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Effect of Different Light Regimes on Pre-Adult Fitness in *Drosophila melanogaste*r Populations Reared in Constant Light for over Six Hundred Generations

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ABSTRACT

Egg to eclosion development time and survivorship were assayed on four laboratory populations of *Drosophila melanogaster* that had been reared for over 600 generations in continuous light (LL) and constant temperature. The assays were performed in three environments: continuous light (LL), periodically varying light/dark cycles (LD 12:12 hr), and continuous darkness (DD). Development time in LL was significantly less than that in LD, which, in turn, was significantly less than that in DD, whereas survivorship did not differ significantly among the three treatments. The results indicate that individuals from *Drosophila* populations routinely maintained in LL do not suffer any deleterious effects of LL treatment on pre-adult fitness. Other studies on these populations have shown that free-running period (τ) of the eclosion rhythm in DD is greater than that in LD. Our results are, thus, also consistent with the notion that development time may be a function of the free-running period.

KEYWORDS: fitness, continuous light (LL), development time, survivorship, free-running period, *Drosophila melanogaster*.

INTRODUCTION

Entrainment to light/dark cycles (LD) is believed to have an intrinsic, or physiological, adaptive value, independent of that deriving from ecological factors such as time-specific predation or availability of food (Pittendrigh & Minis, 1972; Buenning, 1973; Daan & Aschoff, 1982; Recio et al., 1997). This intrinsic adaptive value is thought to stem from the fact that external time cues are needed to

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maintain a set of mutual phase relationships among the many constituent oscillators, each with a different free-running period (τ), that exist within individual organisms. In the absence of external cues, it is thought that the normal phase relationships between the constituent oscillators break down, resulting in deleterious effects to the individual (Pittendrigh & Bruce, 1959; Pittendrigh, 1974). Following current thinking in evolutionary genetics (Rose et al., 1996; Mueller, 1997; Joshi, 1997), the empirical validation of the idea that circadian rhythms have an intrinsic adaptive value would require a demonstration of differential fitness effects of different light regimes, and the further demonstration of adaptive evolution in response to different light regimes in experimental populations. However, relatively few studies thus far have attempted to systematically address the possible intrinsic adaptive significance of circadian rhythms.

There is some evidence for the adaptive significance of circadian rhythms under field conditions from studies on clinal variation in circadian parameters, such as τ, of the eclosion (Lankinen, 1986; Lankinen, 1993) and oviposition rhythms of various species of *Drosophila* (Allemand & David, 1976). However, the observation of clinal variation in τ from natural populations cannot address the issue of the possible intrinsic adaptive value of circadian rhythms. To examine intrinsic adaptive significance, one would have to study the fitness consequences of different light regimes under a situation wherein all time-specific ecological factors could be eliminated, and this has rarely been done systematically on any well-defined experimental system.

A few studies have shown that organisms normally reared in LD conditions tend to perform better in LD cycles of periodicity similar to their rearing conditions. *D. melanogaster* flies, normally reared on a 12:12 hr LD cycle, were found to have the greatest longevity under 12:12 hr LD, as compared to 13.5:13.5 hr LD, 10.5:10.5 hr LD, or constant light (LL) (Pittendrigh & Minis, 1972). Similar observations have been made on the effect of LD cycles of varying periods (T) on growth rates in several plant species (Went, 1959). Moreover, deleterious effects of LL on tomato (*Lycopersicon esculentum*) have also been observed (Highkin & Hansen, 1954; Hillman, 1956), leading to a general assumption that LL conditions exert harmful effects on organisms habituated to LD cycles (Highkin, 1954; Daan & Ashoff, 1982). It is not, however, clear that LL conditions are harmful *per se*, especially in animals, and it is entirely possible that populations routinely maintained in LL may evolve so as to better tolerate any adverse physiological effects of constant light. Indeed, in a recent study, the growth rate in cultures of the cyanobacterium *Synechoccus* was seen to be greater in LL than in various LD cycles (Ouyang et al., 1998).

Unlike most previous studies, the work of Ouyang et al. (1998) examined the reproductive fitness of competing *Synechoccus* strains, with different τ, maintained in LD cycles of different periodicities (T). They found that the strain which

had τ closest to the period of the LD cycle was able to outcompete the other, leading them to conclude that reproductive fitness is improved by resonance between the endogenous clock and the environmental cycle (Ouyang et al., 1998). This study, thus, clearly demonstrates the adaptive value associated with phase locking of circadian rhythms in a controlled laboratory environment, where many time-specific ecological pressures (such as predation etc) commonly found in field conditions are absent. It should, however, be noted that the study by Ouyang et al (1998) does not address the issue of a possible intrinsic adaptive significance of possessing a circadian clock *per se* (*i.e.* the adaptive significance of periodicity, as opposed to that deriving from phasing). In order to address this latter issue, it would also be necessary to examine the fitness consequences of different light/dark regimes and contrast them with the fitness effects of constant environments such as LL and constant darkness (DD).

We have recently initiated a long-term series of studies designed to address the possible intrinsic adaptive significance of circadian rhythms using the methodology of experimental evolutionary genetics, in which the first step would be to assess the effect of different light regimes on pre-adult and adult fitness components in a controlled and replicated system of laboratory populations (*e.g.* see Rose et al., 1987; 1986; Mueller, 1995, 1997; Joshi, 1997). In the present study, we examined two components of pre-adult fitness in four laboratory populations of *Drosophila melanogaster* that have been reared at constant temperature (25 ± 1°C) and humidity in LL for over 600 generations and have, therefore, not been exposed to any environmental time cues. We assayed egg to eclosion development time and survivorship in these populations under three different light regimes: LL, LD (12:12 hr) cycles, and DD, in order to study the effect of different light regimes on these two major components of pre-adult fitness.

MATERIALS AND METHODS

Experimental Populations

This study was conducted on four large (N ~ 1500 breeding adults), outbred, laboratory populations of *Drosophila melanogaster* (JB-1...4) whose origin and maintenance has previously been described in detail (Sheeba et al., 1998). Consequently, we restrict ourselves here to details of rearing pertinent to this study. These populations are maintained in incubators at 25°C (± 1 °C), under constant light and humidity, at moderate larval and adult densities, on a 21-day discrete generation cycle. More importantly, the ancestral populations from which the JB populations were derived (described in Joshi & Mueller, 1996) had also been maintained in the laboratory under constant light and temperature for over 600 generations.

Development Time and Survivorship Assays

From the running culture of each population in plexiglass cages ($25 \times 20 \times 15$ cm^3), eggs were collected by allowing females to lay eggs on a non-nutritive agar medium for exactly one hour. Exactly 30 eggs were collected into 8 dram vials (9.0 mm h × 2.4 mm d) containing ~ 6 ml of banana-jaggery food. In this manner, 24 such vials were set up per population, of which 8 vials were kept under constant light (LL), 8 vials under light dark (LD) cycles of 12:12 hours, and the remaining 8 vials in constant darkness (DD). The light phase in these treatments was achieved by means of fluorescent white light sources, whereas the dark phase was actually a period when the flies were maintained in red light ($\lambda > 640$ nm), to facilitate observation and manipulation without interrupting the dark phase. After pupation, the vials were checked every 6 hours for eclosing adults. At each six-hourly check, the number of male and female flies that eclosed in each vial was recorded, and the flies discarded. This process was continued until no more flies eclosed in any of the vials for three consecutive checks. The entire assay was repeated thrice to ascertain the repeatability of results. The procedure used here for assaying development time is a standard one in fitness component studies in *D. melanogaster* (Rose et al., 1984; Santos et al., 1997; Chippindale et al., 1997). It ensures a moderate and controlled density to minimise environmental variation in development time due to either overcrowding, or hardening of medium due to a very low larval density (Mueller, 1985). It should be noted that the protocol for assaying development time is very different from those used for studying eclosion rhythms in *Drosophila* (Chandrashekaran, 1998); the latter typically involve high larval densities in order to spread out the eclosions through crowding effects, thereby ensuring that several cycles of data can be obtained. In studies such as the present one, such high densities, being very different from the density at which the populations are normally maintained, would increase the likelihood of observing genotype × environment interactions that could make the data very difficult to interpret (Leroi et al., 1994).

Statistical Analyses

From the primary data from each assay, egg to eclosion development time (in hours) and survivorship (the fraction of adults that emerged per vial) were computed. The population mean development times (ln transformed) in each light treatment, for each sex from each experiment were used as data in a mixed model analysis of variance (ANOVA) in which replicate populations were treated as random blocks and the various light dark regimes and sex as fixed factors crossed with blocks. Similarly, the population mean survivorship values (arcsin square-root transformed) in each light regime from each experiment were used as data in a mixed model ANOVA, with replicate populations being treated as random blocks and the various light dark regimes as a fixed factor crossed with block. For both

development time and survivorship, the mean values for each combination of block × light regime × sex (for development time), or block × light regime (for survivorship), from each of the three experiments served as replicate observations in the analysis. We used population means in the analyses because we wish to draw evolutionarily relevant inferences about the differential effects, if any, of light treatment on the development time and survivorship. For such inferences to be drawn, the crucial unit of analysis and interpretation is the population and not the individual (Rose et al 1996).

RESULTS

Survivorship from egg to eclosion did not differ significantly among the various light treatments, indicating that light regime did not affect survivorship in these populations ($F_{2,6} = 1.06$, $p = 0.4$) (Table 1). On the other hand, light regime did have a significant effect on development time, with the fastest development being in LL (Figure 1). The ANOVA on development time data revealed a significant treatment effect ($p < 0.00001$) (Table 2). Across all the three assays, development time in LL, LD and DD were all significantly different from each other (paired *t*-test: $p < 0.0001$ for all pair-wise comparisons) (Figure 1). As expected in *Drosophila melanogaster,* there was a significant effect of sex on development time ($p = 0.00049$), with females developing faster than males (Figure 2). The ANOVA did not reveal any significant difference in development time among the four replicate populations (blocks) ($p > 0.9$), nor were any of the interactions involving block significant (Table 2).

TABLE 1. Mean survivorship from egg to eclosion in the four JB populations under LL, LD and DD in the three experiments. The 95 % confidence intervals are based on variation among the four replicate populations in each experiment × light regime combination.

	Expt 1			Expt 2			Expt 3		
	LL	**LD**	**DD**	**LL**	**LD**	**DD**	**LL**	**LD**	**DD**
JB 1	0.875	0.906	0.875	0.933	0.875	0.870	0.846	0.870	0.870
JB 2	0.954	0.893	0.954	0.854	0.954	0.85	0.82	0.85	0.85
JB 3	0.904	0.823	0.904	0.854	0.904	0.862	0.85	0.862	0.862
JB 4	0.870	0.833	0.870	0.862	0.870	0.854	0.88	0.854	0.854
mean	**0.901**	**0.864**	**0.901**	**0.876**	**0.901**	**0.859**	**0.849**	**0.859**	**0.859**
95% c.i.	**0.061**	**0.066**	**0.061**	**0.061**	**0.061**	**0.014**	**0.039**	**0.014**	**0.014**

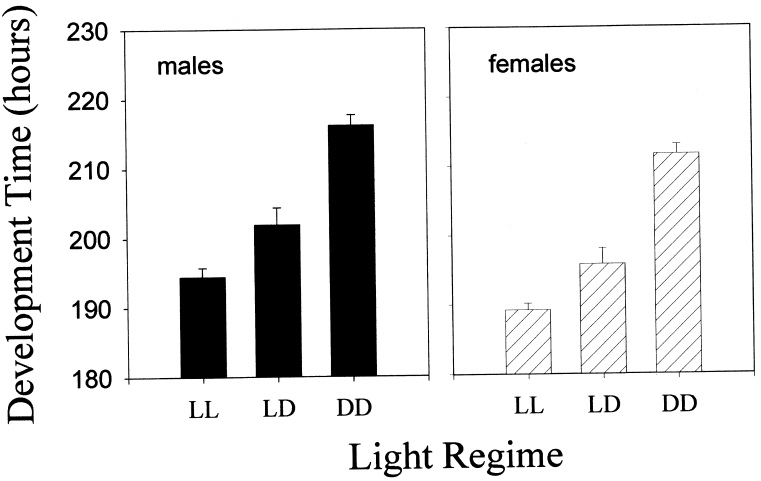

Fig. 1. Mean egg to eclosion development time (averaged across the four replicate populations and three experiments) of males and females under LL, LD and DD. Error bars represent 95% confidence intervals about the mean, and were constructed using the variation among the four replicate populations, after averaging across experiments for each population.

DISCUSSION

In contrast to previous reports that, in general, LL has deleterious effects on organisms (review in Pittendrigh, 1960; Daan & Aschoff, 1982), we found that two major components of pre-adult fitness (survivorship and development time) were not adversely affected by LL in populations of *D. melanogaster* maintained in LL for over 600 generations. Indeed, development time in these populations was shortest in LL, suggesting that maintenance in LL for many generations may

TABLE 2. Results of mixed model ANOVA on ln-transformed development time. The population mean development times from each light regime × sex × experiment combination were used as the units of analysis. Replicate populations were treated as random blocks and the various light dark regimes and sex as fixed factors crossed with block.

Effect	*df*	MS	*F*	*P*
Block (B)	3	0.0003	0.032	0.9922
Light regime (L)	2	0.0681	1403.741	< 0.0001
Sex (S)	1	0.0128	271.509	0.0004
B × L	6	<0.0001	0.004	1.0000
B × S	3	<0.0001	0.004	0.9996
L × S	2	0.0001	8.723	0.0167
B × L × S	6	<0.0001	0.001	1.0000
Error	48	522.4183		

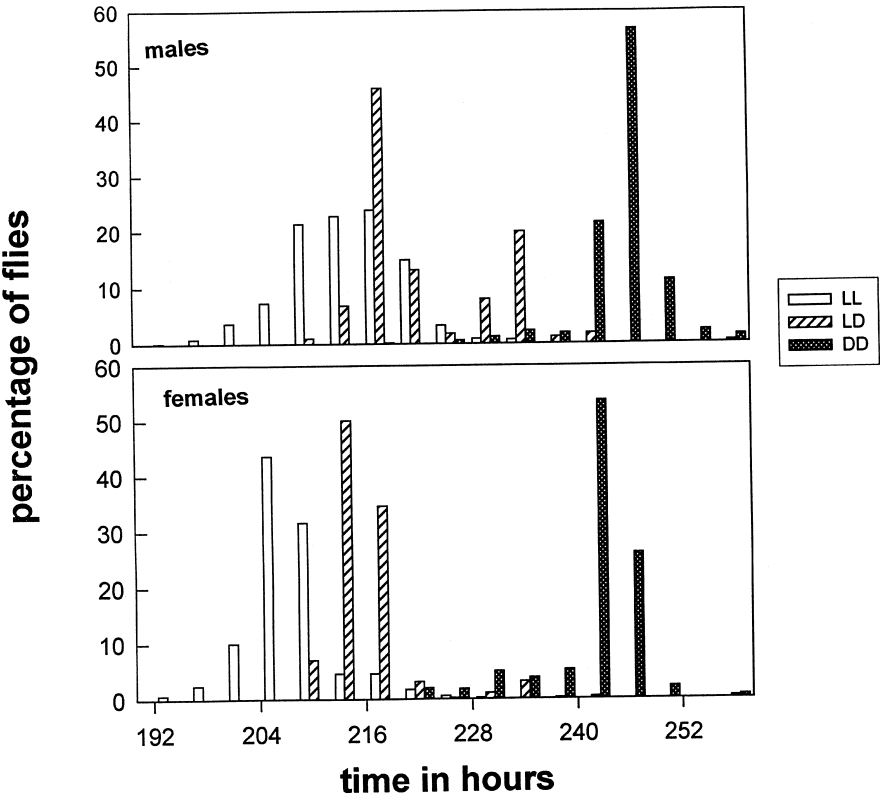

Fig. 2. Frequency distribution of egg to eclosion development time (pooled data from the four populations from one of the representative experiments) showing the degree of separation of the distribution of development time under LL, LD and DD, for both males and females.

have resulted in the evolution of specific adaptations to constant light, thereby allowing flies from these populations to complete development faster in LL, as compared to LD and DD regimes. Thus, our results, while strengthening the idea that organisms perform best in the light regime that they are routinely reared in, extends this logic to organisms that have been maintained in LL, and suggest that there are no intrinsic, deep-rooted deleterious physiological effects of LL on pre-adult fitness in *D. melanogaster* that adaptive evolution cannot overcome.

Development time is known to be a highly labile trait (Mueller, 1985), and the fact that all four populations studied exhibited a similar trend in development time in the three different light regimes, suggests that light conditions exert their influence on some highly conserved aspect of the developmental pathway. This idea is supported by the absence of any significant interactions involving block, which is unusual in studies on fitness components in *Drosophila* (A Joshi, *pers. obs.*). The

fact that the effects of light regime are consistent across independent replicate populations is important because it allows us to rule out fortuitous effects due to either chance or the unique genetic composition of a particular population. Such populational level replication is crucial if one wishes to draw evolutionary conclusions from data on fitness effects of different environmental regimes (Mueller, 1995; Rose et al., 1996; Joshi, 1997).

Why exactly development time in these populations is shorter in LL is difficult to say. There is some evidence that subjective time estimation in organisms may feedback into determining the duration of different life-stages. *D. melanogaster per* mutants with relatively short τ have been seen to have relatively short development time and vice versa (Kyriacou, 1990), suggesting that development time is a function of τ. Similarly, life-span in the prosimian primate *Microcebus murinus* has been seen to be shortened by subjecting the animals to an 8 month year experimentally (Perret, 1997). If the duration of pre-adult development is in fact correlated with subjective rather than chronological time, then the faster development in LL could be a consequence of a direct effect of constant light speeding up the organism's biological clock (Pittendrigh, 1960). If this is indeed the case, then our results suggest that the period of eclosion rhythm in LL may be expected to be shorter than that in LD, and that the period in LD shorter than that in DD. Other experiments in our laboratories have shown that eclosion in LL for these flies is arrhythmic, while it has periodicities of 24 and 26 hr in LD and DD, respectively (Sheeba V, Sharma VK, Chandrashekaran MK and Joshi A, *unpubl. ms.*). A similar observation has been made using cultures of the cyanobacterium *Synochoccus* which exhibit shorter doubling time in LL, as compared to LD (Ouyang et al., 1998). An alternative explanation for our results could be that the faster development of these populations in LL is a consequence of their having adapted to LL conditions, with these adaptations being entirely independent of the biological clock. The present data, obviously, do not permit us to clearly differentiate between these two alternatives. To dissect out the precise way in which LL is causing these populations to exhibit faster development would require a series of studies, merging selection and reverse-selection approaches (Rose et al., 1996) with standard chronobiological methods.

One drawback with the present study is that we do not at this time have matched control populations that were reared in LD (i.e. populations sharing a common ancestry and all other aspects of rearing conditions with our JB populations); such populations will take several years to develop through selection in the laboratory. Comparing the JB populations with LD-reared populations of a different ancestry would be inappropriate because then ancestry and selection regime would be confounded in the analysis. Despite this drawback, we feel that our results on pre-adult fitness in LL-reared populations are, nevertheless, of considerable relevance to the issue of the possible intrinsic adaptive significance of

circadian rhythms, inasmuch as they demonstrate that LL conditions are not necessarily deleterious to organisms that have experienced LL for many generations.

The results reported here are the first step in a detailed ongoing examination of the fitness consequences of different light regimes in *D. melanogaster*. This is an attempt to use the techniques and approaches of modern evolutionary genetics to address the relatively neglected issue of the adaptive significance of circadian organization, and how it may evolve. Although pre-adult fitness components were not adversely affected by LL in this study, it is possible that LL conditions may, nevertheless, have deleterious effects on components of adult fitness, even in populations maintained under LL for many generations. For example, if longevity is positively correlated with development time, mean life span may be expected to be lower in LL than in LD or DD. Moreover, fast development in *Drosophila* is known to trade-off with traits related to adult fitness, such as body size (Zwaan et al., 1995; Chippindale et al., 1997) and fecundity (Hiraizumi, 1961). A clearer picture may, therefore, be expected to emerge after further studies focussing on the effects of different light regimes on components of adult fitness such as size, fecundity and longevity.

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