THE CIRCADIAN RHYTHM
OF FLIGHT ACTIVITY IN THE MOSQUITO *AEDES*
AEGYPTI (L.): THE PHASE-SETTING EFFECTS
OF LIGHT-ON AND LIGHT-OFF

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INTRODUCTION

Various authors have reported 24 hr. cycles of activity in *Aedes aegypti* (L.); these reports include the field observations of Boorman (1961) on biting and flight, and the laboratory studies of Haddow & Gillett (1957) on oviposition and Gillett, Haddow & Corbet (1962) on sugar-feeding. As a result of their laboratory studies, Gillett, Haddow & Corbet (1959, 1962) and Haddow, Gillett & Corbet (1961) came to the conclusion that both oviposition and sugar-feeding are controlled by an inherited endogenous circadian rhythm, which is dependent on the change from light to dark as a time-cue.

The development of an automatic method of recording flight activity in mosquitos (Jones, 1964; Jones, Hill & Hope, 1967) has made possible the present intensive study of the circadian rhythm of flight-activity in *A. aegypti*. This paper confirms the existence of a rhythm in constant dark and the phase-setting effect of light-off. It also draws attention to the possibility of a rhythm in constant light and to the phase-setting effect of light-on. It appears that light-off and light-on may control two different 'clocks' or possibly two 'hands' of the same 'clock'.

METHOD

A breeding colony of a West African strain of *A. aegypti*, from the London School of Hygiene and Tropical Medicine, was maintained in an LD 12:12 regime (alternating 12 hr. light:12 hr. dark) at 25° C. The insects for experiments were reared in a similar regime except where otherwise stated. Adult females were used 5–6 days post-eclosion, as younger adults were relatively inactive; this confirms the findings of Rowley & Graham (1968) that adult *A. aegypti* females show their maximum flight activity 8–12 days post-eclosion. The mosquitos were fed on a 15% solution of an equal mixture of glucose and sucrose. All females examined 5–6 days post-eclosion were found to be inseminated; this was true of only about a third of those 2–3 days after eclosion.

The recording technique was essentially the same as that developed by Jones *et al.* (1967). The activity of individual mosquitos was recorded and a total of five experiments, involving up to thirty-six individuals, could be carried out simultaneously. Four ten-channel event recorders were used in place of the original kymograph apparatus.

At first, the *A. aegypti* females did not survive very well in the recording chambers and intense flight-activity similar to the 'escape' flight described by Haufe (1962) was found to have preceded death in many cases. This problem was overcome by placing a tube, containing water, in each recording chamber as well as the tube containing sugar solution. This gave an R.H. of 85–90% and the mosquitos survived for 1–2 weeks.

The light intensity in all experiments was 70 lux produced by two 15 W. tungsten-filament bulbs. The temperature in the sound-proof boxes was 25° C. increasing to 26° C. towards the end of a 12 hr. light period and to a maximum of 27° C. in constant light.

The hourly flight-activity was assessed by giving each insect a score of 1 for activity in any single minute; the insect was thus given a score between 0 and 60 for each hour of the experiment. Except in the case of Fig. 2, which was an individual result, the histograms show the mean activity scores for each hour.
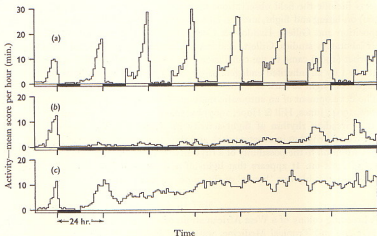

Fig. 1. Mean activity in (*a*) LD 12:12 (14 individuals), (*b*) DD following LD 12:12 (25 individuals), (*c*) LL following LD 12:12 (36 individuals).

RESULTS

1. *Continuation of the LD 12:12 (12 hr. light: 12 hr. dark) rearing regime*

In an LD 12:12 regime (Fig. 1*a*) the main peak of activity occurs 1–2 hr. before light-off and there is a rapid build-up from about the middle of the light period. There may be a small peak following light-on. The insects become relatively inactive within half-an-hour after light-off and remain so throughout the dark period. Male adults are less active but follow a similar pattern.

2. *The rhythm in constant conditions*

LD 12:12 *to DD* (*constant dark*)—*light-off at normal time* (*Fig.* 1*b*)

The level of activity is low in constant dark but the cycle continues with a period of about 22·5 hr.

LD 12:12 *to LL* (*constant light*)—*light-on at normal time* (*Fig.* 1*c*)

There is a marked increase in the level of activity and there appears to be a cycle with a period of about 26 hr.; the first peak comes approximately 13 hr. after light-on.
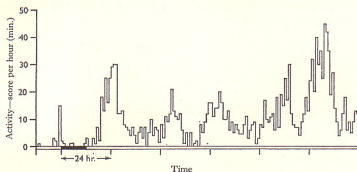

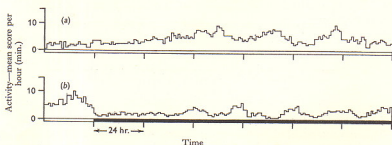Fig. 2. Activity of an individual mosquito in LL following LD 12:12.

Fig. 3. Mean activity (*a*) when reared and recorded in LL (17 individuals), (*b*) in DD following an LL rearing regime (36 individuals).

Individual records show marked variations in activity level, but the most active individuals show a clear cycle (Fig. 2). This is not so clear in the massed data as the individuals are not precisely in synchrony.

Reared and recorded in LL (Fig. 3a)

The overall level of activity is lower than in the previous experiment. There appears to be a weak cycle with a period of about 26 hr. although there was no obvious external time-cue which could have synchronized the activity of the individual insects.

LL to DD (Fig. 3b)

The activity is clearly cyclical with a period of about 23·5 hr: the peaks are in phase with the change from light to dark. The experiment was repeated with four different replicates of nine individuals and the change from light to dark was made at a different time in each replicate.
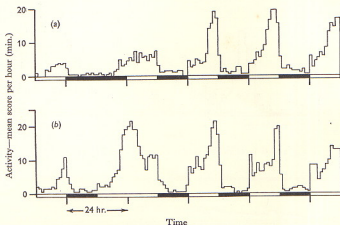

Fig. 4. Effect of reversing an LD 12:12 regime with (a) a 24 hr. dark period (18 individuals), (b) a 24 hr. light period (14 individuals).

3. Phase-resetting experiments

Reversing the LD 12:12 regime (Fig. 4)

This was accomplished by prolonging either a dark period or a light period to 24 hr. In both cases, the main peak was reset in the light period following the first light-off of the new regime; after a 24 hr. dark period the cycle was not reset until the second light period of the new regime. It appears that the cycle is reset by a change in the time of light-off.

Advancing the LD 12:12 regime by 6 hr. (Fig. 5)

The light regime was changed by shortening one light period (Fig. 5a) or one dark period (Fig. 5b). In both cases the first early light-off is followed by a rapid fall in activity level and the normal peak is lost, but in Fig. 5a there appears to be a small peak at about the normal time; in the subsequent light period the cycle has

been reset. When the regime is altered by shortening a dark period, a pronounced peak of activity follows the first early light-on; the absence of a small peak following the first early light-off indicates that the early light-on may have had a phase-advancing effect.
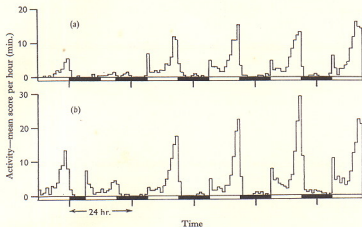

Fig. 5. Effect of advancing an LD 12:12 regime by 6 hr. by shortening (*a*) one light period (9 individuals), (*b*) one dark period (9 individuals).

4. *Changing the relation between light-on and light-off*

LD 12:12 *to LD* 4:20 *(Fig.* 6)

Figure 6*a* shows the effect of changing the regime by delaying light-on by 8 hr. The main effect is a lowering of the level of activity; the apparent position of the peak is unchanged. Figure 6*b* shows the effect of advancing light-off by 8 hr. The result is not very clear because of the low level of activity at the beginning of the experiment. By the sixth period, shown after a break of 48 hr. in the graph, the main peak of activity has advanced to the hour before light-off. In this sixth period a pronounced second peak can be seen some 13 hr. after light-on.

LD 12:12 *to LD* 20:4 *(Fig.* 7)

Figure 7*a* shows the effect of changing the regime by advancing light-on by 8 hr. The main activity peak is rapidly advanced to a point some 13–14 hr. after light-on, that is 6–7 hr. before light-off. There is also a large peak following light-on and some evidence of a smaller peak in the hour before light-off. Figure 7*b* shows the effect of changing the regime by delaying light-off by 8 hr. The main peak of activity is delayed so that again it occurs 13–14 hr. after light-on. As in Fig. 7*a* there appears to be a smaller peak in the hour before light-off.
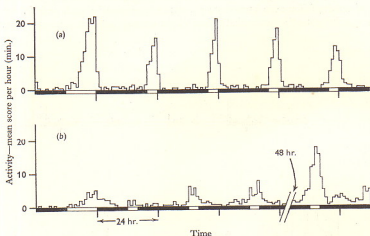
Fig. 6. LD 12:12 to LD 4:20 by (*a*) delaying light-on by 8 hr. (8 individuals), (*b*) advancing light-off by 8 hr. (9 individuals—there is a break of 48 hr. in the record).
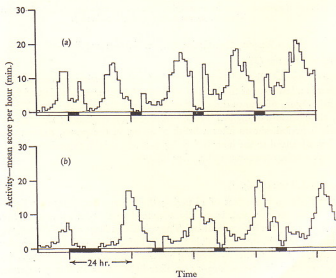

Fig. 7. LD 12:12 to LD 20:4 by (*a*) advancing light-on by 8 hr. (7 individuals), (*b*) delaying light-off by 8 hr. (9 individuals).

5. *The separate effects of light-on and light-off on the phase of the rhythm*

The results of the previous experiments lead to the hypothesis that both light-on and light-off have phase-setting effects on the rhythm of activity. Peaks of activity follow light-off by 22–23 hr. or light-on by 13–14 hr. In an LD 12:12 regime these expected peaks are fairly close, but they are widely separated in LD 4:20 and LD 20:4 regimes. The condition of the underlying rhythm may be exposed by a change to constant dark or constant light. The expected positions for peaks of activity have been plotted under the time-scale of each graph; the symbol $\triangle$ indicates a peak expected from the effect of light-on and the symbol $\blacktriangle$ a peak expected from the effect of light-off. The positions of these theoretical peaks are plotted assuming a free-running period of 22·5 hr. in constant dark and 26 hr. in constant light.
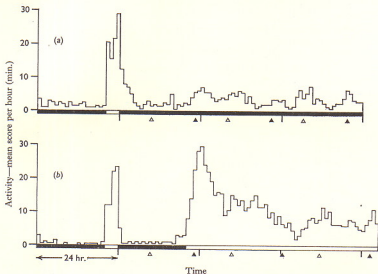

Fig. 8. LD 4:20 to (*a*) DD (9 individuals). (*b*) LL (9 individuals). The expected peaks are shown as $\triangle$ = light-on plus 13·5 hr. and $\blacktriangle$ = light-off plus 22·5 hr. The interval between similar expected peaks is 22·5 hr. in DD and 26 hr. in LLs.

LD 4:20 to DD and LL

The mosquitos were reared and recorded in an LD 4:20 regime and then transferred to constant dark (Fig. 8*a*), or constant light (Fig. 8*b*). Figure 8*a* shows the persistence of 'light-on' and 'light-off' peaks in DD with six peaks occurring in the expected positions. The peaks are not clear in constant light (Fig. 8*b*) except that the first peak appears to have been phase-set by the last change from light to dark.

LD 20:4 to DD and LL

The mosquitos were reared and recorded in an LD 20:4 regime and were then transferred to constant dark (Fig. 9*a*) or constant light (Fig. 9*b*). In constant dark
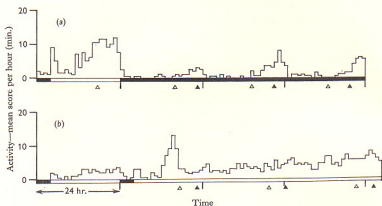
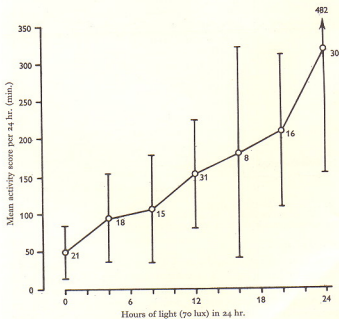Fig. 9. LD 20:4 to (a) DD, (b) LL. Expected peaks plotted as in Fig. 8.

Fig. 10. The effect of length of photoperiod on total activity (mean and standard deviation). The number of individuals is shown to the right of each mean point.

there is no evidence of a bimodal cycle; the peaks are in the 'light-off' position. The first 'free running' peak appears in the expected position (22·5 hr. after light-off) but the subsequent period seems to be 23·5–24 hr.; this corresponds with the period in constant dark following a change from constant light. The first peak in constant light (Fig. 9*b*) is 11–12 hr. after light-on but it is difficult to discriminate subsequent peaks in the record. Some of the individual records show double peaks, but the period varies from 25 to 27 hr. for the different individuals and this leads to the diffuse result when the mean is plotted.

6. *Effect of photoperiod on total activity*

Figure 10 shows the total activity per 24 hr. period (mean and standard deviation) of mosquitos exposed to constant dark, constant light and five intermediate photoperiods. The activity was measured between days 5 and 8 of each experiment; the mosquitos were then 10–14 days post-eclosion and were at their most active stage (Rowley & Graham, 1968). The number of individuals whose activity was measured is shown to the right of each mean point. There is a significant correlation between the total activity and the number of hours of light in the 24 hr. period ($P < 0.001$).

DISCUSSION

Flight is associated with the cyclical activities of oviposition, biting and sugar-feeding and, in our experiments, we appear to be dealing with a different 'indicator process' of the same basic rhythm. In an LD 12:12 regime (Fig. 1*a*) the peak of flight-activity coincides closely with the peak of oviposition observed by Gillett *et al.* (1959) and Haddow *et al.* (1961); the sugar-feeding cycle has a similar main peak (Gillett *et al.* 1962). Flight activity and sugar-feeding both have smaller peaks in the hour following light-on. The activity pattern in the field (Boorman, 1961; McClelland, 1960) is very similar to the pattern in the laboratory.

Haddow *et al.* (1961), Gillett (1962) and Gillett *et al.* (1962) concluded that cyclical activity was controlled by an endogenous rhythm, which was phase-set by a change from light to dark; this is confirmed by our results, but we have also found that light-on may have a phase-setting effect. The greater accuracy of our method has shown that the rhythm has a period between 22 and 24 hr. in constant dark; in this it is very similar to the flight-activity rhythm in *Anopheles gambiae* (Jones, Ford & Gillett, 1966; Jones *et al.* 1967). In constant light there appears to be a weak rhythm with a period of about 26 hr.; this has not been recorded previously. There is evidence of a cycle even when the mosquitos have been reared in constant light (Fig. 3*a*) and the individuals show some synchrony although there were no obvious external time-cues. Possibly in these conditions the insects become very susceptible to any small change in the environment. Gillett *et al.* (1959) concluded that oviposition was acyclic in constant light, but when their results are re-appraised they are consistent with a 26 hr. cycle. In our experiments, following a change from constant light to constant dark, the individual periods are more variable than in constant dark following an LD 12:12 regime. This may be because the individuals are poorly synchronized and the change comes at a different phase in the cycle of each. Lohmann (1967) has described such phase-dependent changes of circadian frequency in *Tenebrio*.

Aschoff (1960) formulated the 'circadian rule' that in light-active animals (i) spontaneous frequency, (ii) the ratio of activity-time to rest-time and (iii) total activity, all increase with increasing intensity of continuous illumination. In *A. aegypti* the spontaneous frequency is lower in constant light than in constant dark; this is the behaviour one would expect in a dark-active animal. Total activity increases with light intensity and thus obeys the 'rule', but only two light intensities have been used, 0 lux (DD) and 70 lux (LL); it also increases with an increase in photoperiod.

The phase-resetting experiments (Figs. 4, 5) show that the cycle is rapidly entrained to a new regime. This may be due to (i) the rapid resetting of the endogenous rhythm, (ii) the over-riding of a weak rhythm by a direct response to strong external signals, or (iii) a combination of the two. It is noticeable that when the dark period begins early, and increased when the light period is prolonged. Light-off appears to be the main phase-setting factor in these experiments.

Under extreme light regimes it becomes apparent that light-on can also have a phase-setting effect. If the photoperiod is long as in an LD 20:4 regime (Fig. 7) the main peak appears 13–14 hr. after light-on, and there may be a minor peak 22–23 hr. after the last light-off. If the regime is changed to constant dark, however, (Fig. 9 a), the cycle is unimodal and the peaks appear to have been phase-set by the last change from light to dark. Thus the light-on phased peaks are lost. When the photoperiod is short as in an LD 4:20 regime (Fig. 6) the activity is concentrated in the light period and appears to be phase-set by the light-off. There is some evidence of a minor peak during the dark period which is phase-set by the light-on (Fig. 6 b). In constant dark both light-on and light-off phased peaks persist to give a bimodal cycle (Fig. 8 a). When an LD 4:20 regime is changed to constant light (Fig. 8 b) the first major peak is in the light-off phased position and a light-on phased peak appears to follow later.

In an LD 12:12 regime the main peak comes just before light-off and this peak persists in constant dark. Experiments in which the light has been prolonged show that the peak would normally last several hours longer and thus include the light-on phased peak; the normal onset of darkness seems to inhibit this activity.

Further evidence for the phase-setting effect of light-on can be found in the work of Gillett *et al.* (1959). In regimes between LD 8:16 and LD 16:8 the peak occurred in the 4 hr. before light-off but in an LD 20:4 regime there is a peak of oviposition 12–16 hr. after light-on; in an LD 4:20 regime the peak occurs after light-off. Corbet, Haddow & Gillett (1960) found that *A. aegypti* adults can adopt non-24 hr. oviposition cycles in artificial days of 16–28 hr. Their conclusion was that there was no evidence that the adopted cycle was being influenced or reinforced by a persistent underlying 24 hr. rhythm and that the inherited endogenous component can be masked completely by non-24 hr. regimes. If the expected peaks, 13–14 hr. after light-on and 22–23 hr. after light-off are plotted on their graphs, however, they correspond very well with the observed peaks.

Flight activity in *A. aegypti* appears to be controlled by an endogenous rhythm which may be phase-set by both light-on and light-off. In addition, the total activity is correlated with the number of hours of light in the 24 hr. period (Fig. 10). The level of activity drops rapidly following the onset of darkness suggesting that light promotes activity or, less likely, that the absence of light inhibits it. Light has a direct

effect in determining the amount of activity and an indirect effect through setting the phase of the endogenous rhythm.

Two alternative hypotheses can be put forward: (i) that there are two independent oscillators, or 'clocks', one entrained by light-on and the other by light-off, or (ii) that light-on and light-off affect different outputs of the same oscillator or two hands of the same 'clock'. Hamner & Enright (1967), working on the house finch *Carpodacus mexicanus* have suggested that their results may be explained by the presence of two independent circadian rhythms, or possibly by two manifestations of a timing system which are apparently not phase-locked under laboratory conditions. They were dealing with two separate physiological processes; in the present case we are concerned with two factors in the control of a single process, flight activity.

It is interesting that the range of photoperiod (LD 12:12 to LD 16:8), in which the theoretical light-on and light-off phased peaks approximately coincide, fits in well with that found in the geographical range of *A. aegypti*, that is 0–40° latitude (Christophers, 1960). Christophers considers that the range of this otherwise ubiquitous species is limited by insufficient summer temperature. The mean July isotherm of 24·8° C., given by this author as the minimum temperature, corresponds with the latitude where there is a maximum of 16 hr. daylight. It is possible that the geographical distribution of this species may be limited to a range of summer day-lengths which permit a state of physiological balance between the factors controlling the timing of flight activity.

SUMMARY

1. The circadian flight-activity of individual, sugar-fed *Aedes aegypti* females has been studied, using the flight-sound as an indicator of activity.

2. The activity appears to be controlled by an endogenous rhythm with a period of 22–24 hr. in constant dark and about 26 hr. in constant light.

3. Both light-on and light-off have phase-setting effects. Under favourable conditions, peaks of activity occur 13–14 hr. after light-on and 22–23 hr. after light-off. Both these peaks present in constant dark following an LD 4:20 regime.

4. The total amount of flight-activity is correlated with the duration of light (70 lux) in the 24 hr. period.

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ADDENDUM

Since this paper was submitted for publication, White (1968) has suggested the involvement of two independent 'clocks' in the timing of egg-hatching of *Cardiaspina densitexta* (Homoptera, Psyllidae). Within the range of day-length experienced in nature by this species, the two 'clocks' remain in phase with each other to produce a strong morning peak of hatching, but, under artificial light regimes, it is possible to separate the effects of the two clocks.

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