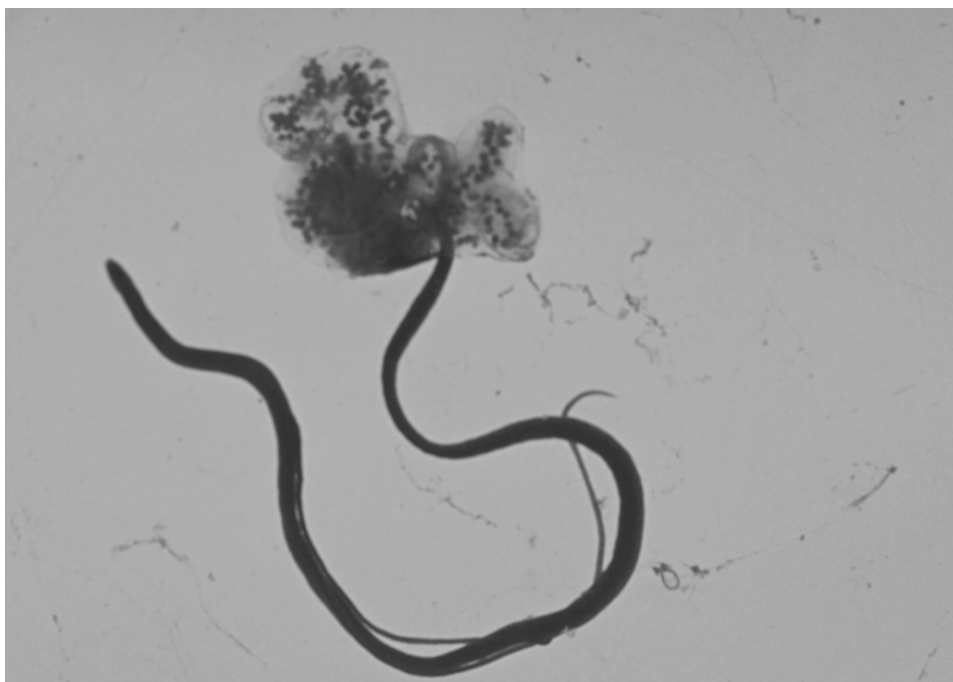
Figure 1. Female *Lineus viridis* with two males immediately after fertilization and deposition of mucus cocoon. Photo courtesy of K. Reise (Wattenmeerstation List/Sylt, Germany).

very interesting morphological characteristics: the individual testes lead via a vas deferens into a seminal vesicle, which opens into the gut (Humes 1941). Roe (1984) mentioned that, in males of *C. epialti* and *C. errans*, the posterior end of the body is highly muscular, which has implications for mating in these species. Franzén (1956) summarized the description by Humes (1941) in the following way: ''The sperms are collected in a vesicula seminalis of the male and are squeezed out through the anus. The slimy envelope around the animals retains the sperm in the vicinity of the female, and at least part of the spermatic mass penetrates into the ovaries.'' Possibly, this morphological adaptation allows males to control sperm investment during individual matings.

According to Bürger (1895) male *C. carcinophila* (reported as *Cephalothrix galatheae*) may have multiple female mating partners: ''.... spermatozoans released in water and immigrate into ovaries. .... males are much rarer than females....''. Based on observations by Dieck (1874), Bürger (1895) remarked that ''one male can spread its spermatozoans simultaneously over five to six females, which cohabit with him in the egg-mass of *Galathea strigosa*.''

Internal fertilization

Members of one sex (typically the male) release or transfer sperm, which are retained in the body of the female, where fertilization occurs. Fertilized embryos may then be kept in the female's body or released either into the water column or in a mucus mass, where further development proceeds. Internal fertilization can also be assumed for all viviparous species. The following list of viviparous species underlines that internal fertilization is not

uncommon in nemerteans: *Cyanophthalma obscura* (Lassig 1964; Norenburg 1986), *Notogaeanemertes folzae* (Crandall et al. 1998), *Pantinonemertes agricola* (Coe 1904, 1939, 1940; Hett 1927; Iwata 1957), *Pheroneonemertes dianae* (Gibson 1990), the species of the genus *Prosorhochmus* (Gibson et al. 1986; Frutos et al. 1998), *Prostoma graecense* (Iwata 1957), *Lineus bilineatus* (Iwata 1957; Gontcharoff 1955), *L. viviparus* (Coe 1943; Iwata 1957), and *Poikilonemertes vivipara* (Stiasny-Wijnhoff 1942). In many of these species, mating occurs also in a mucus mass but, in contrast to the species from the previous category, developing embryos are retained in the female ovary after fertilization. Since several males may occur together in a spawning aggregation, sperm competition is likely to exist.

Argonemertes australiensis. Internal fertilization is common in terrestrial nemerteans from the genus *Argonemertes* and sperm have been observed in female ovaries (Coe 1904; Hett 1924). The worms have been observed to gather in groups, with the bodies of two or more individuals placed side by side, accompanied by the secretion of considerable mucus (Coe 1939). For *A. australiensis*, Dendy (1892, cited in Bürger 1895) reported that "….the mature male was found sitting on the back of the mature female. The spermatozoans, of course, immigrate through the gonopores into the ovaries". Coe (1904) revealed that "the spermatozoa may remain alive for some time after their entrance into the ovary and may fertilize an ovum, which matures some days, or perhaps weeks later". To our knowledge this is the only report on long-term sperm storage and maintenance of sperm functionality in nemerteans.

Fertilization with special sperm-transfer structures

In many pelagic nemerteans, the males feature special structures that have been interpreted as penes or pseudopenes. Many authors debated the function of these structures during sperm transfer but no direct observations have yet been made. Since neither sperm nor developing embryos have ever been found on/in a female, the oocytes must be assumed to be extruded during mating and that development occurs away from the female. While no observations of mating interactions are available, the following reports strongly suggest that the two sexes meet for gamete transfer (Norenburg and Roe 1998). Whether several individuals participate in a single mating interaction is not known and, thus, no inference about the risk of sperm competition can be made.

Nectonemertes mirabilis. Based on Coe and Ball (1920), Franzén (1956) reported: "Each of the testicles, which are concentrated to the head as usually in pelagic nemertines, has its own opening. A vesicula seminalis is, however, differentiated in the vas deferens. The formation of this vesicula and the powerful muscles of the walls of the gonad are by Coe and Ball interpreted as an indication of a storage of the sperms, which during copulation come into direct contact with the eggs in the ovaries. The genital pores can be interpreted as organs of copulation". Brinkmann (1917) suggested that the head tentacles of male *N. mirabilis* are secondary sexual organs, which are used to hold on to the female during mating, and he provides several observations to support this idea, which was later also adopted by Coe (1943).

Phallonemertes murrayi. Brinkmann (1917) provided a detailed analysis of this species and, based on histological sections of several males, he reached the following conclusions: "The

description of the male sexual organs does not hold for all individuals from the collection. In some, which coincide with the remaining in all other characteristics, the penes are completely absent; here the testes open through small pores at the body surface as usually is the case in nemerteans. If one examines these gonopores in sections, one surprisingly finds that the edges of these openings are not intact; one has the impression that something is broken off. It would now be obvious to assume that the penes, which doubtless were located here, had broken off due to rough treatment during collection or preservation; however, I believe that this is not the case. Because then it would hardly be explicable why they are either all present or all absent as I could confirm in all undestroyed individuals. Furthermore, these animals feature partly contracted and often empty testes where the penes are missing. I am therefore of the opinion that, without having to reach risky assertions, one can conclude that the penes are true copulatory organs and that they broke off during copulation or that they possibly function as a type of spermatophore, which are attached on or in the female. This conclusion is supported by the anatomy of this structure in which the very thin-walled compartment between testis and penis facilitates breakage of this penis. However, I should emphasize that I could not find these broken-off penes attached on the females. Thus, this is just a hypothesis, which is, however, supported by several hints''. In summary, Brinkmann (1917) suggests that the penis-like structures in *P. murrayi* function in a similar way as the hectocotylus in some male cephalopods, which separate from the male and attach near the female reproductive tract (commonly reported from pelagic deep-sea cephalopods). Males of *Plotonemertes adhaerens* possess sucker-like attachment organs (Friedrich 1979), which also might ensure direct contact between males and females during gamete transfer in the pelagic environment (Brinkmann 1917). Roe and Norenburg (2001) partly support this suggestion in saying that ''the sexual dimorphism seen in *Plotonemertes* points suggestively to a sex-related function of postero-lateral glands'', but they also emphasize that their function has not been fully clarified. Bürger (1909) noted specialized glandular epithelium surrounding the male gonopores of *Balaenanemertes chuni* Bürger, 1909, now known from several additional species (Norenburg and Roe 1998).

Characteristics of gamete transfer

For several nemertean species, partial gamete release has been reported (Table I), which may be due to the fact that, in large species, maturation of gonads is not synchronized. Some gonads may contain fully developed gametes while others are still in the process of gametogenesis. Regardless of the reasons, members of both sexes may only release part of their gametes and, at present, it cannot be said whether this occurs more frequently in males than in females. One important consequence of partial gamete release is that individuals might mate with multiple mating partners in several subsequent mating interactions. Staged mating interactions with male *Prosorhochmus nelsoni* confirmed that individual males could fertilize the eggs of at least four subsequent females over a time period of 20 days (Vasquez and Thiel, unpublished data). However, also in single mating events, individuals may mate with multiple partners, which has been reported in a wide variety of nemertean species and for all known gamete-transfer mechanisms (Table I). Sperm in the female reproductive tract has been reported for several species with mucus-spawning and for some species with internal fertilization (Gibson 1972; Riser 1974; Cantell 1989). The sperm most likely enter the female ovaries in all viviparous species, but for many species this has not been reported. This strongly suggests that sperm entry into

Table I. Gamete-transfer characteristics reported for nemertean species that spawn gametes freely (FS), in a mucus mass (MS) or that have internal fertilization (IF) – listed in this order. Y – behavior observed directly and reported by authors; (Y) – behavior not observed directly, but strongly suggested based on observations.

Species	Fertilization mode	Partial gamete release	>1 mating partner	Sperm in ovaries	Reference
Cerebratulus lacteus	FS	Y	–	–	Coe (1895); Kline et al. (1985)
Gononemertes australiensis	FS	Y	–	–	Egan and Anderson (1979)
Micrura alaskensis	FS	–	Y	–	Coe (1901)
Nipponnemertes pulcher	FS	–	Y	–	Berg (1972)
Pantinonemertes californiensis	FS	Y	–	–	Roe (1993)
Paranemertes peregrina	FS	–	(Y)	–	Roe (1976)
Amphiporus michaelseni	MS	–	–	–	Joubin (1914)
Lineus ruber	MS	–	Y	Y	Gontcharoff (1951); Bierne (1970, 1975); Riser (1974)
Lineus viridis	MS	Y	Y	Y	Riser (1974); Cantell (1975); Bartolomeaus (1984)
Micrura fasciolata	MS	–	(Y)	–	Gontcharoff (1951)
Procephalothrix filiformis	MS	–	(Y)	–	Iwata (1960)
Prostoma jenningsi	MS	–	(Y)	Y	Gibson and Young (1976)
Tetrastemma phyllospadicola	MS	Y	–	–	Stricker et al. (2001)
Amphiporus incubator	IF	–	–	–	Joubin (1914); Riser (1974)
Argonemertes australiensis	IF	–	Y	Y	Coe (1904, 1939); Moore and Gibson (1985)
Argonemertes hillii	IF	–	–	Y	Hett (1924)
Carcinonemertes carcinophila	IF	–	Y	Y	Coe (1904, 1943); Humes (1942); Riser (1974)
Carcinonemertes epialti	IF	Y	(Y)	Y	Coe (1904); Kuris (1978, 1993); Roe (1984, 1988); Stricker (1986); Stricker et al. (2001); Jespersen (1994)
Carcinonemertes errans	IF	–	Y	–	Wickham (1980)
Carcinonemertes pinnotheridophila	IF	–	Y	–	McDermott and Gibson (1993); McDermott (1998)
Cephalothrix rufifrons	IF	–	(Y)	–	Anadón (1980); Jespersen (1994)
Geonemertes pelaensis	IF	–	–	Y	Coe (1939, 1940)
Pantinonemertes agricola	IF	–	Y	–	Coe (1904)

female ovaries may be more common than presently reported, also in species with mucus-spawning.

Discussion

The reports herein demonstrate that mechanisms of gamete transfer in nemerteans are diverse and that specific tactics have evolved to improve fertilization success. Reports on species with internal fertilization are more numerous in this review than those on free-spawning species. We believe that this reflects an observer bias: it is simply more likely to make direct observations or to find unmistakable evidence of internal fertilization (e.g. in form of sperm or embryos in female ovaries) than of free-spawning fertilization. However, despite this apparent bias, several important points can be made about gamete transfer in nemerteans.

One of the best adaptations in ensuring successful fertilization is by gregarious behavior. In combination with synchronization of gamete release, this will result in high concentrations of gametes, thereby improving the possibility of successful fertilizations (Levitan 1995). Nemerteans have efficient chemosensory capabilities (Amerongen and Chia 1982) that may also be useful to locate mating partners. Aggregations of many conspecifics, which apparently are related to reproduction, are indeed not uncommon in nemerteans (Cantell 1989).

Multiple mating partners in a single mating event have been reported from a wide variety of nemerteans, including free-spawning, mucus-spawning and retention-spawning species (Table I). Gregarious mating might enhance fertilization success but simultaneously it will increase intrasexual competition for fertilization, generating opportunities for sexual conflicts. Recently, it has been shown that polyandrous spawning (multiple males contributing sperm) significantly increases fertilization rates in a free-spawning polychaete, and it was suggested that "polyandry will confer important sexually-selected advantages on females via the ability to select compatible mating partners at fertilization" (Marshall and Evans 2005). Since males typically produce more gametes than females, they are usually considered most sensitive to intrasexual competition for fertilizations during gregarious gamete release. Males might employ different tactics to counteract the risk of sperm competition. Sperm morphology has been invoked to reflect gamete transfer mechanisms in nemerteans (Franzén 1983). Sperm of the primitive type have a compact head and are primarily found in free-spawning nemertean species, while elongated (modified) sperm are found in species spawning in a mucus mass or with internal fertilization (Franzén 1983; Stricker and Folsom 1998) (see Figure 2). Possibly, the variations in shape (and motility?) reflect the different selective environments of free-spawning and mucus-spawning nemerteans. Compact sperm may have an adaptive advantage in direct competition for fertilizations in the water column, while elongated sperm may move more efficiently in a mucus environment. Studies in other species have demonstrated that the risk of sperm competition can have a strong selective effect on sperm characteristics (Hunter and Birkhead 2002; LaMunyon and Ward 2002; Byrne et al. 2003). Also the quantity of sperm invested in a mating event may be a response to sperm competition (Parker 1998).

Besides these traits, male behavior may play an important role. Males of many other species actively attempt to prevent other males from access to females, with the ultimate goal of preventing sperm of other males from fertilizing the female's eggs (Andersson 1994). Several authors (Bierne 1970; Riser 1974; Bartolomeaus 1984) have reported that, in *Lineus ruber* and *L. viridis*, several males may ride on the back of the female, but it has not yet been determined if these males actively attempt to impede other males from accessing preferred gamete-transfer sites. In these species, oviducts open dorsally and spermioducts open ventrally (Bierne 1970), permitting close contacts between the gonads during gamete transfer and opening the possibility of one male pushing the other males away from the oviducts during mating. Similar sexual differences in gonoduct position have also been reported for other species (Riser 1974). Possibly, direct contact between the sexes during gamete transfer, the presence of several males and penetration of ovaries by spermatozoans have provided a selective environment resulting in the evolution of sex-specific position of gonoducts.

Also, partial gamete release has been observed in all three mating categories. At present, the evolutionary significance of this observation is not well known. However, we consider it possible that some individuals save gametes for future mating opportunities, as reported for *Prosorhochmus nelsoni*. Furthermore, it may be possible that, in some large species, not all
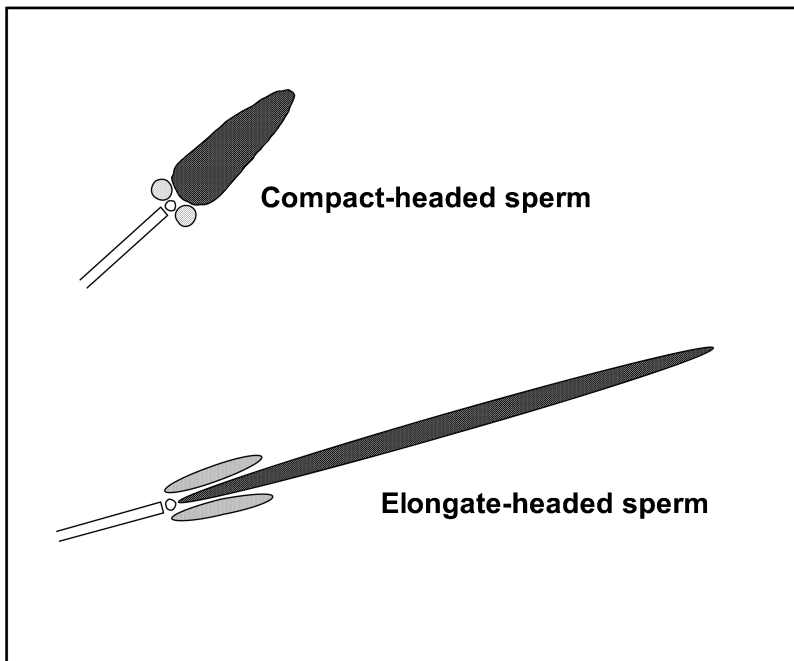
Figure 2. Schematic representation of compact-headed (primitive) and elongate-headed (modified) sperm of nemerteans. Modified after Stricker and Folsom (1998).

gametes mature simultaneously and that, during a given mating interaction, only fully mature gametes are released.

The relatively common observations of spermatozoans in female ovaries appear remarkable. Also, the existence of several viviparous nemertean species (Riser 1974; see also above) demonstrates that the ovarian environment does not impede successful fertilization. Bartolomaeus (1984) emphasized that successful fertilization in *L. viridis* can only occur if spermatozoans enter the ovaries before formation of egg capsules. Internal fertilization has also been reported for *Carcinonemertes epialti* (Stricker 1986) and other mucus-spawning nemerteans. Most likely this has evolved in species that inhabit environments where successful fertilization of freely spawned gametes is difficult, i.e. in symbiotic, pelagic or terrestrial species (Coe 1943; Friedrich 1979). Internal fertilization and subsequent ovovivipary or vivipary could also have evolved in response to unfavorable conditions for development of pelagic larvae (Cantell 1989).

In terrestrial species, internal fertilization appears to be obligatory and even sperm storage has been reported from this group (see above). Sperm storage may primarily be a female mechanism of ensuring fertilization in an environment where encounters with male partners are highly unpredictable.

These considerations suggest that in nemerteans, penetration/uptake of spermatozoa into female ovaries has evolved as a mechanism to ensure fertilization. The fact that sperm penetration into ovaries is reported from a variety of taxa suggests that this gamete-transfer mechanisms has evolved repeatedly and independently in nemerteans, depending on the selective environment. While sperm penetration into female ovaries may facilitate fertilization, it also offers the chance for parasites to enter the female reproductive tract and body. Gontcharoff (1950, 1951) reported high prevalence of gregarine parasites in

ovaries of female *Lineus sanguineus*; Riser (1974) has suggested that this might result in her castration.

Even though sperm penetration into ovaries is not uncommon in nemerteans, sperm transfer structures have only been reported for pelagic nemerteans. Interestingly, many authors have interpreted these structures as having relation to reproduction (Brinkmann 1917; Coe 1926, 1943; Friedrich 1979), but firm evidence for their function is still lacking. Norenburg and Roe (1998) suggested that gamete transfer may involve contact between male and females. We agree with this suggestion, because, as in the terrestrial environment, the conditions in the pelagic realm strongly favor the evolution of direct contact between the sexes during gamete transfer, which apparently is not uncommon in a wide variety of nemertean taxa.

In conclusion, most adaptations in nemertean mating behavior may have evolved to improve fertilization success. This apparently is best achieved via gregarious matings and retention of sperm in or near female gonads. While these may result in an overall increase of successful fertilizations, it also leads to a high risk of sperm competition for males. Based on the results of our analysis and the above observations, we suggest that sexual selection and sperm competition is not uncommon in nemerteans and that future studies on mating associations and the mating process should be conducted.

Acknowledgements

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