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SPERMATOPHORE TRANSFER AND SPERM STRUCTURE IN THE BRACHYURAN CRAB *METOPOGRAPSUS MESSOR* (DECAPODA: GRAPSIDAE)

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ABSTRACT

In the brachyuran crab *Metopograpsus messor* mating occurs only among "hard" (intermolt) individuals. Our present light and electron microscopic studies reveal that the spermatophores received by the females during coitus are discernible within the spermathecae until 24 h postmating. The study demonstrates time-dependent dissolution of the spermatophores of *M. messor* within the spermathecal lumen. The spermatozoa of *M. messor* were subjected to electron microscopic analysis. In addition to decapod and brachyuran features, the spermatozoa of *M. messor* display characters typical of the heterotreme-thoracotreme assemblage crabs, such as acrosomal length : width ratio (1.2), and the concentric arrangement of the acrosomal zones around the perforatorium. While the "onion ring" lamellation of the outer acrosome zone represents a typical thoracotreme character, the occurrence of the accessory opercular ring is indicative of its affinity to the Grapsinae-Sesarminae subfamilies within the Grapsidae.

Decapod spermatozoa are considered atypical and nonflagellate. They are invariably enclosed in sperm packets called spermatophores before being transferred to the female during mating. The spermatophores, when used for internal fertilization as in many brachyurans, are simple degenerate structures, while in others (as in anomurans and macrurans) with external fertilization, they are complex structures and are normally stored epizoically on the body of the female pending fertilization (Adiyodi and Anilkumar, 1988; Subramoniam, 1991, 1993, for reviews). Ultrastructural studies on the decapod spermatozoa are many, thanks to their bizarre structural features which show species-specific variation (Jamieson, 1994; Jamieson *et al.*, 1995; Medina, 1995; Tudge, 1997a). However, the majority of the studies have been made using the sperm enclosed in spermatophores stored in the vas deferens. Although the sperm from the vas deferens as well as from the female seminal receptacles have been assumed to be similar, some studies have indicated a kind of capacitation to take place in the seminal receptacles as in the case of the female shrimp *Sicyonia ingentis* (Burkenroad) (see Lindsay and Clark, 1992). The spermatophores, after their transfer to the female tract, are known to undergo dehiscence in brachyuran crabs, but the details of the spermatophore dissolution to release the sperm within the spermatheca have not been fully investigated. Furthermore, the structural features of the sperm, though presumed to be unchanged inside the spermatheca after their release from spermatophores, have not been elucidated.

In the present investigation, the fate of spermatophores, as received by the spermatheca during mating in the estuarine crab *Metopograpsus messor* (Forskål) has been studied at light and electron microscopic levels in order to evaluate the time-dependent dissolution of the spermatophore wall. In addition, the sperm of *M. messor* have also been subjected to ultrastructural analysis for assessing their phylogenetic relationship with other brachyuran crabs.

MATERIALS AND METHODS

Adults of *Metopograpsus messor* (22–25-mm carapace width) were collected by baiting from Muzhupilangad estuary in Kerala, India. In order to observe the fate of sperm within the spermatheca, female crabs engaged in mating were collected from the wild. They were either sacrificed immediately, or were reared in the laboratory in plastic cisterns laid with wet sand, for further investigation.

The paired, saccular spermatheca (3–5-mm diameter), situated at the junction of the ovarian limb and the oviduct, were dissected out by cutting open the carapace through the dorsal side. For histological studies, the dissection was performed in physiological (0.9%) saline and the tissue was fixed in formol-alcohol. Paraffin sections, stained in hematoxylin and alcoholic eosin (Haskel and Wills, 1968), were used for light microscopic observations.

For electron microscopy, the tissue was fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 24 h, washed with the (cacodylate) buffer and postfixed in 1% osmium tetroxide buffered with cacodylate. The tissue was again washed with the buffer and subsequently

dehydrated in a graded alcohol series. This was followed by en-bloc staining with 2% uranyl acetate. Clearing and embedding were carried out in propylene oxide and Araldite (CY 212), respectively. Ultrathin sections (grey to silver) stained with uranyl acetate and lead citrate (after Frasca and Parks, 1960), were examined in a JEOL Transmission Electron Microscope.

RESULTS

Mating and Sperm Transfer

In *Metopograpsus messor,* the ovarian limbs that extend from the anterior to the posterior part of the cephalothoracic cavity are bridged by a connection at their middle portion, so as to appear like the letter "H." From the middle portion of each ovarian limb, just below the bridging connection, a narrow oviduct arises. This opens to the exterior through the genital pore (vulva), situated at the ventral portion of the third thoracic segment. The saccular spermathecae are attached to the proximal portion of each oviduct.

Incidence of mating could be observed throughout the year, except during June–July (when the females generally abstain from breeding activity). Mating in *Metopograpsus messor* occurs only between a hard male and a hard female. In most instances, the courting pair was found in crevices, underneath large stones which could ensure them sufficient space and protection. Invariably, all the females engaged in mating were unoviposited, but their ovaries were in the late stage of vitellogenesis (Stage 4, described in Sudha and Anilkumar, 1996). During coitus, the male assumes an inferior position to the female. Usually, the mating pair remains in copulo for approximately 15–20 minutes, after which they separate.

Ultrastructure of the Spermatophore from the Male Tract

Semen of *Metopograpsus messor* essentially comprises the sperm packets (spermatophores) embedded in a white, viscous matrix, the seminal plasma. In fresh-smear preparations, the spermatophores appear as oval/globular entities with a translucent wall that encloses up to 106 spermatozoa.

Our ultrastructural observations on intact spermatophores from the vas deferens reveal that the spermatophore wall is noncellular in nature and is composed of outer and inner dense margins with an electron-lucent region in between (Figs. 1, 2B). Interestingly, some portions of the spermatophore wall show a bulged appearance, wherein electron-dense granular materials are found (Fig. 2B). Further, the spermatophore wall shows the presence of "intermittent infoldings" into the spermatophore lumen (Fig. 2A). Spermatozoa are loosely packed within the spermatophore wall (Fig. 1).

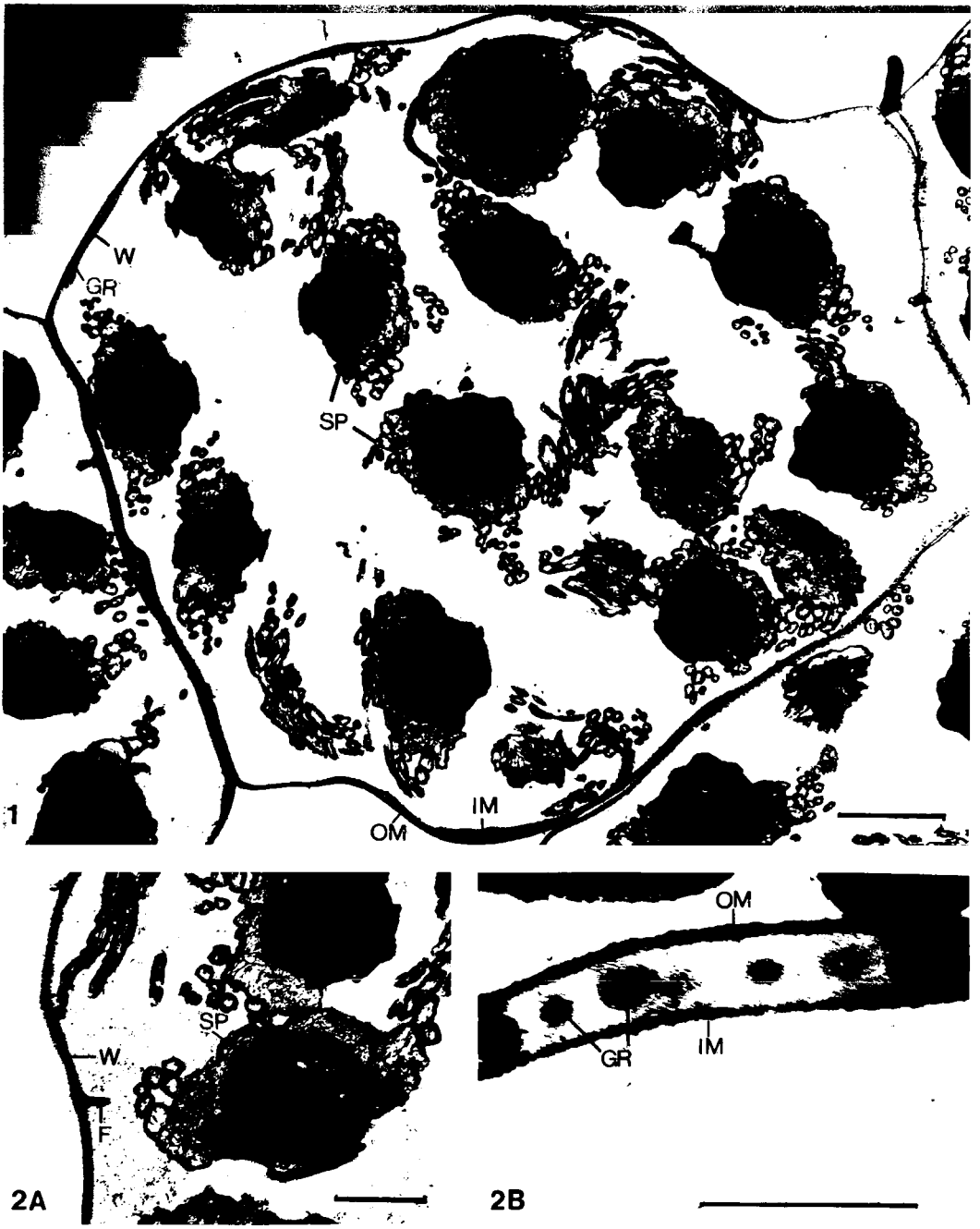
Fate of the Spermatophores within the Female Tract

Our light microscopic examination of the spermathecal smear and paraffin sections, as well as ultrastructural observations reveal that spermatophores are present within the spermatheca of the female up to 24 h after mating (Figs. 3, 4A). Under the electron microscope, the spermatophore wall could be seen as an electron-pale membranous coating over the sperm mass (Fig. 4B). Subsequently, within 24–36 h postmating, signs of dispersion (Fig. 5) and disruption (Figs. 6, 7) of the spermatophore wall begin to appear. As dissolution proceeds, the spermatophore wall loses its discreteness and finally (within 60 h postmating), appears as electron-lucent, inconspicuous entities (Fig. 8). Complete dissolution of the spermatophore wall occurred from 72 h postmating onward and, eventually, spermatozoa were seen dispersed as independent entities in the spermathecal lumen, with no signs of the spermatophore wall (Fig. 9).

Ultrastructure of the Spermatozoa

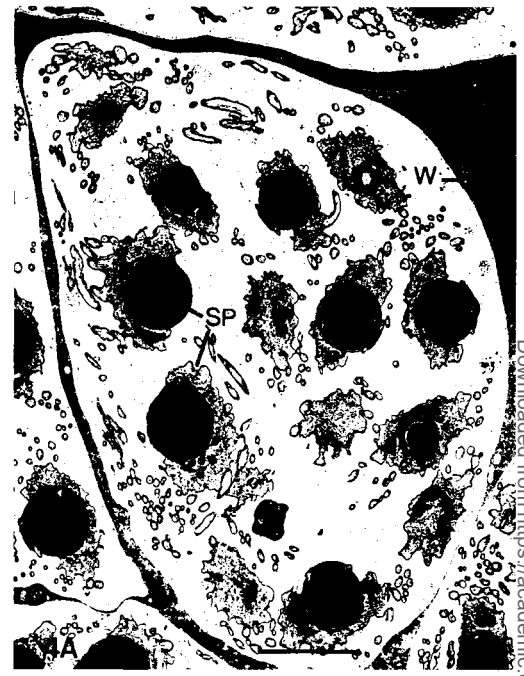
The present study reveals that the spermatozoa collected from the male (vas deferens) and the female (spermathecal lumen) tracts of *Metopograpsus messor* are ultrastructurally identical. In its gross structure, the spermatozoa of *M. messor* show the presence of two distinct regions: a subspheroidal acrosome and an extensive nucleus that surrounds the basal half of the acrosome (Figs. 10A, B, 11A, B).

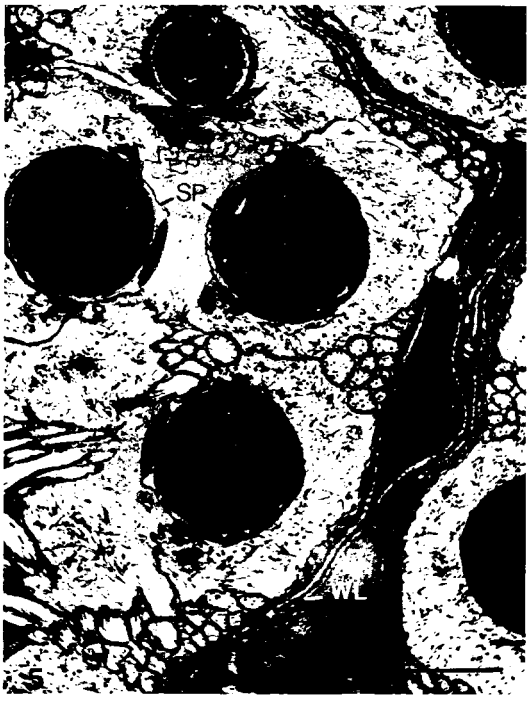
The length to width ratio (L:W) of the acrosome of *Metopograpsus messor* is 1.2. The acrosome is encased by an acrosomal membrane. At the anteriormost portion of the acrosome, there is a conical operculum, underlying which there is a less dense subopercular region. Encircling the periphery of the operculum, there is an electron-pale peri-opercular rim, which is considered to be the expansion of the vesicular layer. Immediately below the subopercular region, and anterior

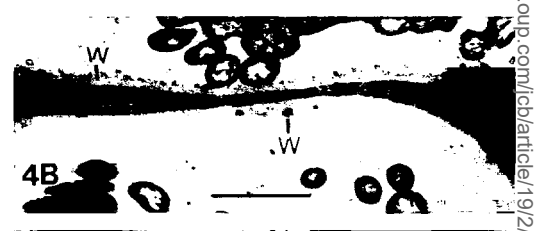


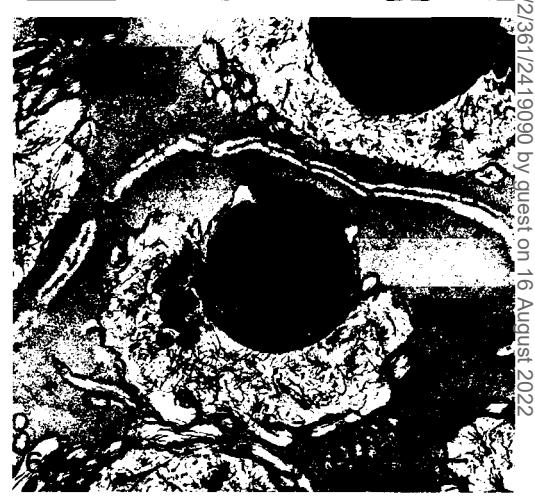
Figs. 1, 2. Spermatophore of *Metopograpsus messor*. Fig. 1. Ultrastructure of spermatophore from the vas deferens. (Scale bar = 2 μm). Fig. 2A. Enlarged view of the spermatophore wall showing "intermittent infoldings" (F). (Scale bar = 1 μm). Fig. 2B. Enlarged view of the bulged portion of the spermatophore wall showing dense granules (GR). (Scale bar = 0.5 μm). F, intermittent infolding; GR, dense granules; IM, inner margin; OM, outer margin; SP, spermatozoon; W, spermatophore wall.

to the acrosome vesicle, there is a dense accessory opercular ring with horizontal orientation. From the base of the acrosome, along its central core, and extending to the subopercular region, there is a longitudinal groove, referred to as the "perforatorium." The perforatorial lumen (subacrosomal chamber of Jamieson, 1993) is occupied by tubu-

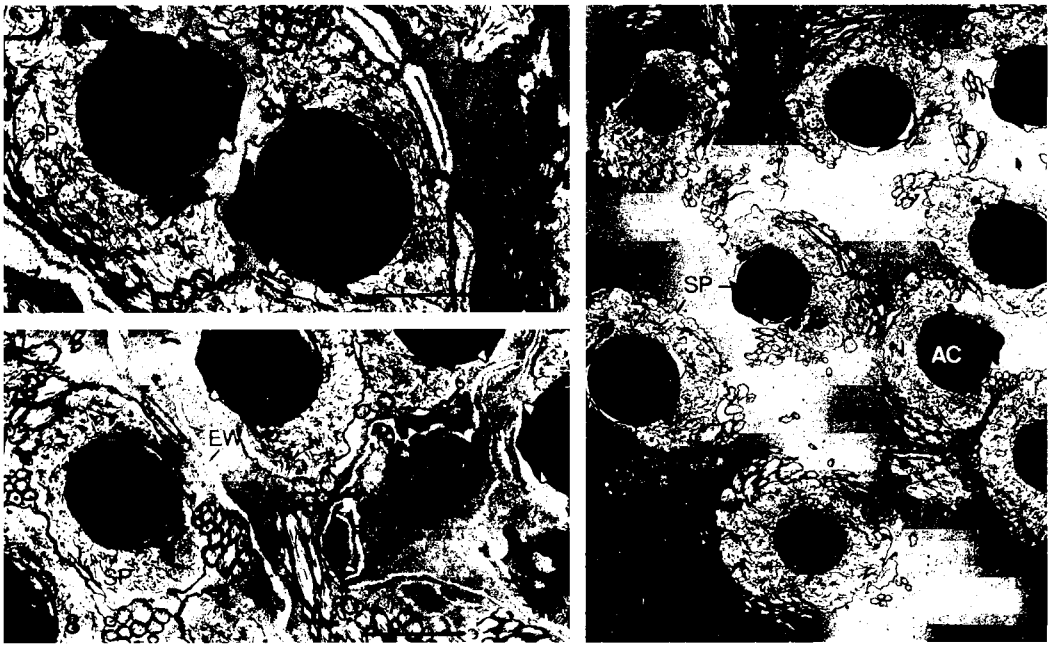








Figs. 3–6. Spermatophore and spermathecal lumen of *Metopograpsus messor.* Fig. 3. Photomicrograph of spermathecal lumen showing the presence of spermatophore (S). (Scale bar = 20 μm). Fig. 4A. Electron micrograph of a spermatophore from the spermathecal lumen within 24 h postmating. (Scale bar = 4 μm). Fig. 4B. Enlarged view of a portion of the spermatophore from the spermathecal lumen (as in Fig. 4A), showing the spermatophore wall. (Scale bar = 1 μm). Figs. 5, 6. Electron micrograph of a portion of the spermathecal lumen of postmated *Metopograpsus messor.* The spermatophore wall shows signs of dispersion and disruption within 24–36 h postmating. (Scale bar = 2 μm). S, spermatophore; SP, spermatozoon; W, spermatophore wall; WL, spermatophore wall material.



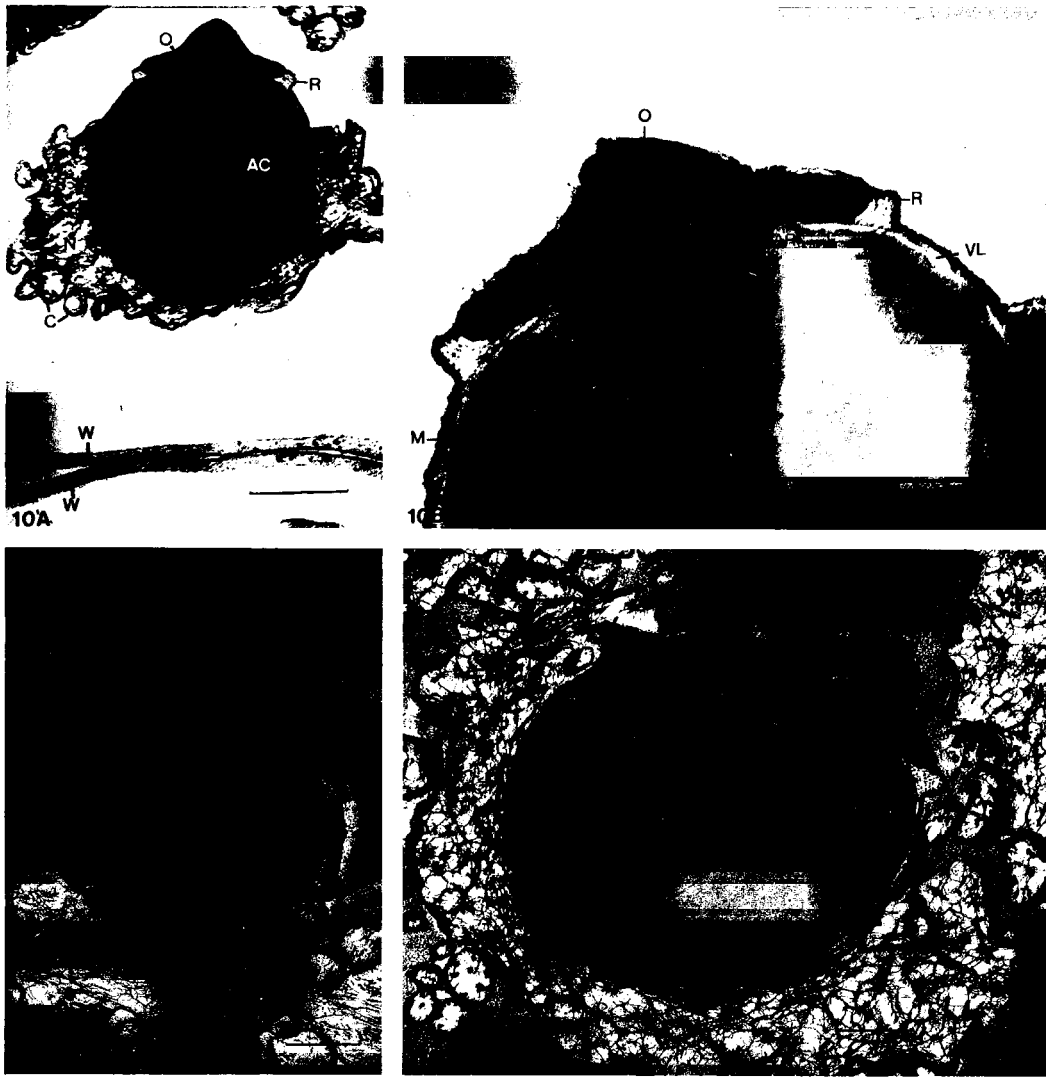
Figs. 7–9. Spermathecal lumen of *Metopograpsus messor.* Fig. 7. Electron micrograph of a portion of the spermathecal lumen of postmated *M. messor.* The spermatophore wall shows signs of dispersion and disruption within 24–36 h postmating. (Scale bar = 2 μm). Fig. 8. Spermathecal lumen 48–60 h postmating. Undissolved portions of the spermatophore wall are visible as electron-lucent entities (EW). (Scale bar = 2 μm). Fig. 9. Spermathecal lumen 72 h postmating, showing the spermatozoa (SP) after complete dissolution of the spermatophore wall. (Scale bar = 2 μm). AC, acrosome; EW, remnants of the spermatophore wall; L, spermathecal lumen; SP, spermatozoon; WL, spermatophore wall material.

lar formations (Figs. 10A, 11A). The contents of the acrosomal vesicle exhibit three zones which are concentrically arranged around the perforatorium. The zones are the outer acrosomal zone with concentric "onion ring" lamellation, a relatively dense middle acrosomal zone, and the inner acrosomal zone apposed to the perforatorium (Figs. 10B, 11B).

The sperm nucleus is enveloped by the nuclear membrane. The chromatin material appears reticulate. Observation of the spermathecal smear under light microscopy reveals that the spermatozoa of *Metopograpsus messor* possess spikelike arms, apparently arising from the nucleus. These spikes are visible in electron micrographs as small circular structures (transverse sections of the spikes) (C in Figs. 10A, 11A, B) in close vicinity of the spermatozoa. The cytoplasmic area in the spermatozoa is restricted to a thin space between the nucleus and the acrosome.

Discussion

In many brachyuran families (such as Portunidae, Majidae, Cancridae, Geryonidae, Parathelphusidae, Menippidae, and Calappidae), only the "soft" (postmolt) females engage in mating (Hartnoll, 1969; Adiyodi, 1988; Orensanz *et al.,* 1995; Saint-Marie and Carrière, 1995; Sainte-Marie *et al.,* 1995). Contrary to this situation, our present study reveals that females of *Metopograpsus messor* receive sperm only when they are "hard" (intermolt stage). Such a mating style, that deviates from those of the "soft maters," appears to be of adaptive significance in this highly fecund species that releases as many as 14–18 broods in a span of about 10 months, i.e., during the reproductive (August–December) and the molt-reproductive (January–May) periods. The population molts only once, however, during the period. The ovary in *M. messor* requires 14–17 days to complete vitellogenesis, after which the eggs are spawned and carried by the mother for 12–14 days. Spawning in most instances is closely followed by another vitellogenic cycle, which leads to oviposition in another 14–17 days, i.e., about 2–5 days after hatching and release of the larvae from the preceding clutch (Sudha and Anilkumar,



Figs. 10, 11. Spermatozoon of *Metopograpsus messor.* Fig. 10A. Ultrastructure of spermatozoon collected from the male tract (vas deferens) of *M. messor.* Note the presence of spermatophore wall (W). (Scale bar = 1 μm). Fig. 10B. Anterior portion of the acrosome of spermatozoon collected from the vas deferens of *M. messor.* (Scale bar = 0.5 μm). Fig. 11A. Cross section of the acrosome of spermatozoon collected from the spermathecal lumen of *Metopograpsus messor,* showing tubular formations (TF). (Scale bar = 0.5 μm). Fig. 11B. Ultrastructure of spermatozoon from the spermathecal lumen of *M. messor,* after complete dissolution of the spermatophore wall. (Scale bar = 0.5 μm). AC, acrosome; AR, accessory opercular ring; C, cross section of spikelike arms; CY, spermatozoal cytoplasm; IZ, inner acrosomal zone; M, plasma membrane; MZ, middle acrosomal zone; N, nucleus; NM, nuclear membrane; O, operculum; OZ, outer acrosomal zone with "onion ring lamellation"; PM, perforatorium; R, periopercular ring; TF, tubular formation; VL, vesicular layer.

1996). This interval of 2–5 days would be insufficient to accomplish molting (which normally requires 14–16 days) (Sudha, 1992), but could serve as the appropriate period for sperm transfer (and subsequent dissolution of the spermatophore wall, judged from the present study). Brood-carrying females of *M. messor* do not engage in coitus. Furthermore, in copulating females of *M. messor* the ovaries are in a late vitellogenic stage pending spawning. A comparable observation has been made in the majid crab *Inachus phalangium* (Fabricius), wherein males prefer to mate with females ready to spawn (Diesel, 1988).

Data on the timing of spermatophore dissolution is inadequate among decapods. In

Scylla serrata (Forskål), Ezhilarasi and Subramoniam (1980) have suggested that the spermatophores are intact until ovulation, when the oviductal fluid presumably digests the spermatophore wall. Further, the externally deposited spermatophores of the mole crab *Emerita asiatica* Milne Edwards are reported to dehisce only after being in contact with the oviductal fluid released during ovulation (Subramoniam, 1977). In *Libinia emarginata* Leach, free spermatozoa are visible within the seminal receptacle shortly after copulation (Hinsch, 1986, 1991). In the majid crab *Inachus phalangium,* on the other hand, intact spermatophores are reported to be present within the seminal receptacle two or three months after mating (Diesel, 1989). However, differing from these patterns, the results of the present study demonstrate the progressive dissolution of the spermatophore wall within three days of sperm transfer into the spermatheca of *Metopograpsus messor.* This timing (three days) is apparently significant in this species, since the spermatophore wall dissolution could precede spawning (the copulating females possess Stage 4 ovaries) and thus ensure the participation of the sperm deposited anew in fertilizing the eggs. A precedence for the "last-male sperm" over the previous (sperm) deposits for fertilization has been suggested in some brachyuran crabs such as *I. phalangium* (Diesel, 1990) and *Chionoecetes opilio* (Fabricius) (see Sevigny and Sainte-Marie, 1996). Brachyuran spermatophores are fragile structures with an envelope made of a protein-bound mucopolysaccharide material (for reviews, see Subramoniam, 1991, 1993). The dissolution of this material would enable the spermatozoa to adhere to the egg surface and accomplish fertilization when ovulated eggs pass through the spermathecal mouth. We hypothesize that the spermathecal fluid of *M. messor* may have the auxiliary function of digesting the spermatophore wall to facilitate sperm-egg adhesion. Our (unpublished) observation of high levels of protease activity (24.21 μmoles/ml/h) within the spermathecal lumen, supports such a suggestion. Thus, the present findings reiterate the role of the spermatheca in facilitating fertilization, not only as a center for sperm storage but also acting as a site for spermatophore (wall) dissolution.

The spermatophore wall is considered to afford protection to the sperm especially during its transport from the male to the female (for reviews, see Adiyodi and Anilkumar, 1988; Subramoniam, 1991, 1993). However, there have been very few attempts to understand the ultrastructure of spermatophore wall in brachyuran crabs. The significance of "intermittent infoldings" seen on the spermatophore wall of *Metopograpsus messor* (Fig. 2A) is not clear. The structure seems to be comparable with the projections on the primary spermatophore layer of *Pacifastacus leniusculus* Dana, which connect to the sperm matter (Dudenhausen and Talbot, 1983). Furthermore, the presence of electron-dense materials within the spermatophore-wall matrix (Fig. 2B) is puzzling. These electron-dense structures could be part of the spermatophore wall, inasmuch as heterogeneity of the spermatophore wall has been shown in other decapods (Tudge 1991, 1997b).

Based on the position of the genital pore and the nature of the seminal receptacle/spermatheca, brachyuran decapods have been divided into three sections (Guinot, 1978). These are the Podotremata, characterized by the coxal location of the genital pores and complete isolation of spermatheca from the oviduct, the Heterotremata with sternal location of the female genital pores, and the Thoracotremata with sternal location of both male and female genital pores and development of the spermatheca as a diverticulum of the oviduct. Using spermatological evidence, the taxonomic and phylogenetic status of a number of podotremes in the Dromiidae (Jamieson, 1990), Dynomenidae, Homolidae, Cyclodorippidae (Jamieson, 1994), Raninidae (Jamieson, 1989a), and several heterotreme families such as the Portunidae (Jamieson, 1989a), Trapeziidae (Jamieson, 1993), Dorippidae (Jamieson, 1994), and Xanthidae (Jamieson, 1989b) have been evaluated. However, with the exception of a few studies (Jamieson, 1991; Jamieson *et al.,* 1995, 1996), the thoracotremes are not adequately studied. In this context, it is significant to note that the spermatozoal ultrastructure of *Metopograpsus messor,* a grapsid in the Thoracotremata, displays several features that deserve phylogenetic comparison.

Previous analyses of brachyuran sperm collected from the vas deferens have shown that the shape and L:W ratio of the acrosome are of phylogenetic significance. Accordingly, in heterotreme and thoracotreme brachyurans,

the L:W ratio would be in the range of 0.8–1.2, while in (primitive) podotremes where the acrosome is anteroposteriorly depressed (as in Dromiidae), the L:W ratio is shown to be as low as 0.3 (Jamieson, 1994). The occurrence of zonation in the contents of the acrosome vesicle is reported in several brachyurans (for reviews, see Pochon-Masson, 1983; Felgenhauer and Abele, 1991; Jamieson, 1991). The zonation is predominantly horizontal in dromiids and dynomenids, while it is chiefly concentric around the perforatorium in the more advanced heterotreme-thoracotreme assemblage (Jamieson, 1994). Thus, the spheroidal shape, L:W ratio of the acrosome (1.2), and the concentric arrangement of the acrosome zones of *Metopograpsus messor* are reminiscent of spermatozoa from heterotreme-thoracotreme stock. The affinity of the spermatozoa of *M. messor* to Thoracotremata (rather than Heterotremata) is shown by the presence of "onion ring" lamellation in the outer acrosome zone (Figs. 10B, 11B) (Jamieson, 1991, 1993).

The opercular cap is very diverse among thoracotreme spermatozoa. In the ocypodid *Uca tangeri* (Eydoux) (see Medina and Rodriguez, 1992), the operculum is relatively flat, while in the grapsid crab *Sesarma haematocheir* (de Haan) (see Honma *et al.,* 1992), it is conical, resembling our present observation in *Metopograpsus messor.* The opercular and subopercular zones of *M. messor* appear larger (in area) than those of *S. haematocheir.* The significance of this fact is not clear.

The perforatorium of *Metopograpsus messor,* in its extent (it extends from the posterior pole of the acrosome to the base of the operculum) and form (pointed anteriorly) (Figs. 10A, 11B), is comparable with those of spermatozoa recorded from crabs in the heterotreme-thoracotreme assemblage (Jamieson *et al.,* 1996). The exact role of the tubular formations within the perforatorial lumen of *M. messor* is enigmatic. However, in *Uca tangeri,* the "perforatorial tubules" were suggested to serve as a membrane source for male pronuclear formation during fertilization (Medina and Rodriguez, 1992).

Among grapsids, the accessory opercular ring (with horizontal orientation) has been reported to be present in two subfamilies, the Grapsinae (*Grapsus albolineatus* Lamarck) and Sesarminae (*Sesarma erythrodactyla* Hess), but absent in the Varuninae (*Varuna litterata* (Fabricius)) (see Jamieson *et al.,* 1996). In this context, the presence of an accessory opercular ring and its horizontal orientation in *Metopograpsus messor* could be considered as a feature signifying its affinity to the Grapsinae-Sesarminae subfamilies.

In summary, the spermatozoal features of *Metopograpsus messor* display typical brachyuran and decapod characters. A L:W ratio of the acrosome of 1.2 and the concentric arrangement of the acrosomal zones around the perforatorium are characters common to the heterotreme-thoracotreme assemblage. The "onion ring" lamellation of the outer acrosome zone represents a thoracotreme character. Furthermore, the presence of an accessory opercular ring (with its horizontal orientation) is indicative of its affinity to the Grapsinae-Sesarminae subfamilies within the Grapsidae. The unusually prominent periopercular rim of *M. messor* appears to be a feature that separates this species from the rest of the grapsid crabs investigated. Its phylogenetic significance, however, is unclear.

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LITERATURE CITED

Adiyodi, K. G., and G. Anilkumar. 1988. Arthropoda Crustacea.—*In:* K. G. Adiyodi and R. G. Adiyodi, eds., Reproductive biology of invertebrates. Vol. 3, Accessory sex glands. Pp. 261–318. John Wiley & Sons, Chichester, England.

Adiyodi, R. G. 1988. Reproduction and development.—*In:* W. W. Burggren and B. R. McMahon, eds., The biology of land crabs. Pp. 139–185. Cambridge University Press, Cambridge, England.

Diesel, R. 1988. Male-female association in the spider crab *Inachus phalangium*: the influence of female reproductive stage and size.—Journal of Crustacean Biology 8: 63–69.

———. 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.

———. 1990. Sperm competition and reproductive success in the decapod *Inachus phalangium* (Majidae): a male ghost spider crab that seals off rivals' sperm.—Journal of Zoology 220: 213–223.

Dudenhausen, E., and P. Talbot. 1983. An ultrastructural comparison of soft and hardened spermatophores from the crayfish *Pacifastacus leniusculus* Dana.—Canadian Journal of Zoology 61: 182–194.

Ezhilarasi, S., and T. Subramoniam. 1980. Spermathecal activity and ovarian development in *Scylla serrata* (Forskål) (Decapoda: Portunidae).—*In:* T. Subramoniam and S. Varadarajan, eds., Progress in invertebrate reproduction and aquaculture. Pp. 77–88. New Century, Madras, India.

Felgenhauer, B. E., and L. G. Abele. 1991. Morphological diversity of decapod spermatozoa.—*In:* R. T. Bauer and J. W. Martin, eds., Crustacean sexual biology. Pp. 322–341. Columbia University Press, New York, New York.

Frasca, J. M., and V. R. Parks. 1960. A routine technique for double-staining ultra-thin sections using uranyl and lead salts.—Journal of Cell Biology 25: 157–163.

Guinot, D. 1978. Principes d'un classification évolutive des Crustacés Décapodes Brachyoures.—Bulletin Biologique de la France et de la Belgique 112: 211–292.

Hartnoll, R. G. 1969. Mating in the Brachyura.—Crustaceana 16: 161–181.

Haskell, G., and A. B. Wills. 1968. Primer of chromosome practice.—Oliver & Boyd, Edinburgh & London, United Kingdom. Pp. 1–180.

Hinsch, G. W. 1986. A comparison of sperm morphologies, transfer and sperm mass storage between two species of crab, *Ovalipes ocellatus* and *Libinia emarginata.*—International Journal of Invertebrate Reproduction and Development 10: 79–87.

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

Honma, Y., M. Ogiwara, and A. Chiba. 1992. Studies on gonad maturity in some marine invertebrates—XII. Light and electron microscopic studies on spermatozoa of the land-crab, *Sesarma haematocheir* (de Haan).—Report of the Sado Marine Biological Station, Niigata University 22: 13–21.

Jamieson, B. G. M. 1989a. Ultrastructural comparison of the spermatozoa of *Ranina ranina* (Oxystomata) and of *Portunus pelagicus* (Brachygnatha) (Crustacea, Brachyura).—Zoomorphology 109: 103–111.

———. 1989b. the ultrastructure of the spermatozoa of four species of xanthid crabs (Crustacea, Brachyura, Xanthidae).—Journal of Submicroscopic Cytology and Pathology 21: 579–586.

———. 1990. The ultrastructure of the spermatozoa of *Petalomera lateralis* (Gray) (Crustacea, Brachyura, Dromiacea) and its phylogenetic significance.—Invertebrate Reproduction and Development 17: 39–45.

———. 1991. Ultrastructure and phylogeny of crustacean spermatozoa.—Memoirs of the Queensland Museum 31: 109–142.

———. 1993. Spermatological evidence for the taxonomic status of *Trapezia* (Crustacea: Brachyura: Heterotremata).—Memoirs of the Queensland Museum 33: 225–234.

———. 1994. Phylogeny of the Brachyura with particular reference to the Podotremata: evidence from a review of spermatozoal ultrastructure (Crustacea, Decapoda).—Philosophical Transactions of the Royal Society of London B 345: 373–393.

———, D. Guinot, and B. Richer De Forges. 1995. Phylogeny of the Brachyura (Crustacea, Decapoda): evidence from spermatozoal ultrastructure.—*In:* B. G. M. Jamieson, J. Ausio, and J.-L. Justine, eds., Advances in spermatozoal phylogeny and taxonomy.—Mémoires du Muséum national d'Histoire naturelle 166: 265–283.

———, ———, and ———. 1996. Contrasting spermatozoal ultrastructure in two thoracotreme crabs, *Cardiosoma carnifex* (Gecarcinidae) and *Varuna litterata* (Grapsidae) (Crustacea: Brachyura).—Invertebrate Reproduction and Development 29: 111–126.

Lindsay, L. L., and W. H. Clark. 1992. Preloading of micromolar intracellular Ca++ during capacitation of *Sicyonia ingentis* sperm, and the role of the pH decrease during the acrosome reaction.—Journal of Experimental Zoology 262: 219–229.

Medina, A. 1995. Spermatozoal ultrastructure in Dendrobranchiata (Crustacea, Decapoda): taxonomic and phylogenetic considerations.—*In:* B. G. M. Jamieson, J. Ausio, and J.-L. Justine, eds., Advances in spermatozoal phylogeny and taxonomy.—Mémoires du Muséum national d'Histoire naturelle 166: 231–242.

———, and A. Rodriguez. 1992. Spermiogenesis and sperm structure in the crab *Uca tangeri* (Crustacea, Brachyura) with special reference to the acrosome differentiation.—Zoomorphology 111: 161–165.

Orensanz, J. M., A. M. Parma, D. A. Armstrong, J. Armstrong, and P. Wardrup. 1995. The breeding ecology of *Cancer gracilis* (Crustacea: Decapoda: Cancridae) and the mating systems of cancrid crabs.—Journal of Zoology 235: 411–437.

Pochon-Masson, J. 1983. Arthropoda-Crustacea.—*In:* K. G. Adiyodi and R. G. Adiyodi, eds., Reproductive biology of invertebrates. Vol. 2. Spermatogenesis and sperm function. Pp. 407–450. John Wiley & Sons, Chichester, England.

Sainte-Marie, B., and C. Carrière. 1995. Fertilization of the second clutch of eggs of snow crab, *Chionoecetes opilio*, from females mated once or twice after their molt to maturity.—Fishery Bulletin, United States 93: 759–764.

———, S. Raymond, and J.-C. Brêthes. 1995. Growth and maturation of the benthic stages of male snow crab, *Chionoecetes opilio* (Brachyura: Majidae).—Canadian Journal of Fisheries and Aquatic Sciences 52: 903–924.

Sevigny, J.-M., and B. Sainte-Marie. 1996. Electrophoretic data support the last-male sperm precedence hypothesis in the snow crab, *Chionoecetes opilio* (Brachyura: Majidae).—Journal of Shellfish Research 15: 437–440.

Subramoniam, T. 1977. Aspects of sexual biology of the anomuran crab, *Emerita asiatica.*—Marine Biology 43: 369–377.

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

———. 1993. Spermatophores and sperm transfer in marine crustaceans.—*In:* Advances in marine biology. Vol. 29, pp. 129–214. Academic Press, New York, New York.

Sudha, K. 1992. Studies on oogenesis and the role of storage tissues in decapod crustaceans.—Ph.D. thesis, University of Calicut, Kerala, India. Pp. 1–311.

———, and G. Anilkumar. 1996. Seasonal growth and reproduction in a highly fecund brachyuran crab, *Metopograpsus messor* (Forskål) (Grapsidae).—Hydrobiologia 319: 15–21.

Tudge, C. C. 1991. Spermatophore diversity within and among the hermit crab families, Coenobitidae, Diogenidae, and Paguridae (Paguroidea, Anomura, Decapoda).—Biological Bulletin 181: 238–247.

———. 1997a. Spermatological evidence supports the taxonomic placement of the Australian endemic hairy stone crab, *Lomis hirta* (Decapoda: Anomura: Lomidae).—Memoirs of the Museum of Victoria 56: 235–244.
———. 1997b. Phylogeny of the Anomura (Decapoda, Crustacea): spermatozoa and spermatophore morphological evidence.—Contributions to Zoology 67: 125–141.

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