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# Humoral Control of Pupal Coloration in the Cabbage White Butterfly, *Pieris rapae crucivora*

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(*Communicated by* K. TAKEWAKI)

In the cabbage white butterfly, *Pieris rapae crucivora*, it is well known that, under varying conditions, pupae of different colors, viz. light green, dark green, light brown, dark brown and black, are produced.

Poulton (1892) was the first to carry out experimental studies on the mechanism concerning the development of pupal coloration in pierid species. He observed that larvae which underwent pupation in boxes lined with either black or red-colored paper became darkened pupae, whereas those which pupated in boxes lined with yellow or orange-colored paper transformed into green pupae. Afterward, Dürken (1916) and Brecher (1917) also reported that greenish pupae were produced if pupation took place on either yellow or orange-colored background. From these observations, it seems likely that background color is the main factor involved in the determination of pupal coloration.

A little later, Brecher (1919, 1924) carried out experiments of blinding pierid larvae either by covering the eyes with an opaque paint or by cauterizing the eyes electrically and concluded that effects of colored lights on pupal coloration were exerted on the larvae through the eyes. Similar conclusion was also reached by Przibram (1922) who showed that decapitated larvae failed to adapt to background color at the time of pupation.

Recently, Hidaka (1956) has shown that pupal coloration in the swallowtails, *Papilio xuthus* and *P. protenor demetrius*, is controlled by a substance or substances secreted by the prothoracic ganglion. It is of great interest that Ohnishi and Hidaka (1956) have demonstrated that, in these papilionid species, environmental stimulus determining pupal color is not background color. Hidaka (1957) has suggested that some volatile substance in green leaves of food plant may play a role in the production of the green color of pupae.

The present paper deals with the results of experiments carried out to disclose the effect of colored lights on pupal coloration in the cabbage white butterfly, *Pieris rapae crucivora*, and of those designed to elucidate a possible humoral mechanism involved in the determination of coloration in pupae of the same species.

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## MATERIAL AND METHOD

Materials used were larvae of the cabbage white butterfly, *Pieris rapae crucivora*, mainly collected from cabbage fields and reared in breeding cages in the laboratory until they had become fully grown. A few of them were obtained from eggs laid in the cages and reared throughout the whole larval period until maturation.

In order to use non-diapausal type larvae as experimental materials, larvae were collected between April and September in 1956, 1958 and 1959.

Larvae kept in breeding cages made from wire gauze painted gray were fed with fresh cabbage leaves. They developed in good health, and pupated normally, the resultant pupae being invariably of the brown type (*vide infra*).

For the sake of convenience of description, the period from the cessation of feeding of the fully grown larvae to actual pupation is divided into three stages.

In Stage I, the fully grown larva ceases feeding and wanders about in search of a suitable site for pupation. Then it crouches in the selected site, and eventually makes a scaffold for pupation with silk. The body becomes shortened, the last brownish excrement being discharged (Stage II). After a while, it begins spinning a silken girdle and makes a small pad of silk beneath the caudal extremity Now it enters the so-called prepupal Stage (Stage III). Several hours later, the larva molts to pupate using the girdle as a support.

If larvae were transferred to complete darkness as soon as they reached Stage I and kept in the dark until pupation, the resultant pupae were invariably dark brown in color (Table 1). In the following experiments, when the larvae attained Stage II, a few hours after transference to the dark, they were taken out, subjected to various operations (*vide infra*) and returned to darkness again.

All operations were performed under ether anesthesia. To ligate the body of larvae at desired levels, 80# nylon thread was used. Removal of ganglion and severing of nervous commissures were done by means of sharpened pincettes.

## OBSERVATIONS ON PUPAL COLORATION

Pupae encountered in the field may be divided into 5 types according to their coloration. (I) The light green type pupae have integument which is green in ground color. In this type, there are no black spots on the back of the abdomen, although a few brown spots may be present. (II) The dark green type pupae show less than 60 yellow or brown spots on each abdominal segment. The integuments is green in ground color although in some parts of the body it is white. (III) Pupae of the intermediate type exhibit more than 60 brown spots on the dorsal side of the second or the third abdominal segment, the spots being mainly distributed in the latero-dorsal parts of the segment. Ground color of the integument is whitish green. (IV) The brown type pupae are provided with brown and black spots approximately equal in number, scattered on the whole surface of the dorsal integument. Ground color is light brown although the intersegmental parts are green. (V) The dark brown or black type pupae have blackish spots on the whole dorsal surface, ground color of the integument being brownish gray.

More than 250 pupae collected on cabbage leaves in the field were invariably of the green type, except for 2 individuals showing a darker tint (the dark green type). Twenty-two pupae which had undergone pupation on a white wall of the laboratory were of the brown or black type, no pupae of the green type being found, while all of 17 pupae produced on a dark brown desk of the laboratory were of the brown type.

## Experimental Results

1) *Effect of environmental color on pupal coloration*

Dyes used to stain filter paper linings for the vessels were as follow: 0.1% naphthol yellow-S for yellow, 0.1% $\alpha$-naphthol orange for orange, 0.05% amarance for red, 0.02% guinea green for green, 0.05% indigo carmine for blue and 0.03% chlorophyllin potassium salt for grayish green, all dissolved in distilled water.

Sheets of filter paper soaked in these dye solutions were dried at room temperature. These sheets of colored filter parer were applied closely to the

Table 1

Color types of pupae produced in glass vessels lined with sheets of colored filter paper

| Lining | Number of pupae showing color type | | | | | Total |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| Yellow paper | 14 | 4 | 3 | 0 | 0 | 21 |
| Orange paper | 20 | 9 | 2 | 1 | 2 | 34 |
| Red paper | 2 | 6 | 3 | 0 | 0 | 11 |
| Green paper | 1 | 4 | 2 | 6 | 1 | 14 |
| Blue paper | 2 | 4 | 6 | 1 | 0 | 13 |
| Grayish green paper | 4 | 5 | 4 | 0 | 0 | 13 |
| Polyethylene film | 7 | 0 | 1 | 2 | 7 | 17 |
| White paper light | 3 | 2 | 5 | 2 | 4 | 16 |
| White paper dark | 0 | 0 | 0 | 6 | 80 | 86 |
| None light | 14 | 0 | 3 | 0 | 1 | 18 |
| None dark | 0 | 0 | 0 | 0 | 7 | 7 |

Color type I: light green; II: dark green; III: intermediate; IV: brown; V dark brown or black.

inner side wall and the bottom of glass vessels as well as the underside of glass covers. For control vessels, unstained filter paper was used.

Several larvae at Stage II were transferred from darkness into each vessel with colored filter paper linings. The vessels were placed by the window on the north side of the room. In addition to such vessels with colored paper linings, those lined with colorless polyethylene sheets as well as those without any linings were also used.

Color types of pupae produced in these vessels are shown in Table 1.

It is seen from Table 1 that pupae obtained on the yellow or orange background, were mainly of the green type, while color types of pupae on the green or blue background, varied from green to brown. On both red and chlorophyllin-K paper pupae of the green type were somewhat few. On white paper and polyethylene sheet in the light, varying color types were produced, whereas in glass vessels without lining a large majority of pupae were of the green type.

2) *Effect of ligation of larval body at different levels on pupal coloration*

Under light anesthesia, a ligature was applied either between the head and the thorax or between the thorax and the abdomen of larvae which had been kept in the dark and which were at Stage II. After ligation, the larvae were again returned to complete darkness.

In one series of experiments, ligature was applied between the head and the thorax in such a way that it interfered with the blood circulation but allowed nervous impulse to pass. In the individuals operated on, the head either became dried up or retained larval characteristics indefinitely, whereas the rest of the body developed pupal characteristics, although many of the larvae failed to molt. The resultant pupal trunks were mostly brown in color (Table 2).

In the other series of experiments, larvae were decapitated after tightly ligating at the neck region. The majority of these larvae produced pupal trunks of the green type (Table 2).

Table 2

Coloration of pupal trunks yielded by larvae ligated at the neck

| Operation | Number of pupal trunks showing color type | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Blood circulation interfered with, passage of nervous impulse not affected | 1 | 2 | 1 | 11 | 0 |
| Decapitation | 3 | 7 | 2 | 0 | 0 |

If a ligature was applied between the thorax and the abdomen in larvae at Stage II in such a way that it affected the blood circulation but allowed nervous impulses to pass, cuticle of the parts both anterior and posterior to the ligature exhibited pupal characteristics.

As shown in Table 3, the anterior part, in the majority of cases, was of the brown type and the posterior part, of the green type. In these animals, each of the anterior and posterior parts was homogeneously colored, never showing two or more types of coloration admixed.

Table 3
Coloration of pupal anterior and posterior parts yielded by larvae ligated between thorax and abdomen

| Anterior and posterior color type combination* | II/III | III/III | IV/IV | III/II | IV/II | V/II | V/III |
|---|---|---|---|---|---|---|---|
| Number of individuals | 1 | 1 | 2 | 1 | 12 | 4 | 2 |

*Other combinations were not encountered.

If larvae were ligatured between the thorax and the abdomen at Stage III, most of the individuals showed the brown/brown color type combination. This appears to indicate that pupal coloration has already been determined at this stage. Therefore, in the following experiments, larvae at Stage II only were used as materials.

3) *Effect of removal of ganglion on pupal coloration*

In larvae at Stage II, one of the following five ganglia, i. e. the supra-oesophageal, suboesophageal, prothoracic, mesothoracic and metathoracic, was removed under ether anesthesia. After operation the larvae were returned to complete darkness.

Cuticle of these animals transformed normally into pupal cuticle. All of the larvae deprived of the brain and the majority of those without the suboesophageal or the prothoracic ganglion transformed into green type pupae, while most of the larvae without the meso- or the metathoracic ganglion, transformed into brown ones (Table 4).

Table 4
Coloration of pupae without ganglia

| Ganglion removed | Number of individuals showing color type | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Brain | 16 | 1 | 0 | 0 | 0 |
| Suboesophageal ganglion | 5 | 8 | 3 | 4 | 0 |
| Prothoracic ganglion | 15 | 5 | 2 | 2 | 0 |
| Mesothoracic ganglion | 2 | 1 | 2 | 15 | 1 |
| Metathoracic ganglion | 0 | 0 | 4 | 2 | 6 |
| Wound only (blank operation) | 0 | 0 | 0 | 0 | 5 |

4) *Severing of nervous commissures*

In this series of experiments, the nervous commissures were cut at different levels, i. e. between the brain and the suboesophageal ganglion, between the suboesophageal and the prothoracic ganglia or between the prothoracic and the mesothoracic ganglia.

Table 5

Coloration of pupae with nervous commissures servered

| Level of severing | Number of individuals showing color type | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Brain–suboesophageal ganglion | 7 | 3 | 1 | 3 | 1 |
| Suboesophageal ganglion–prothoracic ganglion | 4 | 6 | 4 | 0 | 0 |
| Prothoracic ganglion–mesothoracic ganglion | 1 | 4 | 4 | 9 | 1 |

As shown in Table 5 summarizing the results, severing of commissures between the brain and suboesophageal or between the suboesophageal and prothoracic ganglia inhibited the production of brown pupae, while that between the prothoracic and metathoracic ganglia did not.

## DISCUSSION

In the experiments on the development of pupal coloration in response to colored paper background, it was shown that, in accordance with the results of observations by Poulton, Dürken and Brecher, yellow or orange background induced green type pupae, while in complete darkness brown type pupae were produced irrespective of background color. From this, it seems probable that environmental color acts on mature larvae of the cabbage white butterfly at least as a factor determining future pupal coloration. However, the experiments with colored paper yielded much less uniform results than the observations conducted in the field where pupae produced on cabbage leaves were invariably fresh green in color. The problem may be settled by future experiments with monochromatic lights.

From the ligation experiments, it appears likely that brownish coloration of pupal cuticle is caused by a humoral factor originating in the endocrine organ situated at the thoracic region, and that the head plays an important role in releasing the humoral substance. Furthermore, it may be conjectured from the results of removal of a certain ganglion that the endocrine organ producing the above-mentioned factor is, as demonstrated by Hidaka (1956) in *Papillio*, the prothoracic ganglion, and that the organ is stimulated by the brain, via the nervous commissures, to secrete the factor. This hypothesis was supported by the nerve severing experiments. Following severance of the nerves between the brain and the prothoracic ganglion, green type pupae were produced. This seems to show that the operation inhibited the release of the factor. But before drawing a definite conclusion further experiments need be carried out.

## SUMMARY

1. Development of coloration in the pupae of the cabbage white butterfly, *Pieris rapae crucivora*, on various colored backgrounds was studied. Yellow and

orange backgrounds produced in the main green type pupae, while red and grayish green backgrounds mainly dark green pupae. In complete darkness, pupae were invariably colored dark brown.

2. If a loose ligature was applied between the head and the thorax, the parts posterior to the ligature became pupal trunks of the brown type, and, if larvae were decapitated after a similar but tight ligation, headless pupae of the green type were produced even in complete darkness. The ligation between the thorax and the abdomen resulted in pupae with the anterior part of the brown type and the posterior part of the green type.

3. Larvae deprived of either the brain, the suboesophageal ganglion or the prothoracic ganglion became green type pupae. Most of the pupae without the mesothoracic or the metathoracic ganglion were of the brown type.

4. These results seem to show that the brownish coloration of pupal cuticle is brought about by a humoral factor which is released from an endocrine organ in the prothoracic region. Experiments in severing nerves between the brain and the prothoracic ganglion suggested that the release of the factor is stimulated by the brain through the oesophageal commissures.

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