

# Source of the Hormone-like Factor controlling the Manifestation of the Black Pupa in the Silkworm, *Bombyx mori* L.

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The manifestation of the black pupal color of the silkworm is affected by temperature during the period from the end of spinning until pupation. If prepupae of the black pupal strain are kept at 20°C during this period, the black pupal color is manifested, but, if kept at 30°C, the black pupal color is not manifested and the prepupae become normal-colored pupae (Harizuka, 1942; Hashiguchi, 1960).

As regards the mechanism of pigment formation in the black pupa controlled by *bp* gene, Harizuka (1947) proposed that water soluble protein gives a site where chromogen is oxidized by enzyme and, if *bp* prepupae are exposed to high temperature, a slight change occurs in nature of the protein, resulting in the failure of the pigment formation. On the basis of ligature experiments, Hashiguchi (1960, 1962) has concluded that the manifestation of the black pupal color is controlled by a hormone-like factor which is produced by some organs existing in head and thorax.

The study reported here has been attempted to determine the organs which secrete the hormone-like factor by the removal of ganglia, the effect of the severing of nervous commissures and that of the implantation of ganglia. It was found that the complex of brain, suboesophageal ganglion, pro-, meso-, and metathoracic ganglia controlled the manifestation of the black pupal color.

## Materials and Methods

The black pupa strain (bivoltine) used in the present study was the same strain as was used in the ligature experiments (Hashiguchi, 1962). The gene, *bp*, which controls this mutant character is linked with the *K* gene which is located on the XI chromosome (Hashiguchi, unpublished). As it is not yet determined that whether this strain is identical with Harizuka's strain or not the former will be called *bp*(T) in the present paper to distinguish from the latter.

The rearing of *bp*(T) larvae was mainly carried out from February to May. On reaching the mounting (spinning) stage, the prepupae were incubated at 20°C, so that they might become black pupae. After they finished spinning, they were anesthetized with ethyl ether. Operations were carried out on the anesthetized prepupae under binocular dissecting microscope. The operated individuals again incubated at 20°C.

Removal of ganglia and severing of nervous commissures were carried out with sharpened forceps on an anesthetized prepupa. In the case of the implantation of ganglia,

a prepupa was ligatured just behind the second abdominal segment and the anterior portion was cut off. The isolated abdomen was used as a recipient. By means of forceps a ganglion or a complex of ganglia with their commissures intact was removed from a donor prepupa and transferred to Ringer's solution. Implantation was carried out through a small wound which was made on the dorsal side of a recipient in parallel with the dorsal vessel.

After the pupation of the test animals, the pupal color was observed. As there was variation in the pigmentation of pupae, the scoring of pigmentation was made by comparing each pupa with five standard pupae which were chosen according to the distribution and density of black pigment as follows: I, the whole body was normally colored; II, a part of the fore-wing and head was very weakly dark-colored and the dorsal and abdominal parts were normally colored; III, the fore-wing and head were weakly dark-colored and the dorsal and abdominal parts were very weakly dark-colored; IV, the fore-wing and head were dark-colored and the dorsal and abdominal parts were weakly dark-colored; V, the whole body was dark-colored.

### Experimental Results

The results of the removal of various ganglia were shown in Table 1. In most of the test animals from which brain, suboesophageal ganglion, pro-, meso-, or metathoracic ganglion was removed, the manifestation of the black pupal color was inhibited and the normal pupal color (I and II) was observed. On the other hand, the removal of the first abdominal ganglion resulted in the manifestation of the black pupal color (IV and V) in most of the test animals. Similar results were obtained in the case of the removal of the second abdominal ganglion. All the control individuals, which were only wounded, manifested black pupae.

Table 1. Pupal color after the removal of ganglia

Ganglion removed	No. of treated individuals	No. of dead individuals	No. of individuals showing pupal color type				
			I	II	III	IV	V
Brain	20	1	11	3	3	2	0
Suboesophageal ganglion	22	0	9	5	4	3	1
Prothoracic ganglion	20	1	11	5	2	1	0
Mesothoracic ganglion	20	0	10	6	3	1	0
Metathoracic ganglion	22	3	10	6	2	1	0
First abdominal ganglion	20	5	1	2	1	5	6
Second abdominal ganglion	20	4	0	1	2	5	8
Control	15	0	0	0	0	6	9

These results indicate that brain, suboesophageal ganglion, pro-, meso-, and metathoracic ganglion are the source of the secretion of the hormone-like factor, *bp* factor, which controls the manifestation of the black pupal color.

In the second set of experiments, only the nervous commissures were severed, leaving the ganglia intact. As shown in Table 2, severing of commissures behind brain, behind

suboesophageal ganglion, behind prothoracic ganglion and behind mesothoracic ganglion resulted in the normal coloration in most of the test animals. On the contrary, when commissures behind the metathoracic ganglion were severed, the manifestation of the black pupal color took place. Similarly, severing behind the first abdominal ganglion and behind the second abdominal ganglion resulted in the appearance of black pupae.

Table 2. Pupal color after the severing of nervous commissures

Level of severing	No. of treated individuals	No. of dead individuals	No. of individuals showing pupal color type				
			I	II	III	IV	V
Behind brain	20	1	8	3	2	3	3
Behind suboesophageal ganglion	20	0	7	5	4	2	2
Behind prothoracic ganglion	18	0	8	3	4	3	0
Behind mesothoracic ganglion	15	0	10	4	0	1	0
Behind metathoracic ganglion	21	2	1	2	3	8	5
Behind the first abdominal ganglion	20	4	0	1	3	5	7
Behind the second abdominal ganglion	20	2	0	0	2	6	10

From the results mentioned above, it may be postulated that the series of ganglia from brain to metathoracic ganglion manifest their functions only when they are connected with commissures and that the brain itself does not secrete the *bp* factor but controls the secretion of the factor by the other ganglia.

In order to obtain further evidence, the implantation of ganglia was conducted (Table 3). The implanted organs were brain, the complex of brain and suboesophageal ganglion and the complex of brain, suboesophageal ganglion, pro-, meso-, metathoracic ganglion and the first abdominal ganglion. The first abdominal ganglion was included in the latter complex to avoid the accidental breakage of the metathoracic ganglion.

Table 3. Pupal color after the implantation of ganglia

Implanted ganglia	No. of treated abdomens	No. of dead abdomens	No. of isolated abdomens showing	
			normal pupal color	black pupal color
Control	10	0	10	0
Brain	10	0	10	0
Complex of brain and suboesophageal ganglion	10	0	10	0
Complex of brain, suboesophageal ganglion, pro-, meso-, metathoracic ganglion and the first abdominal ganglion	15	1	4	10

Ten isolated abdomens which were only wounded and kept as controls showed the normal pupal color as expected. The implantation of brain and the complex of brain and suboesophageal ganglion had no effect on the pupal color; all the implanted abdomens showed the normal pupal color. On the other hand, the implantation of the complex of a series of ganglia from brain to the first abdominal ganglion resulted in the manifestation of the black pupal color in some of the implanted abdomens: 10 of the 15 abdomens implanted with the complex showed the black pupal color though there was a great

variation in darkness. Of the remaining implanted abdomens, one died before pupal cuticle was formed and 4 abdomens showed normal color. These exceptional abdomens might have been resulted from the breakage of ganglia or commissures during implantation.

The results mentioned above fully confirm the conclusion that the complex of a series of ganglia from brain to metathoracic ganglion is the source of secretion of *bp* factor and the brain itself does not secrete the factor.

Incidentally, it was noticed that the normal-colored pupae which were obtained as the result of the removal of ganglia and the severing of commissures were of vivid amber, while the pupal color of the normal-colored pupae which were obtained after keeping the *bp*(T) prepupae at 30°C was not so vivid.

### Discussion

In a previous paper (Hashiguchi, 1962), the author reported the results of ligature experiments which indicated that several organs which were present in the part anterior to the second abdominal segment controlled the manifestation of the black pupal color. The study reported here attempted to determine the organs which secreted the hormone-like factor by the removal of ganglia and the severing of nervous commissures.

The results indicated that brain, suboesophageal ganglion, pro-, meso-, and metathoracic ganglia controlled the manifestation of the black pupal color. The above-mentioned conclusion was further confirmed by the implantation of complexes of ganglia.

If one of the ganglia existing in the head and thorax was removed from prepupae, most of these treated prepupae became normal-colored pupae. These results as well as the results of severing of nervous commissures indicated that the series of ganglia from brain to metathoracic ganglion manifested their function only when they were connected with commissures. It was probable that the brain itself did not secrete the *bp* factor but controlled the secretion of the factor by the suboesophageal ganglion and thoracic ganglia by way of oesophageal commissures. The environmental temperature might directly affected the brain. These assumptions are based on the results that, when prepupae were ligatured both behind the heads and behind the second abdominal segments, both the anterior and posterior parts showed normal color (Hashiguchi, 1962).

Experiments in which each ganglion was removed at different stages from the end of spinning till the critical period (Hashiguchi, 1962) revealed that the *bp* factor was secreted from the metathoracic ganglion and that the other ganglia served as the communicators of the stimulus for secretion given by the brain (Hashiguchi, unpublished).

From the above-mentioned results, it may be concluded that, if temperature is as low as 20°C during the temperature-sensitive period, the brain is activated and gives a stimulus to thoracic ganglia through the suboesophageal commissures and ganglion. As the result, the *bp* factor is secreted and the black pupal color is manifested. On the contrary, if temperature is as high as 30°C, the brain is inactivated and the stimulus for secretion is not provided, resulting in the manifestation of the normal pupal color.

As regards to the morphological color change of insect, Hidaka (1956, 1957) studied on the pupal color of *Papilio xuthus* as well as *P. protenor demetrius* and reported that the manifestation of the pupal color was determined by the hormone secreted from the complex of brain, suboesophageal ganglion and prothoracic ganglion. Similar endocrine system was found in *Pieris rapae crucivora* (Ohtaki, 1960). On the other hand, Bückmann (1956,

1959) found that the body color of the matured larvae of *Cerura vinula* was controlled by the hormone secreted from prothoracic gland which had been activated by a cephalic center, probably the brain. The present author want to stress the similarlity between these insects and the silkworm on the mechanism of morphological color change.

In the silkworm, two kinds of black pupa are known. One is controlled by *bp* gene which is located on the XI chromosome, and the other is controlled by *so* gene which is not linked to *bp* (Tanaka, 1924; Sasaki, 1953). In order to ascertain if the black pupal color of *so* strain is also controlled by a hormonal factor, ligaturing, removal of ganglia and severing of nervous commissures have been conducted on the prepupae of *so* strains, *osso*, *p-51* and *l-4*. However, no hormonal factor has been found in the *so* strains. Consequently, it may be supposed that the mechanism of the manifestation of pupal color in *so* strains is different from that in the *bp*(T) strain (Hashiguchi, 1961).

### Summary

The removal of ganglia, severing of nervous commissures and the implantation of ganglia were conducted on the prepupae of *bp*(T) strain, which were kept at 20°C after the mounting stage so that they might become black pupae. The results obtained were as follows.

The complex of brain, suboesophageal ganglion, pro-, meso-, and metathoracic ganglion controlled the manifestation of the black pupal color. If temperature is as low as 20°C during the temperature-sensitive period, the brain is activated and gives a stimulus to thoracic ganglia through the suboesophageal commissures and ganglion. As the result, the *bp* factor is secreted and the black pupal color is manifested. On the contrary, if temperature is as high as 30°C, the brain is inactivated and the stimulus to secretion is not provided, resulting in the manifestation of the normal pupal color.

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### Literature cited

- BÜCKMANN, D. 1956 Die Umfärbung der Raupen von *Cerura vinula* unter verschiedenen experimentellen Bedingungen. *Naturwiss.* **43**: 43.
- 1959 Die Auslösung der Umfärbung durch das Häutungshormon bei *Cerura vinula* L. (Lepidoptera, Notodontidae). *J. Ins. Physiol.* **3**: 159-189.
- HARIZUKA, M. 1942 Inheritance of the black pupa in the silkworm (preliminary note). *J. Seric. Sci. Jap.* **13**: 4-8. (in Jap.).
- 1947 Genetical and physiological studies on the black pupa of the silkworm. *Bull. Sericul. Exp. Sta.* **12**: 531-593. (in Jap. with Engl. summ.).
- HASHIGUCHI, T. 1960 On the relations between pupal color and ligature of a black pupa strain in the silkworm, *Bombyx mori*. *Jap. Jour. Genet.* **35**: 269-270. (in Jap.).
- 1961 Two types of black pupae different in the mechanism of manifestation of the pupal color. Abstracts of the Annual Meeting of the Kyushu Branch of Sericul. Society Jap. **5**. (in Jap.).

- HASHIGUCHI, T. 1962 Hormone-like factor controlling the manifestation of the black pupa in the silkworm, *Bombyx mori*. Jap. Jour. Genet. 37 (2): 91-96.
- HIDAKA, T. 1956 Recherches sur le déterminisme hormonal de la coloration pupale chez lépidoptères-I. Les effets de la ligature, de l'ablation des ganglions et de l'incision des nerfs chez prépupes et larves âgées de quelque Papilionides. Annot. Zool. Jap. 29: 69-74.
- 1957 Recherches sur le déterminisme hormonal de la coloration pupale chez l'pidoptères-II. Sur le cas de deux Nymphalides. Annot. Zool. Jap. 30: 83-85.
- OHTAKI, T. 1960 Humoral control of coloration in the cabbage white butterfly, *Pieris rapae crucivora*. Annot. Zool. Jap. 33: 97-103.
- SASAKI, S. 1953 Recurrent mutation of the sooty black pupa gene, *so*. Sanshikenkyu No. 6, 5-6. (in Jap.)
- TANAKA, Y. 1924 A new study on the inheritance of cocoon color. Sangyoshinpo 32: 367, 368 (in Jap.).