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Postembryonic Development of Gonads in *Triops numidicus* (Grube) (Crustacea: Branchiopoda, Notostraca)

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Abstract

The postembryonic development of testes and ovaries of *Triops numidicus* was observed. Gonadal primordia appear first in the fourth instar and stay immature until the eleventh instar, while the sexual differentiation is unclear. In the twelfth or thirteenth instar, spermatogenetic zones including male germ cells and oogenetic zones including female germ cells are histologically distinguishable. Matured sperm and eggs are observed in the testicular and ovarian lumens first in the sixteenth and eighteenth instars, respectively: maturation in the male precedes that in the female by two instars. Development of the male and female gonadal structures in the testis and ovary of gonochoric *T. numidicus* is well consistent with that in the hermaphroditic gonad of *T. longicaudatus*. Thus anatomical comparisons of the reproductive systems of notostracans differing in reproductive mode may be valid.

Introduction

Four *Triops* species are known in the world. Although all resemble each other in many aspects such as general morphology, life cycle and gonadal structures, the sexual types, *i. e.*, female, male, or hermaphroditic, are variable in populations: in some cases there are males and females, but in other cases only females or hermaphrodites (*e. g.*, Longhurst, 1955; Akita, 1971). Comprehensive analyses on the variability in reproductive modes in notostracans may be one way of clarifying the reproductive groundplan and understanding evolutionary changes in crustaceans and arthropods.

As for modes of reproduction in notostracans, a lot of research has been conducted, but few comparative morphological studies exist on the gonadal structures for different reproductive modes (*e. g.*, Martin, 1992), much less their developmental changes (cf. Ando and Makioka, 1992). Under such circumstances, we started comparative morphological and developmental studies on notostracan reproductive systems, and examined the postembryonic development of ovotestes of the hermaphroditic *Triops longicaudatus* (Mitsumoto and Makioka, 2002). In the present study we dealt with the postembryonic development of gonads, *i. e.*, ovaries and testes, in the gonochoric *Triops numidicus*, and compared it with that in the hermaphroditic *T. longicaudatus*.

Materials and Methods

Adults of *Triops numidicus* (Grube) were collected from rice fields in Kashihara, Nara Prefecture, in July, 2001. Juveniles, hatched from eggs laid in the laboratory, were reared in separate bottles, fixed with Bouin's solution in every instar, and processed into serial paraffin sections 4–10 μm thick, which were stained with Mayer's acid hemalum and eosin.

Results and Discussion

Gonadal primordia appeared first in the fourth instar, and therefore observations were described for after the fourth instar. Terms used in the following descriptions are based upon Mitsumoto and Makioka (2002).

Fourth to eleventh instars

The sexual differentiation in the gonads was unclear from the fourth to eleventh instars in *Triops numidicus* as in the hermaphroditic *T. longicaudatus*. In the fourth instar, gonadal primordia appeared as a pair of undifferentiated cellular masses (Fig. 1A) on both sides of the gut. In the eighth instar, these cellular masses consisted of young gonadal cells about 10 μm in diameter, longitudinally elongated (Fig. 1B). These cells were to be gonial, but oogonial or spermatogonial, was not determined. In the eleventh instar, a few lumens appeared along the long axis of the gonads, soon to fuse with each other to form one united gonadal lumen (Fig. 1C).

Twelfth to eighteenth instars

Sexual differentiation becomes obvious in some precocious twelfth instar and most thirteenth instar juveniles. In newly differentiated ovaries, the ovarian epithelium and oogenetic zones were clearly distinguished (Fig. 2A, A'). The epithelium of the ovarian trunk was composed of a single layer of flat cells. Oogenetic zones swelled from the ovarian trunk toward the hemocoel. Oocytes were accompanied by three nurse cells. Primordia of oviducts elongated from around the center of the ovarian trunk, with their exterior openings or gonopores undeveloped.

In the fourteenth and fifteenth instars, ovarian development progresses, while in the sixteenth instar, ovarian branches bearing their own oogenetic zones were observed to elongate from the ovarian trunk, and in some of these ovarian branches, divergence as observed in adults occurred (Fig. 2 B, B'). The ovarian branches increased in number, in length and in the size of their own oogenetic zones toward the adult stage. The cells of ovarian branches were columnar and easily distinguished from the flat cells of ovarian trunks. A single oocyte grew accumulating yolk material in each oogenetic zone, with the progression of oogenesis. In the lumens of ovarian trunks and branches in the eighteenth instar, many eggs, in which the oogenesis had completed, were observed (Fig. 2C, C'). Copulation behavior could not be observed, because all of the specimens were reared separately. However, judging from the fact that the ovaries of the eighteenth instars acquire definitive configurations equivalent to those of adults (see Fig. 3B of Mitsumoto and Makioka, 2002), one may consider that the females of *T. numidicus* become adults in the eighteenth instar, although gonopores were yet to open: the gonopores are likely to open in the first oviposition.

In newly differentiated testes, the epithelium of the testicular trunk and spermatogenetic zones were clearly distinguishable (Fig. 3A, A'). Similarly to the newly differentiated ovaries, primordia of testicular ducts elongated from around the center of the testes. The spermatogenetic zones swelled from the testicular trunk toward the hemocoel. In contrast to the oogenetic zones, the spermatogenetic zones had no branches out from the testicular trunk. Early male

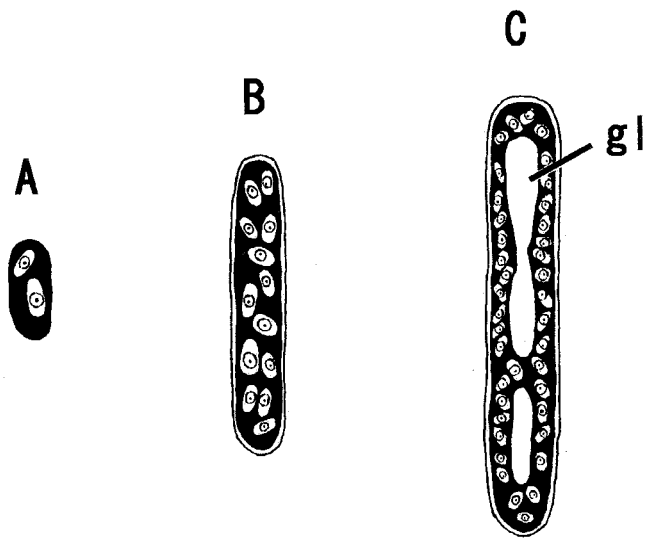

Fig. 1 Diagrammatic representation of one of the paired gonads including young somatic and germ cells in the fourth (A), eighth (B), and eleventh (C) instars. gl: gonadal lumen.

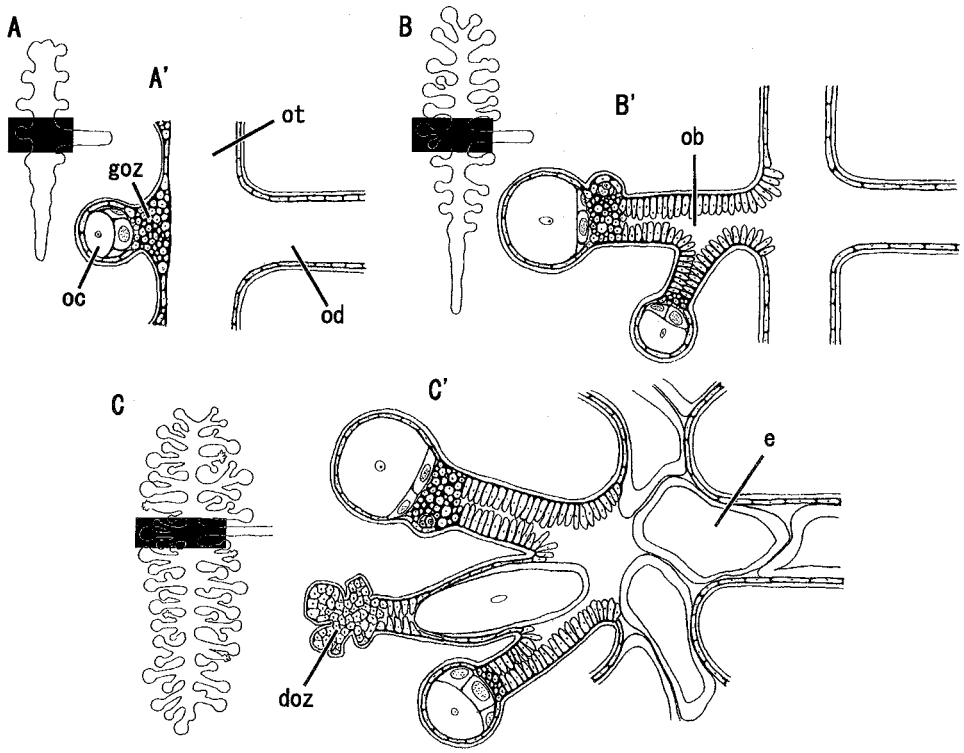

Fig. 2 Diagrammatic representation of ovaries of the thirteenth (A, A'), sixteenth (B, B'), and eighteenth (C, C') instars. A', B', and C' are enlargements of the central parts of the ovaries. doz: degenerated oogenetic zone, e: matured egg, goz: germarium of the oogenetic zone, ob: ovarian branch, oc: oocyte, od: oviduct, ot: ovarian trunk.

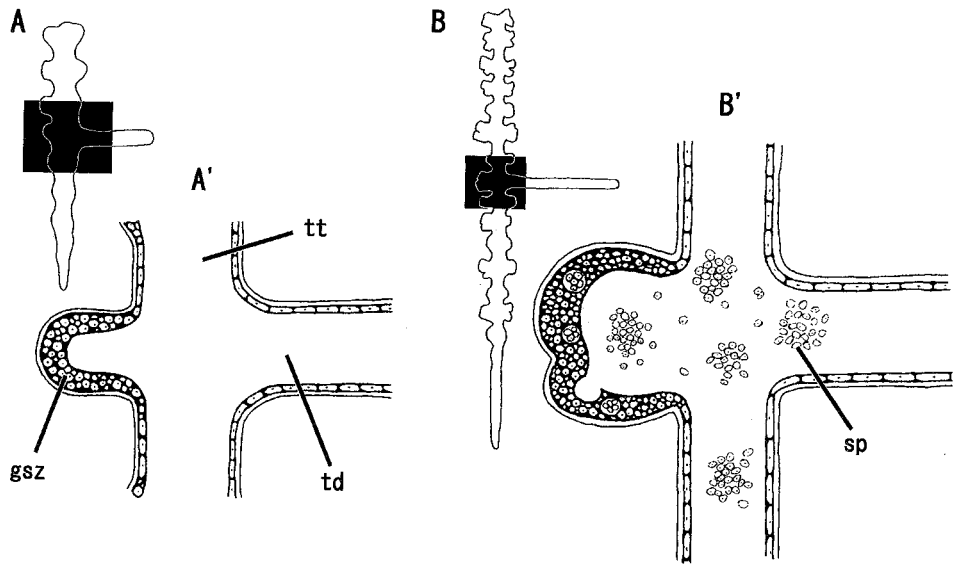

Fig. 3 Diagrammatic representation of testes of the thirteenth (A, A') and sixteenth (B, B') instars. A' and B' are enlargements of the central parts of the testes. gsz: germarium of the spermatogenetic zone, td: testicular duct, tt: testicular trunk, sp: sperm.

germ cells were hardly distinguishable from somatic cells in germaria or walls of spermatogenetic zones.

In the fourteenth and fifteenth instars, development of the testis progresses, and in the sixteenth instar, numerous sperms were observed to be released from the spermatogenetic zones into the testicular lumen (Fig. 3B, B'). The testes acquire their definitive structure, closely resembling that of adults (see Fig. 3A of Mitsumoto and Makioka, 2002), and one may consider that males of *T. numidicus* become adults in the sixteenth instar. Significant differences were not found between the testes of the sixteenth, seventeenth and eighteenth instars.

The gonadal structures and their developmental processes in both sexes of gonochoric *T. numidicus*, which we have described here, agree in every aspect with those of hermaphroditic *T. longicaudatus* (Mitsumoto and Makioka, 2002) for the same sexuality. Also as for the timing of maturation, the male maturation precedes the female one, although the maturation stages for both reproductive organs of *T. numidicus* and *T. longicaudatus* are different from each other by one instar: in the latter, the male reproductive organ matures in the fifteenth and the female one in the seventeenth instar (Mitsumoto and Makioka, 2002). Herewith, we have validated the use of anatomical comparisons of reproductive systems between forms which have different reproductive modes, *i. e.*, the gonochoric *T. numidicus* and hermaphroditic *T. longicaudatus*.

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References

Akita, M. (1971) The reproduction of tadpole shrimps. *Zool. Mag.*, **80**, 242–250. (in Japanese).

Ando, H. (1992) *Comparative Histology of Ovarian Structure and Oogenetic Modes in Some Primitive Crustaceans.* Master's thesis, Institute of Biological Sciences, University of Tsukuba, Tsukuba. (in Japanese).

Longhurst. A.R. (1955) The reproduction and cytology of the Notostraca (Crustacea, Phyllopoda). *Proc. Zool. Soc. Lond.*, **125**, 671–680.

Martin, J.W. (1992) Branchiopoda. *In* F.W. Harrison and A.G. Humes (eds.), *Crustacea: Microscopic Anatomy of Invertebrates, Vol. 9*, pp. 25–224. Wiley-Liss, New Yolk.

Mitsumoto, H. and T. Makioka (2002) Preliminary notes on the structure and postembryonic development of gonads in two tadpole shrimps *Triops longicaudatus* and *T. numidicus* (Crustacea: Branchiopoda, Notostraca). *Proc. Arthropod. Embryol. Soc. Jpn.*, **37**, 13–17.