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Role of Photoperiod in Larval Growth of *Sasakia charonda* (Lepidoptera, Nymphalidae)

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Abstract Larval growth in *Sasakia charonda* was photoperiod dependent, and the magnitude and direction of the response was modified by developmental ages, body size, larval morph and experience of hibernation. Especially, the reversal of response occurred between 3rd and 4th instars; growth retardation by long days being seen at 3rd instar whereas that by short days at 4th instar. Also, larval response to an intermediate photoperiod (LD 14: 10 hr) was unique and delayed growth continued throughout these two instars. Larvae (4th instar) which had experienced hibernation grew rapidly without regaining a sensitivity to photoperiod. Finally, the threshold size at which larvae proceed to metamorphose was examined and its relation to the instar number determination in this species was discussed.

Introduction

Sasakia charonda is a univoltine nymphalid butterfly with larval hibernal diapause. This diapause, which is associated with changes in larval morph and color, occurs usually at 4th instar. After hibernation, larvae undergo two successive molts to pupation. KATO and HASEGAWA (1984) reported that the larval diapause is induced by short days and averted by long days, but growth at the previous instar is accelerated under short days and retarded under long days unlike the case of a related bivoltine *Hestina japonica* (SHIOTSU, 1977). This suggests that in *S. charonda* reversal of photoperiodic response might occur during development.

Life cycle of univoltine insects is under photoperiodic regulation, and the regulation is complicated in contrast to that in multivoltine insects (BECK, 1977; TAUBER *et al.*, 1986). In most cases, photoperiodic changes from short-day to long-day condition or *vice versa* are required for the completion or arrest of development and reproduction. However, it is poorly understood how the insects switch a response to daylength although two-step photoperiodic process has been shown (LUTZ & JENNER, 1964; FERENZ, 1977).

The purpose of this paper is to describe in detail larval growth under various photoperiodic conditions and to elucidate changes in photoperiodic response of *S. charonda*.

Materials and Methods

Animals

Eggs of *S. charonda* were obtained from female adults caught in Yamanashi Prefecture, central part of Japan in July and August. Larvae were kept individually in plastic cups (φ9 cm × 5 cm) from hatching or early time of larval life at 25°C. Also, hibernating larvae (4th instar) were collected in the field in March and provided for photoperiodic treatments. *Celtis* leaves were given as food.

Photoperiodic treatments

First, larvae were placed under 3 different photoperiods (LD 12: 12, 14: 10 and 16: 8 hr) until pupation. Second, larvae were transferred from LD 16: 8 to LD 12: 12 hr or *vice versa* at 3rd and 4th instars. Third, post-diapause larvae were subjected to 3 photoperiods described above when they began to resume development in outdoor conditions.

Assessment of larval growth

Body weight, instar duration and growth rate (gain of body weight per day) were examined at each instar. Head-capsule size was also measured with a calibrator if necessary.

Results

Development at 3 different photoperiods

The results are summarised in Fig. 1. At LD 14: 10 and 16: 8 hr, nearly all larvae underwent 5 successive larval molts before pupation except for individuals kept in LD 16: 8 hr growing quickly without any retardation and then pupating after 4 larval molts. On the other hand, at LD 12: 12 hr all larvae entered diapause following slowed growth at 4th instar. Different larval morphs appeared in 4th instar: At LD 12: 12 hr all larvae had short horns on the head (S-larvae) and at LD 16: 8 hr all ones had long horns (L-larvae). At LD 14: 10 hr, about two-thirds of the larvae were of short horn morph. S-larvae are programmed for hibernal diapause but L-larvae are not (KATO & HASEGAWA, 1984).

Photoperiodic effect on growth was strong in 3rd and 4th instars and weak in 2nd and 5th (penultimate) instars, no effect in 1st and 6th (last) instars. In 3rd instar, instar duration was long at LD 14: 10 or 16: 8 hr, whereas it was short at LD 12: 12. Body weight at the time of a subsequent ecdysis to 4th instar had little difference among 3 photoperiod regimens and growth rate was high at LD 12: 12 hr. Similar results for 3rd instar were obtained in additional experiments (Table 1). In 4th instar, by contrast, instar duration was short and growth rate was high at LD 16: 8 hr whereas at LD 12: 12 hr growth became gradually slow and then feeding cession and brown pigmentation occurring in about 40–60 days. At LD 14: 10 hr, prolongation of the instar duration and lower growth rate were seen, which were more conspicuous in S-larvae than in L-larvae. In 5th instar,

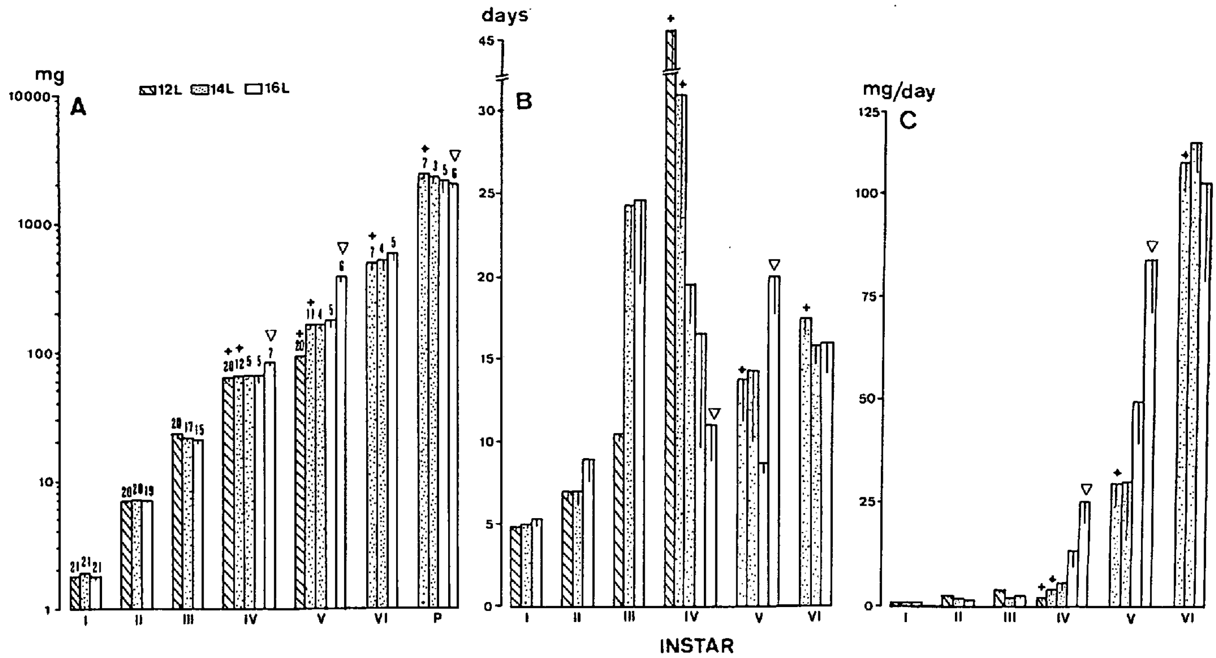

Fig. 1. Growth at each instar of *S. charonda* larvae under 3 different photoperiods. A: body weight on the day of hatching or ecdysis. B: instar duration. C: growth rate. Roman numerals and P indicate instar number and pupal stage, respectively. Arabic numerals above histograms are sample sizes. Cross marks: animals showing short horn morph at 4th instar. Open triangles: animals pupating precociously after 4 larval molts. Duration of 4th instar S-larvae programmed for diapause at LD 12: 12 is from the day of ecdysis to feeding cession. Vertical bars indicate S. D.

Table 1. Effect of three photoperiods on growth of 3rd-instar *S. charonda* larvae.

Photoperiod (LD)	No.	Instar duration (days)	Body weight at the following molt (mg)	Growth rate (mg/day)
12: 12	29	15.1±3.4	59.1± 6.7	2.7±1.7
14: 10	23	28.6±9.3	65.9±12.0	1.7±0.5
16: 8	21	33.4±9.1	55.9±15.6	1.1±0.9

Larvae were reared as a mass during 1st and 2nd instars before experiments. Values are mean±S. D.

growth retardation to a small extent was seen at LD 14: 10 hr.

Transfer experiments from long to short days or vice versa *in 3rd and 4th instars*

In 3rd instar, when the transfer from LD 16: 8 to 12: 12 hr was done on the day of an ecdysis, mean duration of the instar was short, and growth rate was high because body weight at the time of a subsequent ecdysis was large, compared to long-day control (Fig. 2 A). As the time of transfer was late, instar duration was long, and growth rate was decreased although body weight at the time of a following ecdysis increased to a small extent. Also, L-larvae (4th instar) appeared

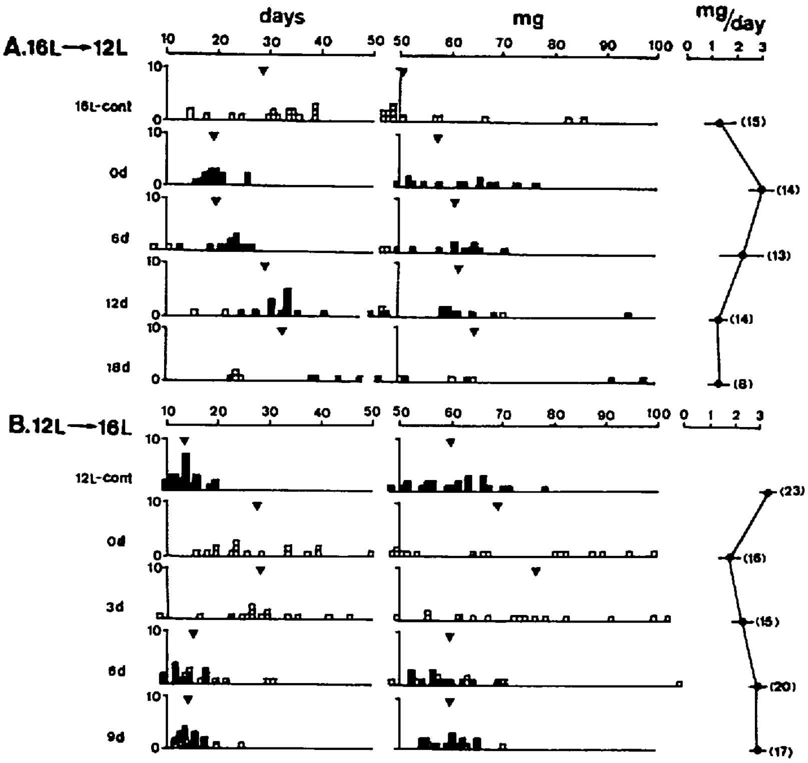

Fig. 2. Effect of photoperiodic transfers from long to short days (A) or *vice versa* (B) at different times of 3rd larval instar on growth in *S. charonda*. Left: duration of 3rd instar. Middle: body weight on the day of an ecdysis to 4th instar. Right: growth rate. Open histograms indicate the number of long horn morph larvae at the following ecdysis and closed ones that of short horn morph larvae. Closed triangles and circles indicate the means and the ones with S. D. (horizontal bar), respectively. Numerals in parentheses are sample size.

with delayed transfer. On the other hand, in the reverese transfer, when the time of transfer was early, instar duration was prolonged and the resulting larvae (4th instar) were relatively large, compared to short-day control (Fig. 2 B). But, due to large prolongation of the instar duration, growth rate became smaller. As the transfer was done on late days, instar duration was shortened and growth rate arose although body weight at a following instar was decreased. And the number of the resulting S-larvae increased with delayed transfer.

For the transfer experiments at 4th instar, S-larvae were used. When the transfer to LD 12: 12 hr was done on early days, larvae exhibited slowed growth and stopped feeding in about 50 days (Fig. 3 A). As the time of transfer was late, larvae was induced to undergo a subsequent molt without entering diapause. Most of them became of short horn morph again. On the other hand, for the transfer to LD 16: 8 hr, all larvae developed to a next instar without diapause and nearly all animals became L-larvae of 5th instar (Fig. 3 B). In this case, when the time of transfer was early, instar duration was shortened (about 26 days) and growth rate

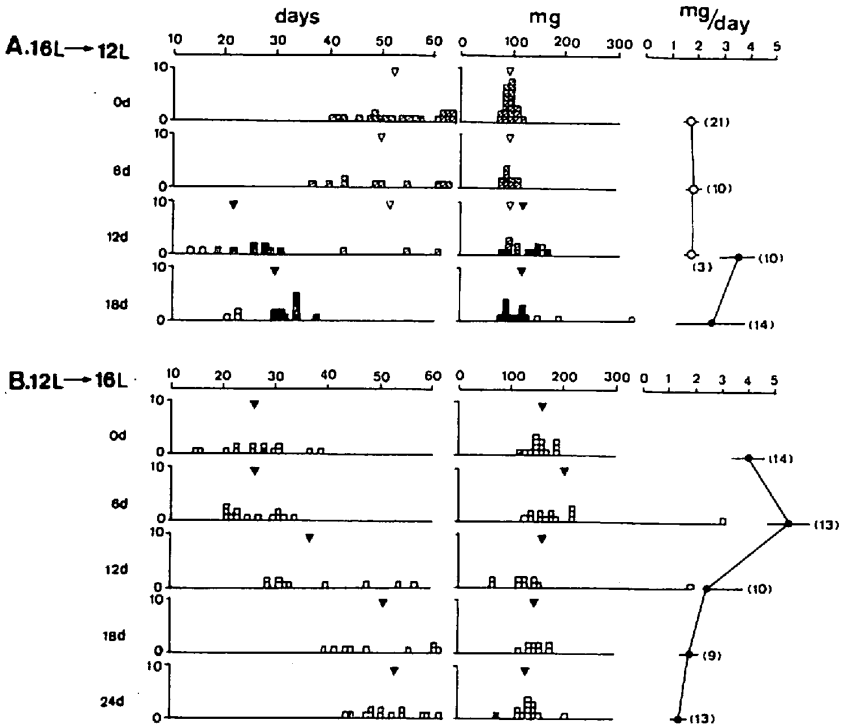

Fig. 3. Effect of photoperiodic transfers from long to short days (A) and *vice versa* (B) at different times of 4th larval instar on growth in *S. charonda*. Left: duration of 4th instar. Middle: body weight on the day of an ecdysis to 5th instar. Right: growth rate. Open histograms indicate the number of long horn morph larvae at the following ecdysis, closed ones that of short horn morph larvae and half-closed ones that of intermediate morph larvae. Histograms with a diagonal line indicate the number of larvae which ceased feeding without a molting. Closed or open triangles and closed or open circles indicate the means and the ones with S. D. (horizontal bar).

was high. As the transfer was late, instar duration was prolonged and the body weight at a subsequent ecdysis was decreased to a small extent with decreased growth rate.

Furthermore, development of 5th-instar animals obtained in each group was observed under LD 12: 12 and 16: 8 hr, which are the same photoperiod as after transfer. For the first group (LD 12: 12 hr), S-larvae (n=20) of 90–120 mg in body weight entered diapause with slowed growth and color change, and L-larvae (n=2) whose weight was 150–200 mg developed rapidly irrespective to short-day condition and then pupated after one more larval molt. On the other hand, for the second group (LD 16: 8 hr) all of L-larvae (n=51) showing 120–160 mg in body weight developed rapidly and pupated after one more larval molt and 2 L-larvae of large size (420–450 mg) pupated precociously at a subsequent molt.

Response of 4th-instar L-larvae to short and long days

The above experiments showed that S-larvae of 4th instar programmed for

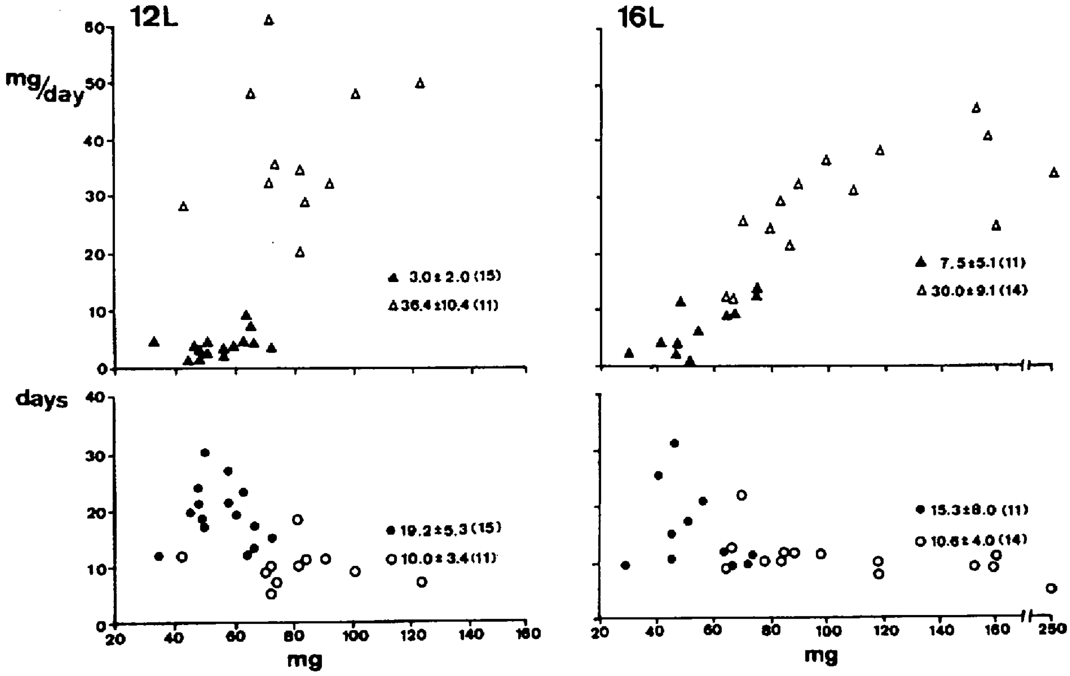

Fig. 4. Growth response of 4th-instar long horn morph larvae of various size to short (left) and long days (right) in *S. charonda*. Upper panel; growth rate. Bottom panel; duration of 4th instar. Open circles and triangles at both photoperiod regimens indicate the animals which underwent a precocious pupation after one more larval molt. Closed circles and triangles at LD 12: 12 hr indicate the animals which developed to become of short horn morph at the following ecdysis, the ones at LD 16: 8 hr those which became of long horn morph at the following ecdysis and then pupated after one more larval molt. Values are the mean±S. D. Numerals in parentheses are sample sizes.

diapause exhibited delayed growth in response to short days. Next, I tested whether 4th-instar larvae of the long horn morph may respond to short days with delayed growth. As shown in Fig. 4, response of the larvae differed according to larval size. Small larvae below about 70 mg in body weight retained a response to photoperiod: Instar duration was slightly prolonged (but not significant; $t=1.59$, $0.1<p<0.2$) and growth rate was significantly low ($t=3.14$, $p<0.01$) at short days, compared to long-day animals. Also, almost all animals of small size molted to become of short horn morph at short days whereas at long days they pupated after two larval molts. But, large size animals grew rapidly without growth retardation irrespective of photoperiodic difference and pupated after one more larval molt.

Development of post-diapause larvae at different photoperiods

In 4th instar, developmental duration from the start of the experiments to the ecdysis into 5th instar was short and identical among 3 photoperiods (Table 2). However, only growth rate was slightly lower at LD 12: 12 hr than those at other

Table 2. Growth of *S. charonda* larvae which had experienced hibernation at three different photoperiods.

Photoperiod (LD)	No.	IV			V			VI		
		Body weight (mg)**	Duration (days)	Growth rate (mg/day)	Body weight (mg)	Duration (days)	Growth rate (mg/day)	Body weight (mg)	Duration (days)	Growth rate (mg/day)
12:12	17*	55.8 ±12.7	8.5 ± 2.4	11.9 ± 3.4	149.6 ±25.5	10.1 ± 3.8	53.6 ±21.2	617.3 ±104.1	15.1 ± 3.3	124.0 ±35.0
14:10	14	57.7 ±10.4	6.7 ± 2.6	16.3 ± 6.6	162.8 ±42.0	8.8 ± 2.9	82.8 ±29.6	765.0 ±147.4	12.7 ± 3.2	153.3 ±34.3
16:8	16	64.1 ±23.0	8.6 ± 3.3	15.1 ± 7.7	179.7 ±58.7	9.5 ± 3.7	63.6 ±26.7	715.3 ±174.9	14.2 ± 4.4	136.1 ±39.7

* Other two larvae became of short horn morph at 5th instar but died without entering diapause again. ** Values show body weight at the start of experiments when larvae began to resume development. Roman numerals show instar number, and values are mean±S. D.

photoperiods, and two short-day animals developed to short horn morph. Other animals became of long horn morph just as those at LD 14: 10 and 16: 8 hr.

In 5th and 6th instars no photoperiodic difference was seen among photoperiods tested.

Determination of instar number

In laboratory rearing conditions, larvae were sometimes seen to undergo a metamorphosis precociously after 4 larvae molts (KATO & HASEGAWA, 1984). Therefore, I determined the critical size at which larvae proceed to metamorphose, based on the data of the animals used above. Figure 5 shows the body weight at the ecdysis to 5th (penultimate and last) and 6th (last) instars in relation to the

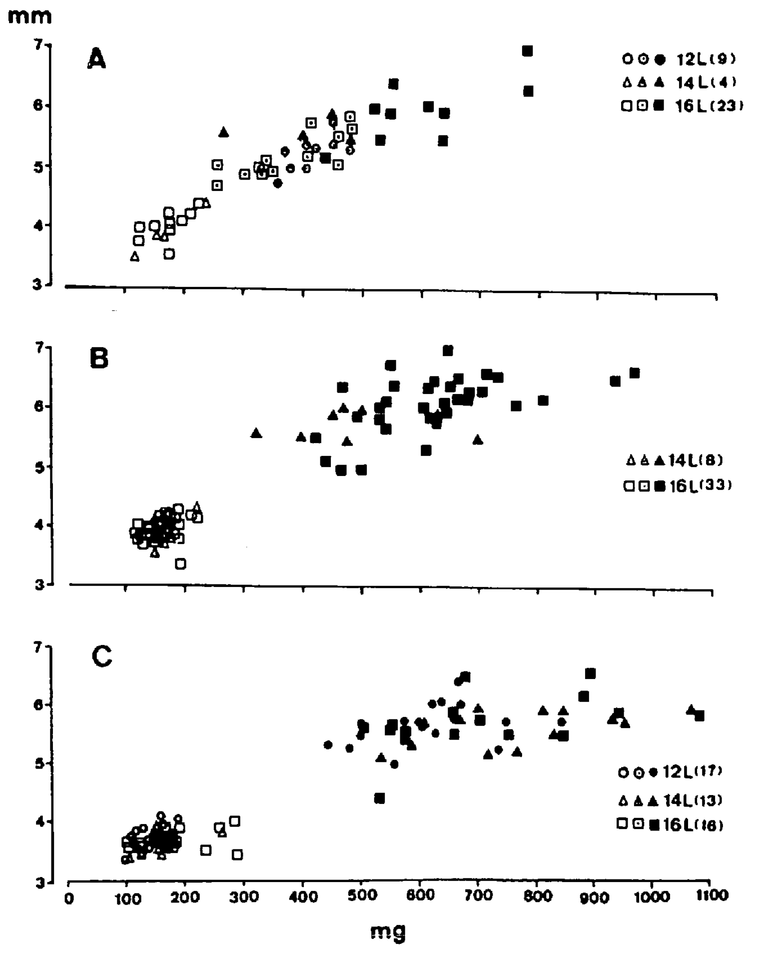

Fig. 5. Relationship between head capsule width and body weight at the time of an ecdysis in 5th (penultimate and last) and 6th (last) instar larvae of *S. charonda*. A: Larvae which had shown long horn morph at 4th instar. B: Larvae having shown short horn morph at 4th instar but developing without diapause. C: Post-hibernating larvae of short horn morph. Open symbols show penultimate 5th instar larvae, open symbols with a dot last 5th instar ones and closed symbols 6th (last) instar ones. Numerals in parentheses are sample size. In B and C, last 5th instar does not appear.

head-capsule size of each instar under various photoperiodic conditions. When 5th-instar larvae attained about 250–300 mg or more in body weight with head capsules wider than 4.5 mm, they pupate at the following molt. However, when they did not attain to this size, the following molt was larval. Difference in body size between such 5th-instar type and 6th-instar type had already been seen at 4th instar (Fig. 1). Also, such precocious pupation occurring at 5th instar was seen frequently in animals exhibiting long horn morph rather than short horn morph at 4th instar.

Discussion

This study elucidated that larval growth of *S. charonda* is photoperiod dependent in part and that the response is modified by factors such as developmental ages, body size, larval morph and experience of hibernation.

Especially, transfer experiments confirmed that growth retardation by long days occurs at 3rd instar whereas the retardation by short days at 4th instar. Also, the findings showed that short-day induced retardation seen at 4th instar was observed in not only S-larvae but also L-larvae although the extent of its retardation is small. In this case, interestingly, only 4th instar larvae of small size less than about 70 mg in body weight responded to photoperiod whereas animals of large size did not. This suggests that larvae may switch the response to photoperiod according to the body size attained. Furthermore, few larvae (4th instar) which had experienced hibernation regained the sensitivity to photoperiod. This is different from the case of a univoltine moth *Spilarctia imparilis*, whose larvae exhibit photoperiodic response even after hibernation (KIMURA *et al.*, 1982).

Sasakia charonda larvae exhibited a unique response to an intermediate photoperiod (LD 14: 10 hr), in which growth retardation was seen at not only 3rd but also 4th instars. This situation resembles the photoperiodic response of a univoltine female carabid (FERENZ, 1977), which exhibits egg maturation around LD 14: 10 hr but not at short or long days alone.

Determination of larval morph and growth retardation in this butterfly seem to be independently regulated. Short days given at not only 3rd but also 4th instars induced short horn morph at a subsequent ecdysis whereas growth retardation occurred at opposite photoperiod between these instars. Also, at LD 14: 10 hr, 3rd instar larvae showed slowed growth but most of the resulting larvae became of short horn morph at 4th instar. These facts support the idea that regulation of growth and diapause phenomena are not causually linked (BECK, 1980).

In *S. charonda*, when 5th-instar larvae have head capsules larger than 4.5 mm and attain body weight of 250–300 mg or more at the time of ecdysis, they proceed to pupate at the following ecdysis. However, occurrence of such precocious metamorphosis is rare in natural conditions and 5th-instar larvae are below the threshold size. Presumably, this may be due to slowed growth at 3rd and/or 4th instars, which is photoperiodically controlled in part. In general, the 5th instar larvae destined to metamorphose precociously were smaller than normal 6th instar (last) larvae. Thus, it seems likely that 5 larval molts linked with slowed growth allowed this insect to keep large body size, which is characteristic of this species. This point is in contrast to the case of *Manduca sexta* (NIJHOUT, 1975), where larvae inevitably have 4 larval molts and when they do not attain a threshold size, an extra-molt occurs.

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