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Short Communication

Studies on the spermatogenesis of two Indian freshwater brachyurans

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Abstract

The events of spermatogenesis in two species of brachyuran crabs [(*Barytelphusa jacquemonti* (Rathbun) and *Oziotelphusa senex* (Fabricius)] were studied by light microscopy. Four different cell types were categorised in the testis, namely 1. Spermatogonia, 2. Spermatocytes, 3. Spermatids and 4. Spermatozoa. Each cell type has been studied in detail, for its structure, morphometry and the process of transformation into the next stage. Comparisons are drawn with the observaitons on the spermatogenesis of other decapods.

Keywords: Spermatogenesis, light microscopy, freshwater brachyuran crabs.

1. Introduction

The class Crustacea is a remarkably successful group, as evidenced by the number of extant species and the diverse habitats colonised by them[1]. This diversity is also reflected in the life history patterns and reproductive strategies[2]. On account of their commercial and/or consumptional importance, over the years, considerable information has been gathered on the biology of crustaceans in general and decapods in particular[3–11]. However, with particular reference to decapods of the Indian sub-continent, much work remains to be done, especially for the freshwater brachyurans—*Barytelphusa jacquemonti* and *Oziotelphusa senex*. As both the species contribute substantially to the local freshwater crustacean biomass, and are of notable commercial value, an understanding of the species-specific events and patterns of spermatogenesis would permit a clearer knowledge of their reproductive cyclicities, a pre-requisite for developing techniques of controlled breeding of the species, in closed aquaculture systems.

2. Material and methods

Specimens of *B. jacquemonti* (Rathbun) were hand-picked from the Byramangala canal system, a perennial reservoir in the Bangalore south taluk[12]. Individuals of *O. senex* (Fabricius) which prefer shallow, lentic habitats, were collected from the paddy fields, near Whitefield, south-east of Bangalore[13]. About 15–20 males were randomly selected and the sampling was done on a fortnightly basis.

Testes and vasa deferentia, excised from either species, were fixed overnight in aqueous Bouin's fluid (18–20 hours). After washing the tissues in distilled water and dehy-

drating in progressive grades of alcohol, the tissues were cleared in xylene and embedded in paraffin (melting point: 58–60°C). Subsequently, they were sectioned at 7–8 μ thickness and stained with hematoxylin-eosin. DPX was used as a mountant and stained sections were observed for morphometric and histological details. Such histological observations on the testis were made over one annual cycle during 1991–92.

3. Results and discussion

Each testis was composite, consisting of many lobules and was covered by a discrete limiting membrane made up of cells with flattened nuclei. A muscle layer, as described by Reger[14] for the testis of the isopod, *Asellus militaris*, was not discernible in the testis of the crabs under study. As the adjacent testicular lobules were closely packed, the inter-lobular space was considerably reduced. Loose connective tissue and distinct blood-sinuses were found in apparent inter-lobular spaces. There was considerable asynchrony in the spermatogenic activity when the different lobules of the same testis were observed, although the spermatogenic activity was fairly well defined with regard to any one lobule (Fig. 1). This was true in the histological observations made throughout the annual cycle. From these observations it can be adduced that males of *B. jacquemonti* and *O. senex* exhibit an extended reproductive activity in natural populations, throughout the year. Such an asynchronous activity with respect to spermatogenesis has also been observed in the testis of another crab, *Menippe mercenaria*[15] and the freshwater prawn *Macrobrachium lanchesteri*[16]. However, in both the species under study, heightened spermatogenic activity was evident between March and October of each calendar year.

The testis of either species of crabs was made up of two kinds of cells, namely, somatic and germinal cells. Somatic cells were large, each with a single voluminous nucleus, and one to several nucleoli. In *Decapoda reptantia*, such somatic cells are generally designated as the 'intercalary' cells[2].

Four distinct cell types of the spermatogenic cycle have been categorised in the testis of the two brachyurans. These are spermatogonia, spermatocytes, spermatids and spermatozoa. The term 'spermatogonia' here refers only to secondary spermatogonia, as primary spermatogonia were not encountered in the histological sections of the testis. Perhaps this stage is rather short-lived and passes off quickly into the next phase. Such a

Table I

Morphometry of different cell types during the spermatogenic cycle

Cell type	*Morphometry (μ) ± S. E.*			
	Longest axes		*Widest axes*	
	B. jacquemonti	*O. senex*	*B. jacquemonti*	*O. senex*
1. Spermatogonia	12.20 ± 0.32	9.06 ± 0.27	8.52 ± 0.21	7.41 ± 0.25
2. Spermatocytes	9.37 ± 0.30	5.34 ± 0.15	7.93 ± 0.30	4.54 ± 0.10
3. Spermatids	6.59 ± 0.23	5.12 ± 0.17	6.20 ± 0.16	4.82 ± 0.18
4. Spermatozoa	4.22 ± 0.07	3.17 ± 0.06	3.47 ± 0.07	2.50 ± 0.07

situation has also been described for another crab, *Potamon koolooense*[17] and the prawn *Macrobrachium lanchesteri*[16]. In contrast, distinct primary spermatogonia have been described in the testis of *Squilla oratoria*[2, 18].

Table I presents the morphometry of all the cell types of the spermatogenic cycle of *B. jacquemonti* and *O. senex*, measured along the longest and the widest axes. Spermatogonia were the largest of all cell types encountered in the histological sections of the testis. Outlines of these cells were not distinct as the adjacent cells were compacted (Fig. 2). The large, more or less elliptical, nucleus occupied almost the entire cell area. The number of nucleoli per nucleus ranged from one to two. Only one nucleolus has been reported for the spermatogonial cell of another crab, *Lophopanopeus bellus*[19]. The uniformly distributed chromatin was positive to hematoxylin. As is characteristic of the process of spermatogenesis in natantians[16, 20], in these reptantians also, cytoplasmic reduction was evident. In the final stages of spermatogonial morphology, the cytoplasm was restricted to a narrow, indistinct rim around the nucleus. Such spermatogonial cells were mostly distributed towards the periphery of each testicular lobule.

The spermatogonia, as above, underwent spermatogonial division to give rise to primary spermatocytes. A distinct cell outline could be noted at this stage and the cells were rather loosely arranged in the matrix of the testicular lobule. Metaphase I was the most commonly encountered stage (Fig. 3). Spindle fibres were clearly visible under the light microscope. Displacement of the diads to the respective poles was also observed. Primary spermatocytes underwent mitosis, to give rise to secondary spermatocytes, which were neary half the size of the former cell type, owing to further cytoplasmic reduction[19]. Golgi granules were fewer in number, as perhaps most of them had undergone disintegration.

FIG. 1. Cross-section of the testis (× 490). Note the asynchrony in the events of spermatogenesis in the adjacent testicular lobules, TL = testicular lobule, FC = flattened cells of the lining of the testicular lobule, a = secondary spermatogonia, b = metaphase plates of spermatocytes, c = spermatids, d = sperms.

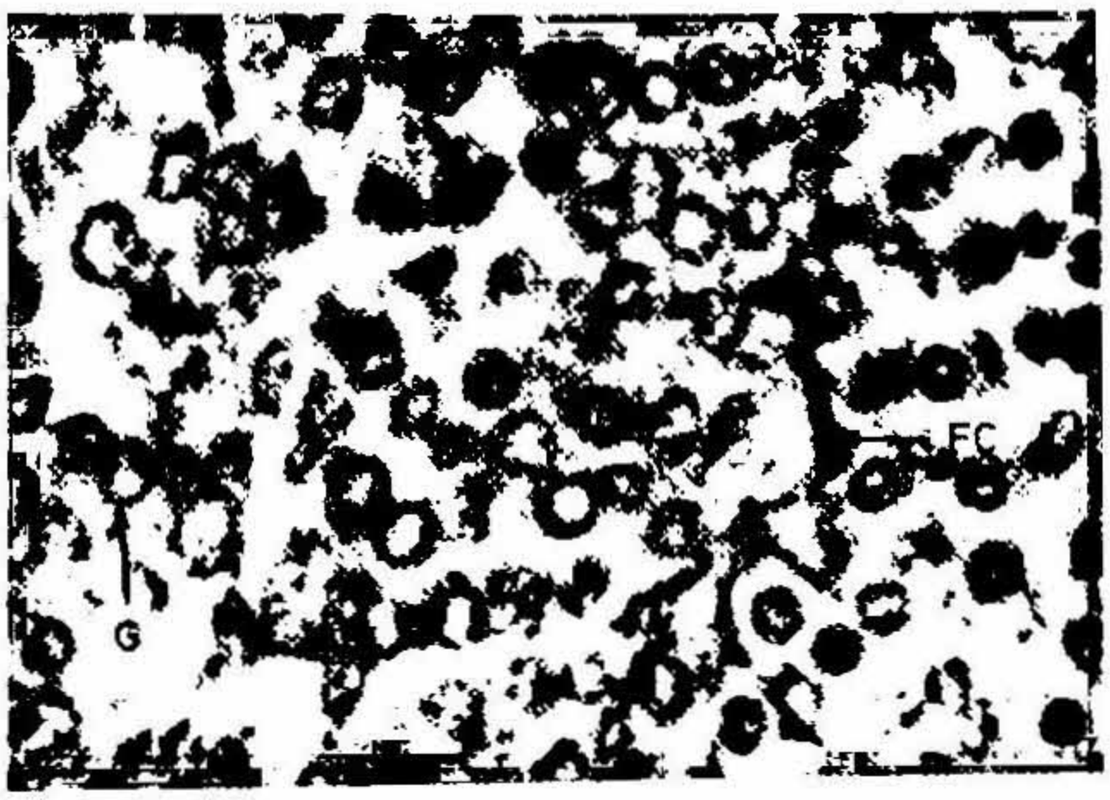

FIG. 2. Higher magnification of Fig. 1 to show the spermatocytes (× 1220). G = apparent golgi material.

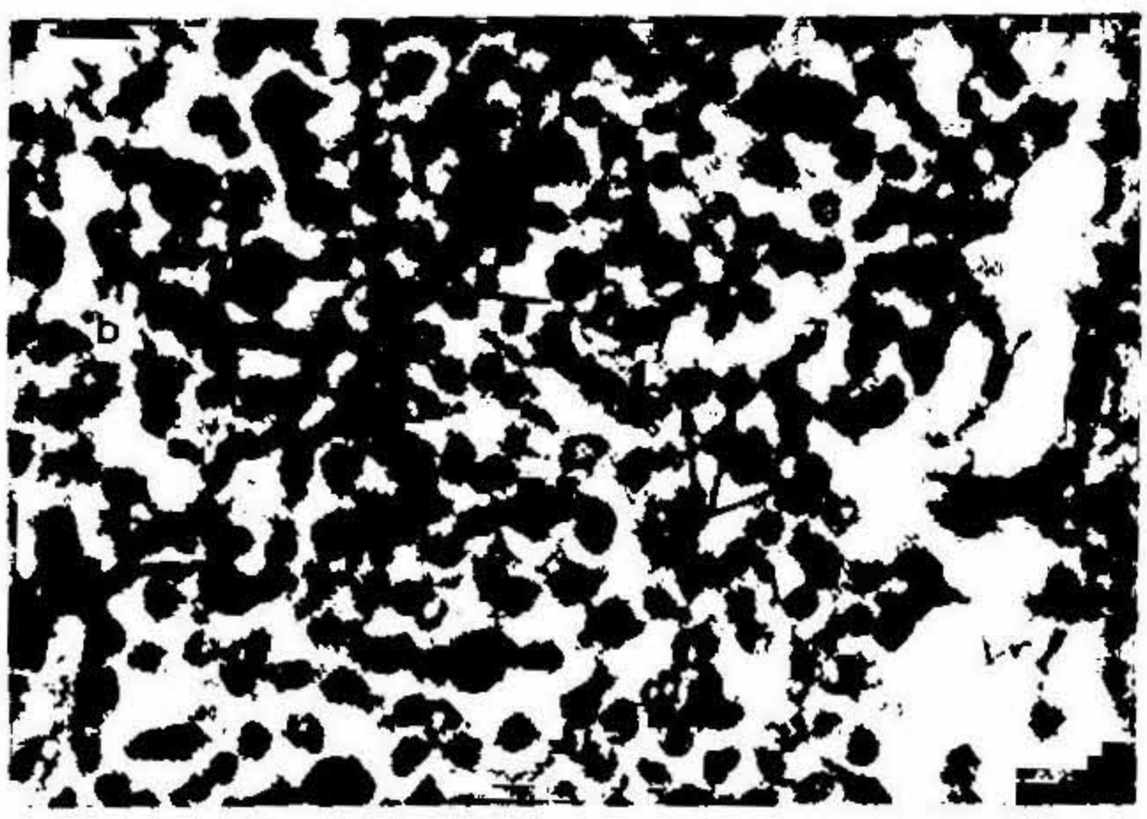

FIG. 3. Higher magnification of Fig. 1 to show the spermatocytes (b) and spermatids (c) (× 1250). Note the metaphase plates.

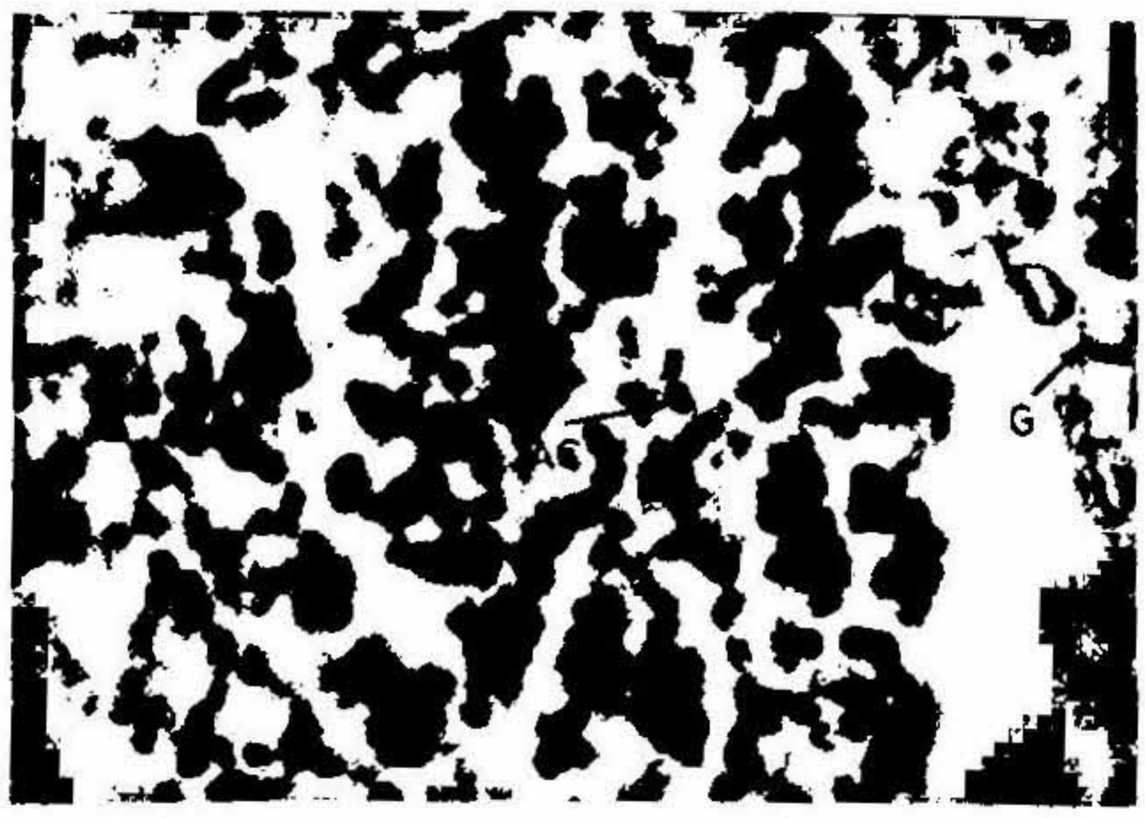

FIG. 4. Higher magnification of Fig. 1 to show the nature of sperms (× 1200). AC = acrosome.

Spermatids were formed following the second meiotic division of the secondary spermatocytes and were accompanied by further cytoplasmic condensation (Fig. 3). Generally, among brachyurans, the mitochondrial vesicle or 'nebenkern' is said to gradually fit into the cavity of the nucleus[21]. Spermatids underwent transformation to give rise to the characteristic, atypical, non-motile, reptantian type of spermatozoa. In the fully formed sperm, the acrosome having fused completely with the lips of the nuclear cup, could not be made out as a separate structure (Fig. 4). No stellae or rays as described for other reptantians[2, 15, 19, 21–25] could be discerned in the sperm of *B. jacquemonti* and *O.senex*. As compared to the structure of the sperm of *Carcinus maenas*[26], *Libinia emarginata* and other reptantians[24, 25], in their external morphology; the spermatozoa of *B. jacquemonti* and *O.senex* bear a close resemblance to that of *Pinnixia* sp[27].

During the process of spermatogenesis, the acroblast of the spermatid increased in size to get transformed into a ring-like acrosome, which finally fused with the margins of the nuclear cup of the sperm. In many of the decapods studied, the origin of the acrosome is a much debated topic. The formation of the acrosome is variously attributed to golgi elements[21, 28–30, 34–37] or even to the nuclear derivatives[28, 38]. In the species under study also, the origin of the acrosome could not be discerned under light microscopy.

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