

INCREASE IN THE LONGEVITY OF ADULT *DROSOPHILA MELANOGASTER* KEPT IN PERMANENT DARKNESS

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INTRODUCTION

NUMEROUS papers have been published on the factors controlling the length of life of adult *D. melanogaster*. In general the flies have been placed in unlighted incubators, which are opened on an aperiodic basis several times per day, or under conditions with a LD 12-12 photoperiod. The authors do not in general appear to attach any importance to the light stimuli received. However, several recent papers have considered the effect of light on the length of life. In particular Erk and Samis (1970) and Pittendrigh and Minis (1972) observed that a permanent illumination caused a shortening of the life of the imago. Furthermore, these same authors have emphasized the importance of the photoperiod rhythm. Pittendrigh and Minis, for example, reduced the length of life, by subjecting the flies to light rhythms with a period different from 24 hr. Up to now, however, the longevity in permanent darkness does not appear ever to have been measured. It therefore seemed worth reconsidering the problem by a study of the effects of the duration of illumination, to which the flies are exposed, and those of the light rhythm.

MATERIALS AND METHODS

All the experiments were carried out at $25^{\circ} \pm 0.1^{\circ}\text{C}$ on F_1 heterozygots, produced by crossing a wild *Champtieres* strain with a *vestigial* mutant strain. The flies were reared in total darkness under low population density conditions (30 eggs per tube) on an axenic medium based on dead yeast (David and Clavel, 1965).

On emergence, the adults were placed under the lighting conditions of the laboratory (approximately 800 lx), anaesthetized with ether and redistributed in groups of 10 females and 10 males in 190 ml plastic cages (David and Clavel, 1968). The feed for the adult flies (axenic medium) was renewed every other day and the dead individuals counted at the same time. The cages were changed every week after subjecting the flies to a slight CO_2 anaesthesia. Each cage was retained until the last adult had died.

Five experimental regimens were used:

- (1) Permanent light: incubator illuminated by a 20 W fluorescent tube, giving an illumination of 1500 lx.
- (2) LD 12-12 light rhythm (same source of light).
- (3) LD 12-12 light rhythm, with inversion of the night-day phase every week, the 12 hr rhythm being displaced by the introduction of a light phase or a darkness phase of 24 hr.
- (4) Darkness, but the nonlighted incubator was opened as required several times per day. The flies spent at least 95 per cent of their life in darkness.
- (5) Permanent darkness.

Ten cages were studied for each experimental regimen. In the case of the first four the handling operation took place under normal laboratory lighting. In the case of the flies, kept in total darkness, renewal of the feed and the cages was carried out using a dark red

light of wave-length higher than 675 nm with an illumination intensity of about 40 lx. This light was obtained by means of a coloured methyl methacrylate double filter, the transmission spectrum of which was analyzed by spectrophotometry (Cary 16). The duration of the illumination received never exceeded 20 min every other day. It is known (Frank and Zimmerman, 1969) that dark red light does not disturb the circadian rhythm of *Drosophila* and this technique has often been used for replacing darkness (Chandrashekar and Loher, 1969; Winfree, 1972).

RESULTS

The results are summarized in Table 1 and shown in graph form in Figs. 1 and 2. In all cases the male lived for shorter periods than the female, which is in fact normal.

TABLE 1. LONGEVITY OF ADULTS AT 25°C UNDER DIFFERENT LIGHTING CONDITIONS

| Conditions | Males | | | Females | | |
|---------------------------|-------------------|-----|-------|-------------------|-----|-------|
| | $m \pm 2\sigma_m$ | n | C.V. | $m \pm 2\sigma_m$ | n | C.V. |
| Constant light | 49.29 ± 1.94 | 85 | 18.13 | 55.33 ± 1.46 | 98 | 13.12 |
| Regular LD 12-12 rhythm | 44.39 ± 2.49 | 88 | 26.32 | 57.44 ± 1.99 | 90 | 16.47 |
| Disturbed LD 12-12 rhythm | 43.95 ± 2.38 | 89 | 25.57 | 54.94 ± 1.56 | 96 | 13.97 |
| 95% darkness | 44.29 ± 2.88 | 77 | 28.57 | 59.25 ± 1.80 | 75 | 13.18 |
| Permanent darkness | 54.72 ± 4.10 | 77 | 33.65 | 79.83 ± 3.09 | 85 | 17.87 |

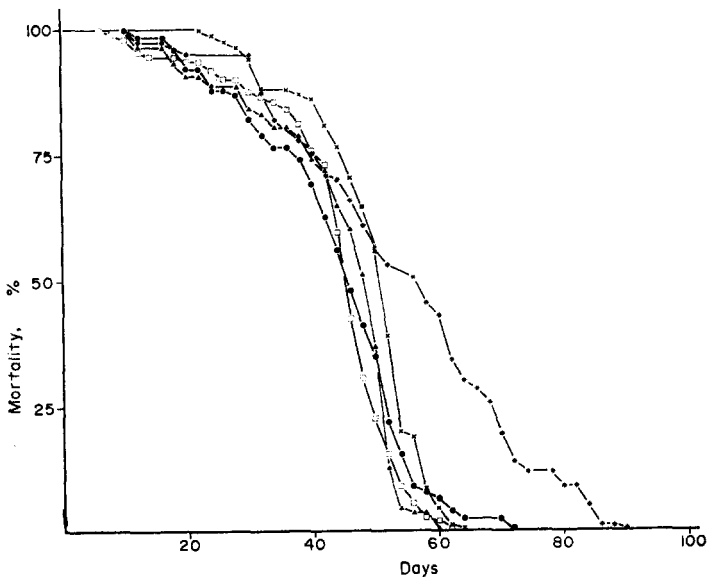
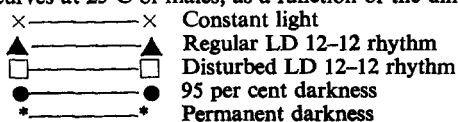


FIG. 1. Survival curves at 25°C of males, as a function of the different light regimens.



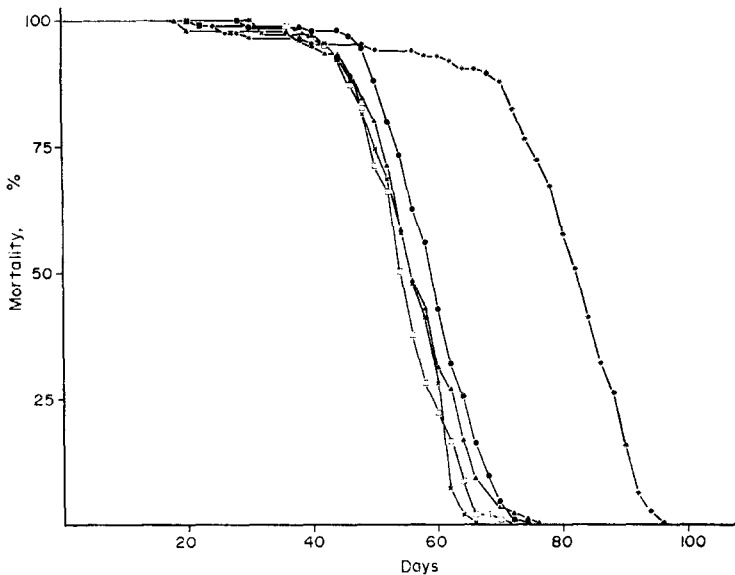
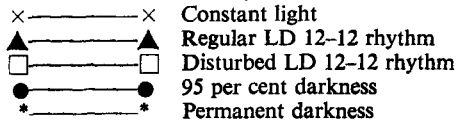


FIG. 2. Survival curves at 25°C of females, as a function of the different light regimens.



The mean values in the four first experimental regimens are close to each other. The variance analysis (Table 2) yielded non-significant values for F , so that the homogeneity of the group can be accepted.

TABLE 2. RESULTS OF VARIANCE ANALYSIS FOR FOUR REGIMENS INCLUDING DAILY ILLUMINATION WITH WHITE LIGHT

| Source of variation | Variance | Degrees of freedom | F |
|---------------------|----------|--------------------|-------|
| Sex | 253.58 | 1 | 34.41 |
| Light regimen | 3.12 | 3 | 1 |
| Residual | 7.37 | 7 | |

In contrast to the above group, the flies which had been placed in permanent darkness (except at the moment of their emergence) exhibited a very clearly increased longevity. The increase in the case of the males was of the order of 10 days or about 20 per cent. The coefficient of variation is particularly high. Examination of the survival curves (Fig. 1) shows that they are all broadly comparable up to the fiftieth day. It is only beyond this point that the effects of the permanent darkness begin to be felt. The general picture is as if only one part of the population was sensitive to the darkness.

For the females, the increase in the duration of life is more than 20 days, corresponding to about 43 per cent. The relative variability (coefficient of variation) is virtually unchanged and examination of the survival curves (Fig. 2) shows that the longevity has been increased for approximately 95 per cent of the individuals.

DISCUSSION AND CONCLUSIONS

Contrary to the observations of Erk and Samis (1970) and Pittendrigh and Minis (1972), permanent illumination of the flies did not cause any particular reduction in their length of life, by comparison with the LD 12-12 alternation conditions. This difference may possibly be due to the type of flies used by the different authors. It does appear as if the sensitivity to light in *D. melanogaster* varies according to the particular strain (Medioni, 1961; Allemand, unpublished).

The weekly phase displacements, involving a shifting of the 12 hour phase, equally caused no significant reduction in the expectation of life, although it was under these conditions that the lowest mean values were obtained. Pittendrigh and Minis (1972) obtained a significant reduction in length of life, by exposing the drosophila to a noncircadian photoperiodic rhythm. It is possible that a phase displacement may give rise to such a result, but only if the phase shifts are sufficiently frequent.

The spectacular extension of the longevity in the flies, placed in total darkness, is a very curious result. It should be recalled first of all that the flies were in fact exposed every second day to illumination with a red light for 10-20 min. The red light is known not to influence the circadian emergence rhythm of *Drosophila* (Frank and Zimmerman, 1969). However, the red light appears to be perceived by the eyes of the adults (Fingerman and Brown, 1953; Medioni, 1961). This could be confirmed by observations on the adults during changing of the feed. Handling of the cages caused them to move toward the light source (positive phototaxis). In no case did the flies take flight, although they flew very easily under the white light. It would therefore appear that the red light exercises qualitatively different effects from those of white light, without the significance of the difference being at present clear.

Two simple explanations can be envisaged for the extended longevity in permanent darkness. In the first place it is known that virgin females regularly live longer (Billewicz, 1953; Kummer, 1960; David and Cohet, 1971) and it could be supposed that insemination had not occurred in the darkness. In fact this interpretation is not valid, since *D. melanogaster* is a species which prefers to copulate in the darkness (Grossfield, 1970; Hardeland, 1971). Furthermore, observation on the eggs laid revealed a normal eclosion, which in fact occurred around the seventieth day of life.

One might also attempt to explain the increased longevity on the basis of the reduction in the activity of the flies and their inability to fly in the darkness, but this interpretation seems hardly probable. It has never in fact been proved in the case of the *Drosophila* that a reduction in the metabolism can produce a direct increase in the longevity (David *et al.*, 1971). Furthermore, if the suggested interpretation were correct, we should expect to find considerable differences, depending on whether the flies spend 95 per cent of their lives in total darkness, or on the other hand 50 or 0 per cent. No such differences have been noted.

It may be said in conclusion that a very brief daily exposure to illumination appears to be capable of considerably shortening the expectation of life. Further experiments will be required, in order to attempt an interpretation of this phenomenon.

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Summary—The longevity of adult *D. melanogaster* has been studied at 25°C under five light regimens: permanent lighting, LD 12–12 circadian rhythm, LD 12–12 circadian rhythm with 12 hr phase displacement every week, total darkness for 95 per cent of the time and, finally, total darkness in conjunction with handling under dark red light. The four first regimens yield comparable and statistically homogeneous results. Total darkness, on the other hand, causes a considerable extension of the expectation of life, of the order of 20 per cent in the males and 43 per cent in the females.

Résumé—La longévité des adultes de *D. melanogaster* a été étudiée à 25°C dans 5 conditions d'éclairage: lumière permanente, rythme circadien LD 12–12, rythme circadien LD 12–12 avec perturbation de phase de 12 heures chaque semaine, obscurité pendant 95 pour cent du temps total, enfin obscurité permanente et manipulation en lumière rouge sombre. Les 4 premiers traitements donnent des résultats comparables et statistiquement homogènes. Au contraire l'obscurité permanente provoque un allongement important de la durée de vie de l'ordre de 20 pour cent chez les males et de 43 pour cent chez les femelles.

Zusammenfassung—Die Lebensdauer erwachsener Exemplare von *D. melanogaster* wurde bei einer Temperatur von 25°C in fünf verschiedenen Beleuchtungsfolgen untersucht: ständige Beleuchtung Licht-Dunkel 12–12 Stundentagesrhythmus. Licht-Dunkel 12–12 Tagesrhythmus mit 12 Stündiger Phasenverschiebung wöchentlich. Völlige Finsternis durch 95 Prozent der Zeit. Völlige Finsternis und gleichzeitige Behandlung der Fliegen unter dunkelrotem Licht. Die vier ersten Beleuchtungsfolgen ergeben vergleichbare, Statisch Homogene resultate. Andere Seit verlängert totale Finsternis in beträchtlichem Masse die Lebenserwartung der Fliegen in der Größenorder von 20 Prozent in den männlichen und von 43 Prozent in den weiblichen Fliegen.