

## Further Study on the Physiological Rôle of Brain in the Imaginal Differentiation of Swallowtail, *Papilio xuthus*<sup>1)</sup>

By

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In the previous paper (1957) we suggested that the brain of the swallowtail, *Papilio xuthus*, is closely related to the phenomenon of metamorphosis in respect of its hormonal stimulation to the prothoracic glands as in other Lepidopterous insects such as *Bombyx*, *Luehdorfia*, *Philosamia*, *Platysamia* and others. This suggestion was derived from the experimental result that the brains of *Papilio*, when implanted into a diapausing pupa of another species of *Luehdorfia*, could induce the precocious emergence of the implanted pupa. It is now evident that the pupal diapause of *Luehdorfia* is solely conditioned by the temporary cessation of the endocrine activity of the brain. Therefore the interruption of diapause of the recipient pupae in the above experiment implies that the implanted brains of *Papilio* secrete their prothoracotropic hormone in the body of *Luehdorfia*. From this it will be natural to infer that the *Papilio*-brain likewise secretes its hormone in its own body.

On the other hand, there is another opinion in which the brain takes no part in the metamorphosis of the very same species, *Papilio xuthus* (OZEKI, 1954).

To substantiate our argument, therefore, the present experiments were carried out, which consisted mainly of the implantation of brains coming from the non-diapausing larvae in the last instar into the diapausing pupae. The results will be described in this paper.

### Materials and Methods

Eggs of *Papilio xuthus* were collected in the field, and the larvae hatched from them were reared under appropriate conditions according to our knowledge of getting the diapausing and non-diapausing pupae (ISHIZAKI and KATO, 1956). But as supplement of non-diapausing material the pupae and larvae collected from the field in the time range from May to August were also used, because such pupae were exclusively non-diapausing and the larvae were completely destined to become non-diapausing in this season.

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The larvae of the commercial silkworm, *Bombyx mori*, reared in our laboratory were also adopted as the source of active brains.

In the series of the *parabiotic experiment*, the rear end of abdomen including 2 or 3 segments was cut off from a couple of pupae which were previously etherized. Pupae thus operated on were joined tail to tail to allow the mixture of body fluid from one partner to another one. The joint was fixed by the melted paraffin.

In the series of the *brain implantation*, brains to be implanted were isolated from the heads in Beadle and Ephrussi's solution or in 0.9 per cent saline solution. Four brains were inserted into each diapausing pupa through a small hole made in the integument mostly at the dorsal part of thorax, but rarely at the tip of head or abdomen. After putting a cut piece of integument on the hole, it was coated with melted paraffin. Before and during the experiments the pupae were kept at 25°C.

### Results of Observations and Experiments

#### 1. Preliminary observation on the termination of diapause

Diapausing pupae were maintained at a constant temperature of 25°C and the duration of diapause was observed. While the non-diapausing pupae continued their differentiation to emerge 9 to 13 days after pupation, the diapausing pupae, as is listed in Table 1, remained unchanged for a considerably longer period of time at this temperature. The actual duration of diapause differed from individual to individual, but it will be seen that there was none which emerged less than 50 days at this temperature.

Table 1. Duration of diapause at 25°C.

Days after pupation	Number of emerged pupae	Number of pupae died without imaginal differentiation
1 - 50	0	0
51 - 60	0	2
61 - 70	1	4
71 - 80	2	5
81 - 90	1	2
91 - 100	1	3
101 - 110	4	3
111 - 120	3	3
121 - 130	2	1
131 - 140	2	2
141 - 150	0	3
151 - 160	1	2
161 - 170	0	2
171 - 180	0	1
181 - 190	0	1
191 -	0	0

Next, the diapausing pupae which have naturally pupated in the field from November 19 to 22 were first kept in an unheated room and some of them were then subjected to a high temperature of 25°C from January 1 on, and the remaining February 15 on, to see whether the wintering affects, in some measure, the duration of diapause of this butterfly.

In the first series it required from 12 to 32 days for the butterflies to emerge, while in the second series the emergence occurred within shorter range of time from 12 to 16 days.

These data so far enumerated indicate that the wintering causes the completion of diapause in *Papilio* just as in other insects which winter in pupal form, and that the pupae which enter naturally in diapause in autumn are completely free from diapause in February so as to start their imaginal development, if they are kept in warm temperature.

## 2. Parabiotic experiment

Diapausing and non-diapausing pupae were joined together one or two days after pupation. All of 28 couples of pupae thus operated on emerged as butterflies at the nearly same time with the allowance of 5 days (Table 3). But, in the control series in which the same union of two diapausing pupae was performed, there occurred none that showed the imaginal differentiation within 50 days, the minimum limit of time necessary for the spontaneous termination of diapause at 25°C.

Table 3. Parabiotic experiments.

Members of couple	Number of couples	Days required for emergence (diapausing partner-non-diapausing one)
{ diapausing pupa + non-diapausing pupa	22	8-6, 8-8, 9-7, 9-8, 10-8, 10-8, 10-10, 11-11, 11-11, 12-10, 12-10, 12-12, 12-12, 12-12, 13-12, 13-12, 13-13, 13-13, 14-9, 15-11, 15-15, 18-13
{ diapausing pupa + diapausing pupa	18	more than 50 days.

A comparison of the two results suggests that the pupal diapause of this species would result from the absence of some principle necessary for adult development and carried by the body fluid from non-diapausing partner to diapausing one, and that the termination of diapause is not induced by the surgical operation alone.

Table 2. Incubation of diapausing pupae from January 1 and February 15.

Date of incubation	Days required for adult development
Jan. 1	12 (2)*, 14 (5), 15 (2), 17 (5), 19 (1), 21 (3), 32 (3)
Feb. 15	12 (7), 13 (6), 14 (5), 16 (3)

\* Numerals in parenthesis indicate the number of individuals.

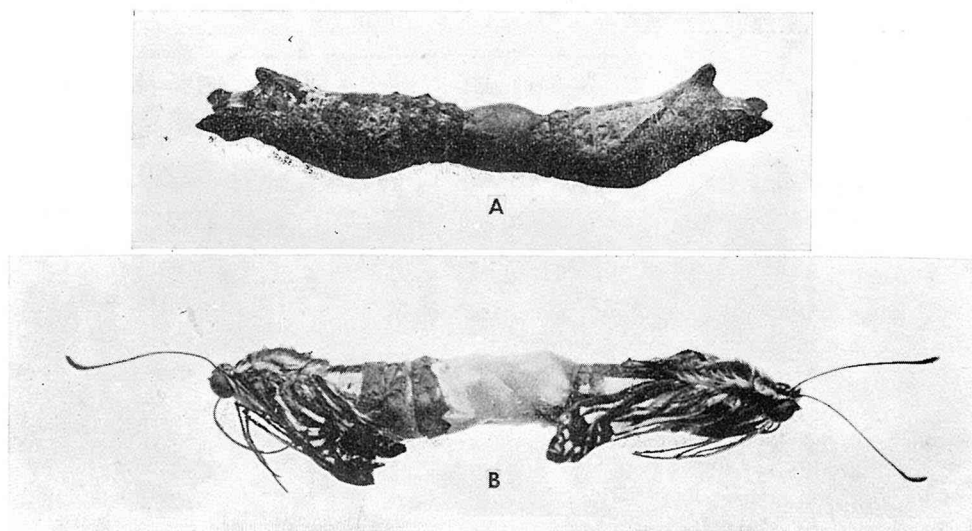


Fig. A. Photograph showing the tail-to-tail union of diapausing pupa (right) and non-diapausing pupa (left).

Fig. B. Photograph showing the simultaneous emergence of butterflies from the pupae joined as above.

### 3. *Brain implantation into diapausing pupa*

According to our experience the principle necessary for adult development is the brain hormone released from the neurosecretory cells in the pars intercerebralis, but the above experiment cannot exclude another possibility that it is the growth and differentiation hormone coming from the prothoracic gland, since it is the latter hormone that is directly responsible for the imaginal differentiation. To answer this question the brain implantation was carried out.

Mature larvae and full grown larvae of the commercial silkworm as well as of the non-diapausing specimens of this species were used as the donors of brain. In these stages the brains of these insects are to be active with respect to the secretion of the brain hormone. Each pupa received four brains. Four subesophageal ganglia isolated at the same time from the swallowtail larvae were also implanted into each diapausing pupa as controls.

The results are shown in Table 4 on the next page.

As is clear from the table, the brain-implanted pupae underwent a precocious metamorphosis, while those implanted with the subesophageal ganglia remained without adult development till the spontaneous emergence occurred later.

Table 4. Brain-implantation into diapausing pupae.

Implants	Number of experiments	Number of emerged specimens	Number of undeveloped specimens	Days required for adult emergence*
4 brains of <i>Papilio</i> larvae	13	7	6	22, 22, 22, 24, 25, 27, 28 average 24
4 brains of <i>Bombyx</i> larvae	9	8	1	14, 15, 15, 15, 16, 17, 17, 18 average 16
4 suboesophageal ganglia of <i>Papilio</i> larvae	17	0	17	more than 50 days

\* positive cases only.

### Discussion

C. M. WILLIAMS (1946) first pointed out that pupal diapause of the giant silkworm, *Platysamia cecropia*, results from the temporary stopping of the brain activity in secreting the prothoracotropic hormone. The same phenomenon has been augmented by himself (1952) in *Actias luna*, ICHIKAWA and NISHITSUTSUJI (1951) in *Luehdorfia japonica* and FUKUDA (1958) in *Samia cynthia*. However, OZEKI (1954) has expressed the opposite view in *Papilio xuthus* in saying that "Decapitation of pupae had no effects on their further development in either the spring, summer or autumn forms. The imaginal differentiation was also not at all affected by the brain implantation. In the swallowtail pupae, therefore, the brain plays no part in stimulating the prothoracic gland to secrete its hormone." As discussed on the previous occasion (ICHIKAWA and NISHITSUTSUJI-UWO, 1957), we are skeptical about his conclusion. We are of the opinion that the brain is involved in the metamorphosis and diapause in *Papilio* as in other Lepidopterous insects. The third experiment described above again gives us reliable evidence favourable to our opinion. But one point still remains. That is why the autoplasmic brain of *Papilio* is less effective than the heteroplasmic brain of *Bombyx* in interrupting of the diapause. One possibility is that the *Bombyx*-brain is more potent in the secretory activity of the brain hormone than the *Papilio*-brain. Another possibility is that the *Papilio*-brain is active as much as the *Bombyx*-brain, but it is more sensitive to some unknown factor which would exist in the diapausing pupal body to make its own brain inactive. This problem awaits a further study.

### Summary

- 1) In *Papilio xuthus* when a diapausing pupa is joined together with a non-diapausing one, the former will emerge simultaneously with the latter.
- 2) Diapause can readily be terminated only by grafting active brains into pupa, irrespective of species by which the brains are supplied.

3) Thus, the brain in *Papilio* is demonstrated to be related to the phenomena of metamorphosis and diapause, as in other Lepidopterous insects.

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