Formation of nervous system and alimentary canal in normal and separate embryos of horseshoe crab, *Tachypleus tridentatus*

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The morphology and embryology of the horseshoe crabs have been studied by many authors, but details of their structures and processes of their formation have not yet been made clear. The examination of formation of the ganglions and stomodaeum, especially the crossing between the nervous system and stomodaeum, has been stayed incomplete. In the present study, we tried to make it clear in Japanese horseshoe crab, *Tachypleus tridentatus.* For this purpose, we observed the processes of formation of the ganglions and stomodaeum in normal embryos, and also examined these processes in the separate embryos (Fig. 1) whose stomodaea did not pass through between the commissures of central nervous system.

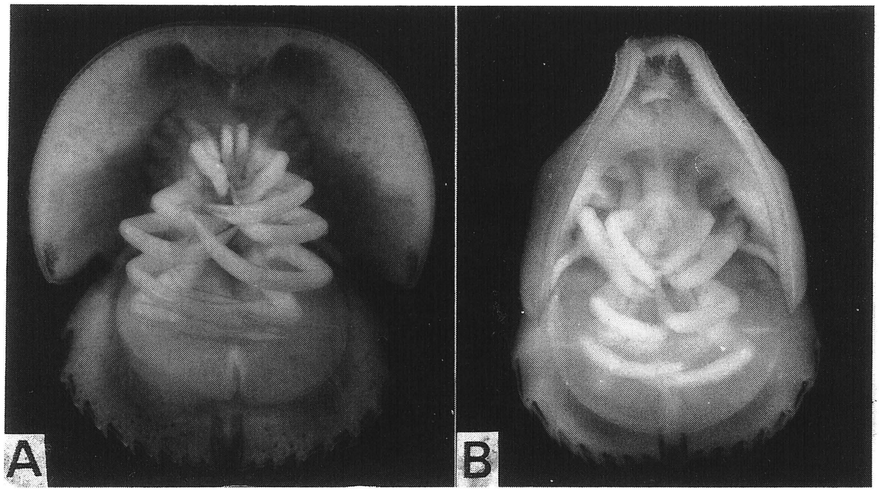

Fig. 1 A. A normal embryo at stage 21. B. A separate embryo whose the ventral plate is separated at the region between the 1st and the 2nd prosomatic segments (The type of Fig. 2-C).

Materials and methods: Adult Japanese horseshoe crabs, *Tachypleus tridentatus* (Chelicerata, Arthropoda), collected in north Kyushu in Japan, were brought to the Shizuoka University where eggs were artificially inseminated. The developmental stages of embryos were determined from the Sekiguchi's normal table (1973). The separate embryos were obtained by 24 hr-treatment with calcium free sea water or inhibitors of DNA synthesis (Itow, 1979, 1982). The sectioned and dissected specimens were examined.

Ⅰ. *The formation of ganglions and stomodaeum in normal embryos*

In the present paper, the ganglions in the front area of the lst prosomatic segment are called as the brain.

The following facts were found and ascertained. 1) The cinemicrographical observations showed that the formation of nervous system and stomodaeum began at the stage of the obvious morphogenic movement (stage 11) (Itow and Sekiguchi, 1980). 2) The stomodaeum was observed as a tubular structure at stage 14. 3) The ganglions could be observed in the embryos fixed at stage 16. The commissures of ganglions were clearly stained by eosin after stage 19. 4) The open of stomodaeum (the developing mouth) began to migrate posteriorly from the position in front of the lst prosomatic segment at stage 18. 5) The stomodaeum passed through the area between the commissures of the lst prosomatic segment and those of the 2nd one. 6) The crossing of nervous system and stomodaeum was thought to be formed at stage 18-19. 7) The all segment primordia and all ganglions finished to be formed till stage 20 (the stage after the 3rd embryonic moulting). 8) All prosomatic ganglions had commissures. 9) In the region between the prosome and opisthosome, there were not any ganglions which had no appendages. 10) The prosome had a brain and 8 pairs of prosomatic ganglions. The prosomatic ganglions are classified into 6 pairs of cephalothoracic ganglions and 2 pairs of abdominal ganglions (the ganglions of chilaria and opercula). The opisthosome had 8 pairs of ganglions (the rest abdominal ganglions). 11) The formation of alimentary canals finished in the 2nd instar larvae (Fig. 2).

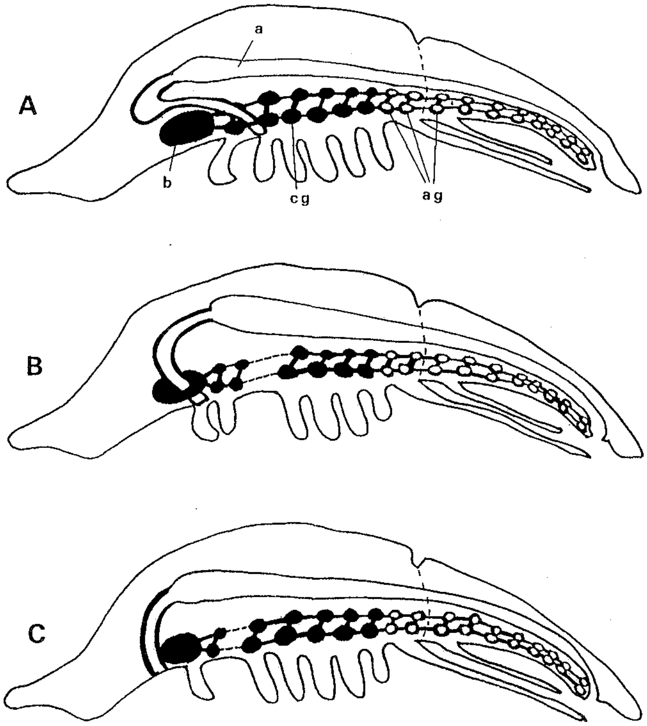

Fig. 2 The nervous system and alimentary canal of the 2nd instar larvae. A. A normal one. B. The separate one whose the ventral plate is separated at the 2nd prosomatic segment. C. The separate one whose the ventral plate is separated at the 1st prosomatic segment. a: alimentary canal, ag: abdominal ganglions, b: brain, cg: cephalothoracic ganglions.

Ⅱ. *The release of crossing between the nervous system and stomodaeum in separate embryos*

Calcium free sea water and inhibitors of DNA synthesis induced the separate embryos. When the embryos at the stage of enlargement of germ disk (stage 7-10) were treated with inhibitors of DNA synthesis, the cell proliferation of germ disk became incomplete and the cell density of germ disk became low in spite of normal spreading of germ disk. The incomplete germ disk was separated mainly at the 2nd and 3rd prosomatic segments in the process of obvious morphogenic movement. During the movement, the embryonic area elongated anteriorly and posteriorly at the region where the transverse furrow was formed. The 2nd and 3rd prosomatic segments were formed later at the region (Itow and Sekiguchi, 1980). Calcium free sea water directly affected the region of the furrow at the stage of obvious morphogenic movement.

The form and structure of the separate embryos depended on the position of the separation, especially the largeness

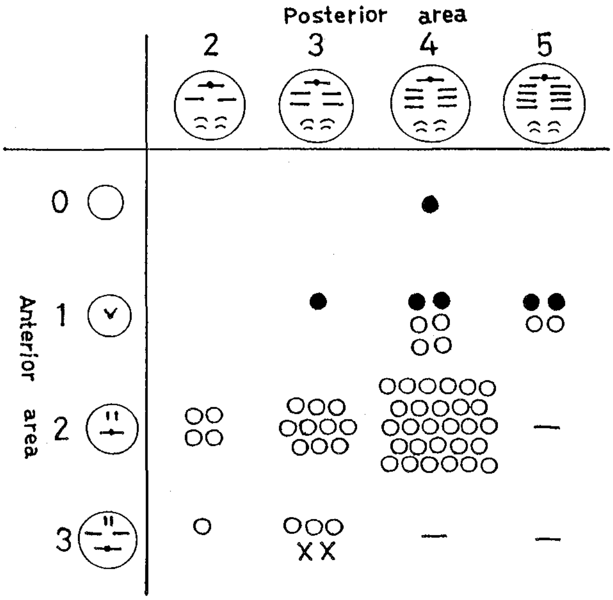

Fig. 3 The position of brains in separate embryos. Each mark shows an embryo. ●: Nervous systems and alimentary canals do not cross each other (The type of Fig. 2-C). ○: Alimentary canals pass through the middle of brains (The type of Fig. 2-B). x: Alimentary canals pass behind the brain. The nervous system and the alimentary canal cross each other (The type of normal embryos). – shows the separate embryos with excess segments. They are never induced.

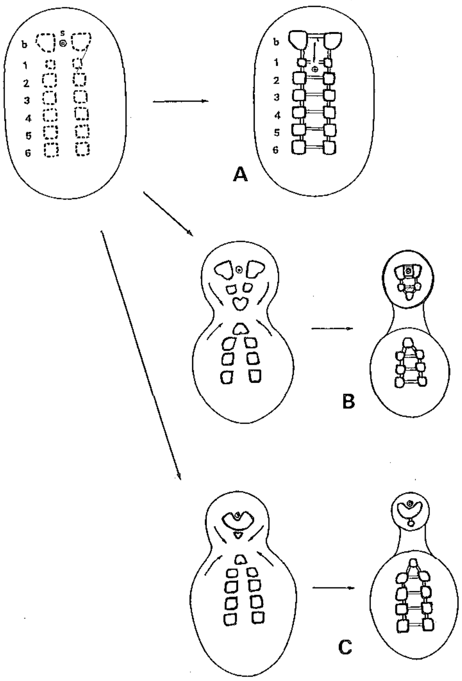

Fig.4 The crossing and no crossing of nervous system and alimentary canal in normal and separate embryos. A. A normal embryo. B. The separate embryo whose the ventral plate is separated between the 2nd and 3rd prosomatic segments (The type of Fig. 2-B). C. The separate embryo whose the ventral plate is separated between the 1st and 2nd prosomatic segments (The type of Fig. 2-C). b: brain, s: stomodaeum, 1-6: each ganglions of prosomatic segments.

of anterior pieces of separate ventral plates (Figs. 2 and 3). When the anterior pieces had more than 4 segments, the stomodaeum passed behind the brain, and the nervous system and stomodaeum were crossed each other, and when they had 1 to 3 segments, the stomodaeum passed the middle of a brain. When the anterior ventral plates of the separate embryos had no or only one pair of prosomatic appendages, the crossing was released (Figs. 2-C and 3-●).

The embryos losing the anterior ventral plates (No-anterior embryos) did not have brains and stomodaea.

The mechanism of release of the crossing was thought as follows (Fig. 4): The prospective cells of stomodaea and brains were determined before the time of separation of ventral plates, because the regulation (the formation of new supernumerary segments) did not occur after the separation. The prospective cells of brains were moved by the separation. When the anterior parts of separate ventral plates were very small, the prospective cells of the brain were situated behind the stomodaeum. As the results, the crossing of nervous system and stomodaeum did not occur (Fig. 4-C).

The release of the crossing revealed the following four points. (1) The crossing was constructed through the process of the gathering of cells of the brain. (2) It was not determined that the cells of the brain gathered anterior to the stomodaeum. That is, the stomodaeum did not determine the gathering point of the brain. (3) The position of mouth is not appropriate for the indicator of determination of homologous segments among arthropodan species. (4) The crossing was susceptible to modifications in at least one representative Protostomia, horseshoe crabs. Attempts to derive Deuterostomia from Protostomia could not be rejected because of the crossing of the alimentary canal and central nervous system.

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