Notes on the Postembryonic Development of the Male Reproductive System in *Eudigraphis nigricans* (Miyosi) (Diplopoda, Penicillata)

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There were various descriptions on the structure of adult male reproductive systems of some diplopods (Newport, 1841; Fabre, 1855; Heathcote, 1889; Rath, 1891; Reinecke, 1910; Seifert, 1932). Kanaka and Chowdaiah (1974) discussed evolution of the male reproductive system in some Indian diplopods. However, no study has been done on the postembryonic development of the male reproductive systems, which is necessary to make certain the origin of various parts of the diplopod male reproductive system and to make up its basic model. In the present study, we observed for the first time the postembryonic development of the male reproductive system in a primitive diplopod, *Eudigraphis nigricans*.

Specimens in various postembryonic stages of *Eudigraphis nigricans* (Miyosi) were collected from supralittoral rocky shores at Shimoda, Izu peninsula, and at Katsu-ura, Boso peninsula, Central Japan. Specimens were divided into eight postembryonic stages, an adult and seven larval, according to Reinecke (1910). They were kept at 25℃ in a plastic container with moistened wiping papers and given some peaces of lichen as food. For the histological observations, each specimen was fixed with Bouin's solution or "Kryofix" fixative (MERCK Co.), after excising the head with a razor blade in a physiological saline. Fixed specimens were then dehydrated in a graded ethanol-*n*-butanol series and embedded in paraffin. Serial sections, 5μm in thickness, were stained with Mayer's haematoxylin and eosin. The number of paired testicular lobes in each specimen was compared with those of paired pedal nerve-protrusions correlating with the body segmentation.

The adult male reproductive system was located between the alimentary canal and the ventral nerve cord, extended from the second to the ninth body segment. Through the fourth to the ninth body segment, it consisted of a thin-walled single median vas deferens and ten pairs of testicular lobes connected to the vas deferens with the short spermatoduct. In the second and the third body segments, the thick-walled vas deferens turned twice or three times to the left and the right, and then bifurcated to be connected with paired gonopores opening on the coxae of the second walking legs (Fig. 1). The lumen of each testicular lobe, surrounded by a thin

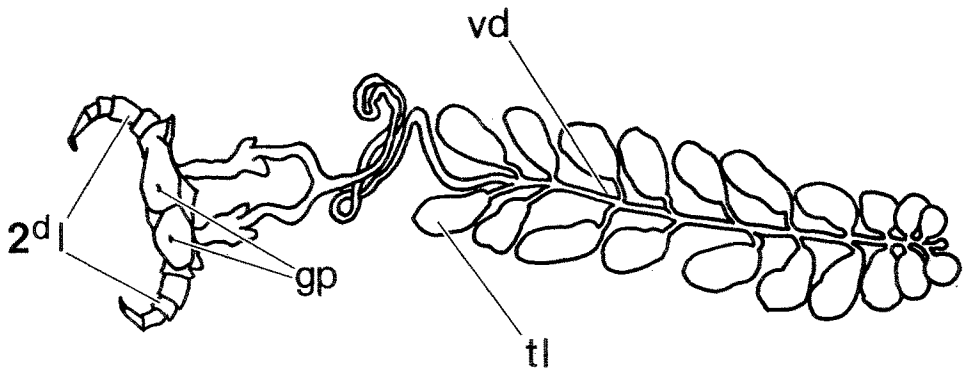

Fig. 1 Diagrammatic representation of adult male reproductive system in Eudigraphis nigricans (ventral view). gp: gonopore, tl: testicular lobe, vd: vas deferens, 2^{d}l: second walking leg.

testicular epithelium, was filled with a large amount of spermatocytes of 10-30μm in diameter and various spermatids of 30-50μm in major length and 20-30μm in minor length. Many eosinophilic spermatids were observed also in the lumen of the median vas deferens (Fig. 2). Male germ cells in anterior testicular lobes were in advanced spermatogenetic stages. Many spermatozoa were found only in the thick-walled vas deferens. No spermatogonia were found throughout the adult male reproductive system.

The paired testicular lobes and pedal nerve-protrusions from the ganglia correlatively increased in number together with the progress of the following postembryonic stages (Table 1):

In the stadium I, a pair of gonad anlagen were found in the 3rd body segment, beneath the hind gut and above the posterior end of the ventral nerve cord. Each gonad anlage consisted of three-six gonial cells of about 10μm in diameter and some young somatic cells with spherical nuclei of about 5μm in diameter (Fig. 3).

In the stadium II, the second pair of the gonads similar to those in the stadium I appeared behind the first pair. Each of these gonads consisted of three-six gonial cells surrounded by a gonadal epithelium.

There were three pairs of gonads in the early stadium III, and four pairs in the late. The gonads formed earlier contained more gonial cells than those formed later, and the posteromost pair of gonads was as young

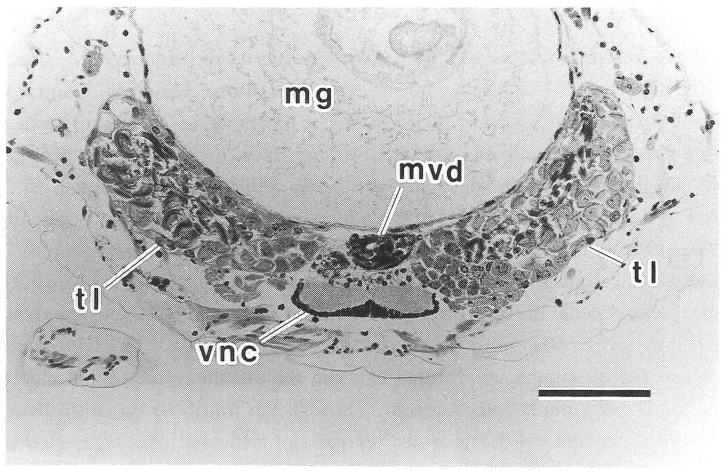

Fig. 2 Cross section of adult male reproductive system of *Eudigraphis nigricans* at fifth body segment showing paired testicular lobes and median vas deferens. Scale = 100μm. mg: mid gut, mvd: median vas deferens, tl: testicular lobe, vnc: ventral nerve cord.

Table 1 Correlation in number of some structures in male *Eudigraphis nigricans*.

Stadium	Body segment	Paired walking leg	Paired pedal nerve protrusion	Paired testicular lobe
I	5	3	3 — 4	1
II	5	4	4 — 5	1 — 2
III	6	5	6 — 7	3 — 4
IV	7	6	8	5 — 6
V	8	8	9 —10	6 — 7
VI	9	10	10—11	7 — 8
VII	10	12	11—12	8 — 9
adult	11	13	13	10

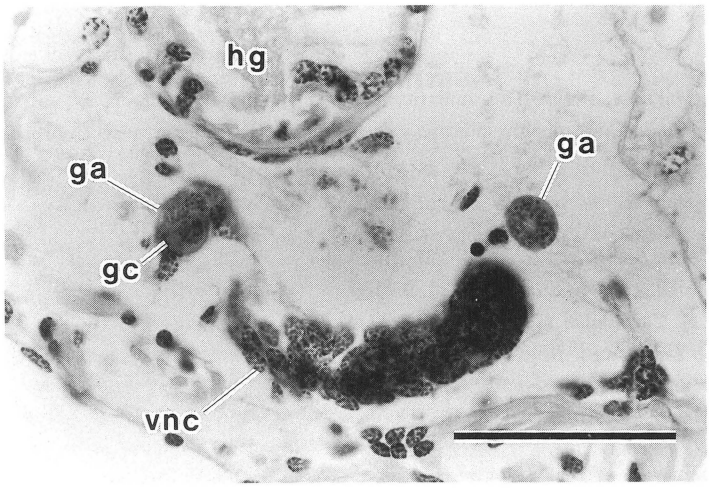

Fig. 3 Cross section of first instar larva of *Eudigraphis nigricans* at third body segment showing paired gonad anlagen. Scale=50μm. ga: gonad anlage, gc: gonial cell, hg: hind gut, vnc: ventral nerve cord.

as the first pair of gonads in the stadium II. A narrow median gonoduct extended forward from the posterior generative zone. It extended paired short lateral branches connected with the paired gonads.

In the stadium IV, several large spermatocytes appeared first in some anterior gonads among the five or six pairs of gonads. Therefore, the paired gonads are regarded as the testicular lobes, and the median and lateral gonoducts connected with the testicular lobes as the vasa deferentia. The median vas deferens extended into the second body segment. Another fine duct also extended upward from a young cell-cluster in the coxa of each second walking leg, and then turned toward the median vas deferens.

In the stadium V, seven pairs of the testicular lobes appeared. Spermatocytes increased in number in several large anterior testicular lobes. In the second body segment, the median vas deferens fused with the fine ducts from the coxae of the second walking legs.

In the stadium VI, eight pairs of the testicular lobes appeared. The anteromost largest lobes contained several tens of young spermatids.

In the stadium VII or the subadult stage, nine pairs of the testicular lobes appeared. A number of eosinophilic spermatids was found in both most of the testicular lobes and the median vas deferens. Spermatogonia were found only in some posterior young testicular lobes. The posteromost lobes stayed generative throughout these larval stages.

In the stadium VIII, the adult stage, almost all testicular lobes were filled with spermatids. The posteromost lobes contained a number of large spermatocytes and spermatids, but no spermatogonia.

Our observations give some conclusions as follows: (1) Ten pairs of the testicular lobes are produced one by one by serial divisions of a pair of the primary gonad anlagen throughout the larval stages. (2) Spermatogonia produced in the gonadal anlagen are transferred forward by the gonadal divisions. The spermatogonia increase in number in the testicular lobes, and then develop into the spermatocytes and spermatids. During the spermiogenesis, spermatids are transferred from the testicular lobes into the median vas deferens, and transformed into spermatozoa in the thick-walled vas deferens. (3) The median vas deferens and its short branches with testicular lobes are originated from the posteromost generative zone of the larval body, and the anterior paired vasa deferentia connecting with the gonopores are produced from the coxal young cell-clusters of the second walking legs. (4) The testicular lobes and pedal nerve protrusions from the ganglia correlatively in-

crease in number throughout the postembryonic stages, evidencing a segmental arrangement of the testicular lobes.

In adult female *E. nigricans*, ten pairs of germ areas were arranged segmentally on the ventral wall of the ovary (Yahata and Makioka, 1991). The similarity in the segmental arrangement between the testicular lobes and the ovarian germ areas presumably indicate that these gonadal structures develop basically reflecting the body segmentation.

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