Eur. J. Entomol. **100**: 275–281, 2003
ISSN 1210-5759

Duration of development and number of nymphal instars are differentially regulated by photoperiod in the cricket *Modicogryllus siamensis* (Orthoptera: Gryllidae)

NORICHIKA TANIGUCHI and KENJI TOMIOKA*

Department of Physics, Biology and Informatics, Faculty of Science and Research Institute for Time Studies, Yamaguchi University 753-8512, Japan

Key words. Photoperiod, nymphal development, cricket, *Modicogryllus siamensis*

Abstract. The effect of photoperiod on nymphal development in the cricket *Modicogryllus siamensis* was studied. In constant long-days with 16 hr light at 25°C, nymphs matured within 40 days undergoing 7 moults, while in constant short-days with 12 hr light, 12~23 weeks and 11 or more moults were necessary for nymphal development. When nymphs were transferred from long to short day conditions in the 2nd instar, both the number of nymphal instars and the nymphal duration increased. However, only the nymphal duration increased when transferred to short day conditions in the 3rd instar or later. When the reciprocal transfer was made, the accelerating effect of long-days was less pronounced. The earlier the transfer was made, the fewer the nymphal instars and the shorter the nymphal duration. The decelerating effect of short-days or accelerating effect of long-days on nymphal development varied depending on instar. These results suggest that the photoperiod differentially controls the number of nymphal instars and the duration of each instar, and that the stage most important for the photoperiodic response is the 2nd instar.

INTRODUCTION

Most insects in temperate zones have life cycles highly adapted to seasonal change. Photoperiod is the most important environmental cue controlling seasonal life cycles. The life cycles of crickets are known to be under photoperiodic control. Nymphal development of crickets that overwinter as eggs, such as *Teleogryllus emma*, *Dianemobius mikado* and *D. nigrofasciatus*, is accelerated by short day or intermediate photoperiods (Masaki, 1967, 1973, 1979). These responses are thought to determine the time of adult emergence and oviposition of diapause eggs in autumn. In univoltine species that overwinter as nymphs, such as *Gryllus campestris* and *Pteronemobius nitidus*, short-days experienced in the early instars induce and maintain diapause but long-days experienced after a period of short-days terminate diapause (Masaki & Oyama, 1963; Ismail & Fuzeau-Braesch, 1972, 1976; Masaki, 1972). Long-days increase not only the duration of nymphal development but also the number of instars in *P. nitidus* (Tanaka, 1979). In a univoltine strain of *Modicogryllus siamensis* (Ibaraki strain, 36.1°N), the early nymphal instars are more sensitive to long-days (Tanaka et al., 1999). In bivoltine species, including *Teleogryllus occipitalis*, *Dianemobius taprobanensis* and *Modicogryllus* sp. (Miyagi strain, 38°N), nymphal development is promoted by long-days and retarded by short-days (Masaki & Ohmachi, 1967; Masaki, 1972; Masaki & Sugahara, 1992). In *Modicogryllus* sp., if the transfer from long-day to short-day or short-day to long-day conditions is made early in nymphal development, the above effects on the duration of nymphal development can be reversed (Masaki & Sugahara, 1992). However, the mechanism by which photoperiod controls the nymphal development in bivoltine species has not been studied in detail.

In this study, the nymphal development of a bivoltine strain of the cricket, *Modicogryllus siamensis*, reared under long-day and short-day conditions, is described in detail. In addition, nymphs were transferred and reared in different photoperiods at different stages in their development. The results show that photoperiod differentially controls the number of nymphal instars and the duration of development, and that the stage most important for the photoperiodic response is the 2nd instar.

MATERIALS AND METHODS

The cricket *Modicogryllus siamensis* used in this study were taken from a colony, which was established from female crickets collected in Yamaguchi City (34°N) in July 1999. This stock culture was kept in short day conditions of 12L : 12D. This cricket was previously referred to as *Velarifictorus parvus* (Tanaka et al., 1999) but was recently identified as *Modicogryllus siamensis* (Ichikawa et al., 2000).

For the experiments eggs were incubated at 25 ± 0.5°C under either 16L : 8D or 12L : 12D. Within 12 hr of hatching, nymphs were transferred to a plastic box (6 × 6 × 9 cm) in which they were reared until the 3rd instar. Newly emerged 4th instar nymphs were reared in a transparent plastic jar (diameter 16 cm; depth 9 cm). Until the 6th instar, 25~27 nymphs were kept in each plastic box or jar, and in the 7th and later instars, less than 15 crickets were placed in each jar. Several pieces of paper folded in an accordion shape were placed in each box or jar. Water and food (Nihon Crea, CA-1) were given ad libitum. The source of illumination was a 15 W cool white fluorescent lamp

* Corresponding author. E-mail: tomioka@cc.okayama-u.ac.jp; tel. & fax.: +81-86-251-8498. Present address: Department of Biology, Faculty of Science, Okayama University, Okayama 700-8530, Japan.

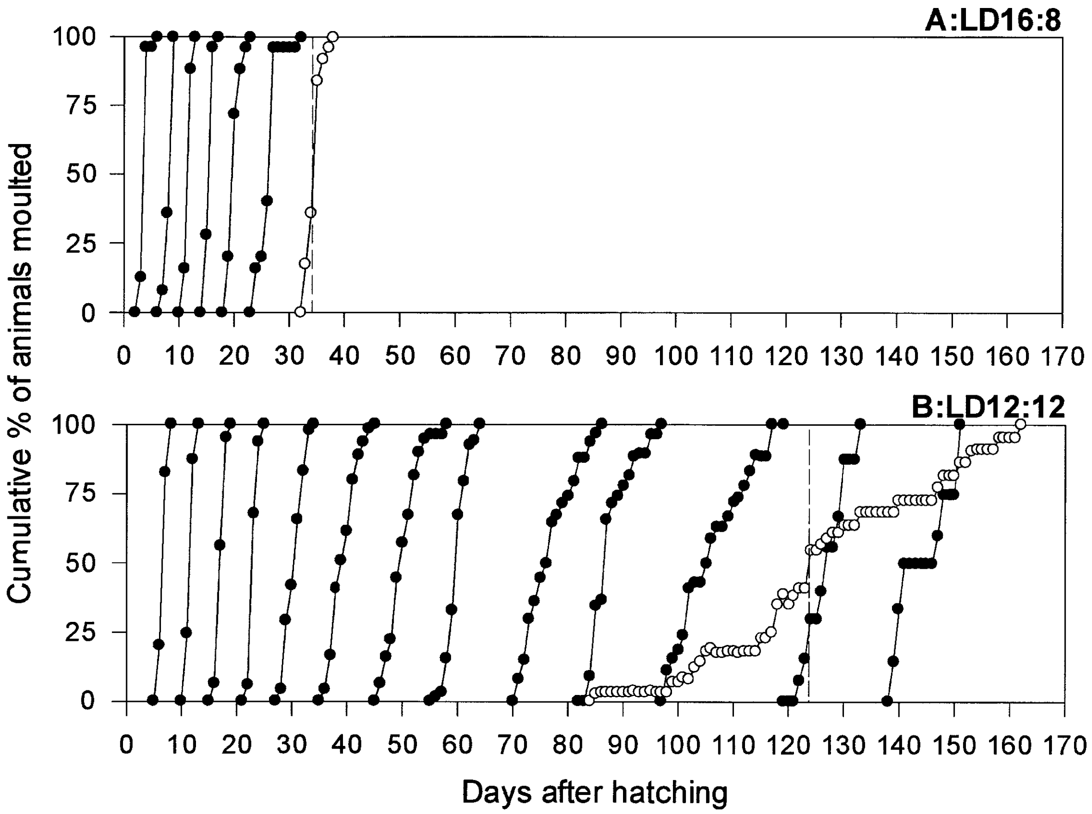

Fig. 1. Nymphal development in *Modicogryllus siamensis* under 16L : 8D (A) and 12L : 12D (B) at 25°C. The vertical broken line indicates when 50% of the crickets were adult. Closed and open circles indicate nymphal and adult moults, respectively.

connected to a timer. The effects of rearing under a constant photoperiod as well as transferring nymphs from short to long day conditions, and vice versa, were examined. An initial number of 25 to 100 nymphs were reared in each photoperiodic regime. The rearing boxes and jars were checked every day for newly moulted nymphs and adults.

RESULTS

Nymphal development under constant photoperiods

Under long day (16L : 8D) conditions all the crickets underwent 7 moults and became adult within 37 days of hatching at 25°C (Fig. 1A). The duration of nymphal development was considerably shorter than that in a univoltine strain (Tanaka et al., 1999). The average duration of each instar was 3~5 days until the 5th instar, increasing thereafter up to about 8 days in the 7th instar (Fig. 2). The average total duration of development was 35 days. Wing pads took the dorsal position in the 6th instar.

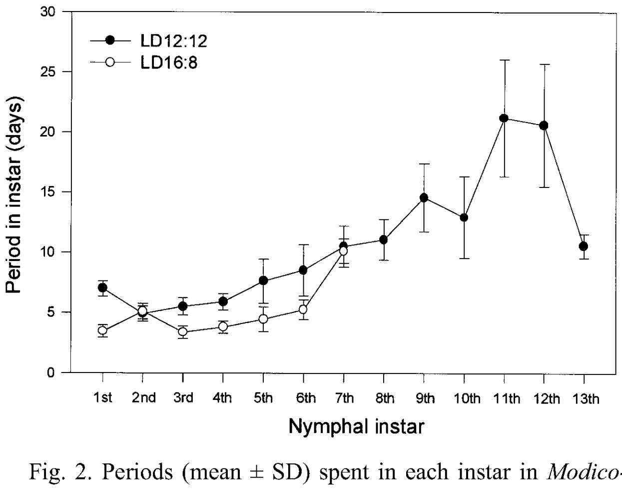

Fig. 2. Periods (mean ± SD) spent in each instar in *Modicogryllus siamensis* reared under 16L : 8D (open circle) or 12L : 12D (closed circle) at 25 °C.

In constant short day (12L : 12D) conditions, the nymphs developed slowly. The average duration of development was 124 days (Fig. 1B). The nymphs moulted at least 11 times and some 14 times or more before becoming adult. The duration of each instar was also substantially longer than under long day conditions (t-test, $P < 0.05$), except that of the 2nd and 7th instars (Fig. 2). In short day conditions wing pads were always dorsal in the penultimate instar.

Response to a change of photoperiod

To determine how nymphal development is regulated by photoperiod, the nymphs were transferred from long (16L : 8D) to short day conditions (12L : 12D) in various instars. Transfers were made on days when nymphs ecdysed. The duration of development varied in response to when the photoperiod was changed (Fig. 3). The nymphs underwent 7 instars when the change occurred on the day of the 2nd nymphal ecdysis or later (Figs 3, 4). The duration of nymphal development became longer the earlier they were transferred to 12L : 12D conditions (ANOVA, $P < 0.001$). The times to when 50% of the nymphs became adult were 48, 46 and 39 days for

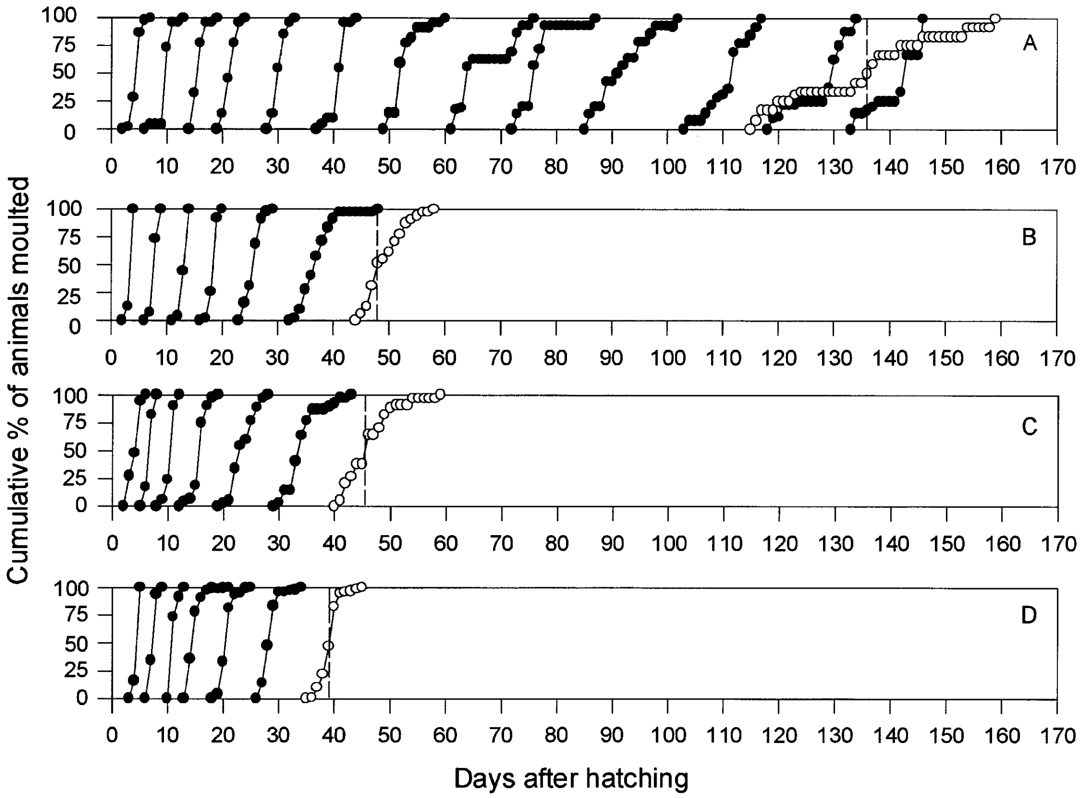

Fig. 3. Effects of transferring various instars from 16L : 8D to 12L : 12D on nymphal development at 25°C in *Modicogryllus siamensis*. The transfer was made in the 2nd (A), 3rd (B), 4th (C), and 5th (D) instars. The vertical broken line indicates when 50% of the crickets were adult. Closed and open circles indicate nymphal and adult moults, respectively.

nymphs transferred in the 3rd, 4th and 5th instars, respectively. However, when transferred on the day of the 1st nymphal ecdysis, both the number of nymphal instars and the duration of development were drastically increased

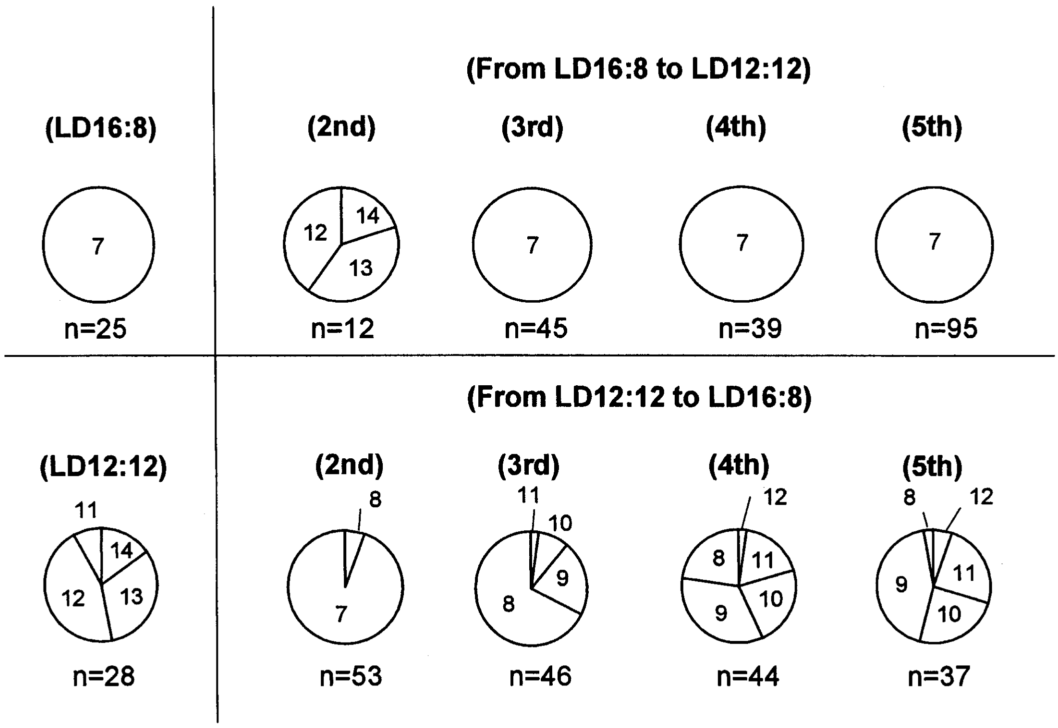

Fig. 4. Pie diagrams of the instar composition at adult emergence in *Modicogryllus siamensis* when exposed to various photoperiods at 25°C. The number in each section indicates the number of moults and its size the frequency of individuals with the indicated number of instars. n indicates the sample size.

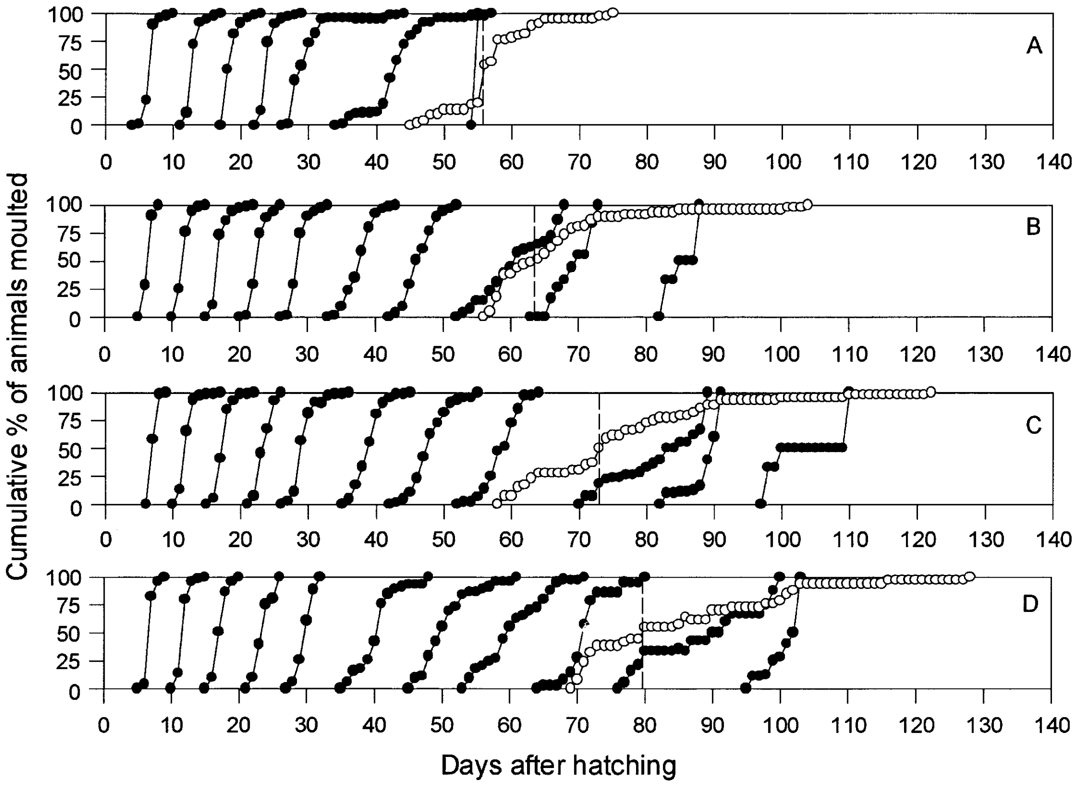

Fig. 5. Effects of transferring various instars from 12L : 12D to 16L : 8D on nymphal development at 25°C in *Modicogryllus siamensis*. The transfer was made in the 2nd (A), 3rd (B), 4th (C), and 5th (D) instars. The vertical broken line indicates when 50% of the cricket were adult. Closed and open circles indicate nymphal and adult moults, respectively.

(Figs 3, 4): the number of nymphal instars was 12 or more, typical of diapausing nymphs exposed to constant short day (12L : 12D) conditions. The average nymphal period (136 days) was slightly longer than under constant short day (12L : 12D) conditions, but not significantly so (Mann-Whitney's U-test, $P > 0.36$).

When transferred from the short- to long-day conditions, nymphs transferred in the 3rd instar or later moulted more than 7 times before becoming adult (Figs 4, 5). Both the number of instars and the duration of development were greater when transferred at later stages of development: the typical number of nymphal instars was 8, 9 and 9, and the average duration of development 63, 73 and 79 days for nymphs transferred in the 3rd, 4th and 5th instars, respectively (ANOVA, $P < 0.001$). When transferred in the 2nd instar, most nymphs became adult after the 7th moult: A few had an additional instar. However, the durations of the 6th and later instars were noticeably longer than those of nymphs transferred in the 3rd or later instars. The average total nymphal duration (56 days) was significantly longer than that of nymphs exposed to a constant long day photoperiod (Mann-Whitney's U-test, $P < 0.001$).

Analysis of the duration of each instar after transfer from long to short day conditions, and vice versa, revealed that the response to a change of photoperiod was stage dependent. When transferred from long to short day conditions, the duration of each instar was significantly lengthened compared to that of nymphs kept in a constant long day photoperiod (t-test, $P < 0.01$), except for the 2nd-instar of crickets transferred in that instar (Fig. 6A). The lengthening effect was greater in the later instars. In nymphs transferred from short to long day conditions, the stage dependency was more prominent. The instar duration mostly increased in the 2nd, 6th and 8th instars, and decreased in the 5th and 7th instars (Fig. 6B).

Response to alternating photoperiodic conditions

The effect of alternating between 12L : 12D and 16L : 8D conditions was determined using a group of nymphs. All nymphs became adult after 7 moults (Fig. 7). The average nymphal period was 40 days, which was 5 days longer than when reared in constant long day (16L : 8D) conditions (Mann-Whitney's U-test, $P < 0.001$).

DISCUSSION

Photoperiodic control of nymphal development

The results of the present study confirmed and extended the earlier report that the nymphal development of the cricket *Modicogryllus siamensis* is regulated by the photoperiod (Tanaka et al., 1999). In the Ibaraki strain, Tanaka et al. (1999) estimated using the frequency distributions of head widths, that there were 7 nymphal instars and at least 9~10, when it was reared under 16L : 8D and 12L : 12D conditions, respectively. Under long day conditions (16L : 8D) at 25°C, the nymphs of the Yamaguchi strain underwent synchronously 7 moults and became adult within 40 days. In short day conditions (12L : 12D) they became adult 12~23 weeks after hatching, undergoing 11 or more synchronous moults (Fig. 1). The large

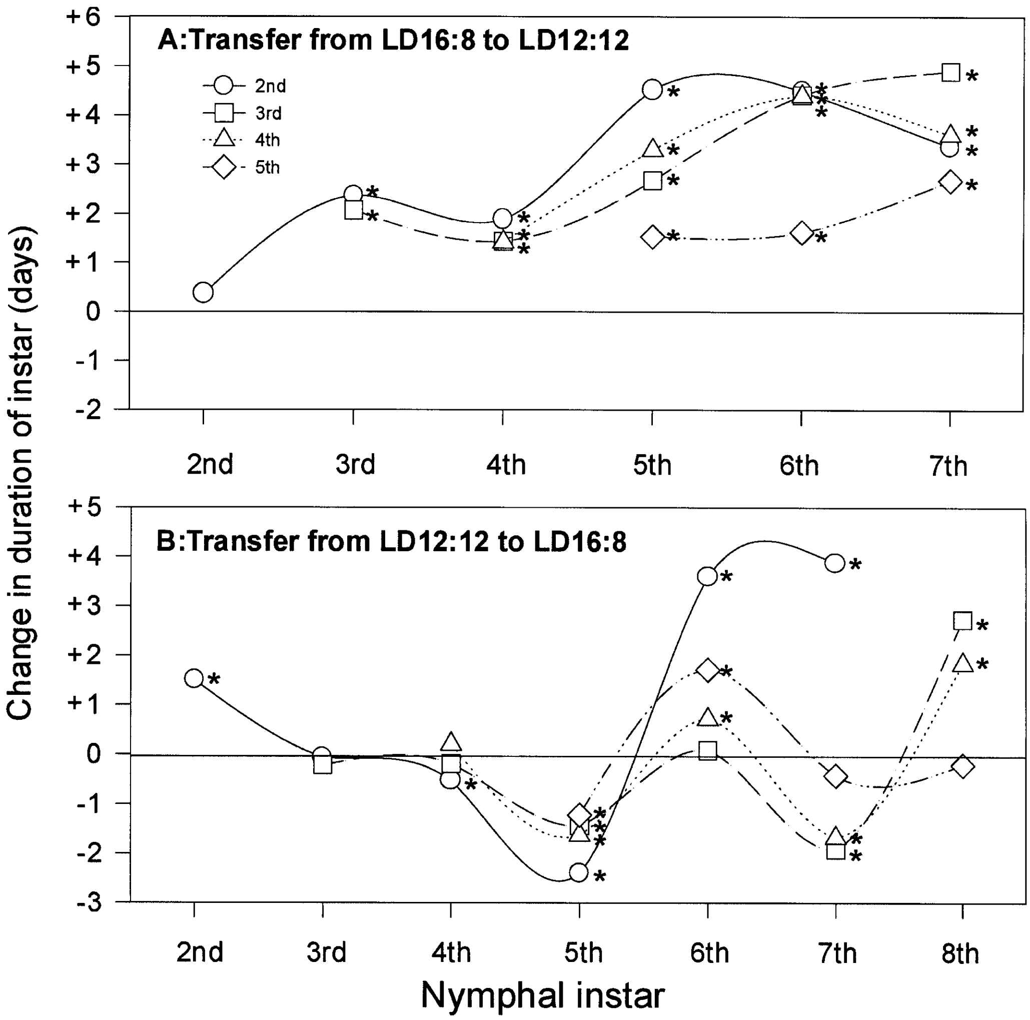

Fig. 6. Change in the duration of each instar at 25°C caused by transferring various nymphal instars of *Modicogryllus siamensis* from 16L : 8D to 12L : 12D (A) or 12 L : 12D to 16 L : 8D (B). The increase and decrease was relative to that recorded for crickets kept in constant 16L : 8D (A) or 12L : 12D (B) and indicated by plus and minus signs. Significant differences between the transferred nymphs and those kept in constant LDs are indicated by asterisks ($P < 0.01$, t-test).

variation in the time to adult emergence under short photoperiods was thus attributable to different numbers of instars, ranging from 11 to over 14. In both 16L : 8D and 12L : 12D the Yamaguchi strain completed nymphal development noticeably earlier than the northern (Ibaraki, 36.1°N) strain, in which the average nymphal duration was about 50 and 150 days when reared in 16L : 8D and 12L : 12D conditions, respectively (Tanaka et al., 1999),

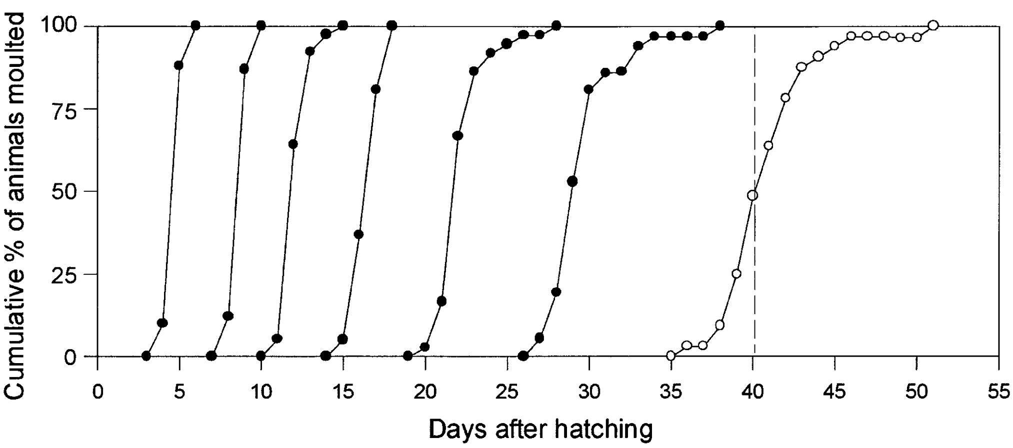

Fig. 7. Nymphal development in *Modicogryllus siamensis* when reared under alternating photoperiodic conditions of 16L : 8D and 12L : 12D at 25°C. The vertical broken line indicates when 50% of the crickets were adult. Closed and open circles indicate nymphal and adult moults, respectively.

but later than in the southern (Tanegashima, 30°N) strain in which adults emerged in 12~19 weeks in short days (Masaki, 1972). This might indicate variation in photoperiodic response related to latitude (Masaki, 1967; Masaki, 1973; Walker & Masaki, 1989).

Sensitive stage

In univoltine species such as *Gryllus campestris* and *Pteronemobius nitidus* in which the nymphs overwinter, long days prolong and short days accelerate development to the instar that goes into diapause. Once this instar is reached, the response is reversed: short days maintain and long days terminate diapause (Masaki & Oyama, 1963; Ismail & Fuzeau-Braesch, 1972, 1976; Tanaka, 1978). This reversal of the photoperiodic response was not observed in *M. siamensis*: Long days and short days consistently accelerated and decelerated, respectively, nymphal development. However, the effect apparently varied with the stage of development. The stage sensitive to photoperiods is an earlier nymphal stage in *M. siamensis* (Tanaka et al., 1999). The present study revealed that the stage most important for the photoperiodic response was the 2nd instar. When transferred from a long to a short photoperiod in the 3rd or later instar, the nymphs became adult after the 7th moult, while those transferred in the 2nd instar underwent 12 or more moults (Fig. 4). Most of the nymphs transferred from short to long day conditions in the 2nd instar became adults after the 7th moult, and the number of moults increased gradually the longer the transfer to long day conditions was delayed (Fig. 4).

The results of transferring nymphs from long to short day conditions, and vice versa, confirm previous reports that short day conditions retard and the long day conditions accelerate nymphal development (Masaki & Sugahara, 1992; Tanaka et al., 1999). They also indicate that short day conditions induce and maintain diapause and long day terminates it. The effect of long day conditions was stronger than that of short day conditions, because when nymphs were alternately exposed to long day and short day conditions their nymphal period was very close to that when kept continuously in long day conditions (Fig. 7). Moreover, the number of nymphal instars was not changed by transferring them from long to short day conditions in the 3rd or later instar (Fig. 3), but it was when changed from short to long day conditions in the 3rd or later instar (Fig. 4).

This cricket can be heard singing in Yamaguchi in early summer, and adults of the second generation can be collected from September to October. Therefore, this cricket is apparently bivoltine in Yamaguchi, and univoltine in Ibaraki (Tanaka et al., 1999). It overwinters as a nymph and the development of overwintering nymphs in late spring might be accelerated by long-day conditions. The nymphs of the second generation occur from late June when the day length gradually decreases, but they become adult within 2 months because their non-diapause development has been largely determined by the long-day conditions perceived during the 2nd instar. The 2nd generation nymphs are exposed to short-day conditions and committed to diapause.

The duration of development was noticeably affected by photoperiod in the later nymphal stages in both those transferred from long to short day conditions, and vice versa, suggesting that the mechanism regulating the duration of nymphal development is different from that controlling the number of moults. The separate control of nymphal duration and moulting has been reported for *Pteronemobius nitidus* (Tanaka, 1979). Our data indicate that the duration of certain instars changes more than that of others (Fig. 6). Thus, the responsiveness to photoperiod depends on the nymphal instar. However, there was a strong correlation between the duration of nymphal development and the number of moultings (r = 0.98), suggesting that there is a limit to the duration of each instar.

After overwintering the responsiveness to photoperiod is sometimes lost in insects (Hodek, 1983; Tauber et al., 1986). Although our results demonstrated that even in the late nymphal instars crickets were sensitive to photoperiod in the laboratory, it is uncertain whether they are responsive to photoperiod after overwintering in the field.

Storage of photoperiodic information

The present results demonstrated that the duration of development was strongly affected by photoperiod experienced in the 2nd instar and only slightly in later instars. This suggests that photoperiodic information is stored for a long time. Since insect development is generally regulated by the endocrine system (Flanagan et al., 1988), it is likely that the information is stored in tissues that regulate the endocrine system. It is also well known that the accumulation of photoperiodic information is necessary to induce photoperiodic responses (Saunders, 1982). Although the 2nd instar is the critical stage for the photoperiodic response, it is not known how many cycles are necessary to induce a response. This deserves to be addressed in the future.

ACKNOWLEDGEMENTS. This work was supported in part by a grant from the Ministry of Education, Science, Sports, Culture and Technology of Japan. The authors are grateful to reviewers for comments on the manuscript.

REFERENCES

Flanagan T.R., Tomioka K., O'Brien M.A., Westbrook A.L., Agui N. & Bollenbacher W.E. 1988: Neuroendocrine regulation of insect development. In: Steffens L. & Rumsey T.S. (eds): *Biomechanisms Regulating Growth and Development.* Kluwer Academic Press, Dordrecht, pp. 119–135.

Hodek I. 1983: Role of environmental factors and endogenous mechanisms in the seasonality of reproduction in insects diapausing as adults. In: Brown V.K. & Hodek I. (eds): *Diapause and Life Cycle Strategies in Insects.* W. Junk, The Hague, pp. 9–33.

Ichikawa A., Murai T. & Honda E. 2000: Monograph of Japanese crickets (Orthoptera: Grylloidea). *Bull. Hoshizaki Green Found.* 4: 257–332. [in Japanese]

Ismail S. & Fuzeau-Braesch S. 1972: Action du photoperiodisme sur le nombre de stades larvaires, la diapause et la morphogenèse chez Gryllus campestris (Orthoptère). *C. R. Acad. Sci. Ser. D* **275**: 2535–2538.

ISMAIL S. & FUZEAU-BRAESCH S. 1976: Programmation de la diapause chez Gryllus campestris. *J. Insect Physiol.* **22**: 133–139.
MASAKI S. 1967: Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution* **21**: 725–741.
MASAKI S. 1972: Photoperiodism in the seasonal cycle of crickets. In: Goryshin N.I. (ed.): *Problems of Photoperiodism and Diapause in Insects*. Leningrad State University, Leningrad, pp. 25–50. [in Russian, English summary]
MASAKI S. 1973: Climatic adaptation and photoperiodic response in the band-legged ground cricket. *Evolution* **26**: 587–600.
MASAKI S. 1979: Climatic adaptation and species status in the lawn ground cricket. I. Photoperiodic response. *Kontyu* **47**: 48–65.
MASAKI S. & OHMACHI F. 1967: Divergence of photoperiodic response and hybrid development in Teleogryllus (Orthoptera: Gryllidae). *Kontyu* **35**: 83–105.
MASAKI S. & OYAMA N. 1963: Photoperiodic control of growth and wing-form in Nemobius yezoensis Shiraki (Orthoptera: Gryllidae). *Kontyu* **31**: 16–26.
MASAKI S. & SUGAHARA T. 1992: Photoperiodic control of larval development and wing form in Modicogryllus sp. (Orthoptera: Gryllidae). *Ecol. Res.* **7**: 25–30.
SAUNDERS D.S. 1982: *Insect Clocks 2nd. ed.* Pergamon Press, Oxford, 409 pp.
TANAKA S. 1978: Effects of changing photoperiod on nymphal development in Pteronemobius nitidus Bolvar (Orthoptera: Gryllidae). *Kontyu* **46**: 135–151.
TANAKA S. 1979: Multiple photoperiodic control of the seasonal life cycle in Pteronemobius nitidus Bolvar (Orthoptera: Gryllidae). *Kontyu* **47**: 465–475.
TANAKA S., ARAI T. & TANAKA K. 1999: Nymphal development, diapause and cold-hardiness in a nymph-overwintering cricket. *Entomol. Sci.* **2**: 173–182.
TAUBER T.J., TAUBER C.A. & MASAKI S. 1986: *Seasonal Adaptations of Insects*. Oxford University Press, New York, 441 pp.
WALKER T.J. & MASAKI S. 1989: Natural history. In: Huber F., Moore T.E. & Loher W. (eds): *Cricket Behavior and Neurobiology*. Cornell University Press, Ithaca, London, pp. 1–42.

Received August 22, 2002; revised October 29, 2002; accepted December 6, 2002