Preliminary Note on the Ovarian Structure in the Penicillate Diplopod, *Eudigraphis takakuwai nigricans* (Miyosi)

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Two different types of the ovarian structures and oogenetic modes are seen among the arthropod groups; one type seems to represent the Chelicerata and the other the Mandibulata (Makioka, 1988). In several diplopods, however, some chelicerate-like ovarian features were described, such as the oocytes extruding from the ovarian surface toward the "haemocoelic" cavity accompanied with stalks or extensions of the ovarian epithelium (Versluys and Demoll, 1923; Crane and Cowden, 1968; Nair, 1981), despite their systematic position as the Mandibulata. Thereafter, the diplopod ovarian structure and oogenesis were studied insufficiently to be generalized for comparison with those in other arthropod groups. In the present paper, we studied one of the primitive states of the diplopod ovarian structure and oogenesis.

Specimens in various postembryonic stages of *Eudigraphis takakuwai nigricans* (Miyosi), belonging to the Penicillata, one of the primitive diplopod groups, were collected from the supralittoral rocky shore at Shimoda, Izu peninsula, Central Japan. Eight postembryonic stages including adult stage were distinguished. The specimens were kept at 25℃ in a plastic container with moistened wiping paper. Some lichens were supplied as food. Each specimen was fixed with Bouin's solution after removing the head part with a razor blade in a physiological saline, dehydrated in a graded ethanol-butanol series and embedded in paraffin. Serial sections, 5 μm in thickness, were stained with Mayer's haematoxylin and eosin.

The adult ovary was a single lengthened sac-like organ consisting of a thin layer of the ovarian epithelium (Fig. 1). It was placed between the alimentary canal and the ventral nerve cord, running along the median axis from the 4th to the 9th body segment. A single oviduct extended forward from the anterior end of the ovary and then bifurcated to be connected with paired gonopores opening on the coxae of the 2nd appendages.

A single germarium, filled with the oogonia, very young oocytes and interstitial cells, was placed medianly on the ventral ovarian epithelium, at about the 6th body segments (Figs. 2 and 3). The oogonia, spherical cells of about 7 μm in diameter with peculiar large nuclei and without nucleoli, were found only in the germarium (Fig. 2). The oocytes, less than 20 μm in diameter, occurred in the periphery of the germarium (Fig.2). They

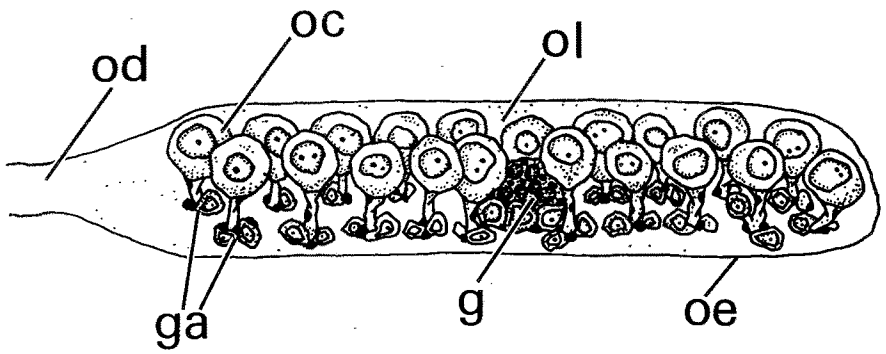

Fig. 1 Diagrammatic representation of adult ovary in *Eudigraphis takakuwai nigricans*. g: germarium, ga: germ area, oc: oocyte, od: oviduct, oe: ovarian epithelium, ol: ovarian lumen.

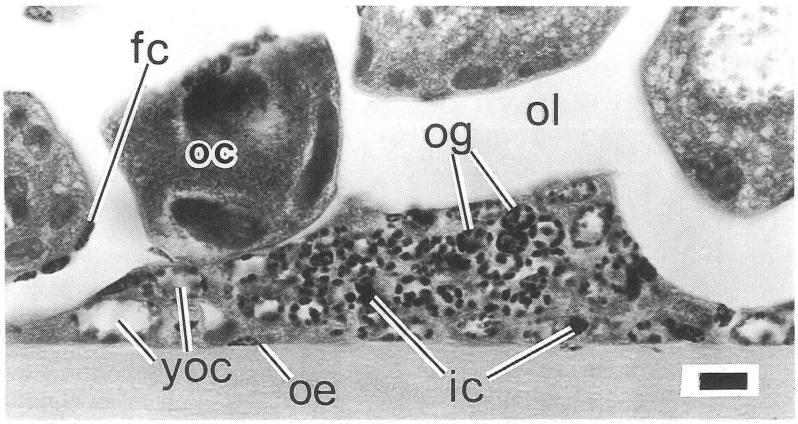

Fig. 2 Sagittal section of ovary showing germarium. Scale =10 μm. fc; follicle cell, ic: interstitial cell, og: oogonia, yoc: young oocyte.

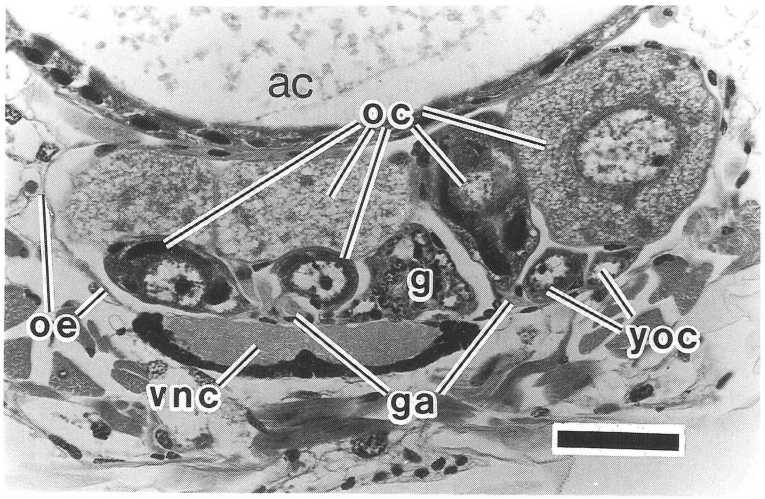

Fig. 3 Cross section of ovary at 6th body segment showing paired germ areas and single germarium. Scale =50 μm. ac; alimentary canal, vnc; ventral nerve cord.

had one or two distinct nucleoli in their large germinal vesicles. The interstitial cells with nuclei of about 5 μm in major length, were found among the oogonia and oocytes in the germarium (Fig. 2). Other than the germarium, a seemingly segmental arrangement of paired small discoidal germ areas was found on the ventral ovarian epithelium (Fig. 4). Each germ area was a cell-cluster consisting of several young oocytes of about 20-30 μm in diameter and the interstitial cells. No oogonia were found in the germ areas. In many adult ovaries, 10 pairs of the germ areas were recognized.

The oocytes attaining about 30 μm in diameter were surrounded by a thin layer of the follicle cells in the germ areas (Fig. 2). These folliculated oocytes more than 35 μm in diameter left the germ areas, but they were connected to their germ areas by elongated follicle cells (Fig. 5). This elongations lasted until the end of the vitellogenic stage. All the follicle cells had no sign to play any role in the vitellogenesis.

The germarium was found first in the larval stadium II, as a young cell-cluster consisting of spherical gonial cells and interstitial cells, entirely surrounded by a thin layer of gonadal epithelium. In the larval stadium III, the youngest oocytes with distinct germinal vesicles occurred in the periphery of the germarium. These

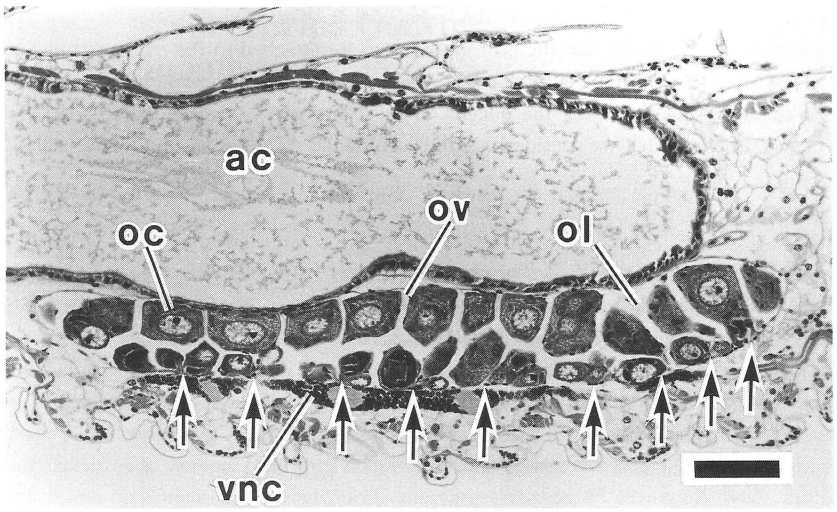

Fig. 4 Sagittal section of ovary parallel with median line, showing seemingly segmental germ areas (arrows). Scale =100 μm.

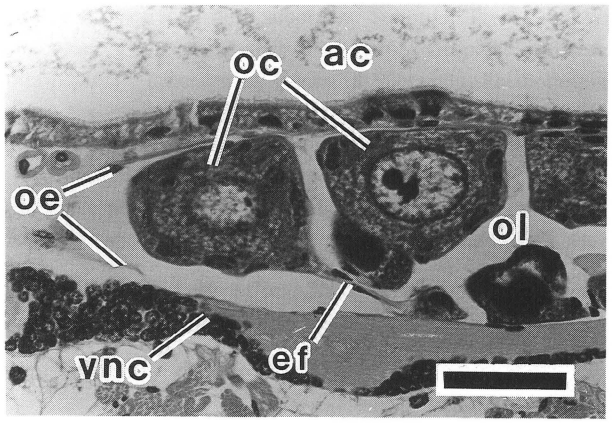

Fig. 5 Sagittal section of ovary showing germ area, follicle elongation connecting between oocyte and germ area. Scale = 50 μm. ef: elongated follicle cells.

oocytes were already surrounded by several interstitial cells developing into follicle cells. During the subsequent stages, some oocytes and interstitial cells were separated forward and backward from the germarium to form a series of new paired germ areas. In the larval stadium VII (the subadult stage), the ovary swelled rapidly. The ovarian lumen was remarkably extended, and the follicle elongations straightened, carrying the large oocytes.

In other penicillate diplopod, *Polyxenus lagurus*, several authors described a single tubular ovary containing a germarium on the ventral epithelium and folliculated oocytes in the ovarian lumen (Heathcote, 1889; Reinecke, 1910; Seifert, 1960), but the paired and seemingly segmental germ areas and the elongated follicle cells connecting between the folliculated oocytes with the germ areas were found for the first time in the present study. The follicle elongations seemingly resemble the cellular stalks bearing the growing oocytes in many chelicerates. Through the postembryonic stages, however, oocytes develop only in the ovarian lumen of the

present species as in many mandibulates, not in the haemocoel as in many chelicerates.

It is known in some myriapods that the growing oocytes are attached to the ovarian epithelium with their follicle layer (Tiegs, 1947; Jangi, 1957; Kubrakiewicz, 1987). In the penicillate diplopods also, Reinecke (1910) described some cellular "stalks" bearing the oocytes in the ovary of *P. lagurus*. Seifert (1960) regarded the "stalks" as artificial structures, but we consider that they are similar to our elongated follicle cells.

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