EFFECTS OF LIGHT: DARK CYCLES ON DEVELOPMENT IN DROSOPHILA MELANOGASTER

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
DALE L. CLAYTON
1968

This is to certify that the

thesis entitled

Effects of light-dark cycles
on development in
Drosophila melanogaster

presented by

Dale L. Clayton

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Zoology

Major professor

Date May 10, 1968

O-169

ABSTRACT

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ON DEVELOPMENT IN
DROSOPHILA MELANOGASTER

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Body of Abstract

A functional role for circadian rhythms in developmental processes has not been described heretofore. This thesis tests the hypothesis that exogenous light cycles act as a chronometer in regulating developmental processes, i.e. longer circadian light cycles retard developmental rate.

One hundred twenty Drosophila melanogaster eggs were placed in each of 40 culture vials. These vials were placed into 6 treatment groups. The treatment groups were 1) long cycle (25 hrs. 9 min.) with 15 second twilight transitions, 2) long cycle with 15 minute twilight transitions, 3) short cycle (22 hrs. 52 min.) with 15 second twilight transitions, 4) short cycle with 15 minute twilight transitions, 5) constant light and 6) constant darkness. Long and short cycle groups were in phase when flies began to eclose at 251.5 hours of incubation, and were continued in constant light thereafter.

Time from oviposition to eclosion was used as a measure of

developmental rate. Flies incubated under short cycles eclosed earlier ($P < 0.01$) than flies incubated under long cycles. Flies incubated under 15 minute twilight transitions eclosed earlier ($P < 0.01$) than flies incubated under 15 second twilight transitions. Flies incubated under constant light eclosed earlier ($P < 0.01$) than flies incubated under constant darkness.

Mortality differed significantly ($P < 0.01$) between flies incubated in constant or in cyclic conditions. Mortality was higher in constant conditions. This difference precludes comparison of eclosion rates between these conditions since eclosion and mortality are probably not independent. Differences in mortality were insignificant ($P < 0.01$) in all other comparisons.

The conclusions are that circadian light cycles do act as a chronometer for developmental processes as indicated by time of eclosion; and furthermore, circadian light cycles increase developmental success as indicated by decreased mortality.

EFFECTS OF LIGHT:DARK CYCLES ON DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

By

Dale L. Clayton

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1968

ACKNOWLEDGMENTS

I am indebted to:

My committee, Dr. M. Balaban, Chairman; Drs. Wm. Cooper, G. I. Hatton, and J. I. Johnson for valuable criticism of this thesis and for meeting somewhat unreasonable demands for their time.

Kay, for long hours of typing and retyping.

Carola Cattani, for making her lab and constant temperature room available.

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INTRODUCTION

The existence of circadian rhythms* in developing organisms is well documented (Rutenfranz 1961, Petren and Sollberger 1953, Johnson 1966, Hellbrugge et. al. 1964). However, a functional role for these rhythms in the developmental process has not been described. Harker (1965a) suggests that competence of embryonic tissues cycles on a circadian basis, but does not describe the functional significance of this. The abundant evidence of light cycles regimenting physiological and behavioral events leads one to suspect they play a role in the chronology of developmental events. Demerec (1950) shows that in Drosophila there is a fixed sequence of developmental events leading from one stage of development to the next. Assuming that the chronology of these events is affected by external environmental oscillations, developmental time should be lengthened or shortened by lengthening or shortening the daily light cycles.

The hypothesis this thesis tests is:

> The developmental period, from ovisposition to eclosion of the Drosophila melanogaster imago, is longer when flies are incubated in long circadian light:dark cycles, than when they are incubated in short circadian light:dark cycles.

This hypothesis implies that the length of the developmental period is correlated with the number of light:dark cycles experienced. Harker (1965a and 1965b) recognizes this possibility but presents

*Circa=about; dian=day, i.e. oscillations of about one day.

data aimed at disproving it. I find no fault with her data but do find them inadequate for the conclusions she draws.

Harker's data are significant in that she points to an important relationship between developmental time spans and the entrainment to daily light:dark cycles. She finds that time taken to complete three stages of Drosophila pupal development is relative to the phase of the light cycle at which each stage is entered. For example, the number of hours required between head eversion and the appearance of yellow eye pigmentation was 19 to 20 hours if the head everted four hours after the lights came on, and 54 to 55 hours if the head everted one hour after the lights went off. These data present a powerful demonstration that circadian light cycles affect development.

She also shows that pupae kept in darkness, once a stage is entered, develop with the same time span as pupae maintained in 24-hour light:dark cycles. Harker (1965a, p. 329 and 327) states, "It is clear from the results that the same developmental periods are involved whether the pupae are in a light cycle or in constant darkness." She further concludes, "There does not seem to be any correlation between the length of the developmental period and the number of bright light:dim light cycles, or any sequence of these cycles, . . . " This conclusion follows only if the light cycles are restricted to a period of 24 hours or very nearly 24 hours. Harker (1965a and 1965b) used only 24-hour light:dark cycles. The free-running period* of Drosophila is very close to 24 hours. Therefore, one

*One free-running period equals the time required for one oscillation of the internal clock under constant environmental conditions, i.e., the period is not entrained to environmental oscillations but is "running free".

would not expect developmental time to differ between one group entrained to a 24-hour light cycle and another group following a 24-hour free-running cycle since the internal clock would maintain the same phase relations in both groups. Harker does not present sufficient data to arrive at a conclusion concerning cycles longer or shorter than 24 hours. In terms of 24-hour cycles, her conclusion is not meaningful because the endogenous cycle continues on a 24-hour free-running basis whether the exogenous cycle is suspended or not.

Data given by Shutze et. al. (1962) and by Minis and Pittendrigh (1968) can be used to argue that there may be a correlation between the number of cycles of the internal physiological clock and developmental rates in chickens and in the moth, Pectinophora gossypiella.

Shutze et. al. found that chicks (diurnal animals) incubated in constant light hatched approximately 16 hours before chicks incubated in constant darkness. Assuming that Aschoff's Rule* is operating in the ontogeny of chick embryos, we would expect that oscillations of the internal clock would occur more rapidly for chicks incubated in constant light.

If it is true that a discrete set of developmental events occur with each oscillation of the internal clock, then knowing the number of oscillations that the internal clock has completed should be useful for predicting stages of development (e.g. hatching). Twenty-one

*Aschoff's Rule (Aschoff 1958 and Pittendrigh 1960) states that organisms maintain relatively constant free-running circadian periods when subjected to constant conditions; and that this period is shorter in constant light than in constant darkness for diurnal organisms, but longer in constant light for nocturnal organisms. This principle was first described by Johnson (1939).

days of incubation (i.e. 21 x 24 hours), normally associated with hatching in chickens, would then be an artifact of the natural vivarium.

Minis and Pittendrigh (1968) find that the median time required for embryogenesis of the moth, P. gossypiella at 20°C. (i.e. oviposition to hatching from the egg) is 244 hours in constant light and 275 hours in constant darkness. If we can think of the moth as being diurnal during embryogenesis, Aschoff's Rule can be applied to these data as it was to the data of Shutze et. al. (1962) for the chick embryo. The adult moth is nocturnal. However, hatching is keyed to the light portion of the light:dark cycle (Minis and Pittendrigh 1968) as is hatching and emergence of Drosophila, a diurnal insect. This suggests that the moth embryo functions on a diurnal basis.

Circadian organization is present in this moth for at least the last half (5½ days) of embryogenesis. This is based on entrainment of hatching to a single light perturbation. During the first 5½ days of embryogenesis, circadian organization is not evident nor is there any apparent difference in hatching times between embryos incubated in either constant light or constant darkness for the first 5½ days and maintained in similar conditions thereafter. During the second half of embryonic development, however, both differential developmental rate (in response to light intensity) and circadian organization are present. This adds credence to the concept of a functional tie-up between developmental rate and circadian organization.

If we accept the assumption that Aschoff's Rule is operating during embryogenesis, the data of Shutze et. al. (1962) and of Minis and Pittendrigh (1968) support the hypothesis that there is a

correlation between the length of the developmental period and the number of circadian oscillations.

The possibility of light intensities acting on developmental rate (via Aschoff's Rule) has been suggested by Thomas and Pizzarello (1967). Their study employed blind and sighted girls as subjects and used age of first menarche as a measure of sexual maturation. Since blind girls experience constant darkness, they were expected to exhibit longer free-running periods and therefore slower development than sighted girls. Thomas and Pizzarello found no difference between blind and sighted girls in time of first menarche.

All subjects were from a school for the blind or from a children's home. Institutional life is highly regimented and both sets of girls followed a rigid 24-hour schedule. This schedule differs from constant conditions assumed by Aschoff's Rule and the prediction would logically be "no difference".

I propose to use light cycles that are either longer or shorter than 24 hours. This allows specification of cycle lengths, and assumptions based on Aschoff's Rule are unnecessary. The endogenous rhythms of Drosophila are sensitive to resetting by light during early stages of development. Brett (1954, p. 176) states that the rhythmic entrainment of emergence "was shown to persist under conditions of constant darkness and temperature provided that the animals [Drosophila] had previously been subjected to day:night illumination changes at some developmental stage other than prelarval." The prelarval period ends approximately 24 hours after oviposition. This means that the endogenous cycle is sensitive to resetting by day:night illumination changes for the last 9 or 10 days of incubation.

METHODS AND MATERIALS

Adult Drosophila melanogaster oviposited eggs on a thin layer of agar media for four hours (7:00 a.m. to 11:00 a.m. EDST). Eleven a.m. was designated as the start of incubation. Small blocks of agar containing 120 eggs* were transferred to glass vials (3 inches long and 1 inch in diameter) into which approximately 8 ml. of media had been poured. These vials were plugged with cotton, divided randomly into six treatment groups and placed in six Light:Dark boxes as illustrated in Figure 1. These boxes were made of 1/4" composition board and painted silver inside and out. A beveled strip of wood was glued down the center of the box. The base of the vials rested on this strip and the tops were tilted against the side of the box.

A black construction paper shutter was glued to a 1/4" wooden dowel. The dowel protruded through 1/4" holes centered near the top of either end of the box. When the dowel shaft was rotated until the shutter was vertical, light entered the box freely; when the shutter was horizontal, light could not enter the box. This provided the conditions of light and dark respectively. Thin,black polyethylene was fastened to, and overlapped, the construction paper shutter to form a light-tight seal.

One-round-per-hour synchronous motors were coupled to the shutter shafts of two of the boxes. One-round-per-minute synchronous motors

*The number of eggs per vial was held constant because eclosion rates are density dependent (i.e. eclosion is slower in vials with higher densities). See Appendix II.

were coupled to the shafts of another two boxes. The remaining two boxes were operated manually.

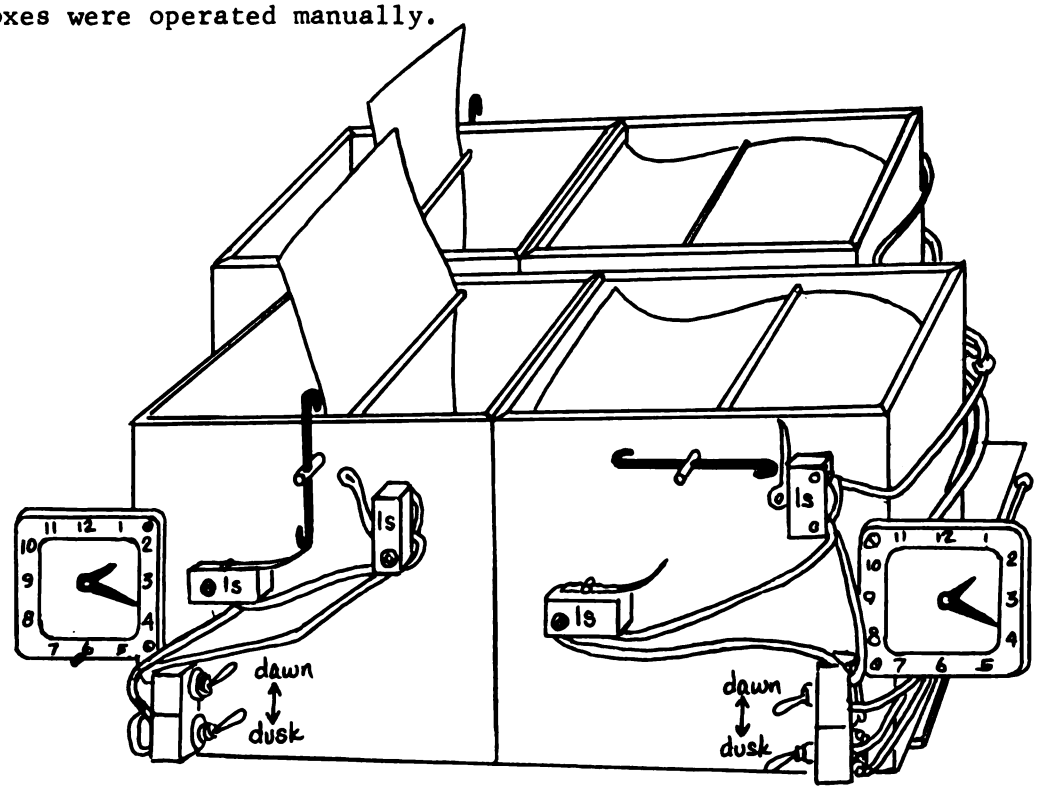

Figure 1. Motor driven Light:Dark boxes. At the time preset on the control clock (cc) the shutter turns 90° simulating either dawn or dusk. Limiting switches (ls) check the travel of the shutter. The clock and the light-dark switches must be reset before the following twilight transition will occur. Each clock controls long or short cycles for shutters driven by one-RPM and one-RPH synchronous motors.

A time clock was set to trigger the onset of each twilight transition (i.e. light-dark or dark-light) and limiting switches checked the travel of the motor-driven shutters. Switches on the front of the boxes were set to indicate twilight transition desired (i.e. dawn, dark to light; or dusk, light to dark). Light and dark phases of each cycle were of equal duration.

Each twilight transition required a 90° turn of the motor-driven shutter. This represents a 15 second twilight transition for the 1 RPM motor (i.e. 90°/360° x 1 RPM = 15 sec.) and a 15 minute twilight transition for the 1 RPH motor (i.e. 90°/360° x 1 RPH = 15 min.)

The six boxes were set up as 3 modules of 2 boxes each. One time clock was set for a short circadian cycle (22 hr. 52 min.) and controlled the shutters of 2 boxes, one with a 15 second twilight transition and the other with a 15 minute twilight transition. A second time clock was set for a long circadian cycle (25 hr. 9 min.) and controlled the shutters of 2 boxes, one with a 15 second twilight transition and the other with a 15 minute twilight transition. The remaining two boxes were set manually; one for constant darkness, the other for constant light.

These particular long and short cycle lengths were chosen so that the cycles would be in phase with each other at 251.5 hours when the flies began to eclose. At this time the short cycle boxes had completed exactly one more light cycle than the long cycle boxes (see Figure 2).

Eclosion is strongly keyed to dawn, therefore light cycles not in synchrony cause differences in eclosion times. If the long and short cycles were not in phase at the onset of eclosion, it could be argued that the cycles being out of phase were responsible for any difference in eclosion time of the treatment groups. It may also be argued that cycles longer or shorter than 24 hours are constantly being reset, and that the endogenous cycle lags or leads the light cycle. This is probably the case. Inspection of Figure 2 will show that the next to the last dawn preceeded the final dawn by 25 hours

9 minutes for the long cycle group and 22 hours 52 minutes for the short cycle group. If eclosion were expected 24 hours* after the next to the last dawn, it would occur 2 hours 17 minutes earlier for the long cycle group. This would not support the hypothesis of this thesis (page 1). Therefore this bias can not be used to argue against data supporting this hypothesis.

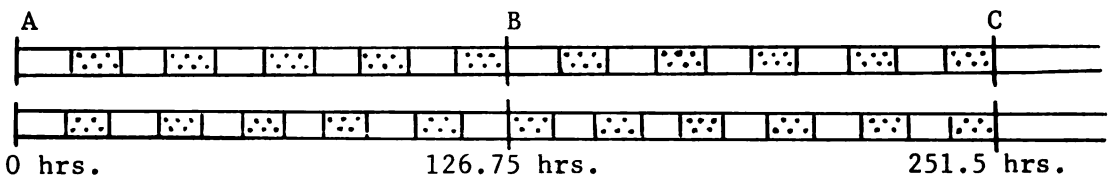

Figure 2. Comparison of the long and short cycle light regimes. The top line represents the long cycle (25 hr. 9 min.) and the bottom line the short cycle (22 hr. 52 min.). Point A represents the onset of incubation; point B, the time at which the treatment groups were 180° out of phase and Point C, the time at which the long and short cycles differ by exactly one light:dark cycle. Point C also represents 1) the onset of constant light, and 2) the onset of eclosion. The first flies were collected at 253 hours of incubation and those eclosing thereafter at 4 hour intervals. Stippled areas indicate the dark phase of the light:dark cycle.

After 251.5 hours of incubation, vials of all treatment groups were subjected to continuous light. Starting at 253 hours of incubation, flies that had eclosed in each vial were removed and counted at four-hour intervals.

Room conditions of light and temperature were held constant at 15 foot candles and 23.5°C. The number of eggs starting incubation

*24 hours is the natural (i.e. free running) period of the endogenous cycle. This is particularly true of population cycles which are the averages of several near 24-hour individual periods.

and the number of adults successfully completing incubation were recorded for each treatment.

The constant condition groups were included in this design to provide baseline data. Intuitively, a group subjected to a 24-hour cycle (i.e., intermediate to the long and short cycles) may seem a more appropriate control group. This is not realistic, however. If incubation (i.e. cycle one) is begun at 1100, the lights come on to begin the 11th long cycle or the 12th short cycle at 2230. This is just 30 minutes before the lights go out to mark 11½, 24-hour cycles. The control group would then be 180° out of phase and could not provide appropriate baseline data.

Table 1 illustrates the experimental design. I shall deal with the following aspects of this design:

I. Effects of light cycles and twilight transitions on mortality.

II. Effects of light cycles on eclosion.

III. Effects of twilight transitions on eclosion

IV. Effects of sex on eclosion.

Table 1. Experimental Design

Long Cycle (25 hr. 9 min.)	15 minute twilight transition*
	15 second twilight transition*
Short Cycle (22 hr. 52 min.)	15 minute twilight transition*
	15 second twilight transition*
Constant Conditions	Constant light*
	Constant darkness*

*Males and females were recorded for each treatment group.

RESULTS

Differences in eclosion rates and mortalities were tested with Chi square contingency tests. A significance level of $\alpha = 0.01$ was chosen. The eclosion curves of the light:dark treatment groups are bimodal and therefore not suitable for testing with parametric tests that assume a normal distribution.

I. Effects of light cycles and twilight transitions on mortality. Differences in mortality rates were tested by comparing the number of flies eclosing and not eclosing. This comparison is based on a known number of eggs (120 per vial) at the start of incubation.

Four independent orthogonal comparisons of mortality can be made of the light:dark and the constant darkness treatment groups. The comparisons chosen were 1) long cycle versus short cycle, 15 second twilight, 2) long cycle versus short cycle, 15 minute twilight, 3) fifteen second twilight versus 15 minute twilight, and 4) cyclic conditions versus constant darkness. These comparisons and their associated χ^2 values are listed in Table 2.

Of these, only the cyclic conditions and constant darkness comparison showed a significant difference in mortality ($P < 0.01$). Mortality was greater in constant darkness. Since mortality was 2.3% higher in constant light than in constant darkness, it can be inferred that mortality of flies incubated in constant light is also significantly higher than mortality of flies incubated in cyclic conditions. A fifth independent comparison of mortalities was made between

constant darkness and constant light conditions. This comparison shows no significant difference (Table 2). The constant light treatment group was excluded from the orthogonal contrasts to allow direct comparison of the constant darkness group with the cyclic groups. Percent mortality for each treatment group is given in Table 3. Table 4 is a summary of the data used for calculating χ^2 and percentage values of mortality.

Table 2. Summary of χ^2 mortality statistics

Treatment Groups	χ^2	P Value	Relative Mortality
Long versus short/15 second twilight transition	$\chi^2_{(1)} = 1.298$	>0.20	No difference
Long versus short/15 minute twilight transition	$\chi^2_{(1)} = 1.448$	>0.20	No difference
15 second versus 15 minute twilight transition	$\chi^2_{(1)} = 3.428$	>0.05	No difference
Cyclic versus constant conditions	$\chi^2_{(1)} = 9.048$	<0.005	Cyclic < Constant darkness
Light versus dark constant conditions	$\chi^2_{(1)} = 1.238$	>0.20	No difference

Table 3. Comparison of mortality for all treatment groups

Treatment Groups		% Mortality
Long cycle	15 second twilight	12.6%
	15 minute twilight	16.9%
Short cycle	15 second twilight	14.5%
	15 minute twilight	14.7%
Conditions	Constant Darkness	19.2%
	Constant Light	21.5%

Table 4. Summary of data comparing mortality rates of all six treatment groups.

Treatment Groups		# Flies Eclosed	# Flies Aborted	Total # Flies
Long cycle (25 hrs. 9 min.)	15 sec. twilight	734	106	840
	15 min. twilight	698	142	840
Short cycle (22 hrs. 52 min.)	15 sec. twilight	718	122	840
	15 min. twilight	716	124	840
Constant Conditions	Light	565	155	720
	Darkness	582	138	720

II. Effects of light cycles on eclosion. Differences in eclosion rates were tested by comparing the number of flies eclosing before and after 269 hours of incubation. Two hundred sixty-nine hours is the best data point for dividing the bimodal eclosion curves of the light cycle groups as can be seen in Figures 3 and 4. Two hundred sixty-nine hours is also closest to the means of the constant light and constant darkness treatment groups (Figure 5). For these reasons, 269 hours was chosen as the most representative point for dividing all curves for χ^2 analysis of eclosion rates.

Three independent comparisons of eclosion rates were made of the four light:dark treatment groups. Since each group was subdivided into flies eclosing before and after 269 hours of incubation, a total of seven independent comparisons could have been made. The other comparisons are not relevant (e.g. those made by combining before and after 269 hour groups) and furthermore do not yield significant differences. The comparisons chosen were 1) long cycle versus short cycle, 15 second twilight, 2) long cycle versus short cycle, 15 minute twilight, and 3) fifteen second twilight versus 15 minute twilight. A fourth independent comparison, constant light versus constant darkness

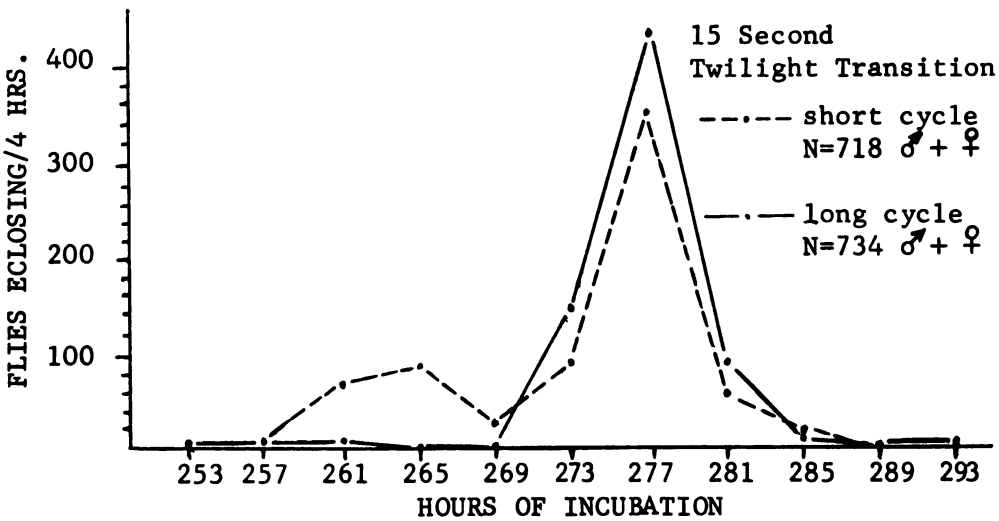

Figure 3. Eclosion curves of long and short cycle light:dark treatment groups, using a 15 second twilight transition (males and females combined).

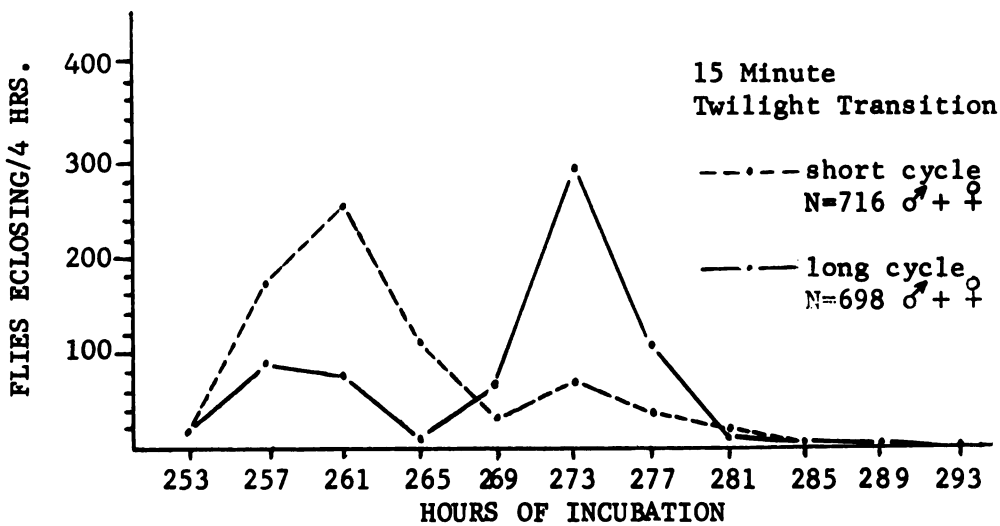

Figure 4. Eclosion curves of long and short cycle light:dark treatment groups, using a 15 minute twilight transition (males and females combined).

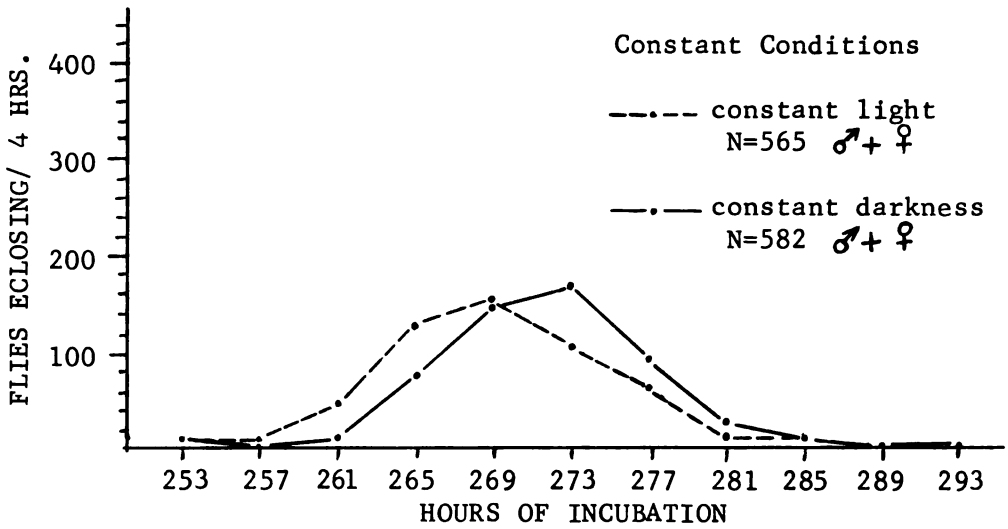

Figure 5. Eclosion curves of constant light and constant darkness treatment groups (males and females combined).

was also tested. It has already been shown that the comparison, cyclic conditions versus constant conditions, exhibits a significant difference in mortality. This comparison in terms of eclosion rate is not valid because eclosion rate and mortality may not be independent; that is, differences in eclosion may or may not be attributed to differential mortality.

Of the four comparisons tested, all exhibit significant differences ($P < 0.01$). These comparisons and their associated χ^2 values are listed in Table 5. Table 6 is a summary of the data used for comparing eclosion rates of the light:dark cycle treatment groups.

Flies incubated under short cycle light regimes eclosed significantly earlier than flies incubated under long cycle regimes ($P < 0.01$). This is true for both 15 second and 15 minute twilight transition groups.

Table 5. Summary of χ^2 eclosion rate statistics

Treatment Groups	χ^2	P Value	Relative Rates*
Long versus short, 15 second twilight transition	$\chi^2_{(1)}=159.648$	P<.001	Short < Long
Long versus short, 15 minute twilight transition	$\chi^2_{(1)}=273.817$	P<.001	Short < Long
15 second versus 15 minute twilight transition	$\chi^2_{(1)}=602.242$	P<.001	15 min.<15 sec.
Constant light versus constant darkness	$\chi^2_{(1)}= 45.935$	P<.001	Const. light < Const. darkness

*Short < Long = flies incubated under short cycles eclose before flies incubated under long cycles.

Table 6. Summary of data used for comparing eclosion rates of long and short cycle groups*

Sex	Long Cycle (25 hr. 9 min.)		Short Cycle (22 hr. 52 min.)		Twilight Transition
	<269 hr.	>269 hr.	<269 hr.	>269 hr.	
♂♂	5	331	9	310	
♀♀	24	374	194	205	15 second
♂♂ + ♀♀	29	705	203	515	
♂♂	53	280	227	98	
♀♀	217	148	358	33	15 minute
♂♂ + ♀♀	270	428	585	131	

*<269=number of flies eclosing before 269 hours of incubation.
>269=number of flies eclosing after 269 hours of incubation.

Table 7. Summary of data comparing eclosion rates of long and short cycle treatment groups*

Sex	Constant Light		Constant Darkness	
	<269 hrs.*	>269 hrs.*	<269 hrs.*	>269 hrs.*
♂♂	112	149	63	232
♀♀	251	53	195	92
♂♂ + ♀♀	363	202	258	324

*<269=number of flies eclosing before 269 hours of incubation.
>269=number of flies eclosing after 269 hours of incubation

The bimodality so evident in eclosion curves of the light cycle groups is not evident in the constant light or constant darkness treatment groups. The eclosion curves of the constant light and constant darkness treatment groups are significantly different ($P < 0.01$, see Table 5). Flies incubated in constant light eclose earlier than flies incubated in constant darkness. Table 7 is a summary of the data used for comparing eclosion rates of the constant condition treatment groups.

III. Effects of twilight transitions on eclosion. Flies incubated with a 15 second twilight transition eclosed significantly later than flies incubated with a 15 minute twilight transition ($P < 0.01$). Data of the long and short cycle treatment groups for a given twilight treatment were combined to form a single twilight transition group. In this way, biases imposed by differences in long or short cycle treatments are accounted for.

IV. Effects of sex on eclosion. Males incubated under 15 second twilight did not conform to the pattern exhibited by all other light: dark treatment groups analyzed as separate sexes (see Figures 6-9). That is, males incubated under short cycles (15 second twilight) did not eclose earlier than males incubated under long cycles (15 second twilight). In this case, there is very close agreement between the long and short cycle eclosion curves (Figure 6). In all other treatment groups, including females incubated under 15 second twilight, both males and females did eclose more rapidly when incubated under short cycles.

Bimodality is lacking in the male long cycle groups for both 15 second and 15 minute twilight transition groups. All other light cycle treatment groups show bimodality.

These data cannot be analyzed for mortality of separate sexes because the sex ratio of eggs was not known.

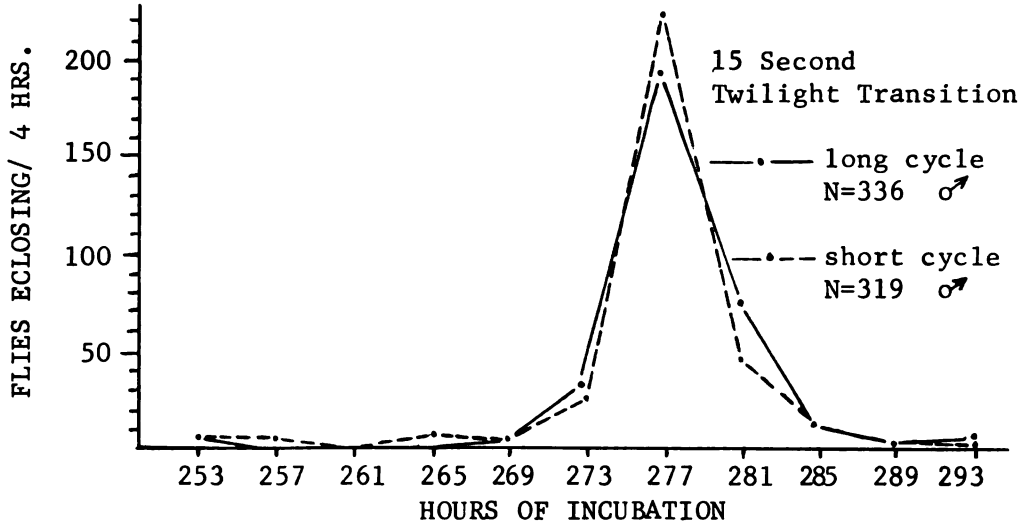

Figure 6. Eclosion curves of long and short cycle light:dark treatment groups, using a 15 second twilight transition (males only).

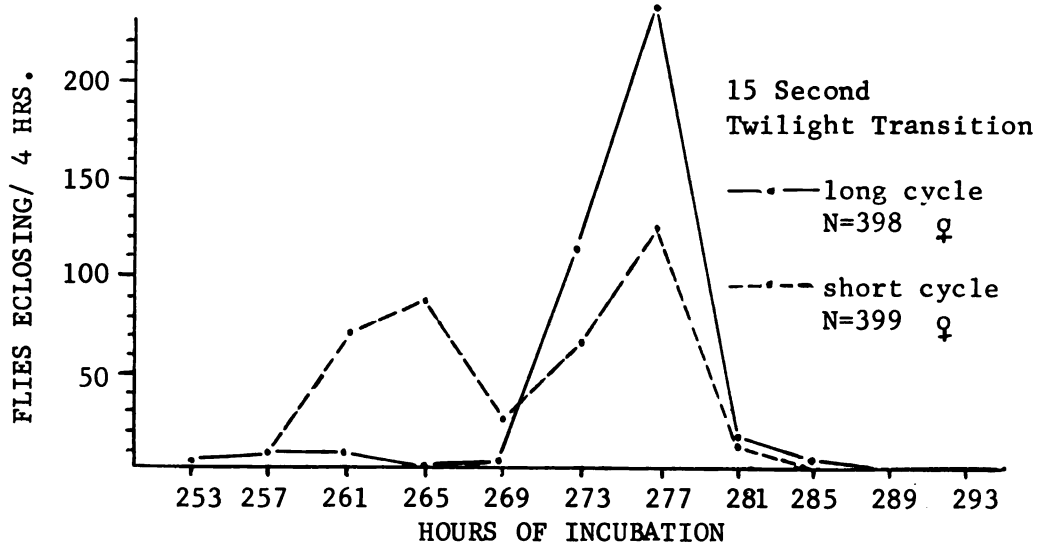

Figure 7. Eclosion curves of long and short cycle light:dark treatment groups, using a 15 second twilight transition (females only).

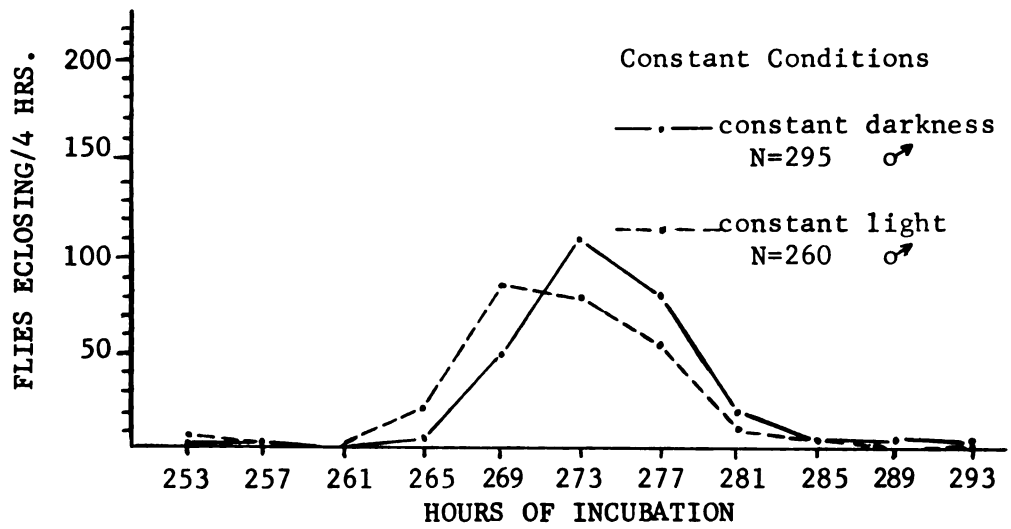

Figure 10. Eclosion curves of constant light and constant darkness treatment groups (males only).

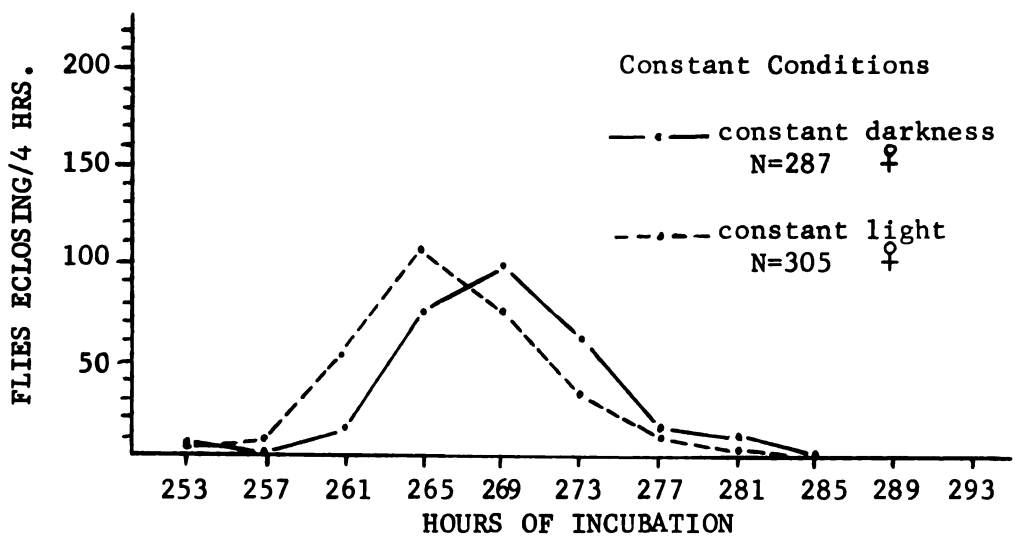

Figure 11. Eclosion curves of constant light and constant darkness treatment groups (females only).

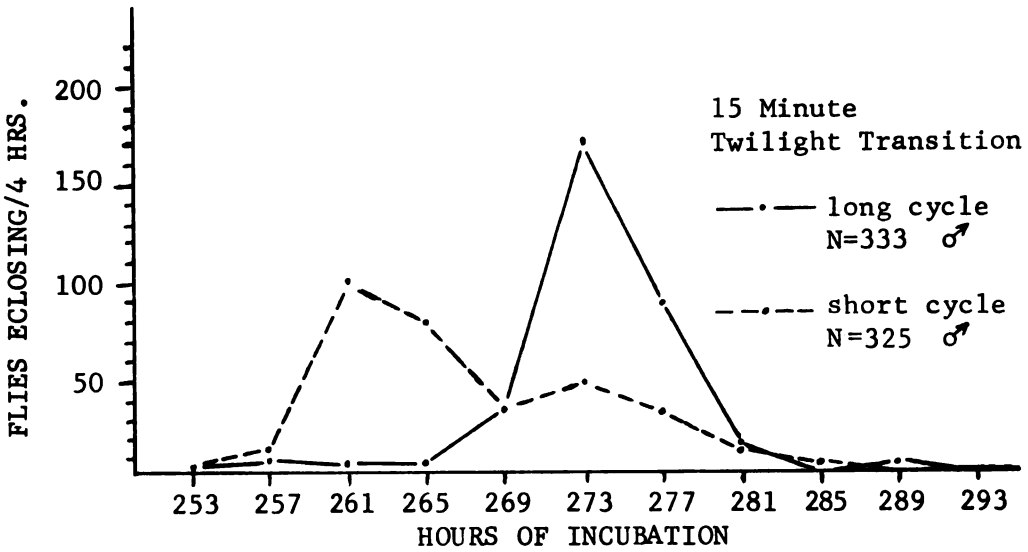

Figure 8. Eclosion curves of long and short cycle light:dark treatment groups, using a 15 minute twilight transition (males only).

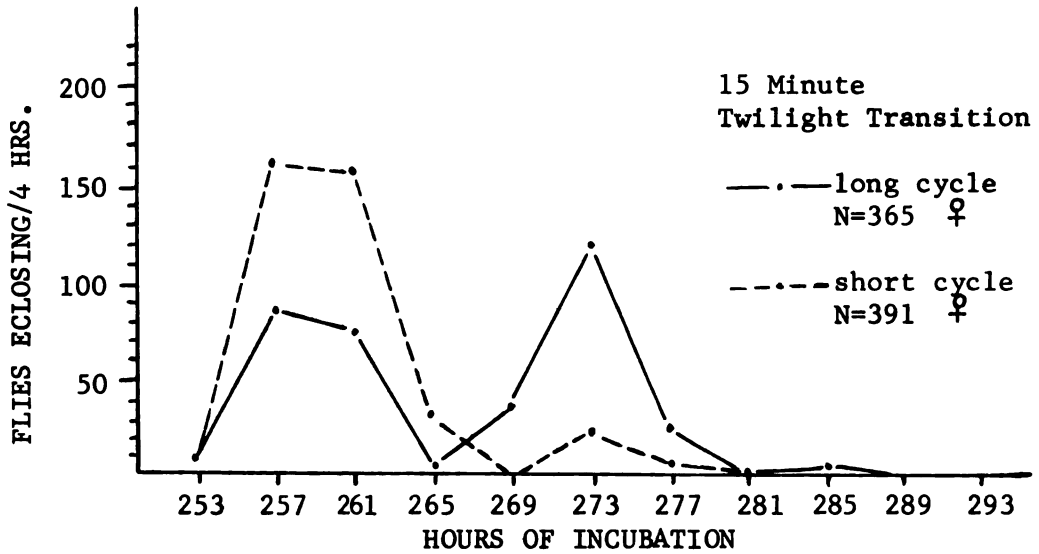

Figure 9. Eclosion curves of long and short cycle light:dark treatment troups, using a 15 minute twilight transition (females only).

DISCUSSION

I. Effects of light cycles and twilight transitions on mortality. Mortality was significantly higher for flies incubated in constant conditions than for flies incubated in light:dark cycles ($P < 0.01$). No significant differences were noted between twilight transition treatments or the long-short cycle treatments ($P < 0.01$). Detrimental effects of constant light have been recognized for some time. Pittendrigh (1960) tested the effects of constant light on the penetrance of the recessive allele tu^g, responsible for melanotic pseudotumors in Drosophila. The penetrance of tu^g dropped from 90% to 40% in the first generation subjected to constant light. Differential mortality of flies carrying the tu^g allele must be assumed.

In the same paper (1960, p. 170) Pittendrigh states, "There is no direct evidence that constant darkness is detrimental to anything but green plants." My data on mortality of fruit flies incubated in constant darkness presents direct evidence for detrimental effects to animals.*

These detrimental effects may result from a desynchronization of

*Another line of evidence is given by Riesen (1951). Riesen reports that chimpanzees reared in darkness do not have normal visual capacity. It is further shown that chimpanzees reared with their heads enclosed in translucent domes exhibit the same abnormalities, implying that it is the lack of patterned vision and not darkness per se. However, since constant darkness excludes patterned vision it must be considered pathogenetic in its own right.

circadian organization. For example, peak times of sodium and potassium excretion bear a fixed relationship when man adheres to a 24-hour activity cycle. If the period of the activity cycle is changed, sodium excretion follows the new cycle, but potassium excretion is much more resistant to change and adheres to the intrinsic 24-hour cycle. In this case the constituent rhythms of sodium and potassium excretion have become desynchronized (Lobban 1960). Desynchrony can be thought of as a change in the juxtaposition of physiological events, including those important in development.

There is ample evidence for adult animals, as well as for plants, that internal rhythms of sodium, potassium, and water excretion, body temperatures, etc., can be desynchronized by 1) constant conditions (Todt 1962, Halberg and Barnum 1961), 2) reversed or shifted light cycles (Lobban 1965), and 3) cycles greater or less than 24 hours (Lobban 1960). Desynchrony as an explanation of the detrimental effects of constant conditions may be tested using desynchronizing stimulations other than constant conditions. For example, if random light cycles or regular cycles that differ radically from 24 hours are given, the constituent rhythms will become desynchronized (Lobban 1960). Assuming that desynchrony becomes greater in proportion to the difference between exogenous synchronizers and the innate 24-hour periodicity, one would predict increased mortality in related proportions.

II. Effects of light cycles on eclosion. Eclosion occured earlier for flies incubated under short cycles than for flies incubated under long cycles ($P < 0.01$). This supports the hypothesis that developmental time is related to the length of exogenous oscillations.

Light cycles do phase developmental events. This is apparent in the differences between eclosion curves of treatment groups subjected to light cycles or maintained in constant conditions. Those flies maintained in constant conditions have unimodal eclosion curves, while flies incubated in cyclic conditions exhibit bimodal patterns of eclosion. Bimodal patterns of insect eclosion and other circadian functions, such as running-wheel activity of rodents and general activity of birds, are common in the literature (Aschoff 1966). Bimodality in eclosion curves may be attributed to the phenomena of "gating" (Minis and Pittendrigh 1968). This phenomena implies that the fly may eclose only at specific phase intervals of the endogenous circadian cycle. These intervals are referred to as gates. If the fly is not ready for the first gate he is obligated to wait for the next one. It would appear that for the cyclic conditions of this experiment, two eclosion gates exist within the final circadian period, the centers of which are separated by approximately 12 hours or one-half a circadian cycle (see Figures 3, 4, and 6 through 9). The absence of bimodal eclosion curves in the constant condition groups indicates that the circadian cycles (and therefore the gates) of individual flies were not in synchrony as a population. The lack of synchrony is to be expected in the absence of a common synchronizer such as a light cycle.

Flies incubated in constant light eclosed significantly earlier ($P < 0.01$) than flies incubated in constant darkness. In absence of exogenous cycles, the endogenous cycle is free running. Aschoff's Rule* would predict shorter free-running rhythms in constant light. Therefore, one would predict earlier eclosion for the constant light treatment group, based on the hypothesis stated on page 1.

The constant conditions were included for comparisons between eclosion of cyclic treatment groups; however, significantly higher mortality rates of constant condition groups preclude this comparison. Mortality and eclosion rates may not be independent. If, for instance, "weak" flies emerge later, and "weak" flies are differentially selected against, then flies in treatment groups with higher mortality would appear to eclose earlier even though the developmental rate of any given fly had not been affected.

III. Effects of twilight transitions on eclosion. Short cycle groups eclosed before the long cycle groups in both the 15 second and 15 minute twilight groups. Both twilight transition groups exhibited bimodal distribution and synchronization to the imposed light cycle. A very real difference exists between these twilight treatment groups however. This difference is best expressed as a delay of 4 hours in the bimodal eclosion peaks and a lower first peak for both 15 second twilight long and short cycle treatment groups when compared with the

*See note at bottom of page 1.

15 minute twilight treatment groups. This results in a significant difference in the means of the two twilight groups ($P < 0.01$). A comparison of Figures 3 and 5 will also show that eclosion of the 15 second twilight group is also later than either constant darkness or constant light. Statistical analysis of this comparison is excluded by the set of contrasts chosen; however, statistical comparison is hardly necessary.

It is clear that the length of twilight transition is the variable involved, since both long and short cycle boxes are affected similarly. Both sets of cyclic treatment boxes were subjected to identical environmental conditions of temperature, humidity, light intensity, barometric pressure, and all other parameters except twilight transitions. Length of light cycles is also accounted for since boxes controlled by both the long and short cycle timer produce 15 second twilight transitions.

The obvious question is: what aspects of twilight transitions caused the shift to later eclosion in the 15 second twilight group relative to the 15 minute treatment group? The answer is not apparent, but three possibilities have arisen. They are:

1) A difference in total light energy. Examination of Figure 12 shows that more light energy reaches the flies at dawn under the 15 second twilight transition, but this is exactly offset by more light energy reaching the flies with the 15 minute twilight at dusk. There is then, no difference in total light energy and this possibility is unrealistic.

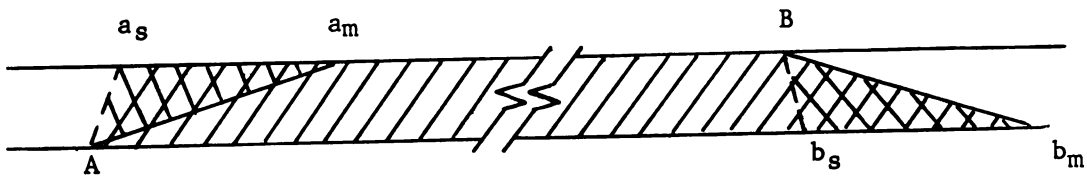

Figure 12. Schematic representation of the twilight transitions used. Point A represents the onset of dawn. Point a_s represents the culmination of the 15 second dawn and a_m, the culmination of the 15 minute dawn. Point B represents the onset of dusk; b_s represents the culmination of the 15 second dusk and b_m the culmination of the 15 minute dusk. The shaded area represents the light phase and the cross-hatching indicates the portions of the cycle that differ under the different twilight transitions. The ratio between 15 second and 15 minute twilight transitions is 1:60. Twilight transitions illustrated in this figure are 1:10.

2) A difference in photoperiod. For any light intensity below one-half "full light" the 15 minute twilight regime presents a longer photoperiod and for any intensity above this point the 15 minute regime presents a shorter photoperiod. Photoperiods for the light intensity one-half way through a transition are equal for both twilight groups. Differences in the photoperiod of the minimal effective light intensity may be the factor involved, but this can only be determined by further research using different photoperiods and equal twilights.

3) A difference in stimulus length. Figure 12 illustrates a longer, more gradual transition for the 15 minute twilight group than for the 15 second twilight group. This is the most obvious difference between the two twilight treatments. The manner in which this might account for the difference noted between twilight treatments is not obvious.

Kavanau (1962) has shown that the period of activity, feeding and drinking cycles of Peromyscus can be manipulated more radically with twilight transitions than with the traditionally used ON-OFF light transitions. Perhaps a similar principle is responsible for both observations. Kavanau does not discuss relative stimulus values of twilight versus an ON-OFF light transition, except to indicate that twilight simulates field conditions.

The effects of twilight on the entrainment of endogenous cycles is probably the most significant parameter of light and certainly one of the least studied or understood.

IV. Effects of sex on eclosion. Figures 6 through 11 show earlier eclosion of females as compared with males. The more rapid development of females is well documented and supported by Bakker and Nelissen (1963), Bonnier (1926), Poulson (1934), and Powsner (1935). In Bakker and Nelissen's study (1963), the sex difference was not evident in the egg and larval periods, but was confined to the pupal period. They also noted that a strong correlation existed between weight and time of eclosion. Heavier males eclosed before lighter males and heavier females before lighter females. Female Drosophila weigh more than males. It appears that weight may be an important factor in determining the earlier eclosion of females. Bakker and Nelissen report that peak female eclosion averages 7.5 hours earlier than male eclosion.

With the exception of the data collected under 15 second

twilight regimes, analysis on the basis of sex does little to alter the relationship between long and short cycle groups. With the 15 second twilight transition regimes, it is apparent that the bimodal nature of the eclosion curves has changed radically and that the females are responsible for any contribution toward bimodality.

OVERVIEW

Prior to this thesis, a functional role of exogenous circadian cycles in ontogeny had not been described. Data presented here support the theory that exogenous circadian cycles act as a chronometer for development. That is, the more rapidly the cycles occur the more rapidly development proceeds. Realistically, limits are to be expected on the extent to which development can be altered. These data are not adequate for speculating on these limits.

In addition, it appears that the presence of such a chronometer maximizes developmental success. In the absence of exogenous cycles, mortality increases. It is possible that the absence of a chronometer (i.e. light cycles) allows component rhythms of the developing organism to become desynchronized, and that such desynchrony is translated into a derangement of developmental events. If the integrity of the developmental sequence is lost, abnormal development and increased mortality can be expected.

Both constant darkness and constant light have been shown to cause desynchrony of constituent endogenous rhythms (Todt 1962). Constant light is the stronger desynchronizer and has been shown by others (Pittendrigh 1960) to be pathological. The significant difference in mortality rates, found between flies incubated in constant conditions or in light cycles, is a demonstration of the pathological effects of constant darkness as well as constant light.

Light cycles longer or shorter than 24 hours have also been shown to desynchronize constituent endogenous rhythms (Lobban 1960). In this case, one would expect mortality of flies incubated in the long and short cycles described here to be greater than mortality of flies incubated in 24-hour light:dark cycles. This question can not be answered from these data because a 24-hour light:dark cycle was not run concurrently.

The two major concepts developed in this thesis are: 1) exogenous circadian cycles act as a chronometer for development, and 2) exogenous circadian cycles, by synchronizing development, maximize developmental success. The hypothesis* I set out to test is the basis of the first concept. The second concept became evident from the mortality data. The concept that exogenous cycles maximize developmental success encompasses the idea that light cycles act as a chronometer and is the more interesting of the two. Indeed, it implies that the presence of exogenous circadian oscillations are important to survival.

These concepts generate several related questions. The series of questions I am most interested in are:

1) What conditions produce the greatest difference in mortality?

2) At what stage of development does highest mortality occur under desynchronizing conditions?

3) What abnormalities lead to abortion?

*The developmental period, from oviposition to emergence of the Drosophila melanogaster imago, is longer when flies are incubated in long circadian light:dark cycles, than when they are incubated in shorter circadian light:dark cycles.

4) What are the processes controlling normal or abnormal development?

5) How are these processes tied into exogenous circadian cycles; and, how do they differ under synchronizing and desynchronizing conditions?

The exceptional case of males subjected to 15 second twilight transitions (Figure 6) suggests sex and twilight as variables requiring more attention than can be given from these data. A consideration of twilight and sex in conjunction with the theory of "gating" (see p. 24) offers a tentative explanation of the late eclosion of males subjected to a 15 second twilight. Two possible gates are indicated by the other curves of flies subjected to cyclic conditions. Slower eclosion rates are correlated with both sex and 15 second twilight. It is conceivable, therefore, that only a few males were competent to eclose at the first gate, whereas practically all were competent by the second gate. This explanation is unsatisfactory, but is the best available at this time.

SUMMARY

The two major concepts developed in this thesis are: 1) exogenous circadian cycles act as a chronometer for development, and 2) exogenous circadian cycles, by synchronizing development, maximize developmental success.

The first concept is based on more rapid development of Drosophila, during the period oviposition to eclosion, when incubated in shorter light:dark cycles.

The second concept is based on lower mortality rates for flies incubated in light cycles as compared to constant light or constant darkness. It is probable that increased mortality results from desynchrony of constituent rhythms in constant conditions.

Males eclose later than females and flies incubated under 15 second twilight transitions eclose later than flies incubated under 15 minute twilight. These statements may be made with certainty; however, further research is required before concepts of functional roles in ontogeny can be developed for these parameters.

Appendix I. Raw Data

The raw data is given in Tables 8 through 13. Each column represents the flies eclosing in a given vial. The columns are further divided on the basis of sex. Flies were collected at 4 hour intervals. The hours of incubation preceding each collection are indicated in the left hand column. The total number of flies eclosing in any one time period is totaled across columns and entered in the right hand column. The number of flies eclosing in any one vial is totaled across time periods and entered in the bottom row. The total of either sex eclosing in any one vial is given in the next to the bottom row.

Table 8. Raw data for the long cycle, 15 second twilight treatment group. Shutterbox #1.

Hours of Incubation	Vial Number														Totals
	1		2		3		4		5		6		7		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
253	1	0	0	2	1	2	0	0	0	1	0	0	0	0	7
257	0	1	0	3	1	1	0	0	0	0	0	2	0	1	9
261	0	2	0	0	0	1	0	1	0	0	0	2	0	3	9
265	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2
269	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2
273	4	13	5	22	5	26	3	11	2	15	3	20	10	6	145
277	27	40	23	23	29	29	19	49	35	37	34	28	27	33	433
281	10	3	8	1	5	2	21	6	11	1	9	0	10	2	89
285	1	0	3	0	0	2	3	1	2	0	0	1	3	1	17
289	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2
293	0	0	0	0	0	0	2	1	1	0	1	0	0	0	5
309	0	1	1	0	1	0	2	0	4	0	1	0	4	0	14/4*
317	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2*
Σ/sex	44	60	40	53	43	63	50	69	56	64	49	53	54	46	734
Σ/vial	104		93		106		119		110		102		100		

Table 9. Raw data for the long cycle, 15 minute twilight treatment group. Shutterbox #2.

Hours of Incubation	Vial Number														Totals
	8		9		10		11		12		13		14		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
253	0	2	1	2	0	7	0	1	1	2	0	1	1	2	20
257	2	14	1	14	2	19	0	15	1	11	1	6	0	6	92
261	0	12	1	13	1	7	0	11	1	15	1	12	0	6	80
265	1	0	1	0	2	1	0	1	0	1	0	0	1	1	9
269	10	4	6	2	1	7	2	5	3	6	8	10	4	1	69
273	28	12	20	21	16	7	27	17	25	20	29	19	24	23	288
277	9	3	10	7	8	1	19	3	13	4	18	3	11	3	112
281	4	0	3	0	1	1	1	0	2	0	1	0	4	0	17
285	0	1	0	0	0	0	0	1	0	0	0	1	0	0	3
289	0	0	0	0	1	0	1	0	1	0	0	0	0	0	3
293	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
309	1	0	0	0	1	0	1	0	0	0	0	0	0	0	3/4*
317	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1/2*
Σ/sex	55	48	44	59	33	50	51	54	47	59	58	52	45	43	698
Σ/vial	103		103		83		105		106		110		88		

* # of flies/# of 4 hr. time periods (i.e. these periods are greater than 4 hrs.)

Table 10. Raw data for the short cycle, 15 second twilight treatment group. Shutterbox #3.

Hours of Incubation	Vial Number														Totals
	15		16		17		18		19		20		21		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
253	0	0	0	0	0	0	0	1	0	1	0	1	0	0	3
257	0	0	1	1	1	3	0	3	0	0	0	0	1	1	11
261	0	9	0	16	1	18	0	8	0	7	0	6	0	5	70
265	2	21	0	9	1	12	0	13	0	10	0	11	0	11	90
269	1	2	0	1	0	3	0	5	0	5	1	8	0	3	29
273	3	9	3	5	3	3	4	12	3	10	8	15	2	11	91
277	32	15	30	24	29	18	32	19	42	9	36	11	22	29	348
281	9	0	8	1	1	1	7	2	4	2	6	1	10	5	59
285	2	0	2	1	1	0	0	0	0	3	1	0	2	0	12
289	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
293	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
309	1	0	0	0	1	1	0	0	0	0	0	0	0	0	3/4*
317	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2*
Σ/sex	50	56	45	58	39	60	43	63	52	44	52	53	38	65	718
Σ/vial	106		103		99		106		96		105		103		

Table 11. Raw data for the short cycle, 15 minute twilight treatment group. Shutterbox #4.

Hours of Incubation	Vial Number														Totals
	22		23		24		25		26		27		28		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
253	1	0	1	1	2	6	0	0	1	1	1	4	0	0	18
257	2	26	0	12	9	30	1	25	0	14	0	26	1	27	173
261	18	24	13	25	24	13	13	26	9	29	19	22	3	16	254
265	9	5	15	4	9	1	12	4	15	5	14	4	4	7	108
269	3	0	9	0	1	0	4	0	6	0	3	1	5	0	32
273	9	1	1	13	3	0	9	1	11	4	3	0	11	3	69
277	3	0	4	0	4	1	6	2	7	2	3	1	5	0	38
281	4	0	0	0	0	0	2	1	0	0	3	0	2	0	12
285	0	0	1	0	0	1	0	0	1	0	0	0	1	0	4
259	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
293	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
309	1	0	0	2	1	0	0	1	0	0	0	0	1	0	6/4*
317	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1/2*
Σ/sex	50	56	44	57	54	52	47	60	51	55	46	58	33	53	716
Σ/vial	106		101		106		107		106		104		86		

* # of flies/# of 4 hr. time periods (i.e. these periods are greater than 4 hrs.)

Table 12. Raw data for constant dark treatment group. Shutterbox #5.

Hours of Incubation	Vial Number												Totals
	29		30		31		32		33		34		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
253	1	2	0	1	0	1	0	0	1	2	1	1	10
257	1	0	1	0	0	0	0	0	0	0	0	1	3
261	0	3	0	1	0	0	0	2	0	5	1	4	16
265	0	7	3	9	2	13	1	11	0	19	1	16	82
269	6	22	4	14	8	8	15	22	14	13	3	18	147
273	9	18	20	15	14	6	24	8	26	5	17	9	171
277	22	5	13	2	13	2	10	2	11	1	10	5	96
281	5	4	2	1	1	3	2	3	6	0	4	1	32
285	2	0	0	1	2	0	1	0	1	0	2	1	10
289	2	0	1	0	0	0	0	0	1	0	0	0	4
293	2	0	0	0	1	0	1	0	0	0	0	0	4
309	0	0	2	0	0	0	0	0	1	0	2	0	5/4*
317	0	0	1	0	0	0	0	0	0	0	1	0	2/2*
Σ /sex	50	61	44	44	41	33	54	48	61	45	42	6	582
Σ /vial	111		91		74		102		106		98		

Table 13. Raw data for constant light treatment group. Shutterbox #6.

Hours of Incubation													Totals
	35		36		37		38		39		40		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
253	1	2	0	2	0	1	0	1	0	0	2	1	10
257	1	3	0	0	0	0	0	3	1	1	0	3	12
261	0	4	0	9	0	13	0	10	0	13	0	5	54
265	4	13	3	20	2	15	2	25	4	13	7	21	129
269	3	16	17	11	19	4	20	11	16	22	10	9	158
273	19	7	15	4	8	6	11	9	15	3	9	3	109
277	13	4	7	3	3	1	10	2	9	0	11	2	65
281	0	0	5	1	1	0	2	1	2	0	2	1	15
285	1	4	0	1	1	0	0	0	0	0	2	0	9
289	0	0	1	0	0	0	0	0	0	0	0	0	1
293	0	0	0	0	0	0	0	0	1	0	0	0	1
309	0	0	0	0	0	1	0	0	0	0	0	0	1/4*
317	0	0	0	0	0	0	0	0	1	0	0	0	1/2*
Σ/sex	42	53	48	51	34	41	45	52	49	52	43	45	565
Σ/vial	95		99		75		107		101		88		

* # of flies/# of 4 hr. time periods (i.e. these periods are greater than 4 hrs.)

Appendix II. Density-dependent developmental rates.

Pilot work for the experiments described in this thesis was done without controlling density of flies developing in each vial. A number of adult flies oviposited in each vial for a four hour period and were removed after that time. The number of eggs oviposited in each vial varied widely as indicated in Figures 13 and 14.

It is clear from these figures that developmental rates are density dependent; and that higher densities result in retarded eclosion.

Figure 13. Density dependence of eclosion rate for flies incubated in short light:dark cycles (22 hr. 52 min.). Each data point represents one vial. The number of flies developing from egg to imago in that vial is represented on the absisa, and the percentage of the total number eclosing by 249 to 252 hours of incubation is indicated on the ordinant.

Figure 14. Density dependence of eclosion rate for flies incubated in long light:dark cycles (25 hr. 9 min.). Other conditions and information same as given for Figure 13.

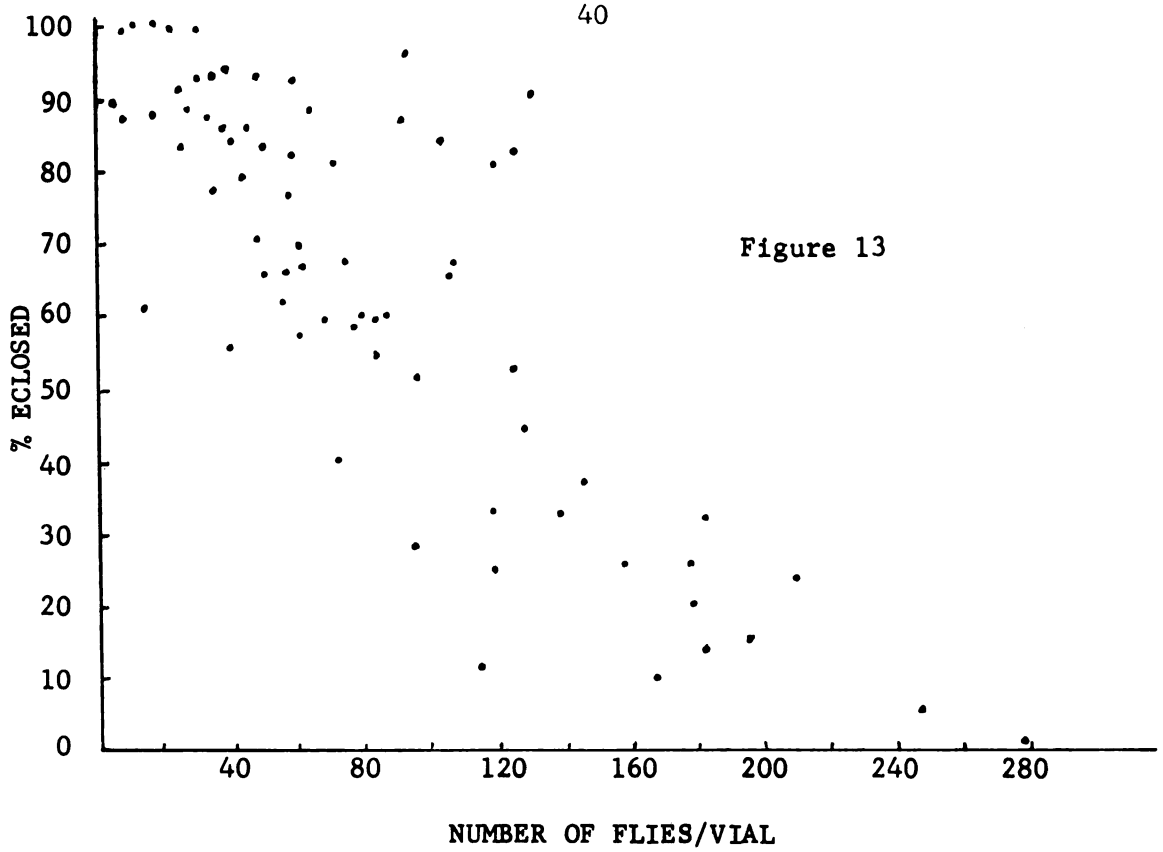

Figure 13

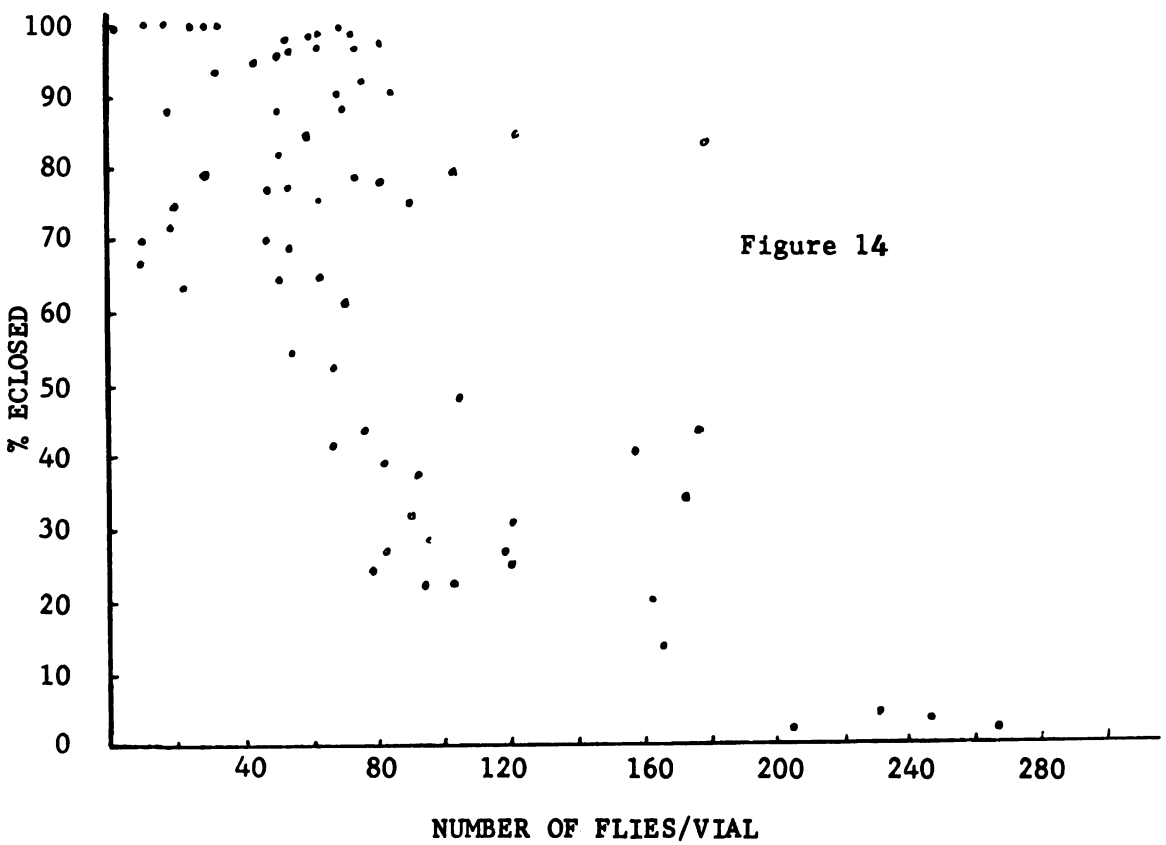

Figure 14

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