Papéis Avulsos de Zoologia

THE POST-EMBRYONIC CHANGES IN *MELIPONA QUADRIFASCIATA ANTHIDIOIDES* LEP. (HYM., APOIDEA). V. DEVELOPMENT OF THE NERVOUS SYSTEM

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ABSTRACT

In this study the condensation of the 11 ganglia of the ventral nerve cord of the larvae into the 7 ganglia of the adult is anatomically and histologically described as well as the differentiation of the brain. The mechanism of post embryonary transformations in the nervous system is discussed in relation to the ventral nerve cord and brain. The modifications that take place in the ganglionar chain occur without changes in cellular numbers, i.e., without mitoses.   The transformations that occur in the brain are in part due to mitoses (optic lobes), in part to cellular differentiation, mainly increase in size.

INTRODUCTION

Considerable work has been done on the nervous system of bees (Kenyon, 1896; Jonescu, 1909; Nelson, 1924; Snodgrass, 1956; Dias, 1958; Wille, 1961; Yousseff, 1968; Cruz-Landim & Höfling, 1971). Much less has been published about the development of the nervous system (Nelson, 1915; Snodgrass, 1956) of these and other insects (Johannsen & Butt, 1941; Wigglesworth, 1953; Chantani & Callahan, 1967; Springer, 1967).

Several aspects of bees have benn studied in this laboratory in recent years (Cruz-Landim, 1968; Cruz-Landim & Mello, 1966, 1967, 1969, 1971; Cruz-Landim & Staurengo-Cunha, 1971), and therefore, it seemed profitable to reinvestigate the post-embryonic development of the nervous system of bees.

Material and methods

The bees used in this study were reared in laboratory in a cage with glass lid, at 28°C.

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The life cycle of the bees employed in the present experiments has been characterized in previous studies (Kerr, 1948; Cruz-Landim, 1966; Cruz-Landim & Mello, 1967). Five larval instars, two pre--pupal stages and 4 pupal stages were observed in the present study. The pupal stages were identified on the basis of compound eye coloration (Cruz-Landim & Mello, 1967).

The specimens were dissected in physiological saline solution under stereoscope for morphological studies. The bees were opened along the middorsal line and the organs removed to obtain views of the dorsal surface of the nervous system.

Fixation of whole individuals with Bouin fluid was used for preparation of microscopic slides for the study of the nervous organs differentiation. The 7 μ paraffin sections were stained with hematoxylin and eosin.

<div align="center">RESULTS</div>

The nervous system of bees consists of the brain and a ventral ganglionar nerve cord linked to the brain by paired connectives.

1 — Anatomical development

The nervous system of the first instar larva of *Melipona* includes a brain in the head, above the stomodaeum, a suboesophageal ganglion in the lower part of the head, and a long ventral nerve cord with eleven ganglia, three of which located in the thorax and the rest in the abdomen (plate I).

The brain consists of two large antero-posteriorly flattened lobes narrowly joined medially (plate II, figs. 1 and 2). The three divisions of the brain present in the adult are much less distinct in the larva. The major part of the larval brain is formed by the protocerebral lobes, with the optic lobes of the future compound eyes on their margins. The deutocerebrum is recognizable only as a pair of slightly differentiated swellings on the anterior surface, merging ventrally into the tritocerebral lobes. There lobes are also poorly developed; they taper into the circumoesophageal connectives that join the brain to the suboesophageal ganglion and are bridged by a thick suboesophageal commissure (plate II, fig. 1).

This configuration of the nervous system remains almost without modfication until the end of the larval phase (plate I). Only two terminal ganglia of the ventral nerve cord fuse during the 2nd larval instar. The variations in length of the connectives until the 4th instar are due to the contracted position of the larvae in the moment of dissection. The larvae rest within the brood alveoli rolled up until the 4th instar, when they stretch out, lying then, with the head to the top. Besides the variations in connective length, the ganglia keep their position unchanged in relation to the body segments, demonstrating that the variations are not real modifications.

During the 4th and 5th instars the number of ganglia is still the same, but the nerve chain shows a shift forwards. In the preceding instars the nervous chain ended in the VIII abdominai segment. From the 4th instar on it ends in the VII segment. This

is due mainly to the shortening of the connectives of the thoracic ganglia (plate I). In this final phase of the larval stage an enlar-gement can already be noted in the brain, but the cerebral structure remains unchanged.

After the quiescent larval period, there is a new spurt of development in the pre-pupa. The brain lobes begin to enlarge and differentiate in the adult direction. The first change noticed is the appearance of the optical nerves (plate II, fig. 2) on the margins of the differentiating optic lobes, in step with the deve-
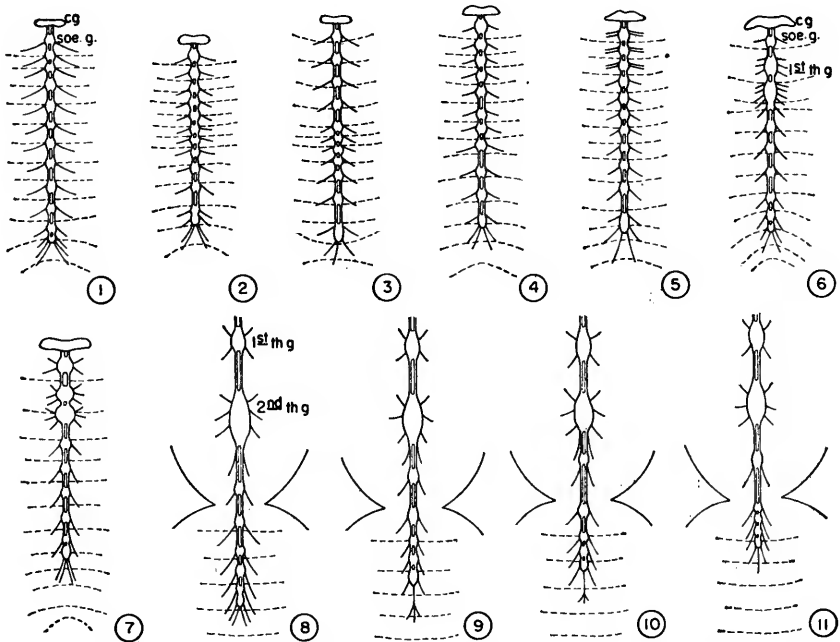

Plate I. 1-5, nervous system of I, II, III, IV and V larval instars; 6, 7, nervous system of prepupae; 8-11, nervous system of white, pink, red and brown eyed pupae. c.g. = cerebral ganglia; soe.g. = suboesophageal ganglion; 1st th.g. = first thoracic ganglion; 2nd th.g. = second thoracic ganglion.

lopment of the compound eyes. The free suboesophageal commis-sure of the tritocerebrum disappears during this phase, buried in the suboesophageal ganglion. The position of the brain in relation to the ventral nerve cord also begins to change. During larval life the brain is dorsal to the suboesophageal ganglion but in the adult it is anterior to this ganglion, because of the displacement of the head in relation to the rest of the body. In larvae the suboe-sophageal ganglion is connected to the brain by long connectives that at first go to the front, bending backwards (plate II) shortly

after.   During the prepupal stage the connectives shorten and the brain begins to rotate to a position perpendicular to the nerve cord.   The tritocerebral lobes enlarge and the optic lobes differentiate.   By the end of this phase, the optic nerves can already be seen on the margins of the lobes (plate II, fig. 2).
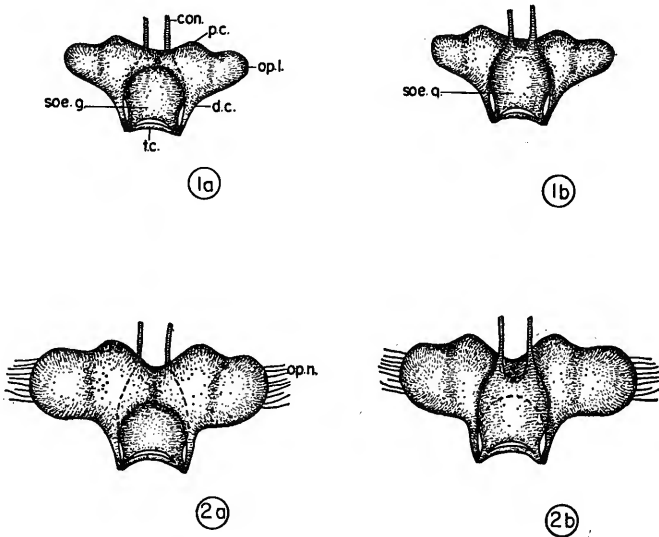

Plate II. 1, brain from a II instar larva (1a, dorsal; 1b, ventral). 2, brain from an early prepupa in dorsal (2a) and ventral (2b) views. p.c. = protocerebrum; d.c. = deutocerebrum; t.c. = tritocerebrum; soe.g. = suboesophageal ganglion; op.l. = optic lobe; con. = connectives; op.n. = optic nerve.

The prepupal stage is also characterized by several transformations in the ventral nerve cord.   In the thorax the 2nd and 3rd ganglia fuse (plate I) and in the abdomen the 10th ganglion fuses with the 9th.   In early prepupae the recent fusion can still be seen anatomically, and the ventral nerve cord ends in the VI abdominal segment.   Later in the prepupal stage the 4th ganglion, i.e., the 1st abdominal ganglion, fuses with the 2nd and 3rd thoracic ganglia and the connectives between the resultant ganglion and the 2nd abdminal ganglion become shorter.   By the end of the pre-pupal stage the ventral nerve cord ends in the V abdominal segment. Besides the fusions the last abdominal ganglion does not seem to have enlarged, but the 1st thoracic ganglion has enlarged during prepupation, even without fusion (plate I).

When the bee enters pupation the ventral nerve cord is constituted by 8 ganglia.   The 3rd abdominal ganglion begins to migrate to the thorax in the white eyed pupa.   In this phase the nerve cord ends in the V abdominal segment.   The next step, in the

pink eyed pupa, is the fusion of the 9th with the 8th ganglion, and the shortening of the connectives between the abdominal ganglia. During pupation the 3rd thoracic ganglion migrates and the connectives are shortened, so that, by the end of pupation, the nerve cord ends in the III abdminal segment. The brown eyed pupa has 3 thoracic and 4 abdominal ganglia (plate I).

The modifications that take place in the brain during the pupal phase, correspond mainly to the differentiation of the parts related to the cephalic sense organs and to the enlargement of the protocerebral lobes.

In the white eyed pupa the brain has rotated to its definitive position. The tritocerebrum projects anteriorly, forming the antennal lobes, and the connectives to the suboesophageal ganglion are displaced from their distal position to a posterior one on the ventral side of the antennal lobes (plate III, fig. 3). With the rotation of the brain the bend of the circumoesophageal connectives disappears, and the suboesophageal ganglion takes a position ventral to the brain. From this phase on, the suboesophageal ganglion is closer to the brain because of the shortening of the connectives. The optical lobes enlarge and the optic nerves become more evident. Each nerve is formed by a branched stalk (plate III). As the optic lobes enlarge, the stalk disappears and the branches arise from the margins of the lobes, as can be seen in the red eyed pupa (plate III, fig. 4). Until this phase the ocelli cannot be seen, their differentiation occurs in the brown eyed pupa, when the brain acquires the adult configuration (plate III, fig. 5). In this brain the fusion between the protocerebral lobes is complete and the ocelli appear on the vertex of the fused lobes. The optic lobes have also finished their differentiation, and their margins are in contact with the cuticular part.

2 — Histological modification

The transformations which occur during the development of the nervous system can be accompanied in histological sections in order to follow the modifications of the tissues and cells.

Basically the histological organization of the nervous system is maintained in all phases of the development, i.e., the neurones stay on the periphery of the ganglia and the nerve fibers in the central part. In the larval brain the neurones are located mainly in the dorsal faces (plate IV, fig. 1), although they are preferentially located on the ventral side in the ventral nerve cord.

From the prepupa on, this situation is changed in the brain (plate IV, fig. 2), but remains unchanged in the ventral nervous system.

In the central region the fibers are interspersed with supporting cells equivalent to the glial elements of the vertebrate nervous system. These cells accompany the nerves that leave the ganglia and the connectives that link them (plate IV, fig. 3). The nerves and connectives originate from the central region of the nervous organs and the neuronal peripheric sheath is interrupted where they leave these organs (plate IV, fig. 4).

The neurones from different parts differ in aspect and size, even inside the same ganglion. In general the ventral nervous system has small neurones (7-10 μ in diameter), but the lst thoracic ganglion has neurones of at least 3 different sizes (plate V, fig. 5): 1) small neurones, 5 to 7 μ in diameter, located in the ganglion periphery; 2) median sized neurones, 12 to 15 μ in diameter, located on the ventral aspect of the ganglion and 3) large neurones (23-30 μ in diameter) laterally located. The nuclei of the small neurones stain more deeply than those of the median and large sized neurones (plate V, fig. 6). The nuclei of the larval neurones, characteristically show a chromatinic mass similar to a nucleolus. The last abdominal ganglion also presents neurones of different sizes (plate V, fig. 7), but in this case, only the small
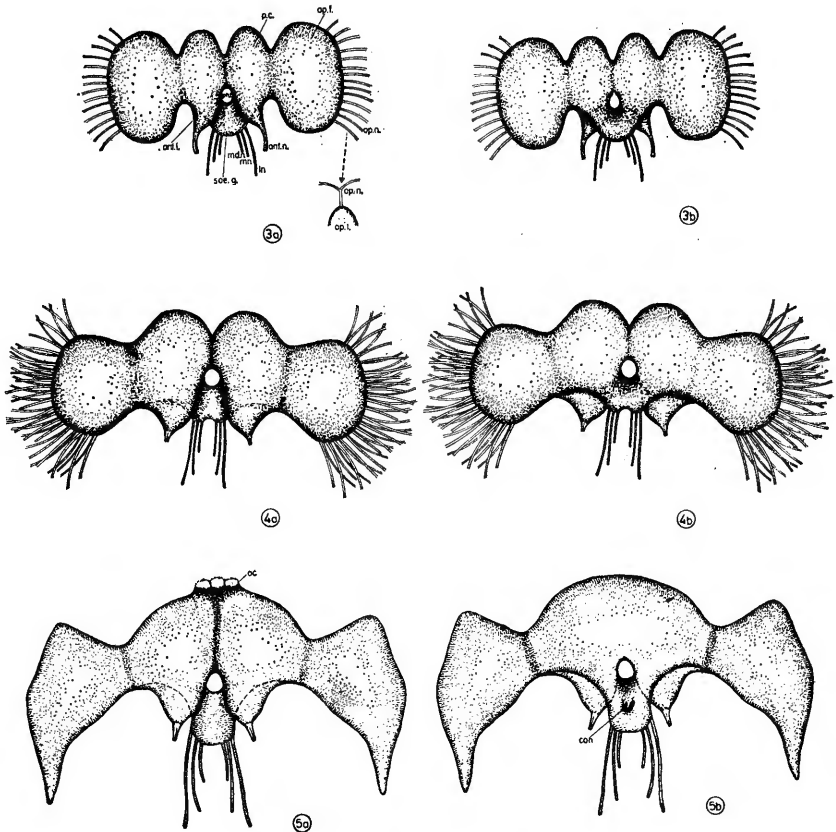

Plate III. 3, brain from a white eyed pupa in dorsal (3a) and ventral (3b) views. 4, brain from a red eyed pupa (4a, dorsal; 4b, ventral). 5, brain from a brown eyed pupa (5a, dorsal; 5b, ventral). ant.l. = antennal lobe; ant.n. = antennal nerve; ln. = labial nerve; m.n. = maxilar nerve; md.n. = mandibular nerve; ph.f. = pharingeal foramen.

and median sized neurones are present. The small neurones are still peripheric and the median sized ones are antero-ventral. The nuclei of these neurones are smaller and more deeply stained than the corresponding ones in the 1st thoracic ganglion (plate V, fig. 7).

These basic characteristics of the nervous system are mostly mantained during all development, but some changes were observed.

The 3rd instar larva has the anterior part of the nervous system as shown in fig. 8. Practically all ventral ganglia are individualized and of the same size and joined by long connectives. In the 5th instar larva there is an enlargement of the 3 first ganglia of the ventral cord (I, II and III), as well as of the last (XI) (plate VI, fig, 9). No mitosis was seen preceding the size increase. In this larva the last abdominal ganglion is already the result of the fusion of two ganglia (plate VI, fig. 10), as seen by the partition in the fibrous medullar region.

In the prepupal phase other transformations occur. In the early prepupa a new fusion takes place at the end of the nervous cord (plate VI, fig. 11) and the migration of the abdominal ganglion to the thorax can be observed in the zone of pedicell formation (plate VI, figs. 11 and 12). In this phase the 1st thoracic ganglion begins its differentiation, but the large neurones that appear later are not yet present (plate VII, fig. 13 — see figure 5 for comparison). As in the abdominal and thoracic ganglia, mitoses were not observed, but they occur in the cerebral ganglia (plate VII, fig. 14), especially in the anterior position of the proto-cerebrum and in the optic lobes. In a frontal section of a prepupal head it can be observed that the brain is enclosed in a membrane or meninge (plate VII, fig. 15). The protocerebrum and the optic lobes are the only parts well discernible in this brain, but it can be already noticed that the head "epidermis" is thickened in the future place of the compound eyes (figs. 14 and 15). In the late prepupa, are noticeable the fusion of the 2nd and 3rd thoracic ganglia (plate VII, fig. 16), and increase in thickness of the cortical (neuronal) sheath in the dorso of the brain (plate VIII, figs. 17 and 18), as well as the progress in the differentiation of the optic lobes (fig. 18).

The fusion of the two last thoracic ganglia, initiated in the prepupa, continues in the white eyed pupa, in which is additionally seen the approximation between the fused ganglia and the preceding ones (plate VIII, fig. 19). Then, 4 ganglia become fused in the last abdominal ganglion in this phase (plate VIII, fig. 20). In the thorax there is the fusion of 3 ganglia to form the 2nd thoracic ganglion (plate IX, fig. 22), and the 1st thoracic ganglion has already assumed its final aspect (plate IX, fig. 23).

From now on the ventral nervous chain remains pratically unchanged, most modifications occuring in the brain.

During the period from the end of the prepupal stage to the white eyed pupa the essential parts of the adult brain are developed, enlargement of the parts only occurring later. The passage from the larval brain to the adult brain is initiated by the differentiation of the optic lobes, through a series of mitoses that last through the white eyed pupa (plate IX, fig. 24).

In the white eyed pupa all parts of the brain are already well developed, including the "corpora pedunculata", the antennal lobes and the optic lobes (plate X, fig. 25). However, the contact between the nervous and epidermal part of the compound eye is not yet perfect (plate X, figs. 25, 26 and 27). Nerve cords project from the edges of the optic lobes to the eye epidermis (fig. 27), but they are still sparse and the contacts few. In the red eyed pupa the epithelial part of the eye is already well differentiated and the contact occurs in a more definitive extension (fig. 26). The epidermal differentiation of the compound eye zone comprehends the hyalination of the corneal cells and the appearance of pigmented cells (plate X, figs. 28 and 29). The limit of the epidermal part of the compound eye may be observed very well as a continuous line, called the "outer limitant". Comparing figures 29 and 31 it can be noticed that from the red eyed pupa to the adult (plate XI, fig. 30 and 31) the epithelial part of the eye thickens and becomes more closely attached to the nervous part. In the adult the different portions of the eye are well discernible in sections (fig. 31).

DISCUSSION AND CONCLUSIONS

The transformation of the larval nervous system into the adult occur through a series of modifications that are distinct in the ventral nerve cord and brain.

During post-embryonary development, the ventral nerve cord of the bee studied bears a centralization that attains its maximum in the brown eyed pupa and goes through fusions of ganglia and shortening of the connectives. Through these processes the larval chain with 11 ganglia is transformed into the adult chain with 7 ganglia. The fusions take place in the abdominal and thoracic ganglia. Those in the abdominal ganglia are more sparse during the development, and always occur in the last ganglion. The first fusion occurs in the XI and X ganglia in the 2nd larval instar. A second fusion takes place during the prepupal phase and a 3rd in the whithe eyed pupa, the last abdominal ganglion of the adult being formed by the fusion of 4 ganglia (XI, X, IX and VIII). These fusions are noteciable histologically because septa of neurones or glial cells divide the fibrous medullar zones of the fused ganglion. In the thorax the fusions occur during the prepupal stage and are continuous. In the early prepupa the 2nd thoracic ganglion fuses with the 3rd and by the end of this stage the new-formed ganglion fuses with the 1st abdominal ganglion. Thus 3 ganglia fuse to constitute the 2nd thoracic ganglion of the adult; one of those ganglia is abdominal, which is understandable, because the first abdominal segment is also fused in the thorax. In most bees the thoracic nervous system is formed only by two ganglia, but in this case the second abdominal ganglion also migrates into the thoráx, a peculiarity of *Melipona* among the meliponids (Cruz-Landim & Höfling, 1971).

Some enlargement of the ganglia may be noticed during the post-embryonary development, even without fusions. This enlargement is more strikings in the first thoracic ganglion. The histo-

logical sections show that its increase is not due to cell division (i.e., to increase in cell number), but to differentiation of neurones. Some of them enlarge 4 times and others 3 times, perhaps by somatic polyploidy.

In most ganglia only one type of neurone was observed. Only in the 2nd last abdominal ganglia more than one category is present during the development.

The centralization due to connective shortening happens mainly in the end of post embryonary development, i.e., during the pupal stage.

The developmental behaviour of the brain is different in the sense that no fusions occur but only increase in size and differentiation of the parts already present in larvae. The enlargement is due to increase in size of cells (perhaps due to somatic poliploydy) and mitoses. Mitoses were mainly seen in the optic lobes; however, the differentiation of the protocerebrum, tritocerebrum and deutocerebrum occurs mainly by cellular modification. This means that all neurones originate in the embryo, except some of the cerebral neurones that originate during the prepuna by division.

As seen in other organs the main modifications during the post embryonary development occur in the prepupa and early pupae. By the end of the pupal stage (brown eyed pupa) the adult conformation is already present.

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