

THE CIRCADIAN FLIGHT ACTIVITY OF THE MOSQUITO *ANOPHELES GAMBIAE*: PHASE SETTING BY THE LIGHT RÉGIME

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INTRODUCTION

The circadian flight activity of sugar-fed females of the Lagos strain of *Anopheles (Cellia) gambiae* Giles has been investigated using a modification of the acoustic technique developed by Jones (1964). The preliminary experiments carried out by Jones, Ford & Gillett (1966) with batches of five or ten females suffered from the possible disadvantage that hyperactivity by one insect individual might (i) mask the comparative inactivity of the others, or, (ii) actually stimulate activity in the others. Because of this, single insects have been used in the present experiments in which the effects of changes in the light régime on the pattern of flight activity have been investigated. Light appears to inhibit activity, and can reset the cycle. The direction of the resetting depends on the time, relative to the phase of the internal 'clock', at which the light acts.

In the description of light régimes and in the discussion of the various aspects of this work which relate to circadian clocks, use has been made of the terminology of Aschoff, Klotter & Wever (1965) and of Corbet (1966).

METHOD

Mosquitoes

Batches of eggs were obtained each week from the Ross Institute of Tropical Hygiene. The insects were reared in LD 12:12 (alternating 12 hr. light:12 hr. dark) at 25° C. The adult females were used 1-4 days after emergence; they were fed on a 15% solution of an equal mixture of glucose and sucrose. They were presumed to have mated as males were present when they emerged.

Mosquito chamber

Individual females were placed in a recording chamber made from the top half of a 200 ml. stoppered bottle. A piece of filter-paper with a thin polythene sheet outside formed the floor of this chamber. This separated the mosquito from the 2½ in., 35 ohm, loudspeaker which served as a microphone with its maximum response in the required frequency range (400-1500 cyc./sec.). A small glass tube of sugar solution, with a cotton-wool 'wick', provided a food supply and maintained a relative humidity

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of approximately 80% in the chamber. The R.H. was checked on several occasions using the cobalt thiocyanate paper technique of Solomon (1957). The polythene prevented the humidity from affecting the 'microphone'. The mosquitoes survived for at least a week in these chambers without apparent ill-effects.

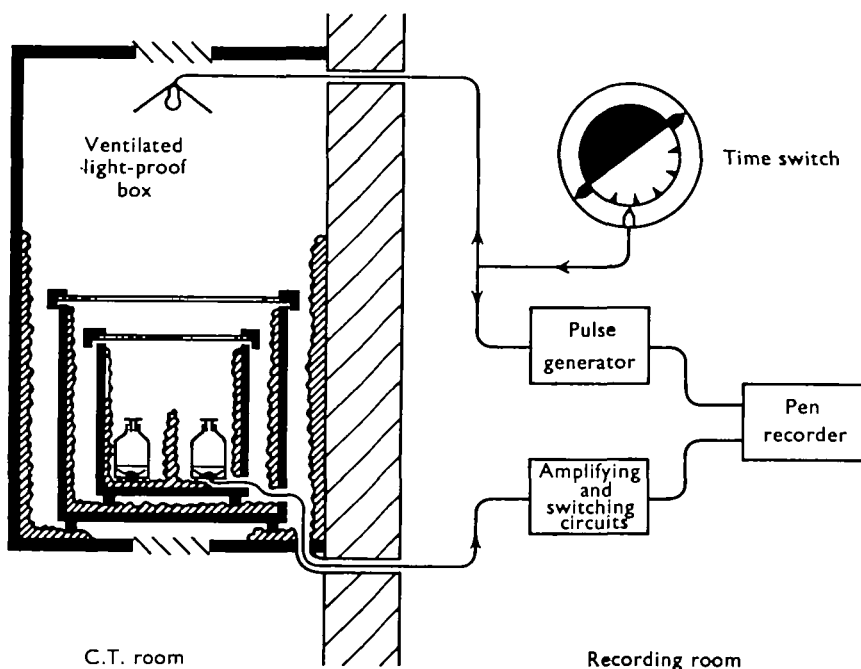


Fig. 1. Arrangement of the apparatus in the two rooms. Diagram not to scale.

Soundproofing, light and temperature

As many as four similar chambers were used in an experiment; one was usually left empty as a control against external noises which might activate the apparatus. The chambers were placed in 'nests' of cotton-wool in a wooden box ($\frac{3}{4}$ in. blockboard, 14 in. cube inside) with a Perspex lid. This box was placed inside a similar, but larger box ($\frac{3}{4}$ in. blockboard, 19 in. cube inside) lined with 'Bondacoust' sound absorbent (see Fig. 1). The outer box was placed inside a light-proof chamber lined with sound-absorbent material in a constant temperature room (25° C.). The mosquito chambers were illuminated with a 15 W. bulb (light intensity = approx. 100 Lux). The bulb was several feet from the Perspex lid of the outer wooden box and ventilators in the top and bottom of the light-proof chamber helped to dissipate the heat produced by the bulb. It was found that the temperature within the sound-proof box fluctuated by less than 1° C. but showed a slight maximum towards the end of the light period in LD 12:12.

The light regime was controlled from the next room by a Venner time-switch. All the recording apparatus was housed in this controlling and recording room. Figure 1 shows the arrangement of the apparatus in the two rooms.

Amplifying and recording apparatus

The output from each microphone was fed into a preamplifier. In approximately half the experiments Levell transistor preamplifiers (type TA 605) were used. It was found, however, that these could be replaced by simple home-made transistor preamplifiers, consisting of two standard earthed-emitter stages and a direct-coupled emitter follower, which gave a low impedance output and a $\times 500$ voltage gain. These latter preamplifiers were used in the rest of the experiments and they gave a very satisfactory performance.

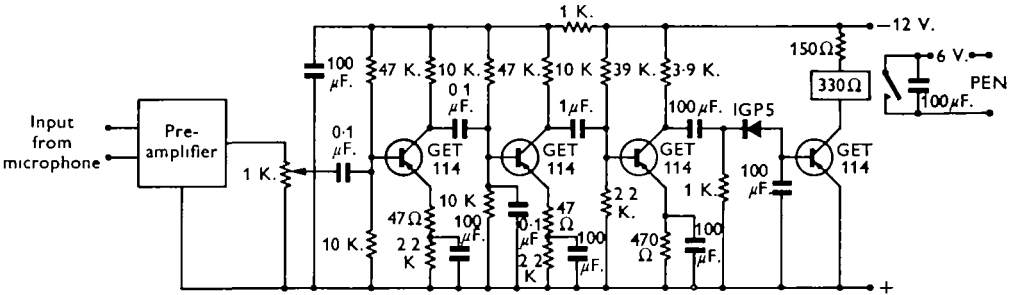


Fig. 2. Recording circuit for one mosquito.

The preamplifier output was fed into the circuit shown in Figure 2. This further amplified the signal, while discriminating against unwanted frequencies, and finally operated a relay which controlled an event-marker pen. In practice it was found that the circuit was most sensitive to frequencies in the range 400–1500 cyc./sec. This range included the first and second harmonics of the flight tone as well as the fundamental frequency (440 cyc./sec).

The event-markers wrote on a paper moving at approximately 3 in. per hour, and each pen recorded simply whether a given insect was flying at any particular time. The light régime was recorded by means of an event-marker pen operated by a pulse clock connected in parallel with the lighting circuit (see Figure 1). Another pulse clock and event-marker provided a time trace for the experiment.

Interpretation of the record and treatment of results

The record was divided into half-hour periods, and each period was further subdivided into minutes. An insect was given a score of 1 for any flight activity shown in any minute and in this way, each insect was given a score between 0 and 30 for each half-hour.

Each experiment was repeated on at least three different occasions until the activity of at least eight individuals had been recorded. The activity scores for comparable periods were then averaged and a histogram was plotted of the mean activity against time. No individual gave results which differed markedly from others which had received the same treatment.

RESULTS

(1) *Continuation of LD 12:12 régime*

Figure 3*a* shows the mean activity when the rearing régime was continued during the recording period. It can be seen that activity peaks occur after the light-off and light-on signals. The insects are moderately active during the dark period; some individual records show periodic short bursts of activity approximately every half-hour during the dark period, but these bursts are not in phase with those of other individuals. The insects are very inactive during the light period after the initial burst of activity at light-on.

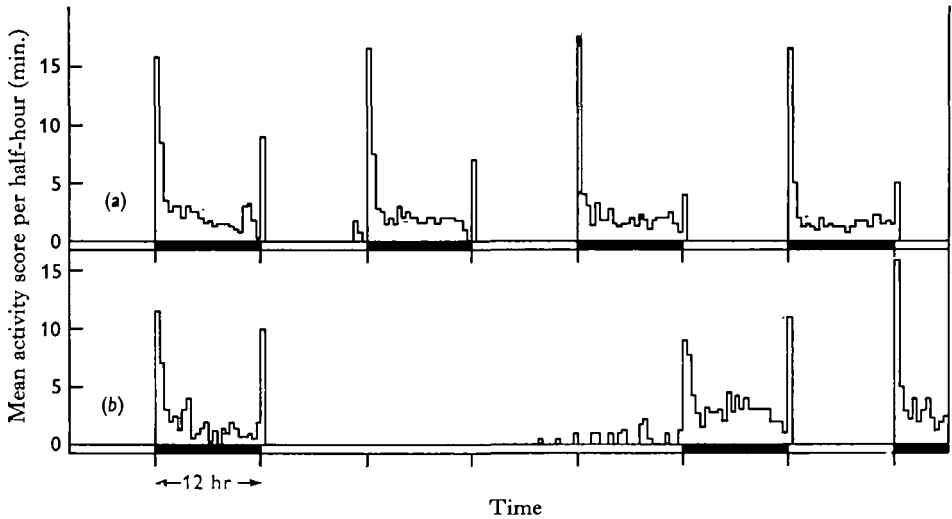


Fig. 3. (a) Mean activity in an LD 12:12 régime. (b) Effect of a 48 h. light period.

The latencies of both the light-off and the light-on 'reactions' have been measured from the records. Definite light-on activity was recorded on 57 occasions out of a total of 72; the activity started within 1 min. of light-on on 16 occasions, and within 3 min. of light-on on 35 occasions. Activity followed light-off on all occasions. The latency had a mode between 5 and 6 min.; on 52 occasions, out of a total of 82, the activity started between 2 and 7 min. after light-off.

(2) *Prolongation of the light period*

Figure 3*b* shows the mean activity when one of the light periods was prolonged to 48 hr. and the insects were then returned to LD 12:12. It can be seen that the light appears to inhibit activity, although some activity reappears towards the end of the long light period. The activity level appears to be a little higher than normal during the first dark period, although the light-off reaction is slightly less pronounced. After the 48 hr. light period the insects are active in a cycle which is 12 hr. out of phase with the old cycle.

(3) *LD 12:12 to DD (constant dark)*

Three experiments were carried out; the results are summarized in Fig. 4. In the first experiment (Fig. 4*b*) the regime was changed to DD at the normal light-off time. In the second (Fig. 4*c*) the change to DD took place 6 hr. before the normal light-off time. In the third (Fig. 4*d*) the change took place after the light period had been prolonged by 6 hr.

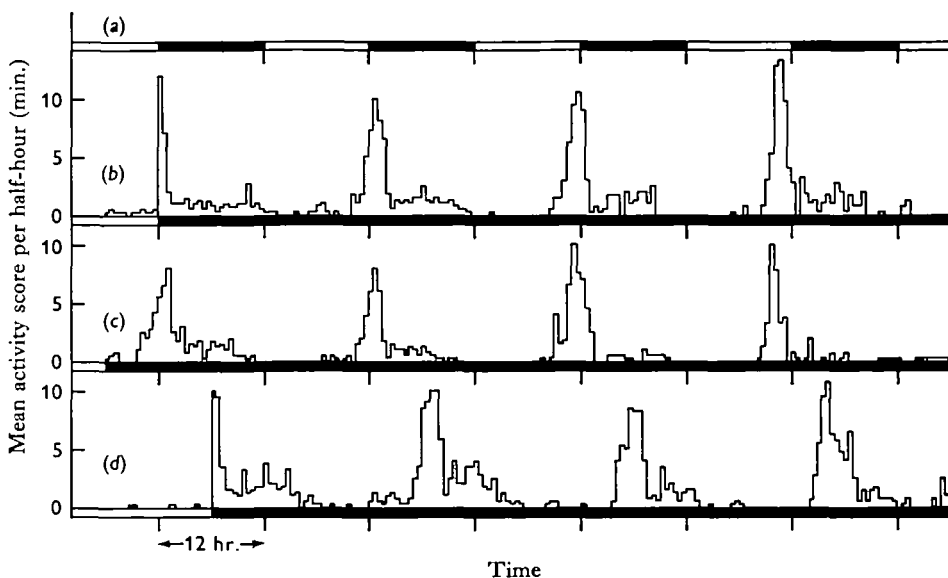


Fig. 4. (a) Rearing régime continued for comparison. (b) LD 12:12 to DD at the normal light-off time. (c) LD 12:12 to DD 6 hr before normal light-off. (d) LD 12:12 to DD after 6 hr. extra light.

It can be seen that cyclical activity continues in constant dark. With three insects in each experiment the activity was recorded for 130 hr. after the normal light-off time. Only a slight spreading of the activity peaks occurred owing to individual mosquitoes getting out of phase. In Fig. 4*b* and *d* the first peak of the free-running cycle appears 24–24½ hr. after the last light-off. The period in DD is about 23 hr. in all experiments.

In the first two experiments, the activity peaks appear in phase with each other; thus the cycle has not been reset by the early light-off. In the third experiment it can be seen that the cycle has been delayed and reset by the late light-off.

(4) *Advancing the cycle by 6 hr.*

Figure 5 shows the results of two experiments in which the LD cycle was advanced by 6 hr. In one (Fig. 5*b*) this was done by decreasing the length of one light period by 6 hr, in the other (Fig. 5*c*) the length of one dark period was decreased. It can be seen that: (i) complete resetting to the new régime takes several cycles; (ii) in both cases resetting is more rapid than would be expected if a free-running cycle of 23 hr. was catching up with the new régime; (iii) resetting is more rapid (by one cycle) when the régime is advanced by decreasing the length of the dark period. It is interest-

ing to note that in each case there is a small burst of activity $2\frac{1}{2}$ –3 hr. after the first early light-on; this appears in only about half the individual records. Also, activity tends to overflow into the light periods for a few cycles after the LD régime is advanced.

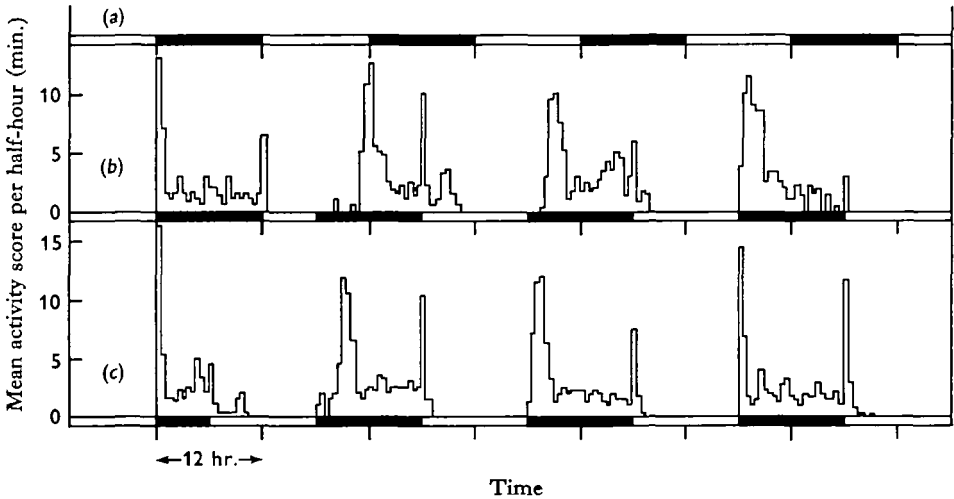


Fig. 5. (a) Rearing régime continued for comparison. (b, c) LD 12:12 régime advanced by 6 hr. by shortening one light or one dark period respectively.

DISCUSSION

Jones *et al.* (1966) found that there was no obvious cyclical pattern of activity in *A. gambiae* kept in constant light, but cyclical activity with a period of about 23 hr. followed a change from constant light to constant dark. They have suggested that the pattern of flight activity is controlled by an endogenous rhythm similar to that which appears to control oviposition and sugar-feeding in other mosquitoes (Haddow, Gillett & Corbet, 1961; Gillett, 1962; Gillett, Haddow & Corbet, 1962). In the present experiments a change from an LD 12:12 régime to DD gave a similar free-running cycle (Fig. 4).

Light appears to have an inhibitory effect on activity, and can reset the cycle by delaying it. A late light-off resets the free-running cycle in constant dark (Fig. 4*d*). An early light-off does not reset the cycle (Fig. 4*c*); the flight activity starts 2 – $2\frac{1}{2}$ hr. earlier than usual but does not build up to a peak until about 1 hr. after the normal time for light-off; subsequent peaks are in phase with the peaks following a change to constant dark at the normal light-off time (Fig. 4*b*). It appears that if it is not inhibited the underlying rhythm initiates activity about 22 hr. after the last light-off and that this activity builds up to a peak at about 25 hr. from the last light-off. Light-off at the normal time may slightly advance the activity peak; possibly this earlier peak is attributable to the release of activity which has been held in check by the inhibitory effect of the light.

The results of the experiments in which the light régime was advanced by 6 hr. (Fig. 5) indicate that light appearing earlier than normal will advance the activity cycle. In this case the light is effective when it appears during what would have been

the last 6 hr. of the dark period. Thus light has a delaying effect at the beginning of subjective night, but an advancing effect at the end. A similar phase-dependence of the effect of light has been found in a number of different organisms, and phase-response curves have been drawn; Aschoff (1965) and Pittendrigh (1965) have both discussed this subject in some detail. It appears that *A. gambiae* may have a similar phase-response curve to those found in the flying squirrel and hamster (De Coursey, 1960, 1961, 1964).

In an LD 12:12 régime peaks of flight activity follow both light-off and light-on. The 'light-off' peak persists in constant dark, but the 'light-on' peak is lost. Jones *et al.* (1966) suggested, because of the latency of the 'reaction' and the similarity with the work of Harker (1964), that a hormonal mechanism is involved in the control of light-off activity and the free-running cycle of activity in the dark; the idea that hormones are involved in mosquito rhythms was first suggested by Haddow & Gillett (1957). The speed of the light-on response made it seem probable that it was a 'startle' response mediated directly by the nervous system. In the present experiments, although the mode was less than 1 min., in many cases the latency of the light-on 'response' was several minutes. Possibly in the earlier experiments with groups of insects these longer latencies were obscured by the faster reactions of other insects. It seems likely that the activity following light-on is also controlled by a hormone.

Rao & Gropalakrishnareddy (1967) have produced evidence for excitatory and inhibitory hormones in the scorpion which affect the activity of the isolated nerve cords. These hormones appear to be secreted at different times of day and their net effect is consistent with the circadian pattern of locomotory activity observed in the intact animal. Strejckova, Servit & Novak (1965) have demonstrated the effect, on the central nervous system of the cockroach, of two neurohormones, C_1 and D_1 , isolated from the brain and corpus cardiacum. D_1 induces a long-term increase in 'spontaneous' electrical activity; C_1 inhibits the existing activity after first inducing a transient rise. A particularly interesting effect was obtained when neurohormone C_1 was applied to prothoracic ganglia which had had their electrical activity raised by previous application of D_1 . The activity was first raised and then decreased or disappeared completely. Possibly the normal dark activity of *A. gambiae* is controlled by an endogenous rhythm of secretion of a hormone similar to D_1 . Light may cause the release of a hormone similar to C_1 . A C_1 type hormone acting at the end of a normal period of secretion of a D_1 type hormone might be responsible for the light-on activity and the subsequent quiescence. The rhythm does not seem to be affected by light during the inactive phase, but continued secretion of an inhibitory hormone under the influence of light could delay the onset of secretion of the excitatory hormone, and reset the phase of the underlying rhythm. Once the activity peak had been passed, inhibition due to the early onset of light might be expected to accelerate the rhythm towards the inactive phase. If the excitatory hormone is fairly persistent, the small bursts of activity seen $2\frac{1}{2}$ -3 hr. after the first early light-on (Fig. 5) may be the result of the complex interaction of this hormone with the inhibitory effect of the light.

The flight activity recorded in these experiments would probably be associated normally with host-seeking behaviour or, once the mosquitoes had fed on blood, with oviposition. Haddow & Ssenkubuge (1962) have recorded that biting activity and oviposition in *A. gambiae* are both essentially nocturnal. There is a sharp oviposition

peak just after sunset, while biting activity is maximal after midnight and in the hours before dawn. They have pointed out that as *A. gambiae* may go through a number of cycles of biting and oviposition in its lifetime, few of the females biting in the hours before dawn are doing so for the first time; indeed many of them have probably oviposited on the same night. It is interesting that in *Mansonia fuscopenmata*, another nocturnal species, the oviposition-cycle shows a small peak of activity early in the night and a major peak before sunrise, while the biting-cycle shows a main peak just after sunset and a lesser peak just before sunrise (Haddow & Gillett, 1958). It seems that the 'dark' activity recorded in our experiments may provide a framework for other activities which involve flight. It is possible that the artificial light régime with its abrupt changes from light to dark and dark to light may have affected the pattern of activity, but Haddow & Gillett (1957) have shown that the oviposition-cycle in *Aedes aegypti* remains unaltered when normal sunrise and sunset are replaced by such sharp changes.

The original technique (Jones, 1964) has been criticized by Powell, Esch & Craig (1966) on the grounds that the elaborate apparatus required makes expansion for simultaneous testing of many mosquitoes most difficult. The apparatus has been improved and simplified during the past 3 years and the circuits now in use are both cheap to construct and are so selective in their response that the problem of external noise is much simplified. The apparatus is currently being expanded to record the activity of up to 20 individuals at the same time. The technique appears to be applicable to other species; it is also being used in our laboratory to record flight activity in *A. aegypti* females.

Powell *et al.* (1966) have also pointed out that flight constitutes only a part of the total activity pattern; their apparatus records walking but only the initiation and termination of flight. In their apparatus flight activity was minimal. Flight is an essential component of such activities as biting, mating and oviposition and would seem to be a more useful indicator than walking.

SUMMARY

1. The circadian cycle of flight activity of individual, sugar-fed, *Anopheles gambiae* females has been studied, using the flight-sound as an indicator of activity.
2. In an LD 12:12 régime (alternating 12 hr. light:12 hr. dark), activity peaks follow both light-off and light-on. The mosquitoes are moderately active in the dark, but are inactive in the light after the first half-hour.
3. If the light period is extended, the activity is delayed until light-off. Light appears to have an inhibitory effect, but the insects may show some activity towards the end of a 48 hr. light period.
4. Cyclical activity continues in constant dark with a period of approximately 23 hr. A late light-off resets the cycle; an early light-off does not.
5. When the LD 12:12 régime is advanced by 6 hr. the cycle is re-entrained within 2-3 days. Light in the second half of the subjective night appears to advance the cycle.
6. It is suggested that the activity is controlled by an endogenous rhythm which possibly controls the release of an excitatory hormone. The effect of light may be mediated through an inhibitory hormone.

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