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The Effect of Light Intensity on the Circadian Rhythms of Eclosion in *Drosophila pseudoobscura*

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Summary. 1. The circadian system in *Drosophila pseudoobscura* responds to a non-recurrent change in light intensity in the same manner as to transitions from light to darkness or from darkness to light.

2. This simulation of the light "on" effect and light "off" effect by light intensities is more efficient when the ratio between the acclimation light intensity and the subsequent test light intensity is higher.

3. The rhythms initiated by the onset of light and continuing in it tend to wane after three or four cycles. Rhythms initiated by onset of darkness and free running in it are persistent over longer periods and better synchronized. In both cases, however, the system undergoes a transient of one cycle in duration.

4. The implications of these observations in the context of earlier work by other authors is discussed.

Zusammenfassung. 1. Das circadiane System von *Drosophila pseudoobscura* reagiert auf einen einmaligen Lichtintensitätswechsel in der gleichen Weise wie auf Übergänge von Licht nach Dunkel und von Dunkel nach Licht.

2. Die Nachahmung des Licht-An-Effekts und des Licht-Aus-Effekts durch Lichtintensitäten ist um so wirkungsvoller, je größer das Verhältnis zwischen der Ausgangsintensität zu der darauffolgenden ist.

3. Die Rhythmen, die durch Licht-An ausgelöst werden und im Licht weiterlaufen, werden nach 3—4 Zyklen schwächer. Durch Licht-Aus hervorgerufene Rhythmen, die in der Dunkelperiode weiterlaufen, werden länger aufrechterhalten und sind besser synchronisiert. In beiden Fällen entwickelt jedoch das System einen "transient", der einen Zyklus lang dauert.

4. Die Bedeutung dieser Befunde wird im Zusammenhang mit früheren Untersuchungen anderer Autoren besprochen.

Introduction

The emergence pattern of the adults of *Drosophila pseudoobscura* is aperiodic when the cultures are raised and studied in continuous light (LL) or darkness (DD) from the egg-stage onwards. A circadian periodicity can be initiated by transferring a pupal population before the onset of eclosion from LL to DD or vice versa. Bünning had systematically studied the phenomenon in 1935 and shown that a singular non-recurrent transfer of the pupae from LL to DD or from DD to LL was sufficient

to set the rhythm into motion. Among plants, the petal movements of *Kalanchoe blossfeldiana* (ENGELMANN, 1960) and the flowering response of *Pharbitis nil* (TAKIMOTO and HAMNER, 1965) appear to exhibit similar sensitivities. ENGELMANN (1966) further studied the rhythms initiated by light and dark steps in *Drosophila pseudoobscura* and showed some differences between the time course of rhythms initiated by light and those by dark. ENGELMANN (1966) explaining the results of dark pulse and light pulse experiments postulated that a "superposition of an on-and off rhythm" could explain the emergence distribution of entrainment and pulse experiments. Subsequently HONEGGER (1968) found support for this explanation on the basis of short duration light pulse studies on *Drosophila pseudoobscura*. In all the experiments cited, light of a given intensity and absolute darkness (or far red light) were employed for the light/dark fractions. Employing this technique it is not possible to study the effect on the rhythm of two or more "on" steps or "off" steps uncomplicated by opposite transitions. The experiments described here were performed to see if the "off" (LL to DD) and "on" (DD to LL) can also be simulated by transition from a relatively high intensity to a lower one and vice versa.

When the difference between the initial or acclimation intensity (I_1) and subsequent test intensity (I_2) of light is of a high magnitude simulations of "on" and "off" seem to be possible. Employing this method the on-and off-rhythm hypothesis can be tested more effectively. Furthermore the quality of darkness (DD) or far red light employed in routine studies is a parameter that does not obtain under normal conditions in nature.

Materials and Methods

The cultures were reared in broad-necked milk bottles of approximately 200 cc. volume in the usual manner (SPENCER in DEMEREC, 1950) at $20 \pm 0.2°$ C in DD (far red in the range of 680 nm) and in the various intensities of light used for the experiments. Five cabins were fitted with cinemoid filters held within wooden frame and glassplate brackets. The light intensity within the individual cabins could be varied by manipulating the number of sheets of cinemoid filters in the brackets. The cabins were illuminated by a common source of Philips fluorescent light tubes from above. The light intensity incident at a level about 10 cm above the floors of the five cabins was adjusted to 0.3, 3, 30, 300 and 3000 lux respectively. Light intensity varied, however, within a cabin at various levels and the corners but the range was never as high as the difference between any two intensities being tested which ranged from ten to ten thousand fold. Thus, the light intensity within the 0.3 lux chamber varied for example between 0.1 lux at the level of the cabin floor to nearly 0.8 lux nearer its ceiling. The culture bottles were always placed within well marked-out areas on the floor of the cabins to minimize possibilities of experiencing fluctuations in light intensity.

The DD cultures were raised in an adjacent darkroom fitted with a safelight (HONEGGER, 1968).

The values given for the emergence maxima are the calculated medians. In all experiments, only those peaks characterized by a synchronization value (D value) higher than 4 were taken. The D-value for a peak is represented by the difference between the percentage emergence during the maximum and emergence during the preceding and following minima. The total number of flies emerging between the two minima is 100% (HONEGGER, 1968).

Results

1. Rhythms Initiated by Transitions from DD to LL (Light "On") and from LL to DD (Light "Off")

The general features of the rhythms initiated by transition from light to darkness and from darkness to light have been reported for *Drosophila pseudoobscura* by ENGELMANN (1966). In the experiments reported in this study various intensities were tried out for the light fraction.

The results are set forth in Fig. 1 and elucidate the conclusions reached by the earlier workers. The rhythms initiated by a transition from any light intensity (LL) to absolute darkness (DD) are better synchronized and more stable over long periods. Only the second, third and fourth peaks of these "off" rhythms are shown in the figure merely for purposes of comparison.

The rhythms initiated by onset of light of all intensities from 0.3 to 3,000 lux tend to wane after the third cycle. There is also a tendency for the peaks to appear progressively earlier i.e., with shorter periods. The period lengths (time duration between one maximum and the next one) in "off" rhythms, however, is fairly constant after the second cycle. These features are presented in Fig. 2.

Table 1. *The degree of synchronization (in "D" values) of the peaks of rhythms initiated by transfers for DD to LL and from LL to DD*

Nature of transition	Peak 1	Peak 2	Peak 3	Peak 4
DD-3,000	6.6	16.3	19.7	7.6
DD-300	5.4	9.8	9.8	4.6
DD-30	3.6	8.7	6.1	5.6
DD-3	3.8	8.0	7.1	5.9
DD-0.3	6.9	10.4	8.1	8.8
L/D-DD	25.0	21.1	23.8	23.0
3,000-DD	3.2	11.2	11.2	14.5
300-DD	5.8	6.1	14.8	10.3
30-DD	1.8	10.9	18.7	18.0
3-DD	4.7	7.6	19.8	12.3
0.3-DD	3.0	6.2	13.5	11.5

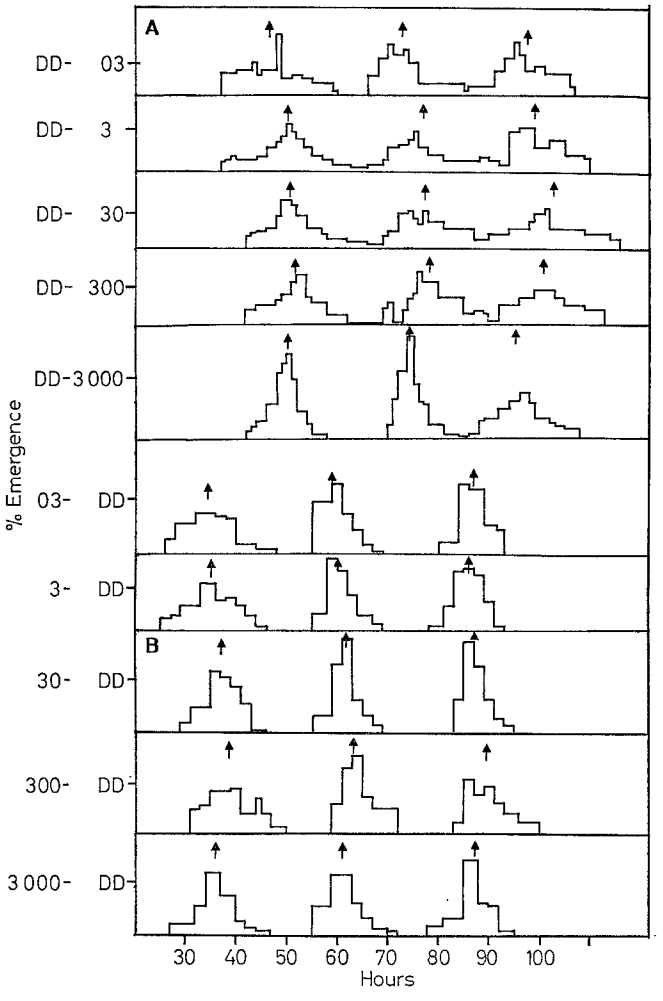

Fig. 1. A: The rhythms initiated by a transfer of the populations of pupae from absolute darkness (DD) to various intensities of light (LL) and B: rhythms initiated by a transfer of the pupae from prior experience in different intensities of light to subsequent darkness. All experiments were performed at $20 \pm 0.2°$ C. Eclosion distribution is presented as % emergence/hour, the flies emerging between two minima representing 100%. Ordinate: A, Intensity of light to which pupae were transferred from DD. B: Intensity of light in which pupae were raised prior to transfer to DD. Abscissa: Number of hours after transfer to test condition. The triangles above the peaks indicate the calculated medians. The first peak appearing after the transition is not shown

The first peak after transition shares one common feature in transitions either from LL to DD or DD to LL in the synchronization being

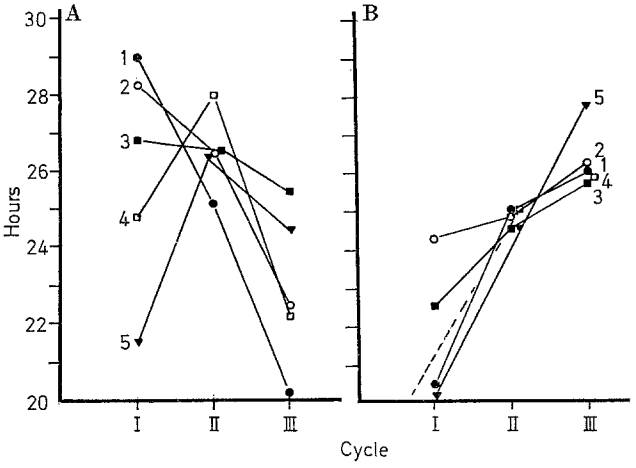

Fig. 2 A and B. Illustrates trends in raw data presented in Fig. 1. The period length of the first three cycles in rhythms initiated by the onset of various intensities of light (A) and by the onset of darkness (B). Ordinate: length of period (the duration between two successive maxima) in hours. A *1*, DD-3,000 lux; *2* DD-300 lux; *3* DD-30 lux; *4* DD-3 lux; *5* DD-0.3 lux. B *1* 3,000 lux-DD; *2* 300 lux-DD; *3* 30 lux-DD; *4* 3 lux-DD; *5* 0.3 lux-DD. Abscissa: Number of cycle. Other details as for Fig. 1

weak (greater variance and a lower D value). This may be seen from the "D" values for the first peaks in Table 1. Obviously the system develops transients of a cycle in duration when set into motion by an "on" or an "off" information.

2. Rhythms Initiated by a Simulation of Light "Off" and Light "On" Effects

Fig. 3. summarises the data for experiments in which the cultures were raised in one light intensity (I_1) from egg-stage on and transferred before the onset of eclosion to another light intensity (I_2). The difference between I_1 and I_2 varied ten, hundred, thousand and ten thousand fold. In Table 2 are given the D values for all four peaks measured and in Fig. 4 the actual emergence distribution of the second and third peaks.

It is evident from Figs. 3 and 4 and Table 2 that a transition from a lower to a higher intensity or from a higher to a lower one, initiates a clear rhythmicity which lasts over 3 cycles. The bigger the ratio between I_1 and I_2 the closer is the simulation to "on" or "off" effect discussed in the previous section. Thus, a $\times$ 10,000 step up from 0.3 to 3,000 lux at transition induces a rhythmicity closer in its time course to the "on" rhythm, than, for example, a $\times$ 10 step up. A similar trend may be

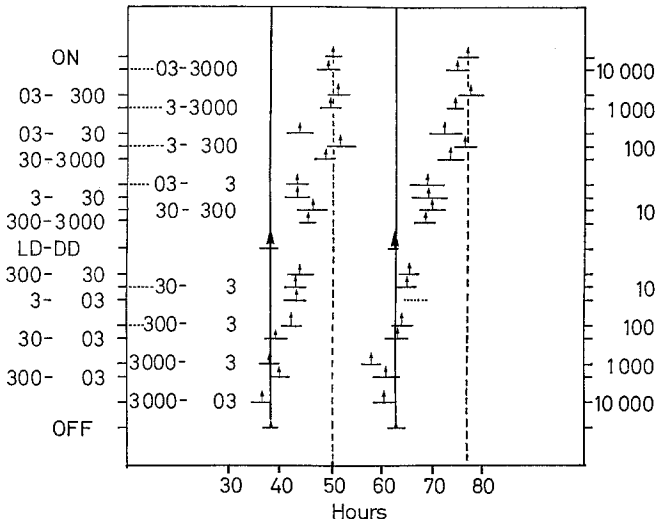

Fig. 3. Calculated data setting forth the medians and standard deviation of the 2nd and 3rd peaks of rhythms initiated by transitions of pupae from one light intensity (I_1) to another light intensity (I_2). The two intensities differed by a factor of: $\times 10$, $\times 100$, $\times 1{,}000$ and $\times 10{,}000$. Given uppermost in the figure are the medians for the 2nd and 3rd peaks of a DD-LL (300 lux) initiated rhythm (on). Similarly in the lowest row are the medians for (3,000 lux) LL-DD initiated rhythms (off). The vertical dashed guide lines indicate the position of "on" maxima and the unbroken vertical lines show the position of "off" maxima presented here to facilitate comparison. Ordinate: the values for I_1 and I_2 and their ratio. In the upper half are simulated "on" steps and in the lower half the simulated "off" steps. Abscissa: Time in hours after transition at "0" hour

noticed in decreasing intensity experiments, intensity drops of higher orders approximating the "off" oscillation closer than those of lower orders.

Another interesting feature apparent in Fig. 3 is that the maxima of rhythms initiated by the same magnitude of transition appear to occur closer in time as though the flies recognized only the relative levels of I_1 and I_2 and not the absolute intensities involved. This point, however, needs further experimentation and study and cannot be conclusively stated on the bais of the experiments reported here.

The response of the system to a transition from LL of whatever intensity — even as low as 0.3 lux — to DD appears to be total and is not simulated as regards synchronization and persistence even by an intensity step down from 3,000 to 0.3 lux. This may be owing to the fact that the difference in level between the weakest light intensity used here and absolute DD is still greater than in the simulation studies.

Table 2. *The degree of synchronization represented by the "D" values of the peaks of rhythms illustrated in Figs. 3 and 4*

Values for I_1 and I_2 in lux and ratio	Peak 1	Peak 2	Peak 3	Peak 4
	Simulated light "on" transitions			
0.3—3,000 (× 10,000)	4.4	13.4	9.8	11.0
0.3—300	5.5	10.6	9.6	2.0
3—3,000 (× 1,000)	9.4	11.4	7.7	7.0
0.3—30	6.7	5.8	4.3	6.6
3—300	6.8	7.2	6.7	[a]
30—3,000 (× 100)	5.1	12.2	10.7	6.0
0.3—3	3.9[a]	9.4	3.8	7.0
3—30	4.1	10.6	6.4	4.7
30—300	4.0	7.6	5.8	5.0
300—3,000 (× 10)	5.2	21.5	10.8	[a]
	Simulated light "off" transitions			
3,000—300	4.3	3.5[a]	3.0[a]	[a]
300—30	4.1	10.8	9.6	[a]
30—3	4.2	11.4	7.0	3.0[a]
3—0.3 (× 10)	3.9[a]	6.5	[a]	[a]
300—3	5.5	10.4	14.4	[a]
30—0.3 (× 100)	4.5	6.3	5.6	[a]
3,000—3	2.7[a]	7.0	7.4	[a]
300—0.3 (× 1,000)	5.6	6.2	7.0	6.7
3,000—0.3 (× 10,000)	5.6	9.0	8.5	4.2

[a] Synchronization very weak and values lower than in light and dark controls.

Discussion

It emerges from our studies, that the circadian system in *Drosophila pseudoobscura* responds to changes in light intensity levels in much the same manner as it does to transitions from LL to DD or DD to LL. The response, its direction and magnitude appear as a function of the direction and magnitude of I_1 and I_2. The rhythmicity though stable and well

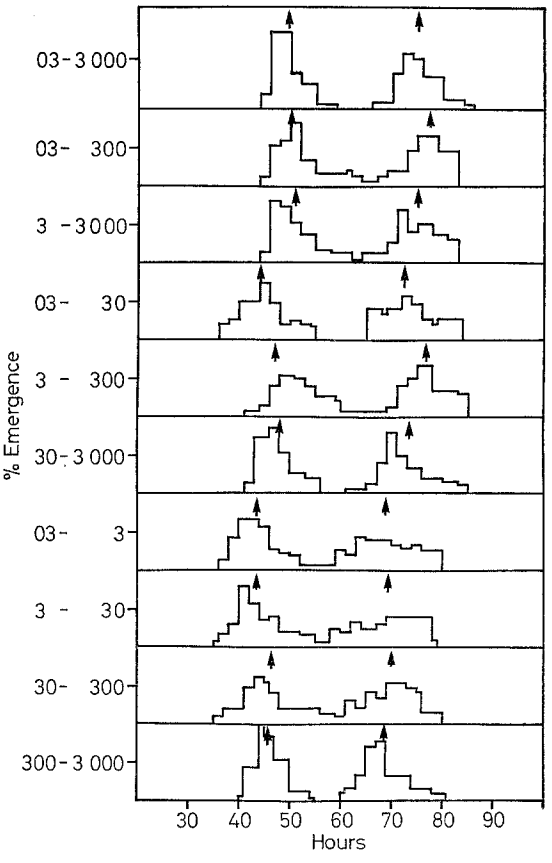

Fig. 4A. The actual emergence distribution of the flies in experiments summarized in Fig. 3.
Emergence distribution in experiments simulating a light "on" step

synchronized over the first 3 cycles tends to wane thereafter in all light intensity experiments reported here. The same is the case with the rhythms induced by transitions from DD to LL of any intensity. Though such a damping is not unknown in plant and animal rythms assayed under constant conditions (BÜNNING, 1963) a similar waning of the rhythmicity is not observed in *Drosphila* when it is initiated by and studied in DD.

PITTENDRIGH (1966) showed that when the final photoperiod is longer than 12 hours in entrained populations before release into DD the peaks appeared subsequently after $n\tau + 15$ hours suggesting that the

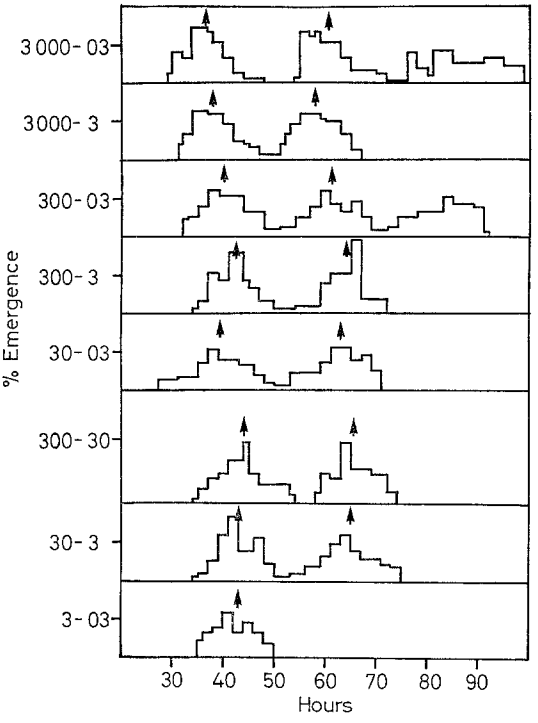

Fig. 4B. Emergence distribution in experiments simulating a light "off" step. Other details as for Fig. 1

basic light sensitive driving oscillation damps out in light lasting longer than 12 hours and resumes its motion in DD. The first 2 peaks appearing after a 24 hour or longer final photoperiod are interpreted by PITTENDRIGH (loc. cit.) as not representing the light sensitive driving oscillation in the flies and that they are transients.

It is difficult to understand on the basis of this interpretation the initiation of a rhythmicity by a transition from one light intensity to another and the persistence of the periodicity for over 3 cycles. If the basic light sensitive oscillation were indeed "held" in a fixed state corresponding to a state at 12 C.T. (PITTENDRIGH, 1966) in light then a subsequent transition of the intensity to a higher or lower order should not initiate a periodicity. Furthermore, the concept of light inhibiting the basic oscillation after 12 hours, does not explain the phase characteristics of rhythms induced by L/D entrainment regimes of the order of 22:2 or 20:4 (vide PITTENDRIGH and MINIS, 1964, Fig. 4; HENGST, 1967).

On the other hand, the super-position of the "on" and "off" rhythms hypothesis of ENGELMANN (1966) does not explain the results of several of the light pulse experiments (CHANDRASHEKARAN, 1967a and b). In several cases any explanation of the maxima on the "on-off" interaction hypothesis is possible (HONEGGER, 1968) only when the "off" component is assumed to play an overwhelming role, which it does.

The responses of the circadian system in *Drosophila* to light pulses and light steps obviously are too complex to be satisfactorily accounted for by any simple model. The role that light plays in entrainment and phase shifting is far from clear. It is, however, evident that the system responds to the onset of light or to its extinction though with some phase differences. The system also seems to oscillate at a different speed and with a different amplitude in light than in darkness. The results of the simulated light "on" and light "off" studies employing transitions from one light intensity to another should help in further experiments planned to elucidate if the "on" and "off" rhythms are qualitively different representing two oscillations with different time courses.

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