CHANGE OF PHOTOREFLECTION FOLLOWING WITH DEVELOPMENT OF THE SILKWORM EGG, *BOMBYX MORI* L.

2. REFLECTION PROPERTIES FROM THE EGG UNDER DIFFERENT THERMAL CONDITIONS

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INTRODUCTION

It is well known that coloration of the overwintered egg of the silkworm (*Bombyx mori* L.), when the egg incubated, becomes gradually darker and after bluish just before hatching. This is caused mainly by the migration of pigments occurring in the serosal cells. According to WATANABE (1919), the pigment migration is accomplished faster in high temperature incubation than in low. Further the migration speed is recognized to be more hastened in light condition than in dark condition (NAGASHIMA 1956). In these investigations, however, a changing rate of the egg coloration was identified only by superficial observation of naked eyes.

In our previous study (TAKIZAWA & KOYAMA 1969), it was ascertained that a gradient of the egg coloration following with the embryonic development could be revealed coincidently by the photoreflection property from the egg. Then in the present study the authors tried to measure photophysically the color change in the hibernated eggs under different thermal incubations by means of the photoreflection meters.

MATERIALS AND METHODS

The eggs of a hybrid, *Nichi-131×Shi-131* were used for the materials.

The eggs were taken out from a chilling room and moved to the incubation condition, which was kept constantly at 20°C and 30°C temperature with 80 % R. H. Further each thermal condition was divided into two photic regimes such as constant light (24L) and constant darkness (24D), respectively.

The photoreflection intensity from the egg was measured by Three Dimentional Goniophotometer (GP-meter) and Automatic Micro-luster Meter (MLm-eter) as reported in the previous paper (TAKIZAWA & KOYAMA, 1969). Simultaneously the eggs in each embryonic stage were fixed with formalin-alcohol solution for observation of the serosal pigment migration and the growth state.

I. REFLECTION INTENSITY IN 24L

1. Measurement by GP-meter

Fig. 1 shows I-θ curves at $\phi_r=45°$ which was obtained by the photoreflection intensity from the egg mass incubated under 20°C and 30°C from the initial day to just hatching.

In 20°C incubation it takes 16 days from the beginning of incubation to hatching. The reflection intensity is 1.80 in the first day, later decreases gradually reaching the minimum value of 1.25 in the 6th day, and thereafter it is raised day by day. At the termination of the embryonic development (16th day) the intensity becomes 2.20. The light reflection from the chorion-only shows a value of 4.10.

In 30°C incubation it takes 8 days for the embryonic growth, which is shorter 8 days than in 20°C incubation. The changing phase of the reflection intensity takes almost the same tendency as in 20°C. The minimum value (1.50), however, appears in the 5th day.

From anatomical observation of the egg, it is recognized that the serosal pigments complete their migration when the reflection intensity falls to the least value either in 20°C inbubation or in 30°C incubation. This time is also coincided with the blastokinesis stage of embryo.

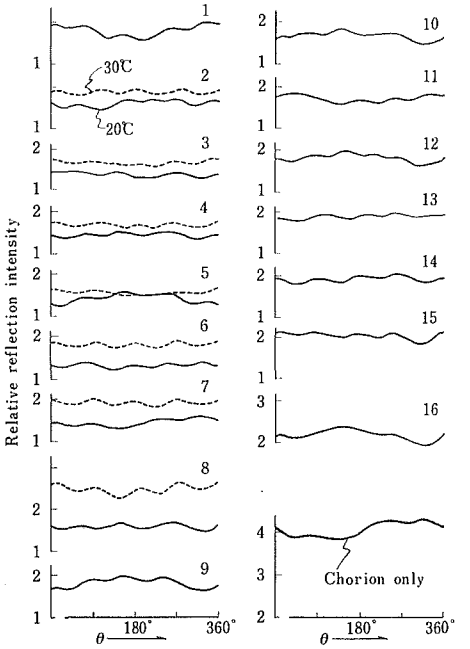

Fig. 1 I-θ curve at $\phi_r = 45°$ in different thermal incubations (24L). The numbers at the right hand show the incubated days. The same expression is taken in the following figures.

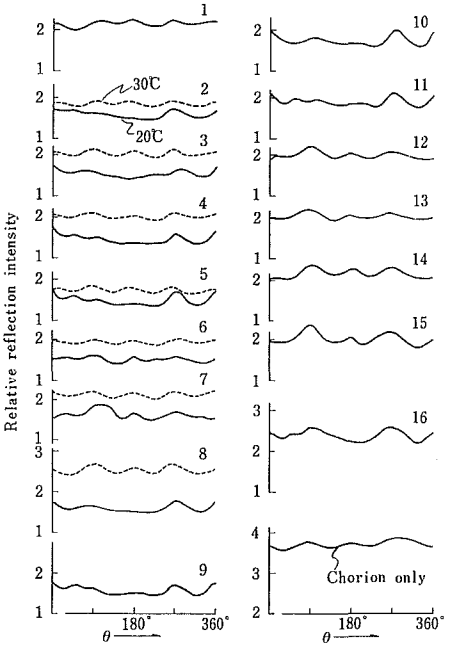

Fig. 2 I-θ curve at $\phi_r=90°$ in different thermal incubations (24L).

In each I-θ curve three rhythmical peaks with 90° intervals can fairly be detected as reported in the previous paper. Compared 30°C condition with 20°C one, the reflection intensity is significantly higher in the former throughout the growth stage.

Fig. 2 denotes I-θ curves at $\phi_r=90°$, which resemble very much those at $\phi_r=45°$, excepting that each intensity value is slightly lower in 45° than in 90°.

2. Measurement by ML-meter

The changing phase of the reflection intensity measured by ML-meter is shown in Fig. 3.

In 20°C incubation duration of the embryonic stage is 16 days as same as in the above experiment. The intensity curve is fluctuated with a trough at about the middle part. The reflection intensity, however, tends to decrease until the 6th day and after increases gradually just before hatching.

In 30°C incubation it takes 8 days for completion of the embryonic development, which is 8 days shorter than in 20°C incubation. The manifestation of the intensity curve is closely similar to that of the former, whereas the

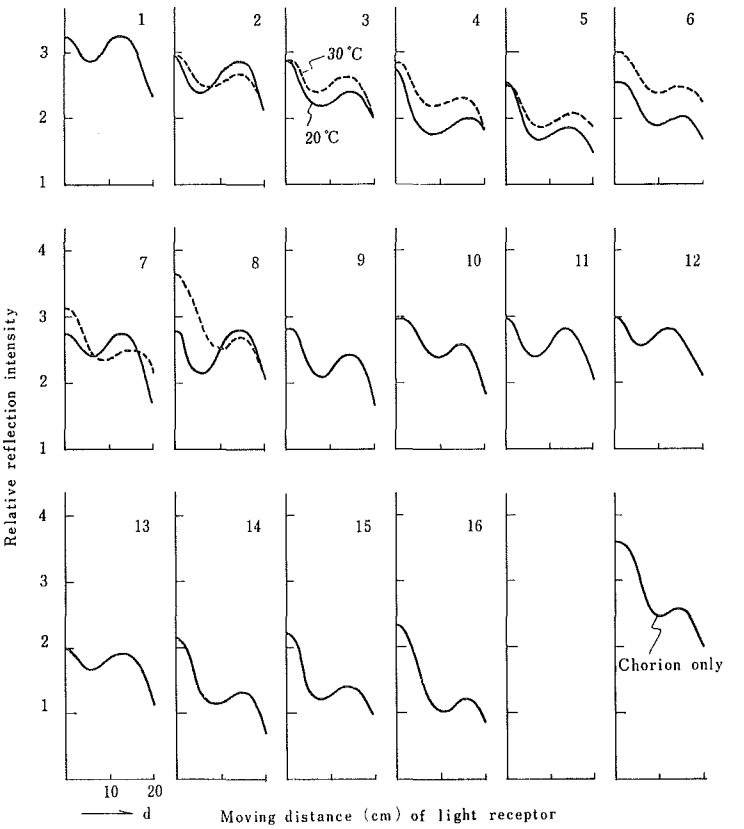

Fig. 3 I-θ curve at $\phi_r=45°$ in different thermal incubations (24L).

lowest value of the intensity appears in the 5th day and each value of intensity is a little larger than in 20°C condition.

Accordingly the changing phase of the reflection intensity by ML-meter is stated to occur in the same manner as by GP-meter. As described in the previous paper, the form of the intensity curve indicates well that of the egg surface, in which a dimpled part is expressed as a depressed part of I-θ curve.

II. REFLECTION INTENSITY IN 24D

1. Measurement by GP-meter

Fig. 4 shows the changing feature of the reflection intensity measured by GP-meter at $\phi_r=45°$.

In 20°C incubation it takes 17 days for completion of the embryonic stage.

The change of the reflection intensity occurs almost in the same feature as in 24L, though the minimum value (1.30) appears in the 7th day being later one day than in 24L.

In 30°C incubation the embryonic development terminates at the 9th day.

The changing phase of the reflection intensity is closely related to that of 24L, but the intensity value falls to the smallest in the 6th day. It is delayed one day than in 24L.

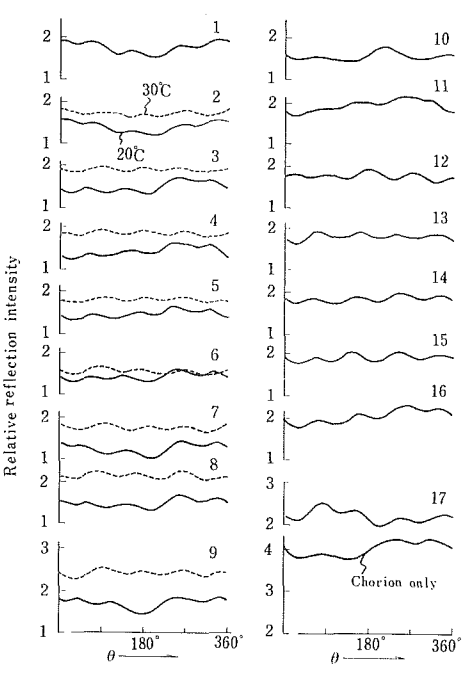

Fig. 4 I-θ curve at $\phi_r=45°$ in different thermal incubations (24D).

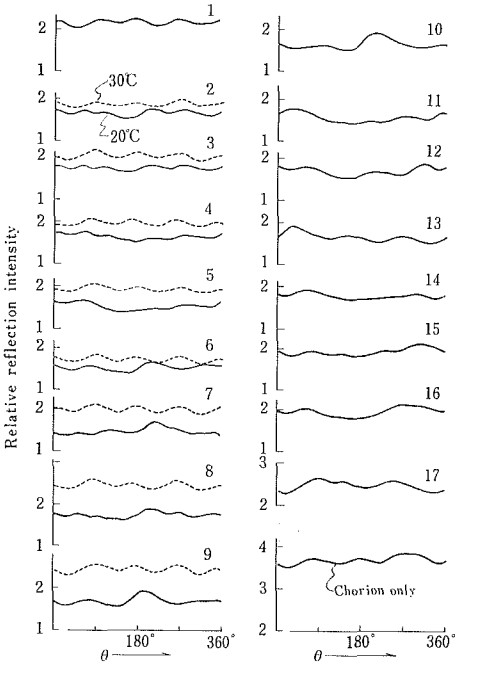

Fig. 5 I-θ curve at $\phi_r=90°$ in different thermal incubations (24D).

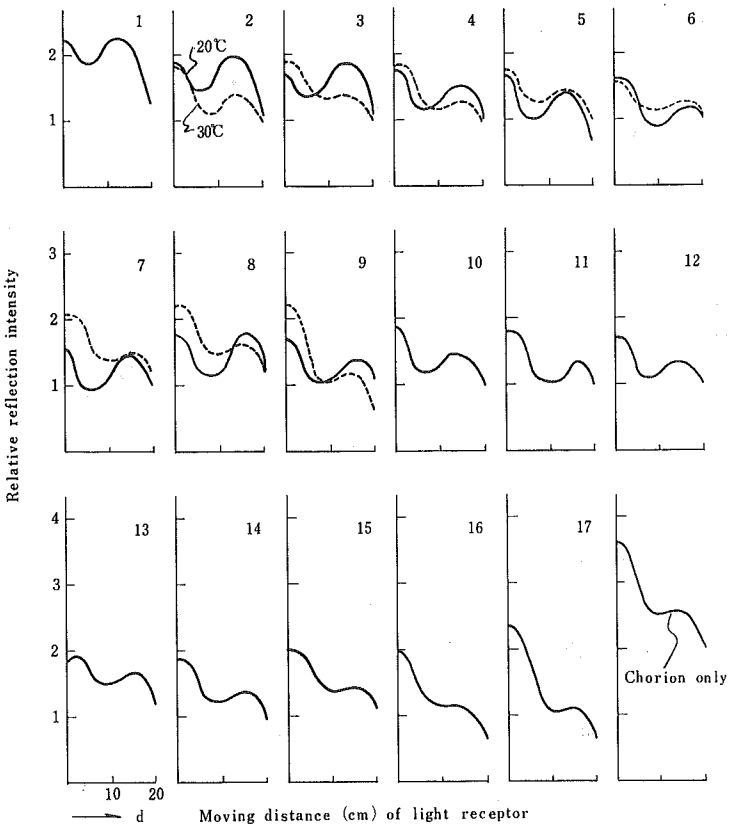

Fig. 6 *I-θ* curve at $\phi_r=45°$ in different thermal incubations (24D).

As shown in Fig. 5, the same tendency as above in the reflection intensity is also recognizable when the intensity measured from another direction that is $\phi_r=90°$, in whcih each reflection intensity is slightly higher than in $\phi_r=45°$.

2. Measurement by ML-meter

The changing feature of the reflection intensity measured by ML-meter is illustrated in Fig. 6.

The growth duration of the embryo is the same as in the above experiment.

In 20°C incubation the reflection intensity falls to the least in the 7th day, while in 30°C incubation the smallest value appears in the 6th day. In 24L the dual intensity curves of 20°C and 30°C run approximately parallel to each other until the 6th day, but in this case they trace different ways especially during the first few days. The reason has not been clear. It might be

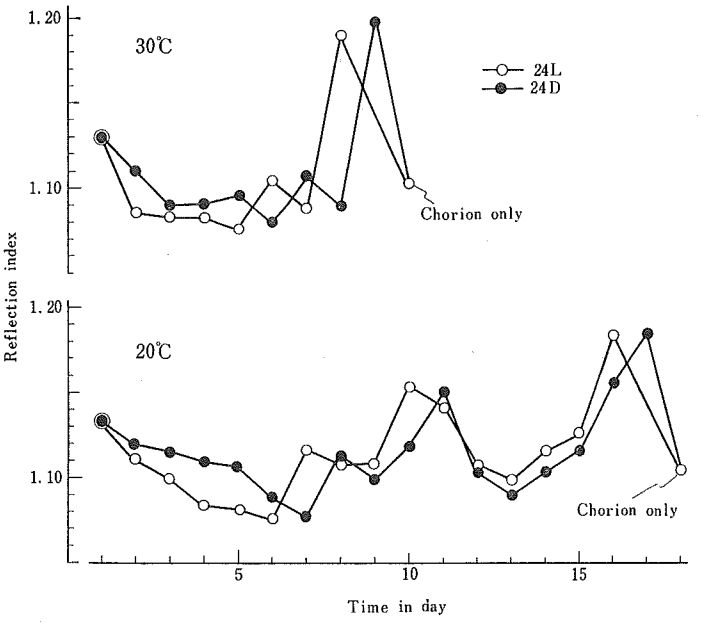

Fig. 7 Change of reflection index at $\phi_r=45°$ (GP-meter)

due to the formal difference in the individual egg.

III. REFLECTION INDEX

1. Measurement by GP-meter

The change of the reflection index calculated from I-θ curve at $\phi_r=45°$ is shown in Fig. 7.

In 20°C incubation the index value is 1.13 in the first day, later gradually declines and at last reaches the minimum value, which occurs at the 6th day in 24L (1.08) and at the 7th in 24D (1.07). Till this time the serosal pigments complete their migration. At the succeeding stage two peaks of the index values are detectable. The first peak (10~11th day) comes out when most of the serosa disappears and the second peak (16~17th day) when the embryonic body is darkly pigmented. A trough seen between the dual peaks corresponds to the stage when the embryonic head becomes darker by pigmentation.

In 30°C incubation, the index curve resembles to some extent that in 20°C incubation. The former's first peak, however, is not so evident.

Fig. 8 shows the changing phase of the reflection index at $\phi_r=90°$. The form of the index curve bears a close similarity to that at $\phi_r=45°$, only excepting that the ascendant curve from the 12th day to the 16th day is different to each other.

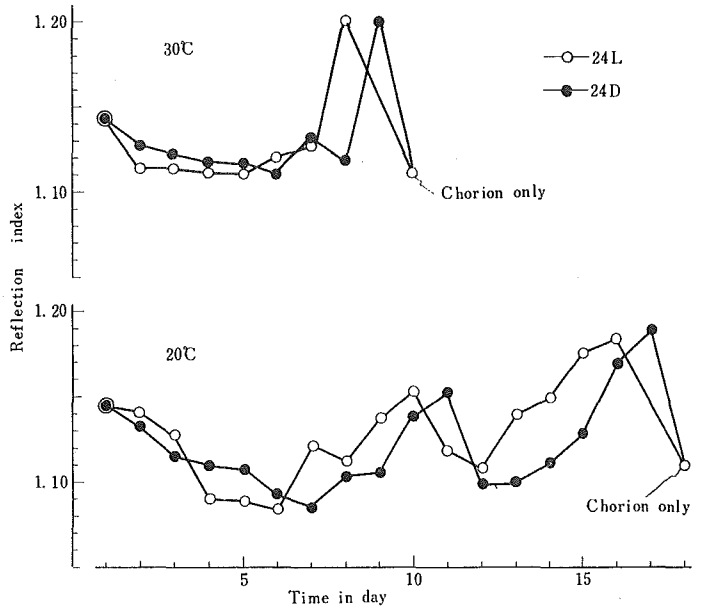

Fig. 8 Change of reflection index at $\phi_r=90°$ (GP-meter).

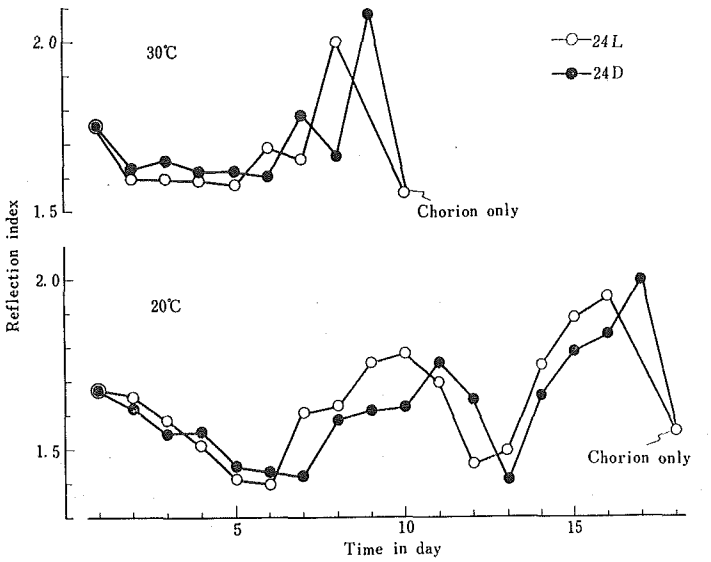

Fig. 9 Change of reflection index at $\phi_r=45°$ (ML-meter).

2. Measurement by ML-meter

Fig. 9 denotes the changing phase of the reflection index at $\phi_r=45°$. The results are almost the same as by GP-meter excepting that each index value is much larger in ML-meter than in GP-meter.

As mentioned above, the changing feature of the reflection index following with the embryonic growth appears to be quite similar between GP-meter and ML-meter, though each index change occurs about one day faster in 24L than in 24D caused by the difference in growth speed of the egg.

CONSIDERATION

The migration speed of serosal pigment in the silkworm egg differs according to incubation condition. WATANABE (1919) reported that it was faster in high temperature than in low, and NAGASHIMA (1956) proved it was hastened in illumination than in darkness.

In these studies, however, a rate of the pigment migration was estimated by naked eyes' observation. As mentioned above, the migration phase was recognized to be catchable quantitatively by the photoreflection property from the egg.

Further the embryonic stage and the egg form were adequately indicated by the changing feature of the photoreflection.

The reflection intensity measured by GP-meter was always higher in 30°C incubation than in 20°C incubation. Especially the intensity value just before hatching when the embryonic body pigmented was larger 0.2~0.3 in 30°C incubation than in 20°C incubation. The fact seems likely based on the coloration difference in the embryonic body, and is almost agreed with the results of several reports, in which newly hatched silkworm larvae take paler color in high thermal incubation than in low (WATANABE 1918, MATSUMURA 1928, NAGASHIMA 1956).

In the reflection index, the first peak which appeared correspondingly to the disappearance stage of the serosa in 20°C incubation was not so evident in 30°C incubation especially of 24L. It might be caused by a great shortening of the growth duration, in which the peak could hardly be catched synchronizingly by one day interval measurement.

SUMMARY

Changes of the photoreflection following with development of the hibernated egg of the silkworm (*Bombyx mori* L.) were measured by Three Dimentional Goniophotometer (GP-meter) and Automatic Micro-luster Meter (ML-meter) under 20°C and 30°C incubations. The results obtained are summarized as follows.

1. It took 16 days and 8 days from the initial day of incubation to just hatching in 20°C condition (24L) and in 30°C condition (24L), respectively. The egg growth in each thermal regime was faster one day in 24L than in 24D.

2. The reflection intensity decreased gradually day by day, at last reaching the minimum value and after increased until hatching. However, each

intensity value by GP meter was clearly higher in 30°C incubation than in 20°C incubation.

3. Anatomical observation of the egg showed that the period when the reflection intensity fell to the least value corresponded to the completion of the serosal pigment migration and to the blastokinesis stage of embryo.

4. In the same thermal incubation the least value of the reflection intensity appeared one day faster in 24L than in 24D. This fact also indicated the change of the reflection intensity occurred in parallel to the embryonic development.

5. Three rhythmical peaks were fairly detectable in I-θ curve by GP-meter. They were related to existence of a dimpled part in the egg surface, but it was more revealable by ML-meter.

6. The reflection index in 20°C incubation declined gradually till the 6～7th day of incubation and subsequently showed two peak values. The first peak and the second peak corresponded to the disappearance stage of the serosa and the pigmentation stage of the embryonic body, respectively. A trough between the dual peaks was just the time when the embryonic head was pigmented darkly.

In 30°C incubation, the changing phase of the reflection index resembled to some extent that in 20°C incubation. The above first peak, however, was not so clear to see. This might be caused by a great shortening of the growth duration, in which the peak could hardly be catched synchronizingly by one day interval measurement.

7. As described above, it was proved that the reflection intensity and index indicated well not only the change of the egg coloration and the egg shape but also the growth stages of embryo.

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