

# Effects of photoperiods on activity level, developmental time, body size and metabolic rate in the nymph of cricket, *Gryllus varius* (Orthoptera: Gryllidae)\*

Xiao-Ting LI\*\* Makio TAKEDA\*\*\*

(Graduate School of Science and Technology, Kobe University, Kobe 657-8501, Japan)

**Abstract** [Objectives] In order to investigate photoperiodic modulation on the behavior and development of the crickets, including the effects on activity level, developmental time, body size and feeding. [Methods] Observation was manipulated by using video camera to monitor the activity of the nymphs and analyzed the behaviors. [Results] Under 12L : 12D, nymphal development took longer, reached a relatively larger body size but the activity level was lower with less defecation than under 16L : 8D. This suggests that the metabolic rate was higher in long photoperiod. The differences between the two photoperiods were statistically significant. The result also indicated that bigger animals tended to live longer at a lower metabolic rate with lower activity level. [Conclusion] The photoperiods definitely affect the development of the crickets. There may be a specific connection that combines the activity level, developmental time, body size and metabolic rate together. The bigger individuals tend to live longer at a lower metabolic rate with lower activity level, while the smaller ones live shorter at higher metabolic rate and activity level.

**Key words** photoperiods, activity, developmental time, body size, metabolic rate, crickets

## 1 Introduction

Photoperiod sometimes affects development in insects (Cymborowski and Giebultowicz, 1976; Ishida *et al.*, 2003; Berkvens *et al.*, 2008). Meanwhile, the causal relationships between behavior, growth, body size and metabolic rate have been examined in some studies (Fernandez *et al.*, 1999; Davidowitz and Nijhout, 2004; Evans *et al.*, 2009; Van Raamsdonk *et al.*, 2010). In general, high metabolic rate supports high activity level and fast development although body size has a complicated relationship with metabolism and longevity (von Bertalanffy, 1951; Davidowitz and Nijhout, 2004; Speakman, 2005).

In this study, we used the Mexican cricket, *Gryllus varius* to investigate photoperiodic modulation on the behavior and development, including activity level, developmental time, body size and feeding.

## 2 Material and methods

### 2.1 Material

The Mexico field cricket, *Gryllus varius* was collected from Teotihuacan, Mexico (19°28'N, 99°09'W) and reared in our laboratory. Rearing of this foreign insect was permitted by Plant Quarantine Service (Shin-shoku 18-220). The eggs were incubated at 27.5°C till the hatching and then were moved to 30°C for nymph breeding under 12L : 12D or 16L : 8D. Nymphs were fed with an artificial mouse food (MF, Oriental Yeast Co. Ltd.) and bottle water.

### 2.2 Activity observation

Observation was manipulated by using video camera (HDR-CX560, Sony Co. Ltd., Tokyo, Japan) to study the activity of the nymphs. One nymph was put in one chamber which is made of

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\*\* First author, E-mail : tatsuya\_711@163.com

\*\*\* Corresponding author, E-mail: mtakeda@kobe-u.ac.jp

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transparent plastic plate (100 mm× 65 mm× 28 mm) and contained solid MF and water. The video recording started from around 40-day old after hatching. Each individual was monitored for 24 h (ZT 0 - ZT 24). Food and water intake, and the movement of body were defined as activity. We divided each hour into 12 phases (0'00'' – 4'59'', 5'00'' – 9'59'', 10'00'' – 14'59''...55'00'' – 59'00'', 5 min per phase; Fig. 1 and Fig. 2), and scored any phase as activity time if the defined activity occurred during the phase. Individual behavior was totally analyzed for 12 h at hourly intervals (ZT0, ZT2, ZT4...ZT22; Fig. 1 and Fig. 2), which was similar to that described by Nishio (2009).

### 2.3 Body size and developmental rate

To simplify the experiment, the pronotum width and the length of metathoracic tibia were measured only for males that emerged from the nymphs reared under 16L : 8D and 12L : 12D, respectively. The developmental time of nymphs from the stock of video observation was analyzed as well, which is the period from hatching to adult emergence.

### 2.4 Feces weight

As an index for metabolic rate, feces of individuals were weighed after the video recording. The analyzed value was the weight of the feces particles during the 24 h activity monitor. We assume that the metabolic rate is proportional to the feces volume, because the defecation was restricted when the energy metabolism decreased in *Caenorhabditis elegans* (van Raamsdonk *et al.*, 2010).

### 2.5 Data analysis and statistics

Independent-sample *t*-Test was used to examine differences in parameter levels under separate photoperiods (12L : 12D and 16L : 8D). The values were based in the text as mean±SE. Fourteen and twelve individuals were tested from stocks under 12L : 12D and 16L : 8D, respectively.

## 3 Results

Fig. 1 and Fig. 2 are the records of behavior for two individuals under 12L : 12D and 16L :

|                 |  |  |  |  |  |  | ZT12 | ZT14 | ZT16 | ZT18 | ZT20 | ZT22 |
|-----------------|--|--|--|--|--|--|------|------|------|------|------|------|
| 0'00''-4'59''   |  |  |  |  |  |  |      |      |      |      |      |      |
| 5'00''-9'59''   |  |  |  |  |  |  |      |      |      |      |      |      |
| 10'00''-14'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 15'00''-19'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 20'00''-24'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 25'00''-29'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 30'00''-34'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 35'00''-39'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 40'00''-44'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 45'00''-49'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 50'00''-54'59'' |  |  |  |  |  |  |      |      |      |      |      |      |
| 55'00''-59'59'' |  |  |  |  |  |  |      |      |      |      |      |      |

Fig. 1 An example of analysis sheet of video recording for the behavior of one nymph under 12L : 12D

White spares stand for activity period, while black ones are motionless period. The animation was analyzed for 12 h with one hour intervals. The number series of the row are 12 phases of one hour recording and each phase contains 5 min. The number series of the line are the 12 phases for the whole one day recording, at hourly intervals. ZT, zeitgeber time. ZT0-ZT10, the light-off period; ZT12-ZT22, the light-on period. The same below.

|                 |  |  |  |  | ZT8 | ZT10 | ZT12 | ZT14 | ZT16 | ZT18 | ZT20 | ZT22 |
|-----------------|--|--|--|--|-----|------|------|------|------|------|------|------|
| 0'00''-4'59''   |  |  |  |  |     |      |      |      |      |      |      |      |
| 5'00''-9'59''   |  |  |  |  |     |      |      |      |      |      |      |      |
| 10'00''-14'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 15'00''-19'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 20'00''-24'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 25'00''-29'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 30'00''-34'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 35'00''-39'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 40'00''-44'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 45'00''-49'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 50'00''-54'59'' |  |  |  |  |     |      |      |      |      |      |      |      |
| 55'00''-59'59'' |  |  |  |  |     |      |      |      |      |      |      |      |

Fig. 2 An example of analysis sheet of the behavior of one nymph under 16L : 8D

8D. The total time of activity was  $(107.07 \pm 6.88)$  min under 12L : 12D, being significantly shorter than that under 16L : 8D, which was  $(150.33 \pm 14.25)$  min ( $t$ -test,  $P < 0.05$ , Fig. 3: A, Table 1).

For the body size, pronotum width and the length of metathoracic tibia were measured to analyze the development under different photoperiods. The pronotum was wider under 12L : 12D ( $5.69 \text{ mm} \pm 0.05 \text{ mm}$ ), compared with that under 16L : 8D ( $5.13 \text{ mm} \pm 0.08 \text{ mm}$ ). Similarly, the length of metathoracic tibia was longer ( $8.89 \text{ mm} \pm 0.18 \text{ mm}$ ) under 12L : 12D than that ( $7.87 \text{ mm} \pm 0.27 \text{ mm}$ ) under 16L : 8D. The difference was significant in both lengths between 12L : 12D and 16L : 8D ( $t$ -test,  $P < 0.01$ , Fig. 3: B, Fig. 3: C, Table 1).

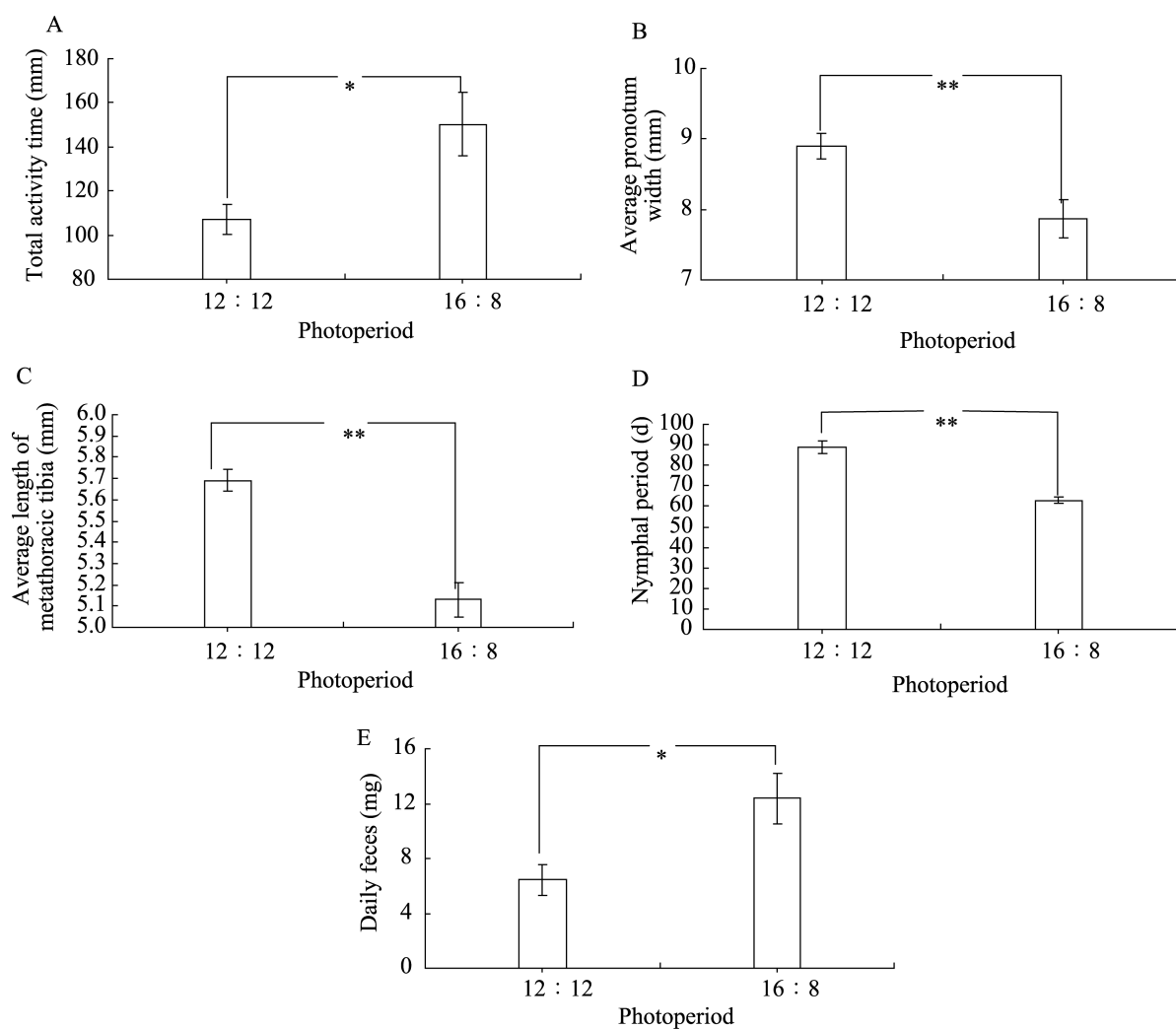
In order to study the effect of photoperiod on the developmental time, the growth period of nymphs was recorded. The individuals grew significantly slower under 12L : 12D than under 16L : 8D ( $t$ -Test,  $P < 0.01$ , Fig. 3: D, Table 1); the periods were  $(88.81 \pm 2.99)$  d under 12L : 12D and  $(63.00 \pm 1.59)$  d under 16L : 8D.

Finally, the daily feces were calculated under two different photoperiods, which were the weight of feces particles collected during the 24 h video

recording. The daily rate of defecation from the individuals under 12L : 12D was significantly less than that under 16L : 8D ( $t$ -test,  $P < 0.05$ , Fig. 3: E, Table 1); the former ( $6.44 \text{ mg} \pm 1.11 \text{ mg}$ ) was approximately half of the latter one ( $12.39 \text{ mg} \pm 1.83 \text{ mg}$ ).

## 4 Discussion

In the present study, the cricket nymphs were reared under two different photoperiods, and they showed significant differences in activity level, body size, developmental time and the rate of defecation. All of the parameters were profoundly affected by photoperiods during the nymph stage. Under 16L : 8D, nymphs grew more rapidly and had longer activity period, which suggests that the crickets were more dynamic under 16L : 8D. The more feces suggest that long photoperiod increased the metabolic rate. The ultimate body size of the adult stage from the tested nymphs was significantly smaller under 16L : 8D. On the other hand, the parameters were affected adversely under 12L : 12D, which showed that the developmental time of nymphal stage was longer. However, the activity time was shorter, fewer daily feces were collected while the body size was bigger under 12L : 12D.



**Fig. 3** Effects of photoperiods on total activity time(A), pronotum width (B), length of metathoracic tibia (C), Nymphal period (D), and daily feces (E)

Vertical bars indicate standard error (SE). \* $P < 0.05$  ( $t$ -test); \*\*  $P < 0.01$ .

**Table 1**  $t$ -test analysis of tested parameters between 12L: 12D and 16L: 8D

| Parameters                | $t$     | $df$   | $P$   |
|---------------------------|---------|--------|-------|
| Activity time             | - 2.735 | 15.997 | 0.015 |
| Pronotum width            | 6.020   | 19.625 | 0.000 |
| Metathoracic tibia length | 3.101   | 19.481 | 0.006 |
| Growth duration           | 7.258   | 24.000 | 0.000 |
| Feces per day             | - 2.772 | 18.496 | 0.012 |

$t$ , the value of the  $t$  statistic;  $P$ , the probability of the  $t$  value having occurred by chance;  $df$ , the degrees of freedom.

There are some reports showing the similar effects of photoperiod modulations on the development in insects. For instance, the larval development of the flour moth (*Ephestia*

*kuehniella*) was faster in the constant light condition than that in the constant dark (Cymborowski and Giebultowicz, 1976). The adult females of western flower thrips (*Frankliniella*

*iccudebtakus*) lived longer under short photoperiod than long photoperiod (Ishida *et al.*, 2003). Berkvens *et al.* (2007) compared the development of *Harmonia axyridis* from different stocks between 16L : 8D and 12L : 12D, which showed that the field population grew faster under the long photoperiod. The cricket, *Velarifictonus ornatus* also developed faster under 16L : 8D than under 12L : 12D (Zhao *et al.*, 2008). Combined with our study, it indicates that various photoperiods definitely affect the development of insects.

On the other hand, the relationships of developmental rate or longevity with metabolism, body size or activity have been studied for a long term to clarify the mechanism of aging and death. The body size has a positive effect on the longevity (Speakman, 2005). In the rats, for instance, the body size is smaller at the higher growth rate (short lifespan) by reducing the body mass that they can accumulate (Davidowitz and Nijhout, 2004). Conversely, the metabolic rate has a negative relationship with the lifespan (Bordone and Guarente, 2005; Evans *et al.*, 2009; van Raamsdonk *et al.*, 2010), and the locomotor activity level showed a similar effect on the lifespan, which suggests that low activity level may extend the lifespan (Fernandez *et al.*, 1999; Evans *et al.*, 2009). In our study, both the metabolic rate and activity level with the developmental time of nymphs-the life period of nymphal stage-presented the similar mutual relationship. Meanwhile, the metabolic rate is higher in smaller animals than in bigger ones (Kleiber, 1947; Speakman, 2005). Particularly, reduced food intake has been shown to slow aging and extend lifespan (Maroso, 2005), which suggests that caloric restriction may retard the growth.

In this paper, we studied activity level, developmental time, body size and metabolic rate systematically and the relationships between these parameters were consistent with other relevant studies. This is the first time to research these parameters together in insects, which broadened the range of phenomena that theory has to explain. We assume that there may be a specific connection that combines the metabolic rate, activity level, developmental time, and body size together. Further study for more other photoperiods is required to clarify the photoperiodic modulation on

the development completely. Finally, investigation of the mechanisms of both the photoperiod effect and hypothesis of networking of the parameters studied here could help us to understand the aging process more deeply.

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# 光照对蟋蟀 *Gryllus varius* (Orthoptera: Gryllidae) 的若虫活动、发育及新陈代谢的影响\*

李晓婷\*\* TAKEDA Makio \*\*\*

(日本神户大学农学研究科 神户 6578501)

**摘 要** 【目的】测试光照对蟋蟀 *Gryllus varius* (Orthoptera: Gryllidae)行为和发育的调节, 包括对活动水平、发育时间、体长以及饮食的影响。【方法】通过摄像机监控蟋蟀若虫的行为活动进行观察并对其行为进行分析研究。【结果】与长光照 (16L : 8D) 相比, 在短光照 (12L : 12D) 下, 蟋蟀若虫发育时间长, 个体大, 但是活动和排泄物都比较少。结果表明, 在长光照下, 若虫的代谢速率快。两种不同光照的影响结果差异性显著。此外, 研究结果表明体型较大的生物存活时间长, 但是代谢速率慢并且活动水平低。【结论】光周期显著影响蟋蟀若虫的发育。活动水平、发育时间、个体大小和代谢速率之间存在着一定的联系, 体型较大的个体存活时间长, 但是代谢速率慢并且活动水平低; 反之, 体型小的个体存活时间短, 代谢速率快并且活动水平高。

**关键词** 光周期, 活动, 发育时间, 体长, 代谢速率, 蟋蟀