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Light-Stimulated Green Coloration of Silk Glands in the Saturniid Moth, *Antheraea yamamai*: Influence of Ligation and Parabiosis

YOSHIOMI KATO

Department of Biology, International Christian University, Mitaka, Tokyo 181, Japan

ABSTRACT—Developmental timing of pigmentation of the silk glands and a possible photoreceptive site responsible for the green coloration were examined in *Antheraea yamamai*. At the time of gut purge, the silk glands became green under high intensity light (5,000 lx) whereas they were yellow under low intensity light (20–30 lx). Green coloration occurred in larval transfer from low intensity to high intensity at gut-purge stage whereas reverse transfer reduced the degree of green coloration, indicating that light of high intensity must be irradiated continuously during the stage. When the neck-ligated larvae or isolted abdomens, both of which had been kept in low intensity and were ligated at the time of gut purge, were wholly or partially irradiated at high intensity, green coloration was seen in any cases. Removal of all ventral ganglia from the isolated abdomens has no adverse effect on green coloration in either light condition. In the parabiosis experiments, in which two decapitated larvae kept in low intensity were connected by a transparent tube and one of the parthers wholly or partially exposed to light and the other shielded, the silk glands of the shielded animal were green as were those of the exposed ones. Local irradiation to the only region of a connecting tube also caused green coloration in both animals. But, when the flow of hemolymph in the tube was disturbed with a plug, no green coloration occurred in the shielded animal. These suggest that the hemolymph has a crucial role in light reception.

INTRODUCTION

Many insects change their body-color or pigmentation according to environmental stimuli. One of the main factors is the intensity of light or the spectrum of the circumstance [1–3]. Light stimuli mostly are perceived by visual organs, and act on the metabolism and accumulation of pigments via neuroendocrine system [4–7]. In some insects, however, extraocular light reception seems to be involved [4].

In the saturniid moth, *Antheraea yamamai*, green coloration of cocoons, which is due to the presence of a blue bilin together with an undertermined yellow compound, is influenced by the intensity of light given during larval life [8]. The larvae, when kept in light of high intensity (e.g., 4,000 lx), produce a green cocoon whereas cocoon color is yellow under light of low intensity (e.g., 40 lx) or darkness. Ocelli-removed larvae respond to light and produce a green cocoon. The occurrence of similar light-induced green coloration has been found in the larval integument and adult wing of the papilionid butterfly, *Graphium sarpedon*, and in the larval integument and cocoon of another saturniid moth, *Rhodonia fugax* (Kato, unpubl.). The goal of our study is to clarify the physiological or biochmical mechanism by which light stimulates the accumulation of a bilin in the tissues (e.g., silk glands where cocoon materials are accumutated).

In the present study, (1) developmental timing of pigmentation of the silk glands, (2) influence of ligation and ganglion removal on the light responsiveness of the larvae, (3) local irradiation on the ligated and parabiosis larvae were investigated in *A. yamamai*.

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MATERIALS AND METHODS

Animals

Larvae of *A. yamamai* were reared on oak leaves (*Quercus actissima* and *Q. serrata*) at 25°C under LD 12:12. They were kept in transparent plastic cases (31×21×10 cm) till 4th-instar. When the larvae became of 5th (last) instar, they were individually kept in small plastic cups (Ø11×6.5 cm). Under these conditions, the duration from the last larval ecdysis till the onset of gut purge, or from the gut purge till pupation was about 11 to 13 days or 5 to 6 days, respectively.

Light irradiation

Till the end of 4th-instar, all larvae were irradiated with the light of low intensity (20–30 lux) during the photo-phase of LD 12:12. Intact developing larvae in 5th-instar were exposed to the light of high intensity (5,000 lx) or remained at the low intensity during the photo-phase according to the experimental regimens. At the time of gut purge, some of those larvae were transferred to continuous darkness or light (5,000 lx), respectively.

White fluorescent tubes (National, FL 40SS·W/37) were used as a light source, and the light intensity was measured at the top level of rearing cases.

Ligation experiments

Feeding larvae (10 days after last larval ecdysis) and gut-purge larvae, which had been kept in light of 20–30 lx, were ligated with cotton threads between head and thorax, or between thorax and abdomen, and then anterior portions were removed. Posterior portions of the larvae were divided into two groups, and one group exposed to 5,000 lx and the other kept in darkness, respectively. Dissections were performed on 4 to 7 days after ligation for the feeding animals, and on 2 to 3 days for the gut-purge ones to check coloration of the silk glands.

Removal of ganglia

Gut-purge larvae kept in light of 20–30 lx were ligated between head and thorax, and the isolated abdomens were prepared. All eight ventral ganglia were removed from each abdomen. As control, a sham-operation was done, where ventral portions of the integument were injured. Operated animals were kept in 5,000 lx or in darkness for 24 hr, and then dissected.

Local irradiation experiments

Larvae kept in 20–30 lx were ligated at the time of gut purge. After removal of the anterior portion, each animal was laid down on its lateral side on a plate, and then fixed at anterior and posterior ends of the body with threads and adhesive tape (Fig. 1). Fixed animals were transversely partitioned into two or three portions at various levels of the body by using a light-tight box (8×10×1.5 cm) with a ⎍-shaped cutting made at its side-wall just large to insert a larva. To prevent light leakage from other sides, the box was wrapped with a sheet of almi-foil. The part outside the box was irradiated at 5,000 lx for 24 hr. In some experiments, neck-ligated animals were again ligated at the other level, where the body was partitioned into anterior and posterior halves, and locally irradiated.

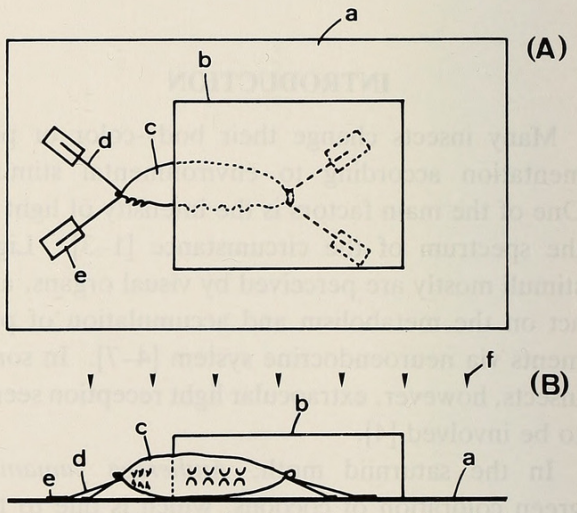

FIG. 1. A schematic drawing of a local irradiation on a ligated larva. (A) upper view; (B) lateral view. (a) plate; (b) box with a hole; (c) larval body; (d) thread; (e) gum tape; (f) light irradiated.

Parabiosis experiments

Two series of experiments were performed. First, the larvae kept in light of 20–30 lx were

decapitated at the time of gut purge. Decapitated larvae were connected with those animals of the same age kept in light of 5,000 lx by using a transparent vinyl tube (Ø 0.8 cm, 2 cm in length), which permits hemolymph flow between each body, and then both of each animal were kept in darkness for 24 hr. As control, the connections were done between the larvae of the same light-condition (5,000 or 20–30 lx).

Second, decapitated larvae kept in light of 20–30 lx were connected each other by the vinyl tube. In one experiment, a connecting tube was plugged with paraffin to prevent the flow of hemolymph through the tube. These animals were locally irradiated at 5,000 lx for 24 hr, as described above.

Examination of silk-gland coloration

For intact animals, the middle and posterior regions of silk glands were examined because the anterior region was scarecely colorless. The degree of coloration was classified into colorless, pale green or pale yellow, and green or yellow.

For treated animals, the middle region was examined because colored material was accumulated there together with liquid silk [9]. Color of the middle region was scored into 4 groups according to the extent of green coloration. Score 3 represents most green coloration whereas score 0 is yellow. Intermediates were scored as 1 or 2 depending on the degree of green.

RESULTS

Developmental timing of silk-gland pigmentation during the last larval instar

Figure 2 shows the timing of pigmentation in the middle and posterior regions of the silk gland under two different light-intensities. Under light of 5,000 lx, the silk glands began to show pale

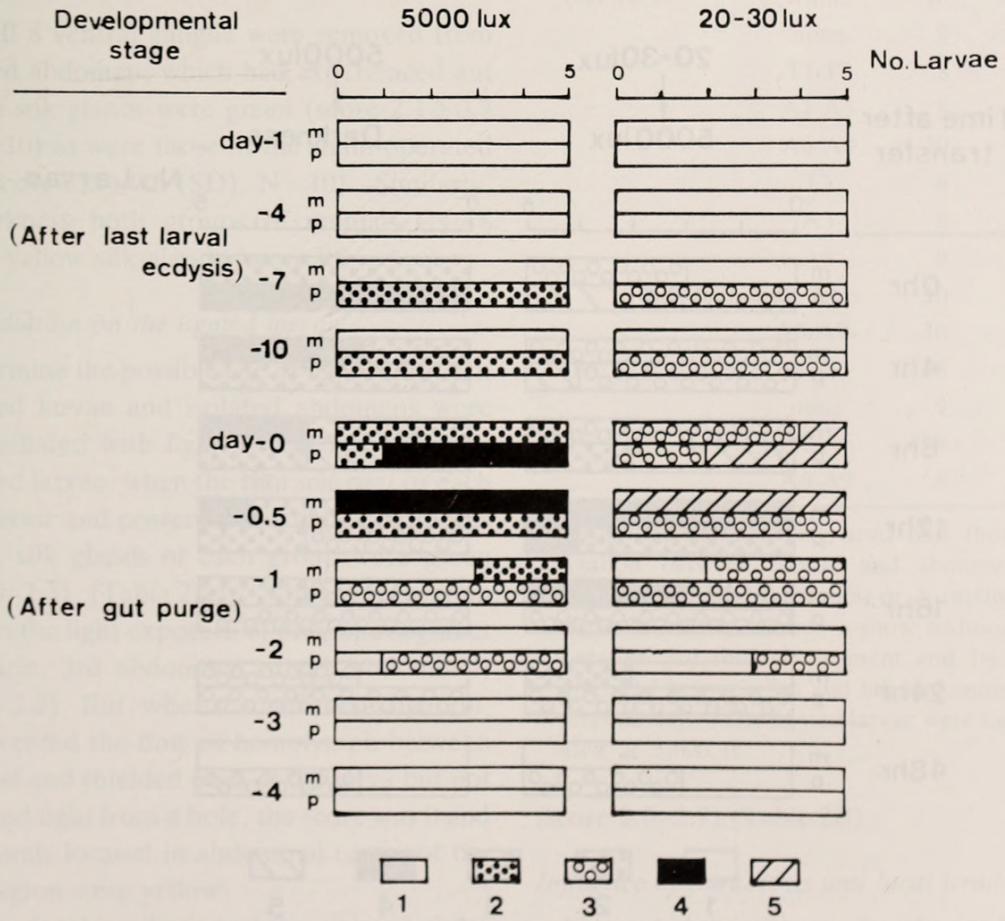

FIG. 2. Timing of silk-gland pigmentation at differnt intensities of light during 5th larval instar. (m) middle region of silk glands; (p) posterior region. (1) colorless; (2) pale green; (3) pale yellow; (4) green; (5) yellow. N=5.

green color on 7 to 10 days in the posterior region (in particular, its anterior half), became green at the time of gut purge in both of the middle and posterior regions, and then became colorless toward pupation. Under light of 20–30 lx, time course of yellow pigmentation was similar to the case in light of 5,000 lx, and yellow coloration was clearly seen at the time of gut purge in both regions of the gland.

When the larvae were transferred from light of 20–30 lx to light of 5,000 lx at the gut-purge stage, the silk glands start to become pale green in the posterior region 8 hr after the transfer, and showed green color in the middle and posterior ones 12 hr later (Fig. 3). On the other hand, when larval transfer was done from 5,000 lx to darkness at the same stage, green color of the silk glands was lost at earlier time of development than in the case of the 5,000 lx condition.

Light responsiveness of the ligated larvae for green coloration

To know whether the head or thoracic part may be responsible for the green coloration of silk glands, ligation experiments were performed. Initially, ligation was dine on feeding (day 10) larvae. When ligated between head and thorax, most of the larvae had the pigmented silk-gland and its color was green (score 1.6) under 5,000 lx whereas under darkness it was yellow (score 0) (Table 1A). For the ligation between thorax and abdomen, no coloration occurred in the silk glands of each group.

Second, gut-purge larvae were used for the experiments because larvae reateined an ability of green coloration till the time of gut purge as demonstrated above. In neck-ligated animals, silk-glands of the larvae exposed to 5,000 lx were of green coloration and the score was higher than that in the neck ligation of the feeding larvae,

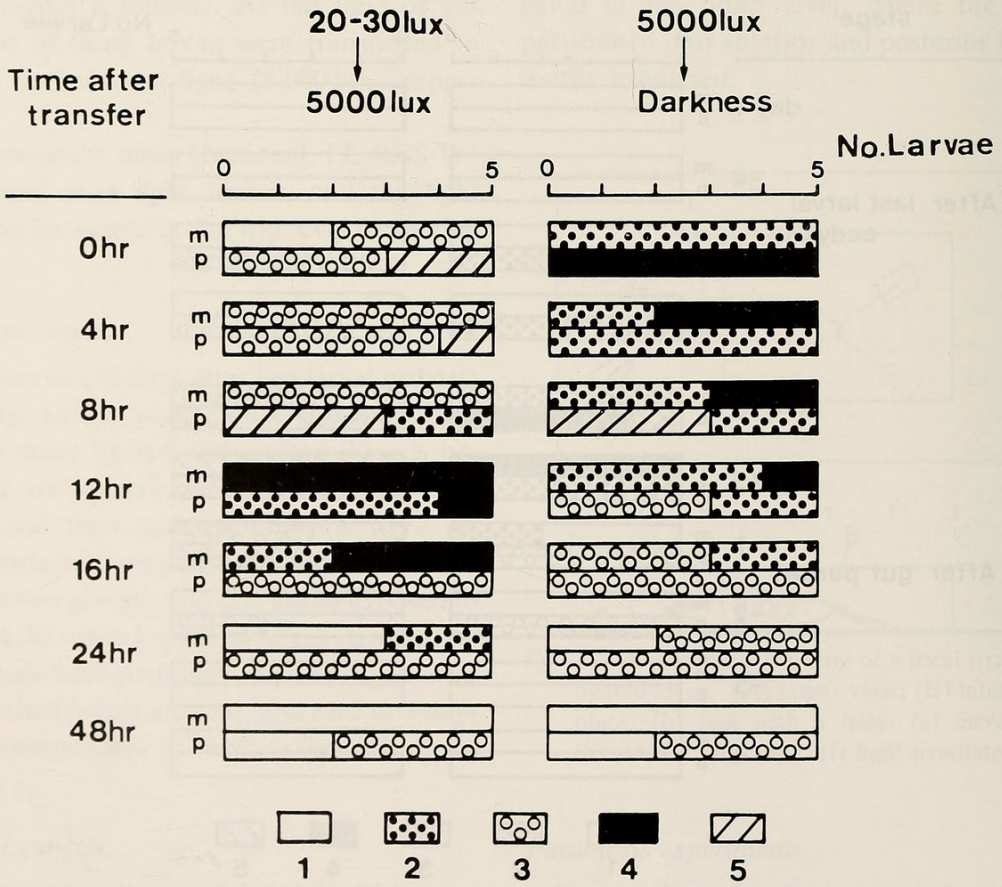

FIG. 3. Pigmentation of the silk gland after from low light intensity (20–30 lx) to high intensity (5,000 lx) (left) or from high intensity to darkness at the time of gut purge (right). Other explanations same as in FIG. 2.

TABLE 1. Light responsiveness of the ligated larvae for green coloration of the silk gland

Stage of ligation	Site of ligation	Light condition	Pigmentation %	Score of green coloration (mean ± SD)
(A)	H/T	5000 lx	63 (11)	1.6±1.0
Feeding		Darkness	75 (8)	0
	T/A	5000 lx	0 (9)	—
		Darkness	0 (8)	—
(B)	H/T	5000 lx	100 (12)	2.3±0.4
Gut-purge		Darkness	100 (9)	0
	T/A	5000 lx	100 (15)	2.7±0.6
		Darkness	100 (10)	0

H/T; ligation between head and thorax. T/A; ligation between thorax and abdomen. After ligation, the larvae were kept in 5,000 lx or darkness. Numerals in parentheses are sample size.

whereas those of the larvae kept in darkness were yellow (score 0) (Table 1B). Similar light-responsiveness was seen in the isolated abdomens.

Influence of ganglia removal on the light responsiveness

When all 8 ventral ganglia were removed from the isolated abdomen, which had experienced gut purge, the silk glands were green (score 2.1±0.7 (SD), N=10) as were those of the sham-operated animals (score 2.5±0.5 (SD), N=10). Similarly, under darkness both groups of animals (N=8 each) had yellow silk-glands (score 0).

Local irradiation on the ligated larvae

To determine the possible site of light reception, neck-ligated larvae and isolated abdomens were locally irradiated with light of 5,000 lx. For the neck-ligated larvae, when the thoracic part or each of the anterior and posterior abdominal parts was irradiated, silk glands of each group were green (score 1.9–2.3) (Table 2A). Green coloration occurred in the light exposure of only one segment (3rd thoracic, 3rd abdominal or 6th abdominal) (score 1.8–2.2). But, when the additional ligation, which prevented the flow of hemolymph between the exposed and shielded ones of the larva but not the scattered light from a hole, the score was 0 and the silk glands located in abdominal cavity of the shielded region were yellow.

Similarly, local irradiation of the isolated abdomens was always effective for green coloration (score 2.0–2.7) (Table 2B).

TABLE 2. Effect of local irradiation on green coloration in the ligated larvae

Site of ligation	Site of irradiation	No.	Score of green coloration (mean ± SD)
(A) H/T	whole	10	2.2±0.8
	none	9	0
	T1-T3	8	2.3±0.9
	A1-A5	8	1.9±0.9
	A6-A9	10	2.1±0.9
	T3	8	1.8±1.0
	A3	8	2.0±0.8
	A6	8	1.8±1.0
	T1-T3/*	10	0
	/A6-A9**	10	0
(B) T/A	whole	8	2.6±0.5
	none	9	0
	A1-A5	8	2.1±0.8
	A6-A9	8	2.0±0.8

H/T; ligation between head and thorax. T/A; ligation between thorax and abdomen. Arabic numerals next to T (thorax) or A (abdomen) show segment order. * and ** show additional ligation between 3rd thoracic segment and 1st abdominal one, and between 5th and 6th abdominal segment, respectively. The ligated larvae were locally irradiated at 5,000 lx.

Influence of parabiosis and local irradiation

Figure 4 shows the results obtained in the first series of the experimets. When the larvae exposed

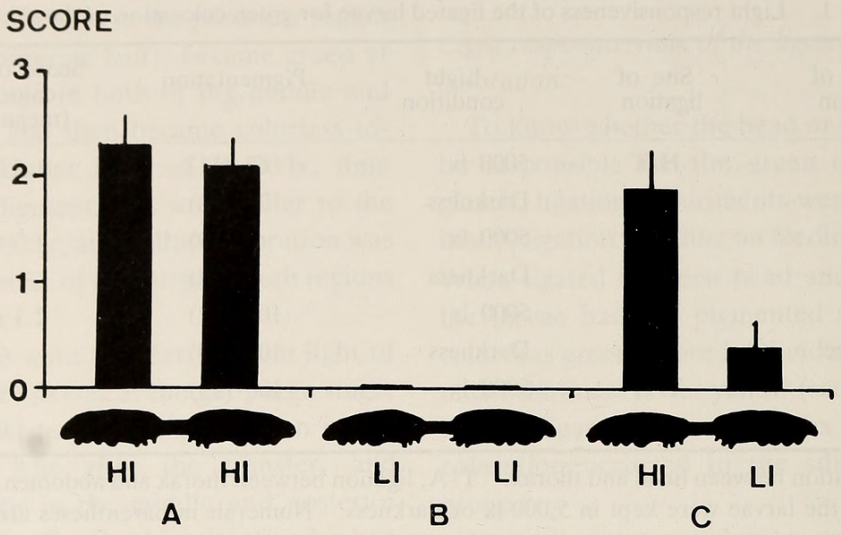

FIG. 4. Induction of green coloration by parabiosis with the light-condiiton larvae. A, parabiosis between the larvae kept in higt intensity of light (HI) (N=8); (B), parabiosis between the larvae kept in low intensity (LI) (N=8); (C), parabiosis between a larva kept in HI and a larva in LI (N=8). After parabiosis. the larvae were kept in darkness. Vertical bars are SD.

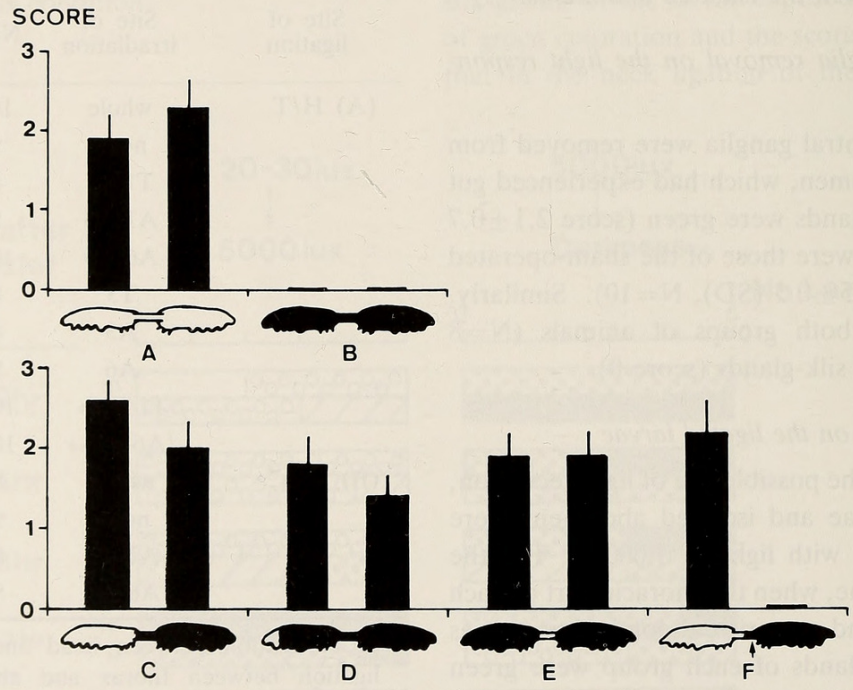

FIG. 5. Effect of local irradiation on green coloraiton in the parabiosis larvae. A, light (5,000 lx)-control (N=8): B, dark-control (N=10); C, one partner was irradiated and the order shielded (N=9): D, only posterior 4 segments of one parther irradiated (N=10): E, only the region of the tube connecting two animals was irradiated and the animals themselves wholly shielded (N=8): F, one partner was irradiated and the other shielded, and the connecting tube was plugged with paraffin (arrow) to prevent hemolymph flow (N=8). Open area of the connected larvae is exposed to light. Vertical bars are SD.

to light of 5,000 lx were connected each other, green coloration was seen in the silk glands of both larvae (score 2.1–2.3). When the larvae kept in light of 20–30 lx were connected with those exposed to the high-intensity light, the degree of green coloration in the former animal was very low (score 0.4) in contrast to that in the partner (light-condition larva) (score 1.9), although it slightly rose as compared to the case of the connection with the dark-condition larva (score 0).

In the second series of the experiments, the larvae kept in light of 20–30 lux were connected each other, and then irradiated locally. When one of the partners was wholly irradiated, the silk glands of the non-irradiated animal were green (score 2.0), as were those of the irradiated one (score 2.6) (Fig. 5). Irradiaiton effect was seen in the case where only several segments of the partner were exposed to light, although the pigmentation level was slightly lower (score 1.4–1.8). Furthermore, local irradiation to only the region of the tube in which the hemolymph was seen flowing, but not to the larval body itself, caused green coloration in each larva (score 1.9 each). But, when the flow of hemolymph in the connection tube was prevented with a plug, no green coloration occurred in the silk glands of the non-irradiated partner (score 0).

DISCUSSION

The present observations demonstrated that silk-gland pigmentation in *A. yamamai* occurs intensively at the time of gut purge under high or low intensity of light, and that its occurrence begins at the posterior region of the glands, in contrast to the case of *Bombyx mori,* in which pigmentation of the silk gland occurs during the longer period of development than in *A. yamamai* and it begin at the middle region of glands [10]. Furthermore, the transfer experiments showed that light of high intensity must be irradiated continuously around at the time of gut purge for giving enough green coloration to the glands.

In *B. mori,* the pigmentation (carotinoid uptake) in the silk gland is controlled by hormones; ecdystaroids and juvenile hormone [10]. The present data showed that green coloration in the silk gland of *A. yamamai,* is more intensive in larvae decapitated at gut-purge stage than at feeding stage, and no isolated abdomens from the feeding larvae had the pigmentaed glands. The timing of the occurrence of gut purge in *A. yamamai* is coincided with the increase of hemolymph ecdystaroid level (Kiuchi, unpubl.). Therefore, pigmentation activity of the silk gland of *A. yamamai* is suggested to be controlled by ecdysteroid level.

The ligation experiments clearly showed that not only the decapitated larvae but also the isolated abdomens retain their photosentisitivity and the silk glands become in green in response to the light irradiation. Furthermore, green coloration occurred even in the ganglia-removed or locally illumintated animals. As the extraocular photoreceptor in insects, the brain has been reported for photoperiodic and circadian systems [11], the prothoracic gland for circadian system [12], the teminal abdominal ganglion for phototactic activity [13], and the genital organ for reproductive behavior [14]. However, the results of the present experiments excluded the possiblity that those organs may be a photoreceptive site responsible for the green coloration.

In the local irradiation of light, it was impossible to completely prevent the intrusion of the scattered light from the hole into the shielded part. However, as shown in Table 2, additional ligations, where the scattered light intruded in but the flow of hemolymph was prevented between the exposed and shielded part, inhibited green coloration in the latter part. Thus, it is thought that the scattered light from the small hole might not contribute to the green coloration.

In the parabiosis experiments combined with light irradiation, local irradiation of light to one of the partner or only hemolymph caused green coloration. But the presence of paraffin plug, which might not prevent the scattered light but the hemolymph flow, between the connected larvae inhibited the coloration in the partner. Thus, it is strongly suggested that hemolymph may have a crucial role in light reception, although whether light may act on a hemocyte or serum was undecided.

Unexpectedly, in the parabiosis experiments, in which the 5,000 lx-conditioned larvae were connected with the larvae kept in the low intensity light, the degree of green coloration in the latter larvae was very low. Preliminary experiments, in which hemolymph of the former larvae was injected into the latter larvae, also gave negative data. This reason is unknown at present. As discussed above, it seems likely that the larval body must be illuminated simultaneously at the time when the pigmentation occurs. Presumably,

unknown light-induced substance which is responsible for the bilin pigmentation (e.g., pigment or pigment-precursor) might be due to be very unstable or rapidly turned over in the hemolymph.

In lepidopteran insects, two types of bilins have been reported [15]: one is biliverdin IX and the other bilins called as neopterobilin. Barbier and his colleagues [16, 17] demonstrated *in vitro* phototransformation of biliverdin IX to a neopterobilin and between neopterobilins. A bile pigment from the green cocoons of *A. yamamai* belongs to the latter group [8], in which absorption peak at about 380 nm is absent. Preliminary report showed in *A. yamamai* that larval hemolymph and integument, which are green irrespective of the light condition, have a bilin of neopterobilin type, and it is spectrophotometrically different from that of the cocoon (silk gland) and larval head capusule, which become green in response to light [18]. Present data showed that the light irradiation to the hemolymph was effective for green coloration. Considering these facts, therefore, the phototransformation of hemolymph bilin and the incorporation of a tansformed bilin into the silk gland may be possibly involved in the light-stimulated green coloration. To prove this idea, more knowledge about biosynthesis and metabolism of the bilins in insects will be required.

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REFERENCES

1 Raabe, M. (1983) Pigment metabolism. In "Endocrinolgy of Insects". Ed. by R. G. H. Downer and H. Lawfer, Alan L. Liss Inc., New York, pp. 493–500.

2 Hoffmann, K. H. (1984) Color and color change. In "Environmental Physiology and Biochemistry of Insects". Ed. by K. H. Hoffmann, Springer Verlag, Berlin/Heidelberg, pp. 206–224.

3 Fuzeau-Braesch, S. (1985) Colour changes. In "Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 9. Behaviour". Ed. by G. A, Kerkut and L. I. Gilbert, Pergamon Press, Oxford, pp. 549–589.

4 Angersbach, D. (1975) The direction of incident light and its perception in the control of pupal melanization in *Pieris brassicae*. J. Insect Physiol., **21**: 1691–1696.

5 Atzler, M. (1931) Untersuchungen über den morphologischen und physiologischen Farbwechsel von *Dixippus morosus*. Z. vergl. Physiol., **13**: 505–533.

6 Bückmann, D. and Maisch, A. (1987) Extraction and partial purification of the pupal melanization reducing factor (PMRF) from *Inachis io* (Lepidoptera). Insect. Biochem., **17**: 841–844.

7 Ressin, W. J. (1980) The effect of jevenile hormone on pupal pigmentation of *Pieris brassicae* L. J. Insect Physiol., **26**: 295–302.

8 Kato, Y., Onuma, Y., Sakurai, K. and Yamada, H. (1989) Role of light in the green pigmentataion of cocoons of *Antheraea yamamai* (Lepidoptera: Saturniidae). Appl. Ent. Zool., **24**: 398–406.

9 Akai, H., Kiuchi, M. and Tamura, T. (1989) Ultrastructures of silk glands and cocoon filaments of wild silkmoths, *Antheraea yamamai* and *Antheraea pernyi*. In "Wild Silkmoths '88". Ed. by H. Akai and Z. S. Wu, Gakkai Shuppan Center, Tokyo, pp. 9–23.

10 Kawai, N. (1976) Hormonal effects on carotenoid uptake by the silk gland in the silkworm, *Bombyx mori*. J. Insect Physiol., **22**: 207–216.

11 Saunders, D. S. (1982) Insect Clocks (2nd ed.), Pergamon Press, Oxford.

12 Mizoguchi, A. and Ishizaki, H. (1982) Prothoracic glands of the saturniid moth *Samia cynthia ricini* possess a circadian clock controlling gut purge timing. Proc. Natl. Acad. Sci. USA, **79**: 2726–2730.

13 Ball, H. J. (1965) Photosensitivity in the terminal abdominal ganglion of *Periplaneta americana* (L.). J. Insect Physiol., **11**: 1311–1315.

14 Arikawa, K., Eguchi, E., Yoshida, A. and Aoki, K. (1980) Multiple extraocular photorereptive areas on genitalia of butterfly *Papilio xuthus*. Nature, **288**: 700–702.

15 Kayser, H. (1985) Pigments. In "Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 10. Biochemistry". Ed. by G. A. Kerkut and L. I. Gilbert, Pergamon Press, Oxford, pp. 367–415.

16 Bois-Choussy, M. and Barbier, M. (1978) Isomerisations and cyclisations in bile pigments. Heterocycles, **9**: 677–690.

17 Barbier, M. (1981) The status of blue-green bile pigments of butterflies and their phototransformations. Experientia, **37**: 1060–1062.

18 Kato, Y. and Yamada, H. (1991) Preliminary report on biliverdins in larval tissues of *Antheraea yamamai*. In "Wild Silkmoths '89. '90". Ed. by H. Akai and M. Kiuchi, International Society for Wild Silkmoths, Tsukuba, pp. 97–99.