

Differentiation of Genital Organs and Androgenic Gland in the Kuruma Prawn *Penaeus japonicus*

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Abstract

For the kuruma prawn *Penaeus japonicus*, histological studies were conducted to reveal the differentiation time of the genital organ and the androgenic gland during postlarval periods from 20th day-stage (P20) to P110. The time schedule of masculinization showed sequent appearances of the vas deferens, external sex character, androgenic gland, and testis. The androgenic gland cells of undeveloped rough surfaced endoplasmic reticula seemed to be unfunctional, indicating their negative role in sex differentiation of the genital organ.

The androgenic gland has been known to be the only hormonal organ in crustaceans, which participates in masculinization inducing secondary sex characters of male appendages in amphipods and commencing spermatogenesis in amphipods, isopods, and decapods¹⁾. It inhibits the vitellogenesis of females by its implantation or causes sex reversal in each sex by its ablation or implantation in species belonging to the above orders^{1,2)}. Especially for *Natantia* species feminization and masculinization have been reported in the Malaysian prawn *Macrobrachium rosenbergii* by androgenic gland ablation from males and its implantation into females, respectively^{3,4)}.

However, regarding its inducing role in sex differentiation of the genital organs some problem seems to be still present especially in decapod species. Namely, concerning the timing of gametogenesis or development of the genital apparatus, there are few reports in respective species. Especially for the kuruma prawn *Penaeus japonicus*, its description has been only referred to the differentiation sequence of the genital organs including androgenic gland¹⁾.

Present study was conducted to reveal the time schedule of differentiations of the genital organ and also of the androgenic gland during larval periods of the kuruma

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prawn, and to reply to a question, whether this gland participates or not in the sex determination.

Materials and Methods

Postlarvae of the kuruma prawn *Penaeus japonicus* were used. First samplings were carried out for larvae at stages from postlarva 20 (20th day-postlarva, P20) to P110 (body weight of P110, 5.7–6.7 g) with intervals of 5 days, during May 28 to August 26, 1991. Each sampling contained 10 individuals. They were fixed in Bouin solution for over 12 h, and supplied to histological preparations stained with hematoxylin and eosin. Besides, a second sampling was conducted for two males of P65 (body weight 2.4 g and 2.5 g) on October 29, 1991. All the above material had been reared in $40,000 \times 1.7$ (m^3) or $5,500 \times 1.7$ (m^3) pond of a semi-intensive prawn farm. For the second sampling, a distal portion of the vas deferens, *i.e.* ejaculatory bulb, was extirpated with neighbouring tissues from each larva and fixed in cold 5% glutaraldehyde buffered with 0.1 M γ -collidine (pH 7.8). After a 2 h fixation, the tissue were rinsed several times in cold 0.2 M γ -collidine with about 1 h intervals. Then, they were postfixated in cold 1% osmium tetroxide buffered with 0.1 M γ -collidine. They were dehydrated with ethanol series and QY1, then embedded in epon 812. They were sliced by a Porter-Blum microtome MT-1, and stained with uranylacetate and lead-acetate. The ultrastructural observation was conducted under 100 kV with an electron microscope of Hitachi 300H.

Results

Differentiation Schedules of Sex Characteristics

The gonad was observed at P20 on the dorsal surface of the midgut gland beneath the pericardium and lateral to the descending aorta. It consisted of many masses of primordial germ cells and mesodermal cells, showing simple layered cords. This simple layer became the multilayer after P40. The male gonoduct, *i.e.* vas deferens, was recognized also at P20. Its distal portion, *i.e.* ejaculatory bulb, was yet slender in shape and linked with the connective tissue under the cuticle of the coxopodite of the fifth pereopod. For that of P30, a slightly developed lumen and a wall of the connective tissue were observed (Figs. 1A and 1B). Externally and opposite to the median line the wall of the ejaculatory bulb showed a distribution of cellular assembly. It may have been a primordium of the androgenic gland. However, differing from that of the completely formed organ its structure showed constitutions of the fibrillar connective tissue and dispersed cells (Fig. 1B).

The endopodite of the first pleopod in males appeared as a large and triangular lobe, *i.e.* petasma, at P50. In females, its endopodite was a small and slender lobe.

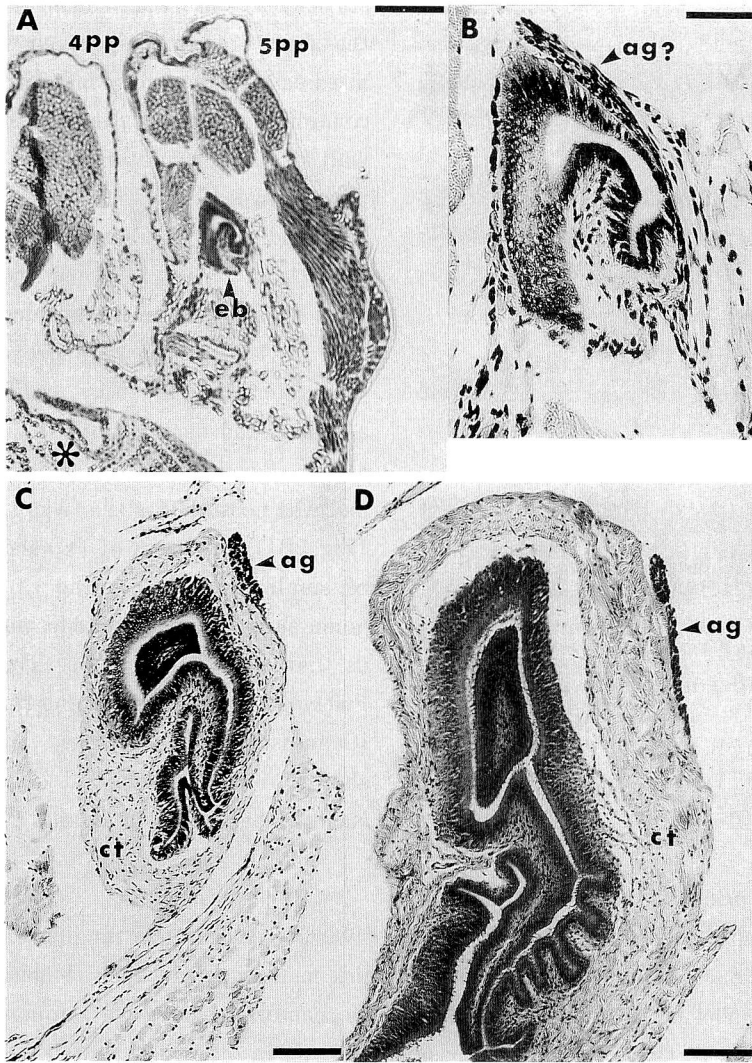


Fig. 1. Cross sections of the right ejaculatory bulb in postlarvae, indicating the position of the androgenic gland. A, 30th day-stage of postlarva (P30) ; B, partial magnification of A ; C, P60 ; D, P100. Scale bar, A = 200 μm ; B = 45 μm ; C and D = 100 μm . ag and ag?, androgenic gland and its probable primordium ; ct, connective tissue ; eb, ejaculatory bulb ; 4pp, fourth pereopod ; 5pp, fifth pereopod ; *, part of the laminar sheath of the fifth thoracic ganglion.

The ejaculatory bulb became to possess thick connective tissue externally and involved lumen surrounded by a simple layered columnar epithelium according to the time lapse (Figs. 1C and 1D). Nuclei of the epithelial cells showed an extensively crowded condition. Muscular system of the external surface of the ejaculatory bulb was not yet

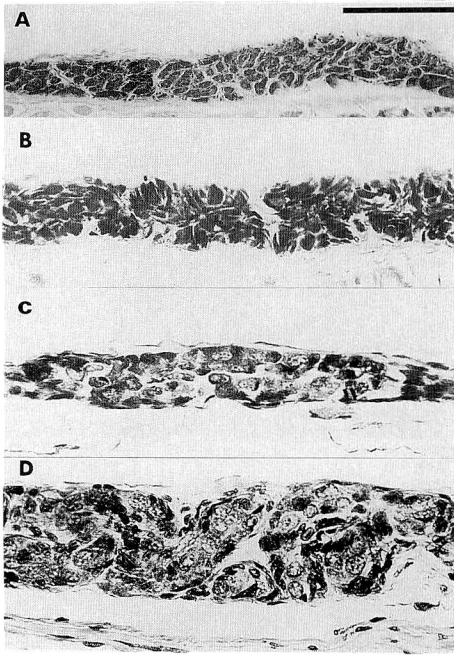


Fig. 2. Postlarval gonads sectioned cross-ly. A, undifferentiated gonad of P40 ; B, testis of P60 ; C, ovary of P85 ; D, testis of P85. Scale bar is 50 μm .

sufficiently observed. During P50 to P60, the slender androgenic gland was recognized as a cellular mass independent of the connective tissue of the postero-external wall of the ejaculatory bulb. Nuclei of these crowded cells were dense, small and flattened. Such characters were maintained at least until P110, and they differed from those round and translucent characters of the pubescent androgenic gland (Nakamura, unpublished), indicating such a young androgenic gland to be unfunctional. Sexual discrimination of the gonad was possible for the first time at P60 (Fig. 2B). The testis of P60 showed many of small blocks consisting of spermatogonia surrounded by mesodermal cells like as that of P85 (Fig. 2D). The ovary of P60 was not presented in the figure due to its indistinct preparations. That of P85 showed centrally arranged oogonia being enclosed by mesodermal cells (Fig. 2C).

Ultrastructure of Androgenic Gland Cells

The young androgenic gland consisted of various cells in size ranging from 10 μm to 20 μm . Each cell was surrounded by a thick membrane of which thickness was peculiar and indefinite (Figs. 3B and 3C). This membrane showed a laminar structure corresponding to that of 'une lame basale' reported in other decapod species^{5,6)}. However, the cell membrane of the latter androgenic gland was thin like the case of common cells.

The nuclear size varied between 4 μm and 10 μm according to cellular size. The shape was elongated or drop-like, sometimes irregularly contoured. This irregular contour of the nucleus has been already reported in the adult prawn, *Penaeus kerathurus*¹⁾. The nucleus possessed much chromatin along the nuclear membrane and the nucleoli which were situated centrally.

The cytoplasm showed distributions of mitochondria, some of which were large, and rough surfaced endoplasmic reticula (rer) (Figs. 3B and 3C). Rer were not so well developed and not arranged lamellarly as in the case of the pubescent kuruma prawn⁷⁾ and crab *Ocypode quadrata*⁶⁾. In the latter case, the gland cells were referred as having a very abundant lamellar rer and also a discrete Golgi apparatus as well

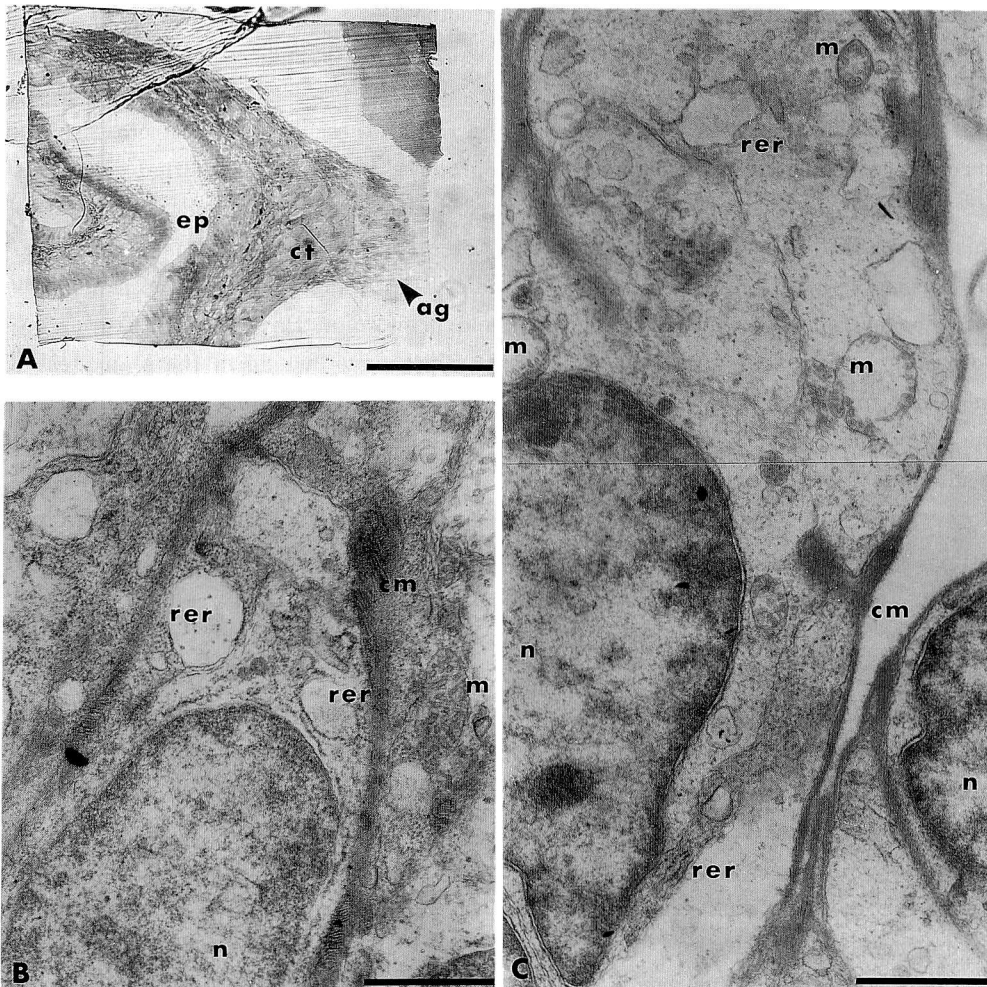


Fig. 3. Electron microscopical specimens of the androgenic gland in P65 postlarvae. A, trimming slice stained with toluidine blue, indicating the position of the androgenic gland ; B and C, electron micrograms of the androgenic gland cell. Scale bar, A = 100 μ m ; B and C = 2 μ m. ag, androgenic gland ; cm, cell membrane ; ct, connective tissue of the ejaculatory bulb ; ep, epithelium of the ejaculatory bulb ; m, mitochondria ; n, nucleus ; rer, rough surfaced endoplasmic reticulum.

as many lysosomes. In the young gland of the present larvae, the Golgi apparatus was not well developed, lacking in lamellar arrangements and dense micro- or large vesicles. Lysosomes were indistinct.

Discussion

The revealed sequence of appearances of the genital organs in this study supported the previous result of the kuruma prawn¹⁾. Their time schedule of the appearance

seemed to suggest that the vas deferens including the ejaculatory bulb and external sexual character differentiated genetically without participation of the androgenic gland.

However, nevertheless the presence of controversial results in crabs, crayfish and kuruma prawn, Charniaux-Cotton and Payen proposed in their review¹⁾ that sexual differentiation of males in malacostracans needed the androgenic gland. The basis of this proposition seemed to depend eventually on a Katakura's hypothesis⁸⁾ that androgenic hormone was first produced by the male determining gene and was responsible for the development of the androgenic gland primordia. However, Katakura's hypothesis⁸⁾ was deduced by isopod experiments which were lacking in the blank test if they were examined from a strict standpoint : namely, the incubation experiment of the primordial gonad without androgenic gland hormone and its negation of the testicular differentiation were not provided. Such an *in vitro* experiment seemed necessary to assert the hypothesis mentioned above. Further, the presence and secretory site (s) of this hormone have been yet unsubstantiated in such a larval period of sexual differentiation in any crustacean species.

In the crayfish *Pontastacus leptodactylus leptodactylus*, primordia of probable (according to the author) androgenic gland appeared at the subterminal of the vas deferens after sexual differentiation of the gonad and before rudimental appearance of the external sexual character⁹⁾. This appearance pattern differed from the cases of crabs¹⁾. In the latter, the primordium of the androgenic gland was recognized generally after or just at the occurrence of the gonadal sex differentiation which was accompanied by complete formation of the vas deferens¹⁾. On the contrary, in the amphipod *Orchestia gammarella* and the prawn *M. rosenbergii*, the following results have been reported¹⁾. Differentiation of the male external characteristics began after differentiation of the androgenic gland. And these results seemed to be the strong ground of Charniaux-Cotton and Payen's proposition.

Therefore, the most important point seemed how to prove the hypothesis of the existence on the androgenic hormone which may have settled the above-mentioned contradictions among malacostracans and how to build a general concept concerning endocrine control of sex or genetic sex determination.

One of the previous contradictions was found also in the relation between the androgenic gland and the testis. The time lag between both differentiations obtained in the present study seemed to indicate that the former participated in inducing the latter differentiation.

However, from the light and the electron microscopical observations, the following considerations supported the autodifferentiation of the gonad in terms of independence from the androgenic gland : 1) beginning of the gonadal sex differentiation may have preceded the morphological discrimination, although its time lag was uncertain in such a morphological study. The ovary and testis were distinguished in the

present study at P60. The histology of the gonad seemed possible to expect that the initiation of their differentiation has occurred far before P60 ; 2) during the period from P50 to P110, the cells of the young androgenic gland did not show so active condition of secretory function as that of the pubescent individuals reported^{1, 2, 7)}.

Then, the differentiation of the testis also seemed to have been induced genetically without participation of the young androgenic gland in the kuruma prawn. This supposition of gonadal sex differentiation differed from the common concept or previous proposition introduced by Charniaux-Cotton and Payen¹⁾.

In the subadult or adult *M. rosenbergii*, masculinization of females was induced by androgenic gland implantation⁴⁾. And also feminization was achieved by androgenic gland ablation³⁾. However, maybe due to technical difficulty, these operations of the gland were always conducted together with removal or implantation of the ejaculatory bulb. And the vas deferens, not the ejaculatory bulb, was implanted into the control group of *M. rosenbergii*. Anatomically, the ejaculatory bulb was certainly a distal part of the vas deferens, though their constructions had some different morphology each other¹⁰⁾ : the convoluted lumen with its glandular epithelium of the ejaculatory bulb developed extremely and expanded, differing from the case of the vas deferens at puberty. This phenomenon and early differentiation of the ejaculatory bulb seemed to indicate its important function related to not only the sperm storage but also masculinization. The following sentences in the previous reference⁴⁾ seemed noticeable : in some masculinized females by implantation of the androgenic gland, living vasa deferentia implants were found but the associated androgenic glands were missing. In these cases, the androgenic glands may have separated from the vasa deferentia in the host and were not recovered with the vasa deferentia at the termination of the experiment. Then, it was possible to consider also function of the ejaculatory bulb on the above-mentioned results.

It has been known that the androgenic gland needed the protocerebral factor of males for its maintenance in Natantia *Leander serratus* and *Crangon crangon*¹¹⁾, although in the isopod *Porcellio dilatatus*¹²⁾, female protocerebrum showed the same action as that of the male. Based on these Natantia references, the disappearance of the implanted androgenic gland in female *M. rosenbergii* was considered as an interpretation to have been caused due to the absence of the brain factor. Then, the androgenic gland ought to have degenerated. However, the masculinization was referred to have occurred.

Therefore, the interpretation of the androgenic gland as the only organ inducing sex determination proposed a re-examination especially in decapod malacostracans.

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