A Preliminary Study of Eclosion Time Selection in Drosophila melanogaster

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Various internal and external environmental factors have an effect in determining or influencing biological rhythms. Light is probably one of the most significant of these influences, and its effect has been studied in various organisms, but no attempt has been made to investigate possible genetic influences on biological rhythms. Such a preliminary study of the genetic influences of a recognized diurnal rhythm is the object of this selection experiment.

The time of eclosion, or emergence of the newly metamorphosed adult *Drosophila* from its pupa case, has a recognized rhythm described by Kalmus (1940), Brett (1955), and others. It has been shown that the fruit fly, *Drosophila melanogaster*, cultured under normal day-night illumination periods has a definite rhythm of emergence with the peak occurring between 6 and 9 AM. As long as the animals have been cultured under conditions of periodic illumination at some period of development other than the prelarval stages, this rhythm will continue. However, there will be no emergence rhythm in flies raised under conditions of constant darkness throughout their development. Various modifications of the normal 24-hr cycle can also be induced by varying the periodicity of the illumination to which the flies are subjected during development. Thus, it is evident that light is the environmental factor with the greatest importance in influencing the diurnal rhythm of eclosion, though temperature also has a slight effect (Kleitman, 1949).

There are several reasons for suspecting that biological rhythms in general, and eclosion time in particular, have a genetic basis. They occur in all levels of organization and development, often with a recognized physiological correlation, and with no evidence or possibility of having been learned. The most interesting approach, however, is to consider what evolutionary benefits would accrue in the accumulation of modifier genes in the gene pool of the population to determine or influence a trait such as the time of eclosion. Though milder temperatures and absence of possible predators at this time may have some effect, the most evident benefit is a result of the physiological conditions of the animal at eclosion. *Drosophila* desiccate easily in the first hour or two after emergence because their exoskeletons are not then completely hardened. If, for example, a fly emerges at noon on a hot day, the heat will be more likely to desiccate the body sufficiently to cause death than if the fly had eclosed under more favorable conditions. There is, therefore, reason to suspect, in natural selection, the accumulation of genes modifying developmental physiology in ways that, with light as a stimulus, may favor eclosion during the hours immediately preceding sunrise.

METHODS

The experimental procedure was designed to select as parents Canton-S wild-type *Drosophila melanogaster* which eclosed during a certain time period, under conditions of constant, dim lighting and constant temperature. Two parallel selection lines, series "A" and "B", were cultured in half-pint bottles on a cornmeal-agar medium (Demerec and Kaufman, 1961) at 28 C. Flies eclosing during the 3-hr morning period from 8 to 11 AM and during the 3-hr evening period from 5 to 8 PM were collected, counted separately as males and females, and used as parents for the next generation. Parents were left in bottles for varying lengths of time so as to approximate equal population density in all lines. The flies eclosing during the intervening periods from 11 AM to 5 PM and from 8 PM to 8 AM were also collected and counted before being discarded. Classification was always made over an integral number of days. The total number of flies eclosing in each of the four categories was then computed as a percent of the flies eclosing in each generation. Contingency tests were run to determine the statistical significance of differences between generations. A listing of *P*-values is given in Table I and II. Graphs of the control, series B (Fig. 1), and of the 8 to 11 AM selection line for series B (Fig. 2 a and b) are also included.

Evening selection, 5 to 8 PM, Series A and B lines, were merged into one line at the F-4 generation because of a lack of offspring. The results of this selection period are consequently difficult to interpret since comparison of the Series A and B controls indicates significant differences at the F-5 ($P < 0.01$).

TABLE I. STATISTICAL COMPARISONS, SERIES A

Generations and lines compared	sample sizes	*P*-values
Series A Parental: Series B Parental	529:487	0.3-0.5
Series A Parental: Series A F-3 Control	529:204	0.90-0.95
Series A Parental: Series A F-5 Control	529:254	0.5-0.7
Series A Parental: Series A F-7 Control	529:139	< 0.01
Series A F-5 Control: Series B F-5 Control	254:247	< 0.01
Series A F-5 (8-11): Series B F-5 (8-11)	529:296	0.1-0.2
Series A Parental: Series A F-7 (8-11)	529:129	0.05-0.10
Series A Parental: Series A F-8 (8-11)	529:125	0.01

TABLE II. STATISTICAL COMPARISONS, SERIES B

Generations and lines compared	sample sizes	*P*-values
Series B Parental: Series B F-8 Control	487:156	0.9-0.95
Series B Parental: Series B F-8 (8-11)	487:143	< 0.01
Series B F-8 Control: Series B F-8 (8-11)	156:143	0.02-0.05
Series B F-8 Control: Series AB F-8 (5-8)	156:115	0.02-0.05

RESULTS

Fluctuations are observed in all four of the categories plotted from the control line, series B (Fig. 1). These fluctuations are expected, however, as a result of the chance reassortment of modifier genes in the offspring from generation to generation. Statistical comparison of the F-8 and parental generations shows no difference, with a *P*-value of 0.9 to 0.95.

Comparison of the parental and F-8 generations in the 8 to 11 AM selection line, series B, gave a quite different result. A *P*-value < 0.01 indicates that these two generations are significantly different. A similar comparison between the F-8 control generation and the F-8, 8 to 11 AM

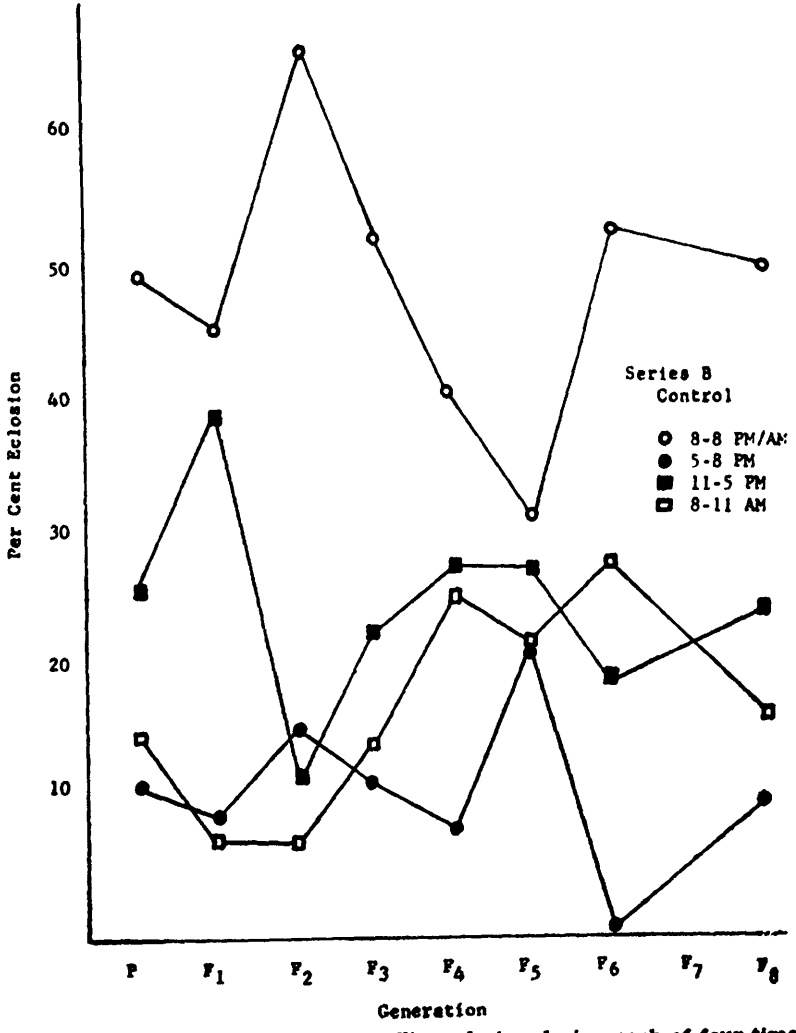

Fig. 1. Per cent of total number of flies eclosing during each of four time intervals in the control line, series B.

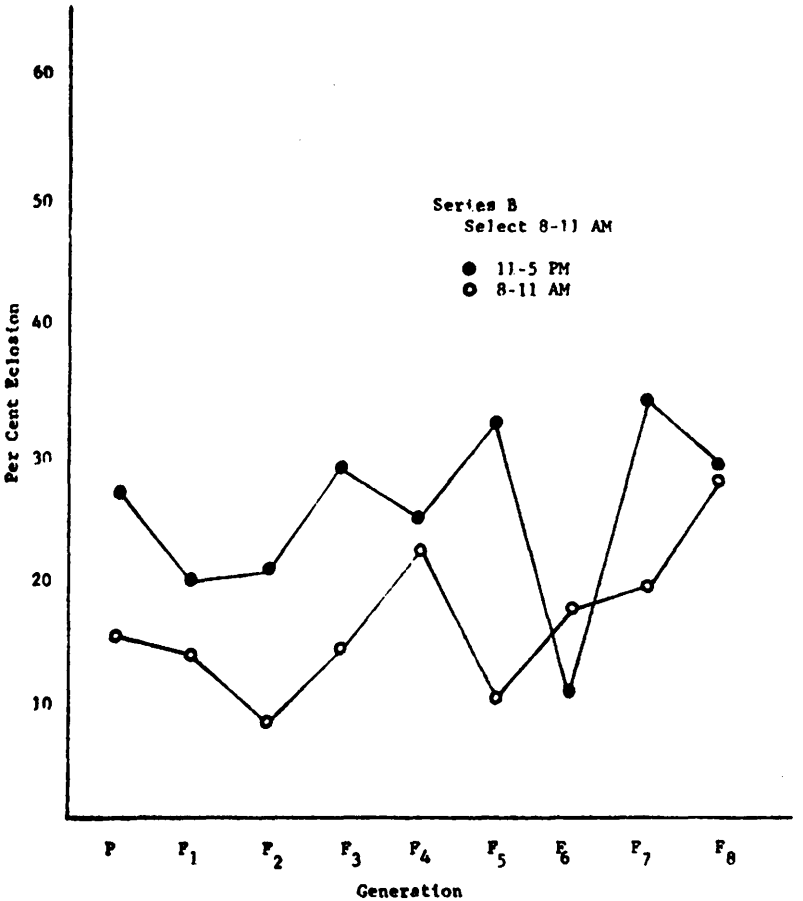

Fig. 2a. Per cent of total number of flies eclosing during two time intervals in the 8-11 AM selection line, series B.

selection line, gave a *P*-value of 0.02 to 0.05, indicating a statistical difference on the border line of significance. The plot of the results of the 8 to 11 AM selection (Fig. 2b) reveals a general increase in the percent of flies eclosing during the period selected for, with a concurrent unexplained decrease in the percent of flies eclosing during the period (8 PM to 8 AM) immediately preceding the one selected for.

Comparisons of other generations are given in Tables I and II. Though significant differences are indicated between certain generations, only a tendency to respond to selection is seen, and longer periods of selection are needed to show whether the significance will continue in further generations.

DISCUSSION

A tendency to respond to selection is evident (see Fig. 2a, 8 to 11 AM). A similar tendency cannot be shown with certainty in the other

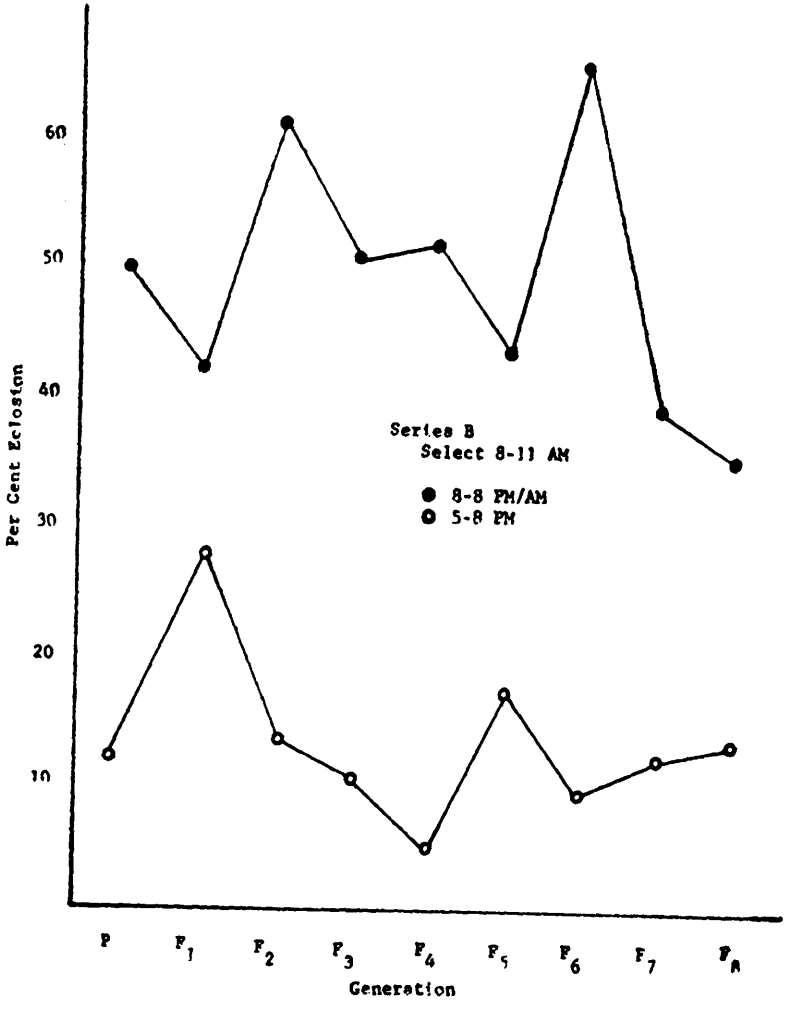

Fig. 2b. Per cent of total number of flies eclosing during two time intervals in the 8-11 AM selection line, series B.

lines, but this is expected from the high degree of variability in the trait and the limited number of generations of selection. This tendency, though not in itself conclusive, provides evidence that artificial modification of a diurnal rhythm by selection is possible, and that potential success exists for the indication of the genetic control of this trait.

Besides a tendency toward selection, other factors recognized should be controlled, or varied, in later repetitions of this experiment. An attempt was made to control population density, but in future studies this factor must be more carefully controlled. In addition, temperature and light intensity and periodicity should be varied in different experiments

to determine their effects on potential selection. Finally, the responses of both laboratory and wild stocks should be compared.

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