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

Formation of Brain of the Normal and the Embryonic Lethal, "Star-spots Duplication" Embryos in the Silkworm, *Bombyx mori* (Bombycidae, Lepidoptera)

Susumu HASHIMOTO

and

Keiichiro MIYA

Synopsis

Formation of the brain is described in the normal and *Dus/Dus* embryos of *Bombyx*. In both types of embryos the brain originates similarly from the neuroblasts in the neurogenic region of the protocephalon and there is no remarkable difference between them. From the appendage formation stage, each neuroblast divides unequally to produce a larger and a smaller daughter cell, and these are organized into three groups of cells, anlagen of the proto-, deuto- and tritocerebrum. The larger cell repeats unequal divisions as the neuroblast and the smaller cell divides equally as the ganglion mother cell, and the number of cells increases remarkably. With formation of the head, these three cerebra migrate and unite to form a brain. In *Dus/Dus* embryo these divisions occur more than in the normal ones, resulting in hypertrophy of the brain and failure of the head capsule formation. This process is discussed comparatively in both types of embryos.

Introduction

The embryonic development of the central nervous system has been studied by many authors in various insect species, but formation of the brain has been reported usually as a part of these studies. The insect brain originates from the neuroblasts which differentiate in the ectoderm of the protocephalon during the early development similarly as the ganglia of the ventral nerve cord. Different from the ganglia of the ventral nerve cord, however, the brain is not only a complex structure consisting of several

groups of nerve cells (see Johannsen and Butt, 1941; Anderson, 1972a, b), but also integrates various informations including those from the sensory organs of the head to control the behavior of the individual (see Chapman, 1969). And the fate map of the central nervous system is made in several species, for example, in *Drosophila* (Poulson, 1950) and in *Tenebrio* (see Anderson, 1972b; Sander, 1976).

Recently, segregation of the neuroblasts in *Drosophila* was studied in detail by Hartenstein and Campos-Ortega (1984) and interesting results were reported. Further, they discussed the function of the genes relating to segregation of the neuroblasts in comparison with mutants affecting the early neurogenesis.

On the other hand, the embryonic development of the central nervous system in *Bombyx* was described already by Toyama (1909) and Ikeda (1913), but there was no detailed description on formation of the brain. Afterwards, the fate map of the neurogenic region was made clear also in *Bombyx* by Takami (1946) with the cauterization experiment and by Katsuki *et al.* (1980) through the analysis of mosaic larvae induced by super-cooling treatment. Furthermore, we found a new embryonic lethal mutant, "star-spots duplication", which affected on development of various organs and tissues, especially on the neurogenesis (Miya and Hashimoto, 1977). The phenotype shows hypertrophy of the central nervous system similarly as in *Drosophila*. Hence we felt that the re-examination of *Bombyx* neurogenesis might elucidate a better understanding of this mutant, but the present paper deals only with formation of the brain in comparison with the normal and the mutant embryos.

Material and Method

"Star-spots duplication" (symbol *Dus*) is a dominant gene and shows additional star-spots on the sixth abdominal segment, and it is a recessive embryonic lethal and the homozygotes represent various abnormalities and die at the setae formation stage. Non-hibernating eggs of *Dus*/+ × *Dus*/+ were used as material.

For light microscopic observation, the eggs were fixed with Carnoy's fluid for 24 hr, stained with carbol thionin, dehydrated with ethanol, cleared with benzene, and mounted in ceder oil for total preparations. The fixed and dehydrated eggs were embedded in paraffin and cut 8 μm thick. The sections were stained with Delafield's hematoxylin and eosin.

For electron microscopy, the eggs were fixed with 2.5% glutaraldehyde in 0.1 M cacodyrate buffer (pH 7.4) at 4℃ for 1.5 hr, rinsed with the same buffer and postfixed with 1% osmium tetroxide in 0.1 M cacodyrate buffer (pH 7.4) at 4℃ for 2 hr. After dehydration with ethanol series, the fixed eggs were embedded in Epon 812 through QY-1. Ultrathin section were stained with uranyl acetate and lead nitrate.

Results

1. *Formation of the brain in the normal embryo*

a. Differentiation of the neuroblasts in the protocephalon

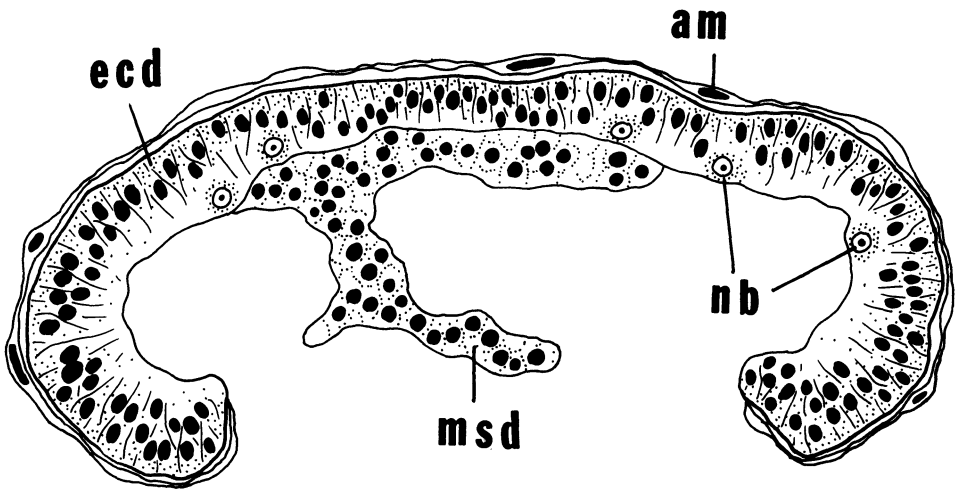


Fig. 1. Cross section of the protocephalon in 36-hour old embryo. am, amnion; ecd, ectoderm; msd, mesoderm; nb, neuroblast.

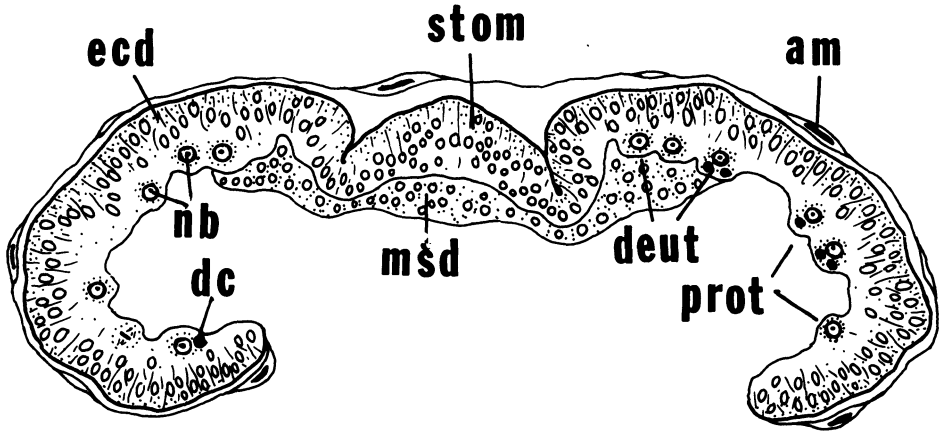


Fig. 2. Cross section of the protocephalon in 48-hour old embryo. am, amnion; dc, ganglion mother cell; deut, anlage of deutocerebrum; ecd, ectoderm; msd, mesoderm; prot, anlage of protocerebrum; stom, stomodaeum.

At 36 hour after oviposition the embryo becomes elongated and the boundary between the protocephalon and the trunk is clearly distinguised, but metamerism of the ectoderm does not occur yet. The mesoderm is arranged segmentally, but in the posterior part of the embryo it does not spread laterally along the dorsal side of the ectoderm. In the middle region of the protocephalon the mesoderm exists as a large

cell group, an oral cell mass, and several cells are being isolated from the oral cell mass into the yolk.

The neuroblasts, anlagen of the future brain, are recognized at first at this stage, earlier than their differentiation in the trunk. The neuroblast has a round larger nucleus than that of the other ectoderm cells composing the protocephalon, and in the nucleus a distinct nucleolus is recognized. The neuroblasts are rich in light-stained cytoplasm and are distributed separately and wide-spreadly in the ectoderm of the protocephalon (Fig. 1). The nucleus of the ectoderm cells is elliptical and the cells are arranged in a layer.

The ultrastructures of the neuroblasts are hardly different from those of the epidermis precursor cells, except for their size. The cytoplasm of the neuroblasts contains rod-like mitochondria, poor-developed rough-surfaced endoplasmic reticulum (rER), free ribosomes, lipid droplets, and lacunae containing glycogen granules (Fig. 3). In the nucleus chromatin granules are dispersed and look less dense than that of the epidermis precursor cells.

b. Proliferation of the neuroblasts

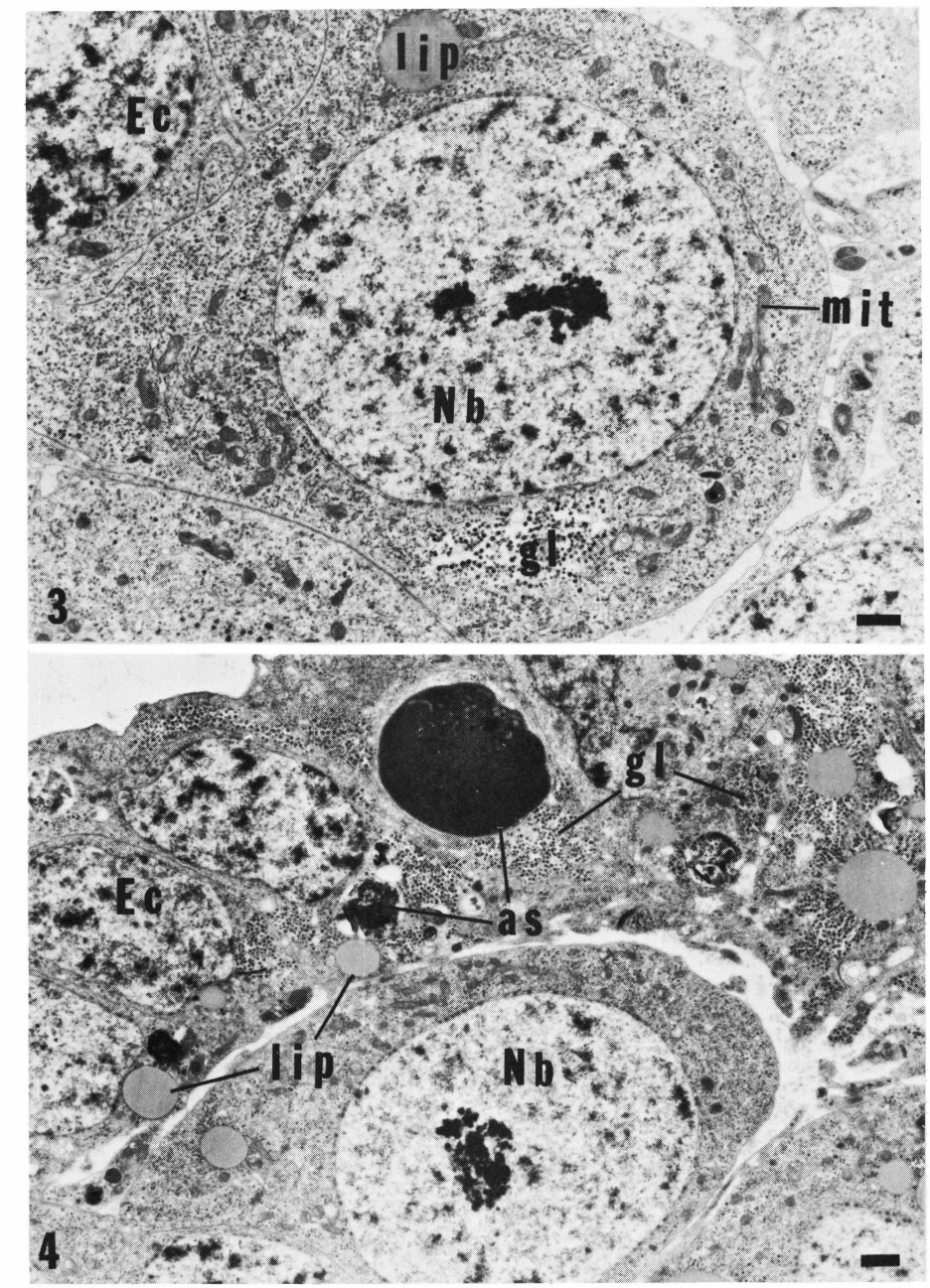
In the 42-hour old embryo, the metamerism of the ectoderm becomes clear, and the embryo consists of the protocephalon, three gnathal, three thoracic, and eleven abdominal segments. Further, the appendages begin to be formed in the gnathal and thoracic segments. From this stage the neuroblasts divide unequally to form a larger and a smaller daughter cells. The larger cell repeats unequal divisions as the neuroblast to produce the smaller cells, and the smaller cell is so-called "ganglion mother cell" and its nucleus is dark-stained.

c. Division of the neurogenic region in the protocephalon

In the 48-hour old embryo, the labral appendages project slightly at the anterior end of the protocephalon. The antennal, gnathal, and thoracic appendages develop more and more, and the appendages also appear in the abdominal segments. At this stage many neuroblasts and smaller ganglion mother cells are distributed widely along the dorsal (inner) side of the ectoderm of the protocephalon. Many neuroblasts, which do not divide yet, also are recognized, and the fact suggests that the segregation of the neuroblasts in the neurogenic region does not occur at the same time, but extends for several hours (Fig. 2).

Then the protocephalon is divided into three areas by occurrence of the slight lobes; that is, a wider area occupying from the anterior end of the protocephalon to the anterior end of the antennal appendages, an area from both sides of the stomodaeum to the base of the antennal appendages, and an area behind the stomodaeum. With the progress of development, the neuroblasts and their daughter cells are aggregated as one pair of groups at the dorsal side of each area. These are anlagen of the proto-, deuto- and tritocerebrum.

In 60—72-hour old embryo, all appendages, and the stomodaeum and the procto-



Figs. 3, 4. Electron micrographs representing neuroblasts in 36- and 48-hour old embryo, respectively. as, autosome; Ec, ectoderm cell; gl, glycogen granule; lip, lipid droplet; mit, mitochondria; Nb, neuroblast. Scales: 1 μm.

daeum develop remarkably. The neuroblasts repeat unequal divisions actively to produce abundant daughter cells, especially in the area of the protocerebrum. The daughter cells produced by divisions of each neuroblast are not arranged radially, but scattered irregularly within the group. The equal divisions of daughter cells also are observed, but it is unclear whether all cells derived from each neuroblast divide or not.

The electron micrographs at this stage indicate several new features. The nerve cells, produced by the unequal division of the neuroblasts and/or the equal divisions of the ganglion mother cells, extend several cytoplasmic projections. In the ectoderm cells autosomes appear, which seem to correspond to differentiation of the ectoderm cells as the anlagen of epidermis forming the head capsule later (Fig. 4).

d. Morphological changes with advance of the head formation and differentiation of constituents of the brain

At about 72 hours after oviposition, the gnathal segments move forward and begin to form the head together with the protocephalon. The deutocerebrum and the tritocerebrum migrate forward with the ectoderm of the protocephalon. The protocerebrum also moves dorsad with ectoderm forming the dorsal side of the future head. Projections of the nerve cells begin to form the distinct nerve fibers (Fig. 5).

In the 78-hour old embryo, the boundary between the head and the thorax becomes distinct. The deutocerebrum and the tritocerebrum go ahead and the former occupies its position under the ventral side of the protocerebrum and contacts with it. The latter connects with the posterior end of the deutocerebrum and lies at both sides of the stomodaeum. In the peripheral region of the brain many neuroblasts are arranged and within the brain several mitotic figures are observed. In the central region the nerve fibers assemble to form the neuropile.

At 84 hours after oviposition, the embryo shortens its length and the mouthparts begin to be formed. The protocerebrum migrates posteriorly along the dorsal side of the head and the deutocerebrum unites with the lower part of the protocerebrum in front of the opening of the stomodaeum. The tritocerebrum occupies its position behind the deutocerebrum. Then each cerebrum continues migration to form the brain on the upper part of the oesophagus. The distinct space appears between the brain and a layer of the ectoderm cells, which secrete later cuticle composing the future head capsule (Fig. 6). Figure 7 represents diagrammatically the process of migration of each cerebrum with progress of the head formation.

At 96 hours after oviposition, the labial appendages unite to form a single labium and the abdominal segments separate slightly from the egg surface to curve into the yolk.

At 120 hours after oviposition, the embryo completes blastokinesis and the appendages of the gnathal segments assemble around the opening of the stomodaeum. The brain is covered by a layer of cells with a flat nucleus, the perineurium, and the glial cells with dark-stained nucleus appear at the boundary between the perineurium and the outer layer of nerve cells and among the nerve cells themselves.

Figures 8 and 9 are electron micrographs representing the periphery of the brain

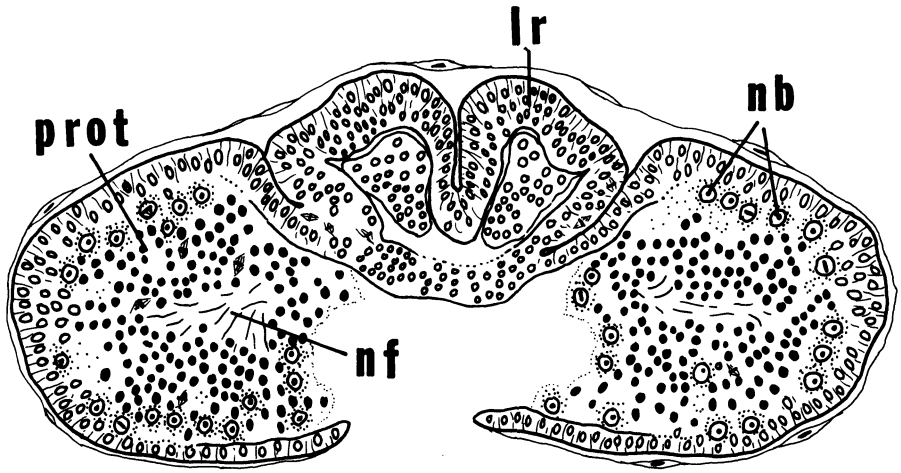


Fig. 5. Cross section of the head in 72-hour old embryo, showing appearance of nerve fibers. lr, labrum; nb, neuroblast; nf, nerve fiber; prot, protocerebrum.

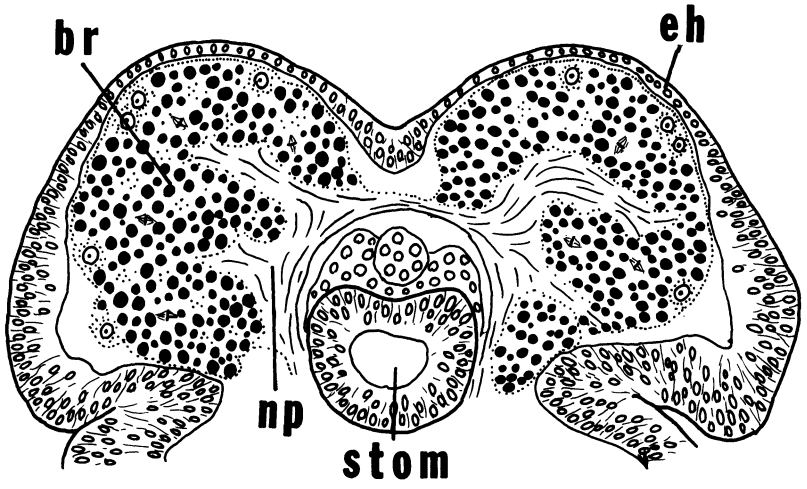


Fig. 6. Cross section of the head in 84-hour old embryo. br, brain; eh, epidermis of head; np, neuropile; stom, stomodaeum.

and the neuropile, respectively. The surface of the brain is covered with a layer of cells, the perineurium, the cell of which is flat and contains autosomes. The periphery of the brain is occupied with the abundant nerve cells, among which the glial cells intervene. The neuropile is constituted with the abundant nerve fibers intermingled complicatedly.

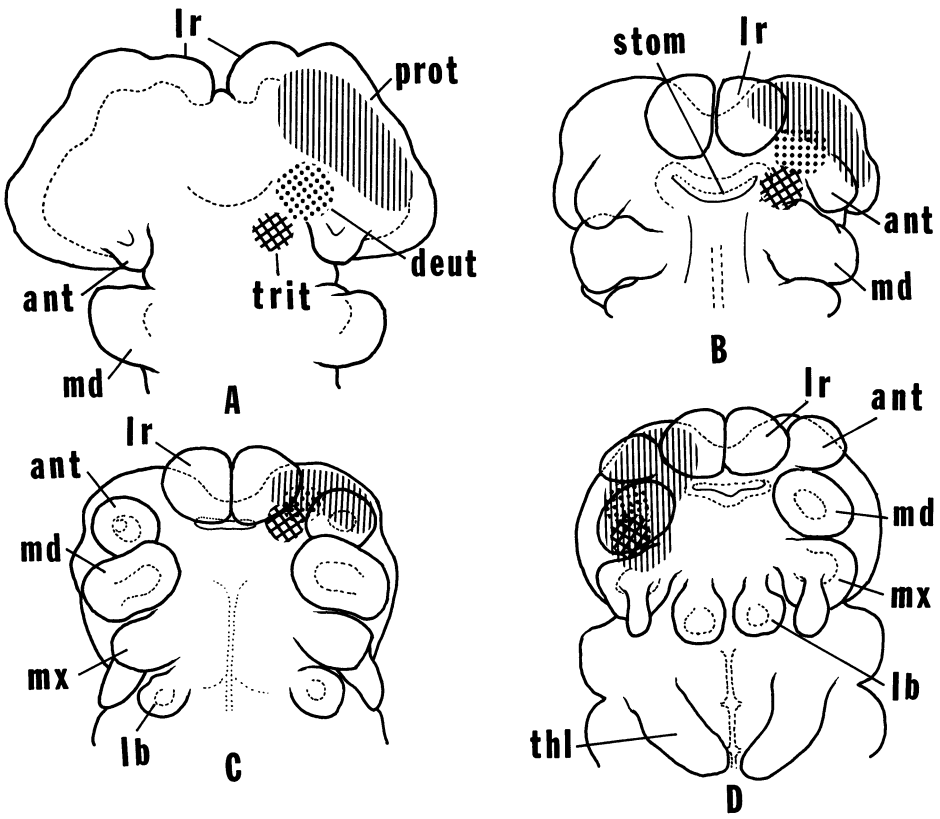


Fig. 7. Diagrammatic figures representing the process of migration of proto-, deuto- and tritocerebrum with progress of head formation. A. 48 hours, B. 72 hours, C. 84 hours, D. 90 hours after oviposition. ant, antenna; deut, deutocerebrum; lb, labium; lr, labrum; md, mandible; mx, maxilla; prot, protocerebrum; stom, stomodaeum; thl, thoracic leg; trit, tritocerebrum.

e. Ultrastructure of the completed brain

Figures 10 and 11 are electron micrographs representing the peripheral and central part of the brain, respectively. As mentioned above, the brain of *Bombyx* also consists of the right and left hemispheres, each of which is complex body of the proto-, deuto- and tritocerebrum. The perineurium secretes a non-cellular membrane, the neural lamella. The neural lamella is a felt-like membrane similarly to the basement membrane of other tissues. The cell of the perineurium is flat and its cytoplasm is filled with many small lipid droplets, mitochondria, and rER parallel to the surface of the brain.

The peripheral region of the brain is occupied with abundant nerve cells, the cytoplasm of which is filled with tubular rER. Some nerve cells represent several different features from the ordinary ones, that is, they contain well-developed vesicular rER and Golgi bodies, larger mitochondria, and groups of small secretion granules (Fig. 12). These cells seem to be neurosecretory cells. Among the nerve cells numerous glial cells intervene and their cytoplasmic processes enclose the nerve cells. In the cytoplasmic processes there are abundant pigment granules. The cytoplasm of the glial cells are denser than that of the nerve cells, because of rich free ribosomes, and contains many vesicles.

The central region, the neuropile, is filled with numerous axons, which contain mitochondria and longitudinal neurotubules. The cytoplasmic processes enclose the bundles of axons and also tracheoles intervene among them.

2. *Formation of the brain in Dus/Dus embryo*

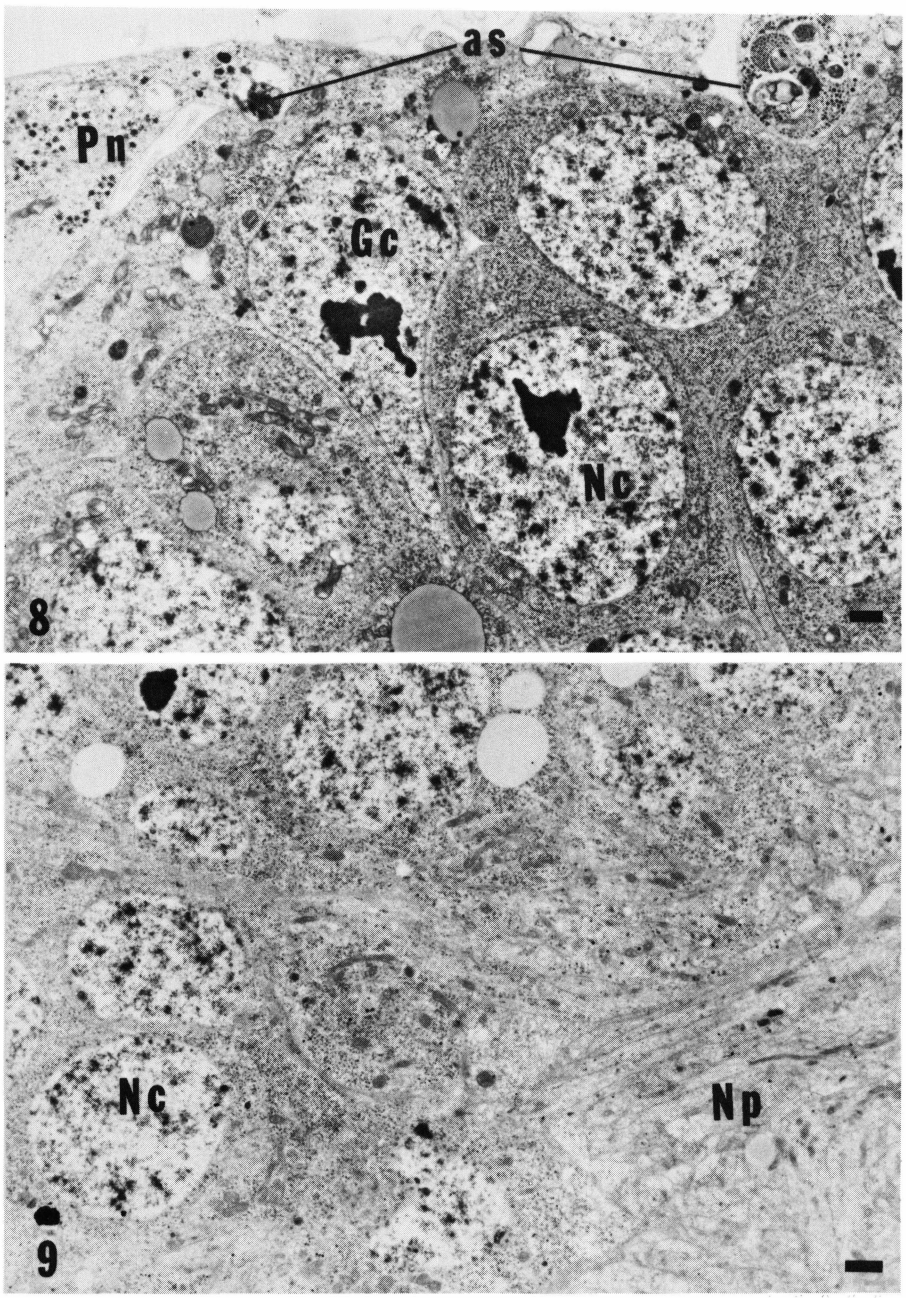
a. Characteristics of "star-spots duplication"

As reported already, "star-spots duplication" gene (symbol *Dus*) is located at 3.9 on the tenth linkage group (Doira *et al.*, 1984) and *Dus*/+ larvae show additional star-spots on the sixth abdominal segment (Fig. 13; Miya and Hashimoto, 1977). Furthermore, this mutant is a recessive embryonic lethal and *Dus/Dus* embryos indicate various conspicuous features: failure of formation of the head capsule and the midgut epithelium, hypertrophy of the central nervous system including brain, supernumerary legs in the seventh abdominal segment, supernumerary spiracles in the ninth abdominal segment, and so on (Fig. 14). The homozygous embryos develop until the setae formation stage, but they cannot carry out blastokinesis because of incompleteness of the dorsal closure.

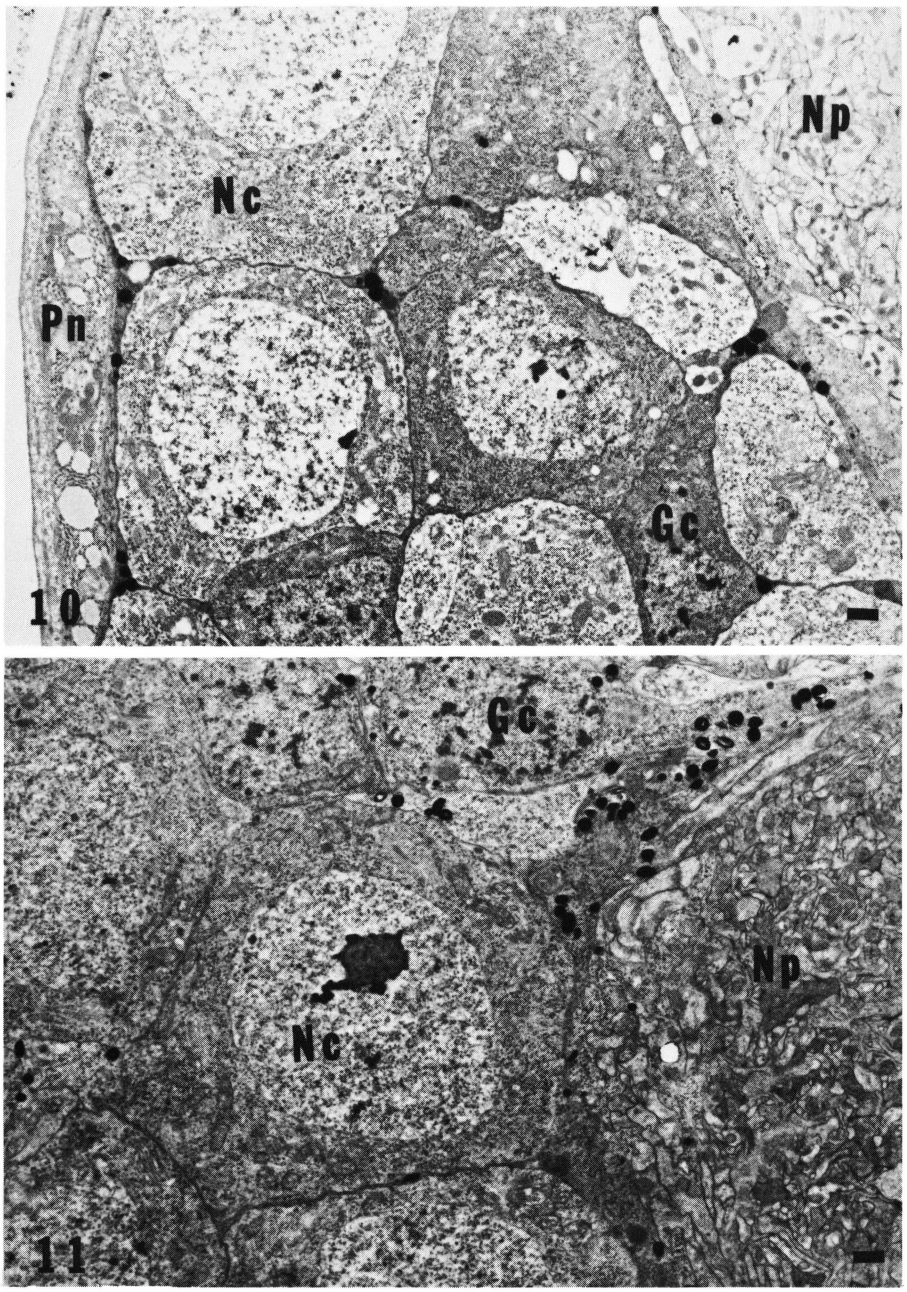
Among the above characteristics, the most conspicuous one is hypertrophy of the central nervous system. Figures 15 and 16 represent the frontal section of the head and the longitudinal section of the abdomen, respectively. The brain is exposed completely without enclosing by the head capsule, and the ventral nerve cord is connected with each other to form a tube-like organ.

b. Segregation of the neuroblasts in the protocephalon

When the *Dus*/+ female is mated with the *Dus*/+ male, it is expected in the laid egg batch that *Dus/Dus* embryos will occupy about one forth of all eggs. Until the early appendage formation stage, however, any distinct difference could not be observed between the normal and *Dus/Dus* embryos even by the histological examination. Therefore, the first segregation of the neuroblasts in the protocephalon and division into three groups seem to occur normally.



Figs. 8, 9. Electron micrographs representing periphery of the brain (Fig. 8) and the neuropile (Fig. 9) in 120-hour old embryo. as, autosome; Gc, glial cell; Nc, nerve cell; Np, neuropile; Pn, perineurium. Scales: 1 μm.



Figs. 10, 11. Electron micrographs representing peripheral (Fig. 10) and central region (Fig. 11) of the brain at body pigmentation stage. Gc, glial cell; Nc, nerve cell; Np, neuropile; Pn, perineurium. Scales: 1 μm.

c. Hypertrophy of the brain

As mentioned above, segregation of the neuroblasts in the neurogenic region of the protocephalon is not carried out at the same time at the definite developmental stage, but continues for several stages. Hence during this period the new neuroblasts not only segregate in the neurogenic region and produce the daughter cells by unequal divisions, but also these daughter cells continue to divide, resulting in increase of the number. In *Dus/Dus* embryos, increase of the neuroblasts might be produced by normal process of segregation, though the exact number could not be calculated (Fig. 17), while the equal divisions of the ganglion mother cells occur more than in the normal embryos, and consequently the size of the brain becomes larger than in the normal (Fig. 18). These results are summarized in Table 1; in the 72-hour old *Dus/Dus* embryo the size of the brain is twice as large as the normal embryo and the mitotic figures are observed three times as many as in the normal brain. Because of hypertrophy of the brain, the lateral epidermis could not spread over the brain, resulting in failure of the head capsule in spite of normal formation of the labral and antennal appendages in the head.

Discussion

The embryonic development of the central nervous system in insects begins at the stage of differentiation of the neuroblasts. The neuroblasts, anlagen of the ganglia, are segregated from the ectoderm on each side of the ventral midline of the embryo behind the stomodaeum, diverging into broader area over the protocephalon. The neurogenic region, from which the neuroblasts are segregated, is traced back the position to a longitudinal strip of blastoderm cells located between the anlagen of the mesoderm and of the ventral epidermis, for example, in *Drosophila* (Poulson, 1950) and in *Tenebrio* (see Anderson, 1972b; Sander, 1976).

Also in *Bombyx* a fate map concerning to the neurogenic region was made by Takami (1946) with cauterization experiment. According to him, the presumptive neurogenic region existed as a pair of slender strips in both sides of the presumptive mesoderm region. A more detailed similar fate map was given by Katsuki *et al.* (1980) through the analysis of mosaic larvae induced by super-cooling method.

As to the embryonic development of the brain, the similar pattern of the differentiation has been described in Holometabola as well as in Hemimetabola, namely the neuroblasts are segregated from the ectoderm of the protocephalon and then are organized into three pairs of groups to form the anlagen of the proto-, deuto- and tritocerebrum, which unite later to produce a compound body, the brain (see Johannsen and Butt, 1941; Ando, 1970, 1978; Anderson, 1972a, b).

Formation of the brain in *Bombyx* embryo proceeds almost similarly as in the other lepidopterans, but several differences are known in given species. In *Pieris* (Eastham, 1930) the areas of the proto-, deuto- and tritocerebrum were distinguishable at the early stage of the neuroblast segregation the same as in *Bombyx*, while in *Antheraea* (Saito, 1937) the neuroblasts distributed evenly in the wide-spread area of the protocephalon, and so distinction of each cerebrum was impossible until the later stage.

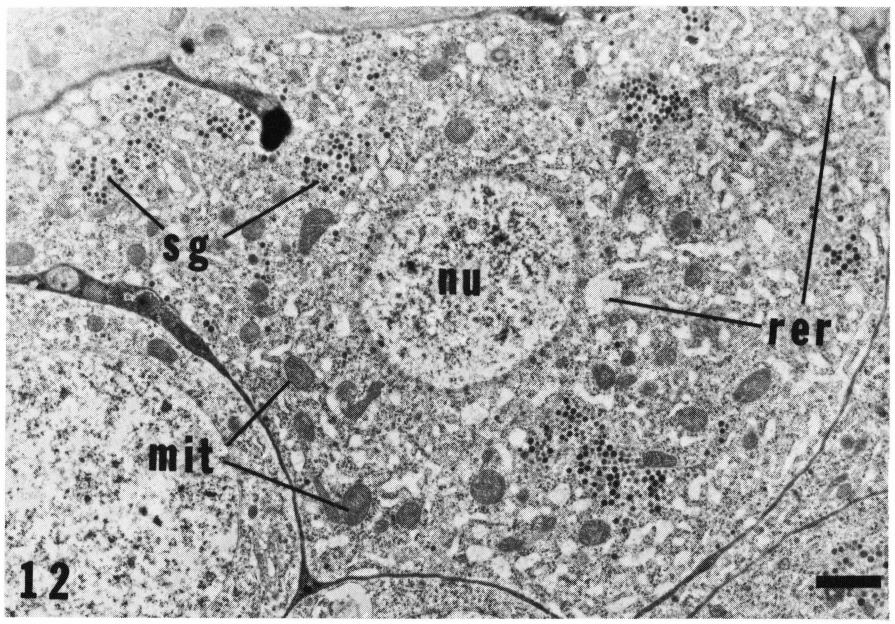


Fig. 12. Electron micrograph representing a neurosecretory cell at body pigmentation stage. mit, mitochondria; nu, nucleus; rer, rough-surfaced endoplasmic reticulum; sg, secretion granule. Scale: 1 μm.

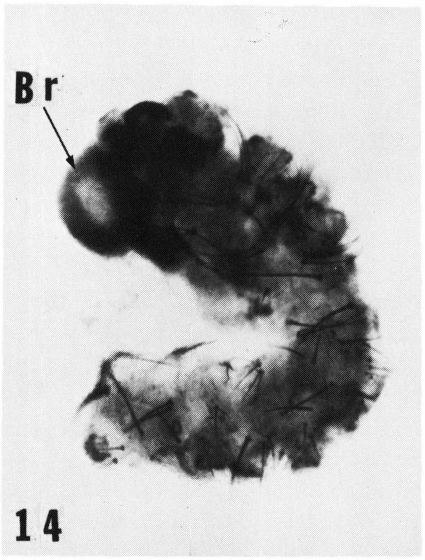


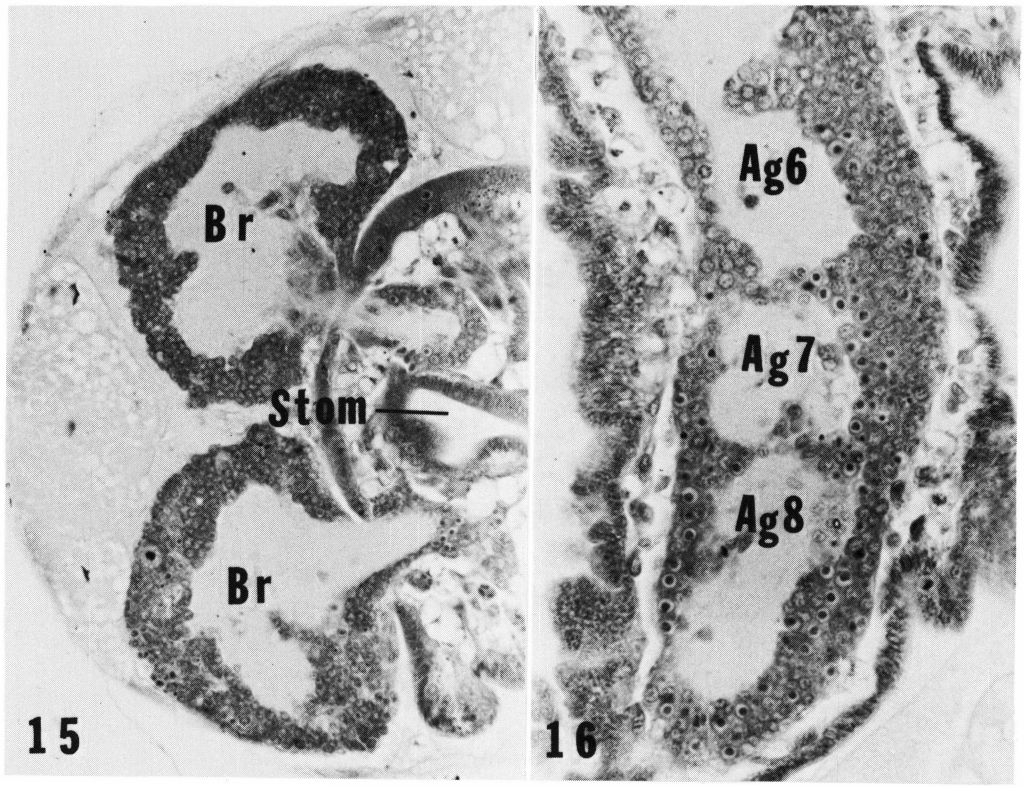
Fig. 13. Dorsal view of fifth instar *Dus*/+ larva (from Miya and Hashimoto, 1977).
Fig. 14. Light micrograph representing *Dus*/*Dus* embryo at setae formation stage (from Miya and Hashimoto, 1977). Br, brain.

Recently Hartenstein and Campos-Ortega (1984) studied in detail on spatial and temporal pattern of the segregation of the neuroblasts in *Drosophila*. According to them, the neurogenic ectoderm contains neural precursors intermingled with epidermal precursors, extending from the midline up to the primordia of tracheal tree along the germ band and latero-dorsally in the procephalic lobe and these two regions are separated from each other by the cephalic furrow, differently from the fate map made by Poulson (1950). Germ band neuroblasts segregate from the neurogenic ectoderm during a period of several hours according to characteristic spatial and temporal patterns, on the other hand protocephalic neuroblasts are segregated continuously during a definite period.

Furthermore, in *Drosophila* several mutants affecting the early neurogenesis have been known and their function are being analysed vigorously (Poulson, 1937, 1940; Lehmann *et al.*, 1981; Jiménez and Campos-Ortega, 1982; Lehmann *et al.*, 1983; Campos-Ortega, 1983; Hartenstein and Campos-Ortega, 1984). Summarizing their assumption, all cells of the neurogenic ectoderm develop into neuroblasts initially, then suppressed in order to allow to become epidermal cells. Therefore, these mutants misroute the ectoderm cells in wild type that would have developed as epidermal cell precursors into the neural developmental pathway, resulting in leading to a conspicuous hypertrophy of the central nervous system and to a lack of several epidermal derivatives.

In *Bombyx* spatial and temporal segregation of the neuroblasts in the neurogenic region could not be observed in detail, but in formation of the brain the following phenomena were confirmed: (1) segregation of the neuroblasts did not occur at the same time, but continuously during a definite period, (2) the newly formed neuroblast soon begins to divide unequally to produce a smaller daughter cell, the ganglion mother cell, (3) these neuroblasts and the daughter cells are organized to three pairs of groups, the anlagen of the proto-, deuto- and tritocerebrum, and (4) during migration and union of each cerebrum, the neuroblasts and the ganglion mother cells divide actively to produce abundant nerve cells, which elongate cytoplasmic processes to form axons.

The embryonic lethal mutant *Dus* affects the early neurogenesis to induce hypertrophy of the central nervous system similarly as the mutants in *Drosophila*. As to hypertrophy of the brain and the related failure of the head capsule formation, the following causes are assumed: (1) abnormal segregation of the neuroblasts in the protocephalon, accompanying with decrease of the epidermal precursors the same as in *Drosophila*, (2) abnormal divisions of the ganglion mother cells, resulting in production of more numerous nerve cells than in the normal brain, and (3) abnormality by both two causes. As shown in Figs. 5 and 17, there is no remarkable difference in number of the neuroblasts in the brain between the normal and the *Dus* embryos, and the fact suggests impossibility of the first cause the same as in *Drosophila*, while Table 1 indicates that abnormal proliferation of the ganglion mother cells might be the principal cause. The tube-like ventral nerve cord in *Dus* embryo is not exposed usually, as shown in Fig. 16. Therefore, from these results abnormality of the head including the brain in *Dus* embryo might be induced chiefly by abnormal proliferation of the ganglion mother cells.



Figs. 15, 16. Light micrographs representing frontal section of the head (Fig. 15) and longitudinal section of the abdomen (Fig. 16) of *Dus/Dus* embryos. Ag6-8, sixth to eighth abdominal ganglion; Br, brain; Stom, stomodaeum. × 160.

Table 1. Size of the brain and number of mitotic figures in the normal and *Dus/Dus* embryos.

Hours after Oviposition	Phenotype	Relative Size	Number of Mitotic Figures*
72	+	1	16.5
	Dus	1.8	52.0
84	+	1.1	24.8
	Dus	2.1	62.7
96	+	1.2	12.5
	Dus	2.2	46.5

(* *Number in hemisphere of the brain*)

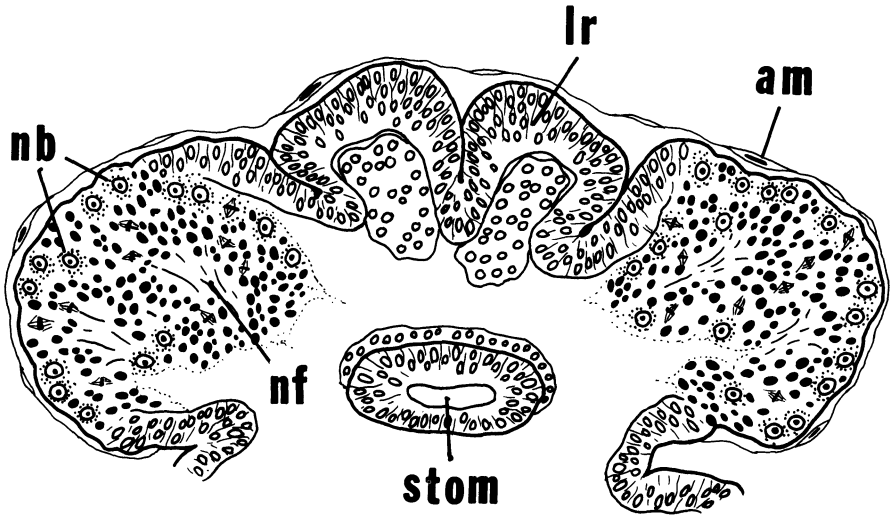


Fig. 17. Cross section of the head in 72-hours old *Dus/Dus* embryo. am, amnion; lr, labrum; nb, neuroblast; nf, nerve fiber; stom, stomodaeum.

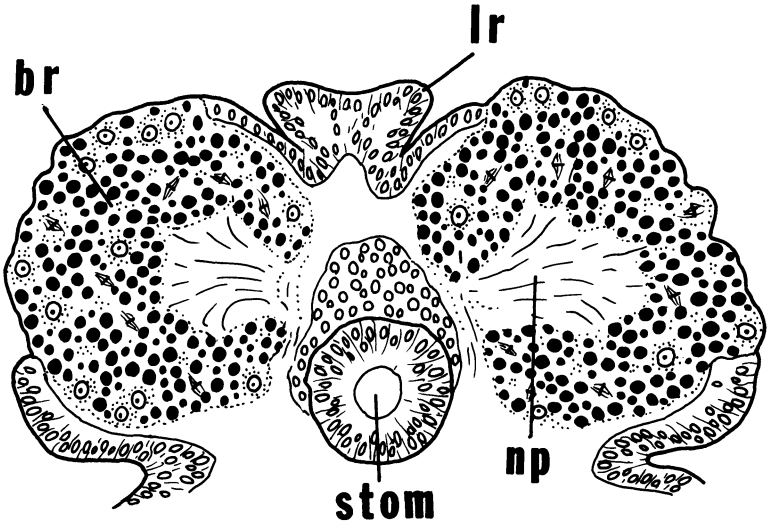


Fig. 18. Cross section of the head in 84-hours old *Dus/Dus* embryo, showing lack of epidermis. br, brain; lr, labrum; np, neuropile; stom, stomodaeum.

References

Anderson, D. T., 1972a. The development of hemimetabolous insects. *In* S. J. Counce and C. H. Waddington (eds.), Developmental Systems: Insects, Vol. 1, 95-163. Academic Press, London, New York.

————, 1972b. The development of holometabolous insects. *In* S. J. Counce and C. H. Waddington (eds.), Developmental Systems: Insects, Vol. 1, 165-242. Academic Press, London, New York.

Ando, H., 1970. Embryonic development (in Japanese). *In* T. Uchida (ed.), Systematic Zoology, Vol. 7-III-A, Arthropoda IIIa, Insecta I, 37-130. Nakayama-shoten, Tokyo.

————, 1978. Embryonic development of insects (in Japanese). *New Entomol.* 27: 1-54.

Campos-Ortega, J. A., 1983. Topological specificity of phenotype expression of neurogenic mutations in *Drosophila*. *Roux's Arch. Devl. Biol.* 192: 317-326.

Chapman, R. F., 1969. The Insects. Structure and Function. English Univ. Press, London.

Doira, H., K. Miya and H. Kihara, 1984. Linkage studies on the "star-spots duplication" mutant of *Bombyx mori* (in Japanese). *J. Seric. Sci. Jpn.* 53: 237-240.

Eastham, L. E. S., 1930. The embryology of *Pieris rapae*. Organogeny. *Phil. Trans. Roy. Soc. Lond, Ser. B* 219: 1-50.

Hartenstein, V. and J. A. Campos-Ortega, 1984. Early neurogenesis in wild-type *Drosophila melanogaster*. *Roux's Arch. Devl. Biol.* 193: 308-325.

Ikeda, E., 1913. Embryonic development (in Japanese). *In* Anatomy and Physiology of the Silkworm, 1227-1337. Meibun-do, Tokyo.

Jiménez, F. and J. A. Campos-Ortega, 1982. Maternal effects of zygotic mutants affecting early neurogenesis in *Drosophila*. *Roux's Arch. Devl. Biol.* 191: 191-291.

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

Katsuki, M., A. Murakami and I. Watanabe, 1980. Fate mapping of some tissues in the genetic mosaics of the silkworm, *Bombyx mori* (in Japanese). *Zool. Mag.* 89: 269-276.

Lehmann, R., U. Dietrich, F. Jiménez and J. A. Campos-Ortega, 1981. Mutations of early neurogenesis in *Drosophila*. *Roux's Arch. Devl. Biol.* 190: 226-229.

————, F. Jimenez, U. Dietrich and J. A. Campos-Ortega, 1983. On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Devl. Biol.* 192: 62-74.

Miya, K. and S. Hashimoto, 1977. Development of an embryonic lethal mutant, "star-spots duplication" in the silkworm, *Bombyx mori*. *J. Fac. Agr., Iwate Univ.* 13: 231-236.

Poulson, D. F., 1937. Chromosomal deficiencies and the embryonic development of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.* 23: 133-137.

————, 1940. The effect of certain X-chromosome deficiencies of the embryonic development of *Drosophila melanogaster*. *J. Exp. Zool.* 83: 271-325.

————, 1950. Histogenesis, organogenesis and differentiation in the embryo of *Drosophila melanogaster* Meigen. *In* M. Demerec (ed.), Biology of *Drosophila*, 168-274. Wiley, New York.

Saito, S., 1937. On the development of tusser, *Antheraea pernyi* Guérin-Meneville, with special reference to the comparative embryology of insects. *J. Fac. Agr., Hokkaido Imp. Univ.* 40: 35-109.

Sander, K., 1976. Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* 12: 125-238.

Takami, T., 1946. Experimental studies on the embryo formation in *Bombyx mori*. V. Presumptive mesodermal and neural regions of the egg (in Japanese). *Seibutsu* 1: 208-211.

Toyama, K., 1909. Sanshu-ron (in Japanese). Maruyama-sha, Tokyo.

Authors' addresses: MAg. S. Hashimoto
Agricultural Administration Department, Iwate Prefectural Government, Uchimaru 10-1, Morioka, Iwate 020, Japan

Dr. K. Miya, Prof. Emeritus (Iwate University)
Yamagishi 3-11-10, Mrioka, Iwate 020, Japan