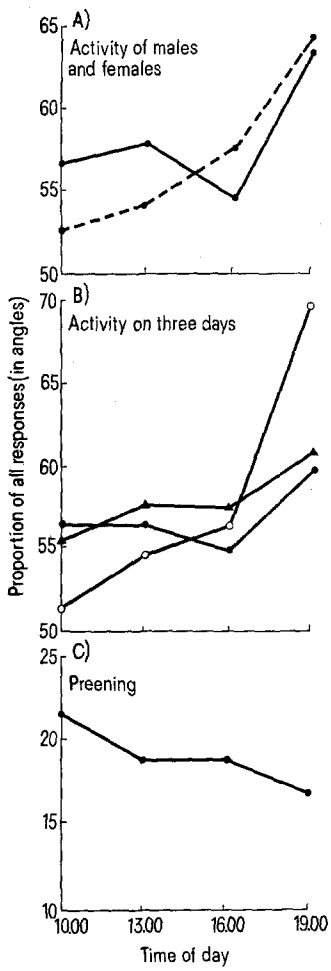
Behavioural Rhythms in Cultures of Immature *Drosophila melanogaster*

Circadian rhythms of locomotor activity have been found in several species of *Drosophila*[1–3]. Apart from their role in adapting to the diurnal cycles of external factors[4], rhythms may also increase reproductive success by synchronising sexual arousal in both sexes[5] and GROSSFIELD[6] inferred a close relationship between locomotor activity and mating from similarities in their circadian rhythms. Using measures of locomotor activity and preening recently developed for the genetical analysis of *Drosophila* behaviour[7], the diurnal periodicities were studied here during the three days after the first pupae emerged, on the hypothesis that changes of rhythm in this period of increasing sexual maturity may also indicate some connection with mating activities.

Methods. The subjects came from Oregon, Samarkand, Florida, 6 C/L, Edinburgh and Wellington, 6 inbred strains of *D. melanogaster* which have already served in a genetical analysis of male mating speed[8]. The stocks were reared by mass-mating in standard half-pint culture bottles at 25 °C ± 2 °C, but, rather than a regular light-dark cycle, the flies were kept in unlit incubators and exposed to light only when the door was opened. This is the standard practice in the laboratory at Birmingham and one to which the strains will presumably have adapted over the several hundred generations of inbreeding.



Changes in behaviour of *D. melanogaster* observed between 10 and 19.00 h. The points show the mean angular-transformed proportions of the particular response during 10 observations in the first 60 sec following stimulation. A) The rhythm of locomotor activity, averaged over the 3 days of testing, in males (—) and females (- - -). $N = 108$ for each point. B) The rhythm of activity averaged over sexes. Here ●, day 1 after eclosion; ▲, day 2; ○, day 3. $N = 72$ for each point. C) The rhythm of preening, averaged over sexes and days of testing. $N = 216$ for each point. Note that the ordinate of C) differs from A) and B).

The parents were removed from the cultures on the day before the first pupae emerged. On the 3 succeeding days, 2 males and 2 females were taken from each of 3 bottles of every strain at 8.30, 11.30, 14.30 and 17.30 h. As the cultures were not cleared of other flies at these times, the age range was increasing with every test. The 72 flies in each sample were introduced under light CO_2 sedation into separate 10 cm lengths of glass tubing, of sufficient diameter (3 mm) to permit all movements except flight, and assigned at random to 12 corrugated trays where each individual was completely screened from its neighbours.

Although recovery from the CO_2 took only about 90 sec, scoring did not start until some 80–90 min later, in deference to the suggestion[1] that such a period of deprivation from food and moisture can substantially reduce the activity rhythm. Then, in turn, each tray of tubes was placed under homogeneous illumination of 50 lux provided by a cool light source (max. temp. 25 °C), the flies stimulated by tapping briskly on the tray and the behaviour of each one recorded as either locomotor activity or preening or inactivity. Observing all 6 flies on the tray took 6 sec and this scoring was repeated 10 times, to give frequencies for each behaviour in the first min after stimulation.

Results and discussion. The changes in activity and preening during the day are presented in the Table and the 3 parts of the Figure. Activity, the predominant response to stimulation, rose towards evening while the frequency of preening declined. The very pronounced periodicities, despite the food deprivation with which CONNOLLY[1] reduced an activity rhythm, probably result from the measures here being ones of reactivity to stimulation, which is far less affected by this form of deprivation than is spontaneous activity (ANGUS, personal communication). Such reactivity is known to impede mating[9] and 6 C/L, whose very high activity was chiefly responsible for the large strain variation (Table), is the slowest to mate of these 6 strains[8].

The magnitude of the activity rhytm increased over the 3 days (the 'time × day' interaction and Figure B); while this change may be due only to the greater synchronization of eclosion, produced in *Drosophila* populations by exposure to light during the pupal stage[10], hormonal factors are needed to explain the more pronounced rhythm in females (the 'time × sex' item and Figure A). The sexual receptivity of female *Drosophila* is very dependent upon the corpus allatum[11], which is linked to

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Summary of the analyses of variance of activity and preening, based on the sum of 10 observations per fly (total $N = 864$), after an angular transformation

Item	d.f.	Activity Mean square	Significance level (P)	Preening Mean square	Significance level (P)
Time of day	3	3639.6	0.1%	697.9	0.1%
Strain	5	3580.4	0.1%	690.3	0.1%
Day of testing	2	127.7	NS	1088.7	0.1%
Sex	1	188.6	NS	380.0	5%
Time × day	6	802.1	1%	150.5	NS
Time × sex	3	634.8	5%	189.8	NS
Error[a]	720	245.1		104.5	

[a] Only items significant in at least one analysis are included; the error terms comprise the bottle interactions pooled with the within-bottle variances.

the corpus cardiacum controlling the rhythm of spontaneous activity and whose own secretions show a rhythm of output[12]. This rhythmicity would increase when the gland develops (the 2nd and 3rd days after eclosion), affecting the activity rhythm of females, and possibly even that of males, since mating behaviour is co-ordinated between the sexes and the presence of flies of the opposite sex can influence the activity rhythm of *Drosophila*[13].

On the other hand, the periodicity of preening was constant over the 3 days, but preening declined significantly between the 2nd and 3rd days, when the first females would be reaching sexual maturity (the means, in angles, on the 3 successive days were 20.81, 19.53 and 16.92 with $S.E._{diff} = \pm 0.85$). When in groups, preening keeps flies a certain minimum distance apart,[7,14,15] and also impedes the male courtship display, so that both this decrease at maturity and the lower level of preening in males (the 'sex' item of the Table) may contribute to mating success.

Further studies are needed to separate the effects of endogenous rhythms, which only become evident some time after emergence, from such population periodicities as the rhythm of eclosion which is influenced by increasing group density[16]. It may then be possible to explain some of the inconsistencies between the existing field and laboratory reports of activity rhythms in *Drosophila*[17].

Zusammenfassung. Die lokomotorische Aktivität von adulten *Drosophila melanogaster* zeigt einen Tagesrhythmus, der während der dreitägigen Reifungsperiode zunimmt und in Weibchen ausgeprägter ist als in Männchen. Die Periodizität der Putzhandlungen ist weniger ausgeprägt und ändert sich nicht, doch nimmt die Frequenz der Putzhandlungen bei den reifen Fliegen ab. Es wird ein reziproker Zusammenhang zwischen Putzfrequenz und sexueller Aktivität festgestellt.

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Tetraethyl Lead Dose Response Curve for Mortality in Laboratory Rats

In view of the extensive use of leaded gasoline throughout the world and the rather recent inception of psychopharmacological investigations of such compounds, it was deemed important to extend the work of previous investigators in a more thorough toxicologic study of tetraethyl lead. As a first step, the LD_{50} value of the toxin was determined from a small sample and presented earlier[1]. This communication represents an assessment of the dose response curve for single dose, oral administration.

Method. 96 Sprague-Dawley male albino rats were employed for these tests. 4 replications of 6 dosage levels were performed using 24 new subjects each time. The animals, which ranged in weight from 350 to 450 g, were randomly assigned to the different dosage groups. As in the previous study, stringent sanitary measures were enforced to prevent Salmonellosis and chronic respiratory disease; 2 diseases to which lead-treated rats appear particularly susceptible. The simplified method of Litchfield and Wilcoxon[2] was the procedure chosen for approximation of the dose response curve.

Procedure. Tetraethyl lead, obtained from the Ventor Corporation, Beverly, Massachusetts, was dissolved in 100% pure peanut oil to produce a solution with a concentration of 7.32 mg/ml. The animals were dosed according to the procedure set forth by Schroeder, Avery and Cross[1].

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