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Environmental Factors Affecting the Formation of Orange Pupa in *Papilio xuthus**

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It is well known that the pupae of *Papilio xuthus* are various in their body colour according to the environment. We classified the colour variation into the following 8 types: green-1, green-2, green-3, brown-1, brown-2, brown-3, orange-1 and orange-2 as is shown in Plate 1.

We are carrying out several experiments with the aim of analysing the environmental factors concerning the formation of those types.

This paper is concerned with the formation of the brown and orange types alone.

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Materials

As material we used the swallowtailed butterfly, *Papilio xuthus*. They appear in Kyoto from the middle of April to the end of October. Eggs used in the following experiments were collected from the field from June to September and larvae hatched from them were reared with leaves of *Poncirus trifoliata* under certain experimental conditions which will be described in the following series of experiments. The mature larvae collected directly from the field from the middle of June to the beginning of September were also used in the experiments on the formation of brown type.

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Experiments

I. *Environmental Factors causing the Formation of Brown Type*

As environmental factors, humidity and light were studied respectively in the following experiments.

(1) *Humidity*

It has been accepted in general that humidity is one of the important factors which affect the metabolism of insects. In their experiments with *Papilio xuthus*, Ohnishi and Hidaka (1956) obtained the brown type of pupae more often when the prepupae were kept in the container with dead twigs of *Poncirus trifoliata* than when with fresh leaves of the same tree. Although they ascribed this result to some unknown environmental factors, the difference of humidity in both containers must not be neglected, that is to say, humidity might be higher in the container with fresh leaves than in the container with dead twigs. So we first examined the effect of humidity on the colour type of pupae. For this purpose we subjected the mature larvae tó two different grades of humidity; one was of 80 percent relative humidity and the other of 100 percent one. The temperature and the light condition throughout this experimentation were 25°C and dark respectively. Two series of experiments were performed. In one series the container ($15\times15\times6\,cm^3$) was made of paper and in the other it ($7.5^2\times\pi\times6\,cm^3$) was made of glass. They contained 10 individuals each. The results obtained in both series are arranged in Tables 1 and 2 respectively.

Table 1. Results of humidity experiments in paper boxes

Humidity (%) at 25°C \ Colour Type	Green-1	Green-2	Green-3	Brown-1	Brown-2	Brown-3
80	2	7	4	7	32	4
	Green 13 (23%)			Brown 43 (77%)		
100	8	7	5	2	20	7
	Green 20 (41%)			Brown 29 (59%)		

Table 2. Results of humidity experiments in glass vessels

Humidity (%) at 25°C \ Colour Type	Green-1	Green-2	Green-3	Brown-1	Brown-2	Brown-3
80	34	9	6	5	13	5
	Green 49 (68%)			Brown 23 (32%)		
100	70	0	0	0	0	0
	Green 70 (100%)			Brown 0 (%)		

As is shown in Tables 1 and 2, the formation of brown pupae is more frequent under the condition of low humidity than of high humidity in paper boxes as well as in glass vessels; and under the same humidity it is more predominant in paper boxes than in glass vessels.

(2) *Light*

There are some evidences that light controls the pigment formation of insects. In our preliminary experiments with *Papilio*, it was assumed that the dark condition was favourable to the formation of brown pupae, so we tried to repeat the same experiment subjecting the prepupae either to constant light or to constant darkness. In this case glass vessels of the same size as previously were used as containers. Humidity and temperature were 80 percent and 25°C respectively. The results are shown in Table 3.

Table 3. Effects of light on the formation of pupal colour types at the time of pupation

Light Condition \ Colour Type	Green-1	Green-2	Green-3	Brown-1	Brown-2	Brown-3
Constant Light	32	10	2	1	4	1
	Green 44 (88%)			Brown 6 (12%)		
Constant Darkness	34	9	6	5	13	5
	Green 49 (68%)			Brown 23 (32%)		

From the results shown in Table 3, it is clear that brown pupae appear more frequently in constant darkness than under constant illumination.

II. *Environmental Factors relating to the Formation of Orange Type*

In the preliminary simple experiments attempting the production of brown pupae not described above, a few orange pupae appeared sometimes among the brown ones only when the mature larvae reared to this stage in comparatively dark condition were subjected to constant darkness. In order to analyse the conditions for producing the orange type more precisely, the present experiments were undertaken.

(1) *Light*

In this series of experiments, larvae in the different instars were subjected to constant darkness for different periods, as is shown in the following Table 4. Glass vessels covered with glass plates were used as containers of larvae. The larvae were supplied with fresh leaves twice a day. Humidity at 25°C in this case was always nearly 100 percent. In every case when the larvae became mature, pieces of wet filter paper were put into the vessel to keep 100 percent of humidity. Results are shown in Table 4.

The data shown in Table 4 reveal that raising of larvae under the dark condition is favourable to the formation of orange type. Especially, when the larvae were kept in the darkness from hatching to the end of the fourth instar (Group 6) or to the end of the last instar (Group 2), appearance of the orange pupae increased up to about 50 percent. But raising of larvae in the darkness from hatching to the third instar (Group 3) or during the fifth instar alone (Group 5) is not so effective as in groups 2 and 6 just mentioned. Therefore, it would be expected that the fourth instar is a sensitive period for the production

Table 4. Frequency of the formation of orange and green pupae under the dark condition of various periods

Periods in darkness and light: □ light ■ darkness

Instars Days	Ist 2	IInd 2	IIIrd 2.5	IVth 4.5	Vth 6.5	prepupa 1	Colour Type: Orange-1	Colour Type: Green-1
Group 1	light	light	light	light	light	light	0 (0%)	65(100%)
Group 2	darkness	darkness	darkness	darkness	darkness	darkness	22(52%)	20(48%)
Group 3	darkness	darkness	darkness	light	light	light	4(8%)	44(92%)
Group 4	light	light	light	darkness	light	light	3(8%)	33(92%)
Group 5	light	light	light	light	darkness	darkness	3(6%)	52(94%)
Group 6	darkness	darkness	darkness	darkness	light	light	23(48%)	25(52%)
Group 7	light	light	light	darkness	darkness	darkness	6(14%)	36(86%)

of orange type. But the fact is contrary to this expectation, i. e., raising the larvae during the fourth instar alone in the darkness produces only 8 percent of the orange pupae (Group 4). Besides, one may surmise from the above data that the longer the period of raising under the dark condition, the more the orange pupae obtained. But the fact is not so simple. Comparing group 6 and group 7 in Table 4, the duration of treatment is the same, 11 days; nevertheless the production of orange type is less frequent in group 7.

Therefore, it may be stated that there is no sensitive period limited to any instar, but that the longer raising of younger larvae in the darkness is more effective for the formation of orange pupae. Here it must be said that there occurred no brown type in this case and that the orange pupae entered the long pupal diapause.

(2) *Humidity*

From the data of humidity experiments described above, it is found that low humidity (80%) causes the appearance of the brown type more often than high humidity does. As the orange type is considered to be a modification in colour of the brown type, the treatments of darkness and humidity were combined in this case; i. e., larvae were kept under the dark, 25°C and 100 percent humidity condition throughout the whole larval period and then transfered to the container of 80 percent humidity that was in constant darkness. The results are shown in the upper row of Table 5. The lower row is rewritten from the data of group 2 in Table 4 for the sake of comparison between low and high humidities. The containers used in this experiment were glass vessels.

Table 5. Frequency of the formation of orange pupae under the dark condition throughout the whole larval period and in two different humidities at the time of pupation

Colour Type / Humidity (%) at 25°C	Orange-1	Orange-2	Green or Brown
80	22	26	3* (6%)
	Orange 48 (94%)		
100	22	0	20** (48%)
	Orange 22 (52%)		

* two are of the green-2 type and the remaining one is of the brown-2 type.
** all are of the green-1 type.

It is clear from Table 5 that in the case in which larvae are kept under constant darkness from hatching to maturity, their exposure to 80 percent humidity at the time of pupation is more effective for the formation of orange type than that to 100 percent humidity. All of the orange pupae entered the pupal diapause.

(3) *Temperature*

According to the field observation on *Papilio xuthus*, the orange pupae were met with more often in the later part of October than in late September. As the reason for this the temperature may be considered one of the important

Table 6. Frequency of appearance of orange type at different temperatures (100 percent humidity, constant darkness)

Colour Type / Temperature	Orange-1	Green-1
20°C	33 (65%)	18 (35%)
25°C	22 (52%)	20 (48%)
30°C	14 (24%)	44 (76%)

factors. Therefore, we tested the effect of temperature for the formation of orange type. Two series of experiments were carried out under the condition of constant darkness, one at 20°C and the other at 30°C. Larvae were kept at these temperatures respectively from hatching to pupation. Humidity in the glass vessel was kept 100 percent throughout the experiments. The results are given in Table 6. The middle row in this Table is rewritten from the data given as group 2 in Table 4.

As is shown in Table 6, low temperature combined with constant darkness is effective to produce the orange type. All pupae of orange type in this experiment entered also pupal diapause. But 18 pupae of green type of 20°C group also entered diapause, whereas other green pupae emerged as butterflies.

Discussion

It is well known that the pupal colour in Lepidoptera is varied in accordance with the surrounding colour. Poulton (1884) was the first who found out this correlation in *Papilio*. Recently, Ohnishi and Hidaka (1956) reported that "pupae which have pupated on green twigs of food plant, *Poncirus trifoliata*, are always of the green type, while those which pupated on dead branches are of the brown type." But they made a denial of the correlation of the pupal colour and the surrounding colour because of the fact that "the same correlation also exists in the pupae pupated in the darkness." They claimed that "the colour of the surroundings cannot be considered as a principal factor" and that "the pupal types are determined by some unknown environmental factors at the time of pupation."

Our experimental data described above indicate that low humidity of the environment where the larvae are going to pupate is one of the factors which determine the brown type of pupae. If Ohnishi and Hidaka's results are inspected closely from the view point of humidity, the difference of humidity may not be negligible between the experiments using green twigs and dead branches. In other words, humidity may be lower in the case of using dead branches than in the case of providing green twigs. If this is true, their results are in good agreement with ours.

The dark treatment at the time of pupation is shown to be also effective for the formation of brown type in our experiments, but the frequency of the production of brown type is greater in the paper box than in the glass vessel under the same conditions. The reason for this difference is yet unknown. One possibility is the relative roughness and smoothness of the surface on which the larvae pupate. Goto (1956) obtained 14 brown and 2 green pupae in a yellowish brown paper box kept in the darkness from the fifth instar. But he said it is uncertain whether roughness of the paper box is involved in the formation of pupal colour.

The orange type of our terminology may be identical to the reddish brown type of Ohnishi and Hidaka's. They included this type in the brown as a mere variant of the latter type determined at the time of pupation. The orange type is sure to be a modification of the brown when viewed from outside. But it is not a mere phenotype produced at the time of pupation. As is clarified by our experiments, this type is determined at the larval period As shown in Table 4, when the larvae were kept in constant darkness throughout the whole larval life or at least to the end of the fourth instar, they pupated into pupae of orange type at the high frequency of about 50 percent. The same treatment at the time of pupation alone yielded no orange type. It faild to reveal the specific sensitive period for the formation of orange type, but when the dark treatment was performed in high humidity (100 percent) throughout the whole larval life and then humidity alone was decreased to 80 percent at the time of pupation, the orange type was obtained at a frequency as high as 94 percent. (Raising of larvae in constant darkness and low humidity is desirable, but it was impossible because of the high mortality.) At any rate, darkness and low humidity are considered as the factors favourable to the formation of orange type. In addition, low temperature is also one of the factors concerning the formation of orange type, as is shown in Table 6. In the natural habitat, Goto (1956) observed that appearance of the orange type (he prefers to call it the red type) increases much in the later part of October. This may be due to the decrease of daytime, of temperature and of humidity in this season.

The brown type was produced when the mature larvae were kept under the condition of low humidity or of low humidity combined with darkness, while the orange type was produced when the larvae were kept in the darkness at the young period. Consequently, the orange type may be separated from the brown type. Moreover, the physiology of the orange type pupa is greatly different from that of the brown type one, i. e., the former enters without exception the long pupal diapause, while the latter dose not. Of course, the pupae of green type emerge without entering diapause except for a few specimens.

Such correlation between pupal colour and diapause was also found in several insects, i. e., the rice leaf-miner, *Agromyza oryzae* (Kuwayama 1950), the rice green caterpillar, *Naranga aenescens* (Iwao, in print) and the wild silkworm, *Antheraea pernyi* (Yamazaki, oral communication).

Summary

1. The environmental factors involved in the formation of various colour types of pupae in *Papilio xuthus* were studied.

2. The colour types of pupae are classified into 8, i. e., green-1, green-2, green-3, brown-1, brown-2, brown-3, orange-1 and orange-2.

3. The formation of brown type takes place often when the mature larvae

are kept in the darkness and in low humidity at the time of pupation. In this case a paper box in which the pupation occurs increases markedly the frequency of the production of this type.

4. The orange type occurs under such conditions as constant darkness and low temperature, to which the larvae are subjected from hatching to at least the end of the fourth instar. In addition, low humidity at the time of p p tion increases the appearance of this type.

5. Pupae of the orange type enter the long pupal diapause without exception, while those of the green and brown types emerge without entering diapause provided that the temperature is sufficiently high.

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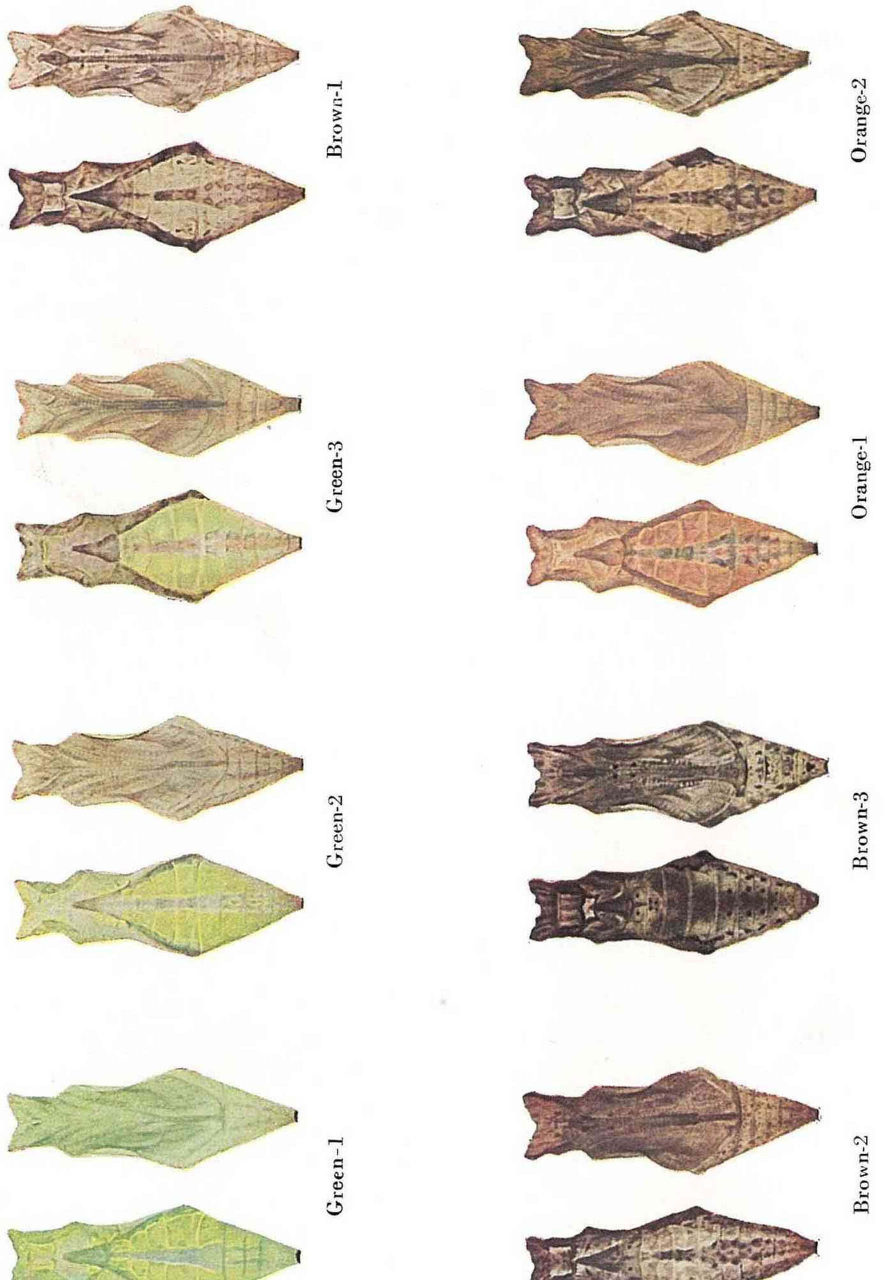

Left : dorsal, Right : ventral.