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ORIGINAL ARTICLE

# Extent of mismatch between the period of circadian clocks and light/dark cycles determines time-to-emergence in fruit flies

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**Abstract** Circadian clocks time developmental stages of fruit flies *Drosophila melanogaster*, while light/dark (LD) cycles delimit emergence of adults, conceding only during the "allowed gate." Previous studies have revealed that time-to-emergence can be altered by mutations in the core clock gene *period* (*per*), or by altering the length of LD cycles. Since this evidence came from studies on genetically manipulated flies, or on flies maintained under LD cycles with limited range of periods, inferences that can be drawn are limited. Moreover, the extent of shortening or lengthening of time-to-emergence remains yet unknown. In order to pursue this further, we assayed time-to-emergence of *D. melanogaster* under 12 different LD cycles as well as in constant light (LL) and constant dark conditions (DD). Time-to-emergence in flies occurred earlier under LL than in LD cycles and DD. Among the LD cycles, time-to-emergence occurred earlier under *T4–T8*, followed by *T36–T48*, and then *T12–T32*, suggesting that egg-to-emergence duration in flies becomes shorter when the length of LD cycles deviates from 24 h, bearing a strong positive and a marginally negative correlation with day length, for values shorter and longer than 24 h, respectively. These results suggest that the extent of mismatch between the period of circadian clocks and environmental cycles determines the time-to-emergence in *Drosophila*.

**Key words** circadian; *Drosophila*; light/dark regimes; period; time-to-emergence

## Introduction

Circadian (*circa* = *about*; *dies* = *a day*) clocks with near 24-h periodicities regulate behavioral and metabolic processes in a wide variety of organisms (Saunders, 2002; Dunlap *et al.*, 2004). In insects including fruit flies *Drosophila melanogaster*, these clocks have also been implicated in the regulation of life-history traits such as preadult development time and adult lifespan (Kyriacou *et al.*, 1990; Miyatake, 1996, 1997; Shimizu *et al.*, 1997; Klarsfeld & Rouyer, 1998; Sheeba *et al.*, 1999b; Paranjpe *et al.*, 2005; Takahashi *et al.*, 2013; Yadav & Sharma, 2013, 2014). It is believed that preadult development time of flies with faster circadian clocks is shorter than those with slower clocks. While this proposal still awaits rigorous and unequivocal empirical support, previous studies in fruit flies *D. melanogaster* (Kyriacou *et al.*, 1990; Paranjpe *et al.*, 2005; Kumar *et al.*, 2006; Takahashi *et al.*, 2013; Yadav & Sharma, 2013), and in melon flies *Bactrocera cucurbitae* (Miyatake, 1996, 1997; Shimizu *et al.*, 1997) have shown that the period of circadian activity/rest rhythm is correlated with the egg-to-adult duration.

Timing of adult emergence in *Drosophila* depends upon a number of factors including developmental state of the fly, phase and period of circadian rhythms, and on

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external light/dark (LD) conditions (Qiu & Hardin, 1996; Saunders, 2002; Paranjpe *et al.*, 2005; Lone & Sharma, 2008). It is believed that LD cycles create a "forbidden zone" which restricts emergence of adults to a narrow window of time called as the "allowed zone" or the "gate of emergence" (Qiu & Hardin, 1996; Saunders, 2002). An adult fly would emerge out of its puparium only if it is mature enough to do so when the emergence gate is open on that particular day, otherwise it has to wait for the gate to open on the next day. Similarly, in insects including *Drosophila*, egg-hatching (Pittendrigh, 1966; Minis & Pittendrigh, 1968; Nayar *et al.*, 1973; Lazzari, 1991), pupation (Bakker & Nelissen, 1963; Nayar, 1967; Pittendrigh & Skopik, 1970; Jones & Reiter, 1975), and wing-pigmentation (Harker, 1964, 1965) have also been shown to be under circadian clock control.

The role of circadian clocks in the regulation of preadult developmental duration has been quite extensively studied in the *period* (*per*) mutants of *D. melanogaster* (short period $per^S$ flies with clock period [$\tau$] of ~19 h, long period $per^L$ flies with $\tau$ of ~28 h and loss of function $per^{01}$ mutants; Kyriacou *et al.*, 1990). The preadult development time of flies is found to be positively correlated with the clock period, under constant darkness (DD) where clocks of flies free-run, or in constant light (LL) and very bright constant light (VLL) where it is known to be dysfunctional, or under LD cycles of 12 : 12 h or in LD cycles coupled with 12 : 12 h temperature cycles where it is entrained. Given that preadult development time of short period ($per^S$) mutants is shorter than wild-type controls, which in turn is shorter than the long period mutants ($per^L$), irrespective of the state of their circadian clocks, suggests that *per* mutation has pleiotropic effects on the preadult development time and the period of circadian clocks (Kyriacou *et al.*, 1990). Such pleiotropic effects are not uncommon at the *per* locus of *Drosophila*, as it is known that *per* mutation also alters the interpulse interval (ipi) in courtship song, almost in the same way as it changes the period of circadian rhythms (Kyriacou & Hall, 1980; Konopka *et al.*, 1996). The $per^S$ males have ipi of ~40 sec, $per^L$ of ~76 sec and $per^{01}$ males are arrhythmic. Taken together, these studies suggest that *per* mutations have similar effect on the length of the circadian cycle, length of courtship song and the preadult duration.

Correlation between $\tau$ and preadult development time has also been reported in studies on wild-type fly populations selected for differences in their preadult developmental duration (Miyatake, 1996, 1997; Shimizu *et al.*, 1997; Takahashi *et al.*, 2013; Yadav & Sharma, 2013), or for morning and evening adult emergence (Kumar *et al.*, 2006). For example, in a study on melon flies *B. cucurbitae*, populations selected for shorter preadult development time were found to have faster running clocks ($\tau \sim 22.6$ h) and advanced time of mating, while those selected for longer development time have slower running clocks ($\tau \sim 30.9$ h) and delayed time of mating (Miyatake, 1996, 1997; Shimizu *et al.*, 1997). Recently, populations of *D. melanogaster* selected for shorter (Yadav & Sharma, 2013) or longer preadult development time (Takahashi *et al.*, 2013) have also been found to display a similar correlation between $\tau$ and preadult development time. Similarly, in a study on *D. melanogaster*, where fly populations were selected for morning (early) or evening (late) adult emergence; morning and evening emerging flies were found to have evolved altered time course and waveform of emergence, faster and slower circadian clocks, and shorter and longer preadult development time, respectively (Kumar *et al.*, 2006). However, in these studies also it is difficult to rule out possible pleiotropic effects of clock genes on circadian rhythms and development time.

In a separate study aimed at bypassing any possible pleiotropic control of clock genes, period of the emergence rhythm of wild-type populations of *D. melanogaster* was manipulated by exposing flies to non-24 h LD cycles of short (20 h) or long (28 h) periodicity (Paranjpe *et al.*, 2005). Since the emergence rhythm of *Drosophila* entrains to a wide range of LD cycles around 24 h (Paranjpe *et al.*, 2003), this would result in altered timing of the allowed zone of emergence, which in turn would cause changes in time-to-emergence. Indeed, flies reared under short (20 h) LD cycles exhibit short period rhythm and take less time to develop than their counterparts maintained under 24 h LD cycles, while flies raised under long (28 h) LD cycles have long period rhythm but take as much time to develop as those maintained under 24-h LD cycles. This suggests a possible effect of the period of LD cycles, and/or of the adult emergence rhythm on the time-to-emergence in *Drosophila*. However, it is still unclear if flies take shorter or longer to develop under short or long LD cycles because their circadian clocks run faster or slower, or because they were raised under short or long period LD cycles. This ambiguity arose because the period of the LD cycles used in this study (20, 24, and 28 h) was well within the range of entrainment of *Drosophila* circadian clocks (Paranjpe *et al.*, 2003). It would therefore be interesting to examine this phenomenon under a wide range of LD cycles with extremely short or long period. This will also ascertain to what extent the length of LD cycles can shorten or lengthen the preadult duration in *Drosophila*.

With the aim to study the effects of LD cycles on the time-to-emergence in *D. melanogaster*, we assayed the egg-to-adult duration of flies under 14 light/dark

regimes–LL, DD, LD cycles of 2 : 2 h (*T4*), 4 : 4 h (*T8*), 6 : 6 h (*T12*), 8 : 8 h (*T16*), 10 : 10 h (*T20*), 12 : 12 h (*T24*), 14 : 14 h (*T28*), 16 : 16 h (*T32*), 18 : 18 h (*T36*), 20 : 20 h (*T40*), 22 : 22 h (*T44*), and 24 : 24 h (*T48*). The results provided interesting insights into the effect of interaction between circadian clocks and LD cycles on the time-to-emergence in *Drosophila*. While the mean time-to-emergence of females is shorter than that of males, across light regimes the mean time-to-emergence of both males and females is shorter under LL compared with LD cycles and DD. Under LD cycles, time-to-emergence of flies is positively correlated with the period length of the LD cycles for day lengths shorter than 24 h, while it is negatively correlated with the length of the LD cycles for day lengths longer than 24 h, suggesting that, the extent of mismatch between the period of circadian clocks and LD cycles determines time-to-emergence in *D. melanogaster*.

## Materials and methods

### *Laboratory population maintenance*

Eggs were collected from running culture of a large outbred population of fruit flies *D. melanogaster*, maintained under LL for several hundred generations (Sheeba *et al.*, 1999a). The baseline population was reared on a 21-d discrete (non-overlapping) generation cycle, inside a cubicle, illuminated with ~100 lux light intensity maintained at ~25 °C temperature and relative humidity of 70%–80%. The population consisted of ~1 200 adults (roughly equal number of males and females) kept in a plexi glass cage (25 cm × 20 cm × 15 cm) containing banana–jaggery food (henceforth will be referred as banana food). To start a new generation, adult flies were provided with banana food supplemented with live yeast paste for 2 d, after which they were allowed to lay eggs for about 12 h on fresh banana food in a Petri plate. From these Petri plates, approximately 60–80 eggs were taken and dispensed into glass vials (9 cm height × 2.4 cm diameter), containing ~4-mL banana food in which larvae develop into adults. Adult flies emerging from 24 such vials were transferred into a plexiglass cage on the 12th day after egg collection, which formed the breeding population for the next generation. To start a new generation, next set of eggs were collected from the cage after 21 d from the previous egg collection date.

### *Time-to-emergence assay*

The assay was performed in 2 incubators maintained at temperature and relative humidity of 25 ± 0.3 °C (mean ± SD) and 70%–80%, respectively. Flies from the running culture of *D. melanogaster* were allowed to lay eggs for a period of 1 h on banana food plate. In order to avoid the antecedent eggs retained in female body, the plate was then replaced by another fresh food plate for the next 2 h. Exactly 30 eggs were collected and introduced into vials containing ~4 mL banana food. Eight replicate vials containing eggs were simultaneously introduced into each of the 14 light regimes–LL, DD and LD cycles of 2 : 2 h (*T4*), 4 : 4 h (*T8*), 6 : 6 h (*T12*), 8 : 8 h (*T16*), 10 : 10 h (*T20*), 12 : 12 h (*T24*), 14 : 14 h (*T28*), 16 : 16 h (*T32*), 18 : 18 h (*T36*), 20 : 20 h (*T40*), 22 : 22 h (*T44*), and 24 : 24 h (*T48*). A total of 112 vials (8 vials × 14 light regimes) were simultaneously introduced into the LD cycles as soon as the lights went off. The LD cycles were created using 2 temperature (~25 °C) and humidity (~75%) controlled incubators. Temperature and humidity inside the incubators were monitored throughout the experiment at regular intervals and were found to be fairly stable. The light phase of the LD cycles and LL was created using fluorescent white light of intensity ~100 lux, and red light of $\lambda > 650$ nm was used during the dark phase of the LD cycles and DD. For creating different LD regimes, 2 incubators–one (*Inc-I*) constantly illuminated by fluorescent light of ~100 lux intensity and the other (*Inc-II*) by dim red light ($\lambda > 650$ nm) were used. Vials with eggs were moved from light to dark and dark to light incubators, at specific times of the day, to create different *T* cycles. For example, to create 12 : 12 h LD cycles (*T24*), flies were first kept for 12 h in *Inc-I* and then in *Inc-II* for the subsequent 12 h, after which they were transferred back to *Inc-I*. This was repeated until the completion of the study. A similar protocol was followed to create other LD cycles. However, vials with eggs remained under LL (in *Inc-I*) or DD (*Inc-II*) throughout the assay. The experimental protocol (shifting of vials from LL to DD or *vice versa*) is followed in order to avoid microenvironmental changes that may occur if 14 different experimental regimes were to be established. Time-to-emergence assay under 14 different light regimes was performed in a single experiment.

Beginning the 6th day after egg collection, that is, after pupae became dark, vials were continuously monitored for emergence, and emerging adults were collected every 2 h, sexed and counted. Time-to-emergence was estimated by multiplying the number of adult flies that emerged in 2 h window with the total duration of time from the egg stage. Time-to-emergence was calculated from the midpoint of the 2-h egg collection window to the midpoint of 2-h window during which the fly emerged as adult. The mean time-to-emergence was estimated by dividing the total egg-to-adult duration of flies in

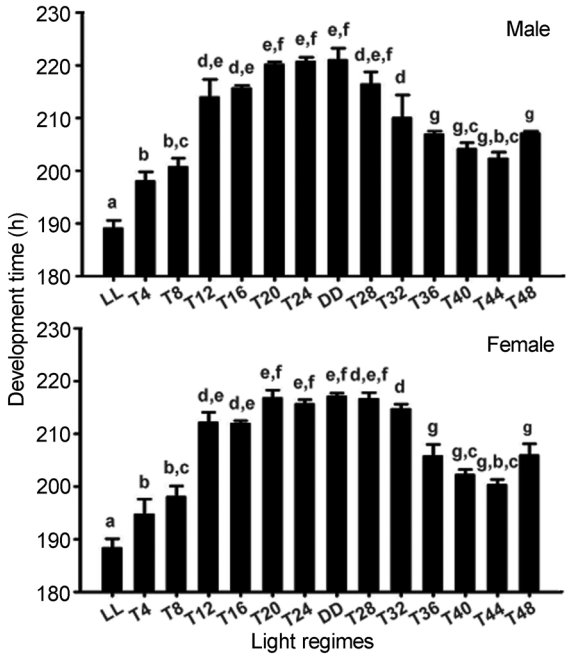


**Fig. 1** Mean time-to-emergence. Mean time-to-emergence of male (upper panel) and female (lower panel) fruit flies under 14 different light regimes, showing effects of light regimes. A total of 8 vials (30 eggs per vial) were used in each of the 14 light regimes. The error bars are standard error around the mean (SEM) estimated from flies emerging from 8 vials used per light regime, obtained from a single experiment. The bars which do not share letters represent statistically significant difference(s).

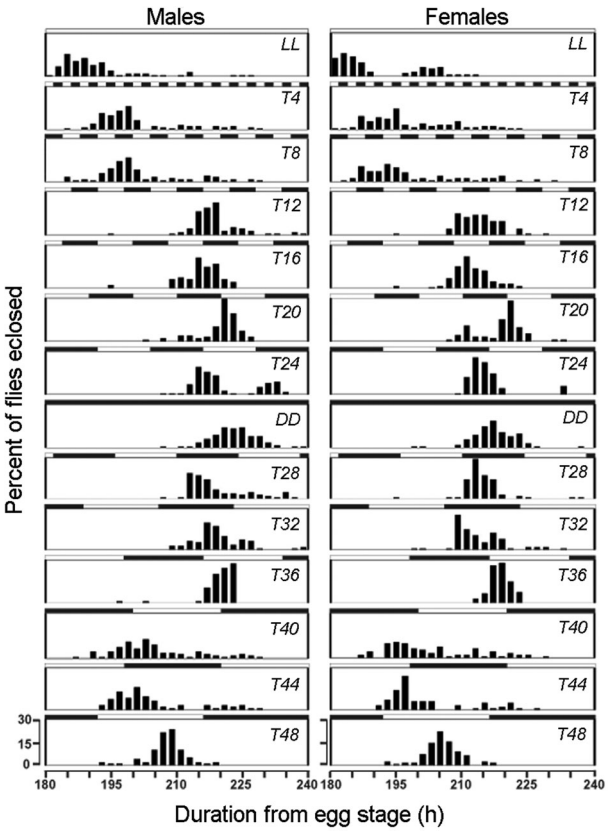


**Fig. 2** Adult emergence profile. Emergence profile of male (left panel) and female (right panel) fruit flies under 14 different light regimes. Time-to-emergence (in hours) is plotted along the *x*-axis and percentage of flies emerged along the *y*-axis. The black and white horizontal bars represent dark and light portions of the LD cycles, while completely white and completely black regions represent LL and DD regimes, respectively.

a vial by the total number of adults that emerged out of it.

*Statistical analyses*

The effect of light regimes on the time-to-emergence was assessed using two-way analysis of variance (ANOVA) considering light regime (*L*) and sex (*S*) as fixed factors, followed by *post hoc* multiple comparisons using Tukey's Honestly Significant Difference (HSD) test. Correlation analyses were performed using Pearson product–moment correlation. All statistical analyses were done using Statistica for Windows (StatSoft Inc., 1995).

## Results

Time-to-emergence of males and females is found to be shorter under LL compared to LD and DD (Fig. 1). Under LD cycles, time-to-emergence of males and females is shorter when flies are reared under LD cycles of period shorter than 24 h (*T4* and *T8*) or longer than 24 h (*T36*, *T40*, *T44*, and *T48*); however, flies take similar length of time to emerge as adults under *T12*, *T16*, *T20*, *T24*, *T28*, and *T32* (Fig. 1). Among the extremely long *T* cycles of *T36*, *T40*, *T44*, and *T48*, time-to-emergence of flies does not differ statistically (Fig. 1). Females developed faster than males under most *T* cycles; however, emergence profile of males and females are similar (Fig. 2). The emergence profile of flies is widely distributed (span of ~40 h) under extreme light regimes (LL, *T4*, *T8*, *T40*, *T44*, and *T48*), whereas it is relatively consolidated (span of ~15 h) under all other conditions (Fig. 2). While time-to-emergence under LL can solely to be attributed to the innate developmental rate of flies, under DD it is likely to be due to circadian gating, and in *T* cycles to emergence gating as determined by the LD cycles.

**Table 1** Results of ANOVA on the time-to-emergence data.

(A) Results of ANOVA on the time-to-emergence data under various light regimes.

| Effect | df | MS effect | df error | MS error | $F$ | $P$ |
|---|---|---|---|---|---|---|
| Light ($L$) | 13 | 1307.26 | 186 | 22.82 | 57.28 | 0.0001 |
| Sex ($S$) | 1 | 191.37 | 186 | 22.82 | 8.39 | 0.004 |
| $L \times S$ | 13 | 21.64 | 186 | 22.82 | 0.95 | 0.504 |

(B) Results of the correlation analyses of time-to-emergence in males

| LD cycles | Mean | $r(x,y)$ | $r^2$ | $P$ | $N$ | Constant $y$ | Slope $y$ | Constant $x$ | Slope $x$ |
|---|---|---|---|---|---|---|---|---|---|
| *T4–T24* | 211.48 | +0.87 | 0.75 | 0.0001 | 47 | 194.44 | +1.23 | −116.14 | 0.61 |
| *T28–T48* | 207.91 | −0.47 | 0.22 | 0.001 | 45 | 226.67 | −0.49 | +132.91 | 0.46 |

(C) Results of the correlation analyses of time-to-emergence in females

| LD cycles | Mean | $r(x,y)$ | $r^2$ | $P$ | $N$ | Constant $y$ | Slope $y$ | Constant $x$ | Slope $x$ |
|---|---|---|---|---|---|---|---|---|---|
| *T4–T24* | 205.08 | +0.81 | 0.64 | 0.0001 | 47 | 192.22 | +1.14 | −103.07 | +0.56 |
| *T28–T48* | 207.73 | −0.66 | 0.44 | 0.0001 | 43 | 234.12 | −0.69 | +170.09 | −0.64 |

ANOVA on the mean time-to-emergence data revealed statistically significant effects of light regime ($L$) ($F_{13,186} = 57.28$, $P < 0.0001$) and sex ($S$) ($F_{1,186} = 8.39$, $P < 0.004$); however, the effect of $L \times S$ interaction is statistically not significant ($F_{13,186} = 0.95$, $P = 0.5$; Table 1, part A). Thus, while females take less time to emerge than males; light regimes alter time-to-emergence of males and females in a similar manner. *Post hoc* multiple comparisons revealed that under LL, time-to-emergence is significantly reduced compared to all the LD regimes and DD. On the other hand, time-to-emergence in DD does not differ statistically from *T24*. Time-to-emergence is shorter under *T4–T8*, followed by *T36–T48*, and then *T12–T32* (Fig. 1). However, time-to-emergence of flies does not differ under LD cycles of period close to 24 h (*T12*, *T16*, *T20*, *T28*, *T32*; Fig. 1).

The mean time-to-emergence of flies is positively correlated with the period of LD cycles for day lengths between 4 and 24 h ($r = +0.87$, $P < 0.0001$ for males and $r = +0.81$, $P < 0.0001$ for females; Fig. 3; Table 1, part B and C), and negatively correlated with the period of LD cycles for day lengths between 28 and 48 h ($r = -0.47$, $P = 0.001$ for males and $r = -0.66$, $P < 0.0001$ for females; Fig. 3; Table 1, part B and C).

## Discussion

The preadult development time of many insects is known to be responsive to changes in the environmental conditions (Saunders, 2002). Time-to-emergence in *Drosophila* is determined by an outcome of the intricate interaction between the developmental state of the fly and its circadian gating (Qui & Hardin, 1996; Saunders, 2002). Since most circadian rhythms of *Drosophila* are abolished under LL, gating of emergence would be expected to be absent, and therefore developing individuals would enter the subsequent developmental stages without any delay. Hence developmental stages would be expected to complete faster. On the other hand, under LD cycles, time-to-emergence is likely to be determined by an interaction between the developmental clocks and gating created by the LD cycles, which is likely to cause advance/delay in the time-to-emergence, depending on the length of the LD cycles. Under DD where circadian clocks free-run with endogenous periodicity close to 24 h, time-to-emergence is likely to be determined by the interaction between developmental states of the fly and circadian gating (Qui & Hardin, 1996), and therefore time-to-emergence would be expected to be comparable to that in *T24*. Indeed, time-to-emergence is found to be shorter under LL compared with *T24* and DD (Sheeba *et al.*, 1999b; Paranjpe *et al.*, 2005; Figs. 1 and 2).

Circadian clocks entrain quite readily to LD cycles, however, this is restricted to LD cycles within a range of periodicity around 24 h. Time-to-emergence of flies maintained under LD cycles of length much shorter than 24 h decreases proportionately with the length of the LD cycles (Fig. 1), suggesting that time-to-emergence is a function of the period of the LD cycles. However, time-to-emergence does not increase when the period of the LD cycles increases beyond 24 h. On the contrary, it shows marginal decrease when the length of the LD cycles increases above 24 h. Hence, it appears, all else being equal, time-to-emergence of *Drosophila* cannot be lengthened beyond its value under the LD 12 : 12 h, at least not by lengthening the period of LD cycles. Perhaps the only way to further slow down development by light, would be

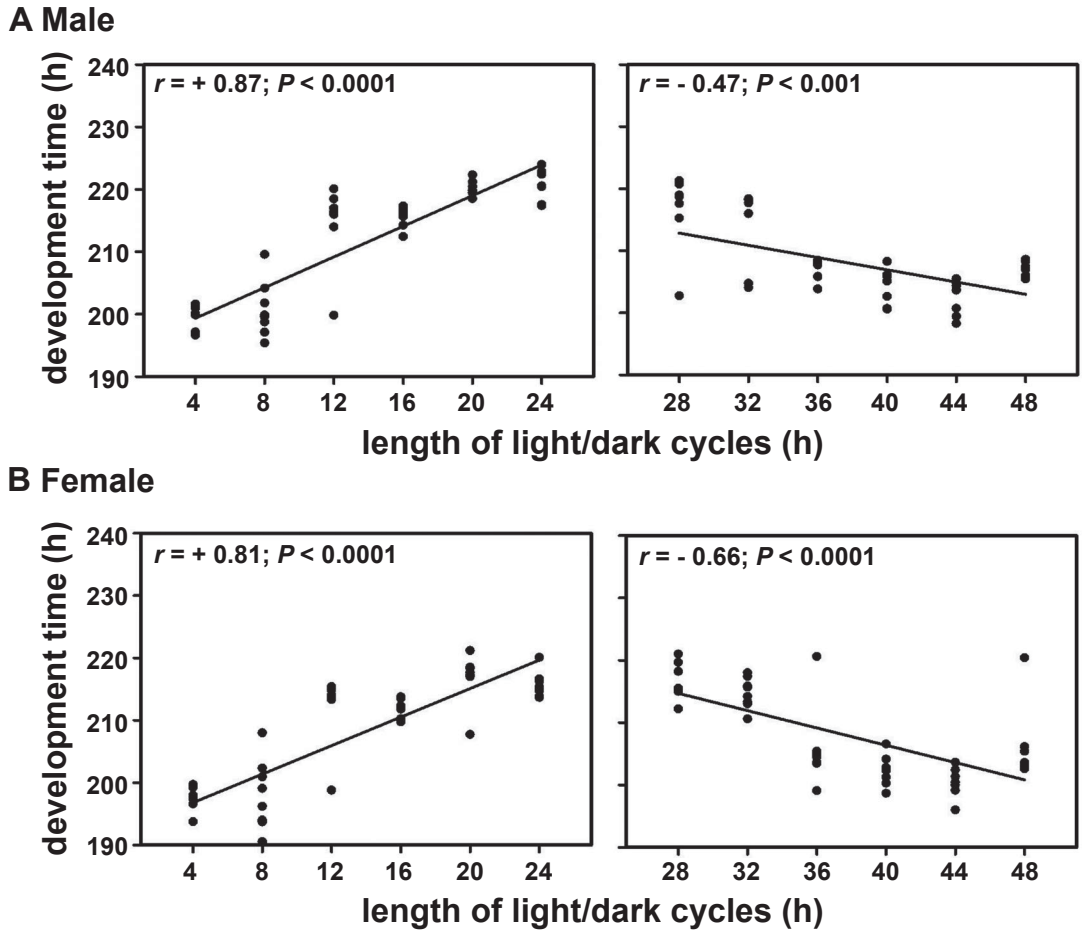


**Fig. 3** Correlation between the time-to-emergence and the length of light/dark (LD) cycles. The time-to-emergence in male (A) and female (B) fruit flies shows a significant positive correlation with the length of LD cycles between *T4* to *T24*, and a marginally negative correlation with the length of LD cycles between *T28* to *T48*. Mean time-to-emergence in hours is plotted along the *y*-axis, and the length of the light/dark (LD) cycles in hours is plotted along the *x*-axis.

either by manipulating the light intensity or by altering the photoperiod.

The frequency demultiplication hypothesis posits that circadian rhythms can be entrained by LD cycles of period lengths that are multiples or submultiples of circadian period (Nayar & Sauerman, 1971; Gwinner 1973; Carmichael & Zucker, 1986; Nisimura & Numata, 2002). Since *T4*, *T8*, *T12*, and *T48* are submultiples or multiples of 24 h, circadian clocks under these LD cycles are likely to entrain, and therefore time-to-emergence would be expected to occur at a rate comparable to that of *T24*. However, in this study we do not find any evidence of frequency demultiplication in the effect of LD cycles on the time-to-emergence except for *T12* (Fig. 1). The limits of entrainment of adult emergence rhythm in *Drosophila* to LD cycles comprise at least *T18* and *T30* (Bünning, 1973; Paranjpe *et al.*, 2003). With the exception of a few light regimes, peak of adult emergence invariably occurs during the light phase of the LD cycles (Fig. 2), which suggests that the phase of circadian rhythm also plays a significant role in the determination of time-to-emergence in *Drosophila* (Paranjpe *et al.*, 2005). Moreover, results from the current study are consistent with those from an extensive study on flesh flies *Sarcophaga argyrostoma*, which reported lengthening of preadult development time under *T24* or *T48* compared with *T36* or *T60* (Saunders, 1972, 2002). In addition, photophase of 12, 14, and 16 h have also been shown to lengthen the preadult development time (Saunders, 1972). Hence lengthening of time-to-emergence under *T24*, *T28* and *T32* in our present study is not entirely surprising (Fig. 1). On the other hand, since LD cycles such as *T36*, *T40*, and *T44* do not comprise of photophases of 12, 14, or 16 h, time-to-emergence is comparatively shortened under these conditions (Fig. 1).

Interestingly, a recent study revealed that the preadult development time of 2 sympatric species of *Camponotus* ants, is in fact shorter under *T24* compared to other non-*T24* regimes (Lone *et al.*, 2010, 2011). Therefore, it is likely that mechanisms underlying light mediated clock regulation of time-to-emergence are not conserved across flies and ants.

Flies used in this study have been maintained for hundreds of generations on a fixed 9-d developmental schedule, and therefore the optimal preadult developmental time of these flies is ~9 d. Any alteration in the development time would be expected to accompany some fitness cost, because of trade-off between preadult development time and survivorship (Prasad & Joshi, 2003 references therein). However, we do not find any difference in the preadult survivorship of flies among any of the light regimes tested, including LL and LD cycles most deviant from *T24* (data not shown). This indicates that the magnitude of changes observed in our study is probably not large enough to cause measurable changes in preadult survivorship; however, it is also possible that there are other fitness components which may bear the consequence of shortening of time-to-emergence.

In *Drosophila*, the steroid eclosion hormone ecdysone released from the prothoracic gland (Gilbert *et al.*, 2002) triggers larval molting and adult emergence (Truman *et al.*, 1983; Riddiford *et al.*, 2010). It is believed that during larval development, opening of emergence gate (lights-off every night) is the signal that regulates the release of prothoracicotropic hormone (Rountree & Bollenbacher, 1986). Studies in tobacco hornworm *Manduca sexta* (L.) have shown that premature release of ecdysone speeds-up development and its delayed release results in slowing down of development (Nijhout & Williams, 1974; Rountree & Bollenbacher, 1986). These studies led to the belief that modulation of preadult development time is possibly due to altered release of prothoracicotropic hormone caused by the mismatch between the period of circadian clocks and that of the LD cycles, and hence due to the time of opening and closing of emergence gates. Although in this study we do not have any estimate of ecdysone levels, the observation that circadian adult emergence rhythm of flies entrain to LD cycles of period ranging between 12 and 32 h (Paranjpe *et al.*, 2003) provides an indirect evidence of differences in the timing of ecdysone release being regulated by LD cycles.

The developmental clocks in nematode *Caenorhabditis elegans* are timed by heterochronic genes and signals that cause expression or repression of genetic switches (Moss, 2007). The expression of the gene *lin4* is responsible for developmental delays, whereas the gene *lin14* prevents such delays by skipping developmental events of L1 (Ambros, 1989; Moss, 2007). In addition, the gene *lin42*, a homolog of the *Drosophila per* gene, whose expression oscillates periodically, is found to regulate the expression of both *lin4* and *lin14*, through yet unknown mechanisms (Moss, 2007). While it is not known how light signals modulate the expression of such stage-specific heterochronic genes, exposure to darkness is known to alter levels of ecdysteroid (Ruei & White, 2003), and ecdysone plays a key role in the molting of larval stages. Thus, it is likely that interaction between light sensitive and circadian clock-controlled processes alter the expression of heterochronic genes and/or their ability to bind to the target sites resulting in an overall change in the time-to-emergence.

Preadult development time and circadian period are polygenic traits, and therefore the possibility of pleiotropic effects of clock genes on circadian clocks and time-to-emergence cannot be ruled out. In this study, we tried to bypass such pleiotropic effects to examine the role of the extent of mismatch between the period of circadian clocks and LD cycles on time-to-emergence in fruit flies *D. melanogaster*. In both males and females, mean time-to-emergence is considerably shortened under *T* cycles of length deviating from 24 h. Further, a positive correlation between the time-to-emergence and length of the LD cycles, as it increases from *T4* to *T24* and a marginally negative correlation when it increases from *T28* to *T48*, suggests that the extent of mismatch between the period of circadian clocks and LD cycles determines time-to-emergence (Fig. 3). Given that we hardly know anything about the developmental timers and circadian clocks operational during the preadult stages in *Drosophila*, it is quite likely that the temporal orders during the early developmental stages are amenable to changes in the period of the LD cycles, and therefore correlation between time-to-emergence and LD period length might be mediated through circadian rhythms present at preadult stages.

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## Disclosure

This article reports the results of research only. The authors do not have any competing interests to disclose. The authors alone are responsible for the content and writing of the paper.

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