Effects of Ambient Temperature and Temperature Cycle on Locomotor Activity Rhythm in the Onion fly, Delia antiqua

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ABSTRACT—The phase of locomotor activity of the onion fly, *Delia antiqua*, in LD12:12 advanced at a low temperature (20°C) as compared with that at a high temperature (25°C). The free-running period (τ) in constant darkness (DD) at 20°C became shorter than that at 25°C, suggesting that the phase advance of locomotor activity in LD cycles at 20°C was caused by the decrease in τ . In constant light (LL), the locomotor activity was arrhythmic at a constant temperature. In both DD and LL, the locomotor activity was entrained to a 12 hr 25°C:12 hr 20°C temperature cycle; the activity occurred in the thermophase and its peak delayed with age. However, the delay in LL was smaller than that in DD. At a cycle of 12 hr cool (20°C) light and 12 hr warm (25°C) dark, the fly showed a similar activity pattern to that in LD 12:12 at a constant temperature (20°C or 25°C); the activity occurred in the light phase. This suggests that LD cycle is a stronger zeitgeber than a temperature cycle to entrain the locomotor activity of *D. antiqua*.

INTRODUCTION

It has been known that in most species of organisms, the free-running period (τ) of circadian oscillation depends on the ambient temperature in constant light conditions. Aschoff (1979, 1981) suggested that with an increase in ambient temperature, τ shortens in most diurnal species and lengthens in nocturnal species. However, it was found that the change of τ by temperature is slight in many organisms including insects; the Q₁₀ is generally very close to 1.0 (Aschoff, 1979). In light-dark (LD) cycles, the ambient temperature influences the phase of the locomotor activity rhythm (Aschoff, 1979; Chiba *et al.*, 1982; Ikeda and Tomioka, 1993; Tomioka *et al.*, 1998).

In constant darkness (DD) or constant light (LL), temperature cycle is also well known to entrain the locomotor activity rhythms (Aschoff, 1960). In insects, for example, entrainment of the locomotor activity to a temperature cycle was reported for *Drosophila melanogaster* in both DD (Wheeler *et al.*, 1993; Tomioka *et al.*, 1998) and LL (Tomioka *et al.*, 1998). Tomioka *et al.* (1998) suggested that temperature cycle forces the clock to move in LL, where the rhythm is believed to be stopped at constant temperature.

At 25°C, in the onion fly, *Delia antiqua*, the phase of locomotor activity in LD cycles (ψ_{RL}) occurs progressively later with age and the free-running period (τ) in DD also changes with age, being shorter than 24 hr until 14–20 days after adult

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eclosion but thereafter longer than 24 hr (Watari and Arai, 1997, 1998, 1999). Watari and Arai (1997) hypothesized that the age dependent change of ψ_{RL} in *D. antiqua* is attributable to the increase in τ . In the present study, the hypothesis was examined by investigating the ψ_{RL} at a low temperature (20°C) where the τ significantly differed from that at 25°C. I also investigated the entrainability of the locomotor activity rhythm to temperature cycles and the age dependent change of the acrophase under the temperature cycles in both DD and LL.

MATERIALS AND METHODS

Insects

A stock culture of *D. antiqua* was originally supplied by Hokkaido Prefectural Central Agricultural Experiment Station in 1981, and thereafter maintained in the laboratory by rearing larvae on fresh slices of onion. Experimental larvae were reared in continuous light (LL) at 25°C. Only males were used for activity recording.

Recording of locomotor activity

All pupae were maintained in LL at 25°C until adult eclosion. Within a day after eclosion, flies were transferred to an activity chamber (Watari and Arai, 1997). The locomotor activity rhythm was recorded individually in a monitoring system comprised of an activity chamber (plastic tube of 3.5×6 cm) flanked with an infrared-light emitter and a detector (GT-1, Takenaka Electronic Industrial Co. Ltd.). When the insect crossed the infrared beam, a signal was fed to a computer (NEC, PC88) that counted movements in each 6 min bin. A bottle (3.2×6 cm) of sugar (about 13%) solution plugged with cotton wool was connected to the activity chamber as a source of food and water. Locomotor activities of six individuals were recorded concurrently under controlled lighting regimens (10 W fluorescent lamp giving more than 400 lx to the fly on the cage floor).

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In LD cycles at 20°C, the major peak of activity (ϕ_A) occurring during the daytime (see Results) was determined by fitting a cosine curve to the activity counts from 3 hr after lights-on to lights-off (the period of major activity) and shown as the time (hours) before dusk (Watari and Arai, 1997). In temperature cycles (12 hr 25°C:12 hr 20°C) in both DD and LL, the activity peak was determined by fitting a cosine curve to the activity counts each day because the activity pattern was different from that in LD cycles (see Results). As the longevity varied among individuals and a few cycles were necessary for entrainment to LD conditions after eclosion, ϕ_A was calculated from days 3 to 30 for flies that gave records for more than 30 days after eclosion. The activity peak in temperature cycles was also calculated from days 3 to 30. τ was estimated by the least-squares spectrum (Tanakadate et al., 1991). The mean t was calculated, based on the data for days 2 to 30 after the LL-to-DD transition on the day of eclosion. Arrhythmicity was judged by a chi-square periodogram (Tanakadate et al., 1991) and if peaks of the periodogram appeared below the 0.05 confidence level, the fly was designated as arrhythmic. Data at 25°C (Watari and Arai, 1997) were cited for comparison.

RESULTS

Effect of low temperature on locomotor activity

Fig. 1 shows a typical locomotor activity record at LD 12:12



Fig. 1. Example of double-plotted locomotor activity rhythm in *D. antiqua* in LD 12:12 at 20°C. Open and solid bars at the top indicate the photoperiod.



Fig. 2. Changes in ϕ_A (upper panel) and activity level (lower panel) in *D. antiqua* with age in LD 12:12 at 25°C (data from Watari and Arai, 1997) and 20°C. ϕ_A is shown as the time (hr) before dusk. Circles, means of ϕ_A and activity level at 25°C. Squares, means of ϕ_A and activity level at 25°C. Squares, means of ϕ_A and activity level at 20°C. Regression lines for ϕ_A are Y=-0.024X+3.971 (d.f.=361) at 25°C and Y=-0.019X+4.839 (d.f.=251) at 20°C, both significant at *P*<0.01. Regression lines for activity level after day 8 are Y=-20.3X+845.7, (d.f.=298) at 25°C and Y=-33.6X+1443.2 (d.f.=206) at 20°C, both significant at *P*<0.01. Vertical lines indicate standard errors.

and 20°C. Adults of *D. antiqua* showed trimodal activity patterns with major and light-on and light-off peaks. The major peak (ϕ_A) was significantly delayed with age (*P* <0.01, regression analysis) (Fig. 2, upper panel) as at 25°C (Watari and Arai, 1997). However, the ϕ_A at 20°C advanced as compared with that at 25°C throughout the adult's life and the two regression slopes were not significantly different (*P*>0.1, *t*test for comparison of regression slopes). The activity level at 20°C was higher than that at 25°C from 1 week after eclosion (Fig. 2, lower panel). The lowering of activity level with age was statistically significant at 20°C (*P*<0.01, regression analysis) as at 25°C.

Free-running rhythm in DD and LL at different temperatures

Fig. 3 shows a typical locomotor activity record of *D*. *antiqua* in DD at 20°C. The free-running rhythm varied with



Fig. 3. Example of change in free-running rhythm in DD at 20°C in *D. antiqua* adults transferred soon after eclosion from LL.

age as in DD at 25°C (Watari and Arai, 1997); τ was shorter than 22 hr until day 19 after eclosion, became closer to 24 hr between days 20 and 27 and increased further to ca. 26 hr between days 28 and 39. Similar tendencies were observed in other flies (Fig. 4). The increase in τ with age was statistically significant at 20°C as at 25°C (P<0.01, regression analysis). At 20°C, however, τ was shorter than that at 25°C throughout the adult's life and the two regression slopes were not significantly different (P>0.1, *t*-test). The activity level at 20°C was lower than that at 25°C from soon after eclosion (Fig. 4, lower panel). The lowering of activity level with age was statistically significant at 20°C (P<0.01, regression analysis) as at 25°C.

To confirm whether the activity rhythm controlled by the circadian oscillation persisted in LL or not, flies were transferred to LL from LD 12:12 at day 8 after eclosion at 25° C (n=9) and 20° C (n=5). Examples of the activity are given in Fig. 5. In LL, the locomotor activities were arrhythmic and



Fig. 4. Change in τ (upper panel) and activity level (lower panel) in DD at 25°C (data from Watari and Arai, 1997) and 20°C in *D. antiqua* adults transferred soon after eclosion from LL. Circles, means of τ and activity level at 25°C. Squares, means of τ and activity level at 20°C. Regression lines for τ (Y, h) on time (X, days) are Y=0.070 X+22.231 (d.f.=483) at 25°C and Y=0.078 X+21.327 (d.f.=372) at 20°C, both significant at *P*<0.01. Regression lines for activity level are Y=-11.6 X+578.0, (d.f.=492) at 25°C and Y=-7.7 X+356.0 (d.f.=372) at 20°C, both significant at *P*<0.01. Vertical lines indicate standard errors.

became rhythmic after transfer to DD at day 23 after eclosion at both 25 and 20°C. All other flies' activities in LL were also arrhythmic at both 25 and 20°C.

Effect of temperature cycles on locomotor activity in DD and LL

To determine whether or not *D. antiqua* can be entrained to temperature cycles, the locomotor activity rhythm was recorded under a temperature cycle consisting of 12 hr high (H, 25°C) and 12 hr low (L, 20°C) (HL12:12). Fig. 6A shows a typical locomotor activity record of *D. antiqua* in HL12:12 and DD. The locomotor activity rhythm appeared soon after temperature rise until day 12 after eclosion and thereafter occurred progressively later with age (P<0.01, regression analysis). Similar tendencies were also observed in other flies; the activity peak delayed with age (P<0.01, regression analysis) (Fig. 7). In LL, the locomotor activity rhythm clearly synchro-



Fig. 5. Examples of locomotor activity rhythm in *D. antiqua* in LL at 25°C (A) and 20°C (B). Flies were transferred to LL from LD 12:12 at day 8 after eclosion and were transferred to DD at day 23.

nized with HL12:12; it was fixed to the late part of H phase throughout the adult's life (Fig. 6B). On the whole (n=7), the activity peak delayed with age (P < 0.01, regression analysis) (Fig. 7) but the regression slope was smaller than that in DD (P < 0.01, *t*-test for comparison of regression slopes).

Effect of combination of photoperiod and thermoperiod

It has been shown that light-active animals mostly show warm-phase activity and dark-active animals cool-phase activity (Aschoff, 1960). To confirm relative importance of photoperiod and thermoperiod in *D. antiqua*, LD 12:12 was combined with HL 12:12 in which a temperature drop from 25 to 20°C was coincided with light-on (Fig. 8). All the flies (n=6) showed the similar activity pattern as that in LD12:12 at constant temperatures (Fig. 1); they showed a diurnal activity.

DISCUSSION

Aschoff (1979) stated that since the phase of locomotor activity in LD cycles (ψ_{RL}) depends on τ , a change of τ due to change in ambient temperature is likely to affect ψ_{RL} . When the ambient temperature was low (20°C) in LD cycles, *Delia antiqua* showed the same activity pattern as that at 25°C; the flies showed trimodal activity pattern with major and light-on and light-off peaks (Fig. 1) and the major peak (ϕ_A) delayed with age (Fig. 2). Furthermore, ϕ_A at 20°C advanced as compared with that at 25°C throughout the adult's life (Fig. 2). These results are consistent with the above Aschoff's statement, because τ in DD at 20°C was shorter than that at 25°C (Fig. 4). However, his statement that most diurnal species shorten τ with rising temperature (Aschoff, 1979) is not supported in the present study.

In *Drosophila melanogaster*, wild type and arrhythmic mutant *per*⁰ flies were entrained to 24-hr temperature cycles in DD, while short period mutant *per*^s and long period mutant *per*^{\perp} flies free-ran under the same conditions, suggesting that a 24 hr period can be outside of their limits of entrainment because of the large period difference between the experimental and their own endogenous periods (Tomioka *et al.*, 1998). In DD, the locomotor activity of *D. antiqua* was entrained to temperature cycles and the activity mainly occurred in the high temperature phase (Figs. 6 and 7). The delay of activity peak with age is probably caused by the change in τ with age and the 24 hr period length (HL12:12) may be within the limit of entrainment throughout the adult's life.

In LL, all strains of *D. melanogaster* were entrained to temperature cycles in which they were arrhythmic at constant temperatures (Tomioka *et al.*, 1998). Judging from recent molecular biological studies in *D. melanogaster* (Price *et al.*, 1995; Lee *et al.*, 1996; Myers *et al.*, 1996), Tomioka *et al.* (1998) suggested that in LL, temperature cycles may force the autoregulatory feedback system of *per* to work through some unknown process. In *D. antiqua*, the locomotor activity in LL became arrhythmic at a constant temperature (Fig. 5) but was entrained to temperature cycles (Fig. 6). Furthermore,



Fig. 6. Examples of locomotor activity rhythm in *D. antiqua* in temperature cycle of 12 hr high temperature (25°C;) and 12 hr low temperature (20°C;) in DD (A) and LL (B). Left panels: actograph records. Right panels: change in the activity peak with age in DD (solid squares) and LL (open squares). Regression of the activity peak (Y) on time (X, days) in DD is Y=0.234X+11.404 (d.f.=27, *P*<0.01).

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Fig. 7. Change in the activity peak with age in *D. antiqua* in temperature cycle of 12 hr high temperature ($25^{\circ}C$;) and 12 hr low temperature ($20^{\circ}C$;) in DD (solid squares) and LL (open squares). Regression lines for the activity peak are Y=0.181X+11.475 (d.f.=195) in DD and Y=0.042 X+19.106 (d.f.=194) in LL, both significant at *P*<0.01. Horizontal lines indicate standard deviations.

the activity peak in the late part of H phase delayed with age although the delay in LL was smaller than that in DD (Fig. 7), suggesting that the entrainment of activity to the temperature cycle may be somewhat dependent on the length of τ . Also in D. antiqua, therefore, temperature cycles in LL may act on the locomotor activity by forcing an oscillator to precess. In the crickets Teleogryllus commodus (Rence and Loher, 1975), Gryllodes sigillatus (Abe et al., 1997) and the cockroach Leucophaea maderae (Page, 1985), it has been reported that the activity rhythm may be driven by a temperature-sensitive secondary oscillator, located outside the optic lobes which believed to be the locus of the circadian pacemaker, that can be forced to precess by temperature cycles. In D. antiqua, further research is necessary to clarify whether or not the entrainment of activity to the temperature cycle in LL is controlled by the temperature-sensitive secondary oscillator.

In *D. melanogaster*, it was suggested that temperature and light cooperate to suppress the activity in one phase and induce activity in the other phase; at warmer temperatures the flies tend to be more active in the dark phase, while at cooler temperatures in the light phase (Tomioka *et al.*, 1998). However, *D. antiqua* showed diurnal activities at both 20 and 25°C (Fig. 1), although the activity level at 20°C was higher

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Fig. 8. Example of double-plotted locomotor activity rhythm in *D. antiqua* in the cycle of 12 hr cool (20°C;) light and 12 hr warm (25°C;) dark . Open and solid bars at the top indicate the photoperiod.

than that at 25°C in LD 12:12 (Fig. 2). Furthermore, in cycles of 12 hr cool (20°C) light and 12 hr warm (25°C) dark, the fly showed a similar diurnal activity pattern to that in LD 12:12 at a constant temperature (Fig. 8). These results suggest that in D. antiqua, LD cycle can induce the locomotor activity in the photophase regardless of the ambient temperature and is a stronger zeitgeber than a temperature cycle to entrain the locomotor activity. However, the amplitude of temperature cycle may be important to entrain the locomotor activity. In G. sigillatus (Abe et al., 1997), for example, although the locomotor activity of crickets with their optic lobes bilaterally removed were rhythmic in the temperature cycle with an amplitude of 3°C [HL 12 (28°C):12 (25°C)] in DD, they turned to be arrhythmic in the cycle with an amplitude of 1°C [HL 12 (26°C):12 (25°C)] in DD. Further research in another temperature cycle in which cryophase is lower (e.g. 15 or 10°C) is necessary to confirm this in D. antiqua.

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