RELATIONSHIP BETWEEN REFLECTION INTENSITY AND PHOTOTAXIS IN NEWLY HATCHED LARVAE OF THE SILKWORM, *BOMBYX MORI* L. (LEPIDOPTERA; BOMBYCIDAE)

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INTRODUCTION

It is well known that newly hatched larvae of the silkworms have a strong phototactic behaviour. YAGI (1923) studied firstly such a response and reported that the silkworm larvae gather at yellowish color. Hereafter a number of investigations have been carried out on this problem (YAMAGISHI, 1926; KOIZUMI, 1950, 1951, 1961; KOIZUMI and HASHIZUME, 1964, 1966; KOIZUMI *et al.*, 1966, 1967; MIYAGAWA, 1951, 1952; MIYAGAWA and SATO, 1953, 1954; YAGI *et al.*, 1959; KOYAMA *et al.*, 1960; Iguchi and KOZAKAI, 1966 etc.).

In the silkworm, phototactic reaction is generally strongest in newly hatched larvae, later becomes weaker, and again becomes somewhat stronger in mature stage.

Such a photo-responsibility seems to be closely related to the coloration of ocelli and body pigmentation as pointed out by MIYAGAWA and SATO (1953) and KOYAMA *et al.* (1960).

The phototaxis is considered that light stimulation acts on the animal behaviour through photoreceptive organ viz. ocelli in the silkworm larvae. The coloration of ocelli therefore will has a close relation to phototactic response of the larvae. Further a possible relationship may exist between dermal pigmentation and phototaxis. Then, the authors pursued the relationship between photoreflection and phototaxis in newly hatched larvae of the silkworm using several strains with different body colorations.

In preparing this presentation, the authors are deeply indebted to Assist. Prof. K. OSHIGANE, Shinshu University, for his helpful support.

MATERIALS AND METHODS

Nichi-1, *Shi-108*, P^s and *w-1* were used for the materials.

The newly hatched but unfed larvae were pasted on black paper just after hatching, and the photoreflection was measured by ML-meter as reported in the previous papers (TAKIZAWA & KOYAMA, 1969, 1970, 1972; TAKIZAWA, 1974).

In the same time, the newly hatched larvae were confined at the temperature of 25±0.5°C and the relative humidity of 75±5% in dark room. After two hours the phototactic response to horizontal light was researched under the room conditions as mentioned above.

The number of the material larvae was 100 in each regime. The light (100V-150W, Toshiba) casted at the larvae was 20, 30, 50, 70, 100, 150, 200, 300, 400 and 500 lux, respectively.

All data obtained by counting the number of phototactic response in each regime after one hour (KOYAMA et al., 1960), and analysed by using the multivariate analysis of variance (KRAMER and JENSEN, 1970; MORRISON, 1982). Further we applied the simultaneous confidence intervals by Roy's method (ROY and BOSE, 1953) to determine which each datum was significantly different.

RESULTS

a) *Phototaxis in different strains*

As illustrated in Fig. 1, the larvae of *Nichi-1* showed positive phototaxis at lower light intensity of 20 lux, in which the number of individuals (44.5±2.47) revealed significant difference between that of other pooled regimes (7.15±1.37) in the same response ($p<0.01$). The larvae were, however, changed markedly to negative phototaxis at more than 70 lux (61.0±7.8), reaching the maximum value (70.0±4.06) at higher light intensity of 200 lux. Therefore, the value of the former were not only significantly different from that of the latter ($p<0.01$), but also from each value of other regimes in both the phototaxis ($p<0.01$), respectively.

As a whole, comparisons of both the total means for two different responses were confirmed that the individuals of negative response were significantly larger than those of positive one ($p<0.01$); the larvae in this strain seem to be negatively responed under intensified light intensities.

Most of the larvae in *Shi-108* appeared typically positive behaviour in all regimes of light intensities (Fig. 2). The larval behaviour towards light showed a tendency to grow in number. In particular, each number of both

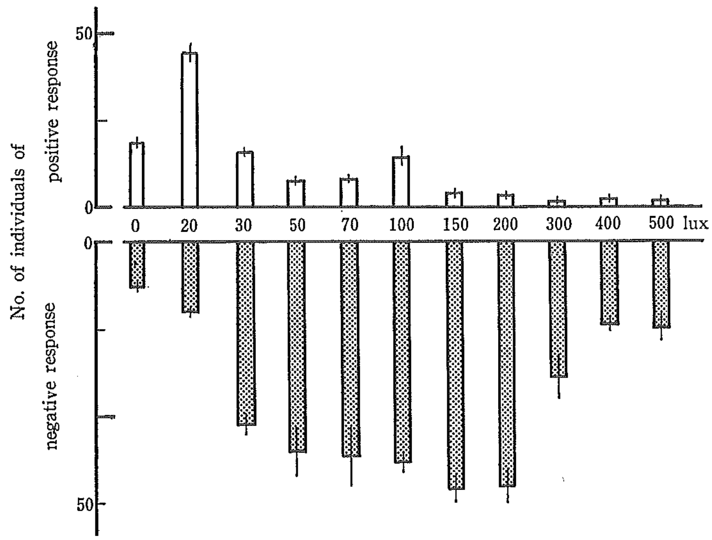

Fig. 1 Phototactic response in newly hatched larvae of *Nichi-1*. The larvae show positive phototxis at lower light intensity of 20 lux, but change to negative at more than 30 lux; the tendency is negatively responsible at higher light intensity. Each column represents a mean value (±SE) of four observations (i. e. in each regime of light intensity 100 animals are used for one observation). Open columns: positive response, stippled columns: negative one.

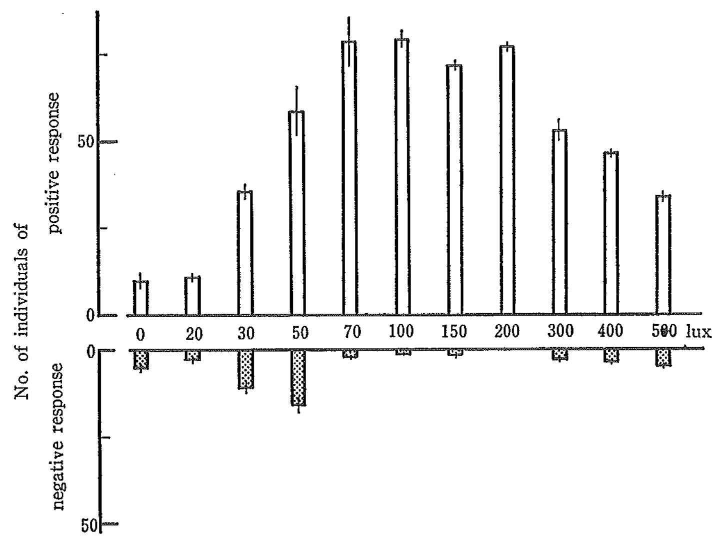

Fig. 2 Phototactic response in newly hatched larvae of *Shi-108*. Most of the larvae are clearly photopositive; especially number of individuals for responding at each regime of 70-200 luxes is the largest of all regimes. Other details the same as in Fig. 1.

the individuals at 70 lux (78.8±7.95) and at 100 lux (78.8±3.12) was the largest of all other regimes, but these values could not be declared significantly to be different from each of both 150 (71.8±1.70) and 200 (77.5±2.47) luxes (p>0.05), respectively. After these regimes, following with an increment of light intensities the reacted individuals toward light decreased, so that the mean value (34.0±1.65) at 500 lux was significantly less than each of other regimes at higher light intensities (p<0.01).

When all data for *Shi-108* and *Nichi-1* were pooled, the trend for the former was significantly different from one of the latter (χ^2=31.047, df=2, p<0.001). The larval behaviour of *Shi-108* which is positively phototactic, therefore, can be suggested to reverse to the case of *Nichi-1*.

In p^s strain, many larvae had been exposed to each light intensity appeared positive response, where a few larvae responded negatively toward a source of higher light intensities (Fig. 3). Moreover it was apparent that the number of positively responded larvae increasing the light intensities had became relatively large at 100, 150 and 200 luxes, in which one of 200 lux showed the maximum value of 77.8±3.35. Although no differences at 200 lux were detected either of 100 or 150 lux (p>0.05, respectively), each value of positive response in these regimes revealed significant differences between

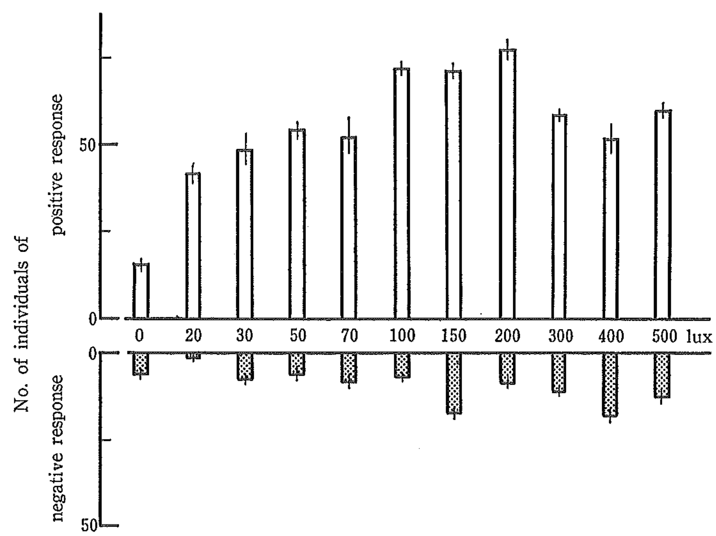

Fig. 3 Phototactic response in newly hatched larvae of p^s. The phototactic response shows positively strongest at higher light intensity. Such a tendency is similar to that of *Shi-108* (see Fig. 2); no difference is found significantly between the former and the latter (p<0.2). Other details the same as in Figs. 1 and 2.

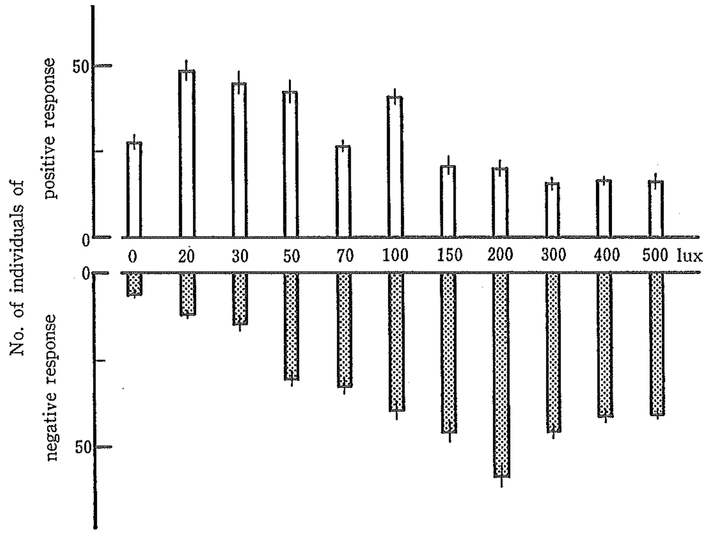

Fig. 4 Phototactic response in newly hatched larvae of *w-1*. The larvae tend to be negatively responsible to higher light intensity even though these appear positive response at a regime of lower light intensity. Therefore the trend responding to light intensity bears a striking resemblance to that of *Nichi-1* (see Fig. 1); there is no significant difference in the pattern of larval response between *w-1* and *Nichi-1* ($p<0.1$). Other details the same as in Figs. 1, 2 and 3.

those of negative one ($p<0.01$). Such a tendency is similar to that in *Shi-108* as shown in Fig. 2.

On the other hand, by comparing all values in both p^s and *Shi-108* with those in *Nichi-1*, the former two strains could be declared significantly to be different from the latter ($\chi^2=27.203$, $df=2$, $p<0.001$). It should be pointed out, therefore, that the trend for positive phototaxis in p^s and *Shi-108* were more intense than that of *Nichi-1*.

As shown in Fig. 4, the larvae in *w-1* appeared more positive response than negative one at lower light intensities ($p<0.01$). In particular, the former mean value (48.0 ± 2.38) at 20 lux was significantly different from that (12.5 ± 0.87) of the latter ($p<0.01$), whereas no differences were between each of the values in both 70 and 100 luxes. However, thses larvae responding to positive but not to negative exhibited adversely negative response under higher light intensities more than 200 lux. In these regimes the larval behaviour at 200 lux was more negative than positive ($p<0.01$); the response values were 59.3 ± 3.09 for negative and 15.5 ± 2.105 for positive, respectively. The former therefore was significantly different from each value in each regime of 300, 400 and 500 luxes ($p<0.01$), respectively. Such a tendency bears a striking resemblance to that of *Nichi-1* as indicated previously; there was no significant difference in the pattern of larval response between *w-1* and *Nichi-1* when all data for each strain were combined ($\chi^2=5.261$, $df=2$, $p<0.1$).

As seen in the above, it is recognized that the phototaxis varies according to strain; *Nichi-1* and *w-1* are positively responsible at lower light intensity and these larvae reverse to negtive at higher light intensity. On the other hand, it is evident that most of larvae in both *Shi-108* and p^s were intensively positive at all regimes of light intensity.

b) *Phototaxis and reflection intensity of light in different strains*

As mentioned above, it has been ascertained that the phototaxis differs remarkably according to strain. Then the number of the phototactic individuals responding at each regime (each of 100, 200 and 300 luxes) of light intensity in Figs. 1-4 was chosen and compared with reflection intensity of light in the newly hatched larvae measured by ML-meter.

As shown in Fig. 5, most of p^s (0.48 ± 0.216, $n=10$) and *Shi-108* (1.05 ± 0.313, $n=10$) with lower reflection intensity appeared a small number of

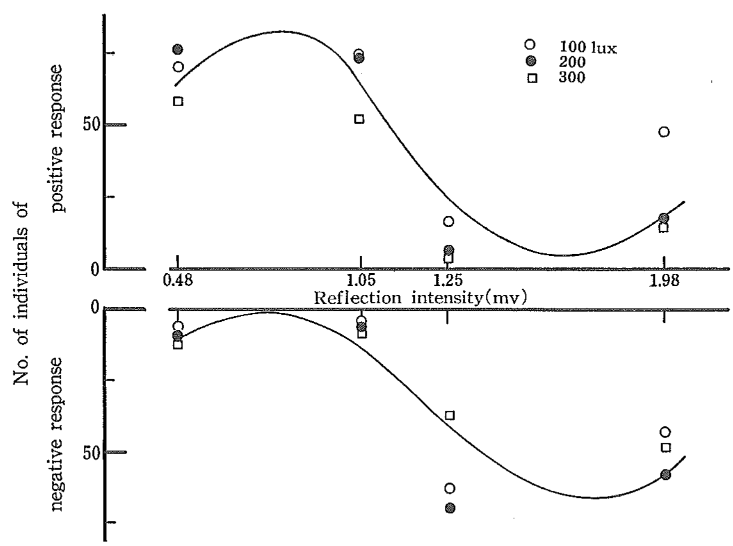

Fig. 5 Relationship between phototactic response within 100-300 luxes and reflection intensity on larval body in each strain. The number of the phototactic individuals is significantly correlated with reflection intensity in both the two responses; in positive response : $R^2=0.844$, $n=48$, $p<0.001$; in negative one: $R^2=0.87$, $n=48$, $p<0.001$, respectively.

negative individuals, while that of negative ones was greater in the larvae with higher photoreflection intensity such as *w-1* (1.98 ± 0.412, n=10). Similarly, in both the cases of 200 and 300 luxes, the phototactic preference of each strain was displaied with photoreflection intensity in the same as described previously.

Consequently the relationship between the numbers of phototactic individuals and the reflection intensity of light in the newly hatched larvae was declared significantly in both positive response ($R^2=0.844$, $p<0.001$) and negative one ($R^2=0.87$, $p<0.001$) i. e. the larger the photoreflection intensity, the smaller the numbers of positive individuals.

CONSIDERATION

In general it is known that the newly hatched larvae of silkworms show the phototaxis including positive response as well as negative one when these larvae are exposed under light intensities, and these larvae may sometimes orientate themselves inversely to sources of light (KOYAMA *et al.*, 1960). The phenomenon of the latter thus was also recognized in each behaviour both in *Nichi-1* and in *w-1* by our present experiment. According to MIYAGAWA and SATO (1953), it has been described that the phototaxis is different by the ocellus color of newly hatched larvae; larvae with red eye tend to be strongly positive to light intensities to which the insects are exposed, but not so in the case of larvae with white eye.

In this experiment, *w-1* bearing the largest reflection intensity of light showed positive response at lower light intensity, and negative one at higher light intensity. Such as *w-1* larvae, their ocellus coloration is whitish, so that their negative behaviour seems somewhat to be large. As a whole, however, the phototaxis of *w-1* was so relatively much positive at lower light intensity than that of p^s and *Shi-108* as to be not large. It would be noted, therefore, that light intensity might well be effective to estimate the significant trends of phototactic behaviour in the newly hatched but unfed larvae.

Conclusively the reflection intensity of light on the body surface has an inverse relationship with the phototactic response, viz. the larger the former, the smaller the latter. The fact seems to be coincident with the phenomenon suggested by MIYAGAWA and SATO (1953), in which the stronger phototaxis is seen in darker body color. The body pigments in the newly hatched larvae, therefore, cannot be overlooked as an important problem on phototaxis.

SUMMARY

In the present paper, an account is given of the results on the relationship between the phototaxis and photoreflection in newly hatched but unfed larvae of the silkworm, *Bombyx mori* L. The results obtained are summarized as in the followings.

1. The phototactic behaviour was shown to be different according to strain. In *Nichi-1*, the larvae responding to negative appeared at 30 lux and became largest at 200 lux. On the contrary, most of the larvae in *Shi-108* displaied typically positive orientation in all regimes of light intensity, where the largest number of individuals was produced at both 70 and 100 luxes, but in p^s the maximum value was at 200 lux, respectively. The phototaxis in *w-1* was negative at higher light intensity even though it was somewhat positive at one regime of lower light intensity.

2. The newly hatched larvae having lower reflection intensity (ex. p^s, *Shi-108*) showed almost photopositive response at each regime of higher light intensity, whereas the larvae bearing higher reflection intensity (ex. *Nichi-1*, *w-1*) tended to be inversely proportional to the same intensity to which the former was exposed.

3. The reflection intensity obtained from the larval body surface was adversely related to phototactic orientation. The fact means that the darker the body coloration of newly hatched larvae is, the stronger the positive phototaxis becomes.

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