Recent Advances in Insect Embryology in Japan and Poland
Edited by H. Ando and Cz. Jura
Arthropod. Embryol. Soc. Jpn.
(ISEBU Co. Ltd., Tsukuba) 1987

Embryonic Development of the Ventral Nervous System of the Stonefly, *Kamimuria tibialis* (Pictét) (Plecoptera, Perlidae)*

Tohru KISHIMOTO

Synopsis

The embryonic development of ventral nervous system in a plecopteran *Kamimuria tibialis* was described, with special reference to the change in number of neuroblasts in the gnatho-thoracic to the abdominal segments during embryogenesis.

Introduction

I have been studying the embryonic development of the stonefly, *Kamimuria tibialis* to understand the basic developmental pattern of the Plecoptera, and to examine the phylogenetic relationship between the Plecoptera and other polyneopteran orders from the embryological point of view.

In our previous papers on the embryogenesis of *K. tibialis* (Kishimoto and Ando, 1985, 1986), we described the changes in the external features of the embryo and the formation of the alimentary canal, but the embryogenesis of other organs remains unpublished.

In the present paper, I describe the embryogenesis of the ventral nervous system of *K. tibialis*.

Materials and Methods

Most of the information concerning materials and methods utilized in the present

* Comprising a part of the doctoral thesis, University of Tsukuba, 1986 (unpublished).
Contributions from Sugadaira Montane Research Center, University of Tsukuba, No. 112.

study were described in a previous paper (Kishimoto and Ando, 1985).

For sectioning, normal paraffin embedding method was employed. Sections, 6 μm thick, were stained with Delafield's haematoxylin and eosin.

Results

Embryonic development in *K. tibialis* divided into 12 stages, based chiefly on changes in external features of the embryo. Stage numbers used in the present study correspond to those in the previous paper (Kishimoto and Ando, 1985).

a) *General development of ventral nerve cord*

Formation of the ventral nerve cord is shown diagrammatically in Fig. 1.

In stage 4, a slight median groove is formed at the ventral surface of the embryo. This groove is said to be a rudiment of neural groove and indicates the sign of the beginning of the neurogenesis, but the neuroblasts could not be found at this time. In stage 5, the gnathal, thoracic and first four abdominal segments of the embryo are formed and several large cells or neuroblasts differentiate among the ectodermal cells in each of the gnathal and thoracic segments. The ectodermal layer on the neural groove invaginates to form the median cord at the dorso-median line of the embryo, but the neuroblasts of the median cord do not differentiate yet. The portion of median cord in the anterior region of each segment becomes thinner than that in the posterior region.

In stage 6, at the time the ectodermal segmentation of the embryo completes to the 11th abdominal segment, the neuroblasts become distinct in the intercalary to the second abdominal segment (Fig. 1 A). They are easily distinguished from the surrounding ectodermal cells, since their nuclei are large (*ca.* 10 μm in diameter) and stained lightly with haematoxylin and the neuroblasts produce small daughter cells which are called as ganglion mother cells by their unequal divisions. The neuroblasts at each lateral plate in a segment are four or five in number in cross and longitudinal sections.

The differentiation of the neuroblasts is delayed in the posterior segments. Early in stage 7, the neuroblasts make their appearance in the four gnathal, three thoracic and 10 abdominal segments. In the gnathal and thoracic segments, the production of the daughter cells or ganglion cells proceeds, so that the columns of the ganglion cells are formed.

As the same time the ganglion cells of the gnathal and thoracic segments form plasmic fibers which in turn form the bundles of the axon or neuropiles, situated independently of each other (Fig. 1 B). As the development proceeds, the neuropiles increase in volume and then extend antero-posteriorly. Late in stage 7, the ganglia of the intercalary to the first abdominal segment are connected longitudinally with the extending neuropiles or connectives (Fig. 1 C).

In this stage a neuroblast differentiates in the posteriormost part of each median cord of the mandibular to the metathoracic segment.

In stage 8, the neuroblasts also appear in the 11th abdominal segment, but they are only two or three in number in the lateral half of this segment (Fig. 1 D). They do

1

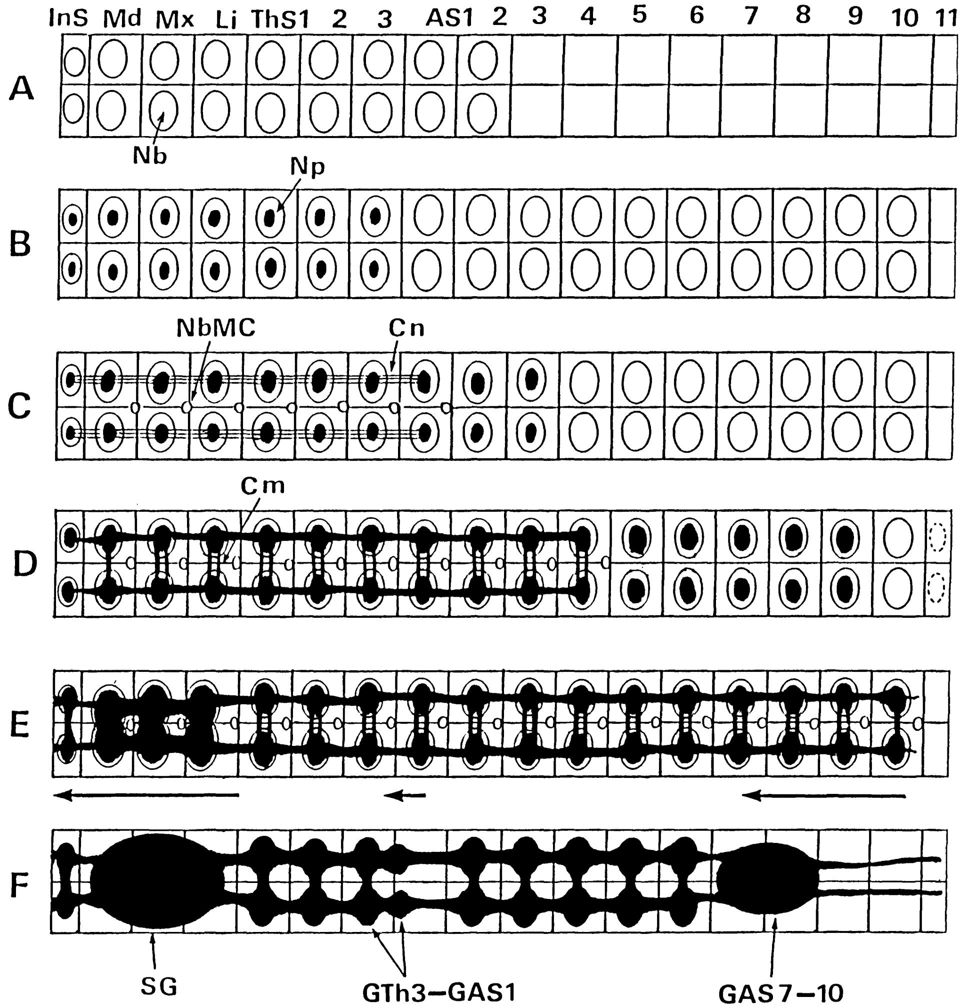

Fig. 1. Schema of the formation of ventral nervous system. A, B, C, D, E, F; stage 6, early in stage 7, late in stage 7, stage 8, stage 9 and stage 12, respectively. Arrows showing the anterior shifting of ganglia. AS1 – 11, first to 11th abdominal segment; Cm, commissure; Cn, connective; GAS7-10, ganglia of seventh to 10th abdominal segment; GTh3-GAS1, ganglia of metathoracic and first abdominal segments; InS, intercalary segment; Li, labial segment; Md, mandibular segment; Mx, maxillary segment; Nb, neuroblast; NbMC, neuroblast of median cord; Np, neuropile; SG, suboesophageal ganglion; ThS1 – 3, pro-, meso- and metathoracic segments.

not produce the daughter cells and disappear in the next stage. Consequently, the ganglion of the 11th abdominal segment is not formed. The neuroblast of the median cord in this segment also does not differentiate.

The columns of the ganglion cells of each segment grow higher, especially in the gnathal and thoracic segments, and each ganglion increases in volume, while the dermatoblasts become attenuated and begin to form the epidermis. The ganglion cells, produced by the neuroblasts at the dorso-lateral part of each segment, do not form the column, but extend medially on the dorsal surface of the ganglia to form the covering of the ganglia. In the median cord of the second to the fourth abdominal segment, the neuroblasts differentiate.

The neuropiles appear in the intercalary to the ninth abdominal segment and increase in volume. The connectives are formed between each segment from the intercalary to the fourth abdominal segment and become thicker than in the previous stage. In this stage the transverse connections of the neuropiles of each ganglion, so-called commissures, are formed in the mandibular to the fourth abdominal segment. Two commissures are formed: the anterior and posterior, in each segment but only one in the mandibular segment.

In stage 9 the dermatoblasts become thinner and are separated from the ganglion. The neuroblasts of the median cord occur from the mandibular to the 10th abdominal segment (Fig. 1 E). The connectives and commissures are completed in the gnathal, thoracic and abdominal segments; two commissures in each segment except in the intercalary, mandibular and 10th abdominal segments, where only one commissure is formed. The neuropiles increase in volume and occupy the most part of the ganglion in each segment.

The neurilemma makes its appearance on the dorsal surface of the ganglion as a thin membrane. The origin of the neurilemma is unclear, but it may be originated from the peripheral cells covering the ganglion, produced by the dorso-lateral neuroblasts.

From this stage the fusion of the ganglia begins in the three regions. With the movements of the cephalization, the mandibular, maxillary and labial ganglia shift forwards to fuse with each other, while the ganglia of the intercalary segment also shift forwards and come to lie laterads of the stomodaeum. The first abdominal ganglion begins to shift to the metathoracic ganglion, and the eighth to the 10th abdominal ganglion begin to shift to the seventh abdominal ganglion. As the development proceeds, the sternum of the first abdominal segment fuses with the metathoracic sternum, but the seventh to the 10th abdominal segment do not fuse with each other.

In stage 12, in the full-grown embryo, the fusion of the ganglia in the three regions completes; the gnathal ganglia fuse to form the suboesophageal ganglion, the seventh to the 10th abdominal ganglion fuse to form the large ganglion (Fig. 1 F). All the neuroblasts in each segment disappear since the neurblasts differentiate into the ganglion cells. The commissures in each segment become thickened, so that the anterior and posterior ones cannot be distinguised from one another.

b) *Number of neuroblasts in each segment*

During the embryonic development of the ventral nervous system the whole number of neuroblasts in each ganglion of the mandibular to the 10th abdominal segment was counted. The cell counts were made on the reconstructions of serial sections, and portions of the one neuroblast could be traced for two or three sections because of its large diameter (*ca.* 10 μm).

Number of neuroblasts in gnathal segments:

The neuroblasts of each gnathal segment (mandibular, maxillary and labial segments) appear in stage 6. They increase in number and reach the maximum number in stage 7: the maximum number of neuroblasts is 42 in the maxillary and labial segments, and 28 in the mandibular segment. The number of neuroblasts in each gnathal segment begins to decrease after this stage and by stage 11 all the neuroblasts degenerate. The time course of change in the number of neuroblasts is similar within the maxillary and labial segments.

Number of neuroblasts in thoracic segments:

The neuroblasts of each thoracic segment appear in stage 6. They increase in number and reach the maximum number in stage 7: the maximum number of neuroblasts in 67 in the prothorax, 65 in the mesothorax and 60 in the metathorax. The number of neuroblasts in each thoracic segment begins to decrease after this stage and then all the neuroblasts degenerate late in stage 11. The time course of change in number of

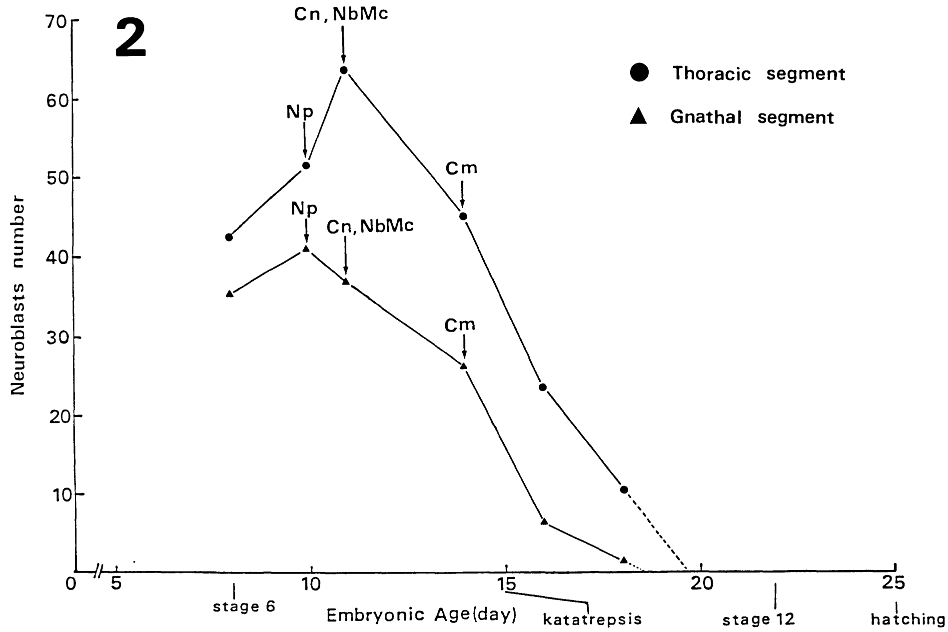

Fig. 2. Change in number of neuroblasts in time course. The mean number of neuroblasts per segment was calculated for the three thoracic and two gnathal (maxillary and labial) segments, and plotted against embryonic age. Arrows showing the differentiating time of neuropile (Np), connective (Cn), commissure (Cm) and neuroblast of median cord (NbMC).

neuroblasts among three segments is quite similar to each other.

Number of neuroblasts in abdominal segments:

The neuroblasts of the first and second abdominal segments appear at the beginning of stage 6, and those of the posterior segments appear late in stage 6 or stage 7. The maximum number of neuroblasts in each abdominal segment is: 43 in the first abdominal segment, 42 in second, 39 in third, 36 in fourth, 32 in fifth, 34 in sixth, 39 in seventh, 34 in eighth, 28 in ninth and 10 in 10th. Apparently, the number of neuroblasts in the 10th abdominal segment is smaller than that of other segments.

Comparison of the time course change in the number of neuroblasts among gnathal, thoracic and abdominal segments:

The time course change in the number of neuroblasts in gnathal, thoracic and abdominal segments is shown in Figs. 2 and 3.

In Fig. 2, the mean number of neuroblasts per segment is plotted at the various stages for the three thoracic segments, and the maxillary and/or labial segments. This figure shows that the neuroblasts in the gnathal segments degenerate more rapidly than in the thoracic segments.

In Fig. 3, the mean number of neuroblasts per segment is plotted for the three

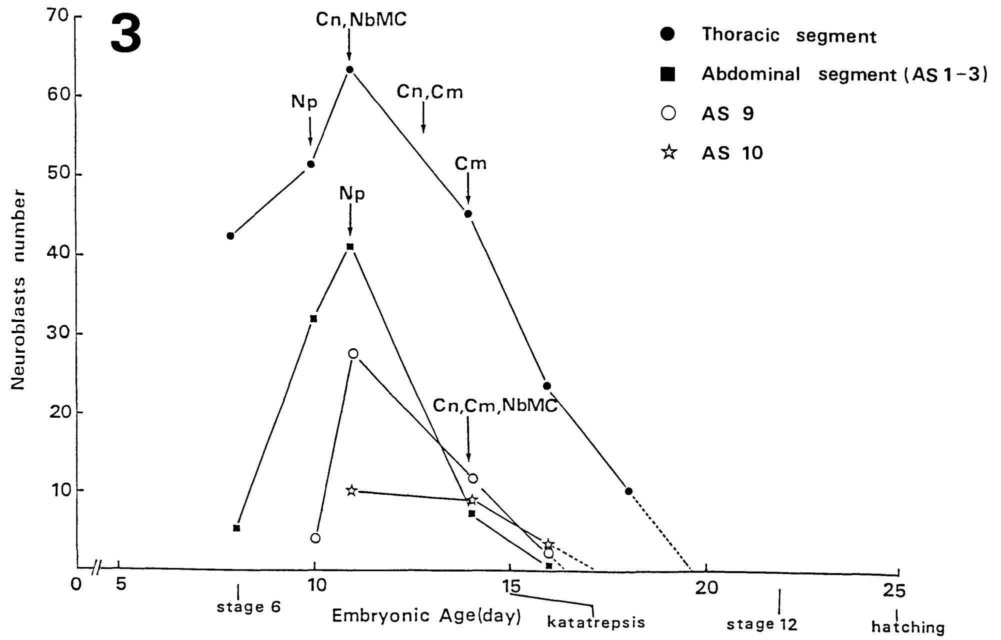

Fig. 3. Change in number of neuroblasts in time course. The mean number of neuroblasts per segment was calculated for the three thoracic and first three abdominal segments, and plotted against embryonic age, also the number of neuroblasts in the ninth and 10th abdominal segments was plotted. Arrows showing the differentiation time of neuropile (Np), connective (Cn), commissure (Cm) and neuroblast of median cord (NbMC).

thoracic segments and first three abdominal segments, additionally, the number of neuroblasts in the ninth and the 10th abdominal segments is also plotted. This figure shows that the neuroblasts of the abdominal segments degenerate most rapidly in the three major regions of the embryo, and that the segmental specificity occurs within the abdominal segments in early neurogenesis.

Discussion

The general development of the ventral nervous system in *K. tibialis* proceeds in the same way as in other insects. In *K. tibialis* the neuroblasts differentiate in three gnathal, three thoracic and 11 abdominal segments, but those in the 11th abdominal segment are transient and do not form a pair of abdominal ganglia. Also *Allonarcys* (= *Pteronarcys*) *proteus*, the 11th abdominal ganglion is not formed (Miller, 1940). According to Johannsen and Butt (1941), during the earlier stages of development, in more primitive insects as well as in some of more advanced ones (*Lepisma*, *Gryllotalpa*, *Periplaneta*, *Gryllus*, *Locusta*, *Leptinotarsa*, *Donacia*, *Calandra*, *Hylotoma*, *Charicodoma*, *Apis*, and others), the 11th abdominal ganglion can be identified but they disappear as the development proceeds.

In the later development of insects, there is a tendency for some of the ganglia, usually the eighth to the 10th (11th) abdominal one, to fuse. Further fusion of the ganglia may also occur, depending on the orders of insects, especially in the short-bodied paraneopterans.

In *K. tibialis* the seventh to the 10th abdominal ganglion fuse and, the metathoracic and the first abdominal ganglia do so also, while in *A. proteus*, only the eighth to the 10th abdominal ganglion fuse (Miller, 1940).

The median cord of *K. tibialis* is derived from the median invagination of the ventral ectoderm, in the same way as in other insects, but the fate of it could not be traced. According to the detail work of Goodman and Spitzer (1979), each neuroblast of the median cord gives rise to the dorsal median neuron.

Concerning the origin of the neurilemma in the ventral ganglia, there are three different opinions: 1) the neurilemma originates from the peripheral ganglion cells (Heymons, 1895; Strindberg, 1913; Nelson, 1915; Eastham, 1930; Roonwal, 1937; Ando, 1962), 2) from the median cord (Nusbaum, 1883; Wheeler, 1893; Tiegs and Murray, 1938; Okada, 1960), 3) or is mesodermal in origin (Baden, 1936). In *K. tibialis*, the neurilemma seems to arise from the peripheral ganglion cells.

The number and arrangement of neuroblasts were investigated in the orthopteran *Locusta migratoria* (Bate, 1976) and *Gryllus bimaculatus* (Miyamoto and Shimozawa, 1983; Miyamoto, 1983), and in the phasmid *Carausius morosus* (Tamarelle *et al.*, 1985) embryos.

Bate (1976) investigated the formation process of thoracic and abdominal ganglia during the embryogenesis of *L. migratoria*. He confirmed that the arrangement and number of neuroblasts are very regular and segmentally homologous: neuroblasts in each of three thoracic segments are arranged in seven rows composed of four to 10 cells plus one median neuroblast, with 60 + 1 cells in each segment. In the abdomen the arrangement of neuroblasts is similar to that of thorax, with 56 + 1 cells per segment.

According to Miyamoto and Shimozawa (1983) and Miyamoto (1983), the number and arrangement of neuroblasts for all segments resemble each other. There are 62 + 1 neuroblasts in each of the gnathal and thoracic segments, and 56 + 1 in the abdominal ones. In *C. morosus*, the map of thoracic neuroblasts shows the presence of 56 elements in each thoracic segment (Tamarelle *et al.*, 1985).

Thus, in the early embryogenesis the anlage of the nervous system shows the segmental homology, not only within the thoracic and the abdominal ganglia, but also between the thoracic and abdominal ones.

In *K. tibialis*, the segmental homology within the thoracic ganglia may be shown in the early embryogenesis by the result of cell counting. However, it should be stressed out that the number of neuroblasts in the mandibular ganglia is smaller than that of the maxillary and labial ganglia. This difference of the number of neuroblasts in gnathal ganglia may be connected with structural differences, that is, the mandibular segment bears a pair of simple appendages, while the maxillary and labial segments bear complicated appeendages with palps.

In the abdominal segments, no appendages are formed, except the pleuropodia in the first abdominal segment, so that the significant structural differences within abdominal segments do not occur. However, the number of neuroblasts in the 10th abdominal segment is extremely small, and it is likely that this segment expresses its own functional specialization or difference.

According to Miyamoto and Shimozawa (1983) and Miyamoto (1983), the segmental homology of the ganglia is expressed in the early neurogenesis, while the segmental specialization of ganglia is expressed in the late neurogenesis, because each segment becomes to express its own functional specialization or difference with the advance of development, in contrast to the structural homologies. In *K. tibialis* the segmental specialization of ganglia, of the mandibular and the 10th abdominal segments, occurs in the early neurogenesis.

Acknowledgements

I wish to express my cordial thanks to Prof. H. Ando, Sugadaira Montane Research Center, University of Tsukuba, for his guidance as well as invaluable suggestion and encouragement given to me in the course of study.

References

Ando, H., 1962. The Comparative Embryology of Odonata with Special Reference to a Relic Dragonfly *Epiophlebia superstes* Selys. Jpn. Soc. Promot. Sci., Tokyo.

Baden, V., 1936. Embryology of the nervous system in the grasshopper, *Melanoplus differentialis* (Acrididae; Orthoptera). *J. Morphol.* 60: 159-188.

Bate, C. M., 1976. Embryogenesis of an insect nervous system I. A map of the thoracic and abdominal neuroblasts in *Locusta migratoria. J. Embryol. Exp. Morphol.* 35: 107-123.

Eastham, L. E. S., 1930. The formation of germ layer in insects. *Biol. Rev.* 5: 1-29.

Goodman, C. S. and N. C. Spitzer, 1979. Embryonic development of identified neurones: differentiation from neuroblast to neurone. *Nature* 280: 208-214.

Heymons, R., 1895. Die Embryonalentwicklung von Dermapteren und Orthopteren unter Besonderer Berücksichtigung der Keimblätterbildung. Gustav Fischer, Jena.

Johannsen, O. A. and F. H. Butt, 1941. Embryology of Insects and Myriapods. McGraw-Hill, New York.

Kishimoto, T. and H. Ando, 1985. External features of the developing embryo of the stonefly, *Kamimuria tibialis* (Pictét) (Plecoptera, Perlidae). *J. Morphol.* 183: 311-326.

————, and ————, 1986. Alimentary canal formation in the stonefly, *Kamimuria tibialis* (Pictét) (Plecoptera, Perlidae). *Int. J. Insect Morphol. Embryol.* 15: 97-105.

Miller, A., 1940. Embryonic membranes, yolk cells, and morphogenesis of the stonefly *Pteronarcys proteus* Newman (Plecoptera: Pteronarcidae). *Ann. Entomol. Soc. Amer.* 33: 434-477.

Miyamoto, T., 1983. Embryonic development of the central nervous system in the cricket, *Gryllus bimaculatus.* II. Segmental specialization in late neurogenesis. *Zool. Mag.* 92: 332-341.

————, and T. Shimozawa, 1983. Embryonic development of the central nervous system in the cricket, *Gryllus bimaculatus.* I. Segmental homologies in early neurogenesis. *Zool. Mag.* 92: 317-331.

Nelson, J. A., 1915. The Embryology of the Honey Bee. Prinston Univ. Press, Prinston.

Nusbaum, J., 1883. Vorläufige Mittheilung über die Chorda der Arthropoden. *Zool. Anz.* 6: 291-295.

Okada, M., 1960. Embryonic development of the rice stem-borer, *Chilo suppressalis. Sci. Rep. Tokyo Kyoiku Daigaku, Sec. B* 9: 243-296.

Roonwal, M. L., 1937. Studies on the embryology of the African migratory locust, *Locusta migratoria migratorioides* Reiche and Frm. (Orthoptera, Acrididae). II. Organogeny. *Phil. Trans. Roy. Soc. Lond., Ser. B* 227: 175-244.

Strindberg, H., 1913. Embryologische Studien an Insekten. *Z. Wiss. Zool.* 106: 1-227.

Tamarelle, M., A. Haget and A. Ressouches, 1985. Segregation, division, and early patterning of lateral thoracic neuroblasts in the embryos of *Carausius morosus* Br. (Phasmida: Lonchodidae). *Int. J. Insect Morphol. Embryol.* 14: 307-317.

Tiegs, O. W. and F. V. Murray, 1938. The embryonic development of *Calandra oryzae. Q. J. Microsc. Sci.* 80: 159-284.

Wheeler, W. M., 1893. A contribution to insect embryology. *J. Morphol.* 8: 1-160.

Author's address: Dr. T. Kishimoto
Kansai Sogô Kankyô Center, Nakazaki-Nishi 2-3-39, Kita, Oosaka 530, Japan