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Effect of Dim Light 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 L (400 lux) L_{dim} (1.0 lux) cycles delayed as compared with that in LD cycles. The free-running period (τ) in constant dim light ($L_{dim}L_{dim}$) was longer than that in constant darkness (DD), suggesting that the phase delay of locomotor activity in LL_{dim} cycle was caused by the increase in τ. At L_{dim}D 12:12 in which the light intensity of the photophase was 1.0 lux, the locomotor activity free-ran with the period shorter than 24 hr until about week 2 after eclosion but thereafter entrained to L_{dim}D in spite of τ might become longer than 24 hr. This suggests that the flies may become more sensitive to light intensity with age.

INTRODUCTION

It has been known that in most species, the free-running period (τ) of circadian oscillation depends on the light intensity. Aschoff (1960, 1981) stated that, τ shortens with an increase in light intensity in many diurnal species and lengthens in nocturnal species. In general, this Aschoff's rule is supported by data from many vertebrates (see Daan and Pittendrigh, 1976; Aschoff, 1981). However, many insects appear to violate Aschoff's rule (Saunders, 1982). For example, in *Drosophila melanogaster* (Konopka *et al*., 1989) and *Calliphora vicina* (Hong and Saunders, 1994) both of which are day-active, τ increases with light intensity and become arrhythmic in light intensity above a certain threshold.

In LL_{dim} (light-dim light) cycles, the light intensity during light period or dim-light period influences the phase relationship between the circadian rhythm and LL_{dim} cycles (ψ_{RL}) (Aschoff, 1965). In several species of bird (Aschoff, 1965), for example, the phase of onset of activity advanced with increasing light intensity in L or in L_{dim}. This aspect of circadian rhythms has seldom been investigated in insects.

In the onion fly, *Delia antiqua*, the phase of locomotor activity in LD (light-dark) cycles occurs progressively later with age (Watari and Arai, 1997). The free-running period τ in constant darkness (DD) also changes with age, being shorter than 24 hr until 14–20 days after adult eclosion but thereafter longer than 24 hr. This suggests that the age dependent ψ_{RL} change in *D. antiqua* would be attributed to the increase in τ. By using D_2O, this hypothesis was tested experimentally (Watari and Arai, 1998). In the present study, we examined the relationship between ψ_{RL} and τ in *D. antiqua* by making the light intensity of LD cycles and constant dim light ($L_{dim}L_{dim}$) 1.0 lux.

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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 at 25°C under controlled lighting regimens. To keep the light intensity at about 1.0 lux (L_{dim}), a 10 W fluorescent lamp was covered by a black polyethylene sheet.

In LL_{dim} cycles as in LD cycles, 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 the acrophase (ϕ_A) appeared before dusk (Watari and Arai, 1997). In L_{dim}D 12:12, the activity peak was determined by fitting a cosine curve to the activity counts each day because the main activity was not fixed to the photophase (see Results). As the longevity varied among individuals and a few cycles were necessary for entrainment to LL_{dim} 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 L_{dim}D 12:12 was also calculated from days 3 to 30. τ was estimated by the least-squares spectrum (Tanakadate *et al*., 1991). The mean τ was calculated, based

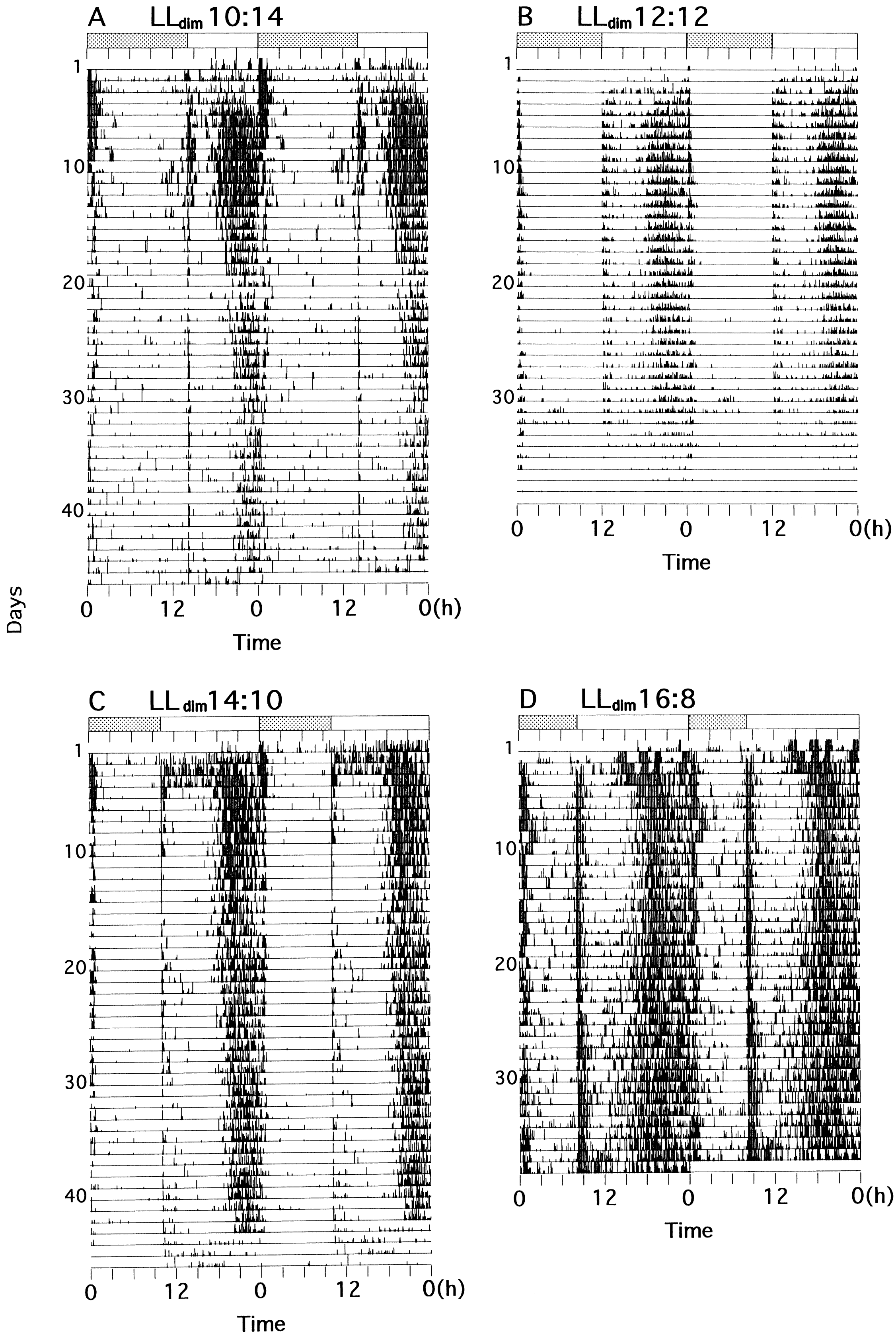


Fig. 1. Examples of double-plotted locomotor activity rhythm in *D. antiqua* in different LL_{dim} cycles at 25°C. A, LL_{dim} 10:14. B, LL_{dim} 12:12. C, LL_{dim} 14:10. D, LL_{dim} 16:8. Open and dotted bars at the top indicate the photoperiod.

on the data for days 2 to 30 after the LL-to-$L_{dim}L_{dim}$ transition on the day of eclosion. Data at LD cycles and in DD (Watari and Arai, 1997) were cited for comparison.

RESULTS

Effect of dim light in scotophase on locomotor activity

Fig. 1 shows typical locomotor activity records at photoperiods ranging from LL_{dim} 10:14 to 16:8 at 25°C in which the light intensity of the scotophase was 1.0 lux. Adults of *D. antiqua* showed trimodal activity patterns with major and light-on and light-off peaks. The major peak (ϕ_A) advanced as the photophase became longer (Fig. 2), like in LD cycles (Watari and Arai, 1997). ϕ_A was significantly delayed with age ($p<0.01$ at LL_{dim} 10:14 and 14:10 and $p<0.05$ at LL_{dim} 12:12 and 16:8, regression analysis). Similar delay has been also observed in LD cycles (Watari and Arai, 1997). The delay in LL_{dim} was larger than in LD for the first 1 to 3 weeks (to day 12 at LL_{dim} 14:10) after eclosion but thereafter became almost the same as that at LD cycles.

At LL_{dim} 4:20 (n=5), the activity after lights-drop increased from about day 10 after eclosion, and in two flies the main activity moved from the photophase to the dim-light phase (Fig. 3).

Free-running rhythm in constant dim light conditions

Fig. 4 shows typical locomotor activity record of *D. antiqua* in $L_{dim}L_{dim}$ at 25°C. The free-running rhythm varied with age as in DD (Watari and Arai, 1997); τ was shorter than 24 hr until day 15 after eclosion and thereafter became longer than 24 hr. Similar tendencies were observed in other flies; τ changed with age (Fig. 5). The increase in τ with age was statistically significant in DD and in $L_{dim}L_{dim}$ ($p<0.01$, regression analysis). In $L_{dim}L_{dim}$, τ was greater than that in DD through the adult's life and the difference became larger with age ($p<0.01$, *t*-test for comparison of regression slopes).

Effect of dim light in photophase on locomotor activity

Fig. 6 exemplifies locomotor activity records of two flies at $L_{dim}D$ 12:12. The locomotor activity rhythm free-ran until day 21 (Fig. 6A) or 15 (Fig. 6B) after eclosion and thereafter

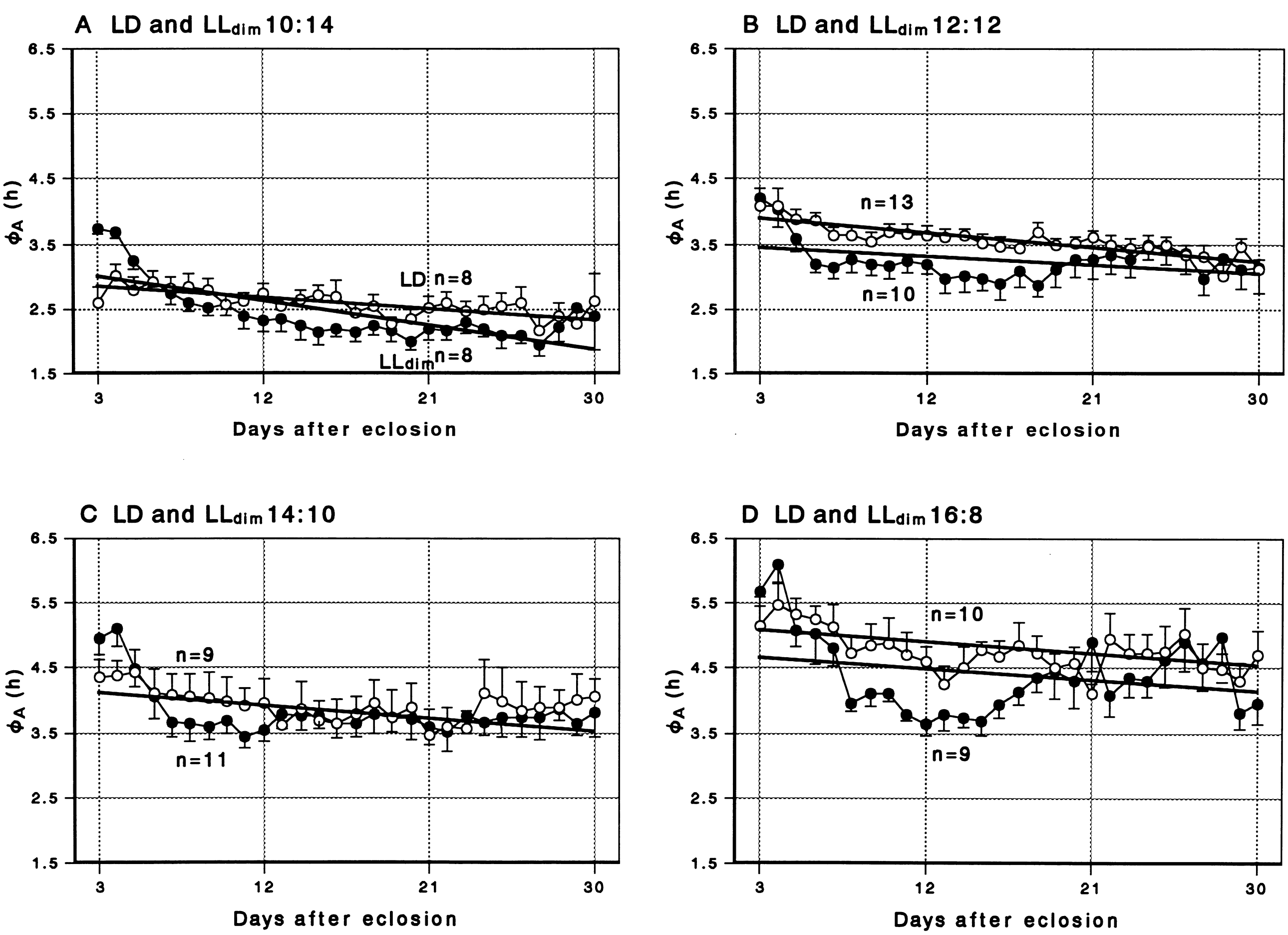


Fig. 2. Changes in ϕ_A as given by the time before dusk in *D. antiqua* given in different LD (data from Watari and Arai, 1997) and LL_{dim} cycles at 25°C. Open circles, ϕ_A at LD cycle; closed circles, ϕ_A at LL_{dim} cycles. Regression lines for ϕ_A (Y) on time (X, days) are Y=–0.018X+2.891 (d.f.=214, $P<0.01$) at LD 10:14, Y=–0.042X+3.118 (d.f.=219, $P<0.01$) at LL_{dim} 10:14, Y=–0.024X+3.971 (d.f.=361, $P<0.01$) at LD 12:12, Y=–0.013X+3.467 (d.f.=279, $P<0.05$) at LL_{dim} 12:12, Y=–0.022X+4.178 (d.f.=305, $P<0.01$) at LL_{dim} 14:10, Y=–0.023X+5.139 (d.f.=259, $P<0.05$) at LD 16:8 and Y=–0.020X+4.730 (d.f.=249) at LL_{dim} 12:12. Vertical lines indicate standard errors.

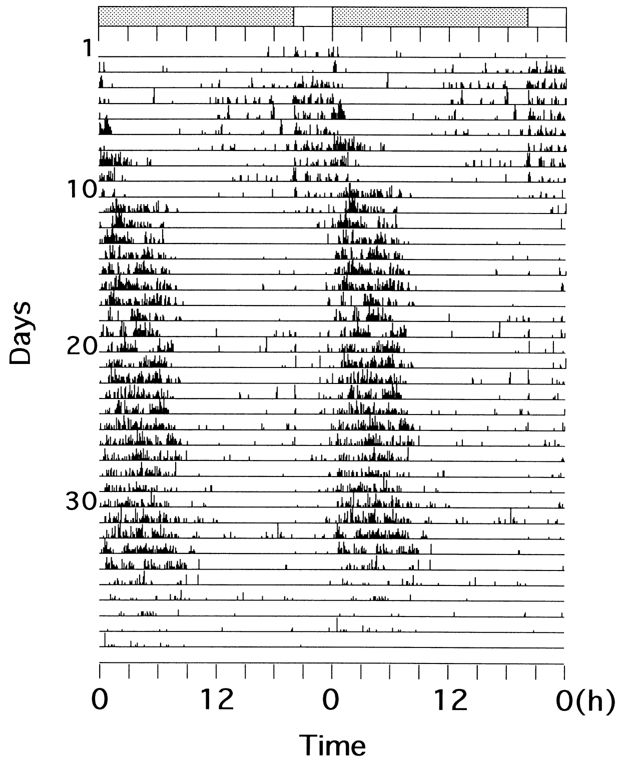


Fig. 3. Example of activity change with age in *D. antiqua* at LL_{dim} 4:20 at 25°C.

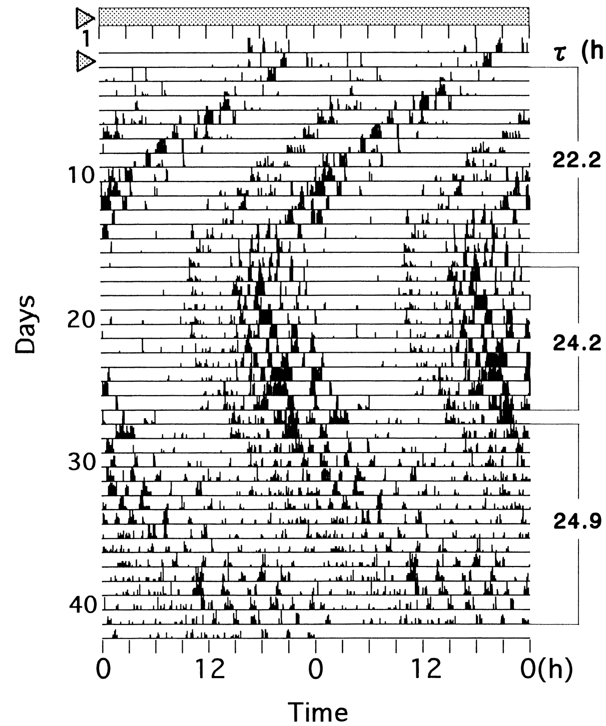


Fig. 4. Example of change in free-running rhythm in $L_{dim}L_{dim}$ (1.0 lux) at 25°C in *D. antiqua* adults transferred soon after eclosion from LL (400 lux). Dotted bars at the top indicate the continuous dim light ($L_{dim}L_{dim}$).

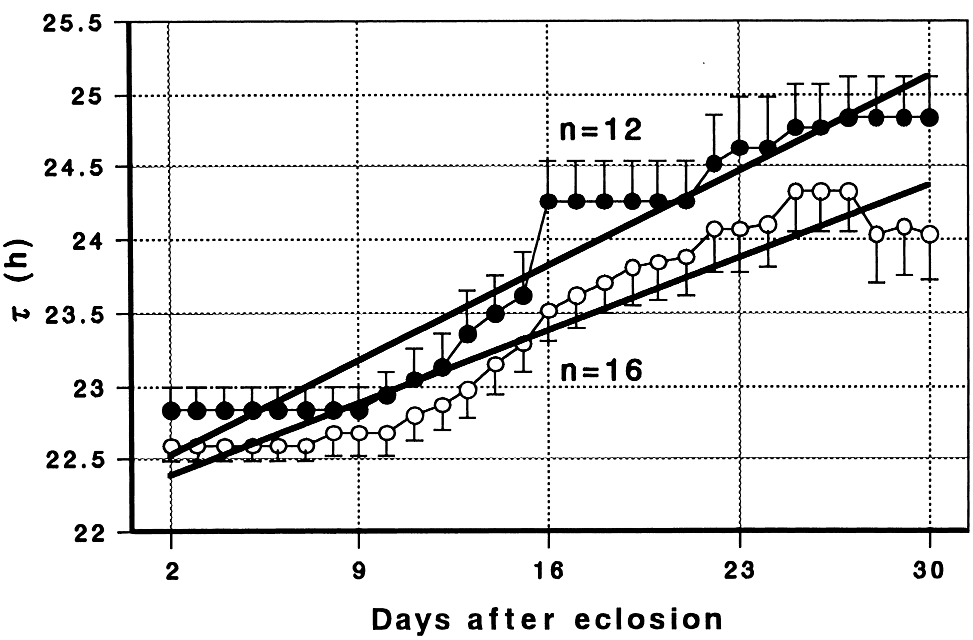
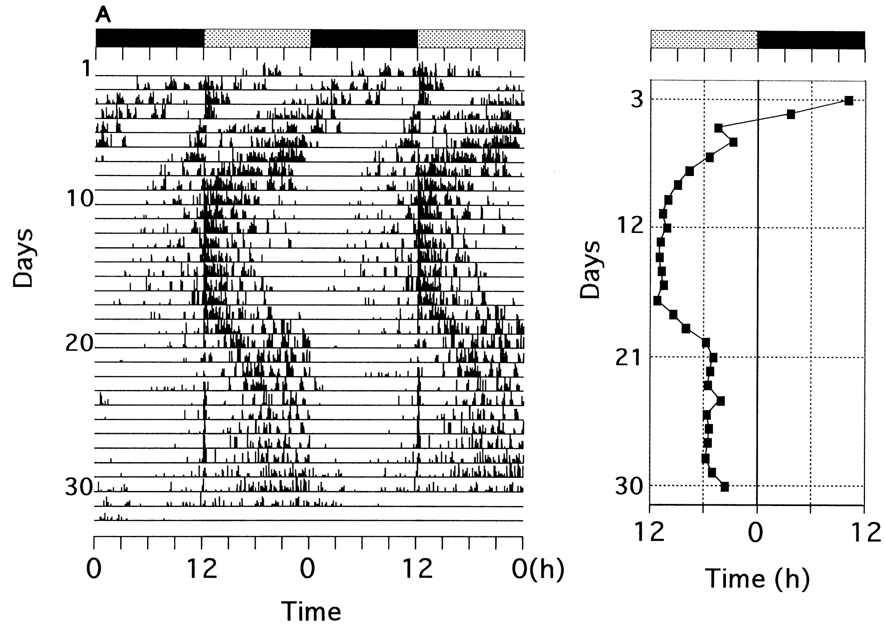


Fig. 5. Change in τ in DD (data from Watari and Arai, 1997) and $L_{dim}L_{dim}$ at 25°C in *D. antiqua* adults transferred soon after eclosion from LL. Open circles, τ in DD; closed circles, τ in $L_{dim}L_{dim}$. Regression lines for τ (Y, h) on time (X, days) are Y=0.071X+22.236 (d.f.=458) in DD and Y=0.093X+22.331 (d.f.=347) in $L_{dim}L_{dim}$. Both significant at $P<0.01$. Vertical lines indicate standard errors. The difference between τ in $L_{dim}L_{dim}$ and that in DD became larger with age ($p<0.01$, *t*-test for comparison of regression slopes).

was entrained with the major peak fixed to the later part of the photophase. Though the light-on peak persisted through the adult's life, the light-off peak did not appear. Similar tendencies were also observed in other flies; the locomotor activity rhythms of 5 of 7 flies free-ran with each period until about 2–3 weeks after eclosion and thereafter was fixed to the later



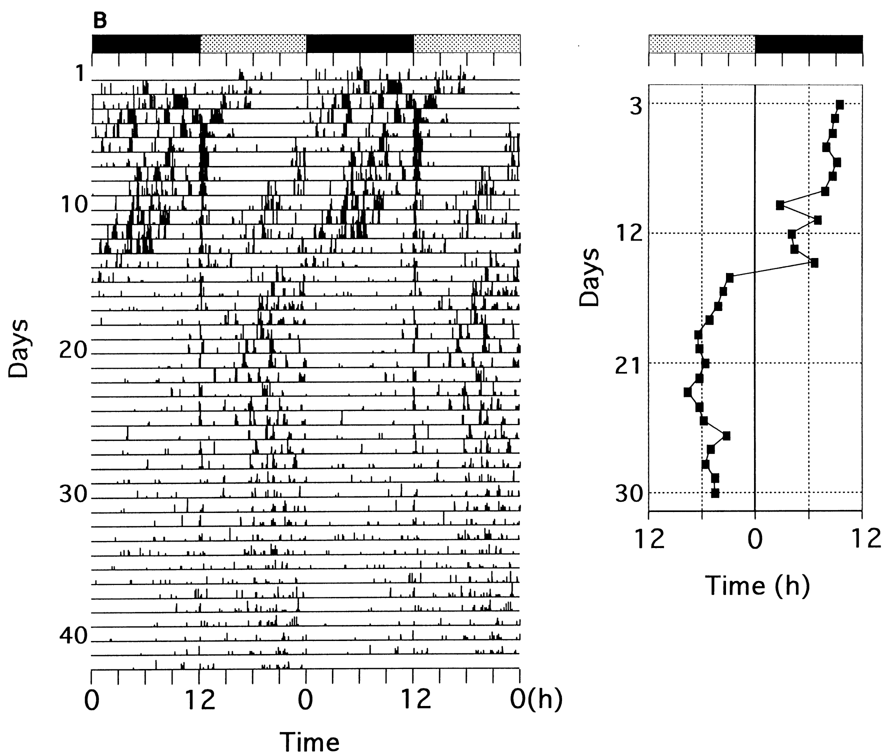


Fig. 6. Examples of change in activity rhythm at $L_{dim}D$ 12:12 in D. antiqua with age at 25°C. Left panels: actograph records. Right panels: change in the activity peak (squares) with age.

part of the photophase. As a result, standard deviation of the locomotor activity peak became smaller from week 2 after eclosion (Fig. 7).

DISCUSSION

In LD cycles, *Delia antiqua* shows a major peak of locomotor activity in the late photophase and also bursts of activity induced by lights-on or lights-off. Only the major peak (ϕ_A) is controlled by the circadian pacemaker and the phase of ϕ_A occurs progressively later with age and this change would be attributable to the increase in τ (Watari and Arai, 1997). Also in LL_{dim} cycles , the fly showed trimodal activity patterns with major and light-on and light-off peaks (Fig. 1) and ϕ_A was delayed with age (Fig. 2). This suggests that the period of 1.0 lux exerts the same effect as a dark period (0 lux) when com-

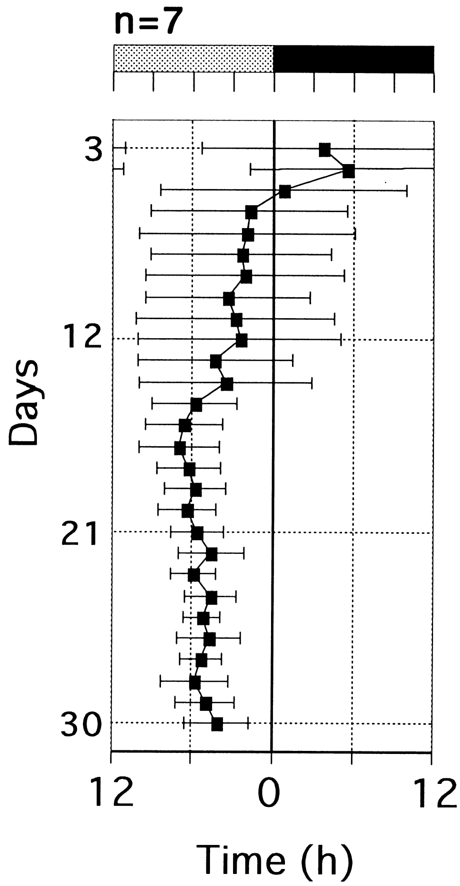


Fig. 7. Change in the activity peak (squares) with age at $L_{dim}D$ 12:12 in *D. antiqua* at 25°C. Vertical lines indicate standard deviations.

bined with a period of 400 lux. However, the phase of the major peak in LL_{dim} slightly delayed as compared with that in LD cycles until about week 3 after eclosion (Fig. 2). This observation implies that the light intensity of 1.0 lux altered the velocity of the pacemaker, because τ in $L_{dim}L_{dim}$ became longer than that in DD (Fig. 5).

When the light intensity of the photophase was 1.0 lux ($L_{dim}D$ 12:12), in *D. antiqua*, the locomotor activity rhythm free-ran until about 2 weeks after eclosion (Fig. 7). This phenomenon is similar to relative coordination. In the presence of a weak zeitgeber such as a small difference in light intensity or a decrease in light sensitivity, external relative coordination would develop (Enright, 1981; Aschoff, 1981; Honma, 1991). *Drosophila melanogaster* could be entrained to $L_{dim}D$ cycle even when the light intensity of dim light was 0.1 lux (Matsumoto *et al.*, 1994). Therefore, it seems that the light sensitivity of *D. antiqua* is weaker than that of *D. melanogaster*. In *D. antiqua*, however, the locomotor activity rhythm was fixed to the later part of the photophase in $L_{dim}D$ 12:12 from about 2 weeks after eclosion; it could be entrained in $L_{dim}D$. Therefore, the light sensitivity of *D. antiqua* may be enhanced with age. At LL_{dim} cycles, the major peak remained almost the same as that at LD cycles after about week 3 (Fig. 2). This might be caused by the change in light sensitivity with age. The wild type and short period mutant, per^S, of *D. melanogaster* was entrained to a short photoperiod LD 4:20 but long period mutant, per^L was not (Tomioka *et al.*, 1997). Based on this fact, it has been suggested that the entrainability of per^L mutant is weaker than the other two strains and these results are consistent with the hypothesis proposed by Saunders *et al.* (1994) that the sensitivity to light is lower in per^L flies than in per^S flies (Tomioka *et al.*, 1997). At LD 4:20 (Watari and Arai, 1997) and LL_{dim} 4:20 (Fig. 3), *D. antiqua* could be entrained but the activity after lights-off increased with age. These results are contradictory to the hypothesis that the light sensitivity of *D. antiqua* may become higher with age. However, the entrainment to short photoperiod may be predicted by the phase response curve (PRC), as shown in *D. melanogaster* (Saunders *et al.*, 1994); the steady state phase relationships of per^S flies to LD 1:23 and LD 6:18 showed entrainment to the LD cycles with a pronounced phase lead as predicted by the PRC. In *D. antiqua*, however, we have not obtained PRC. Further experiments are necessary to determine whether or not the light sensitivity of *D. antiqua* changes with age.

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