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REPRODUCTIVE PROCESSES REVEALED BY SPERMATOPHORE DEHISCENCE EXPERIMENTS AND BY HISTOLOGY, ULTRASTRUCTURE, AND HISTOCHEMISTRY OF THE FEMALE REPRODUCTIVE SYSTEM IN THE SNOW CRAB *CHIONOECETES OPILIO* (O. FABRICIUS)

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ABSTRACT

Histological, histochemical, ultrastructural, and microbiological techniques were used to elucidate the structure and function of the female reproductive system in the snow crab *Chionoecetes opilio* (O. Fabricius). The anatomy of the ovary and oviduct conform to that of other brachyuran species. The spermatheca comprises a dorsal (glandular) and a ventral (chitin-lined) region, with no intervening anatomical separation. Ultrastructural and histochemical bases are presented for the dual role of the spermatheca in secretion and sperm storage. Proteinaceous polysaccharides are the dominant holocrine secretion of the glandular epithelium. A dense, morphologically homogeneous bacterial population was observed in the spermathecae of all of 25 crabs examined. A discrete layer of nonspermatophore-containing acid mucopolysaccharides appears to be transferred to the vagina and ventral spermatheca region by the male after insemination. A functional partitioning of spermatophores seems to occur at insemination; some dehisce immediately (probably due to mechanical forces), while those resisting initial dehiscence are stored in the spermatheca. In vitro experiments show that storage in the spermatheca greatly increases the tendency of spermatophores to dehisce when exposed to sea water. Functional correlates to these data are explored, with emphasis on roles and interactions of secretions, bacteria, spermatophore storage, and dehiscence, and mechanisms of ensuring last-male precedence under conditions of sperm competition.

The rise and fall of fisheries for the snow crab *Chionoecetes opilio* (Fabricius) (Majidae) and congeners has catalyzed research efforts on the basic biology of the genus in Canada, the U.S.A., and Japan (Jamieson and McKone, 1988). Such work highlighted the limited knowledge on brachyuran reproduction in general, and forced a reappraisal of many existing generalizations about *C. opilio* (Conan and Comeau, 1986; Beninger *et al.,* 1988, 1991; Bailey and Elner, 1989; Elner and Beninger, in press).

Contrary to early laboratory observations (Watson, 1970, 1972), recent field (Taylor *et al.,* 1985; Hooper, 1986) and laboratory (R. W. Elner, unpublished data) studies suggest that multiparous mating is a common occurrence in female *C. opilio.* Females are able to fertilize one, or more, subsequent batches of ova after releasing the larvae from the initial egg clutch, using spermatophores stored in the spermathecae from copulation at least 12 months previously (Watson, 1970, 1972; Paul, 1984). However, the relative importance of primiparous versus multiparous copulation and the role that stored spermatophores play, given the fact of subsequent reinsemination, remain uncertain.

Several workers have examined the gross morphology and histology of the brachyuran female reproductive system (Spalding, 1942; Gordon, 1950; Hartnoll, 1968; Johnson, 1980), and the functional anatomy of the male reproductive system of *C. opilio* has been described (Beninger *et al.,* 1988, 1991). However, despite a cursory examination (Beninger *et al.,* 1988), the roles of key features of the female reproductive system, such as the spermatheca, remain poorly known.

The brachyuran spermatheca is thought to be glandular (Ryan, 1967; Johnson, 1980; Bawab and El-Sherief, 1989; Diesel, 1989). However, no ultrastructural studies have been performed to determine the charac-

teristics and mechanism of secretion. Brief histological descriptions of the spermatheca of the majids *C. opilio* and *Inachus phalangium* (Fabricius) were presented by Beninger *et al.* (1988) and Diesel (1989), respectively. Histological data of variable breadth exist for spermathecae of species from other brachyuran families, such as *Portunus sanguinolentus* (Herbst) and *Callinectes sapidus* Rathbun (Portunidae) (George, 1963; Johnson, 1980), and *Paratelphusa hydrodromous* (Herbst) (Potamonidae) (Anilkumar and Adiyodi, 1977).

The present work explores the functional anatomy and histology of the female reproductive system in *C. opilio,* with particular emphasis on the spermatheca, for which ultrastructural, histochemical, and microbiological data are presented. Together with data from spermatophore dehiscence experiments, the results allow new insights into the reproductive biology of this species.

Materials and Methods

Histology and Electron Microscopy.—Female snow crabs for histological and ultrastructural procedures were collected at 28–55 m depth by SCUBA on 28 May 1989 and by beam trawl at 120–140 m depth on 20 July 1989 from Bonne Bay, Newfoundland (49°34′N, 57°56′W). The physical characteristics of Bonne Bay and the biological characteristics of the snow crab population have been reported by various authors (Taylor *et al.,* 1985; Hooper, 1986; Conan and Comeau, 1986). Crabs were kept either overnight in refrigerated coolers or in a submerged crab trap for several days before being flown in refrigerated coolers to the Université de Moncton, New Brunswick. All female snow crabs were ovigerous, and the presence of dark grasping marks indicated that they were also multiparous.

Spermathecae from 3 females from 28 May 1989 and 2 females from 20 July 1989 were removed and dissected using microsurgical instruments. Large segments of the reproductive system including the ovary, oviduct, and spermatheca were dissected from one female for general topological histology. Tissue samples were cut in a grid pattern for examination by histological and both scanning and transmission electron microscopical techniques for all regions of the spermatheca. Tissue for light microscopy was fixed in aqueous Bouin's solution, dehydrated, and cleared in an ascending ethanol–HemoDE (Fisher Scientific Company) series, embedded in paraffin, and serially sectioned at 5, 7, and 10 μm. Sections were stained using the Goldner variation of the topological Masson trichrome technique (Martoja and Martoja, 1967). Several sections were also stained using orcein hydrochloride-picrofuchsin, in order to distinguish between elastic and collagenous fibers in the outer wall of the spermatheca (Gabe, 1968).

Tissue for electron microscopy was initially fixed in 5% glutaraldehyde-cacodylate buffer over melting ice. Since some ultrastructural differences can occur between such samples and those postfixed in osmium tetroxide (Owen and McCrae, 1979), both fixation techniques were used. After 1 h of fixation/postfixation, the tissue was cut into 0.5–1 mm^3 pieces, dehydrated in an ascending ethanol series, embedded in Epon resin, and polymerized. Semithin sections (1–2 μm) were cut and colored with toluidine blue, while thin sections were mounted on TEM grids and contrasted with uranyl acetate and bismuth subnitrate. Grids were examined using either a Phillips 200 or 400 TEM (University of New Brunswick, Fredericton, New Brunswick) operating at 80 or 100 kV, respectively, or a JEOL 100 TEM operating at 80 kV (National Research Council laboratory, Halifax, Nova Scotia). Most of the TEM observations were performed on one crab from the 29 May 1989 sample and one crab from the 20 July 1989 sample; both specimens had distended spermathecae full of spermatophores.

Tissue for scanning electron microscopy (SEM) was fixed as above, dehydrated in an ascending ethanol series, critical-point dried with liquid CO_2, and sputter-coated using either a gold or a palladium-gold electrode. The specimens were mounted on SEM stubs and observed using a Cambridge S4-10 SEM operating at 10 kV.

Histochemistry and Gram Staining.—Three adult female snow crabs were obtained from the fishery off eastern Cape Breton Island on 26 July 1991. Specimens were transported on ice to aquaria at the Halifax Fisheries Research Laboratory. Spermathecae were removed on 29 July and fixed according to the requirements of each histochemical test. Alloxan-Schiff was used to detect proteinaceous substances; controls were deaminated sections treated with alloxan-Schiff and with Schiff only, as well as normal slides treated with Schiff only (Gabe, 1968). Periodic acid-Schiff was used to detect carbohydrates and neutral mucopolysaccharides (Martoja and Martoja, 1967). Alcian blue was used to detect acid mucopolysaccharides following diastase digestion, and Sudan black was used to detect lipids (Vacca, 1985). The Gram staining technique for bacteria was performed on spermathecal matrix smears removed under sterile conditions from 25 other specimens from this sampling (Hucker and Conn, 1923).

Dehiscence Experiments.—Male snow crabs showing mature secondary sexual characteristics (according to the criteria of Conan and Comeau, 1986) and ovigerous multiparous females were collected from the commercial fishery off Cape Breton Island on 23 August 1990. Two males and one female were chosen randomly for study. Seminal fluid from the posterior vas deferens (PVD–males) or matrix from the spermatheca (females) was placed in a petri dish on an ice bath. Spermatophore diameter was measured using a stereoscopic microscope and cold light source for 20 spermatophores chosen at random. Normal O°C sea water was added and the petri dish was gently swirled. Spermatophore diameter was measured after 5, 15, 30, 45, 60, and 90 min elapsed time.

A similar procedure was followed for determining percentage of dehiscence in spermatophores from an additional male and female. Counts of dehisced spermatophores were made at intervals of 5, 15, 30, 60, and 90 min. Total spermatophore numbers selected for observation were 208 (PVD) and 283 (spermatheca).

RESULTS

General Morphology.—The gross morphology of the female reproductive system, including the spermatheca, has been described previously (Beninger *et al.*, 1988). The general anatomical relationships are presented in Fig. 1 for purposes of orientation.

Ovary and Oviduct.—The ovary is a paired structure, each half consisting of a central lumen surrounded by lobules containing developing oocytes. Connective tissue is prominent around developing oocytes, but reduced around mature oocytes (Fig. 2.2). Previtellogenic oocytes are situated closest to the lumen, while vitellogenic and mature oocytes are located at the lobule peripheries (Fig. 2.1, .2). Previtellogenic oocytes are characterized by a nonbasophilic nucleus with prominent nucleolus, and by a basophilic cytoplasm devoid of yolk droplets. Vitellogenic oocytes are strongly basophilic and present nonbasophilic yolk droplets. Mature oocytes are surrounded by a follicular cell monolayer and bounded by a double envelope. Their nuclei are not visible using the Masson technique, and the cell is filled with yolk droplets of various staining affinities (Fig. 2.2).

The oviduct is composed of a columnar epithelium surrounded by abundant smooth muscle fibers embedded in loose connective tissue (Fig. 2.3). The epithelium becomes secretory as it approaches the spermatheca, and secretory products can be seen in the oviductal lumen in this region (Fig. 2.1).

Spermatheca.—Examination of the histology and ultrastructure of the spermatheca reveals two structurally distinct regions: ventral (comprising the pouches described by Beninger *et al.*, 1988) and dorsal (the remainder of the spermatheca), including the oviduct. These divisions are shown in Fig. 1.2; details of their anatomy and ultrastructure are given below.

Dorsal Region.—The dorsal region of the spermatheca consists of two tissue layers: an outer layer of heavily vascularized, folded lacunar connective tissue (Fig. 3.1, .2) and an inner glandular epithelium. The layers are separated by ramified collagenous fibers. The glandular epithelium is subdivided into a germinal zone, a glandular layer, and a squamous layer (Figs. 2.4, 3.3).

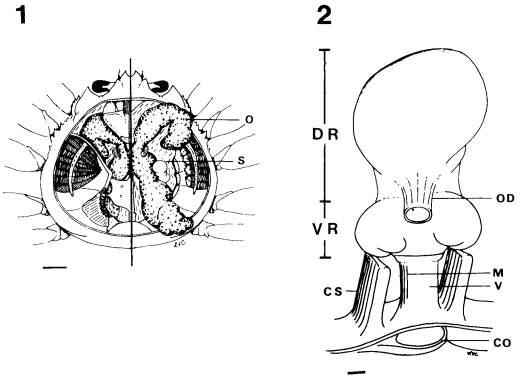

Fig. 1. General anatomical relationships of female reproductive system in *Chionoecetes opilio* (modified from Beninger *et al.*, 1988). Fig. 1.1. Dorsal view, right side shown with heart removed and spermatheca (S) manoeuvered into view from beneath ovary (O). Scale bar = 13 mm. Fig. 1.2. Mediolateral view of spermatheca, showing limits of dorsal (DR) and ventral (VR) regions. CO, insertion of third coxa; CS, cuticular septum; M, sectioned muscles between vagina (V) and carapace; OD, oviduct. Scale bar = 0.6 mm.

The germinal zone presents numerous mitotic figures (Fig. 3.3). These cells give rise to a stratified squamous epithelium composed of secretion-rich gland cells. Toward the lumen, the gland cells become progressively filled with globules of electron-dense secretory products, often adhering to the cell membranes, which become extensively folded, producing an extremely complex appearance in TEM sections (Fig. 4.1). Golgi bodies are numerous and active in these cells (Fig. 4.2). The nuclei contain condensed peripheral heterochromatin and become increasingly irregular in cells closest to the squamous layer (Fig. 4.1). Membrane-bound, variably shaped collecting sites are evident in the glandular layer; these become filled with electron-dense secretions as they approach the spermathecal lumen (Fig. 4.1, .3). Cells and collecting sites in the squamous layer degenerate and slough off, liberating their contents into the spermathecal lumen (Fig. 3.4). Spermatophores in the dorsal lumen had intact pellicles but degenerate contents.

Histochemistry.—Results of the histochemical tests are presented in Table 1. While both the matrix and squamous layer contain substances of a proteinaceous carbohydrate nature, the matrix also contains acid mu-

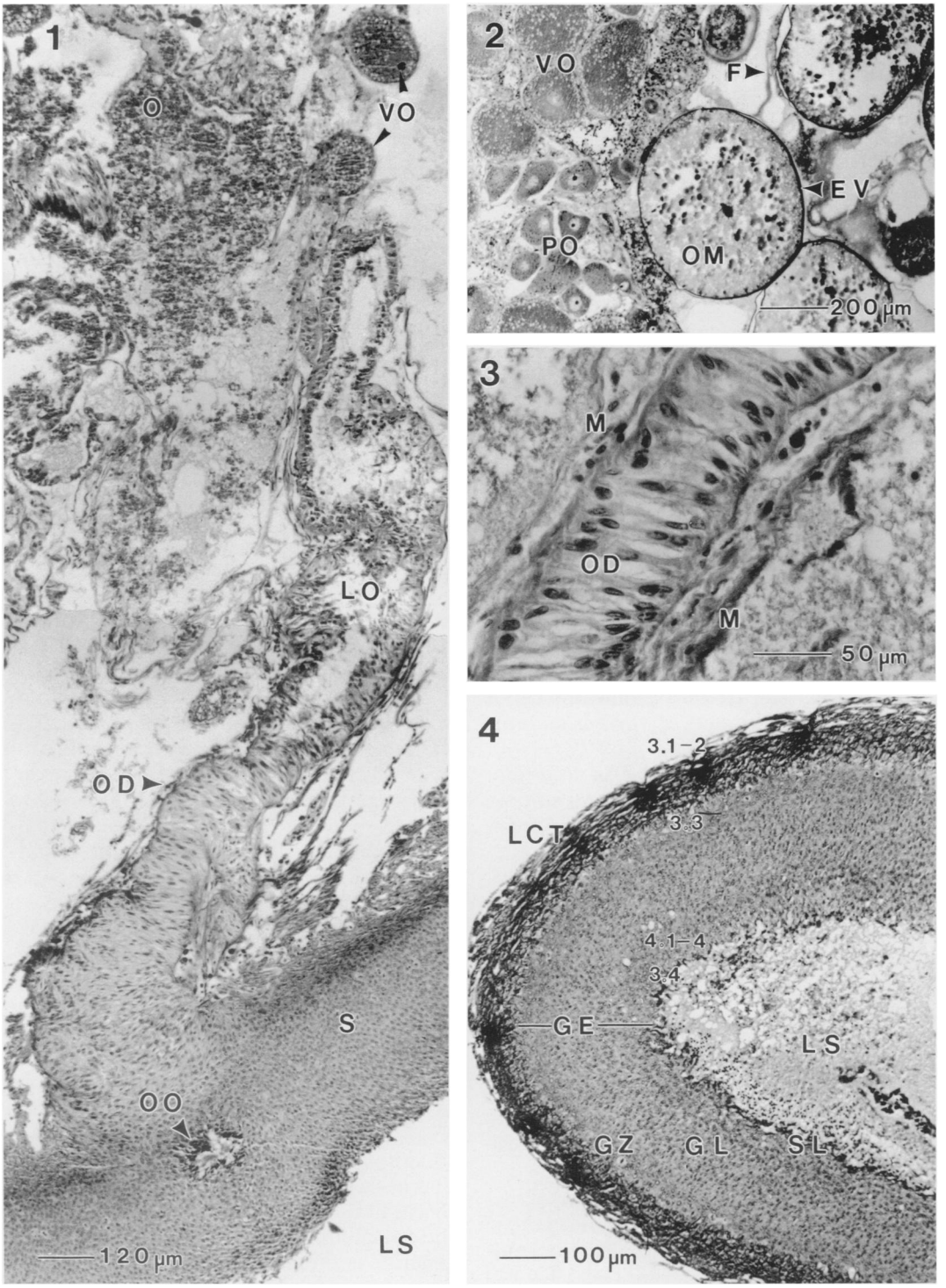

Fig. 2. Histological sections of *Chionoecetes opilio* ovary and spermatheca. All sections stained with Masson trichrome. Fig. 2.1. General organization of ovary (O), oviduct (OD), and spermatheca (S). LO, lumen of oviduct; LS, lumen of spermatheca; M, smooth muscles; OO, oviduct opening into spermatheca (note secretions in oviduct lumen); VO, vitellogenic oocytes. Structural preservation of oocytes is incomplete due to large size of tissue necessary for general view. Fig. 2.2. Detail of ovary and oocytes. EV, oocyte double envelope; F, follicle cells; OM, mature oocyte; PO, previtellogenic oocytes; VO, vitellogenic oocytes. Fig. 2.3. Detail of oviduct (OD),

Table 1. Histochemical tests and results for spermathecae from multiparous *Chionoecetes opilio* from Cape Breton Island in July 1991. − = negative; + = positive; ++ = strong positive reaction.

Test	Target substance	Results	
		Tissue	Matrix
Alloxan-Schiff	proteins	+ (squamous layer)	+
Periodic acid-Schiff	carbohydrates, neutral mucopolysaccharides	++ (squamous layer)	++
Alcian blue, pH 2.5	acid mucopolysaccharides	−	+ (dorsal region) ++ (ventral region)
Sudan black	lipids	−	−

copolysaccharides and lipids not present in the squamous layer.

Ventral Region.—No transverse membrane or velum was observed at the junction of the dorsal and ventral regions. However, the ventral region is characterized morphologically by prominent pouches around the lumen (Fig. 1.2). Abundant muscle fibers are arranged radially around the pouches and muscle fibers also extend into the folded inner epithelium and cuticle (Fig. 5.1). The cuticle is continuous with that of the vagina. The muscle fibers insert onto the cuticle via attachment complexes composed of condensing tonofilaments (Fig. 5.2–.4). Numerous mitochondria are found in proximity to the tonofilaments (Fig. 5.4). Although there is no glandular tissue, matrix and spermatophores extend to this region in distended spermathecae (Fig. 5.1). SEM micrographs of the cuticular folds reveal the presence of a dense, morphologically homogeneous bacterial population dominated by rods (Fig. 5.5). Similarly, all of the 25 crabs used for spermathecal matrix smears showed dense populations of bacteria. These populations were dominated by 0.5–1 μm Gram negative rods, either singly or in chains of up to 10 cells. Occasional 0.3 μm Gram negative single cocci and 3 μm Gram positive rods (either singly or in chains of up to 5 cells) were also found. Spermatophores in the ventral region had a normal histological aspect.

Vagina.—The cuticular folds in the spermathecal ventral region diminish progressively toward the vagina, where the inner face is smooth (Fig. 5.2). Otherwise, the vagina's histology is similar to that of the spermathecal ventral region.

Dehiscence Experiments.—Dehiscence reached 100% within 15 min in spermatophores taken from the spermatheca, whereas dehiscence only reached 10% after 100 min in spermatophores from the posterior vas deferens (Fig. 6). Due to the extremely rapid dehiscence of spermatophores from the spermatheca, it was not possible to measure diameters. However, the size of the spermatophores from the PVD increased rapidly before leveling off after 15–30 min (Fig. 7).

Discussion

Ovary.—The general ovarian and oocyte structure and histology conform to those of other brachyurans (Ryan, 1967; Hinsch, 1971; Charniaux-Cotton, 1973; Adiyodi and Subramoniam, 1983; Armstrong, 1988). The absence of oocyte stages between the primary and late vitellogenic stages has also been reported for the amphipod *Orchestia gammarus* Pallas (see Charniaux-Cotton, 1973). This suggests that in crustaceans, as in the bivalve mollusc *Tapes rhombiodes* (Pennant), maturing oocytes are produced in discrete cohorts rather than continuously (Morvan and Ansell, 1988).

Oviduct.—The oviduct histology is similar to the general description given by Adiyodi and Anilkumar (1988). The assumption of secretory activity as the oviduct approaches the spermathecal dorsal region supports the hypothesis of a common anatomical origin (Payen, 1974; Bauer, 1986). A secretory role for the oviduct has also been reported for

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surrounded by smooth muscles. Fig. 2.4. Cross section of dorsal region of spermatheca. An outer covering of lacunar connective tissue (LCT) surrounds glandular epithelium (GE), which is subdivided into germinal zone (GZ), glandular layer (GL), and squamous layer (SL). Secretions and debris from squamous layer are shed into lumen (LS). Numbers indicate succeeding detail figures from corresponding locations.

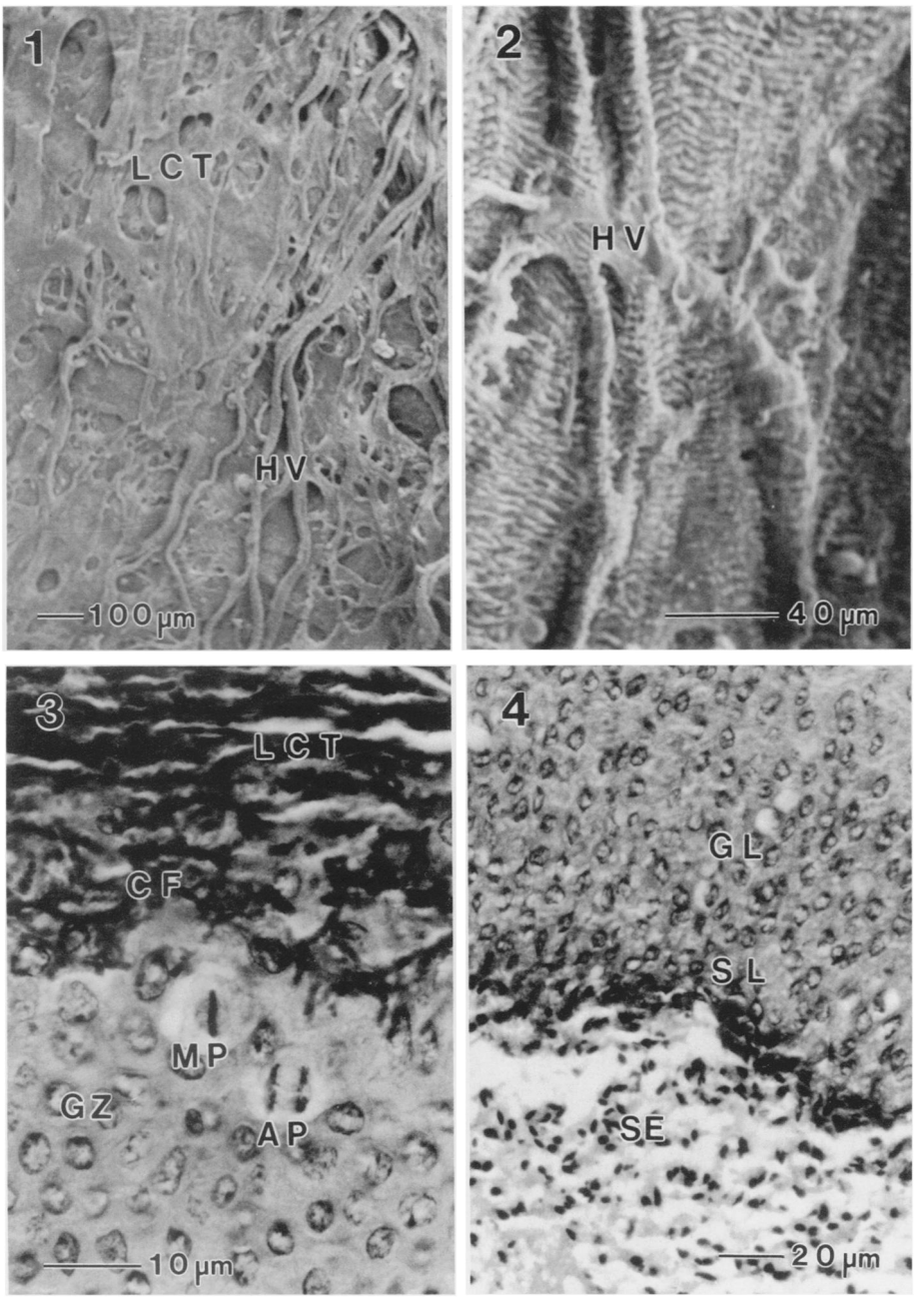

Fig. 3. Spermatheca of *Chionoecetes opilio,* dorsal region. Fig. 3.1. Scanning electron micrograph (SEM) of outer covering of spermatheca, showing lacunar connective tissue (LCT) and hemolymphatic vessels. Fig. 3.2. SEM detail of anastomosed hemolymphatic vessels (HV). Fig. 3.3. Histological section showing detail of transition from lacunar connective tissue (LCT) to germinal zone (GZ). Note insertion of collagenous fibers (CF). Mitotic figures are common; here shown metaphase (MP) and late anaphase (AP). Mallory's trichrome. Fig. 3.4. Histological section showing transition from glandular layer (GL) to squamous layer (SL). Cellular debris and secretions (SE) slough off into spermatheca lumen.

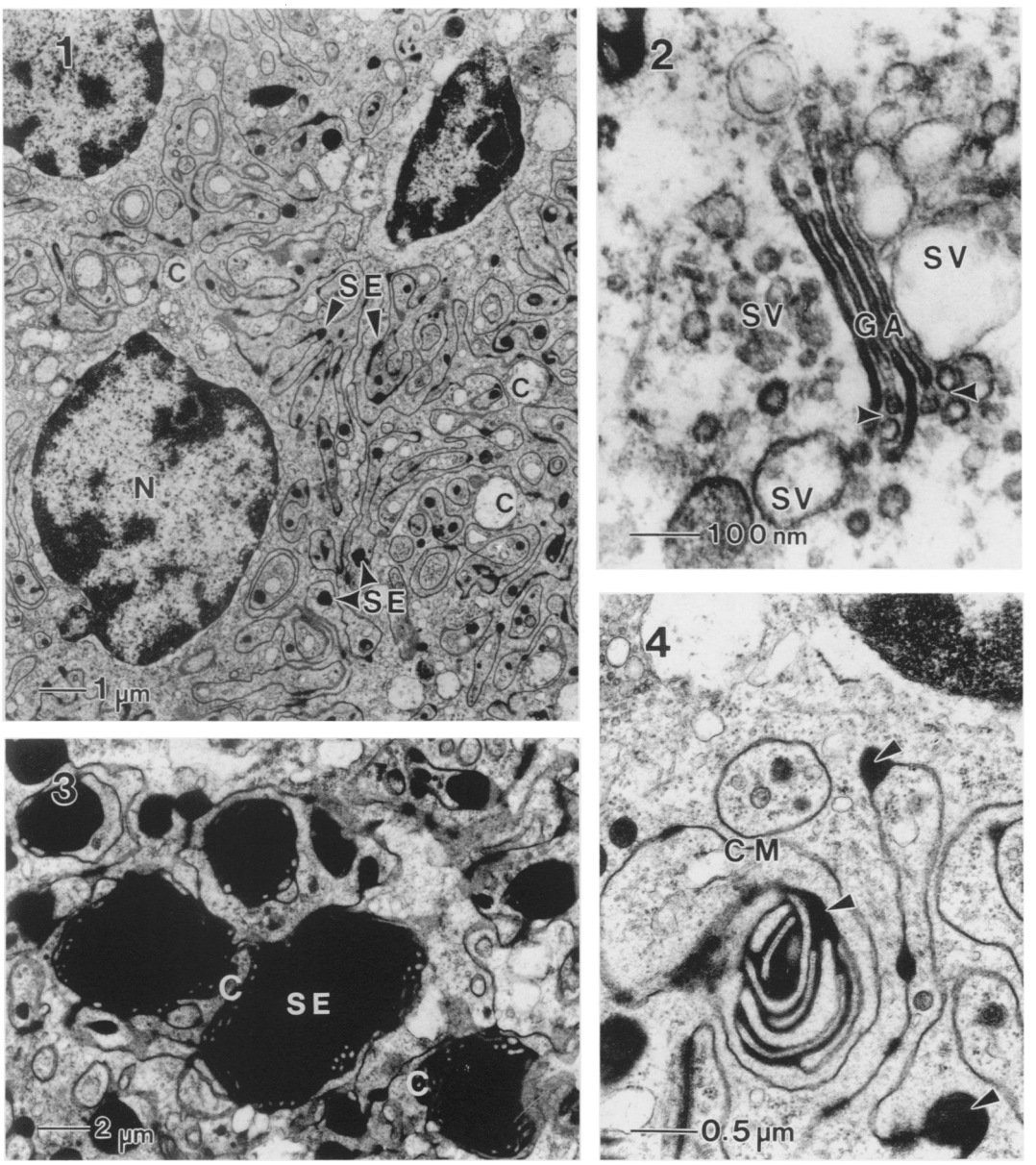

Fig. 4. Transmission electron micrographs of glandular region of spermatheca of *Chionoecetes opilio*. Fig. 4.1. General view of cell structure. Note extremely ramified cell membranes. Nuclei (N) show irregularity indicative of imminent disintegration. Electron-dense secretions (SE) are visible within cells; collecting sites (C) are empty at this point. Fig. 4.2. Detail of one of many active Golgi apparati. Note numerous fully formed secretion vesicles (SV) of various sizes, as well as those in process of formation (arrows). Fig. 4.3. Section through collecting sites (C) filled with secretions (SE). Fig. 4.4. Detail of cell membranes, showing extensive rolling and accumulation of secretions (arrows).

the crayfish *Austropotamobius* (*Astacus*) *pallipes* (Lereboullet) (see Cheung, 1966).

Smooth muscle fibers surrounding the oviduct also occur in penaeid shrimps (Bell and Lightner, 1988) and in the swimming crab *Portunus pelagicus* (Linnaeus) (see Bawab and El-Sherief, 1989). They may function to move oocytes to the spermathecal lumen. Although Ryan (1967) reported a chitinous lining and well-organized layers of circular and longitudinal muscles around the oviduct of *Portunus sanguinolentus*

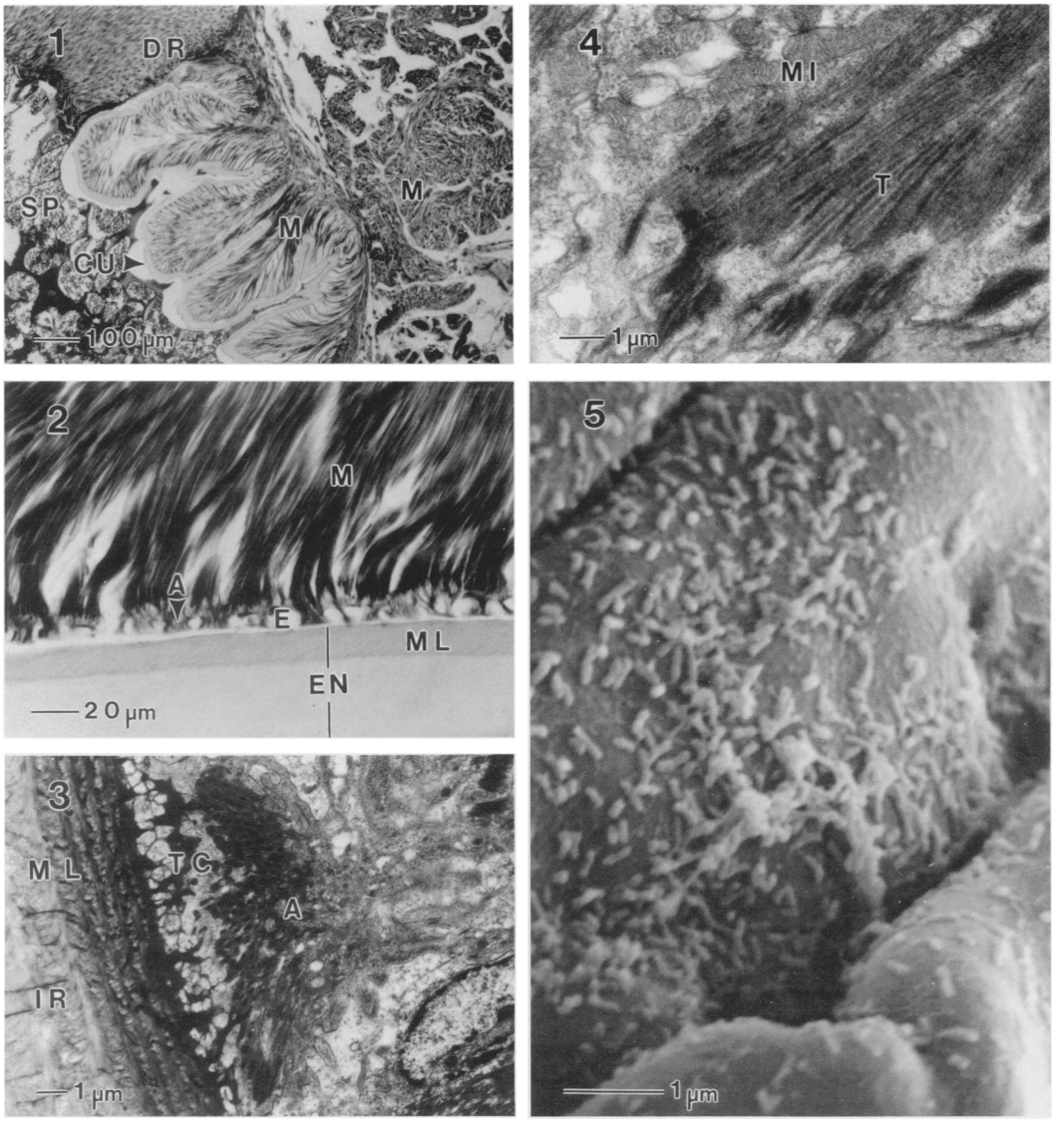

Fig. 5. Spermatheca of *Chionoecetes opilio,* ventral region. Fig. 5.1. Longitudinal histological section. Note abrupt transition from dorsal region (DR), with no intermediate membrane or velum. Muscles (M) surrounding ventral region extend into cuticular folds (CU). Lumen filled with matrix containing spermatophores (SP). Fig. 5.2. Detail of attachment of muscles to cuticle of ventral region and vagina. Muscles (M) insert into cuticle via attachment complexes (A) intercalated with epithelial cells (E). Endocuticle (EN) shows distinct membranous layer (ML). Fig. 5.3. Transmission electron micrograph (TEM) of attachment complex (A), showing condensed tonofilaments (TC) inserting into membranous layer (ML) of endocuticle. Intracuticular rods (IR) are also visible. Fig. 5.4. TEM detail of condensing tonofilament (T) of attachment complex. Note proximity and number of mitochondria (MI). Fig. 5.5. Scanning electron micrograph of cuticle facing spermatheca lumen. Note density and homogeneity of adhering bacteria.

(Herbst), careful reading actually implicates the vagina.

Spermatheca-Dorsal Region.—The anatomical differentiation of the spermatheca into a dorsal (glandular) and a ventral (chitinous) region conforms to the general organization of this structure in the Brachyura. The dorsal region represents a continuation of the oviduct (mesodermal origin), while the ventral region (ectodermal origin) is an extension of the chitinous vagina (Payen, 1974; Bauer, 1986).

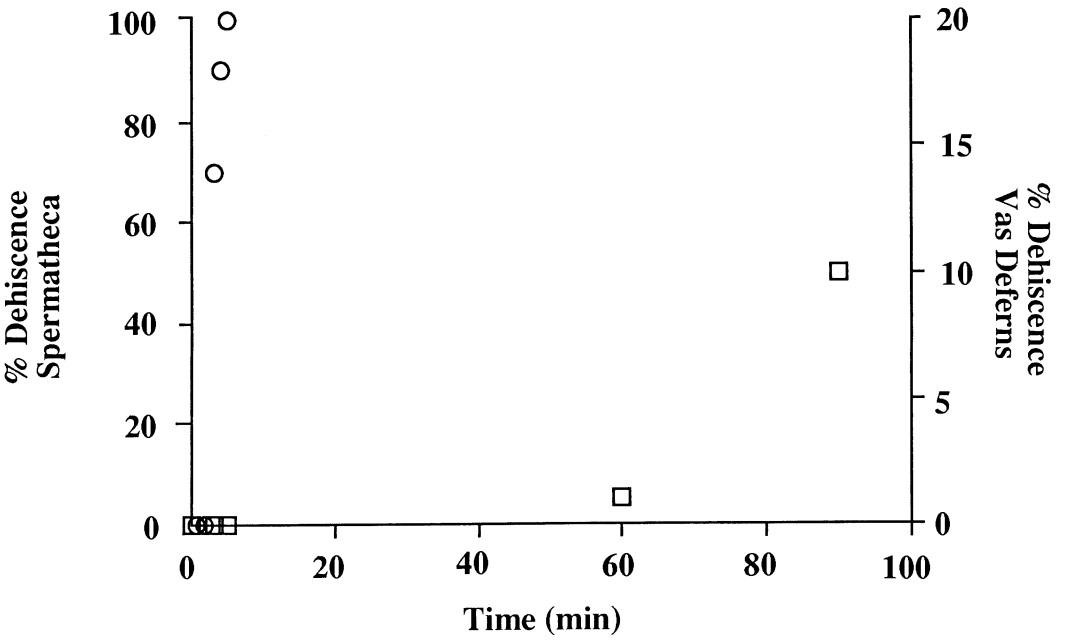

Fig. 6. Percentage of dehiscence versus time after contact with water of spermatophores from spermatheca (○) and posterior vas deferens (□).

Most authors report that the spermathecal dorsal region is glandular (George, 1963; Ryan, 1967—*Portunus sanguinolentus;* Johnson, 1980—*Callinectes sapidus;* Bawab and El-Sherief, 1989—*Portunus pelagicus;* Diesel, 1989—*Inachus phalangium*), although Spalding (1942) failed to find secretory cells there in *Carcinus maenas*. The

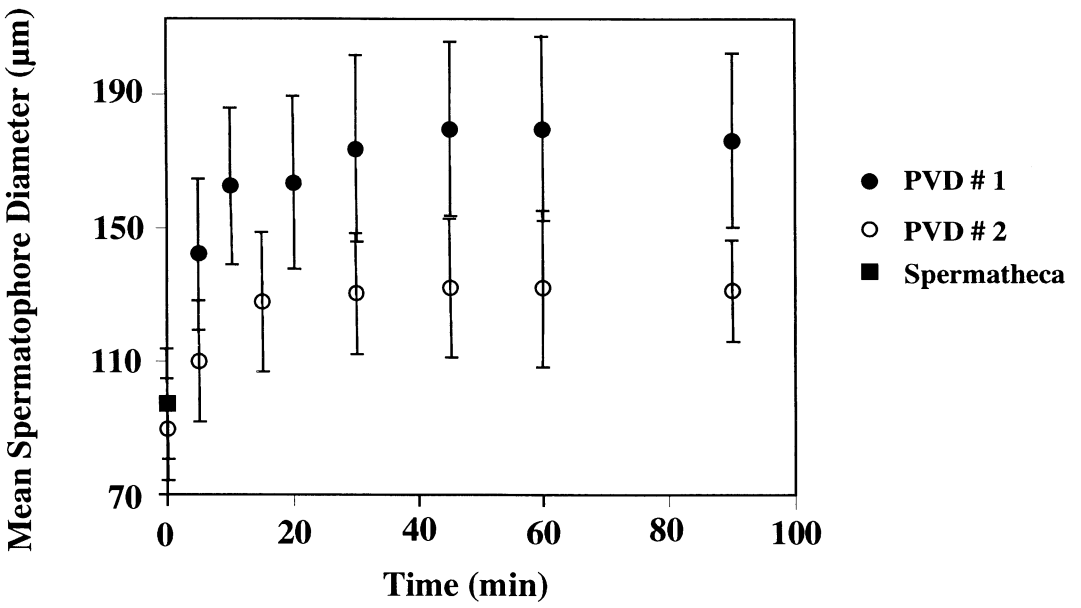

Fig. 7. Mean spermatophore diameter (±standard deviation) versus time after exposure to 0°C sea water for two trials from posterior vas deferens (PVD) and one trial from spermatheca. Initial size measured for PVD–2 and spermatheca trials before adding sea water. Size measurement for spermatophores from spermatheca not possible after addition of sea water, since dehiscence was extremely rapid.

histology of the glandular epithelium of *C. opilio* agrees with that textually described for *Portunus sanguinolentus* (see Ryan, 1967), and also for *Callinectes sapidus* (see Johnson, 1980). In the present work, the "dense layer" of Johnson (1980) has been further subdivided into a germinal zone, a glandular layer, and a squamous layer.

The mechanism of glandular secretion has previously been described as holocrine in *Portunus pelagicus* (see Bawab and El-Sherief, 1989) and *P. sanguinolentus* (see Ryan, 1967), although no ultrastructural evidence has been presented to date. Such data in the present work confirm the holocrine mechanism in *Chionoecetes opilio.* The pronounced folding of the cell membranes and ultrastructural characteristics of the glandular layer resemble those in the male accessory glands of insects such as *Tenebrio* sp. (Happ, 1984).

Microvilli were not observed in the dorsal region of the spermatheca. This contrasts with the observations of Angelhou-Spiliotis and Goudeau (1982) on *Carcinus maenas* (L.) and Johnson (1980) on *Callinectes sapidus,* and suggests that the spermathecal dorsal epithelium of *Chionoecetes opilio* is uniquely secretory, with little capacity for absorption.

Secretions.—Elucidation of the role of the dorsal glandular epithelium secretory products is confounded by mixing of the secretions with those introduced by the male during insemination. However, the data of the present study permit a more precise determination of the nature of the glandular epithelium secretions. Taken together, the histochemical tests indicate that the dominant secretion is an amine-containing neutral polysaccharide (glycoprotein or neutral mucopolysaccharide). These results are supported by the existence of numerous, highly active Golgi apparati in the glandular epithelium which produce mainly protein-containing polysaccharides. These are energy-rich, hygroscopic substances.

Spermathecal secretions have been assumed to nourish stored spermatozoa in crustaceans (see review by Adiyodi and Adiyodi, 1975). However, the studies cited to support this theory (Tulsyan, 1966; Bhatnagar and Musgrave, 1971) refer to terrestrial insects, and neither demonstrates such a relationship. Alternatively, Diesel (1989) stated that enzymes responsible for spermatophore pellicle breakdown are secreted by the spermathecal glandular epithelium, although no supporting data was presented. Similarly, Adiyodi and Anilkumar (1988) cited the results of Krishnakumar (1985) on the presence of a protease in spermathecal homogenates of *Paratelphusa hydrodromous,* although the origin of this enzyme is uncertain.

The high energy and water-binding properties of the glycoprotein/neutral mucopolysaccharide may be important for storage and dehiscence of spermatophores. This point will be considered more fully below.

Anilkumar and Adiyodi (1977) reported similar histochemical profiles for the spermathecal epithelium at all stages of oogenesis in *Paratelphusa hydrodromous.* Nevertheless, a complete seasonal study is desirable for snow crabs, since one of the theories of sperm maintenance in terrestrial insects is that quiescent sperm are activated by alkaline spermathecal secretions just prior to fertilization (Parker, 1970).

The presence of a discrete alcianophilic mass restricted to the spermathecal ventral region suggests that an acid mucopolysaccharide is added by the male in the later stages of insemination. The absence of spermatophores in this substance indicates an origin other than the vas deferens. A likely source for this secretion is the rosette gland complex of the male first gonopod (Beninger *et al.,* 1991), which could add nonspermatophore containing substances independently of the vas deferens.

Ventral Region and Vagina.—In contrast to observations on the majid *Inachus phalangium* (see Diesel, 1989), no transverse membrane or velum was found to separate the dorsal and ventral regions of the spermatheca. Such a structure is thus not a general feature of the Majidae.

The histology of the spermathecal ventral region and the vagina is similar, corroborating the idea of common anatomical origin (Payen, 1974; Bauer, 1986). In the ventral region of the spermatheca, the well-developed muscles which surround and insert on the cuticular lining could create a mixing movement to enhance the probability of spermatophore dehiscence and of

encounters between oocytes and nonmotile sperm at ovulation. The large number of mitochondria associated with the condensing tonofilaments tends to support this idea (providing the energy necessary for such action). Around the vagina, where some of these muscles are also linked to the carapace, contraction could be responsible for opening the lower vagina and vulva during copulation and egg extrusion (Beninger *et al.,* 1988). Diesel (1989) reported that this region must be opened "voluntarily" in order for copulation (and presumably oviposition) to occur in the majid *Inachus phalangium.*

The attachment of muscles to the cuticular lining of the spermathecal ventral region and the vagina is via tendons composed of condensed and condensing tonofilaments. This arrangement is as described for the cuticle of the copepod *Calanus finmarchicus* by Raymont *et al.* (1974), and contrasts with the direct insertion of muscles via conical hemidesmosomes as previously reported for several other malacostracans (Talbot *et al.,* 1972; Halcrow, 1988).

The cuticle layer immediately above the epithelium has been termed a membranous layer (Pütz and Buchholz, 1991). In contrast to this general description for brachyurans, the results of the present study show the laminations of this layer to be well organized. The nature of the lamination is not fully understood. Although Adiyodi and Anilkumar (1988) cited Raymont *et al.* (1974) to show that lamination is due to alternating layers of chitin and protein, the latter study advanced no data other than the proximal composition of the cuticle.

Bacteria.—The presence of bacteria in crab spermathecae has not been reported previously. Although bacteria can be present in any structure exposed (albeit only periodically) to the external medium and to male intromittent organs, the great density and morphological homogeneity of the populations found in the spermathecae of *Chionoecetes opilio* is noteworthy. Rather than suggesting colonization of surfaces, which would produce a very heterogeneous population (Garland *et al.,* 1982), the present observations indicate that this is a rather homogeneous population. Such a situation could be fostered by the chemical characteristics of the spermatheca matrix. Spermathecal secretions and male insemination fluids may thus play an important role in maintaining such a population.

Regardless of origin, the observed density of these bacteria indicates that the effects of their metabolic activity on the chemical environment in the spermatheca and on the reproductive process itself should not be ignored. For example, their metabolic byproducts could render the spermatophore pellicle more susceptible to dehiscence upon contact with sea water, as has been proposed to occur when females use stored spermatophores to fertilize oocytes (Beninger *et al.,* 1988). The results of the dehiscence experiments clearly demonstrate that storage in the spermatheca enhances spermatophore dehiscence when exposed to sea water.

Such a dense, homogeneous bacterial population may also inhibit the growth of other potentially harmful microbes. An analogous situation exists in the mammalian vagina, where glycogen is secreted by epithelial cells (which eventually slough off into the vaginal lumen) and metabolized to lactic acid by the Döderlein's bacteria (Hammersen, 1976). The lowered pH protects the female reproductive system from opportunistic pathogens. It would thus be of interest to study the potential interactions between spermathecal secretions, bacteria, male seminal fluids, and spermatophores in *Chionoectes opilio.*

The presence of a discrete layer of acid mucopolysaccharides in the terminal portion of the transferred male seminal fluids is noteworthy, since Sasikala and Subramoniam (1987) suggested that acid mucopolysaccharides have an antimicrobial activity in the spermatophores of two marine prawns. Such a layer may, therefore, play an additional role in the prevention of subsequent bacterial colonization from the external medium.

The present study shows that attempts to characterize the biochemical and enzymatic dynamics of the spermathecal contents (e.g., Jeyalectumie and Subramoniam, 1991) must consider the effect of bacterial presence on data interpretation. Moreover, unless a technique is found to separate the effects of spermatozoan and bacterial metabolic activity, it will not be possible to determine

whether and to what extent the various matrix substances sustain stored spermatozoa.

Implications for Spermatophore Storage and Fertilization.—Although the spermatheca obviously functions to store sperm, the complexity of this organ suggests that the processes and roles of such storage are complex in *Chionoecetes opilio.* Based on the assumption that in brachyurans the ovaries are not ripe at the puberty molt, Adiyodi and Anilkumar (1988) suggested that the time lag between insemination at the puberty molt and fertilization favored the evolution of spermathecae as specialized regions for sperm storage. The argument would hold for crabs which copulate in the soft-shell state and ovulate later in the hard-shell state, but many brachyurans display a variety of additional possibilities. For example, some species do not attain a terminal molt at puberty, and may retain sperm across successive molts (Ryan, 1967; Cheung, 1966). Still others, such as *Chionoecetes opilio,* have ripe ovaries at the terminal puberty molt and the female may copulate both at the puberty molt and subsequently in a hard-shell state (Hartnoll, 1969; Conan and Comeau, 1986; Elner and Beninger, in press). The spermathecae of many species would, thus, supercede the function proposed by Adiyodi and Anilkumar (1988).

Several lines of evidence indicate that sperm storage may be of value as an "insurance policy" for gravid females. Without a sperm-storage capability, an obvious risk for a female snow crab would not be finding a male with which to copulate in the year following an insemination. Intense natural egg mortality during incubation is another risk (Elner and Gass, 1984; Elner and Beninger, in press). Egg mortality due to parasite infestation appears to be frequent and considerable in *Cancer magister* Dana (see Wickham, 1979; Hankin *et al.,* 1989) and *Paralithodes camtschatica* (Tilesius) (see Kuris *et al.,* 1991). Brood parasites have been identified in several other brachyurans, including *Portunus pelagicus* (see Shields, 1992) and *C. opilio* (see Elner and Gass, 1984; Brattey *et al.,* 1985). Experiments on *Macrobrachium nobili* (Henderson and Matthai) have shown that egg stripping resulted in a greater number of females reovulating using stored sperm (Pandian and Balasundaram, 1982). Pilot experiments on egg stripping of *C. opilio* have also produced a precocious second ovulation (R. W. Elner, P. G. Beninger, J. Tremblay, and M. Eagles, unpublished observations).

Sperm storage may thus be considered a "failsafe" mechanism, allowing females to maximize the probability of successful broods after insemination. This may be important in *C. opilio,* where copulatory behavior can result in injury to the female (Watson, 1972; R. W. Elner, unpublished observation). Sperm storage may not be a major fertilization pathway under normal circumstances. Indeed, a single insemination is insufficient for a second successful egg clutch in primiparous *Chionoecetes bairdi* (see Paul and Paul, 1992), and in multiparous females the effectiveness of stored sperm appears to decrease substantially in the first two years after insemination (Paul, 1984). The observations of the condition of recent and older stored spermatophores in the present study lend support to these empirical data.

Spermatophore Function and Dehiscence.—Despite numerous structural and histochemical studies (see Hinsch, 1991, and Subramoniam, 1991, for reviews), the function and dehiscence mechanisms of spermatophores have remained enigmatic. After observing that the spermatophore pellicle breaks down shortly after insemination in two species of *Geryon,* Hinsch (1988) concluded that the spermatophore pellicle functions as a packaging device for sperm transfer. However, this theory does not address the question of why such packaging is necessary. Furthermore, the spermatophore pellicle remains intact over extended periods within *C. opilio* (see Beninger *et al.,* 1988, and present study).

A possible mechanism for spermatophore dehiscence and function in fertilization was proposed by Beninger *et al.* (1988). In this paradigm, hydration during insemination caused some spermatophores to dehisce (allowing fertilization of oocytes), while others resisted dehiscence and were available for storage. The differential dehiscence characteristics were ascribed to variations in pellicle thickness and folding. Such a paradigm advances a function for the packaging of spermatozoa into spermatophores. The

present dehiscence data show that hydration alone is a weak stimulus for dehiscence in spermatophores from the posterior vas deferens; both mechanical forces and hydration are probably necessary. Such a mechanism was suggested by Hamon (1937) for *Eupagurus prideauxi* Leach, and extended to other crabs by Spalding (1942), who proposed that passage through the narrow canal of the first gonopod could mechanically rupture spermatophore pellicles. Given the narrow opening of the first gonopod of *C. opilio* (approximately 40 μm, Beninger *et al.,* 1991), spermatophores must sustain significant mechanical and pressure forces during transfer, with the largest ones likely to undergo the most stress (spermatophore diameter 50–200 μm). This could represent another mechanism for differential dehiscence of spermatophores, with the largest rupturing at copulation and the smaller ones being available for storage.

The spacious environment of the spermatheca would be unable to generate the mechanical forces encountered at sperm transfer. However, the results of the dehiscence experiments in the present study show that storage in the spermatheca renders spermatophores much more susceptible to dehiscence upon hydration, which is postulated to occur just prior to ovulation (Beninger *et al.,* 1988). Although the cause of this facilitation is not yet understood, it clearly allows the female to use stored, spermatophore-encased spermatozoa for fertilization of a subsequent ovulation. The mechanism of spermatophore hydration is not yet known for *Chionoecetes opilio,* but the imbibition of water by acidic mucopolysaccharides within the spermatophores is postulated to occur in the portunid *Scylla serrata* (Forskål) (see Uma and Subramoniam, 1979).

Although the acrosome reaction is facilitated by contact with sea water (Beninger *et al.,* 1988), it also requires contact with a mature oocyte. Spalding (1942) thus concluded that the spermatophore pellicle is not necessary to protect spermatozoa from untimely acrosome reactions, but rather to "keep them in place until needed for fertilization." It is not clear why a pellicle would be necessary for such a function. However, without a pellicle, all spermatozoa might be facilitated, with excessive numbers undergoing the acrosome reaction upon contact with a single oocyte. Hence, the pellicle could also serve to reduce polyspermy and sperm wastage, enhancing the probability of sufficient numbers of spermatozoa remaining for subsequent fertilization (if needed).

Sperm Competition. — The subject of sperm competition has been recently reviewed, with emphasis on the Majidae (Diesel, 1991). While application of these ideas to crustaceans is relatively new, considerable progress in this field has been achieved in insects. *Chionoecetes opilio* meets three criteria for the occurrence of sperm competition (Parker, 1970): (1) females can mate in a hard-shelled state and are receptive more than once after the terminal molt to maturity, (2) sperm are long-lived (longer than most insect orders), and (3) site of spermatophore storage enhances the probability of fertilization.

While Diesel (1991) emphasized the layering of spermatophores from consecutive inseminations as being of prime importance in ensuring last-male precedence (since most recent sperm are in the ventral region of spermatheca, as is the oviducal opening), Beninger *et al.* (1991) proposed that the male first gonopod could also be used to remove previous insemination products prior to copulation. While the gonopod could probably not remove all stored spermatophores, it could certainly remove the most recent (ventral) ones, which would also be the most viable and competitive. Hence, spermatophores situated in the ventral region would have precedence over more dorsal ones, which may only be used for fertilization in the event that most ventral spermatophores have been used to fertilize a failed brood. This hypothesis could be tested using sterile male inseminations, as has been done for insects (Parker, 1970).

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Literature Cited

Adiyodi, K. G., and R. G. Adiyodi. 1975. Morphology and cytology of the accessory sex glands in invertebrates.—International Review of Cytology 43: 353–398.

———, and G. Anilkumar. 1988. Arthropoda–Crustacea.—*In:* Reproductive biology of invertebrates. Vol. 3, pp. 261–318. John Wiley and Sons, Chichester, England.

Adiyodi, R. G., and T. Subramoniam. 1983. Arthropoda–Crustacea.—*In:* K. G. and R. G. Adiyodi, eds., Reproductive biology of invertebrates. Vol. 1, Oogenesis, oviposition and oosorption. Pp. 443–495. John Wiley and Sons, Chichester, England.

Angelhou-Spiliotis, A., and M. Goudeau. 1982. Analyse au microscope électronique à balayage de la paroi limitant la lumière du conduit génital de la femelle adulte de *Carcinus maenas* (L.), Crustacé Décapode Brachyoure.—Comptes Rendus de l'Académie des Sciences, Paris 294 (Série III): 617–622.

Anilkumar, G., and K. G. Adiyodi. 1977. Spermatheca of the freshwater crab, *Paratelphusa hydrodromous* (Herbst) in relation to the ovarian cycle.—*In:* K. G. Adiyodi and R. G. Adiyodi, eds., Advances in invertebrate reproduction. Vol. 1, pp. 269–274. Peralom-Kenoth, Karivellur, Kerala, India.

Armstrong, J. H. 1988. Reproduction in the paddle crab *Ovalipes catharus* (Decapoda: Portunidae) from Blueskin Bay, Otago, New Zealand.—New Zealand Journal of Marine and Freshwater Research 22: 529–536.

Bailey, R., and R. W. Elner. 1989. Northwest Atlantic snow crab fisheries: lessons in research and management.—*In:* J. F. Caddy, ed., Marine invertebrate fisheries: their assessment and management. Pp. 261–280. John Wiley and Sons, Chichester, England.

Bauer, R. T. 1986. Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans.—Journal of Crustacean Biology 6: 313–325.

Bawab, F. M., and S. S. El-Sherief. 1989. Contributions to the study of the origin, nature and formation of the plug in the spermatheca of the female crab *Portunus pelagicus* (Linnaeus, 1766) (Decapoda, Brachyura).—Crustaceana 57: 9–24.

Bell, T. A., and D. V. Lightner. 1988. A handbook of normal penaeid shrimp histology.—Pp. 38–41. Allen Press, Lawrence, Kansas.

Beninger, P. G., R. W. Elner, T. P. Foyle, and P. H. Odense. 1988. Functional anatomy of the male reproductive system and the female spermatheca in the snow crab *Chionoecetes opilio* (O. Fabricius) (Decapoda: Majidae) and a hypothesis for fertilization.—Journal of Crustacean Biology 8: 322–332.

———, ———, and Y. Poussart. 1991. Gonopods of the majid crab *Chionoecetes opilio* (O. Fabricius).—Journal of Crustacean Biology 11: 217–228.

Bhatnagar, R. D. S., and A. J. Musgrave. 1971. Aspects of histophysiology of the spermathecal gland of *Sitophilus granarius* (L.) (Coleoptera).—Canadian Journal of Zoology 49: 275–277.

Brattey, J., R. W. Elner, L. S. Uhazy, and A. G. Bagnall. 1985. Metazoan parasites and commensals of five crab (Brachyura) species from eastern Canada.—Canadian Journal of Zoology 63: 2224–2229.

Charniaux-Cotton, H. 1973. Description et contrôle de l'ovogenèse chez les Crustacés supérieurs.—Annales de Biologie Animale, Biochimie, Biophysique 13: 21–30.

Cheung, T. S. 1966. The development of egg-membranes and egg attachment in the shore crab, *Carcinus maenas,* and some related decapods.—Journal of the Marine Biological Association of the United Kingdom 46: 373–400.

Conan, G. Y., and M. Comeau. 1986. Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio.*—Canadian Journal of Fisheries and Aquatic Sciences 43: 1710–1719.

Diesel, R. 1989. Structure and function of the reproductive system of the symbiotic spider crab *Inachus phalangium* (Decapoda: Majidae): observations on sperm transfer, sperm storage, and spawning.—Journal of Crustacean Biology 9: 266–277.

———. 1991. Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda, Majidae).—*In:* R. G. Bauer and J. W. Martin, eds., Crustacean sexual biology. Pp. 145–163. Columbia University Press, New York, New York.

Elner, R. W., and C. A. Gass. 1984. Observations on the reproductive condition of female snow crabs from NW Cape Breton Island, November 1983.—Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 84/14: 1–20.

———, and P. G. Beninger. (In press.) The reproductive biology of snow crab, *Chionoecetes opilio*: a synthesis of recent contributions.—American Zoologist.

Gabe, M. 1968. Techniques histologiques.—Masson et Cie, Paris, France. Pp. i–vi, 1–1113.

Garland, C. D., G. V. Nash, and T. A. McMeekin. 1982. The preservation of mucus and surface-associated microorganisms using acrolein vapour fixation.—Journal of Microscopy 128: 307–312.

George, M. J. 1963. The anatomy of the crab *Neptunus sanguinolentus* Herbst. Part IV. Reproductive system and embryological studies.—Journal of the Madras University Section B 33: 289–304.

Gordon, I. 1950. Crustacea: Dromiacea. Part 2. The morphology of the spermatheca in certain Dromiacea.—John Murray Scientific Expedition, 1933–34, Scientific Reports 9: 230–253.

Halcrow, K. 1988. Absence of epicuticle from the repair cuticle produced by four malacostracan crustaceans.—Journal of Crustacean Biology 8: 346–354.

Hammersen, F. 1976. Histology. A color atlas of cytology, histology, and microscopic anatomy.—P. 149. Lea and Febiger, Philadelphia, Pennsylvania.

Hamon, M. 1937. Les mécanismes produisant la déhiscence des spermatophores d'*Eupagurus brideauxi* [sic] Leach.—Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris 204: 1504–1506.

Hankin, D. G., N. Diamond, M. S. Mohr, and J. Ianelli. 1989. Growth and reproductive dynamics of adult female Dungeness crabs (*Cancer magister*) in north-

ern California.—Journal du Conseil International pour l'Exploration de la Mer 46: 94–108.

Happ, G. M. 1984. Structure and development of male accessory glands in insects.—*In:* R. C. King and H. Akai, eds., Insect ultrastructure. Vol. 2, pp. 365–396. Plenum Press, New York, New York.

Hartnoll, R. G. 1968. Morphology of the genital ducts in female crabs.—Journal of the Linnean Society (Zoology) 47: 279–300.

———. 1969. Mating in the Brachyura.—Crustaceana 16: 160–181.

Hinsch, G. W. 1971. Penetration of the oocyte envelope by spermatozoa in the spider crab.—Journal of Ultrastructure Research 35: 86–97.

———. 1988. Ultrastructure of the sperm and spermatophores of the golden crab *Geryon fenneri* and a closely related species, the red crab *G. quinquedens,* from the eastern Gulf of Mexico.—Journal of Crustacean Biology 8: 340–345.

———. 1991. Structure and chemical content of the spermatophores and seminal fluid of reptantian decapods.—*In:* R. G. Bauer and J. W. Martin, eds., Crustacean sexual biology. Pp. 290–307. Columbia University Press, New York, New York.

Hooper, R. G. 1986. A spring breeding migration of the snow crab, *Chionoecetes opilio* (O. Fabr.) into shallow water in Newfoundland.—Crustaceana 50: 257–264.

Hucker, G. J., and H. J. Conn. 1923. Methods of Gram staining.—New York Agriculture Experiment Station Technical Bulletin 93: 1–37.

Jamieson, G. S., and W. D. McKone. 1988. Proceedings of the international workshop on snow crab biology. December 8–10, 1987, Montréal, Québec.—Canadian Manuscript Report of Fisheries and Aquatic Sciences 2005: 1–163.

Jeyalectumie, C., and T. Subramoniam. 1991. Biochemistry of seminal secretions with reference to sperm metabolism and storage in the female.—Molecular Reproduction and Development 30: 44–55.

Johnson, P. T. 1980. Histology of the blue crab, *Callinectes sapidus*: a model for the Decapoda.—Chapter 15, Reproductive system, pp. 327–367. Praeger Publishers, New York, New York.

Krishnakumar, R. 1985. Studies on spermatheca of some decapod crustaceans.—Ph.D. thesis, University of Calicut, Thenhipalam, Kerala, India.

Kuris, A. M., S. F. Blau, A. J. Paul, J. D. Shields, and D. E. Wickham. 1991. Infestation by brood symbionts and their impact on egg mortality of the red king crab, *Paralithoides camtschatica,* in Alaska: geographic and temporal variation.—Canadian Journal of Fisheries and Aquatic Sciences 48: 559–568.

Martoja, R., and M. Martoja. 1967. Initiation aux techniques de l'histologie animale.—Pp. 1–345. Masson et Cie, Paris, France.

Morvan, C., and A. Ansell. 1988. Stereological methods applied to the reproductive cycle of *Tapes rhomboides.*—Marine Biology 97: 355–364.

Owen, G., and J. M. McCrae. 1979. Sensory cell/gland cell complexes associated with the pallial tentacles of the bivalve *Lima hians* (Gmelin), with a note on specialized cilia on the pallial curtains.—Philosophical Transactions of the Royal Society of London, Ser. B 187: 45–62.

Pandian, T. J., and C. Balasundaram. 1982. Moulting and spawning cycles in *Macrobrachium nobilii* (Henderson and Mathai).—International Journal of Invertebrate Reproduction 5: 21–30.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects.—Biological Reviews of the Cambridge Philosophical Society 45: 525–568.

Paul, A. J. 1984. Mating frequency and viability of stored sperm in the Tanner crab *Chionoecetes bairdi* (Decapoda: Majidae).—Journal of Crustacean Biology 4: 374–381.

———, and J. M. Paul. 1992. Second clutch viability of *Chionoecetes bairdi* Rathbun (Decapoda: Majidae) inseminated only at the maturity molt.—Journal of Crustacean Biology 12: 438–441.

Payen, G. 1974. Morphogenèse sexuelle de quelques Brachyoures (Cyclométopes) au cours du développement embryonnaire, larvaire et postlarvaire.—Bulletin du Muséum National d'Histoire Naturelle (Zoologie) 139: 201–262.

Pütz, K., and F. Buchholz. 1991. Comparative ultrastructure of the cuticle of some pelagic, nektobenthic and benthic malacostracan crustaceans.—Marine Biology 110: 49–58.

Raymont, J. E. G., S. Krishnaswamy, M. A. Woodhouse, and R. L. Griffin. 1974. Studies on the fine structure of Copepoda. Observations on *Calanus finmarchicus* (Gunnerus).—Proceedings of the Royal Society of London, Series B: 409–424.

Ryan, E. P. 1967. Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst) (Brachyura: Portunidae). I. The male system.—Proceedings of the Symposium on Crustacea, Ernakulam. Part III. Pp. 506-521. Marine Biological Association of India. Bangalore Press, Bangalore, India.

Sasikala, S. L., and T. Subramoniam. 1987. On the occurrence of acid mucopolysaccharides in the spermatophores of two marine prawns, *Penaeus indicus* (Milne-Edwards) and *Metapenaeus monoceros* (Fabricius) (Crustacea: Macrura).—Journal of Experimental Marine Biology and Ecology 113: 145–153.

Shields, J. D. 1992. Parasites and symbionts of the crab *Portunus pelagicus* from Moreton Bay, Eastern Australia.—Journal of Crustacean Biology 12: 94–100.

Spalding, J. F. 1942. The nature and formation of the spermatophore and sperm plug in *Carcinus maenas.*—Quarterly Journal of Microscopical Science 83: 399–422.

Subramoniam, T. 1991. Chemical composition of spermatophores in decapod crustaceans.—*In:* R. G. Bauer and J. W. Martin, eds., Crustacean sexual biology. Pp. 308–321. Columbia University Press, New York, New York.

Talbot, P., W. H. Clark, and A. L. Lawrence. 1972. Ultrastructural observations of the muscle and modified branchiostegite epidermis in the larval brown shrimp, *Penaeus aztecus.*—Tissue and Cell 4: 613–628.

Taylor, D. M., R. G. Hooper, and G. P. Ennis. 1985. Biological aspects of the spring breeding migration of snow crabs, *Chionoecetes opilio,* in Bonne Bay, Newfoundland (Canada).—Fisheries Bulletin, United States 83: 707–711.

Tulsyan, G. P. 1966. On the glandular functions of the spermatheca in *Laccifer lacca* (Kerr). (Laccifer-

idae: Coccoidea).—Annals and Magazine of Natural History, Series 13, 19: 681–688.
Uma, K., and T. Subramoniam. 1979. Histochemical characteristics of spermatophore layers of *Scylla serrata* (Forskål) (Decapoda: Portunidae).—International Journal of Invertebrate Reproduction 1: 31–40.
Vacca, L. L. 1985. Laboratory manual of histochemistry.—Raven Press, New York, New York. Pp. i–xvii, 1–578.
Watson, J. 1970. Maturity, mating and egg laying in the spider crab, *Chionoecetes opilio*.—Journal of the Fisheries Research Board of Canada 27: 1607–1616.
———. 1972. Mating behavior in the spider crab, *Chionoecetes opilio*.—Journal of the Fisheries Research Board of Canada 29: 447–449.
Wickham, D. E. 1979. Predation by the nemertean *Carcinonemertes errans* on eggs of the Dungeness crab *Cancer magister*.—Marine Biology 55: 45–53.

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ANNOUNCEMENT

In 1993 an international symposium on the systematics, morphology, evolution, and biology of Crustacea Decapoda is scheduled, sponsored by the Senckenberg Natural History Society. Topics include all aspects of decapodology including fisheries. Biochemistry and specialized physiology will not be included unless contributing to the biology of the animals or to solving taxonomic problems (for example, electrophoresis). Contributions in technical aspects of aquaculutre are not encouraged.

The symposium will be held at the Forschungsinstitut Senckenberg, Frankfurt a. M., between 18–22 October. The exact number of symposium days will be determined after response of those interested.

The registration fees will be DM 200.—or equivalent, and will cover most of the costs including the symposium proceedings. Students can register at a reduced price. There will be limited funds available, mainly for participants with nonconvertible currencies and/or students.

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