Original Article

The Spermatozoal Ultrastructure of the Chinese Mitten Crab (*Eriocheir sinensis*)

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Abstract

Background: The Chinese mitten crab (*Eriocheir sinensis*) is an economically important aquatic species in China. The artificial breeding crabs are also increasing in number day by day. However, knowledge about spermatozoal organization of the crab is still very limited. **Aims and Objectives:** In the present study, the spermatozoal ultrastructure of the *E. sinensis* is illustrated for improving artificial breeding technique. **Materials and Methods:** The spermatozoa are observed by light microscopy and transmission electron microscopy. **Results:** Spermatozoa are located in the lumen of seminiferous tubules. The spermatocytes and spermatids are observed in the wall of seminiferous tubules. The spermatophores are both present in the lumen of vas deferens and seminal vesicles. A mature spermatozoon consists of a central electron dense acrosome and a peripheral electron lucent nucleus within structures-organelles complex. The acrosome is divided into three zones, including inner acrosome zone, outer acrosome zone and zonal texture. The centre of acrosome is the perforatorium within parallel arranged perforatorial tubules along vertical axis. The highest electron dense operculum surrounds the head side of perforatorium. **Conclusion:** The ultrastructure of spermatozoa of *E. sinensis* is illustrated. In particular, the outermost part of the acrosome appears as concentric circles and is described as zonal texture.

Keywords: Crab, spermatozoa, transmission electron microscopy, ultrastructure

Introduction

The Chinese mitten crab (*Eriocheir sinensis*) (also known as the big sluice crab) is defined as *Crustacea*, *Decapoda*, and *Grapsidae*. The crab is a native breed to China and has also spread to Europe and America.[1,2] The *E. sinensis* is rich in nutrition.[3] In East Asia, especially in China, a vast amount of the crabs are cooked as a delicacy.[1,3] However, the wild resources of the crab are in short supply. The artificial breeding crabs are increasing in number to meet market demands. The aquaculturists have to breed artificially the crab larva for aquaculture each year. Consequently, the crab is an economically important aquatic species in China. On the contrary, in Europe and America, the crab is an invasive species that destroys the local ecological balance.[1] In addition, Europeans do not have much interest in taste of this crab. Therefore, the number of the crab has to be controlled in nonindigenous regions. The revealing of gamete organization and reproductive characteristics is required to control the number of the crab and protect ecology.

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Previous studies have been involved in morphology of the Chinese mitten crab,[2,4-6] and spermatogenesis-related genes were identified.[7,8] However, ultrastructural knowledge about spermatozoa of the Chinese mitten crab is limited. Moreover, spermatozoa of the decapod crustaceans show distinctive characteristics compared to other farmed animals such as fish. For example, spermatozoa of these animals are immotile.[9] Therefore, the studies of different structures of the spermatozoon in decapod crustaceans can facilitate understanding of reproductive processes, such as spermatozoon capacitation, mechanism of egg–spermatozoon binding, and fertilization,[10] and subsequently can contribute in the further development of techniques for their artificial reproduction. In this work, spermatozoal ultrastructure of the Chinese mitten crab was investigated by transmission electron

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microscopy (TEM). The morphological features of organelles and biometrical quantitative data of the acrosome have been used to distinguish different members of decapods.[11,12] Knowledge regarding the morphology of the spermatozoon can be used for taxonomic and phylogenetic studies of animals. Our results will facilitate to better understand reproductive biology, comparative histology, and evolutionary biology of the crab.

Materials and Methods

Experimental animals

The cultured commercial *E. sinensis* (n = 12) from Junshan Lake in the southeast of China are sampled. This work is approved by the Scientific and Technical Department of Jiangxi Agricultural University. The *E. sinensis* are placed on ice to make them dormant and euthanized, and they are quickly executed and the tissues are excised. All measures are taken to minimize the suffering of the experimental animals.

Histology

The histology protocol is conducted according to a previous study.[13] Briefly, the seminal vesicle samples are fixed in Bouin's solution for 24 h at room temperature. After washing, the samples are dehydrated in ethanol and embedded in paraffin. The samples are cut (5 μm) on a microtome (Yidi, Jinhua, China). After removing the paraffin, the sections are stained with hematoxylin and eosin, and then, the sections are mounted and observed with a BM 2000 Light Microscopy (Yongxin, Nanjing, China). The micrographs are acquired by ScopeImage 9.0 (H3D) Software (Yongxin, Nanjing, China).

Transmission electron microscopy

TEM is conducted according to the method of Zhang *et al*.[13] Small pieces of the testis, vas deferens, and seminal vesicles are excised and fixed in 2.5% glutaraldehyde/phosphate-buffered saline (PBS) for 24 h at 4°C. After wishing with 0.01 M PBS (pH 7.4), the samples are postfixed in 1% OsO_4 for 2 h, and the they are washed in PBS, dehydrated in a series of concentration of ethanol, dehydrated in acetone, and then embedded in Spurr's resin at 37°C for 24 h and at 60°C for 48 h. The samples are sectioned with an ultramicrotome (LKB, Stockholm, Sweden) and are stained with 1% uranyl acetate and Reynold's lead citrate for 20 min. Finally, the stained sections are observed and photographed by a TEM (Hitachi H-7600) with a high-resolution digital camera (Tokyo, Japan).

Results

Microstructure of spermatozoa of *Eriocheir sinensis*

By light microscopy, the spermatozoa of *E. sinensis* are observed in the spermatophore in the lumen of seminal vesicle [Figure 1a and b]. The spermatophores exhibit roundish shape. A basophilous acrosome is observed in the anterior part and nucleus is posterior to the spermatozoon [Figure 1b].

Ultrastructure of spermatozoa of *Eriocheir sinensis*

By TEM observation, the round electron-dense spermatozoa are located in the lumen of seminiferous tubules [Figure 1c].

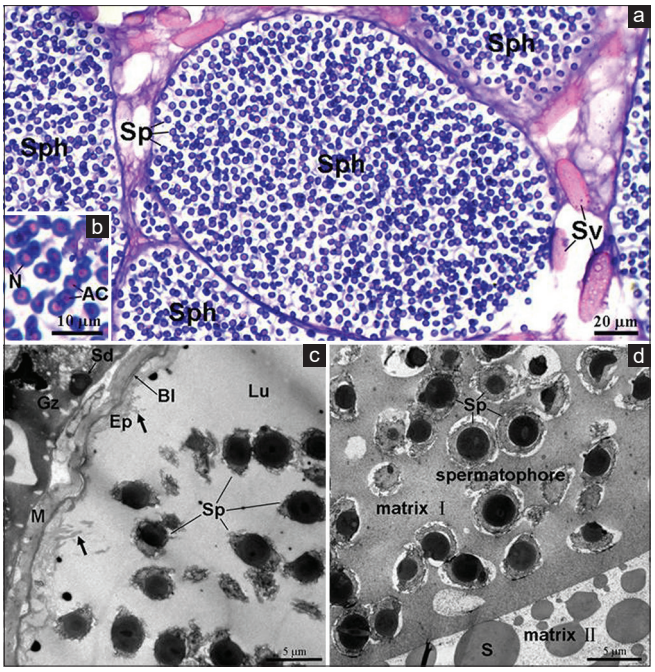

Figure 1: Light microscopy micrographs of the seminal vesicle and transmission electron micrographs of the seminiferous tubule and spermatophore in the vas deferens of *Eriocheir sinensis*. (a) Microstructure of spermatozoa (H and E). The spermatophores (Sph) are observed in the seminal vesicle. Each spermatophore contains a large number of spermatozoa (Sp). Secretory vesicles (Sv) are observed in the matrix between spermatophores. (b) High magnification micrograph for showing the details of spermatozoa (H and E). AC: Acrosome, N: Nucleus. (c) Transmission electron micrograph of seminiferous tubule wall and the lumen within spermatozoa (Sp) of *Eriocheir sinensis*. Epithelial lining of the seminiferous tubule shows short microvilli (thick arrows) in the apical border of epithelial cells (Ep). BL, basal lamina; Sd: Spermatid, GZ: Germinal zone, Lu: Lumen, M: Muscle layer. (d) Transmission electron micrograph of a spermatophore in lumen of vas deferens of *Eriocheir sinensis*. Spermatozoa (Sp) are enveloped by the matrix I. The secretion (S) disperses in electron-lucent matrix II

The wall of seminiferous tubule consists of epithelial cells with sparse microvilli, muscular layer, and germinal zone [Figure 1c]. Many round vesicles are present in apical cytoplasma of the epithelial cells. The round secretion and a big spermatophore containing spermatozoa are located in the lumen of vas deferens [Figure 1d]. The spermatophores are also observed in the lumen of seminal vesicle [Figure 1d].

The ultrastructure of mature spermatozoa is showed by higher magnification TEM images [Figure 2a and b]. The spermatozoa are aflagellate. Whole spermatozoon consists of nearly spherical acrosome and peripheral nucleus with an irregular outline. The acrosome is divided into three zones, including inner acrosome zone, outer acrosome zone, and zonal texture, according to the electron-dense and diverging ultrastructure. The inner acrosome zone is higher electron dense and surrounded the perforatorium, which contains the perforatorial tubules parallel to each other. The zonal texture is located the outermost layer of acrosome. Several visible concentric striations are parallel arranged in zonal texture.

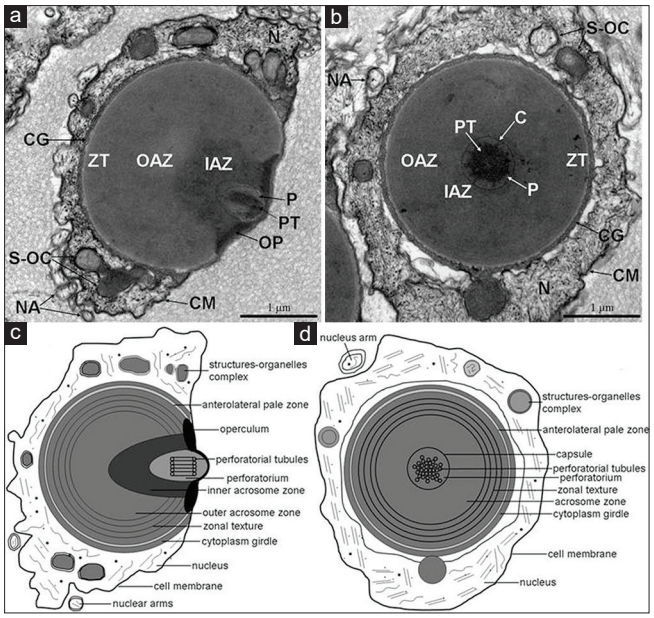

Figure 2: Transmission electron micrographs and schematic diagrams of spermatozoa of *Eriocheir sinensis*. (a) Transmission electron micrograph of tangential section of spermatozoon of *Eriocheir sinensis*. (b) Transmission electron micrograph of transverse sections of spermatozoon of *Eriocheir sinensis*. (c) Schematic diagrams of tangential section of spermatozoon. (d) Schematic diagrams of transverse section of spermatozoon. IAZ: Inner acrosome zone, OAZ: Outer acrosome zone, ZT: Zonal texture, P: Perforatorium, PT: Perforatorial tubules, OP: Operculum, N: Nucleus, CG: Cytoplasm girdle, S-OC: Structures–organelles complex, NA: Nuclear arms, CM: Cell membrane

The highest electron-dense operculum wraps around the head sides of perforatorium. The whole acrosome is surrounded by the electron-lucent nucleus, which contains several electron-dense roundish structures–organelles complexes. The projecting nuclear arms extend from the edge of nucleus. The cytoplasm girdle is present between acrosome and nucleus. The schematic diagrams of spermatozoa are drawn by their TEM images [Figure 2c and d].

Discussion

Previous studies have demonstrated that spermatozoal ultrastructure of different crabs presented some differences, mainly regarding the ultrastructure of acrosome.[14,15] In the present study, the outermost acrosome zone of spermatozoa of *E. sinensis* as "zonal texture" because several concentric striations were present in the region. A structure called "lamellar structure" was observed in the outermost layer of acrosome and was probably the striation structure of zonal texture in a previous study.[2] The regular concentric striations were not present in spermatozoa of others crabs, but similar structure was observed in crayfish spermatozoa.[16]

In the present study, the perforatorial tubules were observed in perforatorium of spermatozoa of *E. sinensis*, but not in perforatorium of *Homolodromia kai, Sphaerodromia lamellate*, and *Dynomenetanensis spermatozoa*.[17] The tubular membranous structures were also present in spermatozoa of European pea crabs and three species of fiddler crab.[15] Acrosome is the most prominent organelle in spermatozoon of decapod crustaceans because of its responsibility for egg–spermatozoon binding and fertilization in the immotile spermatozoon of decapods.[18] It has been demonstrated that during postmating spermatozoon storage on the body of the female, subcellular parts analogous to perforatorium in the spermatozoa of decapods undergo morphological changes such as further development, condensation, and calcification that enables the spermatozoon to fertilize egg.[18,19]

In addition, in the present study, nuclear arms were observed in the periphery of spermatozoa of *E. sinensis*. Microtubular nature of nuclear arms was confirmed by identification of tubulin as a main protein of microtubules in the proteomic studies of decapod male gamete.[18,20] Moreover, a protein tyrosine phosphorylation has been reported in the nuclear arms of the immotile spermatozoon of decapods during capacitation that brings about new evolutionary questions regarding possible origin, roles, and functions of this organelle.[21]

Conclusion

In the present study, we demonstrated the organization of spermatozoa of *E. sinensis*. The ultrastructure of the spermatozoa is roughly the same as that of other crabs, but there are some differences in acrosome structure. In particular, the outermost part of the acrosome appears as concentric circles and is described as zonal texture.

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Conflicts of interest

There are no conflicts of interest.

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