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Studies on the Insect Metamorphosis

V. Implantation of Larval Brains into the Pupae of *Luehdorfia japonica* [1)]

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Concerning the hormonal control of lepidopterous metamorphosis, we have carried out several experiments from the following view point: A hormone released from the brain can stimulate the prothoracic gland so as to secreate the prothoracic gland hormone that is responsible for pupation and emergence. The view has been substantiated by means of a transplantation method even in the commercial silkworm, *Bombyx mori*, in which the action of brain has been neglected or being doubtful in the phenomenon of metamorphosis. When the brains removed from the mature larvae of the silkworm were transplanted into diapausing pupae of a butterfly, *Luehdorfia japonica*, the diapause was interrupted with the induction of a precocious imaginal differentiation of the operated pupae (Ichikawa and Nishiitsutsuji, '51). The same action of brain was demonstrated from the second instar of the Eri-silkworm, *Philosamia cynthia ricini*, which has also a short pupal period like *Bombyx* (Ichikawa, Yashika and Nishiitsutsuji, '53).

However, it has not been ascertained whether or not the larval brain of *Luehdorfia* itself is active enough to interrupt a long pupal diapause of this butterfly, because of the difficulty of collecting suitable materials which differ in the devolopmental stages necessary for the experiment. In 1954 we could collect eggs from Kyoto and Nagoya at different occasions and raised them separately for the present purpose. The result of the experiment will be given briefly in this paper.

Material and Method

Twenty five pupae which pupated earlier than usual from May 25 to May

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27 were used as hosts and 130 of the 5th insters which performed the last larval moult from May 26 to May 28 were selected as donors of brains. The operation was carried out on May 29. After the hosts were anaesthetised with ether, 5 larval brains were kataplastically implanted into each pupa with a fine pincette through a small hole made on the integument of the second thoracic segment, with a care not injuring the prothoracic gland of the host. The hole was covered by a cut piece of integument and coated with melted paraffin. The operated pupae were kept at a room temperature.

Two groups of pupae served as controls. One group comprised 30 pupae which were operated as above only without implanting the brains, and the other group, 61 individuals which were kept intact without any operation.

Experimental results

The pupal integument of *Luehdorfia* is so hard that we can not guess from the outside whether the imaginal differentiation did occur or not. To make sure this point, all pupae were dissected 45 days after the operation (July 13). Results are shown in Table 1.

Table 1. Implantation of larval brains into pupae

	Non-differentiation	Imaginal differentiation				
		Grades of differentiation				
		A	B	C	D	E
No. of survival	3	2	5	7	4	0
Death cases	2	0	2	0	0	0
Total	5	2	7	7	4	0
		20				
Percentage of imaginal differentiation		80 %				

Grades of the imaginal differentiation indicated in the Table are as follows :

A : It is a grade in which the fat-bodies became somewhat dissociable. The brain was apparently larger.

B : It is a grade in which the adult organs such as eyes, legs and antennae were completely formed, but they were not coloured, except for the eyes which turned pale brown. The wings showed no sign of differentiation.

C : It is a grade in which the antennae and legs were coloured in black and the body was provided with numerous scales and hairs. The wings were differentiating and they were milky white in colour.

D : It is a grade in which the wings turned yellow slightly and the black patterns were appearing on them.

E: It is a grade in which the formation of wing patterns was completed and the imago was in a state ready for emergence.

As shown in the Table, 20 pupae indicated more or less imaginal development and 18 out of them almost finished the formation of their adult organs.

Omitting 2 cases in grade A as doubtful, we can still obtain 72 per cent of the positive cases. On the other hand, as indicated in Table 2, all of 61 intact control specimens survived healthily, but they did not show any sign of imaginal differentiation. 9 out of 30 cases in the wound-applied control were possessed of a little larger brain each, but their fat-bodies were as those of the intact pupae. They will be said far from reaching the grade A.

Table 2. Controls

	No. of specimens	Death cases	Date of dissection	Imaginal differentiation
Intact control (1)	30	0	July 10	30 none
Intact control (2)	31	0	July 15	31 none
Wound-applied control	30	1	July 16	20 none 9 A₋ ?

Thus, the effect of the larval brains on the imaginal development of pupae is clearly demonstrated, i. e., the larval brains implanted into pupae are able to release a sufficient amount of the prothoracicotropic hormone for setting the prothoracic glands in action.

Discussion

Luehdorfia japonica has a long pupal period from June to April of the next year and passes naturally through the winter in the pupal form. Referring to this natural habit, we surmise that the low temperature in winter is one of the factors which interrupt the pupal diapause of this butterfly. It is true indeed, because we can obtain the precocious imagines even in summer by subjecting the pupae to the low temperature for two or three weeks. But when the brainless pupae were cooled, they remained unchanged. Moreover, we have succeeded in the interrupting of this diapause by transplanting the heteroplastic brains capable for release of the prothoracicotropic hormone, taken from the mature larvae of the commecial silkworm, *Bombyx mori* ('51). From these facts, we have arrived at a conclusion that the prolonged pupal diapause of this butterfly is completely conditioned by the dormant state of the secretory function of its own brain. In other words, the pupal prothoracic gland is always competent to react upon the hormone from the brain so as to secrete the metamorphosis hormone, if it is supplied somehow or other to the pupae in this state. The pupal imaginal discs are also ready to respond in turn to the hormone from the prothoracic gland without passing through the period of low temperature. Consequently, the mechanism of metamorphosis of our butterfly seems to be the same as that of Williams' *cecropia*-silkworm (Williams, '47, '48).

The result of the present experiment offers a further evidence to the important role of the brain in the imaginal differentiation. When a pupa of *Luehdorfia* received 5 brains active in the secretory function from the 5th instars, it was induced to commence its imaginal differentiation, omitting to enter a long diapausing period, and advanced to a nearly perfect imago by the middle of July. From this fact it is clear that the hormone discharged from the brain of the 5th instar stimulates not only the larval prothoracic gland, but also activates the pupal one. As a matter of course, normal pupae did not indicate any sign of the imaginal differentiation during the same time. Thus we arrive at the same conclusion as before that the pupal diapause of *Luehdorfia*-butterfly should be entirely conditioned by the dormant state of its own brain.

In this connection it becomes important and interesting to investigate the mechanism how the brain does stop its secretory function after pupation. With respect to this question we have nothing to discuss here.

The brains younger than the 5th instar will be available to the present experiment, although we have not yet carried out the experiment with them. Considering the results of our previous experiments with *Bombyx* and *Philosamia*, we will be safe to state that the brain can produce the same hormone throughout the whole larval period in this butterfly, too.

In 9 out of 30 cases of injured control, the state of brain appeared to show an onset of the imaginal development. But their development stopped under the grade A and never advanced more in the course of 45 days after operation. In this connection, Schneiderman and Williams' experiment ('53) will be interesting that an injury applied to a diapausing pupa of *cecropia*-silkworm increased its metabolism as high as 14-folds, although no morphological differentiation started. Referring to this, we are inclined to ascribe a shade enlargement of the brain in 9 aforesaid specimens to a stimulus of injury, i. e., a stimulus of a minor injury applied to the pupal integument of *Luehdorfia* would be strong enough to raise the pupal metabolism to the state sufficient to cause the onset of the earliest development towards imago. In short, it will result not from the prothoracic gland hormone, but from a temporal stimulation of injury.

Summary

1. Five brains isolated from the 5th instars were kataplastically implanted

into a pupa of the same species of *Luehdorfia japonica*.

2. The pupae thus operated can complete the imaginal differentiation by the time of one month and a half after the implantation. Therefore, the brain of the 5th instar is active in the secretory function even within the pupal body.

3. In *Luehdorfia japonica* the brain governs the phenomenon of metamorphosis as in other lepidopterans, and the prolonged pupal diapause of this insect is ascribed solely to the dormant state of the pupal brain.

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