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Circadian Systems: Longevity as a Function of Circadian Resonance in *Drosophila melanogaster**

(light:dark cycle/"oscillating systems")

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ABSTRACT ***Drosophila melanogaster*, which had been reared under standard conditions (25° and a 24-hr light/dark cycle involving 12 hr of light) were exposed, on the first day of adult life, to four environments (all at 25°) as follows: (*i*) a 24-hr day consisting of 12 hr light and 12 hr dark; (*ii*) a 21-hr day (10.5 hr light, 10.5 hr dark); (*iii*) a 27-hr day (13.5 hr light, 13.5 hr dark); and (*iv*) constant light. The experiment was repeated four times. In all four experiments the flies on a 24-hr day lived significantly longer than the flies in the other environments. This result, comparable to other observations on plants, indicates that eukaryotic systems as oscillators perform most effectively when they are driven close to their natural "circadian" frequency.**

The significance of the few observations reported here is that they constitute nearly the only published evidence that animals, like plants (1), function most effectively when as periodic, oscillating systems they are driven at frequencies close to their own innate frequency, which is about 1 period per 24 hr ("circadian"). Background to this proposition was developed by Pittendrigh and Bruce in 1959 (2), and Pittendrigh in 1960 and 1961 (3, 4). Aschoff (personal communication) has recently encountered similar phenomena in the blowfly *Phormia terrae novae*.

MATERIALS AND METHODS

Drosophila melanogaster was raised on standard cornmeal-molasses medium at 25° in a light/dark cycle whose period (T) was 24 hr and whose photoperiod was 12 hr (i.e., T = 24; *LD 12*:12). 24 hr after emergence the flies were etherized, sexed, and separated into standard food vials (4 × 1 inches), each containing 10 flies of one sex. Lots of 10 such vials (for a total n = 100) were then put into cabinets at 25° ± 0.50 in one of the following light regimes: (*i*) T = 24 hr (*LD 12*:12); (*ii*) T = 21 hr (*LD 10.5*:10.5); (*iii*) T = 27 hr (*LD 13.5*:13.5); and (*iv*) constant light (*LL*). The illumination was supplied by 4-W fluorescent ("cool white") bulbs encased in water jackets with continuously circulating water to eliminate any temperature cycle concurrent with the light cycle. Since in each period (T) the light was on 50% of the time, the insects were exposed to essentially the same total illumination over the long duration of their lives. The flies were transferred every 3 or 4 days to fresh food vials, and deaths were scored at the time of transfer. In the first experiment $^1/_2$-pint milk bottles, each containing 50 flies (2 bottles in each environment; n = 100 again) were used instead of the 10 vials, each with 10 flies.

In all, four experiments were performed. The first involved wild-type males from a strain collected in Princeton, N.J. The second involved females of the tumorous strain tu^g. The third involved males of the Princeton wild-type strain; and the fourth involved females of that (wild-type) strain.

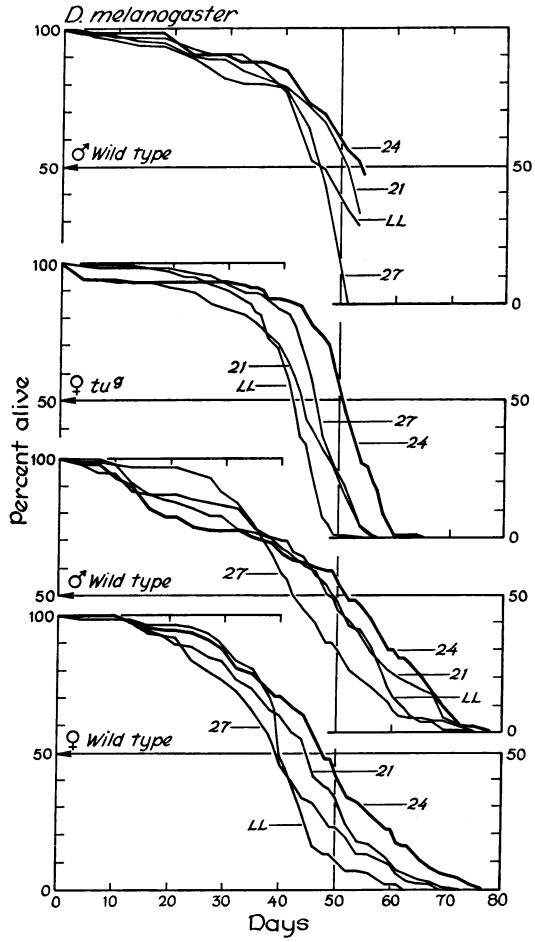

FIG. 1. The time-course of survivorship in all four experiments. The 24-hr population is plotted as a *heavier line* to facilitate comparison with the others. Coordinates are drawn to facilitate comparisons by two criteria: 50% alive and Day 50.

* Preceding paper in series: Pittendrigh, C. S., Eichhorn, J. H., Minis, D. H. & Bruce, V. G. (1970) "Circadian Systems, VI: Photoperiodic Time Measurement in *Pectinophora gossypiella*, *Proc. Nat. Acad. Sci. USA* 66, 758–764.

TABLE 1. *Survivorship, estimated by two criteria, in all four experiments*

	Days to 50% alive				Number alive on Day 50			
	T_{24}	T_{21}	T_{27}	LL	T_{24}	T_{21}	T_{27}	LL
Exp. 1 (wild ♂)*	53.6	51.0	46.5	46.0	60.0	55.0	15.0	38.0
Exp. 2 (wild ♀)† Average	49.9	42.9	45.7	41.4	5.8	2.4	2.5	0.1
Exp. 3 (wild ♂)† Average	51.7	46.8	42.5	45.8	5.4	4.5	3.0	4.8
Exp. 4 (wild ♀)† Average	46.7	43.5	39.9	40.2	4.2	3.4	2.3	1.1

* n = 100/environment.
† n = 10/vial; 100/environment.

RESULTS

The time-course of survivorship in all four experiments is plotted in Fig. 1. Table 1 summarizes, for the four experiments, data on two criteria of survivorship: the number of days to reach 50% mortality and the number of flies alive at Day 50. In experiments 2, 3, and 4 the 10 replicates in each of the four experimental environments were scored separately, and the resulting data were subjected to a one-way analysis of variance. Table 2 summarizes the results of that analysis of variance on the data for experiments 2, 3, and 4, again with respect to both criteria of survivorship (days to 50% dead and the number alive on Day 50). Table 3 summarizes the qualitative results on relative survivorship (by both criteria) under all four environments.

In the analysis of variance we ask two questions: (*a*) Does survivorship in the four environments differ significantly (Table 2, section I); and (*b*) Is the mean survivorship in T = 24 hr significantly greater than the mean of all other environments combined (Table 2, section II)?

The results are unequivocal: in all experiments survivorship is greatest when T = 24 hr whether one uses the criterion of the number of days to reach 50% survivorship or the criterion of the number alive on Day 50. Considering only the three periodic environments, there appears to be a clear indication not only that T_{24} is the most favorable environment but that T_{27} is the worst (Table 3).

The totally aperiodic environment (25° and constant light), while clearly less favorable than T_{24} or T_{21}, is apparently less deleterious than T_{27} in males, but it is clearly the worst environment for females, especially by the criterion of survivorship on Day 50.

TABLE 2. *Results of a one-way analysis of variance of the data for Exps. 2, 3, and 4.*

	Days to 50% alive	Number alive on Day 50
I: Significance of differences between all environments		
Exp. 2: tu^s (♀)	$P < 0.001$	$P < 0.001$
Exp. 3: wild (♀)	$P < 0.001$	$P < 0.01$
Exp. 4: wild (♂)	$P < 0.05$	$P < 0.025$
II: Significance of differences between T_{24} and mean of all others		
Exp. 2: tu^s (♀)	$P < 0.001$	$P < 0.001$
Exp. 3: wild (♀)	$P < 0.001$	$P < 0.025$
Exp. 4: wild (♂)	$P < 0.005$	$P < 0.001$

DISCUSSION

Apart from Pittendrigh's report (3) of the effect of aperiodic versus periodic environments on the viability of semi-lethals in *D. melanogaster*, this is the first clear evidence that the physiological well-being of any animal is affected by the state of its circadian organization. The results, showing the optimal nature of a 24-hr periodicity, are strictly comparable to the pioneering observations of Frits Went (1) on several plants in which growth rates are optimal when T (the period of the light cycle) is close to τ, the freerunning period of the circadian system. Went's observations included the extra, cogent fact that the optimal value of T was slightly temperature-dependent, as is τ in many plant species.

Pittendrigh and Bruce (2) reviewed all the data then available, the most important of which, in addition to Went's [and comparable later findings of Ketellapper (5)] were studies by Highkin and Hanson (6) and especially Hillman (7) on the deleterious action of constant light and constant temperature on tomatoes. Hillman's experiments showed that the adverse effects of constant light could be avoided if a 24-hr temperature cycle was imposed on the plants.

The general proposal that emerges from all these observations, still scant, is that organisms having evolved an innate periodicity in their metabolic functions "perform" most effectively when, as "oscillating systems," they are driven by external cycles close to their natural frequency. Multicellular systems in particular must constitute a population of oscillations comprising the total (circadian) system. Normal function is likely to be contingent on a given set of mutual phase-relationships between constituent oscillations. To a significant extent the system as a whole must rely on external entrainment of all (or most) constituent oscillators for the maintenance of normal temporal organization: in aperiodic environments constituent oscillators, differing in their free-running periods, can be expected to lose normal phase relationships with respect to each other. Indeed Aschoff (8) has recently published the most compellingly clear evidence that such desynchronization does occur. The deleterious action of aperiodic environments is thus very likely due to the loss of an internal temporal organization.

TABLE 3. *Relative survivorship in the four environments. Experiments were pooled. Sexes were treated separately*

	Days to 50% alive				Percent alive on Day 50			
	T_{24}	T_{21}	T_{27}	LL	T_{24}	T_{21}	T_{27}	LL
♂ (Exps. 1 and 3)	52.7	48.9	44.5	45.9	57.0	50.0	22.5	43.0
♀ (Exps. 2 and 4)	48.3	43.2	42.8	40.8	50.0	29.0	24.2	7.0
♂	$T_{24} > T_{21} > LL > T_{27}$				$T_{24} > T_{21} > LL > T_{27}$			
♀	$T_{24} > T_{21} > T_{27} > LL$				$T_{24} > T_{21} > T_{27} > LL$			

The deleterious action of environments whose period (T) is far from 24 hr is also likely to be due to a loss of normal phase-relationships between constituent oscillations whose periods differ. Their phase relations ["normal" when driven by a cycle whose period (T) is 24 hr] will change when each is driven by Ts different from 24 hr. In general, physiological function in organisms, innately periodic in their time-course, is to be expected to be most nearly normal when they are close to "resonance" with the periodic environment in which they operate—when T is modulo τ.

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