SPERMATOGENESIS OF THE FRESHWATER CLAM CORBICULA AFF. FLEMINEA MULLER (BIVALVIA: CORBICULIDAE)

KOOICHI KONISHI,1 KOUCHI KAWAMURA,1 HIROFUMI FURUTA,1 AND AKIRA KOMARU1,2
1National Research Institute of Aquaculture
Naruto, Mie 516-0193, Japan
2Faculty of Bioresources
Mie University, Tsu 514-8507, Japan

ABSTRACT
Spermato genesis of the hemaphroditic freshwater clam, Corbicula aff. fluminea Muller was described from light and electron microscopy. During the spermatogenetic process, difference between primary and secondary spermatocytes was hardly recognized. Mature spermatids consist of an acrosome about 13.7 ± 0.32 μm in length with a rod-like acrosome, indistinct midpiece, and two long flagella, 2.5 times as long as the head, each flagellum with a very thin undulating membrane. Comparison of the gamete morphology in Corbicula species presents two major groups, unflagellated and flagellated. Spermatids of C. levantina and C. aff. fluminea, in spite of their close similarity, were distinguished by their size and number of mitochondria.

KEY WORDS: spermato genesis, ultrastructure, flagella, hemaphroditic, Corbicula

INTRODUCTION
Studies on the reproductive biology of freshwater corbiculid clams, especially detailed studies of their spermatogeny, are few in spite of its commercial importance of all the inland water fisheries in Japan, about 30% of the annual catch is accounted for by Corbicula clams. Ultrastructural studies of the spermatozoa of the Japanese corbiculid clams are published for only two species: Corbicula monodonta Reinhardt and C. leonina (Prune) (Hachiri and Higashi 1970, Komaru and Konishi 1996). These studies distinguished two types of spermatozoa, unflagellated and flagellated, were found within the same penis. Further, only a few previous works have described the ultrastructure of the complete spermatogenetic process in freshwater bivalves (Higashi 1964, Rocha and Asvaduro 1990), and no data are available for Corbiculidae. Recently, in a course of the genetic study on populations of C. leonina in Kyushu, southern Japan, we sampled many specimens that were almost morphologically identical with C. fluminea Muller, and the gonads of these clams were histologically examined to allow a comparison of the spermatogenetic process within Corbicula. The taxonomy of the corbiculid species, however, is complicated and confused at present. Four Corbicula species have been described from Japan (Habe 1977, Masuda and Habe 1988), although some authors listed C. fluminea in the bivalve fauna of Japan (Wong 1988, Hu and Tao 1995). Martin (1979), however, proposed only a two-species-complex, C. fluminea and C. flumana, from the Asian region. We herewith tentatively use the name C. aff. fluminea Muller (for details, see Komaru et al. 1998).

In this paper, we describe the ultrastructure of spermatogenesis of C. aff. fluminea from Japan and compare its morphological characters with those of other freshwater bivalves including Corbicula species.

MATERIAL AND METHODS
Clams were collected from the Tama River, Saga Prefecture, Japan. Intact sperm were obtained by dissecting fresh gonads, and the length of the head and flagella were measured with an ocular micrometer for specimens just after the termination of their movement by adding 1% formalin from the fringe of the cover slip. The soft part of the clams were fixed with Bozin’s solution and processed as for standard paraffin embedding methods. Serial sections in 5 μm thickness were stained with Mayer’s hematoxylin and eosin. The sections were observed with an Olympus BH-2 microscope. Measurements of testicular cells were performed in 20-50 cells, ranging at least two clams, based on these sections using an ocular micrometer.

For electron microscope observations, small pieces of gonads were dissected under a binocular microscope, and prefixed with 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.5) at 4°C. The prefixed tissue was rinsed with the same buffer three times and then post-fixed with 1.0% osmium tetroxide in the same buffer. After fixation, the tissues were dehydrated through a graded acetone series and embedded in an epoxy resin Quetol 812 (Nissin EM Co., Tokyo). Ultrathin sections were stained with aqueous uranyl acetate and lead nitrate, and then observed with a JEOL 1200 EX transmission electron microscope.

RESULTS

Gross morphology of tests
Tests are fixed adjacent to the ovaries, and occasionally taeniata gonads are mingled. From outer wall to inner core of the tests, various stages of germ cells are observable. Mature spermatogonia form spherical or hemispherical clusters in the lumen of the testis (Fig. 1A, asterisk) attaching their head onto a large Sertoli-like cell (Fig. 2C, asterisk). Spermatogenesis (Fig. 1B)

In the peripheral region of the testis, large polygonal spermatogonia are visible, mean value ± S.D. of the nuclear diameter is 5.84 ± 0.47 μm (n = 20), and the nucleus contains a prominent electron-dense nucleus in some sections (Fig. 1B, arrow). Chromatins are not condensed. A number of mitochondria are scattered around the nucleus.

Spermatocytes (Fig. 1C)
Round cells, 3.7 ± 0.47 μm in mean nuclear diameter (n = 50). The nucleus is somewhat condensed and volume of cytoplasm is
Figure 1. Spermatogonial process of Corbicula aff. luminosa Müller. (A) Gross morphology of the testis by light microscopy. Spermatagonia (sg) are found at the periphery of the gland. Asterisk shows Sertoli-like cells onto which mature spermatocytes attach their head forming a cluster. (B) Spermatogonia. Nucleus (ns) is found. (C) Spermatocyte. (D) Early spermatid. Note proximal (pc) and distal (dc) centrioles. Transverse section of flagella (f) is also recognized. (E) Middle spermatid. Acrosomal structure (a) is not apparent. Note numerous fibrils of chromatin arranged with the longitudinal axis (in detail), see inset. (F) Late spermatid. Scale bars = 10 μm for A and 1 μm for B-F.
more reduced than in the spermatagonia. Meiotic figures are frequently recognized (Fig. 1C, asterisk). The primary and secondary spermatocytes are hardly distinguishable in morphology.

**Spermatids (Figs. 1D–F)**

Early spermatid, 3.0 ± 0.11 μm in mean nuclear diameter (n = 20), has scant cytoplasm, and large mitochondria which are displaced to one side of the cell. A pair of centrioles is found near the basal end of the cell, consisting of proximal and distal centriole (Fig. 1D, arrows). A flagellum originates from the distal centriole at this stage in some specimens. In the middle stage, the nucleus becomes elongated, and numerous fibrils are arranged longitudinally in it (Fig. 1E and inset). The acrosomal structure is recognizable at the anterior part of the head (Fig. 1E, arrow). The nucleus of the late spermatids becomes more elongated with condensed chromatin and the acrosome is now conspicuous. The proximal centriole which was perpendicular to the distal one was not recognized. The shape of the mitochondria is elongated and flattened (Fig. 1F).

**Spermatids (Figs. 2A–F)**

The head, 13.9 ± 0.32 μm in length (n = 20), consists of a rod-like acrosome and a long nucleus capped with four tightly packed mitochondria which form a cove-like cluster (Fig. 2D). The length of the flagella is approximately 2.5 times as long as the head. In the head region, the acrosome is of a tapered form and the outer electron-lucent layer and inner moderate dense region are distinguished (Fig. 2B). The chromatin of the nucleus is more condensed and numerous small low electron dense patches are found. Two centrioles are located parallel to longitudinal axis at basal part of the head (Fig. 2E and F) and long flagella originate from each centriole. The basal part of one centriole (Fig. 2E).

---

**Figure 2.** Spermatids of Corbicula aff. fluminea Müller. (A) Mature spermatids, (B) Acrosome on the head, (C) Spermatids which the head attaching to a Sertoli-like cell (asterisk), (D) Transverse section of posterior midpiece, Four mitochondria surround nucleus, (E) Longitudinal section of posterior midpiece, Two flagella (f) originate from each centriole, but not that the basal part of one flagellum is curved forward to the base of another flagellum (asterisk), (F) Transverse section of posterior end of midpiece, Scale bars = 1 μm for A–B, 500 nm for C, and 200 nm for D–F.
**Discussion**

In spermatogenic studies of bivalves, it has been noted that secondary spermatocytes were rarely observed. For this reason, Sazry (1979) stated that the division rate was too rapid to recognize the second meiosis histologically. Two stages of spermatocytes, however, were recognized in C. leucana (Ikeharata and Yamane 1977) and C. japonica (Murakami 1981) by light microscopy. According to Takahashi and Takano (1970), the secondary spermatocytes are distinguishable from the primary spermatocytes by their size. In the present histological observations, no distinct groups were detected in the nuclear size of the spermatocytes. Electron microscopic figures also support this result. On the other hand, the chromosome number and DNA content of the three Corbicula species including C. aff. Flamentia suggest the possibility of first or second meiosis being omitted in the reproduction of these clams (Komatsu et al. 1997). If this omission actually exists in the present species, the absence of a definite mitotic step will be reasonable.

Krämer (1983) distinguished two types of biflagellate spermatids in C. flamentia: wide-headed and slender-head. In the wide-headed type, which was suggested to be a post-mature form, one of the pair of flagella was often relatively motile. In this study, a pair of centricones is recognized in the early spermatid stage, and one of the parallel centrioles in the mature spermatids is curved toward the base of the other centriole. It is most likely that after one flagellum is extruded from the distal centriole, then the proximal one moves its position parallel to the longitudinal axis, and finally the second flagellum originates from it.

Table 1 summarizes the gross sperm morphology and developmental mode in freshwater bivalves including Corbicula species. Two distinct groups are recognized among the corbiculids: uniflagellate and biflagellate spermatids. The groups correspond with other aspects of their reproduction. Biflagellate and hemispheroidal species, includes C. leucana, C. flamentia, and the present species. The spermatids of C. aff. flamentia are different from those of C. leucana in two aspects: 1) the number of mitochondria is four in C. aff. flamentia and five in C. leucana; and 2) the head length of the present species is smaller than that of C. leucana (16.9 μm and 13.9 μm in average, respectively).

Early spermatological studies suggested that spermatozoon of the Bivalvia were classified as a primitive type (e.g., Fränz 1956). Successive works, however, have revealed the presence of modified spermatogenesis in bivalves in different families. Tellina and Codakia (Souza and Freire 1996). Popahan (1979) was the first to review the class from sperm morphology, and suggested that the most useful taxonomic application of comparative sperm morphology in the Bivalvia seems to lie at the species and genus level.

Most spermatozoa of freshwater bivalves are of a primitive type which have a rudimentary acrosome, a short head, five spherical mitochondria, and a long simple flagellum (e.g., Higgins 1964, Trumble and Gaudin 1975, Perez et al. 1990, Rocha and Azevedo 1990, Lynne 1994). The gross sperm morphology of freshwater Corbicula is a modified type among bivalve spermatozoa. This form may be related with their specialized mode of reproduction: hermaphroditic and internal fertilization (Kuruma and Kondo 1996). Fränz (1983) also noted that there was a correlation between the evolution of elongated sperm nucleus and large, yolk-rich yolk. According to Healy's (1996) definition, the sperm of corbiculid clams belongs to an end-aquaperms of which sperm are released into the ambient water and fertilization occurs within the coelom of the mantle cavity. He also commented on the resemblance of sperm morphology between freshwater Corbiculidae and marine Tellinidae in both having an elongated nucleus and similar midpiece structures.

Popahan (1979) pointed out that the presence or absence of acrosomal structures may be indirectly correlated with the brooding of developing young. Perez et al. (1990) compared the sperm morphology and development in freshwater bivalves, and came to a similar conclusion. In fact this is true for the upper six species in Table 1. Based on recent data, however, their category seems to be inadequate for the freshwater bivalves such as found in Japanese corbiculid species. As shown in Table 1, the spermatozoa of C. sansui have a prominent acrosome although the developmental type of this species 's not-brooding: e.g., this clams release developing eggs to ambient water (Mizuyaki 1936, Furukawa and Mizumoto 1953). This suggests that the reproductive strategies in the Corbiculidae species and their sperm morphology are not simply categorized by previous models and classifications.

**Acknowledgments**

This work was partly supported by a grant from the Science and Technology Agency, Japan. We express our sincere gratitude to T. Kagawa for his help in the preparation and sectioning of the material.

---

**Table 1**

Comparison of sperm morphology and developmental mode in freshwater bivalves.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Spem</th>
<th>Ac.</th>
<th>Mit.</th>
<th>Flagellae</th>
<th>Brooding</th>
<th>Sexuality</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unionidae</td>
<td>Hyriopsis elegens</td>
<td>primitive</td>
<td>4</td>
<td>1</td>
<td>Yes</td>
<td>D</td>
<td>Higashi (1984)</td>
<td></td>
</tr>
<tr>
<td>Unionidae</td>
<td>Lepisma sowerbri</td>
<td>primitive</td>
<td>4</td>
<td>1</td>
<td>Yes</td>
<td>D</td>
<td>Trumble and Gaudin (1975)</td>
<td></td>
</tr>
<tr>
<td>Unionidae</td>
<td>Anodonta cygnea</td>
<td>primitive</td>
<td>5</td>
<td>1</td>
<td>Yes</td>
<td>D</td>
<td>Rocha and Azevedo (1990)</td>
<td></td>
</tr>
<tr>
<td>Unionidae</td>
<td>Anodonta concinna</td>
<td>primitive</td>
<td>5</td>
<td>1</td>
<td>Yes</td>
<td>D</td>
<td>Lynne (1994)</td>
<td></td>
</tr>
<tr>
<td>Hyriidae</td>
<td>Diplopleurochiton chiengshih</td>
<td>primitive</td>
<td>5</td>
<td>1</td>
<td>Yes</td>
<td>D</td>
<td>Prezo et al. (1990)</td>
<td></td>
</tr>
<tr>
<td>Mabuiidae</td>
<td>Nezumia penepalmata</td>
<td>primitive</td>
<td>4-5</td>
<td>1</td>
<td>No</td>
<td>D</td>
<td>Fränz (1983)</td>
<td></td>
</tr>
<tr>
<td>Corbiculidae</td>
<td>Corbicula sansui</td>
<td>modified</td>
<td>4</td>
<td>1</td>
<td>No</td>
<td>D</td>
<td>Huchiri and Higashi (1970)</td>
<td></td>
</tr>
<tr>
<td>Corbiculidae</td>
<td>Corbicula flamentia</td>
<td>modified</td>
<td>5</td>
<td>2</td>
<td>Yes</td>
<td>H</td>
<td>Konuma and Kondo (1990)</td>
<td></td>
</tr>
<tr>
<td>Corbiculidae</td>
<td>Corbicula flamentia</td>
<td>modified</td>
<td>5</td>
<td>2</td>
<td>Yes</td>
<td>H</td>
<td>Krämer (1983)</td>
<td></td>
</tr>
<tr>
<td>Corbiculidae</td>
<td>Corbicula aff. flamentia</td>
<td>modified</td>
<td>4</td>
<td>2</td>
<td>Yes</td>
<td>H</td>
<td>this study</td>
<td></td>
</tr>
</tbody>
</table>

Ac. = acrosome; D = dioecious; H = hermaphrodite; Mit. = mitochondrion; + = present; - = absent.