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FOURTEEN

Functional Morphology of Spermatophores and Sperm Transfer in Calanoid Copepods

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Abstract

Spermatophores produced by most calanoid copepods are simple, tube-shaped flasks that contain the spermatozoa and seminal secretions. They adhere to the external cuticle of the female by means of a cementlike substance present on the tapered, open end of the flask. Other calanoids have evolved spermatophore flasks connected to highly complex, chitinlike coupling plates. These have a specific configuration that corresponds to the morphology of the conspecific female's urosome, thus assuring proper placement. Spermatophore transfer is accomplished by a ritualized and precise mating behavior controlled by pheromonal attraction and structurally modified copulatory appendages. The distinctive morphologies of the spermatophores, copulatory appendages, and female genitalia, in combination with species-specific mating behavior, function as controlling factors of reproductive isolation in calanoid copepods.

SPERMATOPHORES PRODUCED by copepods are typically tube-shaped flasks that contain spermatozoa and seminal secretions. In calanoid copepods, a single mature spermatophore is stored within the male's metasome until copulation, at which time it is extruded and attached to the external surface of the female genital segment with the aid of specific male appendages. The spermatophoric contents empty into internal chitin-lined spermathecal sacs where they are stored until the oocytes are spawned.

Published information concerning spermatophore morphologies and mating behaviors in calanoids is very limited relative to the large number of species in this group. This paper will combine information from the literature with new observations on the functional morphology of spermatophores, copulatory appendages, and female genitalia of some marine calanoid copepods. The significance of these reproductive structures and mating behavior in establishing reproductive isolating barriers will be discussed.

MORPHOLOGY OF REPRODUCTIVE STRUCTURES

Copulatory Appendages

In numerous calanoid families (e.g., Pontellidae, Centropagidae, Temoridae, Metridiidae, Pseudodiaptomidae, Lucicutiidae, Heterorhhabdidae, Augaptilidae, Arietillidae, Candaciidae, Bathypontiidae, Acartiidae, and Tortanidae [Brodskii 1967a,b]), the antennule (A1) of the male (generally the right one) is geniculate and prehensile, modified with one or two hinged joints that enable the antennule to fold back upon itself (fig. 14.1). Segments on either side of the hinge are lined with cuticular teeth and equipped with sensory setae. Intergeneric differences include the relative location of the hinges along the length of the antennule, extent of development of cuticular teeth, and number of sensory setae or aesthetascs. This appendage is used for the initial capture of the female (fig. 14.9A).

Calanoid copepods typically have five pairs of thoracic swimming legs. Pairs 1–4 are biramous, symmetrical, and in the majority of species essentially identical. However, in many calanoid families the fifth pair of swimming legs (P5) on both the male and female rarely resembles P 1–4 and its structure is used often as a taxonomic feature of males. The female P5 is symmetrical but usually reduced or absent. This appendage has been observed in females of *Labidocera aestiva* to function in the removal of the empty spermatophore (Blades & Youngbluth 1979).

The male P5 is asymmetrical to varying degrees, but often is strongly modified to hold the female and transfer the spermatophore. Structural modifications of the male's P5 range from a slight reduction or thickening of spines and setae to the development of a chela with powerful musculature on one leg of the pair (fig. 14.2A). Development of this clawlike structure is notable in some calanoid families such as the Pontellidae and Centropagidae. In *Labidocera aestiva*, for example, the right leg is uniramous and the terminal segment is modified into a large chela (fig. 14.2A). The chela is equipped with several spines, presumably sensory in function (Blades & Youngbluth 1980). The left leg is biramous, con-

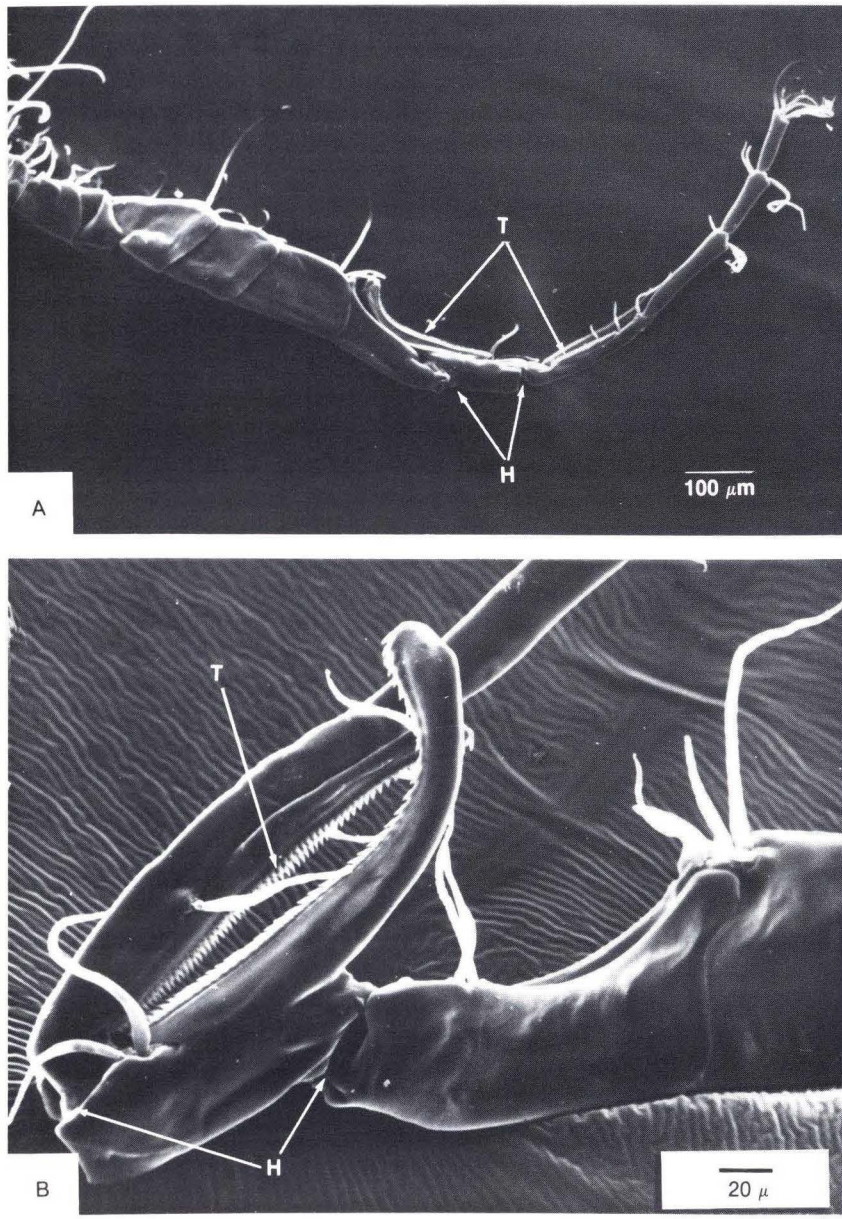


FIGURE 14.1. Distal segments of geniculate antennule from male *Labidocera aestiva*. A. Antennule open. B. Antennule closed. H, hinges; T, toothed margins. Note numerous hairs. Figure 14.1B from Blades and Youngbluth 1979.

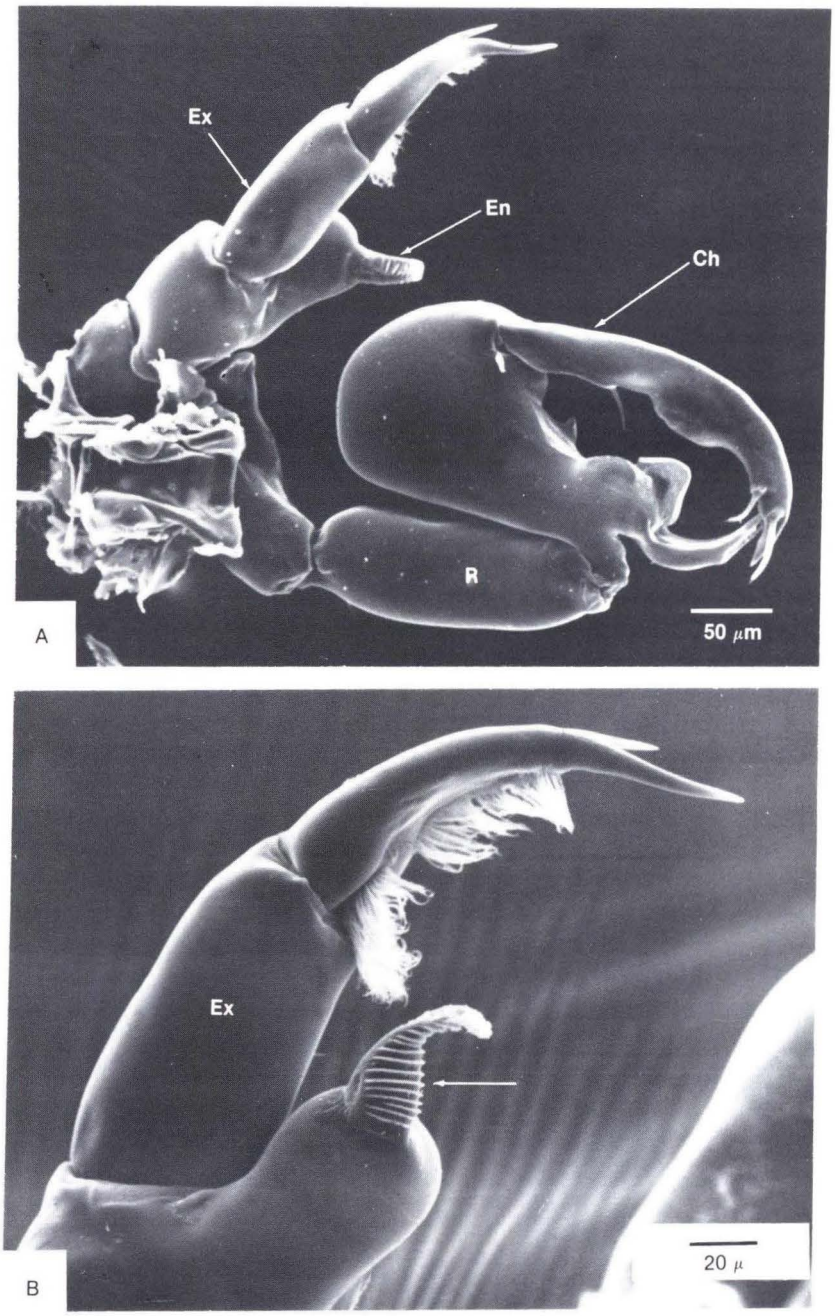


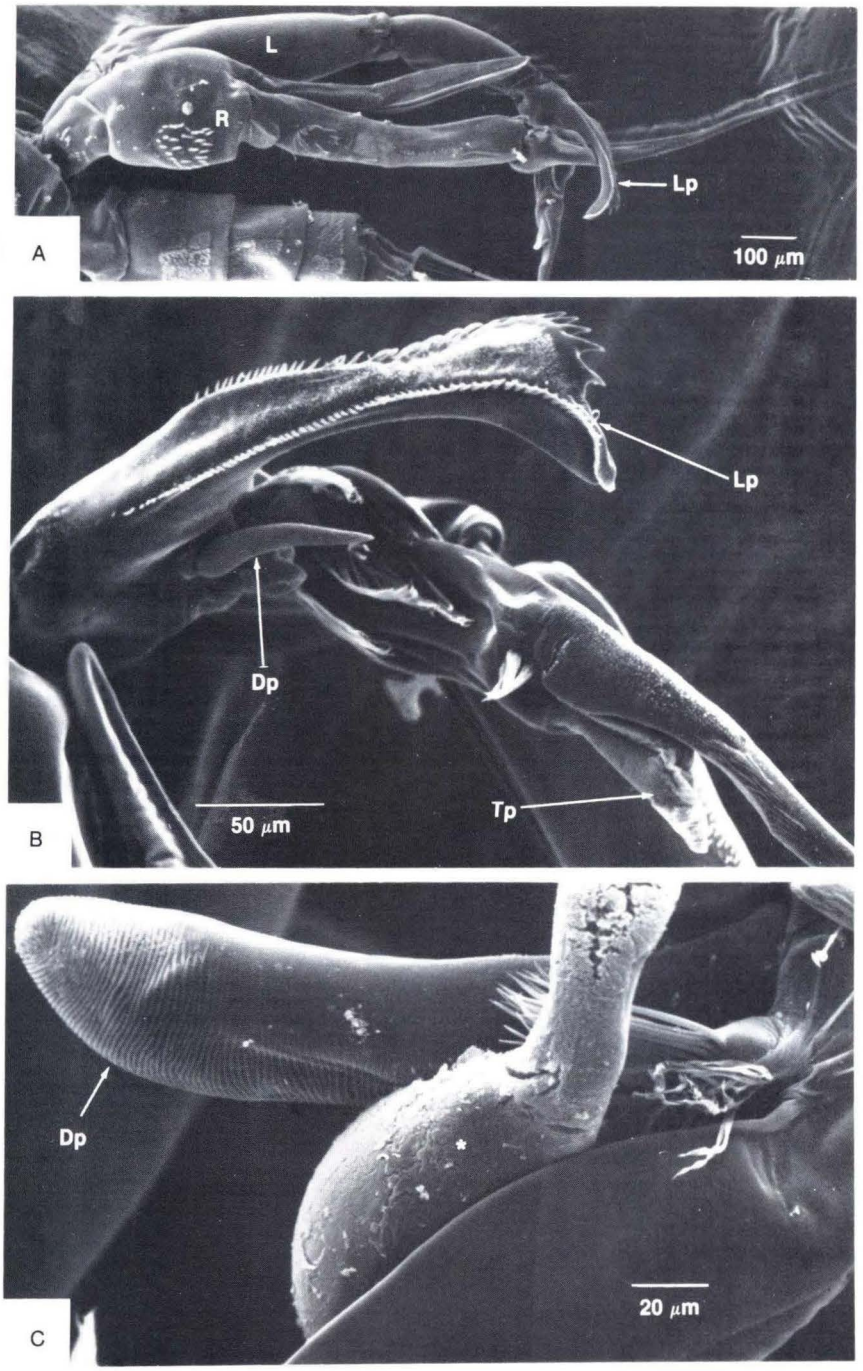
FIGURE 14.2. A. P5 from male *Labidocera aestiva* showing large chela (Ch) of right leg (R). En, endopod; Ex, exopod; L, left leg. B. Closer view of left leg showing details of exopod and corrugated surface on distal tip of endopod (unlabeled arrow). Both figures from Blades and Youngbluth 1979.

sisting of a unisegmented endopod and a double-segmented exopod (fig. 14.2). The distal section of the exopod terminates in a bifurcation of one short and one long spine and has two distinct patches of long setae located along the medial margin (fig. 14.2B). The distal acuminate portion of the endopod has a distinctly corrugated surface (fig. 14.2B). The specific functions of this pair of legs have been described by Blades and Youngbluth (1979, 1980). The chela holds the female's urosome in the copulatory position (Figs. 14.9B–D). The left leg has a dual role: it functions in the tactile examination of the female's genital field and "cleaning" of the spermatophore attachment site by the corrugated endopod, and it transfers the spermatophore that adheres to the exopodal hairs.

In some calanoid genera, the chelate structure of the male's fifth leg is much less pronounced, but this appendage may assume other unusual forms. For example, the P5 on the male *Euchaeta* is characterized by its length (nearly that of the metasome) and the complex morphology of the left leg's distal segments that function to hold the spermatophore (fig. 14.3; Park 1975, 1978; Hopkins, Mauchline & McLusky 1978; Ferrari & Dojiri 1987). Variable morphological features of the left P5 include: (1) a serrated lamelliform process off segment 2 that may be flat and bladelike (*E. marina* [figs. 14.3A, B] or partially toothed and dagger-shaped (*E. norvegica*); (2) a digitiform process off segment 2 of variable size and length that may be reduced and tapered (*E. marina* [fig. 14.3B]; *E. antarctica*), but more often is club-shaped with a corrugated surface (*E. norvegica* [fig. 14.3C]); (3) an assortment of short stiff spines and tufts of thickened setae along the medial margin of segment 3 (figs. 14.3B,C). At the base of the third exopodal segment of the left leg in *E. marina* is a small, thumblike process that appears to be "fleshy" in texture and covered with papillae (fig. 14.3B). This process was also noted in *E. rimana*, *E. marinella*, and *E. indica* by Bradford (1974), who termed it a "thin-skinned lobe." Male *Euchaeta* are frequently observed in plankton collections holding a spermatophore between the digitiform process and the lamelliform structure (fig. 14.3C).

A most unusual P5 is present in the male calanid *Undinula vulgaris* (fig. 14.4). The right leg is greatly reduced, consisting of a tiny 3-segmented endopod and a larger 3-segmented exopod. In contrast, the left leg is extremely long; when it is extended, its length equals nearly that of the metasome (fig. 14.4A). The leg hinges at two locations, enabling the male to carry it folded against the metasome. The last two segments each terminate in long stout spines, and together they form a shape that may be considered clawlike (figs. 14.4A,B). The last segment is very complex, composed of a large knob with a partially corrugated surface (fig. 14.4C). Extending from this knob is another elongate process that appears to be composed of soft, flexible tissue (figs. 14.4B, C, D). The length of this fleshy protuberance is covered with papillae, and its broadened tip is covered with numerous fine setae. A short, pointed digitiform process projects off one

FIGURE 14.3. A. Left (L) and right (R) legs of P5 from male *Euchaeta marina* extending along length of urosome. Lp, lamelliform process. B. *E. marina*, higher magnification, terminal segments of left leg showing serrated lamelliform process (Lp) and digitiform process off segment 2; and thumblike process (Tp) off base of segment 3. C. *E. norvegica*, male P5. Spermatophore (*) held between club-shaped, digitiform process (Dp) and lamelliform process. Note corrugated surface of digitiform process.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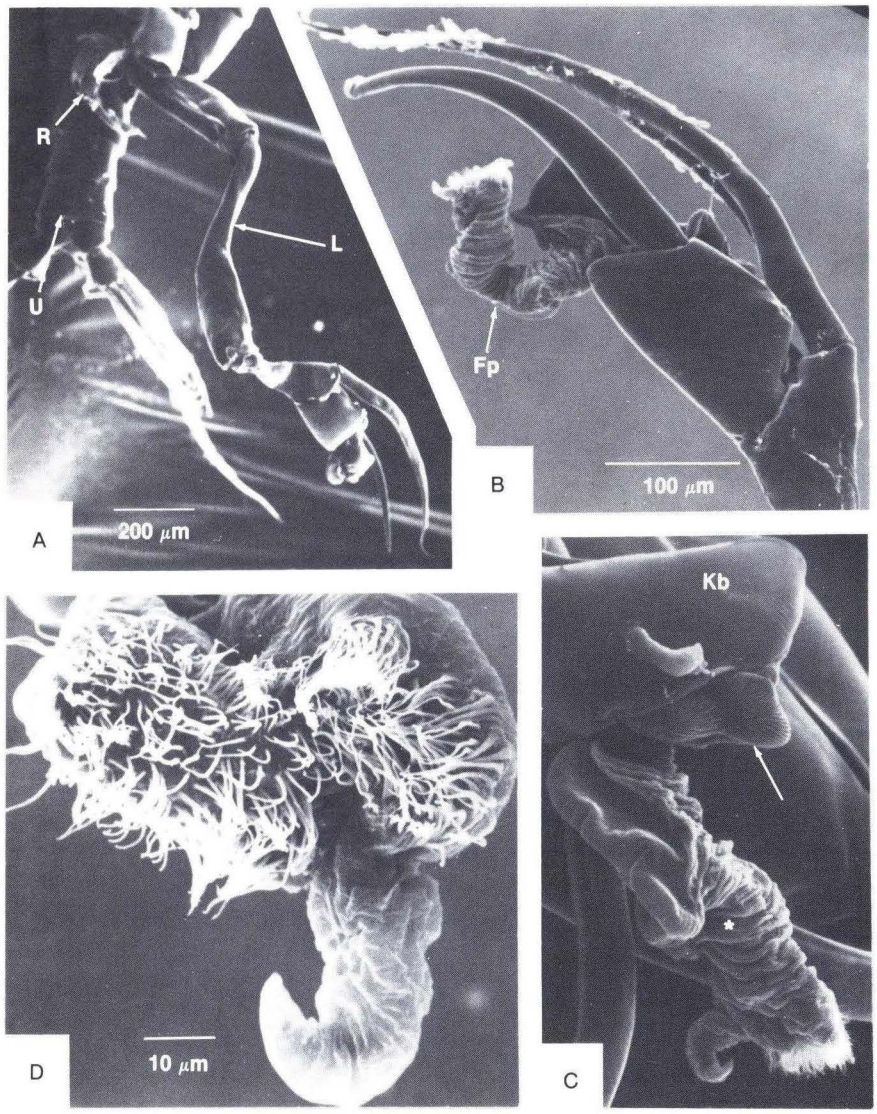


FIGURE 14.4. P5 of *Undinula vulgaris* male. A. Left leg (L) extended. R, right leg; U, urosome. B. Distal segments of left P5 showing clawlike shape and flexible protuberance (Fp). C. Last segment of left P5 showing knob (Kb) with corrugated surface (unlabeled arrow) and flexible protuberance (*). Scale bar = 30 μm. D. Distal tip of flexible protuberance showing hairs and thumblike projection.

edge. The manner in which the male *U. vulgaris* uses this leg during spermatophore transfer remains unknown.

Female Genitalia

In female calanoids, the external and internal morphology of the genital region are notably variable and often used as taxonomic characters (Williams 1972;

Park 1975, 1978). The opening of the genital tract, the genital pore, is positioned on the ventral surface of the first urosomal segment, the genital segment (figs. 14.5, 14.10C). The genital pore in most genera is covered by a flaplike structure of variable morphology, referred to as the genital plate or operculum, that articulates along its anterior margin (figs. 14.5B, 14.10C). The cuticular area surrounding the genital pore, the genital field, may be smooth (figs. 14.5A,D) or adorned to various degrees with cuticular spines, hairs, and glandular pores (fig. 14.10A,C). In some species, (e.g., Euchaetidae) the morphology of the genital field is very complex and species-specific, exhibiting paired lateral "flanges" and an assortment of grooves and cuticular infoldings (fig. 14.5C and Geptner 1968).

Internally, the paired oviducts terminate at separate gonopores that lie just beneath the genital pore. The gonopores typically merge at a common atrium, although in a few species, e.g., *Scolecethrix danae* (fig. 14.5D), the gonopores open to the exterior separately, a condition that supposedly is primitive (G. Boxshall, personal communication). Paired, chitin-lined spermathecal pouches, usually referred to as spermathecae or seminal receptacles, are also present beneath the genital opening on either side of the genital plate. Depending on the species, the spermathecae merge with the oviducts separately or open into the common atrium.

Spermatophores

Spermatophores of calanoid copepods are simple, tube-shaped flasks, sometimes referred to as the spermatophore flask or spermatophore proper (figs. 14.6A,B, 14.7A,B,D, 14.10A, 14.11A, 14.12C), that store the spermatozoa and associated seminal secretions. Toward its open end, the flask narrows into a spermatophore neck of variable length (figs. 14.6A, 14.7A,D). In the majority of calanoids, the spermatophore adheres to the female by a cementlike secretion present on the outside of the spermatophore neck or by secretions extruded from the spermatophore itself (figs. 14.6A, 14.10A,B, 14.12C). However, in some calanoids, most notably members of the Pontellidae and Centropagidae, the spermatophore flask is connected to one or more chitinlike plates (figs. 14.6B,C, 14.7A,B,D, 14.11A). These plates, termed coupling plates, the coupling apparatus, or coupler (after Koppler, Heberer 1932), carry an adhesive secretion that, in combination with their unique shape, secures the spermatophore to the urosome of the female. The morphology of the coupling plates, therefore, is unique in each species and corresponds to the external morphology of the conspecific female's urosome and genital region. This has been called a "key-and-lock" relationship (Fleminger 1967; Lee 1972), implying that the coupler assures attachment of the spermatophore at a precise location on a conspecific female (figs. 14.6B,C, 14.7A,B). The configuration of the coupling apparatus within a single genus may be highly variable, as reported by Fleminger (1967, 1975, 1979) for the pontellid *Labidocera*. For example, the plates of the coupler form simple ventral shields in *L. aestiva* (figs. 14.6B, 14.7D), whereas they encapsulate the urosome totally in *L. scotti* (fig. 14.6C). Even more elaborate examples were reported for *L. barbadiensis* (Fleminger & Moore 1977) and *L. barbudae* (Fleminger 1979), in which the spermatophores are connected to several coupling plates arranged in a complex

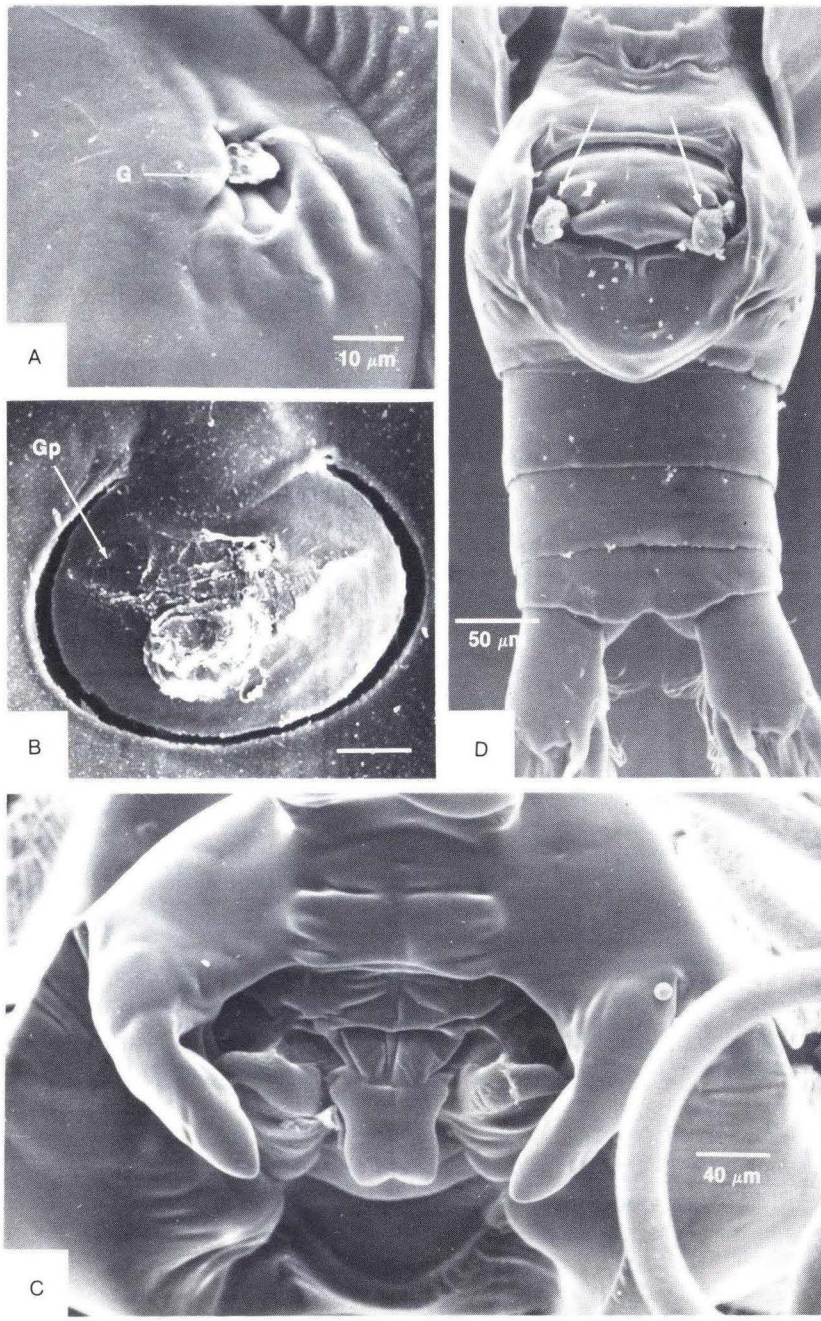


FIGURE 14.5. Morphological diversity of female genital pores (G). A. *Pleuromamma gracilis*. B. *P. abdominalis*. Gp, genital plate. Scale bar = 20 μm. C. *Euchaeta antarctica*. D. *Scolecethrix danae*. Unlabeled arrows indicate two separate genital openings, both with remnants of spermatophore contents.

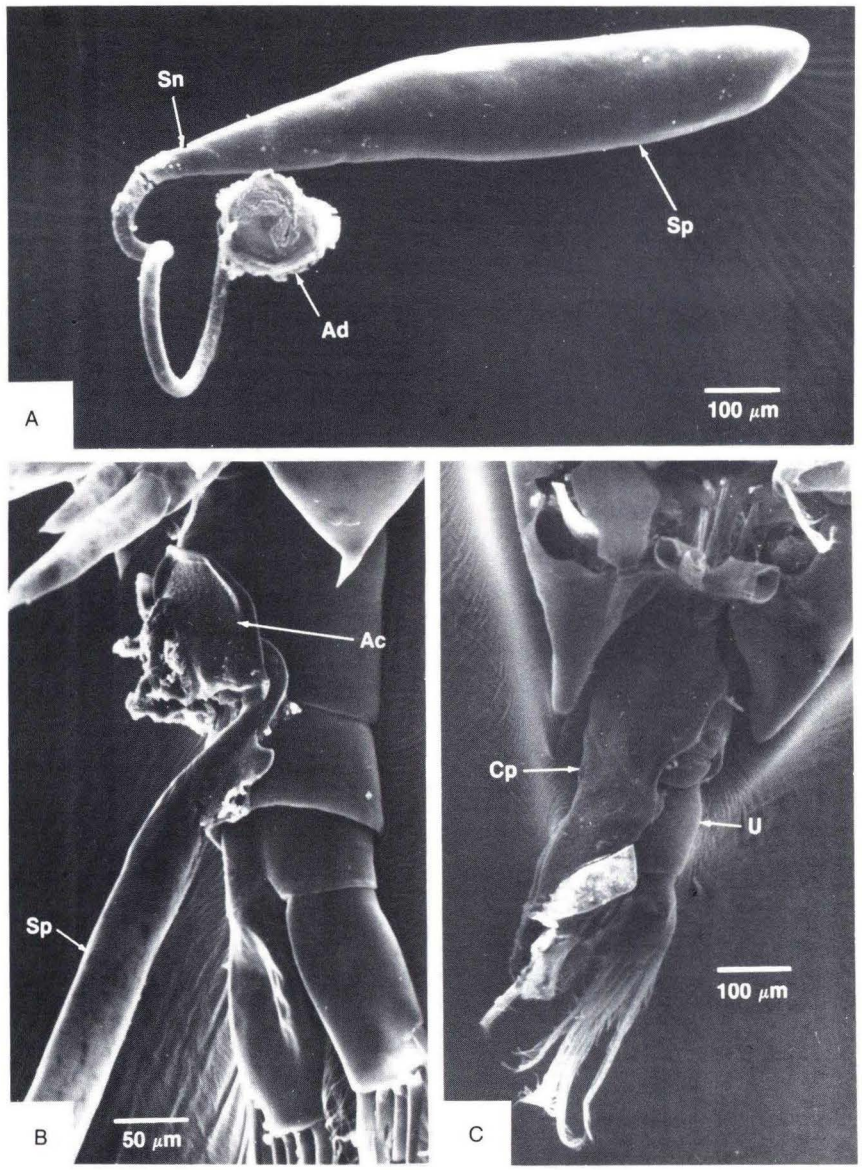
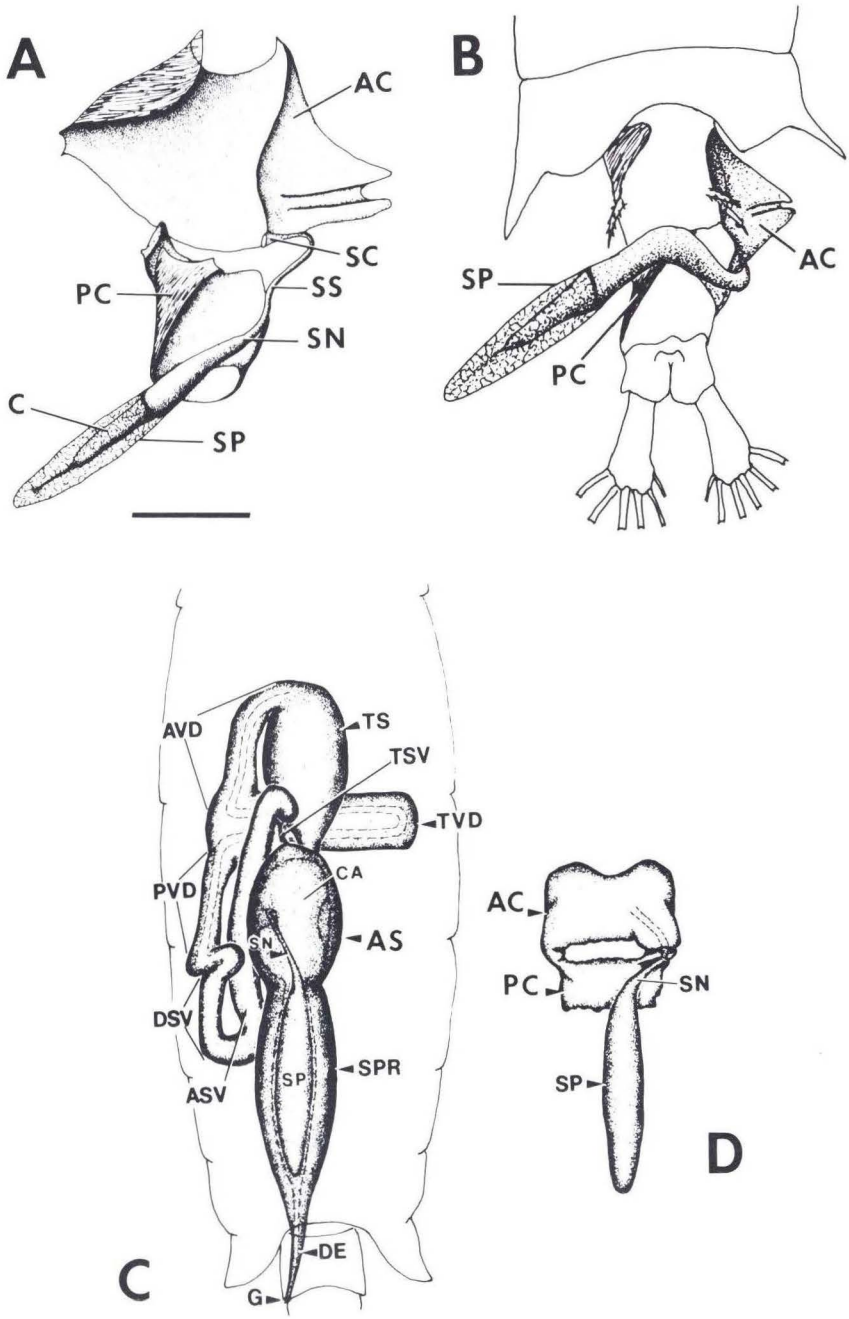


FIGURE 14.6. Spermatophore morphology. A. Simple spermatophore from *Euchaeta norvegica*. Ad, attachment disc; Sn, spermatophore neck; Sp, spermatophore proper. B. *Labidocera aestiva* female, urosome with spermatophore attached to ventral surface; simple coupler showing anterior coupling plate (Ac) covering genital pore. Sp, spermatophore proper. C. *L. scotti* female urosome (U) with spermatophore attached. Note encapsulating coupler (Cp).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manner around the urosome, with the dorsal plate sending a long extension over the dorsal surface of the cephalothorax.

Male Reproductive System and Spermatophore Formation

The spermatophore is produced entirely within the male reproductive system. The morphology of the male genital system and the process of spermatophore formation have been studied previously for a few calanoid copepods using light microscopy (Heberer 1932; Park 1966). Ultrastructural studies on copepods that produce simple spermatophores are available for *Calanus finmarchicus* (Raymont et al. 1974) and *Euchaeta norvegica* (Hopkins 1978). Ultrastructural details of the more complex system in *Labidocera aestiva*, which produces a spermatophore with coupling plates, are found in Blades and Youngbluth (1981) (fig. 14.7C,D).

The reproductive system of male calanoids contains organs comparable in function to the male accessory glands of other invertebrates (see Adiyodi & Adiyodi 1975 for review) where the secretions produced by the glands contribute to the internal and external components of the spermatophore. The reproductive tract of male calanoids consists of a single testis and a long, sinuous genital duct that terminates at a gonopore on the first urosomal segment (fig. 14.7C). The genital duct is morphologically divided into the vas deferens (ductus deferens), seminal vesicle, spermatophore sac, and chitin-lined ductus ejaculatorius. As immature spermatozoa pass from the testis through these regions, the various components of the seminal fluid, spermatophore wall, and coupling apparatus (if present) are produced and secreted into the lumen.

In general, the first part of the vas deferens produces secretions that compose the seminal fluid, often referred to as the core secretion, that surrounds the spermatozoa inside the spermatophore. These secretions take on the form of flocculent material and granules of varying size and density. The last part of the vas deferens continues to produce seminal secretions as well as material that forms the surrounding spermatophore wall. The seminal vesicle is much less glandular yet may produce additional material for the spermatophore wall.

The large, elongate, and highly glandular spermatophore sac is divided into two morphologically distinct but contiguous regions, the anterior spermatophore sac (="former" or "molder" of Heberer 1932) and the posterior sac proper

FIGURE 14.7. A. Spermatophore complex of *Centropages typicus*. Scale bar = 100 μm. B. Diagramatic illustration, dorsal view of properly positioned spermatophore on urosome of *C. typicus* female (not drawn to scale). C. Diagrammatic illustrations of male reproductive system, dorsal view. D. Spermatophore complex of *Labidocera aestiva*. AC, anterior coupling plate; ASV, ascending seminal vesicle; AVD, anterior vas deferens; C, core secretion; CA, coupling apparatus (inside lumen of anterior spermatophore sac [AS]). DE, ductus ejaculatorius; DSV, descending seminal vesicle; G, gonopore; PC, posterior coupling plate; PVD, posterior vas deferens; SC, spermatophore cup; SN, spermatophore neck; SP, spermatophore proper; SPR, spermatophore sac proper; SS, spermatophore stalk; TVD, transverse vas deferens; TS, testis; TSV, terminal part of seminal vesicle. Figures 14.7A and 14.7B from Blades 1977, 14.7C and 14.7D from Blades-Eckelbarger 1986.

(fig. 14.7c). Depending on the species, simple adhesive secretions or more complex plates of a coupling apparatus are produced within the anterior spermatophore sac and connected to the neck region of a mature spermatophore flask that lies in the lumen of the sac proper. The size and cellular composition of the anterior spermatophore sac apparently are related to the final configuration and complexity of the coupling apparatus, if present. For example, *Euchaeta norvegica* produces a spermatophore without coupling plates (Hopkins 1978). Consequently, the anterior spermatophore sac is represented by a simple gland that secretes an adhesive substance onto the spermatophore neck. The next level of complexity is observed in *Undinula vulgaris* (Blades-Eckelbarger personal observation), in which the anterior spermatophore sac is composed of 4 to 6 morphologically distinct secretion types. Favorable sections through this region (fig. 14.8A) reveal that once released into the lumen, these secretions form a large, indistinct mass that adheres to the spermatophore neck (fig. 14.12c).

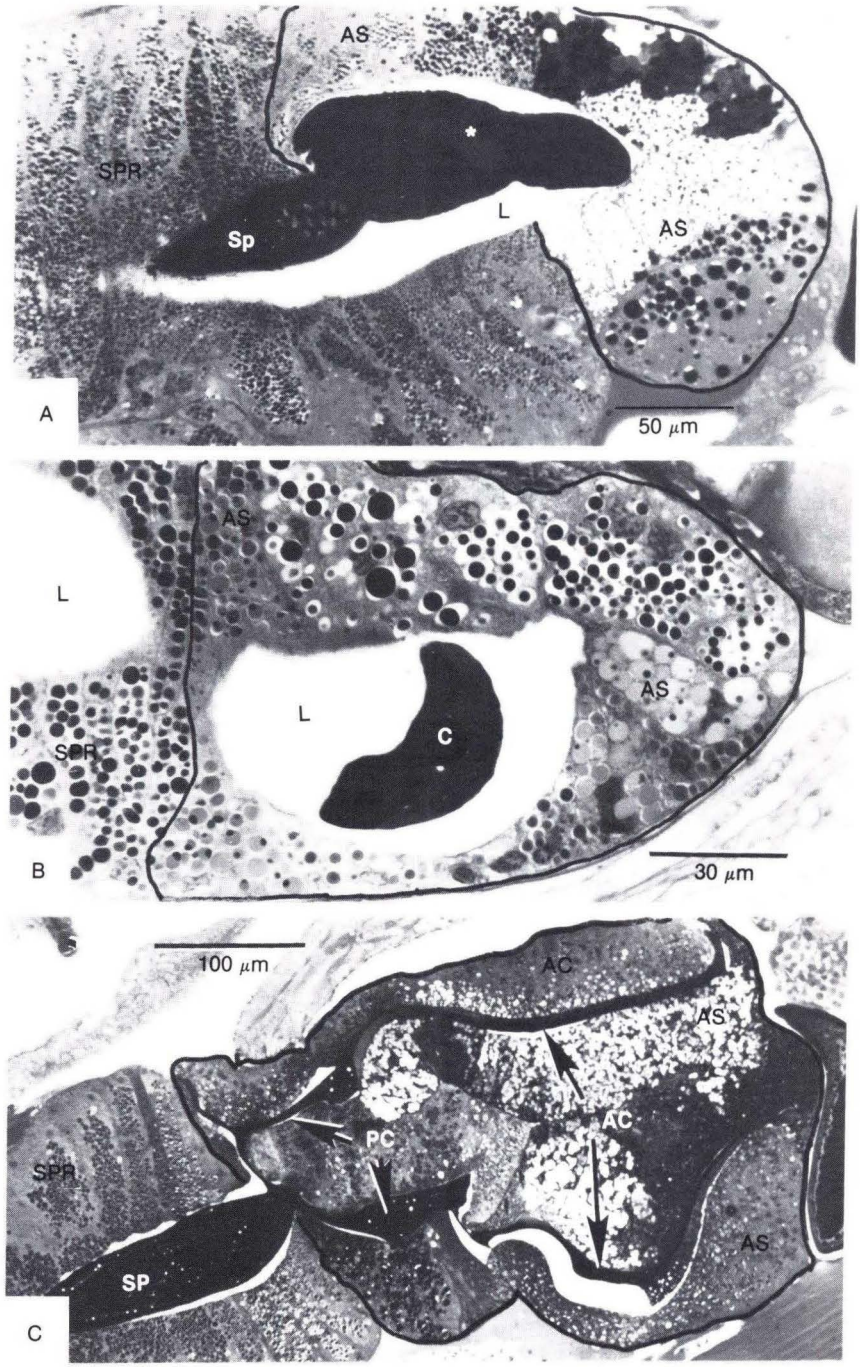
With respect to calanoids that form spermatophores associated with couplers, *Pleuromamma abdominalis* produces a small cup-shaped coupler within a much reduced anterior spermatophore sac composed of 4 to 6 different types of secretion (fig. 14.8B; Heberer 1932; Blades-Eckelbarger, personal observation). In contrast, this glandular region in the pontellids *Epilabidocera amphitrites* (Park 1966) and *Labidocera aestiva* (Blades & Youngbluth 1981) produces 7 to 8 different secretion types that are molded by the shape of the lumen into the highly complex plates of the coupling apparatus (figs. 14.6B, 14.7D, 14.8c).

MATING BEHAVIOR

Reports of copulatory behavior in calanoid copepods as observed on living specimens (Gauld 1957; Jacobs 1961; Katona 1975; Blades 1977; Blades & Youngbluth 1979; Jacoby & Youngbluth 1983), in addition to other descriptions of reproductive morphology and spermatophore attachment sites (see Vaupel Klein 1982 for review), provide convincing evidence that the mating behavior of calanoid copepods is precise, ritualized, and species-specific. In general, the mating behavior of calanoids appears to follow the same sequence of events: (1) attraction of the male to the female, (2) capture of the female by the male, (3) movement into the copulatory position, (4) spermatophore transfer and attachment, (5) release of the female, and (6) discharge of the spermatophoric contents.

Upon receiving chemical stimulation, probably by perception of pheromones emitted by the female (Katona 1973; Griffiths & Frost 1976), the male performs a distinctively different swimming behavior often referred to as "searching

FIGURE 14.8. Cellular diversity of the anterior spermatophore sac (AS) outlined region, (light micrographs, 1 μm thick sections). A. *Undinula vulgaris*, sagittal section. Note adhesive mass (*) in lumen connected to partial section of spermatophore proper (Sp). B. *Pleuromamma abdominalis*, sagittal section. Note cup-shaped coupler (C) in lumen (L). L', lumen of spermatophore sac proper (SPR). C. *Labidocera aestiva*, frontal section. Note shape of lumen that "molds" anterior coupling plate (AC) and posterior coupling plate (PC).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movements" or "mate-seeking behavior" (Parker 1902; Jacobs 1961; Katona 1973, 1975; Jacoby & Youngbluth 1983). The male's erratic swimming movements bring him in close proximity to a potential mate, where he may receive further chemical or mechanical cues from the swimming wake of the female (Strickler & Bal 1973). The male uses the geniculate antennule to grasp the female by her caudal rami or caudal setae (fig. 14.9A). During this initial physical contact, specific hairs on the male's antennule (fig. 14.1B) may be stimulated when it closes, thereby signaling the male to move into the copulatory position (Blades & Youngbluth 1980).

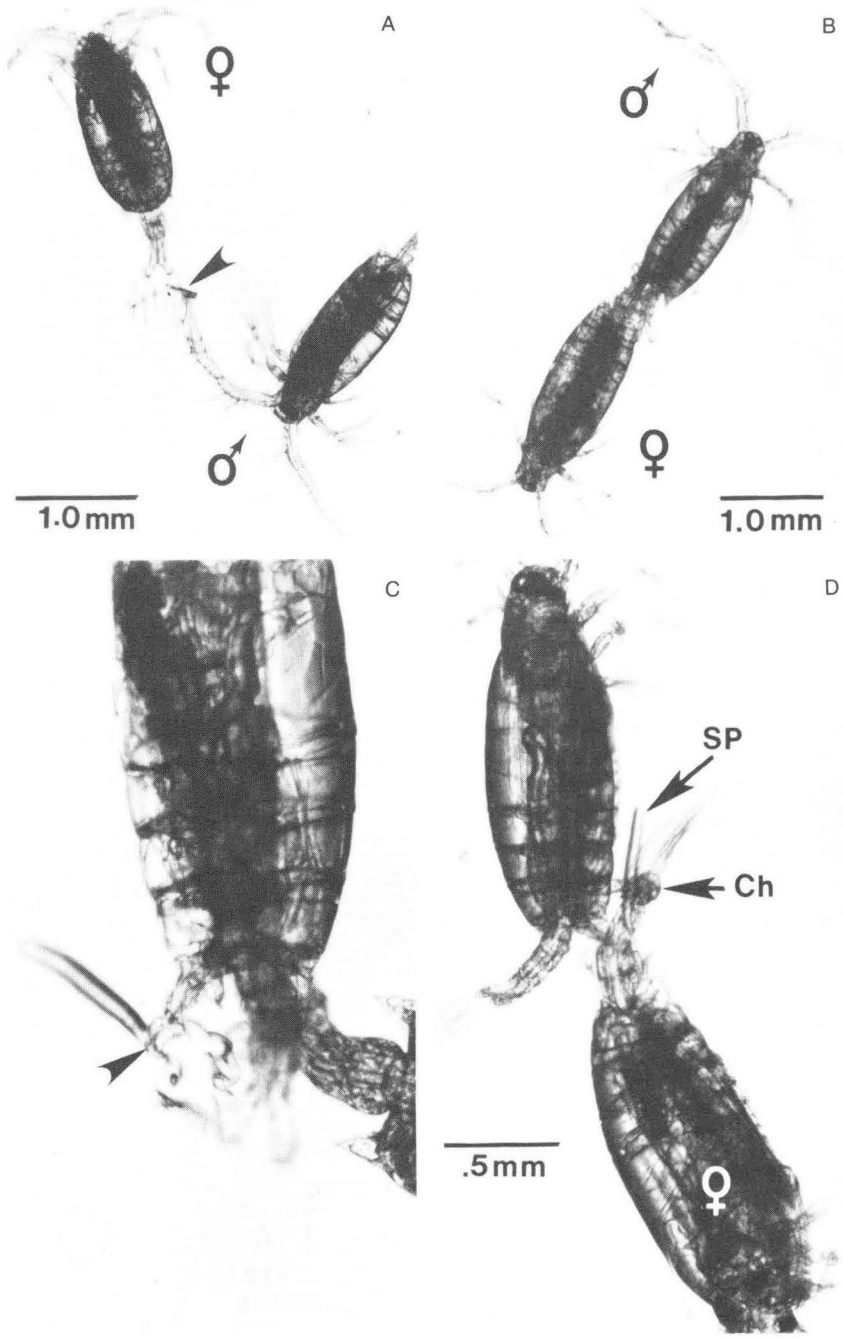
In the copulatory position (fig. 14.9B), the male secures the female with the chelate part of his fifth leg and releases the antennular hold. Now the mating pair lie in the same plane with heads facing in opposite directions. The male extrudes a spermatophore (fig. 14.9C) and, using the other part of his P5, attaches it to the female (fig. 14.9D). The male then releases the female, and the spermatophore discharges. The above is a generalized description of the mating behavior in calanoids using *Labidocera aestiva* as an example (Blades & Youngbluth 1979). Reported variations in this behavioral sequence involve (1) timing between and within phases, (2) additional tactile inspection of the female by the male and, (3) the moment of spermatophore extrusion relative to attachment (see "Importance of Reproductive Morphology in Copepod Speciation").

SPERMATOPHORE ATTACHMENT AND DISCHARGE

The development of spermatophores in calanoid copepods provides an efficient mechanism by which the aflagellate and immobile spermatozoa can be transferred to the female. The spermatophore is attached to the external surface of the female but may or may not be in direct contact with the genital pore. In the latter case, the sperm must exit the spermatophore and be transported over the cuticle of the female to the spermathecal sacs.

In those calanoid species in which the spermatophore is attached to the genital opening, referred to as direct placement (Hopkins & Machin 1977, Ferrari 1978), the spermatophoric contents empty almost completely into the spermathecal sacs. Direct placement of a simple spermatophore is illustrated here in *Acartia tonsa* (fig. 14.10A), (Hammer 1978; Blades-Eckelbarger personal observation). The attachment sites of spermatophores with coupling plates (figs. 14.6B,C, 14.7A,B, 14.10B) may also be considered as direct placements because the configuration of the coupling apparatus conforms to the morphology of the female's urosome and, when attached properly, brings the open end of the spermatophore flask in close proximity to the genital pore (Heberer 1932; Fleminger & Tan

FIGURE 14.9. Mating behavior of *Labidocera aestiva*. A. Initial capture of female by male. Arrow indicates grasp with geniculate antennule. B. Copulatory position, ventral view. C. Spermatophore extrusion, dorsal view of male showing left leg exopod holding coupling plate (arrow) of fully extruded spermatophore. D. Spermatophore attachment. Ch, chelate P5 of male holding female's urosome; SP, spermatophore proper. From Blades & Youngbluth 1979.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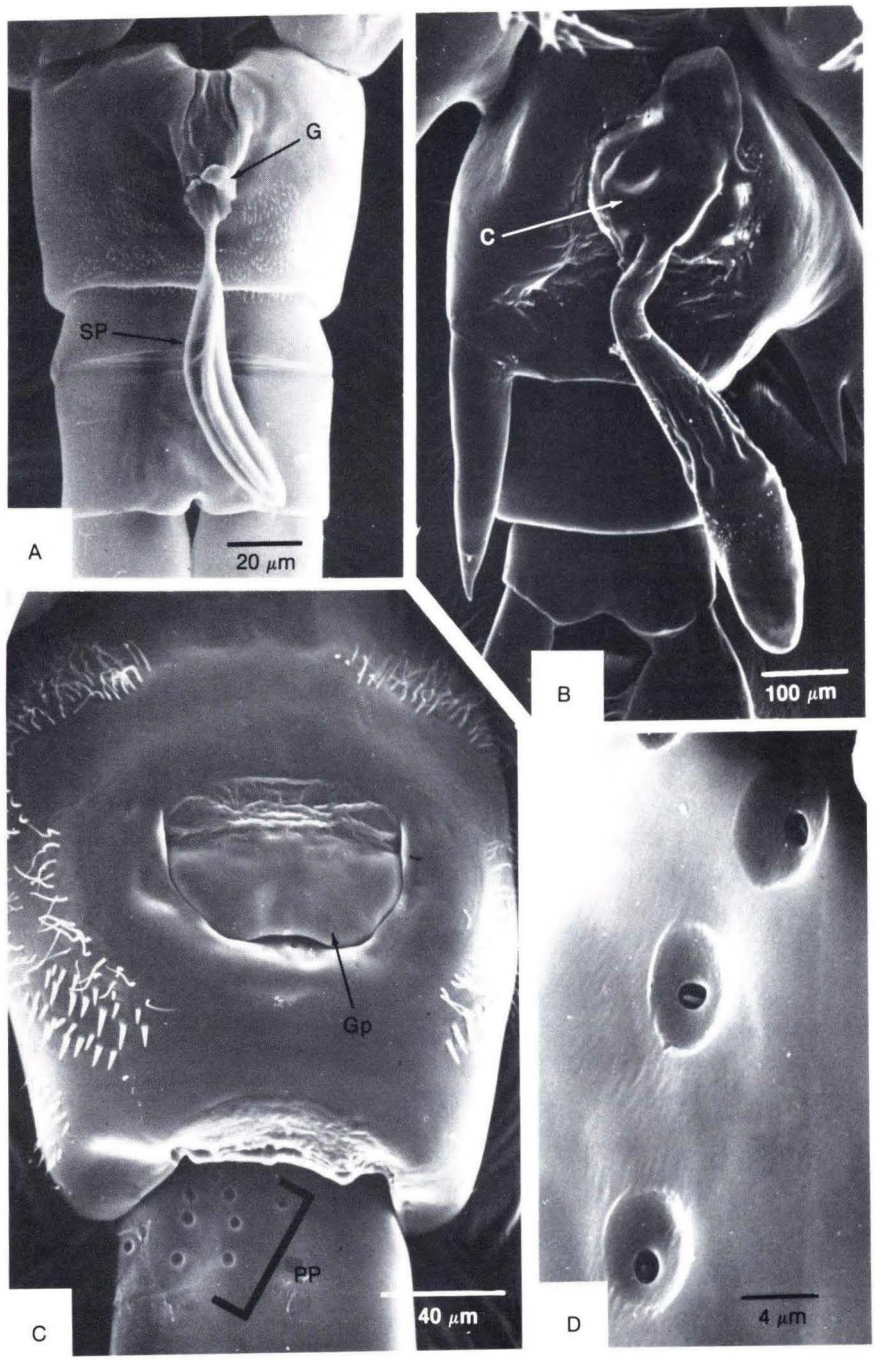
1966; Fleminger 1967, 1975; Lee 1972; Fleminger & Moore 1977; Blades 1977; Blades & Youngbluth 1979). In *Labidocera aestiva* the spermatophore attachment site corresponds to a distinct field of cuticular pores or "pit-pores" that are present on the second urosomal segment of the female (figs. 14.10C,D). TEM examination revealed that these pores connect to secretory cells that may produce a substance that dissolves the adhesive holding the spermatophore to the female (Blades & Youngbluth 1979).

In *Euchaeta norvegica*, which possesses a simple spermatophore without coupling plates, two common sites of attachment, direct and nondirect, have been described (Hopkins & Machin 1977; Ferrari 1978). Ferrari and Dojiri (1987) reported also that both "correct" and "alternate" attachment sites are common in *E. antarctica*. Furthermore, these authors found two morphologically distinct spermatophores attached to the two different areas on the genital segment of the females. They suggested that the males may be able to purposefully produce either type of spermatophore depending upon where it will be attached.

Hopkins and Machin (1977) observed with *Euchaeta norvegica* that when the spermatophore was attached directly over the female genital cavity the contents of the spermatophore emptied into the spermathecae. However, if the attachment site was a distance from the genital area, the initial contents of the spermatophore flowed onto the surrounding cuticle to form a circular mass of cementlike material, which they referred to as the attachment disc (fig. 14.6A). In approximately 30 percent of the nondirect spermatophores observed on *E. norvegica* a narrow "fertilization tube" (fig. 14.2C–F) extended from the attachment disc to the female's genital opening. Transport of the sperm to the female's genital opening, therefore, appears to be accomplished by the physical properties of the seminal secretions within the spermatophore. The granules and flocculent material that compose the core secretion solidify on contact with seawater and form a tube (fig. 14.12F) through which the liquid interior carrying the sperm continues to pass en route to the female's gonopore (Blades 1977; Hopkins & Machin 1977; Hopkins 1978).

An example of extensive fertilization tube formation is observed in *Undinula vulgaris*, where the simple spermatophore is attached to the right dorsolateral surface of the last thoracic segment (fig. 14.12C). A fertilization tube extends from the adhesive mass and traverses a distinct field of cuticular pores (figs. 14.12A–D) to what appears to be an attachment disc. From the attachment disc, the tube passes between the junction of the metasome and urosome to ultimately connect with the genital opening. TEM observations of the area beneath the pore field revealed an extensive glandular tissue (Blades-Eckelbarger personal observation). The function of the secretory product contained within the associated cells remains unknown.

FIGURE 14.10. A. Simple spermatophore (SP) attached directly to genital pore (G) of *Acartia tonsa* female. B. Spermatophore with simple coupling plate (C) covering genital opening of *Anomalocera ornata* female. C. Genital segment and second urosomal segment of female *Labidocera aestiva* showing genital plate (Gp) and field of pit-pores (PP). Note hairs and spines over genital field. D. Higher magnification of pit-pores. Figures 14.10C and 14.10D from Blades & Youngbluth 1979.


G
SP
A
20 μm
C
B
100 μm
Gp
PP
C
40 μm
D
4 μm

Discharge of the spermatophoric contents is accomplished by means of mechanical or hydrostatic pressure resulting from water uptake and consequent swelling of modified cells located immediately within the wall of the spermatophore flask (fig. 14.11). These cells were originally called swelling spermatozoa ("Quellspermatozoen" or "Q-sperm") by Heberer (1932), later referred to as foamlike bodies in *Euchaeta norvegica* (Hopkins 1978), and more recently identified as modified spermatozoa in *Labidocera aestiva* (Blades & Youngbluth 1981). In *L. aestiva,* these modified cells are present in the distal half of the spermatophore (fig. 14.11A), whereas in *Undinula vulgaris* they extend throughout the length of the spermatophore (fig. 14.11B).

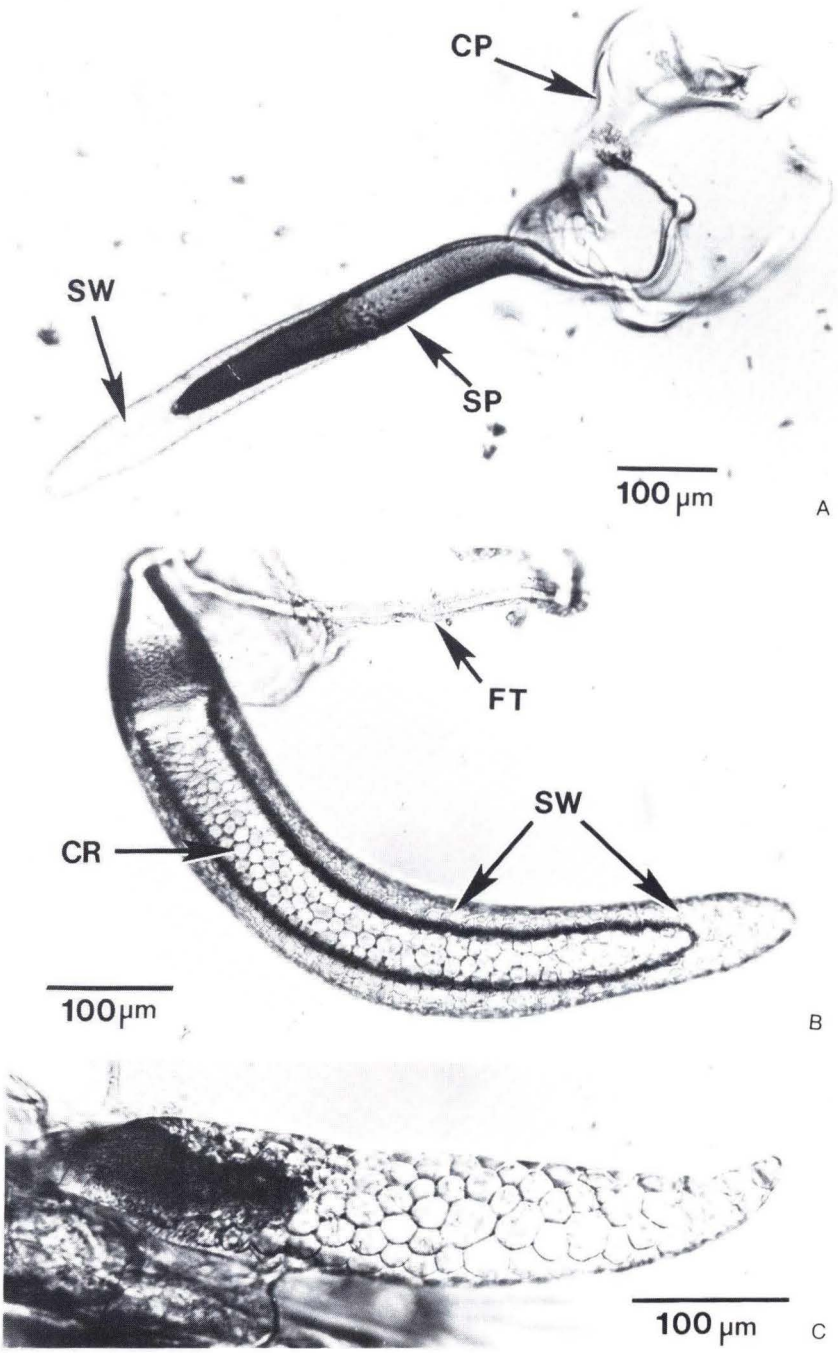
The mechanism by which the fertilization tube flows in the correct direction to the gonopore is still unclear. Chemical attraction has been discounted because the spermatophore and its secretions are of acellular nature and incapable of "sensing" chemical gradients (Vaupel Klein 1982). The present observations of *Undinula vulgaris,* along with those on *Euchaeta norvegica* (Hopkins & Machin 1977, Ferrari 1978), indicate that these tubes follow a distinct trail to the female genital area. Ferrari (1978) and Vaupel Klein (1982) suggested that general body shape in combination with mechanical stimuli from the configuration and cuticular structures on the female genital region would control the path of growth of the tube while it was forming.

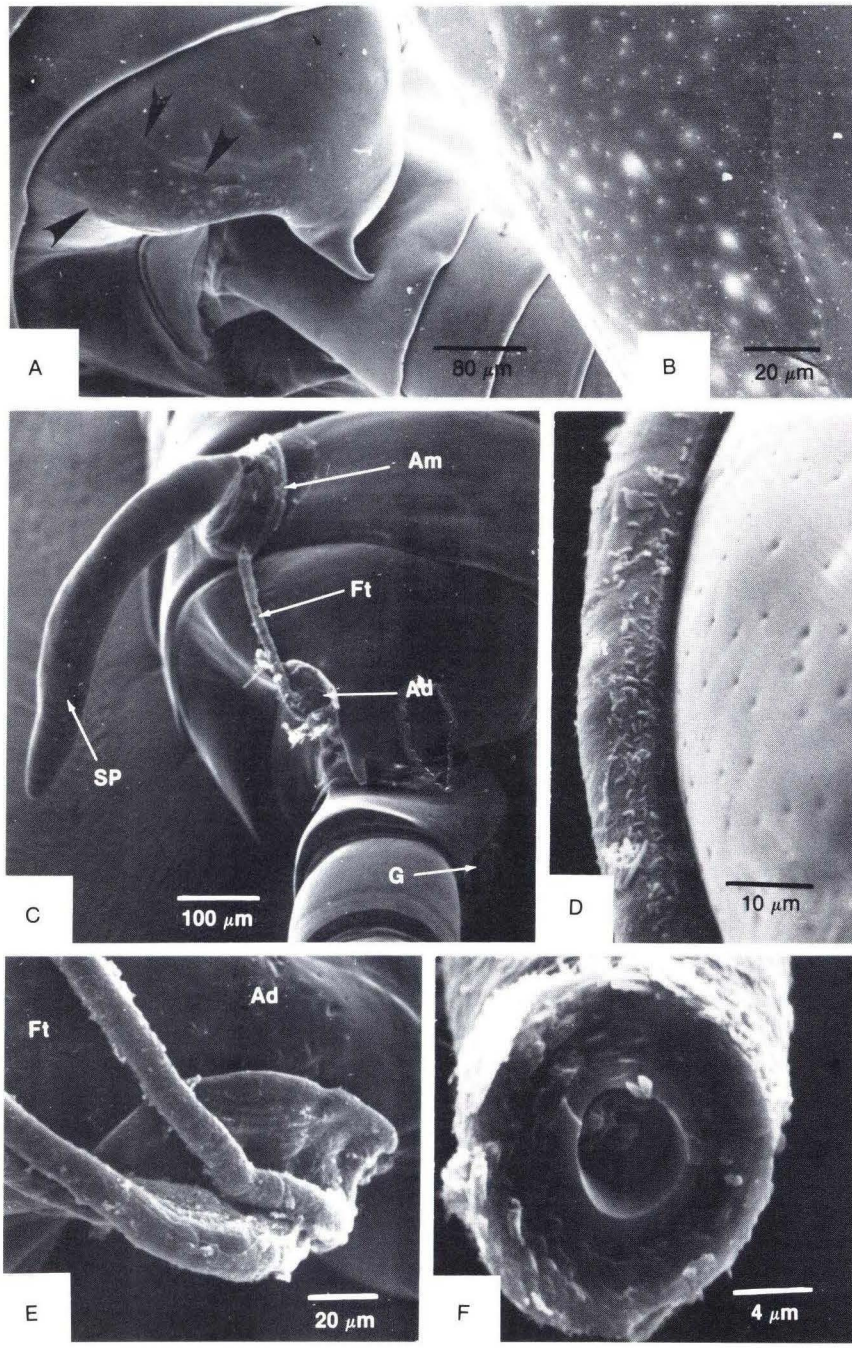
The quantity of core secretion is notably variable among genera and may correspond to the distance traveled by the fertilization tube to the female's genital pore. Favorable sections through the seminal vesicle or spermatophore proper of a male calanoid can provide a simple means by which relative quantities of core secretion can be compared. This type of morphological information may be used to determine direct or nondirect attachment sites for species on which spermatophores are not easily observed. For example, the spermatophores of *Undinula vulgaris* (Blades-Eckelbarger personal observation) and *Euchaeta norvegica* (Hopkins 1978) contain large quantities of seminal secretions that can form long fertilization tubes. In contrast, the spermatophore of *Pleuromamma abdominalis* contains very little core secretion and most of its volume is filled with spermatozoa (Heberer 1932, 1937; Blades-Eckelbarger personal observation). Although the site of spermatophore attachment has not been reported for *Pleuromamma,* the lack of abundant core secretion suggests that the spermatophore is attached directly to the female's genital opening.

IMPORTANCE OF REPRODUCTIVE MORPHOLOGY IN COPEPOD SPECIATION

Interspecific reproductive isolation in calanoid copepods is considered to be the result of a combination of the following factors: (1) differences in mate recognition due to species-specific pheromones (chemical), (2) morphological diversity

FIGURE 14.11. Spermatophore discharge. A. Partially discharged spermatophore of *Labidocera aestiva* showing coupling apparatus (CP), spermatophore proper (SP), and swelling cells (SW). B. Partially discharged spermatophore of *Undinula vulgaris.* Note swelling cells (SW) extend length of spermatophore proper. CR, core secretion; FT, fertilization tube. C. Fully discharged spermatophore of *Centropages furcatus.*


CP
SW
SP
100 µm
A
FT
SW
CR
100 µm
B
100 µm
C


A
80 μm
B
20 μm
Am
Ft
Ad
SP
G
C
100 μm
D
10 μm
Ad
Ft
E
20 μm
F
4 μm

in the primary and secondary sexual structures (mechanical) and (3) deviations in mating behavioral patterns (Fleminger 1967, 1975; Frost & Fleminger 1968; Fleminger & Hulsemann 1974; Vaupel Klein 1982; Jacoby & Youngbluth 1983). These barriers to hybridization may be expressed at varying stages of the mating encounter depending on the species and degree of geographical overlap between sympatric species. The present review defines premating barriers in calanoids as those that are expressed prior to spermatophore extrusion, whereas postcopulation or prezygotic barriers are those that occur after spermatophore extrusion.

Chemical premating barriers have been recognized in some calanoids during the initial attraction or search phase, where the male acts upon pheromonal signals from the female (Parker 1902; Jacobs 1961; Katona 1973; Griffiths & Frost 1976; Jacoby & Youngbluth 1983). Jacoby and Youngbluth (1983) found chemical cues to be specific among three species of *Pseudodiaptomus*. When attempting to cross heterospecific pairs, the authors noted that males performed fewer searches and fewer copulations.

Species-specific morphological differences, which can act at either the premating or prezygotic level, are most obvious with respect to the male fifth leg, female genital area, and spermatophores. A critical step in the mating encounter involves the exact coupling of the male's P5 with the female's urosome, which is controlled by the morphology of these parts (see review by Vaupel Klein 1982). The male's grip on the female must be firm, and it must be positioned precisely to enable the male to place the spermatophore correctly. Such precision has been observed in *Centropages typicus* (Blades 1977), *Labidocera aestiva* (Blades & Youngbluth 1979), and *Pseudodiaptomus* spp. (Jacoby & Youngbluth 1983). The male may receive mechanical signals through sensory hairs or spines on the clasping part of the P5 (Blades & Youngbluth 1980), confirming that he has captured a conspecific female and that his fifth leg is grasping the right area. Furthermore, tactile inspection of the female genital region by the male has been described for *L. aestiva* in which the male performs a deliberate "stroking behavior" with the left fifth leg over the spermatophore attachment site prior to extrusion of the spermatophore. Such behavior may inform the male of an existing spermatophore on the site and may prepare the site for attachment of his spermatophore (Blades & Youngbluth 1979).

Literature on calanoids that produce spermatophores with complex coupling plates, i.e., members of the Pontellidae (Fleminger 1967, 1975; Blades & Youngbluth 1979) and *Centropages* spp. (Lee 1972; Blades 1977), strongly indicates that a species-specific "key-and-lock" mechanism is formed by the morphology of the

FIGURE 14.12. A–E. Spermatophore attachment site on *Undinula vulgaris*. A. Site corresponds to distinct field of pores (within arrowheads) on right dorsolateral surface of last thoracic segment. B. Higher magnification of pores. C. spermatophore attached to this site. Note fertilization tube (Ft) traversing pore field to attachment disc (Ad). G, genital opening of female. D. Higher magnification of fertilization tube crossing pore field. E. Fertilization tubes from three spermatophores joining at one attachment disc. F. Transverse break in fertilization tube from *Euchaeta norvegica* spermatophore showing solidified flocculent and granular components of seminal secretion surrounding central canal through which liquid core secretion and sperm flow.

coupling plates, the conspecific female's genital configuration, and the specific movements of the male while holding and orienting to the female. Due to the structural specificity of the coupler, the spermatophore of one species will not accurately "fit" the urosome of a heterospecific female. Futhermore, the degree of divergence of these morphological characters appears to be closely related to the extent of geographical overlap (Fleminger 1967, 1975). Therefore, hybridizing barriers in species with elaborate coupling plates may be more effective during the copulation stage rather than the premating search and capture stage (Vaupel Klein 1982).

The "key-and-lock" mechanism is not applicable to those species that produce simple spermatophores lacking couplers. Some studies suggest that males of these species are capable of quickly and accurately attaching the simple spermatophore directly onto the genital opening of the female (Gauld 1957; Katona 1975; Hammer 1978; Jacoby & Youngbluth 1983). Indications are that these species have become more efficient in mate recognition and/or the actual act of placement of the spermatophore, a result perhaps of highly evolved pheromonal or mechanical cues. Additional observations of other calanoids with simple spermatophores are needed to determine the dominant character or characters that may function as a block to hybridization.

Less obvious morphological differences may be found with respect to body proportion and total body size. These characters also have been cited as playing a role in reproductive isolation (Fleminger 1967; Lawson 1977). In addition, species-specific patterns of cuticular setae and pores may provide important chemical and mechanical cues in those calanoid species that have not developed highly modified secondary sexual characters (Fleminger 1975; Fleminger & Hulsemann 1974; Blades & Youngbluth 1979; Fleminger 1986).

Behavioral reproductive barriers may be expressed in combination with chemical and mechanical cues during one or more phases of the mating encounter: (1) timing between and within phases, as observed for three species of *Pseudodiaptomus* by Jacoby and Youngbluth (1983), (2) additional tactile inspection of the female by the male, as noted in the pontellid *Labidocera aestiva* by Blades and Youngbluth (1979), and (3) the moment of spermatophore extrusion relative to attachment. For example, in *L. aestiva* and three species of *Pseudodiaptomus* (Blades & Youngbluth 1979; Jacoby & Youngbluth 1983), the male extrudes the spermatophore only after attaining a firm grasp with the modified P5. In *Centropages typicus* the male extrudes the spermatophore prior to this, while holding the female with his antennule (Blades 1977), whereas in *Eurytemora affinis* and *Euchaeta* spp. the male swims about with a spermatophore gripped in the P5 before making physical contact with a female (Katona 1975; Hopkins, Mauchline & McLusky 1978; Ferrari & Dojiri 1987; Blades-Eckelbarger, personal observation). Once the male extrudes a spermatophore from the spermatophore sac, there remains the chance that he may drop it or fail to attach it correctly to a conspecific female and thus risk wasting sperm. Therefore, premating barriers functioning prior to spermatophore extrusion are considered to be the most efficient (Mayr 1963) serving to conserve energy and gametes.

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Literature Cited

Adiyodi, K. G. & R. G. Adiyodi. 1975. Morphology and cytology of the accessory sex glands in invertebrates. In G. H. Bourne & J. F. Danielli, eds., *International Review of Cytology* 43:353–399. New York: Academic Press.

Blades, P. I. 1977. Mating behavior of *Centropages typicus* (Copepoda: Calanoida). *Mar. Biol. (Berl.)* 40:47–64.

Blades, P. I. & M. J. Youngbluth. 1979. Mating behavior of *Labidocera aestiva* (Copepoda: Calanoida). *Mar. Biol. (Berl.)* 51:339–355.

Blades, P. I. & M. J. Youngbluth. 1980. Morphological, physiological, and behavioral aspects of mating in calanoid copepods. In W. C. Kerfoot, ed., *Evolution and Ecology of Zooplankton Communities*, pp. 39–51. Hanover, New Hampshire: University Press of New England.

Blades, P. I. & M. J. Youngbluth. 1981. Ultrastructure of the male reproductive system and spermatophore formation in *Labidocera aestiva* (Crust.: Copepoda). *Zoomorphology (Berl.)* 99:1–21.

Blades-Eckelbarger, P. I. 1986. Aspects of internal anatomy and reproduction in the Copepoda. *Syllogeus* 58:26–50.

Bradford, J. M. 1974. *Euchaeta marina* (Prestandrea) (Copepoda, Calanoida) and two closely related new species from the Pacific. *Pac. Sci.* 28:159–169.

Brodskii, K. A. 1967a. *Calanoida of the Far Eastern Seas and Polar Basin of the USSR*. Moskva-Leningrad: Isdatel'stvo Akademii Nauk SSSR. (Note: Translation from Russian by A. Mercado in 1950. Israel Program for Scientific Translations.)

Brodskii, K. A. 1967b. Types of genitalia and heterogeneity in the genus *Calanus* (Copepoda). *Dokl. Akad. Nauk SSSR* 176:222–225.

Ferrari, F. 1978. Spermatophore placement in the copepod *Euchaeta norvegica* Boeck 1872 from the deepwater dumpsite 106. *Proc. Biol. Soc. Wash.* 91:509–521.

Ferrari, F. & M. Dojiri. 1987. The calanoid copepod *Euchaeta antarctica* from southern ocean Atlantic sector midwest trawls, with observations on spermatophore dimorphism. *J. Crustacean Biol.* 7:458–480.

Fleminger, A. 1967. Taxonomy, distribution, and polymorphism in the *Labidocera jollae* group with remarks on evolution within the group (Copepoda: Calanoida). *Proc. U.S. Natl. Mus.* 120:1–61.

Fleminger, A. 1975. Geographical distribution and morphological divergence in American coastal-zone planktonic copepods of the genus *Labidocera*. In L. E. Cronin, ed., *Estuarine Research. Vol. 1, Chemistry, Biology and the Estuarine System*, pp. 392–419. New York: Academic Press.

Fleminger, A. 1979. *Labidocera* (Copepoda, Calanoida): New and poorly known Caribbean species with a key to species in the Western Atlantic. *Bull. Mar. Sci.* 29:170–190.

Fleminger, A. 1986. The pleistocene equatorial barrier between the Indian and Pacific oceans and a likely cause for Wallace's line. *UNESCO Tech. Pap. Mar. Sci.* 49:84–97.

Fleminger, A. & K. Hulsemann. 1974. Systematics and distribution of the four sibling species comprising the genus *Pontellina* Dana (Copepoda, Calanoida). *U.S. Natl. Mar. Fish. Serv. Fish. Bull.* 72:63–120.

Fleminger, A. & E. Moore. 1977. Two new species of *Labidocera* (Copepoda, Calanoida) from the Western North Atlantic region. *Bull. Mar. Sci.* 27:520–529.

Fleminger, A. & E. Tan. 1966. The *Labidocera mirabilis* species group (Copepoda, Calanoida) with descriptions of a new Bahamian species. *Crustaceana (Leiden)* 11:291–301.
Frost, B. & A. Fleminger. 1968. A revision of the genus *Clausocalanus* (Copepoda: Calanoida) with remarks on distributional patterns in diagnostic characters. *Bull. Scripps Inst. Oceanogr. Univ. Calif.* 12:1–235.
Gauld, D. T. 1957. Copulation in calanoid copepods. *Nature (Lond.)* 180:510.
Geptner, M. V. 1968. Structure and taxonomic significance of the genital complex in copepods of the family Euchaetidae (Calanoida). *Oceanology* 8:543–552.
Griffiths, A. M. & B. W. Frost. 1976. Chemical communication in the marine planktonic copepods *Calanus pacificus* and *Pseudocalanus* sp. *Crustaceana (Leiden)* 30:1–8.
Hammer, R. M. 1978. Scanning electron microscope study of the spermatophore of *Acartia tonsa*. (Copepoda: Calanoida). *Trans. Am. Microsc. Soc.* 97:386–389.
Heberer, G. 1932. Untersuchungen uber Bau und Funktion der Genitalorgane der Copepoden. I. Der mannliche Geitalapparat der calanoiden Copepoden. *Z. Mikrosk. Anat. Forsch. (Leipz.)* 31:250–424.
Heberer, G. 1937. Weitere Ergebniss euber Bildung und Bau der Spermatophoren und Spermatophorenkoppelapparate bei calanoiden Copepoden. *Verh. Dtsch. Zool. Ges.* 39:86–93.
Hopkins, C. C. E. 1978. The male genital system and spermatophore production and function in *Euchaeta norvegica* Boeck (Copepoda: Calanoida). *J. Exp. Mar. Biol. Ecol.* 35:197–231.
Hopkins, C. C. E. & D. Machin. 1977. Patterns of spermatophore distribution and placement in *Euchaeta norvegica* (Copepoda: Calanoida) *J. Mar. Biol. Assoc. U. K.* 57:113–131.
Hopkins, C. C. E. , J. Mauchline & D. S. McLusky. 1978. Structure and function of the fifth pair of pleopods of male *Euchaeta norvegica* (Copepoda: Calanoida). *J. Mar. Biol. Assoc. U.K.* 58:631–637.
Jacobs, J. 1961. Laboratory cultivation of the marine copepod *Pseudodiaptomus coronatus* Williams. *Limnol. Oceanogr.* 6:443–446.
Jacoby, C. A. & M. J. Youngbluth. 1983. Mating behavior in three species of *Pseudodiaptomus* (Copepoda: Calanoida). *Mar. Biol. (Berl.)* 76:77–86.
Katona, S. K. 1973. Evidence for sex pheromones in planktonic Copepods. *Limnol. Oceanogr.* 18:574–583.
Katona, S. K. 1975. Copulation in the copepod *Eurytemora affinis* (Poppe, 1880). *Crustaceana (Leiden)* 28:90–94.
Lawson, T. J. 1977. Community interactions and zoogeography of the Indian Ocean Candaciidae (Copepoda: Calanoida). *Mar. Biol. (Berl.)* 43:71–92.
Lee, C. M. 1972. Structure and function of the spermatophore and its coupling device in the Centropagidae (Copepoda: Calanoida). *Bull. Mar. Ecol.* 8:1–20.
Mayr, E. 1963. *Animal Species and Evolution.* Cambridge, Massachusetts: Harvard University Press.
Park, T. S. 1966. The biology of a calanoid copepod, *Epilabidocera amphitrites* McMurrich. *Cellule* 66:1–251.
Park, T. S. 1975. Calanoid copepods of the family Euchaetidae from the Gulf of Mexico and Western Caribbean Sea. *Smithson. Contrib. Zool.* 196:1–26.
Park, T. S. 1978. Calanoid copepods (Aetideidae and Euchaetidae) from Antarctic and sub Antartic waters. *Antarct. Res. Ser.* 27:91–290.
Parker, G. H. 1902. The reactions of copepods to various stimuli and the bearing of this on daily depth migrations. *Bull. U.S. Fish Comm.* 21:103–123.
Raymont, J. E. G., S. Krishnaswamy, M. A. Woodhouse & R. L. Griffin. 1974. Studies on the fine structure of Copepoda. Observations on *Calanus finmarchicus* (Gunnerus). *Proc. R. Soc. Lond. B Biol. Sci.* 185:409–424.
Strickler, R. & A. K. Bal. 1973. Setae of the first antennae of the copepod *Cyclops scutifer* (Sars): Their structure and importance. *Proc. Natl. Acad. Sci. U.S.A.* 70:2656–2659.
Vaupel Klein, J. C. von. 1982. A taxonomic review of the genus *Euchirella* Giesbrecht, 1888 (Copepoda, Calanoida). II. The type-species, *Euchirella messinensis* (Claus, 1863). A. The female of *F. typica*. *Zool. Verh. (Leiden)* 198:1–131.
Williams, R. 1972. A further character for identification of the adult females of *Calanus finmarchicus, C. helgolandicus,* and *C. glacialis* (Crustacea: Copepoda). *Bull. Mar. Ecol.* 8:53–60.