THE EFFECT OF LIGHT REGIMES ON THE CIRCADIAN RHYTHM OF FLIGHT ACTIVITY IN THE MOSQUITO *AEDES TAENIORHYNCHUS**

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INTRODUCTION

The bimodal patterns of flight activity in populations of *Aedes taeniorhynchus* (Wiedemann) (Nayar & Sauerman, 1969) resemble the patterns of circadian flight activity demonstrated in *Anopheles gambiae* (Jones, Hill & Hope, 1967) and the diurnal activity in *Culex pipiens pallens* (Chiba, 1964). Since Aschoff (1966) had clearly demonstrated that the two-peak pattern of locomotor activity present in finches and some mammals is a persistent property of the circadian oscillating system, it was thought pertinent to make a further study of bimodal patterns of flight activity in populations and individuals of *A. taeniorhynchus* as revealed with the apparatus designed and modified in this laboratory (Nayar & Sauerman, 1969). It was also necessary to establish whether the diurnal rhythms demonstrated during developmental stages (Nayar, 1967) are continued during the adult stage, where through some modification they might be manifested as endogenous bimodal rhythms of flight activity.

MATERIAL AND METHODS

Larvae of *A. taeniorhynchus* were reared from eggs collected from wild-caught adults in the vicinity of Vero Beach as described by Nayar (1967). Groups of larvae were reared at 27 °C under LD 12:12 as follows: 200 larvae/standard pan (24 × 34 × 6 cm enamel pan) containing 350 ml of 1/10 dilution of sea water, and fed as follows: at 0 hr 320 mg of dry brewers yeast, at 24 hr 40 mg of liver powder, at 48 hr 160 mg of dry brewers yeast, and 160 mg of yeast every subsequent 24 hr until 50 % of the larvae had pupated. Only virgin females were used for these studies. Twenty females, either pupated within 1 or 2–3 hr old as adults, were placed in the sound-proof boxes as previously described (Nayar & Sauerman, 1969).

The sound-proof boxes and interpretation of the records as described earlier (Nayer & Sauerman, 1969) were found to be adequate for studying the flight behaviour of 20 females as a group, without complications from hyperactivity of one insect over the other, because of the large size of *the inner chamber*. The mosquitoes survived up to 4 weeks in the inner chamber without any apparent ill effects.

For individual adults smaller boxes were made to the same pattern as the boxes described earlier (Nayar & Sauerman, 1969). *The inner chamber* was cylindrical in shape (25 cm long and 25 cm in diameter) and the square outer chamber was made to enclose it. This inner chamber was illuminated with one 7-W bulb. This bulb raised the inside temperature by about 5° C, so in order to maintain the desired temperature in these small boxes an air-flow system was provided. Two small holes (2 cm diameter) were made in the walls of the outer chamber, an inlet on the side near the bottom and an outlet near the centre on the top. A sharply angled blackened acrylic pipe with cotton plug filter was attached to the inlet hole and another pipe was inserted in the top end with a plastic hose attached. The plastic hose was then attached to a small suction Rotron motor (Model NTP 4, 115 V, 60 c.p.s., 7 W, manufactured by Rotron Mfr. Co., Woodstock, N.Y., U.S.A.) to withdraw warm air from the outer chamber. The motors performed quietly and did not interfere with the recording of the flight sound. A sugar vial was suspended from the top of the inner chamber for *ad libitum* feeding on 10 % sucrose solution. All experiments were conducted for 12 days after the newly emerged adults had been introduced into the boxes.

The sound of mosquito flight was amplified and recorded using two sets of recorders, and this helped to define any interference in the records produced by surges in current or by other electrical disturbances: (*a*) with the Rustrak recorder system as previously described (Nayar & Sauerman, 1969), and (*b*) with an electrical multiple-point Chopper-bar recorder (Manufactured by Dr B. Lange GMBH, Berlin, West Germany), for which a small sensitive preamplifier and amplifier system was constructed to activate the recorder. These systems worked simultaneously and thus duplicated the data while also providing cross-references to eliminate any electrical disturbances not detectable by one system alone.

RESULTS

In the initial experiments, when newly emerged females reared under the conditions described above were placed in boxes during the dark or light periods of the LD 12:12 regime and then maintained at the next change of light to dark or vice versa under DD or LL regimes respectively, no flight rhythmicity was observed during the next 10 days, i.e. no original endogenous rhythms of flight activity were discernible after the first change of light. There were sporadic individual flights, none of them lasting over 2 min. A light or dark stimulus of 12 h during the first 24 h after emergence did not initiate a sustained diurnal flight activity. When a similar group of females was continued under the rearing regime of LD 12:12, however, a first peak of flight activity occurred at the third change from light to dark or vice versa i.e. about 24–36 h after emergence. This suggested that even though a slight response to light and dark could be detected within the first 24 h, sustained entrainment by light or dark stimuli could not take place within that time. This also indicated that diurnal rhythm of pupation and emergence which were exhibited as population phenomena did not continue as a flight activity rhythm which would in fact have to be exhibited by individuals. Independent flying rhythms establish themselves about 36 h after emergence if the desired light cycles are continued during this time. It is quite likely that the compound eyes, which are probably responsible for the distinct response to light and dark changes, have not matured enough to respond to such changes before 1 or 2 days after emergence,

Thus, in all the following experiments, either the new light regimes were imposed immediately after emergence or a change in the light regime was imposed when adults were at least 4 days old, and so for all the data reported herein recordings are given from the beginning of the 5th day to the end of the 10th day after emergence. The patterns of flight activity of groups of 20 female mosquitoes did not differ substantially from those of individual females, so figures were drawn from group data only. Data from individuals were nevertheless given equal consideration, but mention of them will be made only when necessary.

(A) *The rhythm in constant conditions*

(a) *Continuation of the LD* 12:12 *rearing regime (Fig. 1a)*

The pattern of mean flight activity as shown in Fig. 1a is from females when the rearing regime of LD 12:12 was continued beyond the 5th day of emergence to the end of the recording period. Flights showed two peaks of activity which can be designated as an 'alternans' activity pattern (Aschoff, 1957), in which the first peak at the
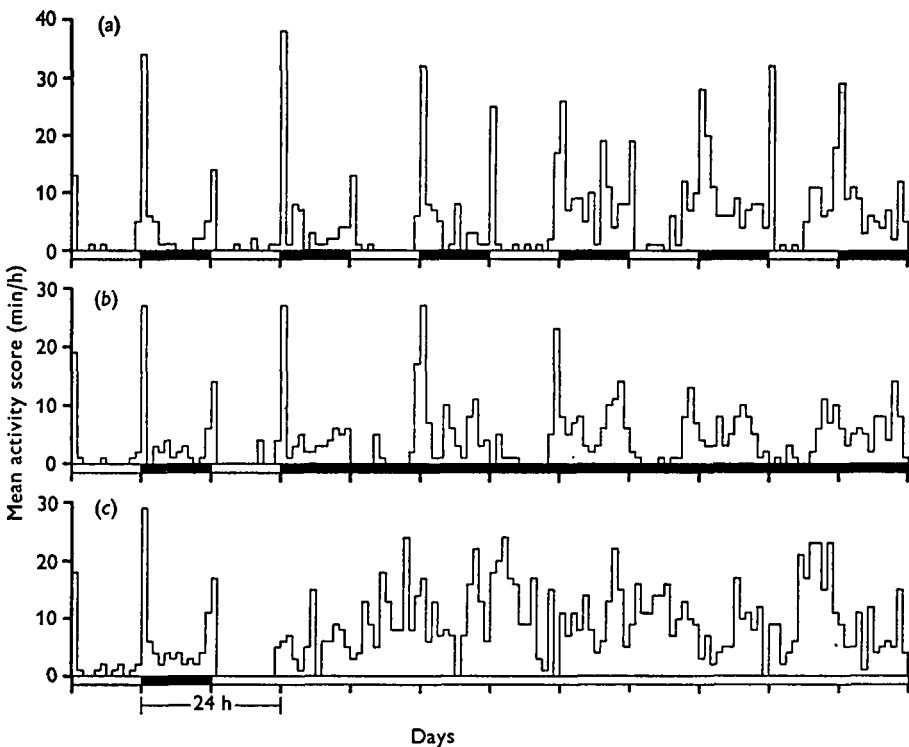

Fig. 1. Mean flight activity in (a) LD 12:12, (b) continuous DD following LD 12:12, and (c) continuous LL following LD 12:12

change from dark to light is lower than the second peak at the change from light to dark. Each of the second peaks lasted about 1 h, with maximum activity lasting 35 min, whereas the first peaks lasted about 30 min with maximum activity for about 20 min only. The latencies of the light-on and the light-off reactions were a maximum of 2 min. Even though there were flight activity periods less than 15 min in an hour quite

often during the dark period of the cycle, the peak of the activity occurred distinctly at the change of light to dark or vice versa with a period of 12 h showing a distinct entrainment to the LD 12:12 cycle with two-peak activity. There was very little activity during the light period the first 8 days after emergence. During the 9th and 10th days and thereafter, there was a marked increase in flight activity in the latter half of the light period. This increase in flight activity during the second week after emergence is probably related to physiological ageing of the mosquitoes, as described in *Aedes aegypti* by Rowley & Graham (1968).

(b) *The LD* 12:12 *regime to continuous DD, light-off at normal time (Fig. 1 b)*

The data in Fig. 1 *a* indicate that the peaks of activity are a direct response to the light-on or light-off stimulus, but this does not appear to be so much the case in Fig. 1 *b*. In this case mosquitoes were entrained under LD 12:12 regime for 5 days after emergence and then subjected to continuous DD. The results show that an 'alternans' pattern of flight activity persists during continuous DD for at least 5 days and perhaps longer, and is thus endogenously determined, i.e. it is not dependent on any concurrent changes in the environmental conditions. Slight spreading of the activity peaks occurred, probably due to individual mosquitoes getting out of phase. The period between the second peaks during every 24 h became 23·5 h under continuous DD. There are some indications of disappearance of the first peak during the first day after mosquitoes were transferred to continuous DD but it soon reappeared.

(c) *The LD* 12:12 *regime to continuous LL, light-on at normal time (Fig 1 c)*

When mosquitoes entrained to LD 12:12 regime were subjected to continuous LL, they seemed to become hyperactive and did not show the two-peaked flight activity rhythm of an 'alternans' pattern but instead displayed short irregular periods of increased activity after the first 12 h period of light, which corresponds to the 'old' dark time.

(B) *Phase-resetting experiments*

(a) *Reversing the LD* 12:12 *regime (Figs. 2 a, b)*

In the first experiment the mosquitoes entrained to LD 12:12 were given an extra stimulus of 12 h of light on the 6th day after emergence followed by DL 12:12 regime (Fig. 2 a), whereas in the second experiment an extra stimulus of 12 h of dark was given on 7th day after emergence and then followed by LD 12:12 (Fig. 2 b). Flight activity became adjusted to the new light regime rather quickly. In the first case (Fig. 2 a) an 'alternans' flight pattern continued except for the last day of recording, where it seemed to indicate reversal; whereas in the second case, for the first day into the new LD 12:12 regime, the 'bigeminus' flight pattern (Aschoff, 1966) was evident, i.e. where the second peak was lower than the following first peak, but soon reversed to an 'alternans' flight activity pattern. This short appearance of the 'bigeminus' flight pattern might indicate the presence of phase-shift which is one of the characteristics of endogenous circadian rhythms (Bruce, 1960). In general, there were no distinct transients, suggesting that mosquitoes respond and adjust to new light cycles almost immediately.

(b) Continuous DD to 12 L to continuous DD (Fig. 2c)

As during the first 36 h after emergence a 12 h light or dark stimulus did not produce rhythmic flight activity, it was concluded that the eyes of the insect as such had not matured enough to respond to the stimulus. So a 12 h light stimulus was given on 6th day after emergence to adults maintained under continuous DD (Fig. 2c). This almost immediately established an 'alternans' flight activity pattern with a 23·5 h periodicity in the second peaks. Stimuli of shorter durations than 6 h did not lead to rhythmic flight activity even at this time. This again demonstrated that flight activity was endogenously determined.
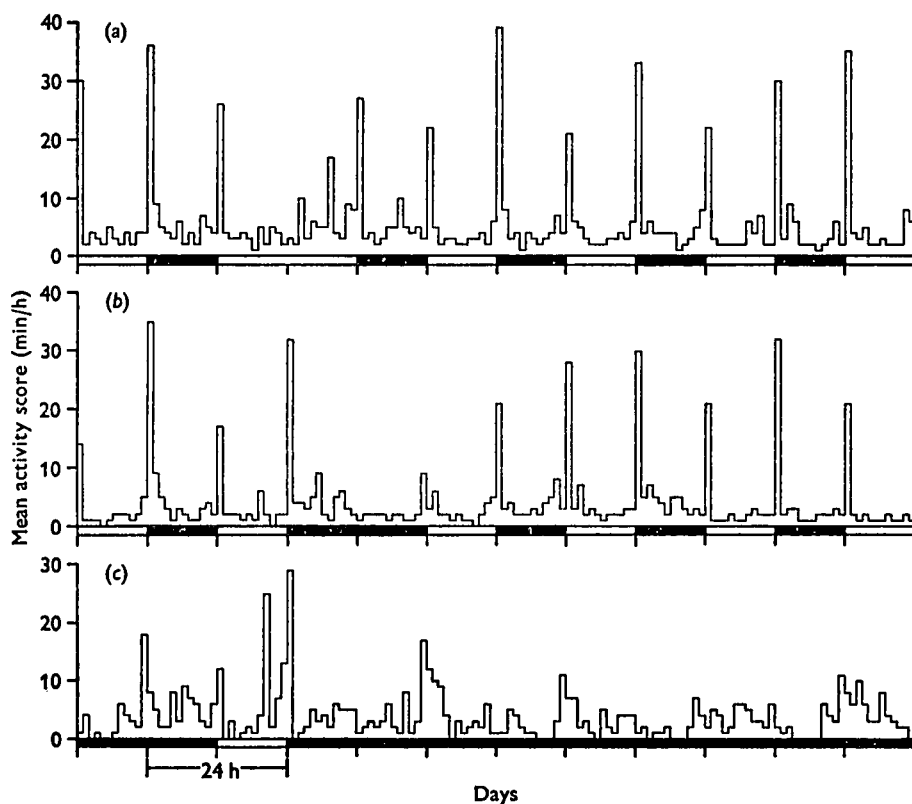

Fig. 2. (*a*) and (*b*). Effect of reversing an LD 12:12 regime by giving a 24 h period of either LL or DD, (*c*) effect of a 12 h light stimulus in DD.

(c) Advancing the LD 12:12 regime by 6 h (Fig. 3a, b)

These experiments were conducted to establish that there is in fact a short period during which phase-shift takes place before the flight activity rhythm adjusts to the new light cycle. In the first experiment the mosquitoes entrained to LD 12:12 were given 6 hr of light instead of the regular 12 h on the 6th day after emergence, followed by a new DL 12:12 regime (Fig. 3a); in the second experiment 6 h of dark was given instead of the regular 12 h on the 6th day and followed by a new LD 12:12 regime (Fig. 3b). In the first experiment (Fig. 3a) as soon as light was turned off after 6 h,

there was a distinct peak of flight activity followed by another much larger peak after another 6 h and then a very small peak at light-on. Subsequent to that an 'alternans' flight pattern marked the new LD regime. This demonstrated that phase-shift was restricted exclusively to the first day after a light change. In the second experiment (Fig. 3b), light-on after 6 h of dark produced a distinct peak of flight activity which was followed by an 'alternans' flight activity pattern after 12 h. There were indications of slightly more flight activity at the first light-off signal and during the first dark period in the new LD 12:12 regime. This was probably due to some phase-shift adjustments.
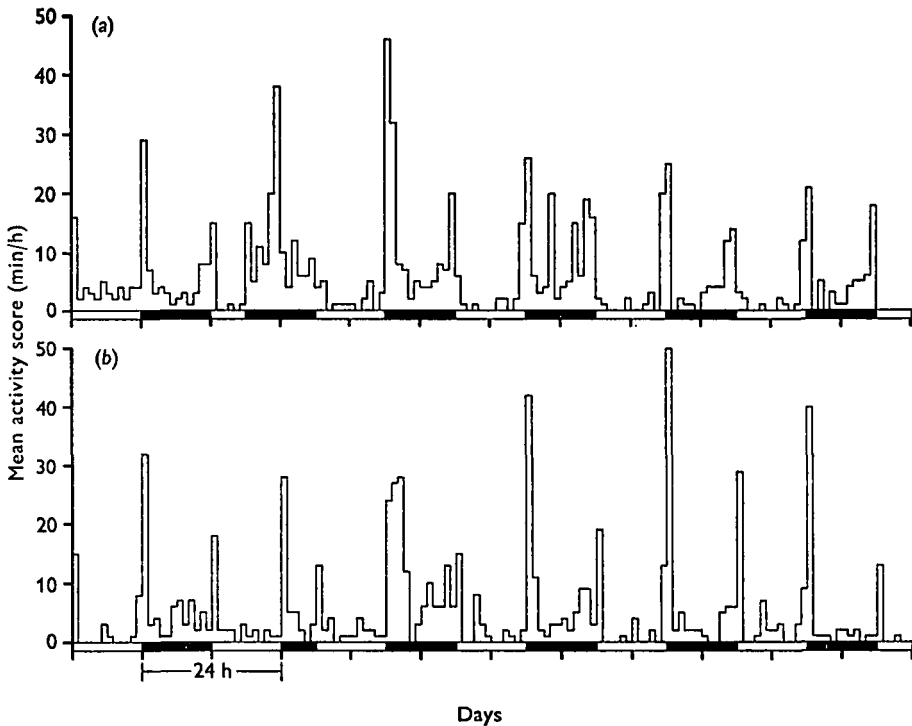

Fig. 3. Effect of advancing an LD 12:12 regime by 6 h by shortening (a) one light period, (b) one dark period, followed by a new LD 12:12 regime.

(d) *Shortening or lengthening of the light period of the LD* 12:12 *regime by* 6 *h followed by continuous DD* (Fig. 4a, b)

In the first of the two experiments mosquitoes entrained to LD 12:12 for 5 days after emergence were given only 6 h of light on the 6th day instead of the regular 12 h and then subjected to continuous DD (Fig. 4a); in the second experiment a similar group was given 18 h of light on the 6th day instead of the regular 12 h and then subjected to continuous DD (Fig. 4b). Rhythmic activity continued in continuous DD, but in the first experiment (Fig. 4a) at the earlier light-off a small insignificant peak of flight activity was observed followed by a larger peak of flight activity 6 h later. This was followed by an 'alternans' flight pattern with about a 23 h periodicity, thus showing very slight or no phase-shift as compared to the group entrained at LD 12:12

which was subjected to continuous DD at regular time on 6th day (cf. Fig. 1*b*). However, in the second experiment a distinct flight peak was observed at the expected light-off and then followed 6 h later by another large peak of flight activity at the delayed light-off. This was then followed by an 'alternans' pattern with a periodicity of 23·5 h under DD, and with a phase-shift of 6 h as compared to an 'alternans' pattern described in Figs. 4*a* and 1*b*. This experiment also suggested that a new rhythm of flight activity could be established with a delayed light-off.
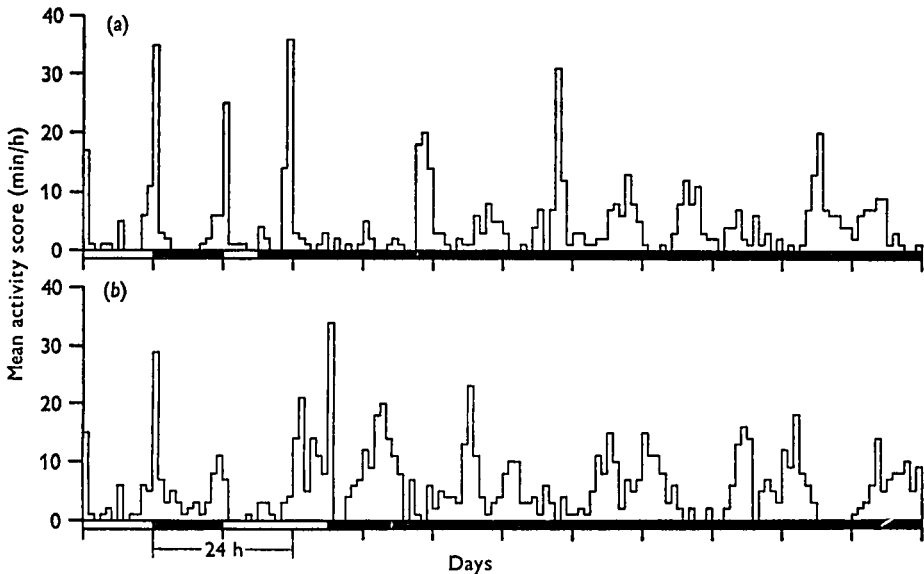

Fig. 4. Effect of (*a*) shortening or (*b*) lengthening the light period of the LD 12:12 regime by 6 h followed by continuous DD.

(C) *Entrainment by 24 h light cycles other than LD* 12:12

(a) *The LD* 16:8 *and LD* 8:16 *regimes followed by continuous DD* (*Figs* 5*a, b*)

In these two experiments, where adults were subjected to LD 16:8 and LD 8:16 for 7 days after emergence, entrainment was complete and the peaks of flight activity occurred almost always at the change from light to dark and dark to light, exhibiting an 'alternans' flight activity pattern, even though under LG 8:16 (Fig. 5*b*) there was a sharp peak of activity in the middle of the dark period. When both experiments were then subjected to continuous DD at the normal times, the rhythmic pattern with bimodal peaks continued, and it took at least 48 h before the period length between the second and the first peaks of the 'alternans' pattern became 12 h, thus exhibiting transients even between the second and the first peaks of the flight activity rhythm.

(b) *The LD* 23:1 *and LD* 1:23 *regimes* (*Figs.* 6*a, b*)

It was stated earlier that a stimulus of less than 12 hr was not enough to entrain the flight activity rhythm, so these experiments were conducted to see if a shorter stimulus repeated every 24 h would entrain the flight activity rhythm. Two groups were entrained from emergence under LD 23:1 and LD 1:23 regimes (Figs. 6*a, b*). The

results show that entrainment under both conditions did in fact take place, and the patterns of flight activity produced were mirror images of each other. The main peak of activity occurred at the light-off under LD 23:1 with considerable activity before the light-off and very little after the light-on, for several hours. Under LD 1:23 the main peak of activity occurred at the light-on, followed by considerable flight activity.
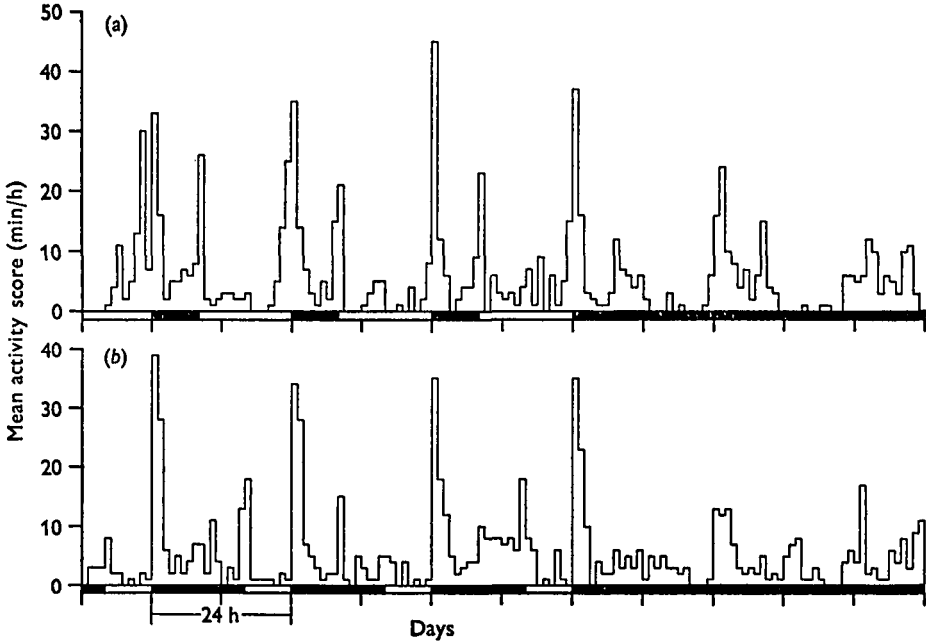
Fig. 5. Mean flight activity in (a) LD 16:8, and (b) LD 8:16 both followed by DD.
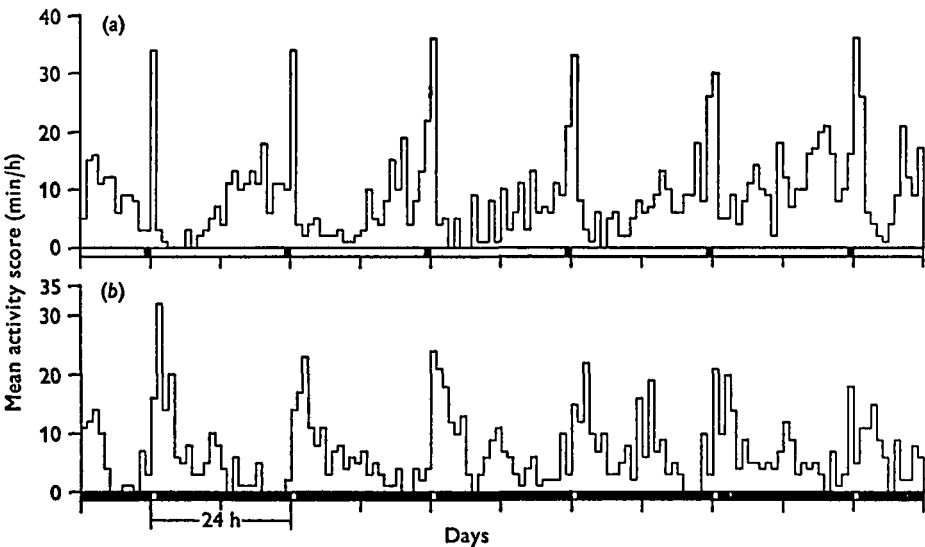
Fig. 6. Mean flight activity in (a) LD 23:1, (b) LD 1:23.

Thus it is clear that even though one short stimulus was not enough to entrain the rhythm, yet when repeated every 24 h it would entrain the flight rhythm perfectly.

(D) *Entrainment by frequency demultiplication*

One of the other properties of the circadian rhythm is that in most of the cases, especially in higher animals, the rhythm can be entrained to a 24 h period by some LD cycles whose periods are whole submultiples of 24 h. Two experiments were therefore conducted, where newly emerged females were subjected to LD 6:6 (Fig. 7*a*) and LD 3:3 (Fig. 7*b*) for 6 days, then on the 7th day were subjected to continuous DD. The results show that under LD 6:6 (Fig. 7*a*), the flight activity rhythm was entrained to 24 h with bimodal peaks, showing an 'alternans' pattern, and when the mosquitoes
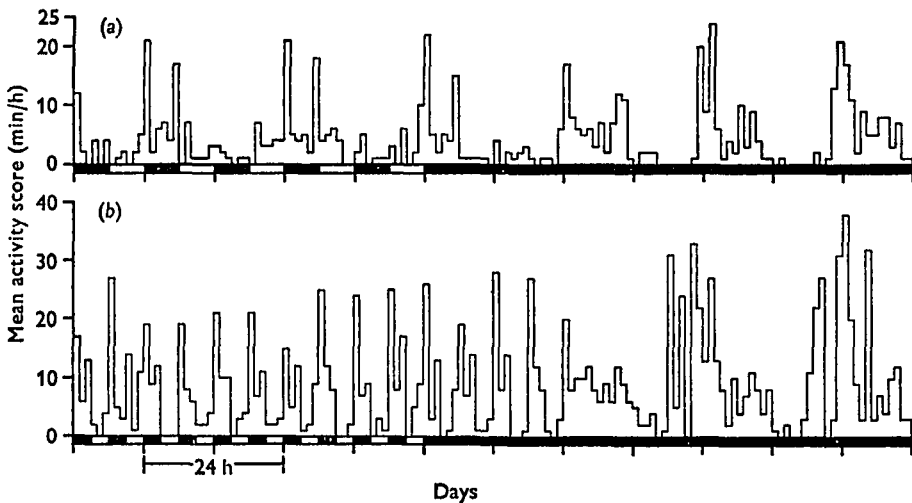

Fig. 7. Mean flight activity in (*a*) LD 6:6, and (*b*) LD 3:3, both followed by DD.

were subjected to DD, it continued with 23·5 h periodicity. The periodicity between the two peaks was 6 h as the response to light and dark changes occurred every other LD 6:6 cycle, but under DD the periodicity reverted to 12 h within 24 h.

On the other hand under LD 3:3, flight activity occurred with almost every change of light (Fig. 7*b*) producing a 6 h flight activity rhythm with an 'alternans' flight pattern. When these females were subjected to DD on the 7th day the 6 h rhythm, continued for at least 24 h before any indications of reversion to 24 h cycle were evident.

DISCUSSION

These studies clearly demonstrate that the basic bimodal (double-peaked), 'alternans' pattern of flight activity in wild *A. taeniorhynchus* is a persistent property of the circadian oscillating system. Under continuous darkness the basic bimodal pattern of flight activity persists with a 23·5 h periodicity, which characterizes it as a circadian rhythm of flight activity. In continuous light the basic pattern is masked over by excessive flight activity, which occurs at short irregular intervals. It may be pointed out that when colonized *A. taeniorhynchus*, which are highly autogenous, were used

there were no clear-cut indications of a persistent bimodal rhythmicity under continuous DD. The persistent bimodal 'alternans' pattern of flight activity in *A.* *taeniorhynchus* resembles the basic bimodal pattern of flight activity demonstrated in *Anopheles gambiae* (Jones *et al.* 1967) and in *C. pipiens pallens* (Chiba, 1964). The only clear-cut difference between *A. taeniorhynchus* and the other two species is that in *A.* *taeniorhynchus* continuous light has an excitatory effect on flight activity in contrast to the inhibitory effect on flight activity in the other two species.

The bimodal pattern of flight activity in *A. taeniorhynchus* originates for the first time in the adult stage, and it is not carried over from larval or pupal stages. It takes at least 36 h before a response to a light–dark change different from the larval-rearing light–dark regime appears. This differs from the case of *Culex pipiens pallens*, where a single transition from light to dark is effective in initiating the diurnal rhythm when it is given after 48 h from pupation during the development of the mosquito, i.e. about 12 h after emergence (Chiba, 1966 c).

Under the LD 12:12 regime the peaks of flight activity appear at the light-off and light-on signals with a latency of a maximum of 3 min.

A 6 or 12 h phase jump of the 'Zeitgeber' caused by either shortening of the light or dark period of the LD 12:12 regime (Fig. 3 *a*, *b*) or prolongation of light or dark periods (Fig. 2 *a*, *b*) resynchronizes the flight activity rhythm to the new cycle rather quickly, i.e. the transient phases last only 24–36 h. Shortening the light period by 6 h produces only a short peak of activity at light-off, but a very strong flight activity peak lasting more than half-an-hour 6 h later (Figs. 3 *a*, 4 *a*). The rhythm under the new LD 12:12 regime entrains within 24 h, but under continuous DD, it persists with a periodicity of 23·5 h, and the main peak is in phase with that originally entrained under LD 12:12 rather than in phase with the light-off. When the 12 h light period of the LD 12:12 regime is lengthened by 6–18 h and then subjected to continuous DD, the persisting rhythm is then set by the new delayed light-off, with a phase-shift of 6 h from the originally entrained phase under LD 12:12. Thus light has a delaying effect at the beginning of the subjective night, but an advancing effect at the end. This type of phase-dependence of the effect of light has been observed in *Anopheles gambiae* (Jones *et al.* 1967), in *A. aegypti* (Taylor & Jones, 1969) and in a number of different organisms (Bünning, 1967).

The persistent bimodal flight activity rhythm of *A. taeniorhynchus* can be easily entrained by LD 16:8, 8:16, 23:1 and 1:23 regimes. One h of light or dark repeated every 24 h can entrain the rhythm but a single stimulus of 1 h of light or dark given even 6 days after emergence did not entrain the rhythm. Only a stimulus of 12 h of light given 5 days after emergence to adults maintained under continuous DD could effectively initiate a persistent circadian rhythm with a 23·5 h rhythmicity, resembling the activity behaviour observed in *C. pipiens pallens* (Chiba, 1966 b). This limits the entrainment of the flight activity rhythm by a single stimulus of light or dark, and makes it difficult to work out the phase-response curves demonstrated in other animals (Bunning, 1967). This also demonstrates that the flight activity pattern in *A. taenio-* *rhynchus* is relatively refractory to phase-shift by single discrete light signals less than 12 h and is similar to the locomotory activity demonstrated in cockroaches (Roberts, 1962).

The persistent bimodal flight activity rhythm of *A. taeniorhynchus* can be entrained

through frequency demultiplication (LD 6:6) to precise 24 h periods with an 'alter-
■ns' pattern, and under DD continues with 23·5 h periodicity. Entrainment of the
rhythm to 24 h through frequency demultiplication has also been demonstrated in
plants, micro-organisms and higher animals (Bruce, 1960; Bunning, 1967), in cock-
roaches (Roberts, 1962) and in *C. pipiens pallens* (Chiba, 1966a), and is one of the
properties of circadian rhythms. However, under LD 3:3 the flight activity rhythm
entrains to a 6 h period with an 'alternans' pattern with the result that flight activity
occurs at every change of light, i.e. either from light to dark or dark to light, and this
6 h rhythm continues under DD for about 24 h before a persistent flight activity
rhythm reappears (Fig. 7b). Similar results were obtained with the sporulation
rhythm in *Pilobolus* (Übelmesser, 1954, cf. in Bruce, 1960). Bruce (1960) considers
these results as exceptional. Therefore, in *A. taeniorhynchus*, entrainment through
frequency demultiplication can probably take place within certain limits only.

It is concluded from this study that the basic two-peak 'alternans' pattern of flight
activity provides a framework for other activities which involve flight, like mating,
host-seeking, sugar feeding and oviposition, and which seems to be true in the case of
Anopheles gambiae (Haddow & Ssenkubuge, 1962). *A. aegypti* (Haddow, Gillett &
Corbet, 1961; Gillet, Haddow & Corbet, 1962) and some other arthropods (Danilevsky,
Goryshin & Tyshchenko, 1970).

SUMMARY

1. The flight activity patterns of groups and individuals of sugar-fed *A. taenio-
rhynchus* females have been studied under different light regimes, by recording of
flight sound as an indicator of flight activity.

2. In an LD 12:12 regime, flight activity occurs both at light-off and light-on,
forming a bimodal 'alternans' pattern. This basic pattern of flight activity persists
with a periodicity of 23·5 h under continuous DD, but under continuous LL is
masked over by irregular excessive outbursts of activity.

3. The flight activity rhythm originates for the first time in the adult stage, and it is
not carried over from rhythms of developmental stages.

4. Flight activity rhythms can be entrained to a new light regime within 24–36 h
which is rather fast. An early light-off does not reset the phase of the rhythm, but a
delayed light-off does.

5. The flight activity rhythm can be entrained to 24 h light regimes other than LD
12:12. But a single stimulus of less than 12 h is not effective in initiating the bimodal
circadian rhythm.

6. Frequency demultiplication within certain limits can entrain the flight activity
rhythm to 24 h.

7. This flight activity rhythm of *A. taeniorhynchus* is compared with the activity
rhythms of *Aopheles gambiae* and *Culex pipiens pallens*.

8. It is concluded that the basic bimodal alternans pattern of flight activity is a
persistent property of the circadian oscillating system, which suggests that other
activity rhythms involving flight are dependent on the same rhythm.

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