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SHORT COMMUNICATION

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Locomotor activity rhythm in *Drosophila melanogaster* after 600 generations in an aperiodic environment

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Abstract The locomotor activity rhythm of flies from four populations of *Drosophila melanogaster*, maintained under constant light for more than 600 generations, was recorded in continuous light (LL) and continuous darkness (DD) using four different protocols. The main objective behind these experiments was to estimate the proportion of flies exhibiting circadian rhythm of locomotor activity in LL, and to investigate whether this could be increased by subjecting the flies to various light regimes. About 26% of the flies exhibited a circadian rhythm of locomotor activity in LL, and the proportion increased to about 48% after an exposure to 12 h of darkness. About 77% of the flies exhibited a circadian locomotor activity rhythm in DD. Persistence of circadian locomotor activity rhythm in a considerable proportion of these flies suggests an intrinsic adaptive value to possessing circadian rhythmicity, derived, perhaps, from the need to synchronise various processes within the organism.

Introduction

In an environment where most biotic and abiotic factors oscillate with a periodicity of 24 h, any mechanism by which organisms can appropriately time their behavioural and physiological functions could confer an adaptive advantage. It is hypothesised that organisms possess biological clocks that can measure passage of time on a circadian time scale, and also allow them to appropriately time their functions with respect to environmental cycles (Sharma and Joshi 2002). Laboratory studies on *Drosophila* indicate that traits that do not confer any fitness advantage to an organism under a given environment are fairly rapidly (within 100–150 generations) affected adversely by mutation accumulation (Mueller 1987). If the trait in discussion involves some cost, the decline can be even faster (within 20–50 generations) (Service et al. 1988; Teotónio et al. 2002). Therefore, one may expect that organisms living in aperiodic environments for several hundreds of generations may have lost the capacity to measure time on a circadian scale. On the other hand, persistence of circadian rhythmicity in a number of physiological and behavioural processes in organisms living in such aperiodic environments over protracted time may suggest an intrinsic adaptive value in possessing circadian periodicity, deriving, perhaps, from the need to synchronise various processes within the organism (Pittendrigh and Minis 1972; Ouyang et al. 1998; Sheeba et al. 1999, 2001a; Sharma and Joshi 2002). We have four populations of fruit flies *D. melanogaster* that have been reared under an aperiodic environment where light (of about 100 lx intensity), temperature (24±1°C), and humidity were constant for over 600 generations (described in detail in Sheeba et al. 1998). We have previously reported that circadian rhythms in eclosion and oviposition by individual females persisted in these populations in constant light (LL) and constant dark (DD) regimes, and entrained to a light/dark (LD) cycle of 12:12 h (Sheeba et al. 1999, 2001a). The periodicities of eclosion rhythm in LL and DD were 21.80±2.72 h (mean ±95% CI) and 22.72±4.7 h (mean±95% CI), respectively.

Locomotor activity of *D. melanogaster* is among the best studied circadian rhythms and most studies indicate that the rhythm is robust in DD and LD but damps out in

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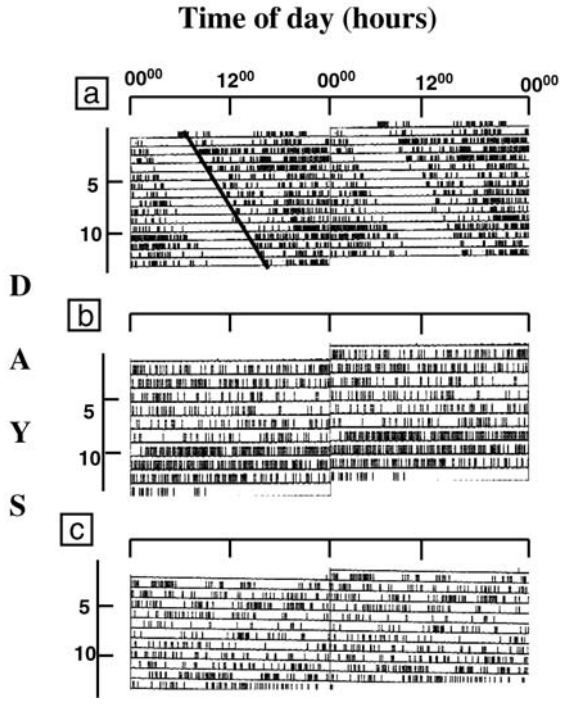

Fig. 1 Representative locomotor activity records of flies assayed in LL regime exhibiting **a** circadian rhythm, **b** arrhythmicity and **c** ultradian rhythm. *Abscissa* shows time of day while ordinate shows number of days. *Thick bars* indicate activity, while *horizontal lines* indicate rest

LL (Konopka et al. 1989; Zerr et al. 1990; Qui and Hardin 1996; Emery et al. 2000). Moreover, three separate oscillators are thought to control eclosion, oviposition and locomotor activity rhythms in *D. melanogaster* (Engelmann and Mack 1978; Sheeba et al. 2001b). Here we report results on the locomotor activity rhythm of individual flies from four populations of *D. melanogaster* maintained under LL for several hundred generations. We estimated the fraction of individuals exhibiting circadian rhythmicity in locomotor activity in LL, LD and DD regimes after being subjected to one of four different light treatments. We also examined the effect of light regimes in which the rhythm was assayed, as well as the light regime experienced by the flies during the first 15 days of adult life, on the locomotor activity rhythm of adults.

Materials and methods

We assayed the circadian rhythmicity in locomotor activity using four different experimental protocols (henceforth referred to as experiments 1, 2, 3 and 4). In all four experiments, the flies were maintained in LL during the pre-adult stage. In experiment 1, the locomotor activity of adults was monitored in LL (intensity of about 100 lx) for 30 days immediately after eclosion. In experiment 2, freshly laid eggs were subjected to 12 h of darkness after which the same protocol as in experiment 1 was followed. In experiment 3, the locomotor activity of adults was first monitored under LD 12:12 h for 15 days and then in DD for the next 15 days, while in experiment 4 the adult locomotor activity was monitored in DD for the first 15 days after eclosion. In experiments 1, 3 and 4, both males and females were assayed, while in experiment 2 only male flies were assayed.

From the running cultures of the four populations, eggs were collected at densities of approximately 50 eggs per vial into vials containing ~6 ml of banana-jaggery (a coarse brown sugar). The locomotor activity of virgin flies was assayed using infra-red beams which detected the vertical movements of individual flies in narrow vertical glass tubes (80 mm height, 6 mm diameter). When a fly cut the IR beams, the event was recorded in 5 min bins by a computerised recording and display system (Sharma 2002).

Flies were classified as being rhythmic with circadian periodicity, arrhythmic, or showing ultradian rhythmicity, based on visual observation of the actograms (Fig. 1). The activity rhythms were clear enough and did not require any time series analysis to demonstrate circadian patterns. In this paper, we have discussed the presence of circadian rhythms in the LL-raised populations and have exercised stringent visual criteria. Unless the activity records showed a convincing pattern, we did not consider it to be rhythmic. Use of a time series analysis program is not expected to change the results. The locomotor activity of a fly was assumed to follow a circadian pattern only if it occurred with a periodicity ranging between 12 and 36 h; patterns with periodicities below 12 h were considered to be ultradian. The proportion of flies in each population that were rhythmic was estimated for each of the four experimental protocols, and the arcsine square root transformed fractions (Freeman and Tukey 1950) were used as data in two separate mixed-model analyses of variance (ANOVA). In both analyses, replicate populations were treated as random blocks. In one analysis, only data from experiments 1, 3 and 4 were used, and experimental protocol and sex were treated as fixed factors crossed with block. In the other analysis, data from only males in all four experiments were used, and experimental protocol was treated as a fixed factor crossed with block.

Results

The regime in which locomotor activity rhythm was assayed influenced the proportion of flies exhibiting circadian rhythm in locomotor activity (Fig. 2). The percentage of flies exhibiting circadian rhythm in locomotor activity was 26.4% and 77% when assayed in LL and DD, respectively. The ANOVA on male and female data from experiments 1, 3 and 4 revealed significant main effects of experimental protocol ($F_{2,6}$=21.096, P<0.0002) and sex ($F_{1,3}$=38.422, P<0.0008). In all experiments, more males than females were rhythmic

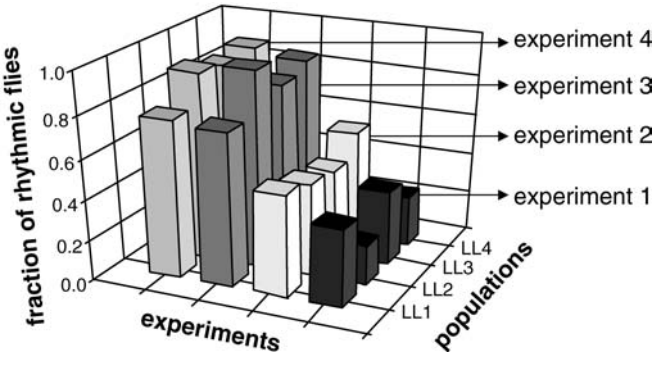

Fig. 2 Proportion of flies that exhibited circadian locomotor activity rhythm when assayed after subjecting them to four different experimental protocols

(0.30 vs 0.22 in experiment 1; 0.87 vs 0.70 in experiment 2; and 0.85 vs 0.67 in experiment 4). The proportion of flies exhibiting circadian rhythm of locomotor activity in experiment 2, in which only males were used, was 0.48 (Fig. 2). Multiple comparisons by Tukey's test revealed that fewer flies were rhythmic in LL, as compared with those assayed in the other regimes. The ANOVA using data from only males in all four experiments revealed a significant main effect of experimental protocol ($F_{3,9}$=33.8, P<0.0001), and multiple comparisons (Tukey's test) revealed that the number of rhythmic flies when assayed in LL without dark pulse (experiment 1) was significantly less than in the other three protocols, while for those assayed in LL with dark pulse (experiment 2) it was significantly less than for those assayed in DD (experiments 3 and 4) (Fig. 2). The proportion of flies exhibiting circadian locomotor activity rhythm when assayed in DD did not differ between groups first maintained for 15 days in LD 12:12 h, and those that were directly assayed in DD (experiments 3 and 4) (Fig. 2).

Discussion

Several studies of the molecular mechanisms of circadian organisation suggest that a system of interlocked feedback loops involving genes such as *per*, *tim*, *dclk*, *cyc* and *cry* and their protein products control many overt rhythms in *D. melanogaster* (Glossop et al. 1999). The protein TIM degrades rapidly in the presence of light and, therefore, it is believed that circadian rhythms that are regulated by the molecular mechanisms involving TIM would damp out in LL (Emery et al. 2000). The protein CRY is believed to be responsible for circadian photoreception, in addition to playing some role downstream of the photoreception mechanism, and a mutant cry^b exhibits circadian rhythm in locomotor activity even when assayed under LL regime, and entrains to LD cycles (Emery et al. 2000).

Although LL (intensity ~100 lx) did have some direct suppressive effect on the expression of the circadian rhythm of locomotor activity (Fig. 2), a considerable proportion of our flies (23–48%) showed distinct circadian rhythmicity. Some previous studies on *Drosophila* raised in LD 12:12 h reported that only about 5% of the flies continued to exhibit circadian locomotor activity rhythm in LL (intensity ~10 lx) (Konopka et al. 1989; Zerr et al. 1990). Our results show that a large proportion of flies in populations that have been reared in an aperiodic environment for more than 600 generations have retained the capacity to exhibit locomotor activity rhythm, even in bright LL. This suggests an intrinsic adaptive value in possessing circadian periodicity, deriving, perhaps, from the need to synchronise various processes within the organism, even in aperiodic environments such as bright LL.

The unique property of exhibiting circadian rhythm in eclosion, oviposition and locomotor activity in LL, in the populations of flies used in our experiments, could be the result of their prolonged exposure to LL. Further studies of the molecular mechanisms underlying the circadian rhythms of these flies could lead to a better understanding of the interaction between circadian pacemakers and light.

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