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# Independence of genetic variation between circadian rhythm and development time in the seed beetle, *Callosobruchus chinensis*

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ABSTRACT

A positive genetic correlation between periods of circadian rhythm and developmental time supports the hypothesis that circadian clocks are implicated in the timing of development. Empirical evidence for this genetic correlation in insects has been documented in two fly species. In contrast, here we show that there is no evidence of genetic correlation between circadian rhythm and development time in the adzuki bean beetle, *Callosobruchus chinensis*. This species has variation that is explained by a major gene in the expression and period length of circadian rhythm between strains. In this study, we found genetic variation in development time between the strains. The development time was not covaried with either the incidence or the period length of circadian rhythm among the strains. Crosses between strains suggest that development time is controlled by a polygene. In the $F_2$ individuals from the crosses, the circadian rhythm is attributable to allelic variation in the major gene. Across the $F_2$ individuals, development time was not correlated with either the expression or the period length of circadian rhythm. Thus, we found no effects of major genes responsible for variation in the circadian rhythm on development time in *C. chinensis*. Our findings collectively give no support to the hypothesis that the circadian clock is involved in the regulation of development time in this species.



## 1. Introduction

Endogenous time-keeping mechanisms, which produce rhythms in biological processes with a period close to a day, are called circadian clocks, and are present in a wide variety of organisms (Edmunds, 1988; Panda et al., 2002). In insects, these circadian clocks regulate timing of various daily events such as mating, reproduction, foraging, feeding, or rest (Miyatake, 2002a; Saunders, 2002; Tauber and Kyriacou, 2008). It is hypothesized that the circadian clocks are key elements controlling the timing of development, with faster or slower ticking of the clocks speeding up or slowing development, respectively (Kyriacou et al., 1990; Shimizu et al., 1997; Paranjpe et al., 2005). Evidence for this hypothesis comes primarily from the positive genetic correlation between period length of circadian rhythm and development time.

This genetic correlation between circadian and developmental periods has been shown in two fly species. In *Drosophila melanogaster*, mutation on the *period* (*per*) locus markedly alters the circadian periods ($\tau$) of eclosion and adult locomotor activity (Konopka and Benzer, 1971). Compared to those of the wild type ($\tau$ = 24 h) a study of *per* mutants found that a mutant ($per^S$) with short circadian period ($\tau$ = 19 h) developed faster, whereas another mutant ($per^L$) with long circadian period ($\tau$ = 28 h) developed slower under constant light, constant darkness, and light–dark cycle of 12:12 h (Kyriacou et al., 1990). In the melon fly, *Bactrocera cucurbitae*, artificial selection for faster and slower development produced a drastic divergence between the selection lines in development time under light–dark cycle of 14:10 h (Miyatake, 1995). The circadian period of adult locomotor activity in constant darkness was shorter in the lines with shorter development time ($\tau$ = 22.5–22.6 h) than in the lines with longer development time ($\tau$ = 26–31 h) in this species (Shimizu et al., 1997). The genetic correlation between circadian rhythm and development time may be ubiquitous because the core molecular mechanisms underlying circadian clocks are highly conserved (Dunlap et al., 2004). However, it is impossible to evaluate whether such genetic correlation indeed occurs more broadly in insects as thus far all empirical evidence has been restricted to those two species of Diptera.

Genetic variation in the circadian rhythm of adult locomotor activity has been demonstrated between strains of the adzuki bean beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae) (Harano and Miyatake, 2010). Beetles in some strains clearly retained circadian rhythms in constant darkness whereas most beetles in other strains were arrhythmic. Where present the circadian period

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varied from approximately 19 to 23 h between the strains. Crosses between strains revealed that the circadian rhythm was inherited autosomally and under a major gene, one or a few genes having a major effect, in *C. chinensis* (Harano and Miyatake, 2010). This major gene may have an effect on the timing of development and thus cause a genetic correlation between circadian rhythm and development time, as in the dipteran species described above. If this is the case, development time is expected to covary with circadian rhythm among the strains. Moreover, development time is expected to be associated with circadian rhythm, which is attributable to allelic variation in the major gene, across individuals in the second ($F_2$) generation of crosses between different strains.

In this study, we found variation in development time between the *C. chinensis* strains. We tested whether development time was correlated with the incidence (the proportion of individuals that expressed circadian rhythm) or period length of circadian rhythm among the strains. We also tested whether development time was correlated with expression or period length of circadian rhythms in $F_2$ individuals from crosses between the strains.

## 2. Materials and methods

### 2.1. Study animals

*C. chinensis* is a broad-based pest of stored legumes, such as the adzuki bean, *Vigna angularis* and the cowpea, *V. unguiculata* (Kiritani, 1961). Adult females lay eggs on the surface of host beans. Hatched larvae burrow into the bean in which they complete their development, pupation and eclosion. As adults can reproduce without feeding, in the laboratory the adults generally have no access to either food or water and are simply provided with beans as oviposition substrate. We used seven strains of *C. chinensis* (Table 1). For adult males from each of these strains, the circadian rhythms of locomotor activity in constant darkness were measured by Harano and Miyatake (2010) and are given in Table 2. Before experiments, stock cultures of these strains had been maintained as mass cultures on adzuki beans under 25 °C, 50% relative humidity and photoperiod cycle of 14:10 h light–dark conditions.

### 2.2. Assay of development time

To reduce maternal effects, all mothers were reared at a density of up to three larvae per adzuki bean and allowed to oviposit for 24 h after they mated once during 48 h post-emergence. To exclude effects of larval competition on development, larvae were allowed to develop at one egg per bean by scraping excess eggs from the bean. The beans were maintained under 25 °C, 60% relative humidity and photoperiod cycle of 16:8 h light–dark conditions and checked every day for adult emergence. Egg-to-emergence development time was recorded for all adults that emerged.

### 2.3. Crosses

We made reciprocal crosses of two combinations of strains. Cross 1 was between smC02, which had the shortest period and a low incidence of circadian rhythm, and jC-S, which had the longest circadian period of all of the strains (Table 2). Cross 2 was between yoC02, which had the shortest circadian period among the strains with a high incidence of circadian rhythm, and jC-S as above (see Table 2). In the first ($F_1$) and second ($F_2$) generations of the crosses, egg-to-emergence development time was examined as described above. The circadian rhythm of adult locomotor activity in the $F_2$ males was measured by Harano and Miyatake (2010).

### 2.4. Statistical analysis

We analyzed development time using an analysis of variance (ANOVA), with strain, sex, and their interaction as factors, and used the Tukey's HSD method for the post hoc comparisons between strains. An ANOVA was used to compare development time between the $F_1$ of reciprocal crosses. Because of unequal variances, Welch's ANOVA was used to compare development time between

**Table 1**
The rearing history of *Callosobruchus chinensis* strains used in this study.

| Strain | Locality of population | Collection date | Number of founder adults | Reference |
|---|---|---|---|---|
| jC-S | Kyoto, Kyoto, Japan | 1936 | Unknown | Utida (1941a,b) |
| mC | Morioka, Iwate, Japan | 1960s | Unknown | Nakamura (1969) |
| isC | Ishigaki, Okinawa, Japan | 1997 | About 200 | Yanagi and Miyatake (2003) |
| rdaCmrkt | Rajshahi, Bangladesh | 1998 | More than 50 | Harano and Miyatake (2007) |
| yoC02 | Akaiwa, Okayama, Japan | 2002 | 26 | Harano and Miyatake (2005) |
| smC02 | Izumo, Shimane, Japan | 2002 | More than 20 | Harano and Miyatake (2005) |
| kiC07 | Inami, Hyogo, Japan | 2007 | More than 100 | Harano and Miyatake (2010) |

**Table 2**
Egg-to-emergence development time in strains that differ in the incidence and period length of circadian rhythm.

| Strain | Circadian rhythm[a] | | Development time (day) | | | | |
|---|---|---|---|---|---|---|---|
| | Incidence (%) | Period (h) | Female | | Male | | Least square |
| | | Mean ± SD | *N* | Mean ± SD | *N* | Mean ± SD | Mean ± SE[c] |
| rdaCmrkt | 11.1 | (21.4)[b] | 81 | 28.0 ± 2.1 | 61 | 26.7 ± 1.9 | 27.4 ± 0.2ab |
| mC | 28.6 | (18.8 ± 1.1)[b] | 48 | 30.9 ± 2.1 | 42 | 30.6 ± 2.3 | 30.7 ± 0.3d |
| smC02 | 33.3 | 18.7 ± 0.9 | 57 | 28.8 ± 2.5 | 64 | 28.0 ± 2.4 | 28.4 ± 0.2c |
| yoC02 | 100.0 | 20.1 ± 1.7 | 44 | 28.5 ± 3.0 | 50 | 27.9 ± 2.4 | 28.2 ± 0.3bc |
| isC | 100.0 | 20.0 ± 1.0 | 39 | 31.8 ± 3.4 | 45 | 31.3 ± 2.7 | 31.6 ± 0.3d |
| kiC07 | 84.2 | 21.0 ± 1.1 | 77 | 27.7 ± 2.4 | 65 | 26.3 ± 2.3 | 27.0 ± 0.2a |
| jC-S | 95.2 | 22.6 ± 0.7 | 95 | 29.5 ± 2.7 | 103 | 28.2 ± 2.3 | 28.8 ± 0.2c |

[a] Data were derived from Harano and Miyatake (2010).
[b] Sample size were extremely small: rdaCmrkt, $N = 1$; mC, $N = 2$ (Harano and Miyatake, 2010).
[c] Different letters indicate significant differences at $P < 0.05$ by the Tukey HSD method.