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Zoomorphology

Spermiogenesis and sperm structure in the crab *Uca tangeri* (Crustacea, Brachyura), with special reference to the acrosome differentiation

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Summary. Early spermatids of the crab *Uca tangeri* consists of the nucleus of granular chromatin and the cytoplasm, which contains a proacrosomal vesicle in close association with membrane lamellae. In the mid spermatids an invagination of the acrosomal vesicle membrane gives rise to the formation of the perforatorium, a spindle-shaped tubule which encloses tubular membranous structures. The pair of centrioles located at the base of the acrosome is not directly involved in perforatorial differentiation. The acrosomal vesicle shows a heterogeneous content composed of the operculum, the thickened ring, and three layers of different materials concentrically arranged around the perforatorium. During the late spermatid stage the nuclear profile differentiates numerous slender arms and the chromatin arranges into fibers. Membranous tubules from the cytoplasm become incorporated into the tubular structures of the perforatorium. The mature spermatozoon has the typical structure of the branchyuran sperm, with a complex acrosome, cupped by the nucleus, and a thin cytoplasmic band intervening between the former main elements. The centrioles are degenerate. The nuclear arms are unusually numerous (more than 20) and lack microtubules or microtubular derivatives.

A. Introduction

In Brachyura, the spermatozoon is a bizarre cell consisting mainly of an uncondensed nucleus with radial arms and a large acrosome. The fine structure of this sperm type is well known in various crab species from different families (Yasuzumi 1960; Langreth 1965, 1969; Pochon-Masson 1965, 1968; Brown 1966; Hinsch 1969, 1973, 1986, 1988; Reger 1970; Jamieson 1989a, b, 1990; Jamieson and Tudge 1990), but only a few accounts have dealt with the process of differentiation of the sperm cell (Moses 1960; Yasuzumi 1960; Langreth 1969; Reger 1970; Pearson and Walker 1975). The study of the origin and formation of the gamete constituents would help, however, to clarify some aspects of the sperm biology during fertilization, a process which shows interesting peculiarities in crabs (Brown 1966; Hinsch 1971; Goudeau 1982).

Comparative studies on sperm ultrastructure are considered to be useful in establishing phylogenetic relationships between different taxa in Brachyura (Hinsch 1973). Recently, Jamieson (1989a, b, 1990) and Jamieson and Tudge (1990) have used morphological features of the spermatozoa as phylogenetic criteria. This paper describes the spermiogenesis of the fiddler crab, *Uca tangeri*, and compares the sperm structure with previous data from other species of Brachyura. For nomenclature unification, the sperm constituents will be named according to the terminology employed by Jamieson (1989b, see Table 2).

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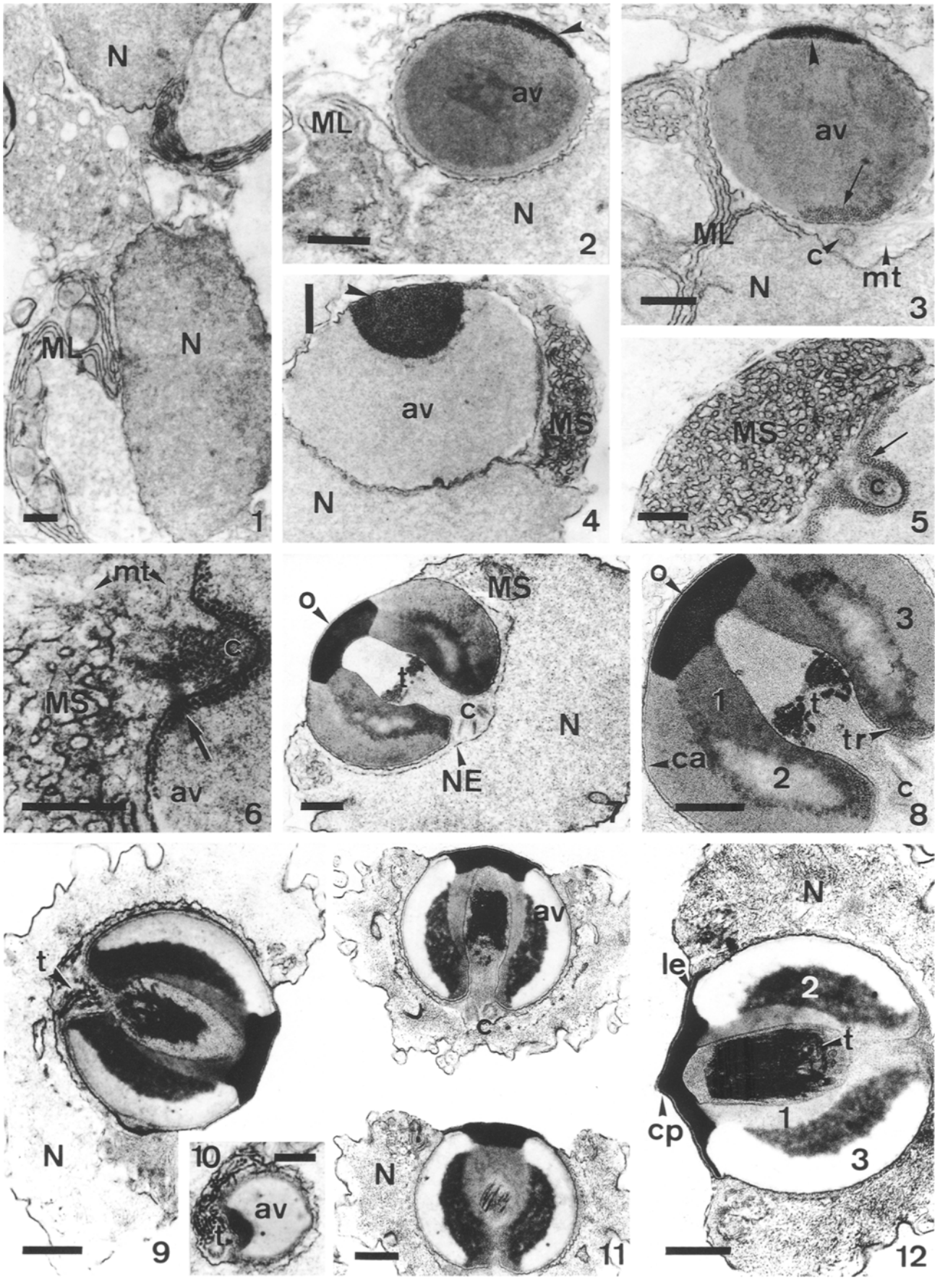
B. Materials and methods

Adult male specimens of *Uca tangeri* (Eydoux, 1835) were collected from salt marshes in the San Pedro Canal (Puerto Real, Southern Spain). Small fragments of testicles and vasa deferentia were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), supplemented with 3.5% sucrose, for 3 h at 4° C. After two rinses in cacodylate buffer for 30 min, the samples were post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, washed in distilled water, dehydrated in acetone, and embedded in Spurr. Thin sections were picked up on copper grids, stained with uranyl acetate and lead citrate, and viewed in a Jeol JEM 1200 EX microscope.

C. Results

1. Early spermatids (Figs. 1–6)

The early spermatids of *U. tangeri* are polarized cells measuring about 6 μm which possess a homogeneous, finely granular nucleus and a reduced cytoplasmic mass.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Most of the cytoplasm is located in a position adjacent to the anterior side of the nucleus and largely consists of membrane lamellae arranged in concentric layers around extensive areas of low electron density (Figs. 1–3). At the proximal side, the membrane lamellae are adjacent to the nucleus. However, continuity of the nuclear envelope with the outer membrane sheet has not been observed. Mitochondria with very few cristae may be occasionally identified between the membrane lamellae.

In association with the lamellar membranes and nuclear envelope (Fig. 2), a proacrosomal vesicle measuring 1.5–2 μm begins to form. It shows a dense core and a thin peripheral band (Fig. 2). As spermiogenesis proceeds, the size of this organelle increases and two condensations arise at both its poles (Fig. 3). The proximal condensation is made up of a granular material that accumulates next to the nucleus in the basal region. The distal condensation consists of more densely packed granules underlying the apical region of the vesicle membrane. Occasionally, centrioles accompanied by microtubules are seen between the nuclear envelope and the proximal side of the acrosomal vesicle (Fig. 3).

At the end of the early spermatid stage, the membrane lamellae arrange into an anastomosing tubular pattern constituting a membrane system that is located at one side of the cell. The spermatid now can be divided into three parts which correspond to the nucleus, the enlarged acrosomal vesicle, and the membrane system (Fig. 4). The acrosomal vesicle reaches 3–4 μm. The distal condensation has increased in size, whereas the proximal condensation appears to become oriented toward the lamellar region. At this point, the acrosomal vesicle membrane shows a shallow indentation where a single centriole is lodged (Figs. 5, 6). Microtubules radiating from the centriole disappear into the membrane system (Fig. 6).

2. Mid spermatids (Figs. 7, 8)

The mid spermatid stage is characterized by the formation of the perforatorium, a long tubule which runs through the acrosomal vesicle from the base to nearly contact the apex (Fig. 7). The lower portion of the acrosome is cupped by the nucleus. The cytoplasm is reduced to a thin band between the acrosome and nucleus which enlarges at its anteriormost end, where remnants of mitochondria and other membranous organelles are located. The nucleus has a more or less regular profile and a granular chromatin that is bounded externally by the nuclear envelope and the plasma membrane. The nuclear envelope shows discontinuities adjacent to the acrosomal complex (Fig. 7).

The structure of the acrosome in mid spermatids does not greatly differ from the one in mature sperm. The perforatorium differentiates from a deep indentation of the acrosomal membrane, which stretches to contact the distal condensation. Neither of the pair of centrioles accompanies the invaginating acrosomal membrane in its upward movement, but both remain at the base of the perforatorium (Figs. 7, 8). The slightly swollen mid region of the perforatorium is filled with vesicular or tubular structures. The upper half has a finely granular content and the lower portion is filamentous. The granular material that surrounds the basal opening of the perforatorial tubule (thickened ring) derives from the proximal condensation of the acrosomal vesicle. The distal condensation differentiates in the operculum, an extremely electron-dense structure underlined by a slightly paler subopercular zone (Figs. 7, 8).

The inner structure of the vesicle is heterogeneous (Fig. 8). Under the boundary membrane, a thin dense deposit, known as the capsule, surrounds the vesicle contents. The vesicle contains three layers (labeled 1–3 in Fig. 8) arranged concentrically around the perforatorium. The inner layer is wider under the apical cap. The mid layer may or may not include electron-lucent areas. The outer layer fills the remainder of the vesicle.

3. Late spermatids (Figs. 9–12)

The passage from mid to late spermatid is marked by the appearance of invaginations and pseudopod-like prolongations in the nuclear profile. The chromatin begins to arrange into fibers that become more elongated and coarser. As the chromatin arranges into fibrillar elements, some areas of nucleoplasm appear electron lucent (Figs. 9–12). These chromatin-free areas are preferentially located in the peripheral regions. In the latest spermatids the nuclear prolongations are numerous (Figs. 11, 12). These appendages or nuclear arms are filled with a low number of chromatin fibers and lack microtubules.

The inner organization of the acrosomal vesicle remains as in the previous stage, excepting that the opercu-

Figs. 1–12. Spermatids of *Uca tangeri*. Scale 500 nm

Figs. 1–6. Early spermatids. **1** Stage previous to the appearance of the proacrosomal vesicle. **2, 3** A conspicuous proacrosomal vesicle is present close to the nucleus and membrane lamellae. **4–6** At the end of the early spermatid stage, the acrosomal vesicle is swollen and shows a dense apical condensation. At its base, a small indentation lodges a single centriole. The membrane lamellae form an anastomosing membrane system

Figs. 7, 8. Mid spermatids. The perforatorium is formed. Vesicular or tubular structures fill the perforatorial tubule. The acrosomal vesicle shows a heterogeneous content

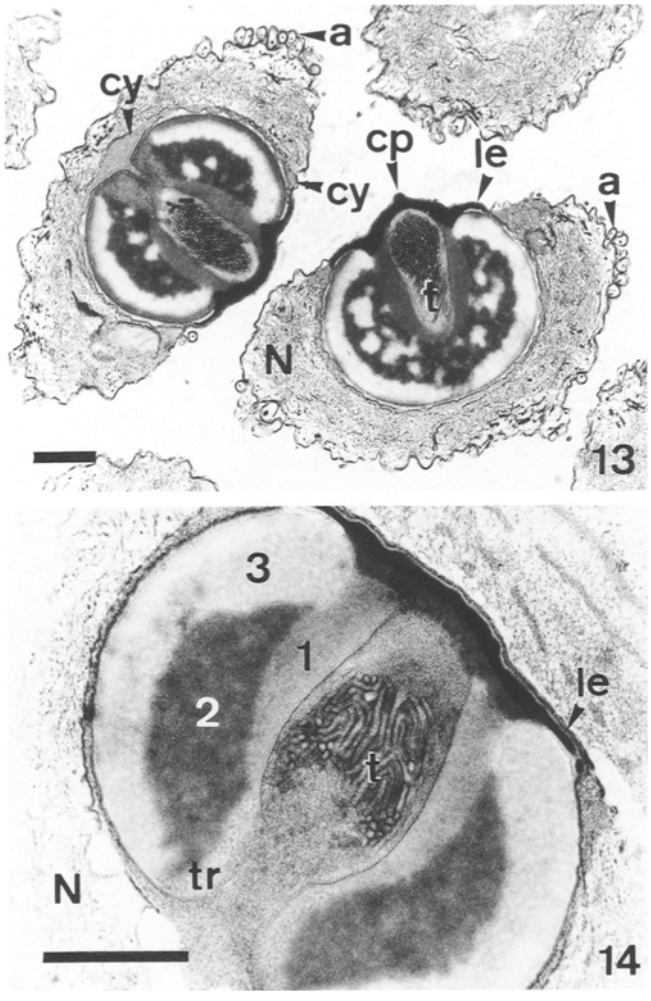
Figs. 9–12. Late spermatids. The nucleus progressively forms pseudopod-like prolongations which are precursors of the nuclear arms. The chromatin becomes arragned into fibers. During this stage tubular membrane formations migrate from the cytoplasmic band into the perforatorium and join with the pre-existing tubular structures (9, 10)

Abbreviations used in the figures: *arrows*, proximal condensation; *arrowheads*, distal condensation; *a*, nuclear arms; *av*, (pro) acrosomal vesicle; *c*, centrioles; *ca*, capsule; *cp*, central prominence of operculum; *cy*, cytoplasm; *le*, lateral expansions of operculum; *ML*, membrane lamellae; *MS*, membrane system; *mt*, microtubules; *N*, nucleus; *NE*, nuclear envelope; *o*, operculum; *t*, tubular membranous structures; *tr*, thickened ring; *1, 2, 3*, different materials of the acrosomal vesicle

lum differentiates a central prominence and lateral expansions (Fig. 12). The membranous component of the perforatorium increases (Figs. 9, 11, 12). Tubular membranous structures invade the perforatorium from the cytoplasm through its basal opening (Figs. 9, 10 appear to show this in spermatids sectioned at different angles). This tubuliform membrane system would actually coalesce and mingle with the pre-existing membranous structures to form a compact mass (Fig. 9).

4. Mature sperm (Figs. 13, 14)

The mature sperm is lens-shaped, with a voluminous acrosome and a cup-shaped nucleus which contains fibrous chromatin and possesses numerous radial arms wrapped around the cell at the equatorial plane (Fig. 13). The cytoplasmic region is very reduced, although some membrane materials, sometimes including mitochondria, are retained anteriorly. The lower acrosome surface is cupped by the nucleus, but its anterior face (the opercular region) is free. The definitive perforatorium consists of convoluted (Fig. 14) or helically oriented (Fig. 13) membrane tubules followed basally by a filamentous or granular material, which is, in turn, in continuity with the cytoplasmic subacrosomal band. Centrioles are not present in the mature spermatozoon.



Figs. 13, 14. Sperm of *Uca tangeri* from the vas deferens. Scale 500 nm

D. Discussion

1. Membrane lamellae and (pro)acrosomal vesicle

A remarkable feature of early spermatids of *U. tangeri* is the presence of membrane stacks located adjacent to the nucleus and to a moderately electron-dense proacrosomal vesicle. The presence of membrane complexes formed from or in association with the nuclear membranes is common in spermatids of decapod crustaceans (Moses 1960, 1961; Kaye et al. 1961; Anderson and Ellis 1967; Pochon-Masson 1968; Langreth 1969; Reger 1970; Pearson and Walker 1975; Arsenault et al. 1979; Koehler 1979; Arsenault 1984; Haley 1986; McKnight and Hinsch 1986; Shigekawa and Clark 1986). As in *Carcinus maenas* (Linné 1758) (Pochon-Masson 1968), the proacrosomal vesicle in *U. tangeri* spermatids forms in close proximity to the membrane complex. However, the precise origin of such an organelle is obscure. Further rearrangements leading to the definitive acrosome structure start with the formation of two opposite condensations which are the precursors of the operculum and the thickened ring. This is followed by the differentiation of a long perforatorium and the arrangement of the inner contents of the vesicle in three distinct layers.

2. Centrioles and perforatorium

A pair of conspicuous centrioles lie at the base of the perforatorium in mid and late spermatids of *U. tangeri*, but they degenerate in the mature spermatozoon. Neither of the mid spermatid centrioles accompanies the invaginating acrosomal membrane during perforatorial development. Rather than a direct induction of membrane infolding, the centrioles may provide a guide for the incorporating tubuliform membranes. The centriole-associated microtubules may constitute tracks for the moving membranes, thus contributing indirectly to acrosomal membrane invagination. A considerable portion of the perforatorium in brachyurans is occupied by an anastomosing or helically arranged mass of tubular formations (Brown 1966; Pochon-Masson 1968; Hinsch 1969, 1973, 1986, 1988; Langreth 1969; Reger 1970; Jamieson 1989a, b; Jamieson and Tudge 1990). The term "microtubules" frequently assigned to this constituent appears not to be reliable, because, as already pointed by Reger (1970) and described in the present paper, it apparently derives from membrane systems. Furthermore, micrographs shown in many previous reports suggest that the structure of the perforatorial tubules does not correspond to the usual microtubule bundle pattern. These elements are not arranged into a rigid pattern, but its conformation is tortuous and rather irregular. According to Reger (1970), it may serve as a membrane source for the male nucleus prior to fusion with the female pronucleus.

3. *Nucleus*

Nuclear differentiation comprises the arrangement of chromatin into fibers and the elaboration of arms, which serve to attach the sperm to the oocyte surface (Brown 1966). The number of the arms is variable and characteristic for each species. There is usually a small number of them, although in *U. tangeri* more than 20 slender processes may be present. In some brachyurans (Pochon-Masson 1965, 1968; Hinsch 1969, 1973, 1986), the arms are packed with microtubules which may derive from the spermatid centrioles. In contrast, the nuclear arms lack microtubules in many other species (Yasuzumi 1960; Langreth 1965, 1969; Brown 1966; Reger 1970; Hinsch 1986, 1988; Jamieson 1989a, b, 1990; Jamieson and Tudge 1990; present study). Thus, it is clear that microtubules are not essential for the activity of the spermatozoon, but their significance, when present, is so far unknown.

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