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Breakdown of selection-mediated correlation between development time and clock period

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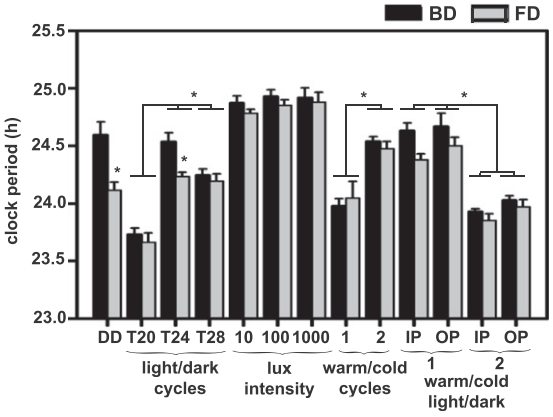
HIGHLIGHTS

- Selection for faster development shortened circadian clock period (τ) by ~0.5-h.
- Exposure to LD and WC cycles elicits after-effect.
- Exposure to LD and WC cycles breaksdown correlation between development time and circadian clocks.

GRAPHICAL ABSTRACT



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ABSTRACT

Previously we have reported that selection for faster pre-adult development in fruit flies speeded-up development by ~29-h and shortened the clock period (τ) by ~0.5 h, which suggests that development time and τ are correlated. Since it is known that τ is altered following exposure to light/dark (LD) cycles, we asked whether this correlation persists in the faster developing (FD) and control (BD) flies by examining the τ of the activity/rest rhythm and its difference between the two stocks following exposure to a variety of cyclic conditions. We assayed the activity/rest behavior of FD and BD flies under DD, following a week-long exposure to (a) LD cycles of 10:10 h, 12:12 h and 14:14 h, or (b) LD12:12 h with different light intensities (10, 100 and 1000 lx), or (c) 12:12 h warm/cold (WC) cycles of 25:18 °C (WC1) and 29:25 °C (WC2), or (d) WC1 or WC2, in-phase or out-of-phase with LD. The results revealed that both LD and WC altered the τ of FD and BD flies, and considerably reduced the selection-mediated difference between the two stocks. LD10:10 caused more severe after-effects on τ compared to LD12:12 and LD14:14. Among the WC cycles, WC1 which had a higher contrast caused period shortening. Irrespective of the phase relationship, imposition of LD cycles on WC cycles made no difference to the extent of after-effects; however, interestingly there was a reversal in the trend, in that, now WC2 with LD caused most drastic reduction in τ. These results suggest that cyclic environments modulate the circadian organization of *Drosophila melanogaster* altering the selection-mediated correlation between pre-adult development time and clock period.

1. Introduction

Circadian clocks regulate a variety of behavioral and metabolic processes in a wide range of organisms [1,2]. These clocks entrain

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to light/dark (LD) or warm/cold (WC) (also referred as Thermophase/Cryophase or TC) cycles, free-run under constant darkness (DD), and are disrupted under constant light (LL) [1,2]. The period (τ) of circadian clocks varies depending on the nature of prior environmental exposure (a phenomenon called as "after-effect" [2–4]). Prior exposure to LD cycles of varying light durations, skeleton photoperiods, light pulses or LL, is known to cause after-effects in the circadian clocks of a wide range of organisms [4–7]. For example, in crickets *Teleogryllus commodus*, prior exposure to continuous red light first lengthened and then gradually shortened the τ of the activity/rest rhythm [3]. In weta *Hemideina thoracica*, prior exposure to long period LD cycles (e.g., LD8:23) lengthened the τ of the activity/rest rhythm during the early days of DD after which it eventually returned to its intrinsic value [6]. Similarly, in freshly emerged blowflies *Calliphora vicina*, prior exposure to short (LD4:20) or long (LD20:4) photoperiods for as few as 3–7 days caused an initial shortening of τ (<24 h) before abrupt lengthening to values greater than 24 h [7]. Similarly, entrainment to complete or skeleton photoperiod, or phase-shifts caused by single light pulses, or exposure to LL was found to alter the τ of the activity/rest rhythm in mice *Mus musculus* and in hamsters *Mesocricetus auratus* [5]. The τ of circadian rhythms in humans was found to vary according to the period length of previously experienced LD cycles [8,9]. Thus, it is clear that in a wide variety of organisms, τ of circadian rhythms shows history-dependent effects of prior exposure to LD cycles.

After-effects of exposure to cyclic environments on circadian rhythms have been observed for light; however, to the best of our knowledge, such effects have never been reported for temperature. Moreover, large phase-shifts induced by cold temperature pulses, or exposure to 12:12 h WC (WC12:12) cycles of 30:20 °C did not cause any detectable after-effect in the circadian activity/rest rhythm of cockroaches *Leucophaea maderae* [10]. Although the mechanisms underlying after-effects on circadian clocks are not yet known, recent studies in mice involving modifications of core clock genes *Period* (*Per1*, *Per2* and *Per3* [11]) and *Clock* [12] have suggested that circadian after-effects do not depend on epigenetic modifications in the core clock genes, suggesting that after-effects are independent of clock gene regulation. However, the possibility of epigenetic modifications of non-*Period* and non-*Clock* clock genes mediating circadian after-effects cannot be ruled out. Moreover, it is believed that after-effects help organisms in maintaining stable phase-relationships with environmental cycles in the face of fluctuations in environmental and behavioral conditions [13,14]. It is also believed that after-effects on circadian clocks may help frequent travelers to rapidly adjust to new time schedules [9]. Furthermore, based on the findings of previous studies it is likely that after-effects have important implications in the treatment of sleep disorders, such as delayed sleep phase disorder and advanced sleep phase disorder, and in the development of counter measures for adaptation to sleep/wake schedules encountered by shift-workers, during jet lag, in submarine naval operations and during short-term and long-term human space exploration [15–18].

Circadian clocks of some insect species are developmentally plastic. For example, exposure to LD cycles during the pre-adult developmental stages alters the τ of the activity/rest rhythm in the wild-type and *period* (*per*) mutant flies of *Drosophila melanogaster* [14,19]. Such plasticity in activity/rest rhythm has been reported to significantly improve the ability of circadian clocks in three wild-type strains of *D. melanogaster* to entrain to cyclic environmental conditions comprising of simulated natural twilights and ambient temperatures [20]. Furthermore, it is known that rearing fruit flies under DD alters several of its physiological and behavioral processes such as phototactic ability, copulation, longevity and fecundity [21–25]. In addition, exposure to varying photoperiods during the pre-adult stages modifies the τ of the activity/rest rhythm of adult cockroaches *L. maderae*, and alters its light induced phase response curve [26,27].

In a recent study, we reported that selection for faster pre-adult development in fruit fly *D. melanogaster* populations, maintained for several generations under DD, speeded-up pre-adult development by ~29 h and shortened the τ of its activity/rest rhythm by ~0.5 h, suggesting a correlation between pre-adult development time and τ [28]. Since these flies have not been exposed to any cyclic condition for ~55 generations, we asked whether this correlation would be affected by prior exposure to cyclic light and temperature conditions.

Here we report the results of our study where we assayed the activity/rest behavior of faster developing (FD) and control (BD) flies under DD, after a week-long exposure to (a) either LD cycles of 10:10 h (LD10:10), or 12:12 h (LD12:12), or 14:14 h (LD14:14), or (b) LD12:12 with 10, 100, or 1000 lux light intensity during the light phase (LD_{10}, LD_{100}, LD_{1000}), or (c) WC12:12 h of 25:18 °C (WC1) or of 29:25 °C (WC2), or (d) WC in-phase (WC1-IP or WC2-IP), or out-of-phase (WC1-OP or WC2-OP) with LD12:12 cycles. Since our study involves two sets of stocks of *Drosophila*, we used the regime-mediated modulation in τ within a given stock and the selection-mediated difference between the FD and BD flies, to assess the extent of after-effects of exposure to cyclic conditions. The results suggest that prior exposure to light and temperature cycles considerably alters the τ of activity/rest rhythm, thus reducing the selection-mediated difference, which suggests that the genetic correlation between pre-adult development time and clock period breaks-down due to after-effects of exposure to cyclic conditions on circadian clocks.

2. Materials and methods

2.1. Laboratory population maintenance

In this study we used two sets of four outbred replicate *D. melanogaster* populations — FD (Faster Development — experimental stocks selected for faster pre-adult development, $FD_{1–4}$) and BD (Baseline Development — control stocks $BD_{1–4}$). While the origin and maintenance of these populations are discussed in details elsewhere [28]; briefly, four FD populations were initiated from four populations of BD controls by imposing selection for faster pre-adult development, using the fastest (20–25%) of the emerging flies in every generation. Four BD populations were also maintained alongside the selected populations, which were not subjected to any conscious selection pressure. Each population consisted of ~1200 adults (roughly equal number of males and females) maintained in a plexiglass cage ($25 \times 20 \times 15$ cm^3) supplemented with banana–jaggery food medium (henceforth referred to as banana medium). These populations were maintained in a cubicle, at constant temperature (~25 °C) and humidity (~80%), on a 21 day discrete generation cycle. The FD_1 population was derived from the BD_1 population, FD_2 from BD_2, FD_3 from BD_3 and FD_4 from BD_4 populations. Thus, selected and control populations bearing identical numerical subscripts are likely to be more closely related to each other than the populations with which they share selection regime. Such pairs of populations, referred as blocks, were maintained as genetically separate entities, which were never interbred and therefore no gene flow occurred between them. To start a new generation, adult flies were provided with banana medium supplemented with live yeast paste for two and half days prior to egg collection. Flies were allowed to lay eggs for about 12 h on fresh banana medium in a petri plate, from which 60–80 eggs were dispensed into glass vials (9 cm height × 2.4 cm diameter), in which larvae developed as adults. Flies emerging from 24 such vials were transferred into plexiglass cages on the 12th day after egg collection, which formed the breeding population for the next generation of the control (BD) stocks, whereas only the fastest (first 25%) of the freshly emerging flies from 80 vials contributed to the next generation of the selected (FD) stocks. Eggs were collected after 21 days from the previous egg collection date to start the next generation.