# Effects of Unilateral Compound-Eye Removal on the Photoperiodic Responses of Nymphal Development in the Cricket *Modicogryllus siamensis*

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The cricket, *Modicogryllus siamensis*, shows clear photoperiodic responses at 25°C in nymphal development. Under long-day conditions (LD16:8), nymphs became adults about 50 days after hatching, while under short-day conditions (LD8:16) the duration of nymphal stage extended to more than 130 days. Under constant dark conditions, two developmental patterns were observed: about 60% of crickets became adults slightly slower than under the long-day conditions, and the rest at later than 100 days after hatching, like those under the short-day conditions. When the compound eye was unilaterally removed on the 2nd day of hatching, an increase of molting and an extension of the nymphal period were observed under the long-day conditions, while under the short-day conditions, some crickets developed faster and others slower than intact crickets. These results suggest that this cricket receives photoperiodic information through the compound eye, that a pair of the compound eyes is required for a complete photoperiodic response, and that interaction between bilateral circadian clocks may be also involved in the response.

**Key words:** cricket, nymphal development, photoperiodism, circadian clock, compound eye

## INTRODUCTION

Most insects in temperate zones have life cycles highly adapted to seasonal changes in climate. Photoperiod is the most important environmental cue that regulates various physiological responses, including diapause and seasonal forms. These photoperiodic responses hypothetically consist of the following steps: reception of photoperiodic information, photoperiodic time measurement, counting the number of photoperiodic cycles experienced, and output of the final regulatory signal (Beck, 1980; Saunders, 2002). The mechanism of photoperiodic time measurement is a challenging problem and has been studied for a long time. Some experimental results (Saunders, 2002) have suggested circadian clocks to be involved in the mechanism. One such result was obtained under light-dark conditions, with a light period combined with a dark period of various durations. The diapause ratio of *Tetranychus urticae*, for example, varied periodically depending on night length, decreasing when the period of the light-dark cycle was 24 h or a multiple of 24 h (Vaz Nunes and Veerman, 1986). A similar result was found for the cabbage white, *Pieris rapae*, and the cricket *Pteronemobius fascipes* (Kono, 1970; Takeda, 1986). The other case was obtained under light-dark cycles of multiples of 24 h, with a light period combined with a long night interrupted at various times. In the flesh fly *Sarcophaga argyostoma*, for example, diapause was interrupted when

the light pulse was given at 24-h intervals (Saunders, 1976). However, no direct experimental evidence exists for the involvement of circadian clocks in insect photoperiodism.

Crickets are a good model with which to address this issue, since they often show robust photoperiodic responses and since the loci of the circadian clock and the circadian photoreceptor are known. A circadian clock that regulates both locomotor and electroretinographic rhythms resides in each optic lobe, and the photoreceptor for photic entrainment is in the compound eyes (Loher, 1972; Tomioka and Chiba, 1982, 1984, 1992). The bilaterally paired clocks are known to be mutually coupled to stabilize the output of the system (Tomioka, 1993; Tomioka *et al*., 2001).

In this study, we used the cricket *Modicogryllus siamensis,* which shows a robust photoperiodic response in nymphal development (Tanaka *et al*., 1999; Taniguchi and Tomioka, 2003). We investigated the effects of unilateral removal of the compound eye on its photoperiodic responses, to clarify the relationship between the circadian clock and the photoperiodic time-measurement system. The operation resulted in altered photoperiodic responses, and the results are discussed in relation to photoreception and the involvement of the circadian system.

## MATERIALS AND METHODS

**Animals and observation of nymphal development**

The crickets (*Modicogryllus siamensis*) used in this study were taken from a colony established from animals collected in the city of Yamaguchi (34°N), Japan. The stock culture was maintained under a long-day condition, 16 h light: 8 h dark (LD16:8) at 25°C. To collect eggs, a dish with moist cotton was placed in a colony of adult crickets. The next day, the cotton with eggs laid was trans-

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ferred to a plastic box and placed until hatching in an incubator (MFD-153; Sanyo Medical Co.) in which experimental conditions were set.

For observation of nymphal development, nymphs were held in a box (7.5×7.5×9 cm) for the first three instar stages, then transferred to a larger box (16×12×12 cm). Fifteen to 16 nymphs were housed in each box. They were kept in an incubator (MDF-153; Sanyo Medical Co.) under either a long day (LD16:8) or short day (8 h light: 16 h dark; LD8:16) condition at a constant temperature of 25°C. The rearing boxes were checked daily for newly molted nymphs and adults. Final-instar nymphs were easily discriminated by having wing pads on their dorsal side.

For observation of nymphal development under constant darkness (DD), crickets were held in a box supplied with food and water immediately after hatching. The box was placed in a light-tight box covered with a dark bag to exclude light and kept in an incubator at 25°C. Adult emergence was first checked on the 20th day of hatching, and thereafter every 7 days, under dark-red light (>570 nm, 20–50 lux). Water and food were exchanged on the day of checking.

### Unilateral removal of the compound eye

In *M. siamensis*, the sensitive stage for photoperiod is limited to the first- and second-instar nymphal stages (Taniguchi and Tomioka, 2003). Therefore, unilateral removal of the compound eye was carried out on the 2nd day of hatching. Nymphs were fixed onto soft clay, and one or the other of the compound eyes was removed with a fine, stainless-steel needle under a dissecting microscope. To test the effect of damage associated with the surgical operation, a sham operation was also performed in which only the edge of a compound eye was cut. After the operation, crickets were placed in humid conditions in a box for 1 day for recovery and then transferred to a rearing box (7.5×7.5×9 cm).

### Histological analysis

Histological examination of the optic lobe and compound eye was performed on crickets with the compound eye unilaterally removed on the 2nd day of hatching. Nine and 14 days after the operation, when they were in the 2nd and 3rd instars, respectively, the crickets were fixed in Bouin's fixative, dehydrated, and embedded in paraffin. Eight-micrometer serial sections were cut and stained with hematoxylin-eosin by conventional methods. They were observed and photographed with a light microscope (Eclipse E600; Nikon) equipped with a digital camera (DXM1200; Nikon).

## RESULTS

### Nymphal development under long-day and short-day conditions

Under long-day (LD16:8) conditions, all of 22 crickets observed underwent seven molts and became adults within 53 days of hatching at 25°C (Fig. 1a). The average duration of each instar was about 5 days until the 5th instar, increasing thereafter up to about 14 days in the 7th instar. The average total duration of development was 50 days, longer than that in our previous study (35 days; Taniguchi and Tomioka, 2003). This difference might have been caused by the density of nymphs in a cage, as has been suggested for some insects (Wharton *et al*., 1967; Fescemyer and Hammond, 1988). In the previous study, the density was
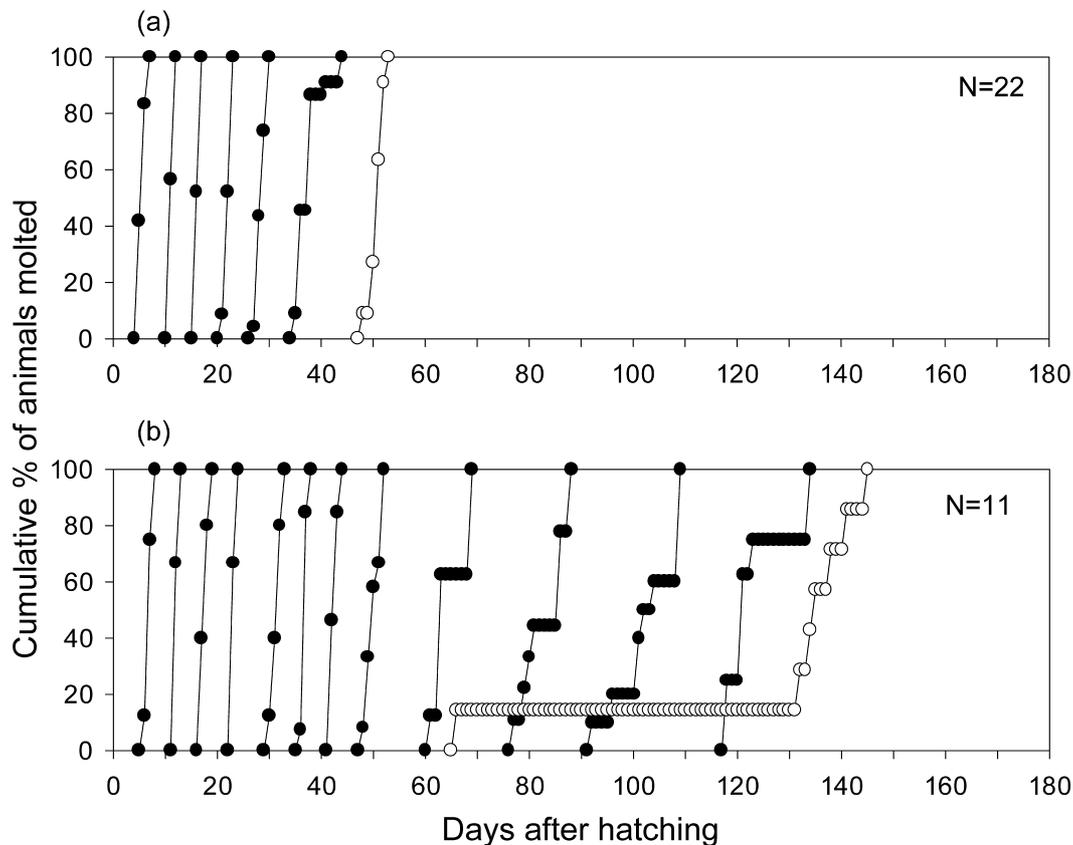


**Fig. 1.** Nymphal development in the cricket *Modicogryllus siamensis* under **(a)** long-day conditions (LD16:8) and **(b)** short-day conditions (LD8:16). Closed and open circles indicate nymphal molting and adult emergence, respectively. N indicates the number of crickets that became adults.

25–27 nymphs per box (6×6×9 cm) for the first six instars, and less than 15 crickets in a jar (diameter 16 cm, depth 9 cm) for the 7th or later instars. Although the duration was different, the proportion of animals molting was the same as in the previous study.

Under short-day conditions (LD8:16), 14 crickets were initially used but three of them died during the experiment.

A single exceptional cricket became an adult on day 65, undergoing nine molts. The remaining 10 crickets developed slowly and became adults after molting 12 or 13 times, taking 132–145 days (Fig. 1b), as has been reported for LD12:12 (Taniguchi and Tomioka, 2003). The average nymphal duration of the slowly developing animals was 137.4±4.2 (SD) days.
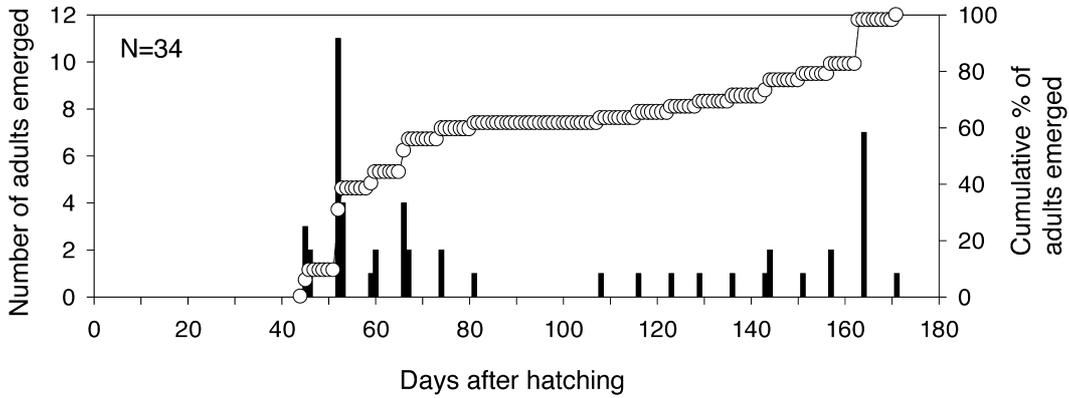


**Fig. 2.** Nymphal development of *Modicogryllus siamensis* under constant dark conditions (DD). Black bars show the number of adults emerged. Open circles indicate the cumulative percent of adults emerged. N indicates the number of crickets that became adults. Note that adult emergence occurred with two peaks, one between days 40 and 82 and the other between days 107 and 170.
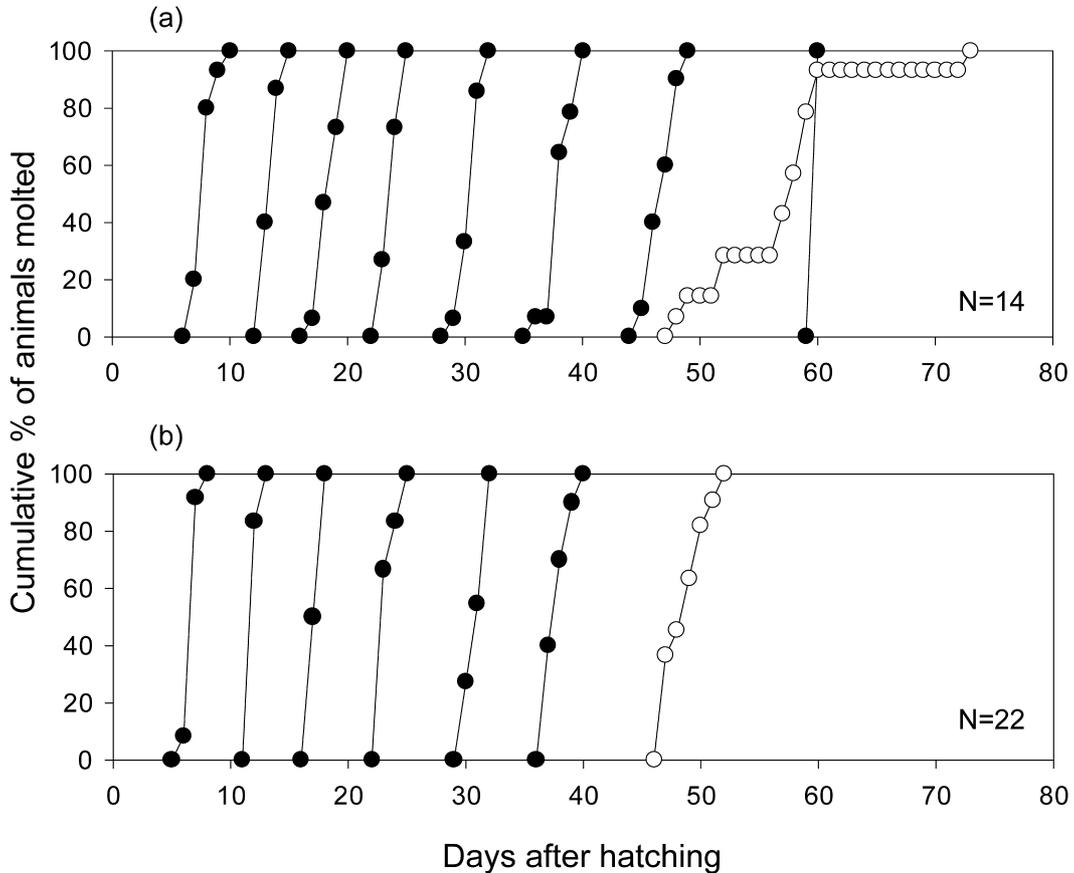


**Fig. 3.** Effects of **(a)** unilateral removal of the compound eye and **(b)** sham operation on nymphal development of *Modicogryllus siamensis* under long-day conditions (LD16:8). Closed and open circles indicate nymphal molting and adult emergence, respectively. N indicates the number of crickets that became adults. Note that extra molts occurred in crickets with the compound eye unilaterally removed.

### Nymphal development under DD conditions

The nymphal development of 34 crickets was examined under DD conditions at 25°C. The observed pattern of adult emergence was quite different from that under short-day or long-day conditions (Fig. 2). Adult emergence showed two peaks. Sixty-two percent of the nymphs became adults between days 40 and 85 after hatching, with a peak around day 50; the average nymphal period for this group was 56.6±9.4 (SD) days. The peak was significantly later than that of intact crickets kept under long-day conditions (Mann-Whitney U-test, P<0.01). The remaining crickets developed slowly, and their nymphal duration was longer than 107

days, with a peak around day 160 days and averaging 148.9±18.6 (SD) days. The nymphal duration was also significantly different from that under the short-day conditions (Mann-Whitney U-test, P<0.05). The medians of adult emergence for the early and late groups were days 60 and 140, respectively. No adult emergence was observed during the period between days 82 and 106.

### Development of crickets with the compound eye unilaterally removed

Under long-day conditions, 14 and 22 crickets were used for observation of nymphal development, receiving uni-
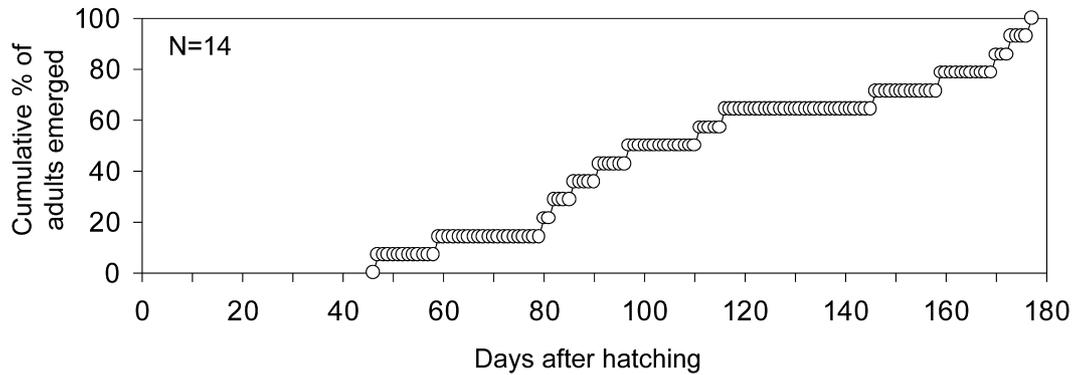


**Fig. 4.** Effect of unilateral removal of the compound eye on nymphal development under short-day conditions (LD8:16). N indicates the number of crickets that became adults. Note that adult emergence occurred with two peaks, one between days 80 and 120 and the other later than day 160.
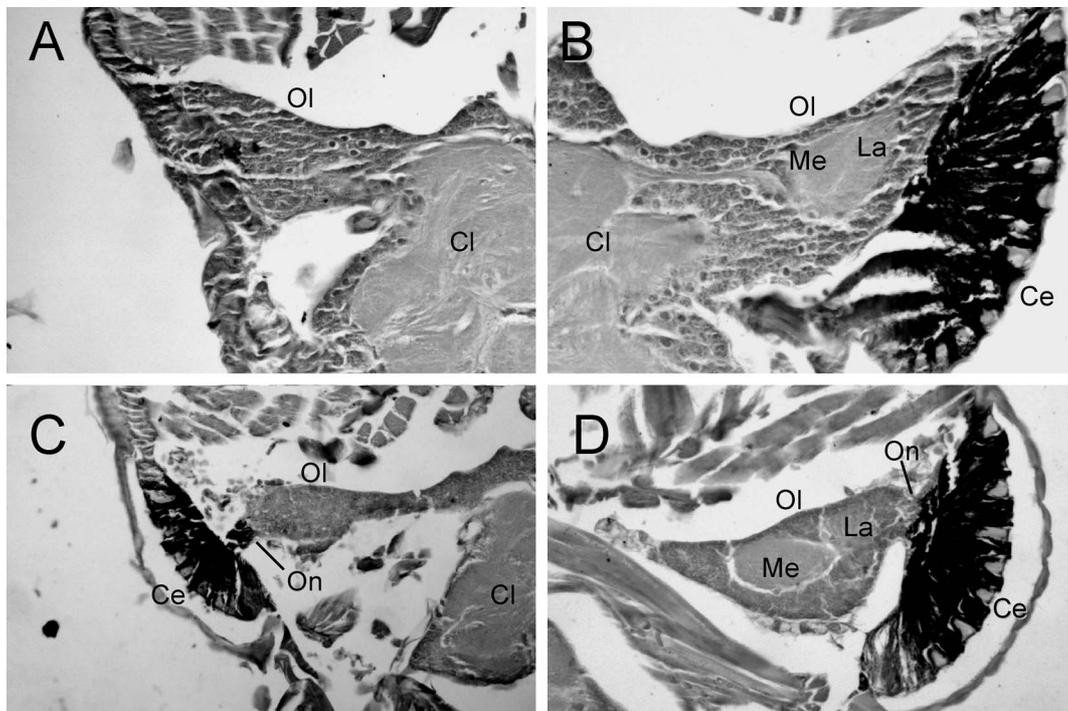


**Fig. 5.** **(A, B)** Frontal sections of a 2nd instar and **(C, D)** horizontal sections of a 3rd-instar cricket head with the compound eye unilaterally removed on the 2nd day of hatching. **(A, C)** Operated (right) side. **(B, D)** Intact (left) side. A and B are oriented with the dorsal side at the top, C and D with the posterior at the top. Ce, compound eye; Cl, cerebral lobe; La, lamina; Me, medulla; Ol, optic lobe; On, optic nerve. Note that the compound eye and the optic nerve were substantially regenerated in the 3rd instar, but that there was no neuropil structure in the optic lobe on the operated side. Scale, 100 µm.

lateral compound eye removal and the sham operation, respectively, on the 2nd day of hatching. All sham-operated crickets showed seven molts, and nymphal durations ranged from 47 to 52 days (Fig. 3b), as with intact animals. Four of the crickets with the compound eye unilaterally removed showed a developmental pattern similar to that of intact crickets, and the duration of the nymphal stage ranged from 48 to 52 days. In the remaining crickets, however, the number of molts increased by one or two times (Fig. 3a): nine crickets underwent eight molts and one cricket nine molts, and the duration of the nymphal stage ranged from 56 to 72 days. The average duration of the nymphal period was 57.1±5.9 (SD) days, significantly longer than that of sham-operated animals (48.1±1.7 days; Mann-Whitney U-test, P<0.01).

Under short-day conditions (LD8:16), 14 crickets with the compound eye unilaterally removed had a mean duration of nymphal stage of 113.9±43.8 (SD) days. The earliest adult emergence occurred on day 47, after seven molts, and the last on day 177. There were two peaks in adult emergence; one occurred between days 80 and 120 and the other later than day 160 (Fig. 4). The average nymphal period of the early-emerged group was 85.4±22.3 (SD) days, significantly later than that of intact animals under DD (Mann-Whitney U-test, P<0.05) but significantly earlier than that of intact animals under short-day conditions (Mann-Whitney U-test, P<0.01). The average nymphal period of the late-emerged group was 165.2±12.4 (SD) days, significantly longer than that under short-day conditions (Mann-Whitney U-test, P<0.01). In most crickets, the number of molts was apparently greater than that under long-day conditions: the number of crickets that underwent 7, 8~12, and more than 12 molts was 1, 5, and 8, respectively. It was difficult to determine the exact number of molts that each nymph underwent until emergence, however. This is because inter-individual variability in inter-molt periods increased after the 12th molt.

Gross anatomical observation showed that during the course of development, the compound eye on the removed side gradually regenerated. The regenerating compound eye was observed only as a tiny spot in the 2nd instar and reached two-thirds the diameter of the intact eye in the 3rd instar. In adults, it was almost the same size as the intact side, and the optic lobe was only slightly smaller than that of the intact side. Histological examination revealed that the optic lobe was not damaged by the operation, but no lamina or medulla neuropil structures were clearly observed in the optic lobe on the operated side, in either 2nd- or 3rd-instar nymphs (Fig. 5). The neural connection between the retina and the optic lobe may be necessary for formation of normal neuropil structures, as has been suggested for locusts (Anderson, 1978). There was slight regeneration of the nervous connection between the regenerating compound eye and the optic lobe by at least at the 3rd instar (on the 9th day after the operation) (Fig. 5).

### DISCUSSION

Nymphal development of *M. siamensis* is regulated by photoperiod. Under long-day conditions, nymphs grow faster and become adults within 8 weeks at 25°C, whereas nymphal development takes much longer, 12~23 weeks,

under short-day conditions (Tanaka *et al*., 1999; Taniguchi and Tomioka, 2003). The present study not only confirmed these previous results but also revealed that under DD conditions, the crickets show two types of developmental pattern (Fig. 2). Some showed early development, but the duration of the nymphal stage was significantly longer than under long-day conditions. The rest developed slowly, as under short-day conditions. In *M. siamensis*, it has been proposed that photoperiodic control consists of two steps: choice of the faster or the slower developmental rate, and regulation of the number of molts (Taniguchi and Tomioka, 2003). According to this view, although we could not determine the exact number of molts under DD, the more slowly developing group probably underwent more molts.

An explanation of the induction of two different responses in *M. siamensis*, i.e., long-day-like early and short-day-like late development under DD, may be that there are two genetic types corresponding to the response types, as in the photoperiodic response in *Pectinophora gossypiella* (Pittendrigh and Minis, 1971). Long-day-like effects of DD have also been reported in other insects, such as *Pieris brassicae, Acronycta rumicis,* and *Neodiprion sertifer* (Danilevskii, 1965; Sullivan and Wallace, 1965). The effect of DD conditions might be predicted by the internal coincidence model (Pittendrigh, 1972), where two circadian clocks normally synchronizing to either lights-on or lights-off determine the response type according to their phase relationship. However, no anatomical or neurophysiological evidence is so far available for this model.

Since the photoreceptor for the circadian clock is the compound eye in crickets (Loher, 1972; Tomioka and Chiba, 1984; Tomioka *et al*., 1990; Waddel *et al*., 1990), removal of the compound eye or severance of the optic nerve makes the clock run free, even under LD conditions. Partial removal of the compound eye (Tomioka *et al*., 1990) could cause a similar response. However, unilateral removal of the compound eye or unilateral severance of the optic nerve usually allows the clock on the blinded side to be synchronized to the light-dark cycle through a coupling signal from the contralateral clock under LD12:12 (Tomioka *et al*., 1991). The present study revealed significant changes in the time course of nymphal development after unilateral removal of the compound eye (Figs. 3, 4). Since no damage associated with the operation was observed in the optic lobe, and since the sham operation had no apparent effect on nymphal development, the alteration is attributable to the removal of the compound eye. The compound eye gradually regenerated after removal on the 2nd day of hatching, however. This indicates that the removal was incomplete and that some proliferation area remained in the compound eye. The size of the regenerating eye was about two-thirds that of the intact side at the 3rd instar, and the optic lobe on the operated side was almost the same as on the intact side and had a neural connection with the compound eye. These observations suggest that during the first two instar stages, the operated side might acquire the capacity to perceive photic information. However, the neuropil structure was not observed in the optic lobe on the operated side, suggesting that the processing of photoperiodic information would be quite incomplete. Thus, the altered photoperiodic responses in the operated crickets would be attributable to the incom-

plete perception of photoperiods during the stage sensitive to photoperiod, i.e., the 1st- and the 2nd-instar stages (Taniguchi and Tomioka, 2003).

Under long-day conditions, most crickets receiving the unilateral removal of the compound eye showed an increased number of molts and slight but significant lengthening of the nymphal period (Fig. 3). The occurrence of adult emergence was not simply intermediate between those of DD and long-day conditions. Under short-day conditions, there were two peaks in adult emergence of the operated animals; both were significantly later than those of intact animals under DD, and the latter peak even occurred significantly later than that under short-day conditions (Fig. 4). These results could be primarily attributable to the lack of photic input on the operated side, since the sham operation had no apparent effect on nymphal development under long-day conditions. The altered photoperiodic responses of nymphal development might be explained in either of two ways. One is that a pair of compound eyes is required for a complete photoperiodic response, although each compound eye would receive light information and affect the photoperiodic response. Similar importance of the compound eye in the photoperiodic response has been reported for the cricket *Pteronemobius nigrofasciatus* (Shiga and Numata, 1996). In *P. nigrofasciatus*, unilateral removal of the compound eye affected the responses only under short-day conditions: the percentage of diapause eggs laid by adults whose compound eye had been unilaterally removed was intermediate between those laid by intact adults and adults with the compound eyes bilaterally removed. However, the present results were not consistent with the prediction that unilateral compound eye removal would yield responses intermediate between those under DD and the long-day or short-day conditions of intact crickets. It is thus somewhat difficult to explain the present results by a simple summation of the two different photoperiodic inputs.

The other explanation is that the different photoperiodic conditions given to the two compound eyes affect the output of the circadian system through interaction between the circadian clocks (Koga *et al*., 2005). In crickets, a circadian clock is located in each optic lobe, and the bilaterally paired clocks mutually interact through a neural pathway (Tomioka *et al*., 1991; Tomioka, 1993; Yukizane and Tomioka, 1995; Ushirogawa *et al*., 1997). The clocks control the locomotor activity rhythm to be stably nocturnal, probably by excitatory and inhibitory action to the locomotor center located in the central brain (Tomioka, 1993). The coupling between the clocks also plays an important role in determination of the circadian waveform in response to a given photoperiod, and in its maintenance (Koga *et al*., 2005). Unilateral removal of the compound eye disturbs this interaction and results not only in considerable disorganization of the circadian temporal structure (Tomioka *et al*., 1991; Tomioka, 1993; Ushirogawa *et al*., 1997), but also in changes in the waveform of the circadian clock (Koga *et al*., 2005). The significant effects of unilateral compound-eye removal on the post-embryonic development in *M. siamensis* may result from a similar disorganization of the circadian system and changes in the waveform of the optic-lobe circadian clock. An important question related to this hypothesis is whether the circadian clock is functional in the optic lobe despite the

considerable developmental abnormality caused by compound-eye removal. To address these issues, locomotor rhythms in animals with the compound eye unilaterally removed should be carefully examined in future studies.

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