CHANGE OF PHOTO-REFLECTION IN THE LARVAL INTEGUMENT FOLLOWING WITH THE GROWTH OF THE SILKWORM, BOMBYX MORI L. (LEPIDOPTERA:BOMBYCIDAE)

By

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INTRODUCTION

As well known, the lightness and gloss of the silkworm body is superficially different according to strain and growth stage. Such a difference is caused mainly by the structure and pigmentation of the integument. Previously many investigations were carried out on the morphology of the larval integuments of the silkworms (KUWANA, 1932; ARUGA, 1943; ITO, 1951 a·b; KOBAYASHI, 1951 a·b, 1955; TAKAHASHI, 1955, 1956 a·b; KAWASE, 1959, 1960; MORI, 1965; TANAKA, 1970 a·b, 1971 a·b; AKAI, 1974; etc.).

Almost all investigations in the above, however, were dealt with the integuments from histological point of view. The author measured the reflection property of light from the integuments following with the larval growth of *Bombyx mori* L. and researched photophysically the lightness and gloss of the integument using the reflection intensity and the reflection index as indicators. The results obtained are shown in the present paper.

In preparing this presentation, the author is especially gratefull to Prof. N. KOYAMA, Shinshu University, for his kind guidance and reading the original manuscript. Thanks are also due to Prof. E. NAGASHIMA, Ass. Prof. K. TANAKA, who assisted throughout the experiment.

MATERIAL AND METHOD

The silkworm larvae of Nichi-1, Shi-108 and Nichi- $1 \times Shi$ -108 were used for the materials. The larvae were reared under a temperature of 28°C with R. H. of 80% and photoperiodic condition of 8L16D.

The material silkworms were fixed on a black paper just after anesthetized by chloroform $(CHCL_3)$ for about few minutes and the reflection intensities of light from

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the bodies which contained newly hatched or newly exuviated stage and molting stage in each instar larvae, were measured by the Automatic Micro-luster (ML meter) as described in the previous paper (TAKIZAWA & KOYAMA, 1969, 1970, 1972).

The position of the larval body casted by spot light was the 4th abdominal noturm, because there was no marking affecting the reflection intensity of light. The reflection index (gloss) was calculated by the reflection intensity curve ($I \cdot \theta$ curve).

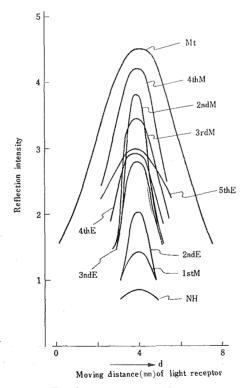
RESULTS

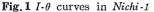
A. REFLECTION INTENSITY

1. Nichi-1

Fig. 1 shows an example of the reflection intensity curves $(I-\theta \text{ curves})$ in different growth stages of the strain.

The moving distance of the light receptor becomes larger and the relative reflection intensity (RI value) becomes higher following with the development of the





NH: newly hatched larva M: molting larva E: newly exuviated larva Mt: matured larva These abbreviations are used in the following figures.

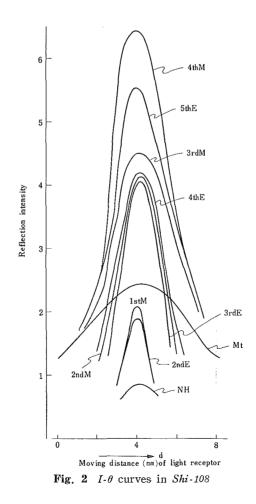
larva.

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The shape of each $I \cdot \theta$ curve resembles to each other, excepting that in the 1st molting larva, the 5th instar larva and, especially in the newly hatched larva, in which it is greatly flattened.

Fig. 4 shows changes of the maximum values of the reflection intensity in each larva.

RI value is 0.7 in the newly hatched larva, being as large as $1.4 \sim 3.9$ from the 1st molting larva to the 2nd molting larva. Thenceforth the value in either the newly exuviated larva or the molting larva increases as growth. The highest value (4.5) appears in the 4th molting larva. However, each value in the newly exuviated larva is so smaller than that in the molting larva, though it keeps almost a constant range of $3.0 \sim 3.5$ except in the 2nd newly exuviated larva (2.2).



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RI value (4, 2) in the matured larva becomes larger than that (3, 2) in the 5th newly exuviated larva. Such a tendency differs from the cases of the other strains, in which the values decrease greatly.

2. Shi-108

As shown in Fig. 2, the features of $I \cdot \theta$ curves are similar to those in Nichi-1, but the shape of the curve becomes flattened evidently in the matured larva.

The changing phase of RI value takes the same tendency as in Nichi-1, though RI value of each larva in this strain is generally larger than in Nichi-1 (Fig. 4). Especially, the highest value (6.0) occurs samely in the 4th molting larva as in Nichi-1. RI value in the matured larva (3.0), however, becomes remarkably lower than that in the 5th newly exuviated larva and in the 4th molting larva. Such a significant decrease of RI value in the matured larva is not seen in the case of Nichi-1 as above-mentioned.

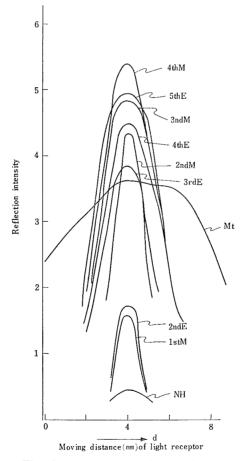


Fig. 3 $I \cdot \theta$ curves in Nichi-1×Shi-108

3. Nichi-1×Shi-108

 $I \cdot \theta$ curves take an intermediate form between those in *Shi-108* and *Nichi-1* (Fig. 3).

RI value changes with some resemblance of that in *Shi-108*, even though it (about 5.3) is larger than that in the other strains in the 3rd molting larva (Fig. 4).

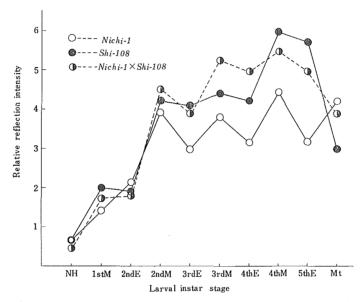


Fig. 4 Change in the maximum value of relative reflection intensity in each instar larva

The value of RI is about 0.5 in the newly hatched larva and in the 4th molting larva reaches the highest value (5.5) which is somewhat smaller than that in *Shi-108*. It is recognized that the highest value of RI appears samely in the 4th molting larva of each strain.

In the matured larva, RI value (about 4.0), though being almost the same as that in *Nichi-1*, falls into a smaller value than that in the 5th newly exuviated larva (5.0) as seen in *Shi-108*.

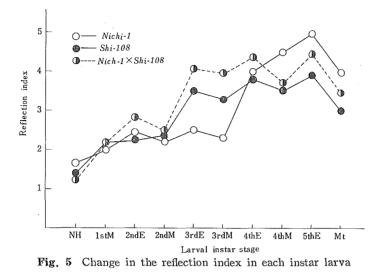
B. REFLECTION INDEX

1. Nichi-1

The changing phase of the reflection index (gloss) calculated from $I \cdot \theta$ curve is illustrated in Fig. 5.

The index value is lowest (1.7) in the newly hatched larva. Successively it takes

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 $2.0 \sim 2.5$ from the 1st molting larva to the 3rd molting larva. The value raises suddenly in the 4th newly exuviated larva (about 4.0) and reaches the maximum (about 5.0) in the 5th newly exuviated larva. In the matured larva, the index value falls into 4.0, which is highest among the material strains.

2. Shi-108

The change of the reflection index resembles somewhat that in Nichi-1 from the newly hatched larva to the 2nd molting larva, but the value in the 3rd newly exuviated larva (3.5) becomes higher than that in Nichi-1 (2.5). Afterwards the reflection index increases gradually as growth and takes the maximum value (about 4.0) in the 5th newly exuviated larva. It (3.0) falls into the smallest value in the matured larva among the sampled strains.

Each index value in *Shi-108* can be said as a whole to be smaller than that in *Nichi-1*, except in the 3rd newly exuviated larva and the 3rd molting larva.

3. Nichi-1×Shi-108

The changing feature of the index value is similar to that in Shi-108.

The index value is as large as 1.3 in the newly hatched larva, which is lowest among the three strains (Fig. 5). It reaches the highest value (about $4_{1}5$) in the 5th newly exuviated larva and later falls into a lower value of 3.5 in the matured larva. Such a changing tendency of the index value is seen also in the other strains.

CONSIDERATION

In the light of the results obtained, it is recognized that the changing feature

in the reflection intensity curve $(I \cdot \theta \text{ curve})$ measured by ML meter on the integument of the silkworm larvae (*Bombyx mori* L.) is different according to strain.

Observing from the shape of $I-\theta$ curve in the matured larva of Nichi-1×Shi-108 takes as a whole an intermediate form between those in Shi-108 and Nichi-1, though being somewhat similar to that in Shi-108. Further $I-\theta$ curve in the newly hatched larvae takes a flattened shape in each strain, and also their relative reflection intensity (RI) is considerably small as compared with that in the larvae of the other stages. It is assumed that the above fact is caused by strong diffusion and absorption of light in black and long bristles and purplish pigments which are richly distributed at the integument of the newly hatched larvae, and also it has a close coincidence with the results reported by TAKIZAWA and KOYAMA (1969, 1970), in which the reflection intensity of the silkworm egg was greatly effected by the pigmentation of the serosa and the structure of the chorion.

In all strains RI value tends to increase in zigzag form following with the larval growth. Especially it rises greatly in the 2nd molting larva than in the 2nd newly exuviated larva. The phenomenon that the value is higher in the molting stage than in the newly exuviated stage seems to be attributed by some differences in the surface characters of the integument between the both stages, as reported by SHIMIZU (1932), ARUGA (1943), ITO (1951 $a \cdot b$), KOBAYASHI (1955), TANAKA (1970 $a \cdot b$), and AKAI (1974), who found the structural difference in the larval integument between the both stages. The increasement of RI value in the molting larvae is similar to that of lightness observed by the naked eyes.

RI value in the matured larva becomes smaller than that in the 5th newly exuviated larvae in *Shi-108* and *Nichi-1×Shi-108* differing from *Nichi-1*.

Such a decrease in RI value is reasonably thought to be brought by transparency of the matured larval body. The adverse case, however, occurring in *Nichi-1*, remains unexplained.

The reflection index (gloss) obtained from $I \cdot \theta$ curve tends to change with some similarity to RI value following with the larval development (Fig. 5).

In the newly exuviated larva, however, the index value becomes higher than that in the molting larva, suggesting that the changing phase of the gloss is completly adverse to that of RI value. This difference seems due to the fact that the gloss indicating quantitatively the light reflection is expressed by the ratio of the maximum reflection intensity to the minimum one regardless of lightness, and the ratio is smaller in the molting larva than in the newly exuviated larva.

Taking the present results into consideration, the photo-reflection property of the larval integument can be said to be more exactly estimated by RI value than the reflection index. Tatsuo TAKIZAWA

The racial difference in the highest value of the reflection intensity appears significantly at the 3rd molting stage and later. The above value, therefore, can be used as an indicator of the photophysical character of the larval integument in the silkworm.

SUMMARY

In the present paper, an account is given of the results on the photo-reflection from larval integument following with the growth of the silkworm, Bombyx mori L.. The photo-reflection from the larval integument were measured by Automatic Micro-luster (ML-meter). The results obtained are summarized as in the followings.

1. The reflection intensity curve $(I \cdot \theta \text{ curve})$ with mono-modal peak tended to change as a whole to the same form in all strains (*Nichi-1*, *Shi-108* and *Nichi-1*× *Shi-108*), though that in *Nichi-1*×*Shi-108* took an intermediate form between those in *Shi-108* and *Nichi-1* in the matured larva.

2. The relative reflection intensity (RI value) obtained from the maximum values of $I \cdot \theta$ curves increased following with the larval growth in all strains, but in Nichi-1 RI value from the 2nd molting larvae were smaller than that in the other strains, except that in the matured larva RI value (4.2) became higher than that (3.2) in the 5th newly exuviated larva.

In the molting larvae, however, RI value became higher than that in the newly exuviated larvae from the 3rd instar stage in all strains, amongst which *Shi-108* took the highest value (6.0) in the 4th molting stage when the maximum value appeared in each strain.

Such a phenomenon seems to be attributed by some difference in the surface characters of the integument between the molting larva and the newly exuviated larva.

3. The reflection index (gloss) calculated from $I \cdot \theta$ curve was apt to change with some similarity to RI value following with the larval growth. In all strains, the index value became higher in the newly exuviated larva than in the molting larva. The changing phase of the index value, however, was completely adverse to that of RI value. This difference seems due to the fact that the index value indicating quantitatively the light reflection is expressed by the ratio of the maximum reflection intensity to the minimum one regardless of lightness, and the ratio is smaller in the molting larva than in the newly exuviated larva.

4. In the light of the results, the photo-reflection property of the larval integument was more adequately estimated by RI value rather than the reflection index (gloss), especially by that in the 3rd molting stage and later when the racial difference became significantly. RI value, therefore, can be utilized as an indicator of the photophysical character of the larval integument in the silkworm.

LITERATURES CITED

- AKAI, H. (1974) On the surface structure of the larval integument in the silkworm (in Japanese); Sanshikagaku and Gizyutsu, 13:66-69.
- ARUGA, H. (1943) Histological studies on mutant integument regarding larval markings in the silkworm, *Bombyx mori* L. (in Japanese); Bull. Sericul. Exp. Stat., 11 (4):387-425.
- ITO, T. (1951a) Studies on the integument of the silkwrom, Bombyx mori L. (in Japanese); Bull. Sericul. Exp. Stat., 13(6):305-328.
- ITO, T. (1951b) Studies on the structure of the larval integument of Bombyx mori L. (in Japanese); J. Appl. Zool., 16(3-4):132-134.
- KAWASE, S. (1959) Studies on the pigmentation in the integument of silkworm (in Japanese); Rep. Res. Minist. Educ. Agr. (III), 147-148.
- KAWASE, S. (1960) Electron microscopical observation in the integument of silkworm (in Japanese); J. Sericult. Sci., 29(3):284.
- KOBAYASHI, M. (1951a) Cyto-histological studies on the dermal gland of the domestic silkworm (I) (in Japanese); J. Sericult. Sci., 20(4):226-231.
- KOBAYASHI, M. (1951b) Cyto-histological studies on the dermal gland of the domestic silkworm (II) (in Japanese); J. Sericult. Sci., 20(5):351-355.
- KOBAYASHI, M. (1955) The relationship between the secretion from the dermal glands and the surface colour of the body of the newly moulted silkworm larva, *Bombyx mori* L. (in Japanese); J. Sericult. Sci., 24(5-6):383-388.
- KUWANA, Z. (1932) Distribution of papilla on the surface of the larval integument in the silkworm (in Japanese); J. Sericult. Sci., 3(2):189.
- MORI, S. (1965) The electron microscopical studies on the fine structure of the integument in the silkworm, *Bombyx mori* L. (in Japanese); Kontyu, 33(4):385-426.
- SHIMIZU, S. (1932) Origin of crystal substances on the surface of integument in the newly exuviated silkworm (in Japanese); Bull. Sericul. Exp. Stat., 8:103-108.
- TAKAHASHI, Y. (1955) Histological and histochemical observation on the larval cuticle of silkworm (in Japanese); J. Appl. Zool., 20(4):199-202.
- TAKAHASHI, Y. (1956a) Studies on the cuticle of the silkworm, Bombyx mori L. II. Histological and histochemical observations on the larval cuticles of sclerite and intersegmental membrane (in Japanese); J. Appl. Zool., 21(1):9-14.
- TAKAHASHI, Y. (1956b) Studies on the cuticle of the silkworm, Bombyx mori L. III. Comparative observations in the larval cuticles in some silkworm strains; J. Appl. Zool., 21(2):50-52.
- TAKIZAWA, T. and N. KOYAMA (1969) Change of photoreflection following with development of the silkworm egg, *Bombyx mori* L. I. Reflection properties from the egg under different photic conditions; J. Facul. Text. Sci. & Technol. Shinshu

Univ., No. 51, Ser. A, 14:53-68.

- TAKIZAWA, T. and N. KOYAMA (1970) Change of photoreflection following with development of the silkworm egg, *Bombyx mori* L. II. Reflection properties from the egg under different thermal conditions; Ibid., No. 53, Ser. A, 15:17-25.
- TAKIZAWA, T. and N. KOYAMA (1972) studies on the reflection property of light in the silkworm egg, *Bombyx mori* L.; Ibid., No. 58, Ser. A, 16:1-11.
- TANAKA, K. (1970a) Studies on the structure on the integuments of Bombyx mori L. and several other kind of the Lepidoptera. I. On the surface structure of the larval integuments of the silkworm (in Japanese); J. Sericult. Sci., 39(2):94-100.
- TANAKA, K. (1970b) Studies on the structure of the integuments of *Bombyx mori* L. and several other kind of the Lepidoptera. II. On the histological structure of the cuticle of silkworm larva (in Japanese); Ibid., 39(4):231-238.
- TANAKA, K. (1971a) Studies on the structure of the integument of Bombyx mori L. and several other species of Lepidoptera. V. Comparison of the size and number of the hypodermic cells among the different silkworm races, and between the P₁worm and F₁-hybrid of silkworm pupa (in Japanese); Ibid., 40(2):69-73.
- TANAKA, K. (1971b) Studies on the structure of the integuments of *Bombyx mori* L. and several other species of Lepidoptera. VI. Investigation on the increasing feature of the surface area of the body following with the larval growth of the silkworm (in Japanese); Ibid., 40(3):231-235.

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