CHAPTER 6

Circadian Rhythms in Insects

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Extensive studies have been made of insect circadian rhythms, and have contributed largely to our knowledge of those basic characteristics which are common to such rhythms in all animals. Laboratory studies have, in the main, been concerned with defining these characteristics and most recently the majority have been concerned with the exploration of the basic controlling system. Running alongside these experimental studies, but on the whole parallel with, rather than converging upon them, have been the even more extensive studies made in the natural environment.

Despite the advances in both of these fields each remains so complex that we are still a long way from dealing with either in terms of the other. Nevertheless it is important from time to time to consider where the evidence from each of the two fields interrelates, and where indeed it appears to be in direct conflict. Such an attempt is made in this chapter. In doing so it is neither possible to cover all the extensive literature in each field, nor to discuss in detail certain aspects which would be of major importance in a discussion confined to only one of these fields. Neither has it been possible to discuss the role of circadian rhythms in orientation phenomena or photoperiodism. In the last section one group of insects, the mosquitoes, whose natural behaviour has been studied more extensively than that of any other group, is considered separately in the light of the evidence presented in the rest of the chapter.

J. N. Mills (ed.), *Biological Aspects of Circadian Rhythms*

Circadian rhythms are apparent in the total locomotory activity of many insects; activity which may include walking, flight and various taxes. Rhythms are also apparent in the patterns of feeding, respiration, excretion, reproduction (including copulation and oviposition), and in the timing of ecdysis and the emergence of many larvae, or pupae, as adults. Associated with these rhythms are others of change in cell or nuclear volume, of laying down of materials, and of various biochemical events.

Some of these rhythms may not, in themselves, be of great biological importance to the insect; they may perhaps only be produced as a consequence of other rhythms with more adaptive significance. Some rhythmical functions which, in the field, appear to be under endogenous control, may in the laboratory show only a correlation with environmental fluctuations; on the other hand some field rhythms which are apparently influenced strongly by environmental fluctuations, may, in the final analysis, prove to have an underlying endogenous circadian component.

Perhaps the most difficult rhythms to interpret are those of physiological processes which are greatly affected by the total activity of the animal. Yet there is some evidence that the rhythmical fluctuation in the concentration of some metabolic substances, for example blood sugar, is independent of either locomotory or feeding rhythms. Such rhythms need to be considered against the background of homoeostatic control.

Despite the fact that insects are cold-blooded, and therefore have little control over the major physical factor of temperature, they have, like other animals, a high degree of homoeostatic control. Like other animals too, many of the fluctuations in the so-called stable state occur about a mean which varies with a circadian periodicity. The adaptive significance of such rhythms can be seen when homoeostatic control is regarded as a system which enables the insect to receive information from the external environment, and to react in such a way as to stabilize the internal environment. Many major environmental changes occur, however, at regular times of day, and, since biological processes take finite time, it is clearly advantageous to the insect to have a system which fluctuates appropriately and enables the control mechanism to be prepared for such changes [1].

The converse of this situation should also be considered, for if the homoeostatic control system is rigidly tied to a circadian rhythm then fluctuations in the more irregular environmental variables at the "wrong" time of day might affect the animal deleteriously. The separation of some behavioural rhythms which need to be suppressed in unfavourable environments, from metabolic rhythms, may thus be advantageous. In addition, although all circadian rhythms may be entrained by environ-

mental variables, major changes in phase frequently show a time-lag so that an occasional variation in the environment does not, on the whole, reset a rhythm to an inappropriate time for the following day.

6.1 LOCOMOTORY RHYTHMS

By far the greater number of laboratory experiments on circadian rhythmicity in insects have been concerned with locomotory activity. Since such activity affects, or is affected by, most of the physiological systems, and virtually defines the way of life of the animal, an understanding of activity rhythms is an essential step towards the ultimate aim of biology, an understanding of living organisms in their natural environment.

6.1.1 Walking Rhythms

Although relatively few adult insects confine their movements to walking, for practical reasons it is this type of activity, rather than flight, which has gained most attention. Possibly because so many flightless insects are nocturnal rather than diurnal, there has also been a tendency to concentrate on nocturnal insects.

Detailed studies have been made, for example, of the activity rhythms of several species of cockroach [2-11], the cricket *Gryllus* [12] and the beetle *Tenebrio* [13]. In all these insects active walking begins close to the start of the dark phase in LD 12 : 12, when the temperature is held constant, although even between individuals of the same species there may be considerable variation in the phase-relationship to the light-off signal.

In natural conditions, of course, such environmental conditions are rarely, if ever, present, so that it is of considerable interest to follow the effect of different photoperiods and varying temperature on such rhythms. But before considering these environmental variables some attention should be given to another variable which occurs in laboratory experiments.

The Effect of the Actograph

One aspect of rhythmical activity in laboratory conditions which has received little attention is the effect which the container in which the animal lives, or the method of recording activity, has on the form of the rhythm displayed by the animal. It was earlier suggested [14] that animals might, by their own behaviour patterns, reinforce the effect of

weak Zeitgeber, or even compensate for a lack of variability in their environment. For instance, in LL the presence of a shelter into which the animal can move and be shielded from the light might result in the animal receiving an effective LD cycle, should it actively seek darkness at one phase of its endogenous behaviour rhythm. Since this suggestion was made such a rhythm of light preference has been found in the crab *Uca* [15], and self-selected rhythms have been studied in the canary [16], but studies on insects are still badly needed.

Lipton and Sutherland [17] have tested the variability of rhythms in the cockroach *Periplaneta americana* in two different types of recorder, one being a capacitance monitor and the other a running wheel. Although the authors have not entirely clarified the point, it appears that in the capacitance monitor a rhythm entrained by LD 12 : 12 was characterized by greater activity during the first 2 or 3 h of darkness, followed by a relatively quiescent stage during the rest of the dark period, and by intermittent activity during most of the light period. Perhaps too much emphasis should not be placed on these results as only seven cockroaches tested in this way were rhythmic, and the record as illustrated might not be accepted by some authors as showing a normal activity rhythm. In the running wheel, of the 126 animals which showed an entrained rhythm, 31.8% showed a marked peak of activity at the beginning of the light period in addition to the peak associated with the onset of darkness. The great majority of insects continued to show a biphasic rhythm when placed in DD, although in most animals the secondary peak moved closer to the main peak, and some animals lost it altogether. A similar loss of the secondary peak has been reported in the cockroach [18] when its activity is measured by means of a thread attached from the pronotum to a writing lever.

It is also worth noting that Lipton and Sutherland present their results in a different form for each type of recorder; the activity in the capacitance monitor is shown by the use of histograms, whereas activity in the running wheel is shown by the direct record from an event-recorder. Because the activity occurring in any one hour is summed for the histogram presentation a great deal of information is lost, and indeed if some of the event-recorder figures are put into histogram form no rhythmical activity at all can be seen.

There can be little doubt that the now common practice of presenting results by direct event-recorder traces has led to a considerable increase in our knowledge of rhythmical activity, and the results of some earlier work needs revision in the light of the more detailed records now obtainable with modern equipment.

An initial study of the role of the recorder itself in reinforcing, or stimulating, the rhythm has been made by Brady [8] working in this laboratory. He found that when cockroaches are removed from the

actograph and subjected to enforced periods of activity, the subsequent circadian activity peak is markedly increased, the peak being much more affected than is the background activity during the rest of the 24 h.

Animals placed in a running-wheel are able to run more actively than animals in a restricted box of the type used in tipping actographs, capacitance monitor devices, or in conjunction with photo-cells; furthermore any active movement in a running-wheel tends to stimulate the animal to further activity, so that a rhythm of activity becomes more marked. From the point of view of the observer this type of recorder is therefore far superior, and certainly gives much clearer evidence of both entrained and free-running rhythms; but more work is needed before any relationship can be established between the natural behaviour of the animal in the field and the type of rhythmicity recorded in the laboratory. Indeed Kavanau [19] as a result of his experiments on the behaviour of *Peromyscus* in a running-wheel of a type which could be motor-driven, motor-driven but controlled by the animal, or free-turning, concludes that records from running-wheels may be misleading.

Photoperiod and Light Intensity

Turning now to the effect of the more frequently studied environmental variables, those of photoperiod and light intensity are of particular interest.

The cockroach *Leucophaea,* when in an LD 23 : 1 regime, shows a peak of intense activity about 7-8 h after the usual peak associated with the onset of darkness: this secondary peak of activity lasts, under these conditions, for about 4-5 h. Similar secondary peaks occur whenever the length of the dark period is less than between 7-8 h, that is whenever light falls on the animal 7-8 h after the initiation of the dark-active peak. This secondary peak must represent a phase of extreme sensitivity to light, as is clearly shown when the insect is kept in LD 1 : 1 (Fig. 6.1). In this latter regime the LD cycle does not entrain the activity rhythm and the cockroach free-runs, but when the light comes on during the light-sensitive phase intense activity occurs, ceasing however during the alternate hours when the light goes off [11]. The conditions under which these experiments were performed do not enable any conclusion to be drawn about whether the intense activity during the light period is a vigorous escape reaction, or whether such activity would normally occur in natural conditions. Should the latter be the case then the light-sensitive phase may play an important role in the life of nocturnal animals in temperate climates, causing them to be more active during the early morning hours than during the night.

It seems likely that at least some of the rhythms which become biphasic under certain conditions do so because of a similar

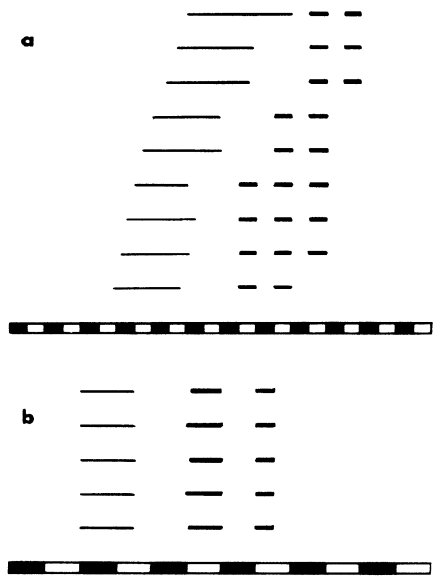

Figure 6.1. Diagrammatic representation of the activity of *Leucophaea* in (a) LD 1 : 1; (b) LD 2 : 2. The LD regime is indicated by black and white enclosed bars.

light-sensitive phase, and it would be of interest to know whether in these cases the two peaks keep the same phase-relationships under different light intensities.

Although the effect of the intensity of the light, in both LL and LD, has been studied extensively in other animals, few such studies have been made with insects. Lohmann [13] has, however, shown that the period of the free-running rhythm of the nocturnal beetle *Tenebrio* increases as the light intensity increases, in accordance with Aschoff's Rule. He has also made the interesting observation that when the light intensity is increased by a factor of 100 (within the range 0.01-100 lux) the period lengthens by about 50 min; on the other hand when the light intensity is decreased by the same factor the period decreases by only about 30 min. Lohmann [20] has also found that when a light-step, from lower to higher intensity, is given to a free-running *Tenebrio* the magnitude of the consequent change in period length is related to the circadian time at which the intensity change took place. Maximum changes in period occur when the increase in intensity is given 2-3 h after the onset of activity, and minimum changes in period occur when the light-step comes about 12 h later than this. A similar phase-dependent shift-response was shown earlier in cockroaches [21]. Lohmann points out, however, that since both light-on and light-off stimuli were included

in the latter experiments the "two directional" response curve found for cockroaches may be the product of a combination of the two stimuli: only a light intensity increase was given to *Tenebrio* and a "one directional" response curve was obtained.

Lohmann found similar effects at much lower intensities, that is when the light-steps were only from 0.01 to 2 lux, and an animal in its natural environment must frequently experience intensity changes of this order. Normally, however, an animal would also be under the much more effective stimulus of the change from day to night, and in such conditions the phase-relationship between Zeitgeber and the response-curve might act towards stabilizing the system.

The intensity of the light in LD 12 : 12 also has a clear effect on the timing of the activity peak in *Tenebrio* [13]. Activity normally starts before the light-off signal, and with low light intensity during the light phase the time-difference between this onset of activity and the light-off signal is greater than it is at higher intensities. This intensity effect may be quite significant in a natural environment; observation of nocturnal moths, for example, suggests that they become active earlier after an overcast day.

The effect of alterations in the timing of the LD cycle on the activity rhythms of cockroaches has been studied by a number of authors [4, 5, 9, 11]. Some differences appear in their results, partly because of the different types of recorder used in the different experiments. Harker [4, 5] showed that when the timing of an LD 12 : 12 cycle was altered so that the onset of darkness came 7 h earlier than usual, the major peak of activity in *Periplaneta* did not phase-shift immediately to the new time of onset of darkness, but that after small phase-shifts had brought the activity peak to within about 5 h of the beginning of darkness a rapid phase-shift occurred. Brady [9] observed that, out of 7 animals given an environmental phase-shift of more than 5 h, possibly three showed what he calls "slight sign of an acceleration in phase-shifting" when the activity peak came to within about 5 h of the new LD transition. Examination of his text-figures however, shows that at this stage there is at least a three hour jump, followed by another on the following day, in contrast to shifts of considerably less than an hour at other times. However his point that the final steady state is reached by gradually decreasing phase-shifts, when the onset of activity is within about an hour of the LD transition, is clearly supported by his figure. It is here that once again the advantages of the method of recording in a running wheel and the use of direct traces can be seen, for with the methods used originally [4, 5], involving the summation of the activity within each hourly period, such small shifts could not be seen. It is also clear that the use of the running wheel, which, as discussed earlier, appears to induce a very sharp onset of activity, allows for a different type of measurement

of phase-shifts. This can also be seen clearly in Brady's figures, for when he recorded from a photo-cell box, although he states that no jump can be seen in the phase-shift, such a jump can be seen if the results are summed in hourly groupings. Phase-shifts showing jumps at the time when onset of activity comes to within about 4 h of the new LD transition can also be seen in records published by Roberts [11] showing the resetting of phase by *Leucophaea.*

Environmental phase-shifts in which the onset of darkness comes later than previously cause an immediate, or in the case of very large shifts a very rapid, phase-shift in the activity rhythm of both *Periplaneta* and *Leucophaea.* In another cockroach, *Blaberus,* the rate of phase-shift has been shown to depend on the light-intensity during the light phase [22, 23].

As has been mentioned earlier the reaction of an insect to both LL and LD cycles is related to the intensity of the light, and further studies like those made of *Blaberus* are badly needed, particularly in relation to phase-setting.

One interesting aspect of phase-shifting has hardly been considered, and that is the question of stability. When the peak of some particular activity has been phase-shifted the question must arise as to whether the *total* rhythmicity of the animal has also been phase-shifted, or whether it is possible that different processes phase-shift at different rates.

Although dissociation of different physiological processes has been shown in the case of man [24] it has not, to my knowledge, been closely studied in insects, although some of the evidence on the instability of phase-shifts suggests that it might occur. Warnecke [25], for example, has shown that when the beetle *Geotrupus* is placed in a reversed LD cycle the activity cycle is phase-shifted in about 8 days (depending on the light-intensity in the light-phase), but if the LD cycle is reversed again a very rapid phase-shift back to the old phase-setting occurs. Such a rapid reversal takes place even after a new cycle has been maintained for a considerable time, and only ceases after about 4 weeks of the new conditions.

Since most of the work described in this section deals with nocturnal animals it is of interest that Warnecke has also studied three closely related species of *Geotrupus; G. silvaticus* and *G. vernalis* being diurnal in habit, while the third *G. stercorarius* is nocturnal. When kept in DD from the egg onwards all three show the same pattern of activity, with a bimodal rhythm in the adult stage. However the free-running frequency shows a basic difference, being longer than 24 h in the diurnal species, and shorter in the nocturnal. This is an interesting example of a fundamental difference between the biological clock of closely related species, a phenomena which will be discussed further in a later section.

Temperature

In common with other organisms the period of circadian rhythms in insects is only very slightly, if at all, affected by temperature. On the other hand fluctuating temperature may act as a Zeitgeber in the same way as does light. For most insects, however, temperature is a very weak Zeitgeber compared with light, and may act only in the absence of any fluctuation in other environmental factors.

Roberts [11] has shown that the locomotory activity rhythm of *Leucophaea* may be entrained by a 24-h sinusoidal temperature cycle with a 5° C range, the onset of activity occurring at the high point of the temperature cycle. It is interesting that the peak of activity should be phased to the time of maximum temperature since in a nocturnal animal activity would normally come at a time of falling temperature, although Roberts states that sunset and the high point of the temperature cycle are roughly coincident in nature.

Single low temperature "pulses" will cause a phase-shift in the activity rhythm of both *Leucophaea* [11] and *Periplaneta* [5, 26], the magnitude of the phase-shift being related to the circadian time at which the low temperature treatment occurs. Roberts found that the maximum phase-shift resulting from a 12-h temperature drop from 25° to 7° occurred when the return to the higher temperature came 14 h after the previous peak of the activity rhythm, and the minimum shift occurred when the temperature rise came 18 h after the previous activity peak. When the low temperature was continued for 48 h, however, the phase-shift which followed was strictly correlated with the time of the temperature rise.

Insects living in areas with large temperature differences between day and night experience the range used by Roberts and for a nocturnal insect the day-time increase in temperature would come about 14 h after its activity peak. Field observations relating to this aspect would be of interest.

In contrast to the effect of low temperature steps high temperature "pulses", during which the temperature is increased from 21° C to 31° C for 4 or 8 h, do not induce phase-shifting in *P. americana* [26].

The temperature range to which an insect is subjected may affect the phase-setting of an activity rhythm in relation to the LD cycle, and this type of effect must be of great importance in natural conditions. Edwards [27] has made a very careful study of the activity rhythm of two Lepidoptera from hatching through to the adult stage, and finds that in LD the larva of one, *Halisidota argentata,* undergoes a temperature-related phase-reversal, becoming diurnal at low temperatures and nocturnal at higher temperatures. A similar phase-reversal is seen in

the ant *Messor semirufus*; it too is diurnal at low and nocturnal at high temperatures [28]. Some insects show unimodal activity rhythms at one temperature and bimodal rhythms at others; for example *Calliphora stygia* shows a single-peak curve in winter, but has a morning and an evening peak in summer [29].

6.1.2 Flight Activity

Studies on flight activity have been made mainly in the field rather than the laboratory, in contrast to those on walking activity. The results from field work on flight periodicities are so extensive that it is not possible to discuss them here in any detail: Lewis and Taylor [30], for example, analysed collections of about 5 million insects from 46 habitats, Williams [31, 32] trapped insects over a 4-year period, and many other very detailed studies have been made of particular genera. Laboratory studies have been related to field studies in a few cases, in particular with reference to *Drosophila,* aphids and mosquitoes: discussion will be confined here to the first two, since mosquitoes are dealt with in a separate section (p. 000).

Aphids

In a series of papers Johnston, Taylor, and their colleagues, have analysed the factors involved in the flight activity patterns of *Aphis fabae* [33, 34].

The number of aphids in flight above a summer breeding site shows a diurnal fluctuation which is bimodal in form (Fig. 6.2). The bimodal curve must result from single acts by a succession of individuals, since the winged aphids fly away from the crop a few hours after ecdysis to the adult stage and do not return: the curve therefore represents a true population periodicity.

Analysis of the sequence of events leading to flight has shown that the length of the teneral period (when the wings are expanding and hardening) is dependent on temperature. Once this developmental period is over flight itself is affected by temperature, light and the presence of adjacent insects. The afternoon peak is thus produced by a combination of two factors: a shortening of the teneral period as the temperature increases, and an increase in the rate of take-off. The morning peak appears to be due to aphids which have matured in the late evening, or overnight, all of which start to fly when the rising temperature makes this possible. If the light-intensity remains high late in the evening the number of flying insects still drops because the falling temperature lengthens the teneral period, although the number flying the next morning is lower than usual because all the flight-mature insects will have

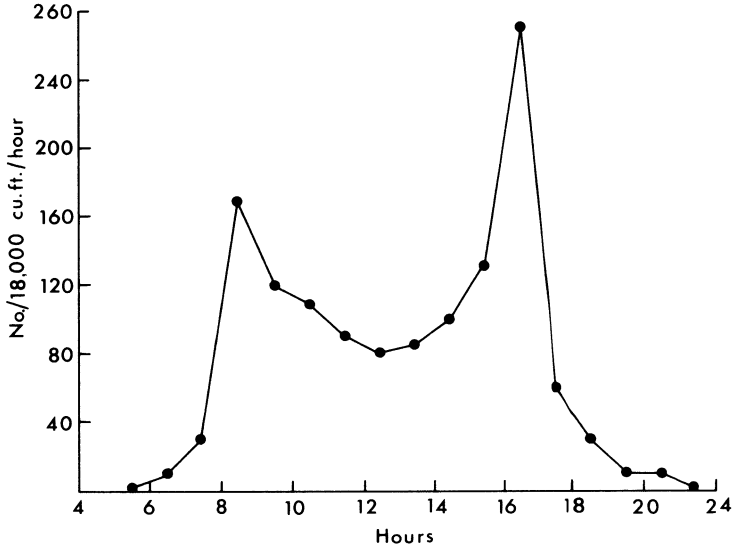

Figure 6.2. Aerial density of aliencolae of *Aphis fabae* flying above a bean crop on which they were produced, on a July day in England (after Johnson and Taylor [34]).

flown the previous evening. By contrast, if the temperature remains high, but light-intensity decreases, then take-off is inhibited but large numbers of flight-mature aphids are available for the next morning peak.

It seems from these results that the entire sequence of events leading to flight periodicity is under the control of exogenous environmental factors, and that circadian rhythmicity is not involved. Yet by comparison with other studies a circadian rhythm of ecdysis might be expected, and would underlie the subsequent events. Haines [35] studied the periodicity of moulting in constant light and temperature, and found that although peaks of ecdysis were maintained during the morning and afternoon these were not statistically significant unless the population had been maintained in LL for weeks beforehand. This of course still suggests a true rhythmicity. It should be noted that these experiments were not however confined to the imaginal ecdysis, but dealt with a population of mixed age groups, so that unless moulting has the same phase-setting in all instars any periodicity would be blurred. In further experiments a very clear periodicity appeared in LD 12 : 12, but this virtually disappeared when the light cycle was reversed; again since nothing is known about how different instars react to phase-shifting it is difficult to draw any conclusions. It might, however, be unwise to rule out any circadian rhythmicity in the flight of aphids.

This species has been discussed as an example of the way in which an interrelation of exogenous factors may produce a clear rhythm, and to show that even if there is an underlying endogenous rhythm it may have little obvious influence in the field.

Drosophila

Many species of *Drosophila* show a bimodal dawn and dusk flight pattern [36, 37, 38]. The time of flight is closely related to light-intensity, and indeed Pavan *et al.* have shown that this bimodality disappears in rain forests, particularly on cloudy days. *D, subobscura* also shows a bimodal periodicity in open areas of meadows and open woodland, but in dense woodland the closely related *D. obscura* shows only a unimodal peak [39]. It has been suggested [40, 41] that the dawn and dusk flight of *Drosophila* is an ecological adaptation to avoid dessication, but humidity is not a factor governing its flight in nature. Lewis and Taylor [30] postulate that this bimodal flight is in fact an adaptation to the type of feeding rather than due to susceptibility to dessication. Taylor and Kalmus [41] earlier suggested that *Drosophila* has become adapted to dry conditions, not by an increased resistance to dessication, but by the increased visual efficiency in low light intensities, thus allowing for a crepuscular burst of activity.

It is perhaps curious that despite the very detailed analysis of the periodicity of adult eclosion in *Drosophila* there does not seem to have been a related study on the time of flight, although Roberts [43] has shown that there is unimodal rhythm of flight activity in the laboratory, in contrast to the bimodal rhythm described above.

6.1.3 The Influence of Non-physical Factors on Locomotory Rhythms

The biotic, as distinct from the physical, environment has received relatively little attention in either field or laboratory studies on circadian rhythmicity. This is hardly surprising in view of the complexity of the results obtained after varying even the relatively easily controlled physical factors, yet if the role of rhythms in the life of animals is to be considered we cannot ignore the interaction between individuals of the same species, let alone interaction between species.

Competition

In equable climates, in which the environment is most predictable, and most permissive for many activities, there is a high adaptive value in a rhythm which can reduce inter-specific competition by temporal diversification, as Corbet has pointed out [44]. It is in such situations that laboratory and field studies tend to reveal similar activity patterns,

whereas in harsher climates laboratory and field studies often reveal dissimilar patterns. Perhaps this discrepancy arises because in the field the requirements for an immediate response by the animal to any permissive physical condition when it occurs, regardless of the time of day, may override endogenous rhythms which may indeed even have a rather low adaptive value.

Differences in phase-setting of activity rhythms of individuals of the same species living in adjacent habitats has frequently been recorded. Light trap catches, for example of male *Chrysops centuriones* and *Tabanus thoracinus,* have revealed a biphasic activity rhythm in open woodland, with peaks in the early night and before sunrise, whereas in forests the activity is confined to a single hour before sunrise [45]. Complete reversal of phase-setting in the beetle *Feronia madida* occurs between open ground and adjacent woodland, the insect being diurnal in the former and nocturnal in the latter [46]. Changes in pattern in *Drosophila* flight rhythms have been mentioned earlier, those occurring in mosquitoes are discussed in the last section, and many other instances could be quoted.

It is probable that in many cases the differences in phase-setting are due to physical factors, in particular to light-intensity, operating at different levels in the different environments. Yet when individuals remain within the particular habitat, and do not pass in and out of adjacent areas, it is possible to distinguish local populations with differences in phase-setting in the laboratory under identical physical conditions.

The number of animals in the population may also affect the phase-setting, both of individuals and of the population as a whole. In some cases this may be due to changes in the physical environment brought about by the animals themselves; for example the eclosion of *Drosophila* adult occurs later in the day as the population increases [47] and it is possible that this is due to changes in food, or gas, concentration with increasing numbers. On the other hand this type of competition may not always be a major factor, and it would be interesting to investigate the role of mutual stimulation, or stress, in such situations, particularly in view of the results obtained from the use of different types of activity recorder in the laboratory.

Closely related species living in the same habitat frequently show specific differences in their time of activity, a feature of obvious adaptive value. A very clear example of such a species difference has been shown in the males of Doryline ants [48] (Fig. 6.3), and the extensive work on the eclosion of *Drosophila* has revealed characteristic emergence patterns, both for different species and for mutant strains. Surprisingly little study has been made of the genetical control of patterns of phase-setting, particularly since such detailed information on each of

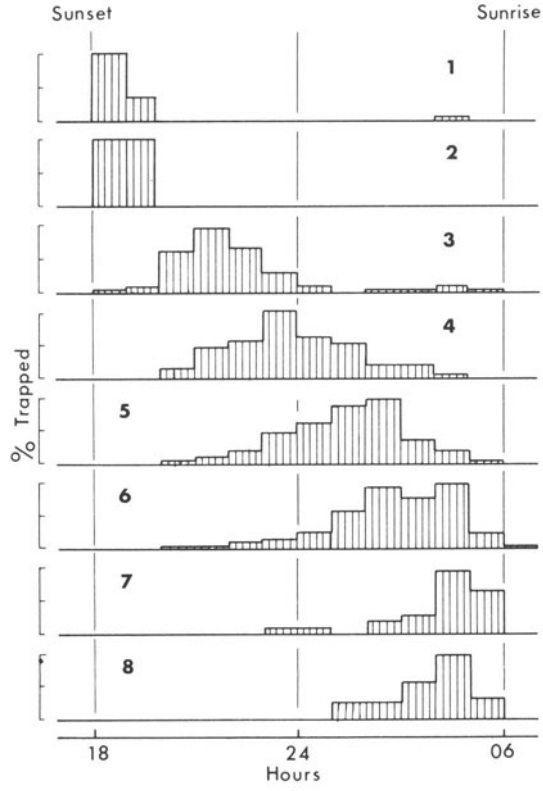

Figure 6.3. The entry of Doryline male ants into light traps 1. *Dorylus moestus* 2. *D. nigricans* 3. *D. affinis exilis* 4. *D. fulvus* 5. *D. alluaudi* 6. *D. katanensis* 7. *D. burmeisteri* 8. *D. fimbriatus laevipodex* (after Haddow, Yarrow, Lancaster and Corbet [48]).

these aspects is available for *Drosophila;* the results of one such investigation are however considered in a later section (p. 206).

On the whole the two sexes are active at the same time of day in many animals, and Lewis and Taylor [30], in their large-scale study of flight times, found this to be so for the vast majority of insects. There are, however, some exceptions. The male of the moth *Lithocolletis messaniella* is found predominately in the morning flight peak, and the female in the sunset peak; a similar dissociation occurs in another moth *Anagasta kuhniella* [49]. The hymenopteran *Neuroterus* shows even more dissociation, with a dawn peak consisting entirely of males and a day time peak entirely composed of females. The males of *Drosophila subobscura* predominate in the dawn peak and the females in the dusk,

despite the fact that both sexes respond to the same light intensity. On the other hand there is some evidence that in the laboratory groups of *Drosophila,* in which only one sex is present, show a bimodal rhythm, whereas when both sexes are present only an evening activity peak occurs [50].

Activity records of cockroaches show differences between adult males and females [3, 17], but to what extent these differences are related to reproductive activity is not yet entirely clear.

Further study of behaviour patterns during reproductive cycles is needed in relation to all those rhythms which show sex differences, but it does seem that it is not only the reproductive cycle which is responsible for the sexual distinction in phase-setting, for it is also found in the eclosion rhythm of various species [51, 52, 53].

Age

Very different patterns of activity may be shown by insects at different stages in their life cycle, and although this is certainly not unexpected considering the great difference between adult and larval habits and habitats in so many insects, it is perhaps less expected in the exopterygotes in which all stages may occupy the same environmental niche. Remarkably little attention has been paid to this changing pattern, and yet it poses some fascinating problems, for it implies that the alteration in phase-setting is produced by some process within the developing animal.

The commonest type of change in pattern is probably a "sharpening" of the rhythmicity, involving a change from a rather broad peak of activity to perhaps a clear single peak or to even a biphasic pattern. Such a change is seen in *Carausius* which is active over most of the 24 h in the first instar with only a slight increase around midnight, whereas in later instars activity during the day virtually ceases and a peak of activity occurs at dawn and dusk [54, 55]. The moth *Halisidota,* when in a regular LD cycle and constant temperature, shows in the second and third instars a peak of activity around the time of light-off, and another small peak just after light-on. In the fourth and fifth instars the evening peak moves earlier and activity declines more sharply after light-on, and by the eighth instar the activity peak is occurring during the afternoon. After emergence as an adult the activity peaks again occur near the light-on and light-off signals.

In some insects the activity rhythm may become very weak, or even disappear altogether at times. This can be seen to happen in the last nymphal instar of *Gryllus domesticus;* since the rhythm reappears in the adult it seems likely that it is also maintained, but is not overt, at the earlier stage [12].

Even within the same instar the pattern of activity may change with age; for instance the adult dragonfly *Anax imperator,* in addition to the sunset activity peak, shows a peak at dawn when it is newly emerged, this peak moving to midday by the time it is more than a week old [56], and several species of Ephemeroptera are active at different times of day according to their age [57].

Effect of Feeding

Relatively few experimental studies have dealt with the question of whether feeding, or the presence of food, can act as a Zeitgeber. It is, however, well known that bees can be trained to feed at certain times of day, and the presence of food is undoubtedly a Zeitgeber in this insect. For bees, and carnivorous insects, the presence of food is dependent on the rhythmicity of other species, and there may be an adaptive value in a close relationship between feeding and the phase-setting of activity rhythms. On the other hand the food of many insects is constantly present; yet it is to such insects that experimental work has been almost entirely confined.

Green [58, 59] has made an extended study of the relationship between feeding and the locomotor rhythm of the fly *Phormia regina.* In the absence of food and water the amount of activity increases as a function of the deprivation time, but when sucrose solutions are supplied the amount of activity is immediately reduced. Further operational procedures give results suggesting that the amount of activity is controlled by a hormone released from the corpus cardiacum, the release of the hormone being under the control of receptors in the foregut. The circadian rhythm however overrides these effects, so that only the amount and not the timing of the rhythm is affected. A similar effect on the degree of activity of food-deprived *Drosophila* has been observed by Connolly [42]. The beetle *Geotrupus silvaticus* also becomes increasingly active in the absence of food [25], but in this species it is interesting that only one peak of the bimodal rhythm is affected. In all these examples it appears that food does not have any affect on the entrainment of the rhythm, but the results are worth noting in relation to weak or obscure rhythms in the field, for it may be that the presence of abundant food may reduce activity to a level at which a rhythm is difficult to measure.

The effect of feeding on activity rhythm of the cockroach *P. americana* has been studied by Harker [3] and Lipton & Sutherland [17]. The latter measured the feeding activity by recording the movement of a pendulum on which the food was suspended; use of this method reveals a feeding rhythm which is very similar to the locomotor

rhythm in terms of time of onset and period length, in its ability to be reset by a change in the LD cycle, and most important, in its persistence in DD. Unfortunately these authors did not test the effect of giving food at only one time of day during DD to establish whether a phase-shift could be effected, so that the question of whether the presence of food can act as a Zeitgeber is still an open one. In the earlier experiments [3] an attempt was made to answer this question by restricting food availability to a few hours during the light phase of an LD cycle. Although the insect became active in the presence of food the normal activity rhythm continued, and the activity during the light phase appears to be only an immediate response to the presence of food; when food was not presented on any particular day there was no increase in activity at that time of day. The experiments were repeated in LL with the same result, but in view of the results of further work in recent years it is now apparent that the presentation of food during a free-running rhythm in DD might have been a more appropriate test, particularly as an activity recorder of the confined type was used.

An interesting observation has been made concerning the relationship of feeding behaviour to locomotion in the cricket *Gryllus domesticus* [12]. Feeding activity reaches a maximum about 4 h before the onset of darkness in LD, the number feeding then drops, but rises again about an hour before dusk; thus the major feeding activity comes before a minor activity peak in the afternoon, and only a minor feeding peak comes at the time of the major activity peak.

Feeding behaviour of the milkweed bug *Oncopeltis fasciatus,* again an insect whose food is always available, follows the LD cycle with maximum feeding occurring at the end of the light period. This rhythm only persists for one cycle in LL, and not at all in DD [60]. It is not possible to tell from these results whether this is really a persistent rhythm, for the one cycle shown in LL could be a direct hunger effect.

Information is still needed about the phase-shifting of feeding rhythms in insects, particularly in those like bees for which the presence of food appears to be a strong Zeitgeber. A valuable extension of the work on feeding cycles of bees might come from a study of the effect of a phase-shift in the feeding rhythm on its phase-relations with some other function; the work of Bennett & Renner [61], on the use of a bee laboratory, suggests that it might now be possible to make this type of study under controlled conditions.

An effect of the time of feeding of the larvae on the actual period of the rhythm of pupation of the mosquito *Aedes taeniorhynchus* has been shown by Nayar [62]; it is possible however that this results from an effect on the synchronization of the population rather than an effect on the periodicity of the individual larva.

6.2 RHYTHMS OF PUPATION AND ECLOSION TO THE ADULT STAGE

Many insects, particularly those with aquatic larval stages, emerge as adults in very large numbers over a limited area, and during only a rather confined time of day. It is not surprising therefore, that many field studies have been made of this event [51, 52, 63-67].

The most extensive of the laboratory studies are those made of the eclosion of *Drosophila*. Kalmus [68] first showed that a definite peak of emergence occurred just after the change from darkness to light; this was later confirmed by other workers, and a careful study by Brett [69] showed the effect of light and temperature on the timing of eclosion. Pittendrigh and his associates, in a long series of papers (see [70]) have since then explored this phenomenon in depth, particular attention being paid to experiments designed to give information about the fundamental properties of the circadian system: experiments which include studies of the effect of "skeleton" photoperiods and of single light periods on the timing of emergence some days later. In the final analysis all information about the circadian system must come into our picture of the role it plays in the life of the animal, but while our understanding of this system is still not clear it is not always possible to consider it in perspective against the rhythms we observe. Since this chapter concerns the role of rhythms in the normal life of insects, not all of Pittendrigh's results will be discussed here, valuable though they may be in the interpretation of the properties of biological clocks.

Although in LD 12 : 12 the eclosion peak occurs just after the beginning of the light period the position of the peak changes with different photoperiods. For example, in LD 4 : 20 the peak occurs during, although towards the end of, the dark period [69], whereas in LD 14 : 10 it occurs some hours after light on, and in LD 18 : 6 there are two emergence peaks, one soon after dawn and the other about 9 h later [71,72].

The timing of the eclosion peaks is virtually temperature-independent, having a Q_{10} of 1.02 [73], despite the fact that the actual length of the developmental period of the pupa is of course very temperature sensitive. The rhythm of eclosion can also be entrained by a temperature cycle when the insects are kept in LL, in which they do not otherwise reveal an eclosion rhythm [72].

By following the development of over 16,000 individual pupae it has been shown that the timing of the eclosion peak is related to a circadian rhythm which affects the development of the pupal stage, and which results in the synchronization of the population [71]. Pittendrigh & Skopik [74] have however also followed the development of three

species of *Drosophila,* using a different procedure, and have concluded that there is no circadian factor in the development of the pupa which can affect the eclosion. A detailed analysis of Pittendrigh & Skopik's paper in relation to the previous papers will be made elsewhere.

A clear rhythm of puparium formation has been described in *D. victoria,* and a less distinct one in *D. hydei* and *D. melanogaster* [76, 77]; Pittendrigh & Skopik, however, state that there is no rhythm in *D. melanogaster* although no evidence is cited to support this and they accept Rensing's results for *D. victoria.* The two strains of *D. melanogaster* used in Harker's study underwent pupation at all times of day, but it occurred very rarely at some times and most commonly at others.

Rensing & Hardland [77] have also shown that the number of pre-pupae formed reaches a maximum at dusk, and a minimum in the morning, and that this rhythm is controlled by the LD cycle: the rhythm of puparium formation is at least partly due differences in the length of the developmental time, for pre-pupae formed in the morning pupate 18 h later at 18.5° C, whereas those formed in the afternoon take 19-20 h to pupate. It is interesting to note, in relation to the studies on the development of the pupa itself, that Rensing also finds that in LD 20 : 4 puparium formation occurs earlier in the day than it does in LD 12 : 12 [76].

A phase-relationship has been shown between the puffing-pattern of the salivary gland chromosomes in the 3rd instar larva, and the time of puparium formation, the pattern associated with puparium formation appearing 4-6 h earlier than the actual formation of the puparium [78]. On the day before puparium formation takes place the nuclear size of the cells in three endocrine systems show a rhythmical variation, and the size of the cells of the corpus allatum, prothoracic gland, and the neurosecretory brain cells all reach a maximum, in LD 12 : 12, three hours before light-on and three hours before light-off. On the day of puparium formation the maximum occurs 3-4 h *after* light-on with the change in the puff-pattern occurring immediately afterwards [79], suggesting that ecdysone release has taken place [80, 81].

Several authors have shown that the emergence rhythm of the mosquito *Aedes taeniorhyncus* is dependent on the pupation rhythm, and that the two events are separated by an interval affected by temperature and not by photoperiod [62, 82, 83].

6.3 CUTICULAR DAILY GROWTH LAYERS

One of the most fascinating manifestations of a circadian rhythm has been revealed by the work of Neville on the growth layers in the cuticle

of a variety of insects. This work does not seem to have attracted the attention it deserves by workers on circadian rhythmicity, despite the fact that it now makes available a near-ideal system for fundamental work on the biological clock.

Neville [84, 85] first showed that the endocuticle of *Schistocerca gregaria,* which is deposited after each ecdysis, can be seen to display daily growth layers when viewed in section with a polarizing light microscope. The layer which is deposited during the night has the chitin organized into several lamellae, whereas that deposited during the day is non-lamellate. More recent work [86], using electron microscopy, has shown that the night-deposited layer appears lamellate under polarized light because of the helicoidal orientation of the planes of the microfibrils. The day-deposited cuticle appears non-lamellate since all the microfibrils run in one direction.

The deposition of these growth layers in *Schistocerca* continues when the locust is kept in DD and constant temperature, so that it can be regarded as showing a true circadian rhythmicity. The free-running rhythm in DD has a period of about 23 h [87], and as with other rhythms the free-running period is virtually temperature-independent. In LL the rhythm disappears, only non-lamellate cuticle being produced.

One of the most interesting results from this work has been the discovery that in the locust, epidermal cells in different parts of the insect differ in their response to LL. Even cells which are spatially very close may show quite different responses; for instance in constant low temperature and high intensity LL the endocuticle of the thickened proximal region of the hind tibia is laid down continuously in the lamellate form, despite the fact that the endocuticle of the rest of the hind tibia is, under these conditions, non-lamellate. This suggests that either each epidermal cell is controlled by its own biological clock, and the clocks differ in their response to LL, or, as Neville suggests, that many cells are controlled by a single clock, but the way in which individual epidermal cells respond to it, or are coupled with it, differs.

Although it has previously been suggested that different processes may be differently affected by environmental phase-shifts [21], this very clear demonstration at the level of closely related epidermal cells is a striking development, particularly when the pleuripotency of the insect epidermal cell is considered.

In this respect it is interesting that the deposition of endocuticle layers in the beetle *Oryctes rhinocerus,* which is rhythmical, although perhaps not truly circadian, shows a variation in the *frequency* of deposition of the different layers in different parts of the body [88], again suggesting some degree of autonomy for different epidermal cells.

Light does not act through either the compound eyes or the ocelli of the locust, nor does it appear to act directly on the neurosecretory cells

of the brain, since layering of the cuticle is lost in LL even after cauterization of eyes and ocelli and the blackening of the entire head capsule. Neither is there an indirect effect via the food plant. Neville [89] concludes that these results provide evidence against neural timing of this rhythm, but it could be argued that the clock itself continues to function under these conditions, but that the epidermal cells themselves are sensitive to light, and in its presence cease to react to fluctuations in the internal environment. Neville suggests this, in effect, when he speaks of uncoupling the epidermal cell from the clock, and he concludes that different epidermal cells by reacting differently to LL are showing different degrees of uncoupling from the clock. Furthermore the way in which epidermal cells react to the environment may show a time-sequence; the same epidermal cells which produce non-lamellate endocuticle in LL will, before ecdysis, produce lamellate exocuticle, so that there is evidence here that the cell itself may change in its reactions.

There does not, however, appear to be any direct control of the rhythm by the nervous system, or by neurosecretion being passed directly to the cells by axons. This is clearly shown when small cylinders of hind tibia are implanted into the haemocoele; after an initial period, during which the epidermal cells of the implant migrate and encapsulate the whole implant, new endocuticle is laid down and appears in the banded form. This banding continues in DD.

Not all insects lose their rhythmicity in LL, both *P. americana* and the pharate adult of the bug *Oncopeltis fasciatus* continue to show a rhythm in both LL and DD [90, 91].

Very small changes in light-intensity may be sufficient to entrain a rhythm, as has been suggested by Neville [89] in the cave cricket *Dolichopoda linderi.* This cricket lives in an environment of darkness and almost constant temperature, yet still shows a rhythm of lamellogenesis; it is possible that its nocturnal excursions outside the cave expose it to sufficient moonlight (or temperature change?) for this to act as a weak Zeitgeber.

The effect of light intensity on the loss of rhythmicity is marked. Those epidermal cells of the locust which do lose their rhythmicity in LL take longer to do so the lower the light intensity; at intensities below 1 ft candle it may take up to 6 days before lamellate cuticle production ceases, whereas at 75 ft candles lamellate cuticle is only produced during the first experience of LL. As Neville points out this suggests that there is an accumulation of a hormone or metabolite which is regulated by light intensity.

The adaptive features of the system, whereby a regime which induces non-lamellate endocuticle in the major part of the locust but does not prevent lamellate endocuticle production in some specific regions, is of interest. It seems likely that the unaffected parts are those in which

lamellae are functionally indispensible, including as they do the ends of the tibia which are involved in the stresses of jumping, the edge of wing veins, the rubber-like cuticle of the pre-alar arm and wing hinge ligaments, and the cuticle of the median ocellus and compound eye.

6.4 PHYSIOLOGICAL RHYTHMS

Insects showing clear activity rhythms might also be expected to show rhythmical variation in at least some metabolic substances. Nowosielski and Patton [92] have measured the blood sugar concentration in the cricket *Gryllus,* and find a circadian rhythm in the total blood sugar concentration which can be related, in the main, to a fluctuation in trehalose. In LD 12 : 12 the concentration shows a peak about 3 h before light-on, but surprisingly this peak is not related to the feeding rhythm, which has its peak in mid-afternoon, nor does it appear to be dependent on locomotor activity since the blood sugar rhythm is maintained during the last larval instar in which there is no overt locomotory rhythm. By contrast, no circadian fluctuations appear in the protein, amino acid or lipid fraction of the haemolymph [93]. Glycogen levels in adults of the mosquito *Culex tarsalis* fluctuate rhythmically, with the highest levels occurring towards the end of the light period [94]. The relative levels of the principal hydrocarbons in the cockroach *P. americana* are also said to fluctuate in a circadian manner, with greater fluctuations in the male than in the female [95].

Haemolymph potassium and sodium concentration has been measured in *P. americana* at intervals covering about 9 h spanning the time of onset of darkness in an LD 12 : 12 cycle, and the results suggest that there is a change in concentration over the day. Analysis of 166 samples showed a fall of about 10% in the potassium concentration during the first hour of darkness and a gradual decline of 2% in the sodium concentration, although when successive samples were taken from individual cockroaches no daily change was recorded [96]. Wall [97] has shown that the volume of fluid secreted by the malphigian tubules in *P. americana* is greater in the dark period, although there is a good deal of variation, and suggests that the drop in haemolymph potassium might be related to increased tubular secretion, since tubules secrete a fluid that contains about six times as much potassium as that in the haemolymph.

The part played by a circadian rhythm in the behavioural response of male noctuid moths to the sex pheromone of the female has been described by Shorey and co-workers [98, 99]. The female releases the pheromone rhythmically, and the male responds to it rhythmically being maximally responsive 6 to 12 h after light-on in LD 12 : 12, but electroantennograms from pherome-stimulated antennae of males show no change in olfactory response related to the LD cycle. In this case,

therefore, the rhythmical behaviour must be related to a central inhibitory mechanism.

A bimodal rhythm of oxygen consumption has been shown to occur in *Drosophila* throughout larval, pupal and adult life, although the phase-setting of the peaks changes, the maxima moving earlier in the day [100] with age. There is also a difference between the sexes, the male maxima being about equal in morning and evening, but the female showing a smaller morning than evening peak.

Rensing *et al.* [101] have found that, in some mutant stocks, the female shows only a single, evening, peak. By a series of experiments, in which mutants with different ratios of X chromosomes to autosomes were used, they showed that as the ratio of X chromosomes to autosomes decreases so the oxygen consumption curve shows a relative decrease in the evening maximum and an increase in the morning maximum. From the results of crossing experiments they suggest that there may be two circadian oscillations, with a phase-difference of 12 h, and that these are quantitatively dependent on the X chromosome and the autosomes respectively.

Rhythmical changes in nuclear, nucleolar and cell volumes have been investigated by a number of workers. Klug [102] found a rhythmical fluctuation in nuclear volume in the cells of the corpora allata of carabiid beetles, and in the relative numbers of two types of neurosecretory cells in the brain. Rensing has also found a rhythmical change in the nuclear size of the corpora allata and the brain neurosecretory cells of adult female *Drosophila*. In larval *Drosophila* a bimodal rhythm has been found in the nuclear size of corpora allata, brain neurosecretory cells, prothoracic gland cells and the fat body cells, and in the nuclear and nucleolar volume of salivary gland cells [78], although not all the curves appear to be statistically significant. Brady [103] found a statistically significant increase in nuclear size of the neurosecretory cells of the suboesophageal ganglion of cockroaches during the peak of the locomotor activity rhythm. Associated with these observations on volume changes attempts have also been made to estimate the amount of neurosecretory material in brain and suboesophageal ganglion neurosecretory cells, and some suggestion of a rhythmical fluctuation has been reported from the suboesophageal ganglion cells in the phasmid *Clitumnus* [104], and the cockroaches *Leucophaea* [105] and *Periplaneta* [103]. In *Periplaneta* there is some suggestion that the neurosecretory granules may be slightly more aggregated peripherally before the onset of darkness, although no obvious migration of neurosecretory material has been seen. More recently the conclusion that the fluctuations in nuclear volume reflect the activity of neurosecretory cells has been questioned, for fluctuations in RNA synthesis are not always related to fluctuations in nuclear size [106].

By means of autoradiography, estimates of the level of RNA synthesis in the neurosecretory cells of the male cricket *Acheta domesticus* have been made at different times in its locomotor activity cycle [106]. The highest level of RNA synthesis in the pars intercerebralis cells occurs just after the light-on signal, this is followed by a decrease in intensity until another peak appears at about the middle of the light period, coinciding with the time of a small peak of locomotor activity. During the period of maximum locomotor activity, that is, after the onset of darkness, the lowest level of RNA synthesis is seen. The level of RNA synthesis in the suboesophageal ganglion follows a different pattern, with a low level after the light-on signal, and with the maximum occurring half way throught the light period. At the time of peak locomotor activity no synthesis was observed, but another high level was reached in the middle of the dark period. Insects cultured in LL, which show no activity rhythm, also show no fluctuation in the level of RNA synthesis. Unfortunately it is not clear exactly which cells in the suboesophageal ganglion were concerned in these experiments, for both ventral cells and neurosecretory cells are mentioned and pictured separately, but the calculated figures are for ventral cells, so that it is not possible to know whether any, or how many, neurosecretory cells are included.

In a later paper [107], although no figures are given, it is said that in the female the most intensive RNA synthesis in the suboesophageal ganglion neurosecretory cells occurs during the dark period, with the maximum about 3 h after the onset of darkness (i.e. at the time when no synthesis took place in the male), and that during the light period there is a far lower level of synthesis. The authors do not comment on this apparent reversal of the cycle compared with the male, nor have they measured RNA synthesis in the brain of the female but they assume that it follows the same pattern as in the male.

Cymborowski and Dutkowski [106] argue from the results obtained from the male that the sudden increase in RNA synthesis in the pars intercerebralis after the onset of light, which is followed by an increase in synthesis in the suboesophageal ganglion 6 h later, suggests a relationship between the two systems; they also suggest that the suboesophageal ganglion synthesis is being stimulated by the passage of neurosecretory material down a nerve to that ganglion, the combination of these two events leading to the locomotor activity rhythm (Fig. 6.4). However, since the relationship between the two events appears to be quite different in the female, until further details are published it is difficult to draw any conclusion. Furthermore the increase in RNA synthesis in the pars intercerebralis, after the onset of light, would seem to point to this onset of light as the Zeitgeber for locomotor activity, yet the onset of activity is entrained to the onset of darkness.

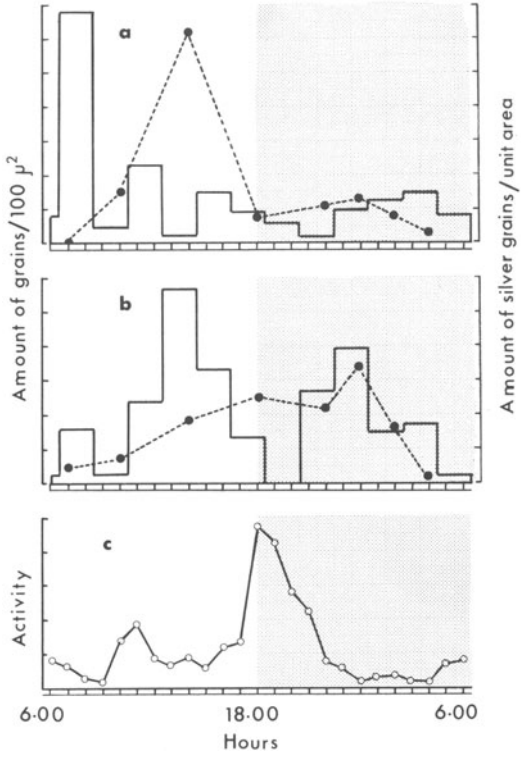

Figure 6.4. House crickets, *Acheta domesticus*, originating from LD 12 : 12. a Intensity of RNA synthesis in neurosecretory cells of the pars intercerebralis—histograms; intensity of protein synthesis from same cells – – ● – –. b Intensity of RNA synthesis in the ventral cells of the suboesophageal ganglion—histograms; intensity of protein synthesis in the neurosecretory cells of the same ganglion – – ● – –. c Locomotor activity expressed in number of movements per hour (after Cymborowski and Dutkowski [106, 108]).

After cauterization of the neurosecretory cells of the pars intercerebralis no RNA synthesis was observed in the suboesophageal ganglion neurosecretory cells on the third day after the operation [107]. Dutkowski and Cymborowski conclude that there can be no further RNA synthesis in these cells at any time once the pars intercerebralis has been destroyed. It is, perhaps, premature to draw this conclusion however, since trauma may suppress RNA synthesis, and after many different types of operation a cessation of rhythms has been observed for a period of some days before they reappear [8, 28]. Indeed even in normal conditions rhythms may cease to be overt for a period of time,

although when they reappear their phase-setting is such that it is apparent that they have continued at a subthreshold level.

In contrast to the post-operative lack of RNA synthesis in the suboesophageal ganglion, synthesis in the follicular epithelial cells of egg vesicles is increased, although here too the rhythmical fluctuations cease, as they do in the fat body cells.

A further study has been made on the normal fluctuations in protein synthesis in the same nerve cells [108]. In the pars intercerebralis cells maximum protein synthesis occurred about 7 h after both RNA synthesis and accumulation of neurosecretion reach their maxima. In the suboesophageal ganglion, in which protein synthesis does not reach such a high level, a gradual rise continues during the light period and, after a slight drop at the beginning of the dark period, reaches its maximum about half way through the dark period. The fluctuation in the number of cells filled with neurosecretion is not as distinct as in the pars intercerebralis, and the results are difficult to interpret, but the accumulation reaches its maximum towards the end of the dark period, and its minimum before the onset of darkness.

In view of the number of types of cell which have been shown to vary in a rhythmical manner in respect of size, content, or synthetic activity, it is difficult to draw any conclusions about the sequence of events, since all are likely to be affected by the total physiological and behavioural rhythmicity of the animal. Indeed Rensing [109] has found that the volume of the nuclei of the salivary glands of *Drosophila* shows rhythmical fluctuations in culture solution, for up to ten days after the removal from the insect, suggesting that a metabolic circadian rhythm may indeed be present in all cells [110], for it seems extremely unlikely that the salivary gland cells would be the basic biological clock responsible for maintaining the circadian rhythmicity of the entire organism.

Perhaps the rhythmical changes which have been observed are more significant in relation to the way in which insects, like other organisms, show differing responses to various stimuli at different times of day. Rensing [109], for example, has found that the rhythm of the cultured salivary gland cells is affected by pulses of ecdysone, but when this is added to the medium at one time of day a phase-shift of between 3 and 9 h occurs, whereas its addition at other times of day is not followed by any phase shift. This clearly has implications for the way in which insects may react to ecdysone during moulting, and an extension of this type of study might give information about moulting rhythms. However it must be emphasized that it seems very likely that a multiplicity of cycles may be involved in any physiological rhythm, and that different cells may be "sensitive" at different times, so that only certain combinations, or sequences, of sensitivity may give any particular physiological event.

6.5 THE CONTROL OF THE LOCOMOTOR ACTIVITY RHYTHM

The search for a specific system which might control locomotor activity rhythms has been diligently pursued for a number of years, and considerable controversy has arisen over the results.

The first suggestion that hormones might be involved in the control of these rhythms came from experiments in which apparently arrhythmic cockroaches, after being joined in parabiosis with rhythmic cockroaches, took up a clear locomotory rhythm [110]. It now appears possible that this result may have been affected by the conditions under which the activity of the insects was recorded, for while the insects appear to have become arrhythmic in LL in the type of recorder then used, rhythmicity is maintained in LL in running-wheel recorders. It might thus be argued that while the cockroaches before the operation showed no overt rhythm sub-threshhold rhythms become overt under the stimulus of wounding. Little is known about the effect of wounding; Brady [8] has found a tendency for a decrease in the amount of activity at the peak of the circadian rhythm after one type of wounding, although other types of stress are followed by an increase in the activity at the peak period. However nothing is known about the particular set of conditions relevant to parabiotic experiments, and further work is clearly needed. On the other hand rhythms free-run in LL, so that the phase-setting of the rhythms of animals maintained in LL would not always be identical with that of animals maintained in LD 12 : 12, yet in the parabiosis experiments the arrhythmic animals took up a rhythm in phase with that of their parabiotic partner. In any case over the following 16 years a number of hormones have been found to affect activity rhythms.

To verify the assumption that an endocrine system is involved in the control of activity, the main endocrine systems were removed by beheading the insect; cockroaches can still walk after this operation, but are arrhythmic [3], as has since been confirmed by a number of authors [8, 111, 112, 113, 114]. Transplantation of the brain, corpora cardiaca, corpora allata, or thoracic or abdominal ganglia together with their associated neurohaemal organs, from rhythmic donors into headless cockroaches is not followed by any rhythmic activity [3, 5, 8, 115]. On the other hand the transplantation of a suboesophageal ganglion from a rhythmical donor into a headless cockroach is followed by an overt activity rhythm, in phase with the previous rhythm of the donor.

This latter result has been particularly controversial. Roberts [112] attempted to repeat these experiments, using a running-wheel recorder, and in 19 trials reported no successes, although Brady [9] comments that the method of presenting the results might obscure some rhythms. A film has since been taken of the movements of headless cockroaches in

a running-wheel, similar to that used by Roberts, and it can be seen that, owing to the postural stance of a beheaded animal, the insect tends to tip forward on a curved surface, pushing down on to the severed neck and frequently struggling violetly. Brady [9] also repeated the experiments, using a number of different types of recorder, and concluded that in 2 out of 29 trials suboesophageal ganglion implants appeared to induce rhythms in their hosts, with a further eight possibly becoming rhythmic. While this does not provide certain confirmation it does not rule out this method of control, for *any* positive result is important in showing whether a rhythm can be induced by a particular method.

However the question remains as to what differences in method have been involved in the operations performed by the different authors. Apart from the fact that the present author has always left the nerves of the ganglion as long as possible for easier handling, and that when Brady did this he observed some evidence of an induced rhythm in four out of nine trials, no obvious difference in technique has been discovered.

Nishiitsutsuji-Uwo & Pittendrigh [114] claim to have found positive evidence that the suboesophageal ganglion cannot control the activity rhythm by a hormonal agent in the cockroach *Leucophaea.* They found that "severance of the ventral cords between the first thoracic and the suboesophageal ganglion caused nearly total inactivity"; they also found that "the animal is so inactive that the presence or absence of rhythmicity cannot be resolved". However they later state that the arrhythmia of 10 out of 14 animals in which the ganglion was present, but in which the nerve cord had been severed anterior to either the first or second thoracic ganglion, is incompatible with any humoral control from the suboesophageal ganglion. It is difficult to reconcile all these statements.

In another experiment these authors cut the circum-oesophageal connectives between the brain and the suboesophageal ganglion, and found that five out of six animals immediately resumed their normal rhythm of activity. This result rules out the control of rhythmicity by a nervous pathway from the brain. Brady [116] has questioned this result; he finds that in *Periplaneta* this operation is followed by such a high level of activity that any rhythmicity would be masked, he also quotes a personal communication from Roberts stating that rhythmicity is lost in *Leucophaea,* and Brady suggests that Nishiitsutsuji-Uwo & Pittendrigh had in fact cut the maxillary nerves rather than the circum-oesophageal connectives. It is difficult to see how such skilled operators could, however, have made such an error. Furthermore in the cricket *Acheta domesticus* the entire brain can be removed and rhythmical activity is still maintained [118]. In this latter insect not even hormonal, let alone nervous, control from the brain can be involved, and since it has been confirmed that the thoracic and abdominal ganglia play no part in the

control of the rhythm only the suboesophageal ganglion can be doing so.

The possible control of rhythmicity by a humoral agent from the brain has been explored by a number of workers, and again the results are contradictory. Roberts [112] found that surgical lesions bisecting the pars intercerebralis of *Leucophaea* produced arrhythmicity, although lesions lateral to this area did not do so, and in a few insects rhythmic activity reappeared some weeks after the former operation. These results were later questioned by Nishiitsutsuji-Uwo and Pittendrigh [114], who found that many animals remained rhythmic after protocerebral bisection, or even when one whole lobe of the brain was removed. In an earlier paper [119] it was claimed that removal of the neurosecretory cells of the protocerebrum caused loss of rhythmicity. On the other hand only 19 out of 47 animals became arrhythmic in these circumstances, and the authors concluded that in the remaining animals some neurosecretory cells must have remained intact. Brady [9] cauterized the medial neurosecretory cells *in situ* and found that the animal remained rhythmically active thereafter.

From the results of later experiments Nishiitsutsuji-Uwo & Pittendrigh [114] conclude that the lateral neurosecretory cells are involved, for when a lateral-sagittal cut has been made through both protocerebral lobes all of the seven operated animals failed to recover any rhythmicity before they died. This conclusion must be based on the assumption that the critical affect of this operation is the cutting of the axons of the neurosecretory cells. The authors note however that the brain of the one animal examined histologically was completely deformed, and that it was impossible to recognize normal tissue relationships, so that the question remains as to what effect such an operation has on the total nervous control of the animal: from the results of this experiment alone it would not be possible to conclude that it must be the cutting of the neurosecretory axons which stops the locomotor rhythm. Indeed it is well known that destruction of the pars intercerebralis is followed by degeneration of the central body, and that the central body is the co-ordinating centre for pre-motor interneurones, so that such degeneration interferes with movements of all kinds. The endocrine role has, however, been argued by the authors because they have shown that cutting the circum-oesophageal connectives does not stop the rhythm, and they therefore assume that hormonal control must be derived from the brain, since they have also assumed that it cannot come from elsewhere. As noted above there is still no certainty about any of these assumptions, and the question has also been raised as to whether the connectives had actually been cut.

In yet another set of operations the optic nerves were cut between the compound eyes and the optic lobes, and although the animals remained rhythmic the rhythm could no longer be entrained by LD. When the

optic tract between the optic lobes and the rest of the brain was cut on both sides, thus isolating the optic lobes, 14 out of 18 animals became permanently arrhythmic; when the optic lobes were removed completely all six animals became arrhythmic. From this interesting result it has been concluded that the circadian rhythmicity of locomotor activity is ultimately caused by an autonomous self-sustaining oscillator in the nervous output of the optic lobes, and that this causes a non-autonomous circadian rhythmicity of secretion by the pars intercerebralis, which in turn imposes a circadian periodicity on the activity of the thoracic ganglia.

This attractive theory must still be questioned however, for, as has been pointed out, it is still not proven that the secretion from the pars intercerebralis controls the activity of the thoracic ganglia, and although removal of the optic lobes caused arrhythmia in all animals, the severance of the nervous connection between the brain and the optic lobes did not prevent three animals from retaining a weak rhythm and one from retaining a perfectly normal rhythm. No nervous control from the optic lobes could have been involved in these rhythmical animals, and for them the optic lobe could not be the primary clock.

Despite the divergence of opinion about which regions are involved in the control of activity rhythms it seems very likely that at least two systems are involved, one endocrine and one nervous, as was originally proposed by Harker [5], but whether either, or both, are autonomous for any length of time, or whether there is considerable feedback from other systems remains to be seen.

The questions which have been raised by the different types of activity shown in different types of recorder may in the end lead to a further understanding of the whole problem. Already the results from two types of recorder have brought to light an endocrine system which, although not controlling the rhythm, plays a part in the intermediary processes. In a photocell recorder cockroaches became arrhythmic after the nerve carrying neurosecretion between the corpora cardiaca and the suboesophageal ganglion is cut [115]. Cockroaches in a running-wheel, in which there is considerable feedback from the environment, do not however become arrhythmic even when the corpora cardiaca are removed altogether [8]. These results suggest that the corpus cardiacum hormone is involved in the actual control of the level of activity in the absence of other types of stimulation, but that in the presence of other types of feedback (perhaps nervous) the corpus cardiacum system is bypassed.

Curiously little attention has been paid to the role of the endocrine system in relation to the metabolic processes in the insect. As has been noted earlier, the trehalose concentration in crickets fluctuates rhythmically, but the peak concentration does not coincide with the

peak of either the locomotor or feeding rhythm. In general carbohydrates are transported in the blood of insects in the form of trehalose, and it is utilized as a source of energy during flight and almost certainly during other locomotor activity. An independently maintained rhythm of trehalose concentration would seem to be an excellent example of the way in which rhythmical activities could aid in the preparation for events which may be triggered by predictable fluctuations in the environment.

The conversion to trehalose of the glycogen stored in the fat body is under the control of the corpus cardiacum hormone, the major effect being produced by the secretion from the glandular lobe (that is, the region of the corpus cardiacum which is intrinsically secretory, and not concerned with the storage of hormones coming from the brain neurosecretory cells). The secretion acts on two enzyme systems, converting the fat body phosphorylase to its active form, and effecting the breakdown of glucose-6-phosphate to trehalose-6-phosphate. The secretion also seems to effect the actual transfer of trehalose across the cell membrane into the haemolymph [120, 121]. The neurosecretory storage region of the corpus cardiacum also contains a hyperglycaemic factor, and it is well known that stress causes release of both secretions: these hormones also affect the rate and amplitude of heart beat, gut peritalsis and the movements of the malphigian tubules. When an insect is in a running-wheel it may be under a certain amount of stress once it begins to move; perhaps in these circumstances the corpus cardiacum is stimulated and its secretions heighten the level of activity, thus producing the very intense activity so often seen in these recorders. In a cardiectomized insect under stress the secretion which would normally pass from the brain down the axons to the cardiaca may still be released from the ends of the cut axons, and it may be in this way that the high level of activity is maintained in such animals.

The rhythmical fluctuations of a number of other physiological systems have been noted earlier in this chapter, some of which do not appear to be directly related to the rhythm of locomotor activity, but it would be surprising if they too did not affect such activity, which must in the final analysis be a function of the overall condition of the total animal. In addition the role of proprioceptive stimulation has not yet been considered at all. Perhaps the rather simple systems which have so far been postulated have only touched the surface of the problem.

6.6 CIRCADIAN RHYTHMICITY AND MOSQUITO BEHAVIOUR

Now that many of the major lines of work on circadian rhythmicity in insects have been outlined, it is interesting to take one group, with complex circadian behaviour patterns, and consider how far we have proceeded towards an understanding of this rhythmicity.

Adult mosquitoes have been chosen, despite the multiplicity of species and their vast geographical range, for this group has probably been more extensively studied in the field than any other (over 60 papers have been published by Haddow and his colleagues alone), and very careful attention has been paid to their different behavioural activities.

The flight activity of the adult, from the time of eclosion, can be divided into four categories: (a) flight associated with host-seeking; (b) flight associated with mating; (c) flight associated with oviposition after a blood meal has been taken; (d) what may be loosely termed "free flight", that is flight which has not yet been correlated with any other activity.

Haddow [122] suggests that the different types of biting behaviour fall into two main classes: (i) that in which there is a single, very short and very pronounced wave of biting activity, although there is always scattered biting at other times; (ii) that in which biting activity goes on over a prolonged period. This latter type is strikingly irregular apart from being either diurnal or nocturnal, and comparison of counts from different days shows very marked differences in timing. Because of the considerable differences between the two types of biting cycle the published records need careful attention for, as Haddow points out, where observations have been grouped into longish periods, for example 4-h periods, not only may the sharpness of a pronounced wave of biting activity be concealed, but minor peaks may be completely eliminated.

By analogy with both laboratory experiments and field studies in other animals it would be expected that changes in light intensity might play a major role in defining the timing of an activity peak. It is difficult to relate changing light intensity to biting cycles in forests, however, because of the continuous intensity fluctuations caused by movement of the vegetation. To avoid this difficulty a study has been made on a tower in Zika Forest at a height of 80 ft, above the surrounding low canopy, but no relationship between the onset of biting time and absolute light intensity was revealed: on the other hand a clear relationship appears between the onset of biting and the angle of the sun [123]. When Crep Units [124] (a measure of the length of time between sunset and civil twilight, when the sun is 6° below the horizon) are plotted against biting times the form of the waves of activity are clearly revealed. As the authors comment these results strongly suggest that the *rate* of change of light intensity is the critical factor which determines the onset of crepuscular biting. For the seven species observed in this study onset of biting falls within the very short period when the rate of change is reaching its maximum.

Few experimental studies have been made with insects on the effect of rate of change of light intensity, but it has been established that the threshold control of swarming of *Culex pipiens fatigans* is not simply one

of light intensity, but requires a gradual change of intensity towards the permissive range [44].

Clear as these results are, however, it is difficult to correlate them with records from a tower in Mpanga Forest [125] which show that although at the canopy level the main wave of biting of *Mansonia fuscopennata* begins when the light intensity falls to about 0.5 ft candles and ends when it drops to 0.002 ft candles, the biting activity at ground level continues throughout the day although the light intensity is at times as high as 300 ft candles.

These results suggest that the level at which mosquitoes bite may also be related to light intensity, and indeed very clear vertical movements are seen in, for example, *Aedes ingrami,* and in *Taeniorhychus* which bite almost exclusively at ground level by day and in the trees at night [122].

The preferred level at which biting takes place has not been studied in the laboratory, yet this aspect must have a marked effect on laboratory results, for in some species the precision with which levels are maintained is extraordinary. *Eretmapodites chrysogaster,* for example, bites from 6 to 18 in above the forest floor; if a human bait lies down flat hardly any of the mosquitoes will bite, whereas increasing the bait level by lying on the side brings immediate biting [126]. Clearly here again further studies are badly needed.

A further complexity revealed by studies of biting cycles is the change in biting pattern with the type of habitat. McClelland [127] for instance, found that the domestic population of *A. aegypti* on the Kenya coast shows a very ill-defined biting cycle, whereas an outdoor population of the same species in Uganda has a very sharply defined cycle. Furthermore in the latter area the time of biting by the same species found inside huts differs by 12 h from the biting time outside the huts. Although light intensity must be a factor involved in such phase-differences there must also be many other factors concerned, and again there is little experimental evidence on this point. The recent concern with variation between activity records of insects in different types of recorder in the laboratory suggests that many significant variables may have been ignored in other laboratory experiments in the past.

Flight activity apparently unassociated with biting cycles has also been studied, and it is useful here to consider whether biting activity can be associated with a general activity rhythm. Haddow [125] has examined the relationship between a number of activities of *M. fuscopennata* (Fig. 6.5). It can be seen that the biting patterns vary from level to level in the forest, but at all levels in the forest they differ from the pattern in huts; the general flight activity pattern in the forest also differs from that outside the forest. It might also be expected that the flight activity of males would influence that of females, but this does not appear to happen, and the swarming of males is confined to the hour after sunset

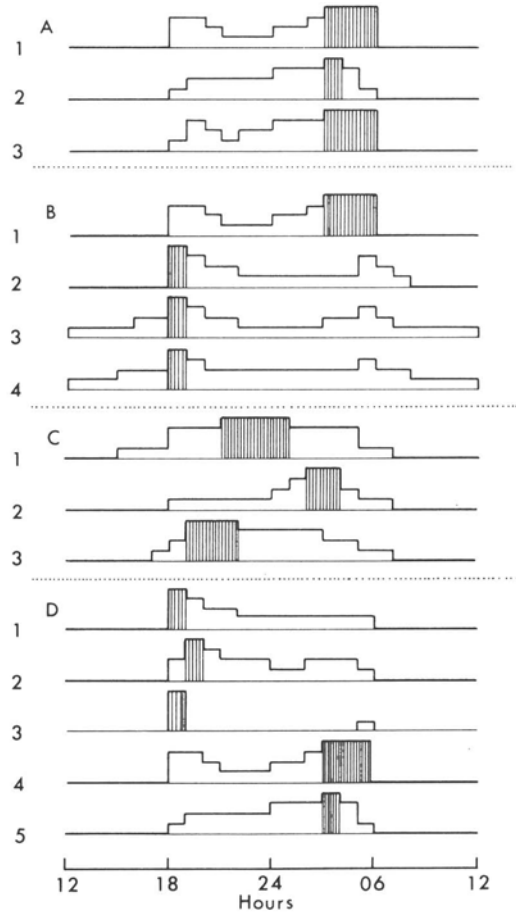

Figure 6.5. Synoptic figure of the circadian rhythms shown by *Mansonia fuscopennata* in the Entebbe area. Hatched areas represent maximal activity of each function. *A* (1) Attraction of females to mercury-vapour light in forest; (2) Attraction of females to light in open; (3) Oviposition. *B* (1) Oviposition; (2) Biting activity above forest canopy; (3) Biting activity in the canopy and understory; (4) Biting at ground level in forest. *C* (1) Biting activity in banana plantation; (2) Biting activity in huts; (3) Probable entry into huts. *D* (1) Attraction of males to light in forest; (2) Attraction of males to light in the open; (3) Swarming activity of males; (4) Attraction of females to light in the forest; (5) attraction of females to light in the open (after Haddow [125]).

and the hour before sunrise. Within the forest, apart from a close correlation between oviposition and flight activity, which will be discussed later, there does not seem to be any relationship between the biting cycle and the general activity rhythm: this is rather surprising since it might be expected that when the insect is most active there would be more frequent contact with the host.

Haddow in discussing these rather unexpected findings points out that Harker's [110] theory of multiple clocks, each of which may be phase-set to a different time in relation to an entraining factor, does not explain the difference in timing of a single activity in different habitats, and he postulates strong environmental influences as the causative factor. Environmental factors must of course be setting the phase in any case, but it is possible that the expression of some rhythms is inhibited by exogenous factors, and peak activity occurs only when the inhibitory factor ceases to act; what is then seen as a peak would not appear as such if the true circadian peak had not been suppressed. Corbett [128] has, for example, shown that in exposed sites outside a forest *A. africanus* oviposits at evening twilight, 4 h after the time dictated by the rhythm, because exogenous factors do not become permissive until then.

We still really need to know whether the effect of an environmental factor on one activity affects all other rhythmical processes in the same way, or whether the phase-setting of different activities can be dissociated. Examination of Fig. 6.5 suggests that although there is a major phase-shift in biting time between forest, banana plantation and huts, there is no such major shift in time of flight activity: perhaps there is here some dissociation of the rhythms. Another possibility is that small differences in genotype control the phase-setting in relation to the Zeitgeber; little is known of this aspect, although it is known that females of the butterfly *Colias,* differing by one sex-linked gene, fly at different times of day [21]. It is clear that we need to know more about the exogenous factors which operate in different types of locality, and their effects on the actual expression of each type of activity before the problems mentioned above can be solved.

Laboratory experiments on mosquito flight have shown that in LD 12 : 12 individual sugar-fed female *Anopheles* show a peak of activity after both light-on and light-off, but in DD only that peak associated with light-off persists. Light itself appears to have an inhibitory effect on this endogenous activity peak, and if the insects are exposed to light at this time virtually no activity takes place [130]. This finding is particularly relevant for many field studies in which flight activity is measured by the attraction of insects to light, for if the presence of light actually inhibits flight at the usual time of maximum activity then totally false conclusions could be drawn from collecting

records. A high-intensity light may also cause an abnormal degree of activity at certain sensitive times in the circadian cycle, as has been discussed in relation to cockroach activity. Nothing is known about this in relation to the attraction of insects to light-traps, but again it is possible that such an effect may give erroneous ideas about the patterns of normal flight activity.

Flight activity of individual *Aedes aegypti* in the laboratory under LD 12 : 12 shows a main peak of activity 1 to 2 h before the onset of darkness. Although the amount of activity is decreased in darkness the rhythm is maintained in DD, as it is in LL [131]. It was earlier suggested that the rhythm was not overt in LL [132], but examination of the figures suggests that a free-running rhythm with a period of about 26 h is present. In this connection it is interesting that the period is about 22.5 h in DD, so that this insect does not follow Aschoff's Rule, which demands that the period would be shorter in LL than in DD, although the total activity is in this case higher in DD in accordance with Aschoff's Rule.

Aedes aegypti shows a bimodal rhythm of flight in certain photoperiods: in LD 4 : 20, although activity is concentrated within a short light period, the phase-setting appears to be achieved by the light-off signal, although a very small peak may occur in the dark, being phased by the light-on signal. Both peaks are maintained on transfer to DD. In LD 20 : 4, however, although the rhythm is still bimodal, the minor peak which appears 22 to 23 h after light-off, and so here occurs during the next light period, is not maintained when a transfer is made to DD [131]. It is possible that this minor peak may represent a phase of the rhythm in which the insect is very light-sensitive, as we saw in the cockroach.

It is interesting that a LD 12 : 12 regime which is advanced by 6 h, through shortening one dark period by 6 h, causes the flight activity of *Anopheles* to be entrained very rapidly to the new cycle, peak activity shifting after just one exposure to the new light-off signal. By contrast if the LD regime is advanced 6 h by shortening one light period, several cycles of the new regime are necessary before the steady state is reached [131].

In natural conditions it is likely that heavy cloud may cause a decrease in light intensity unexpectedly early in the day; a circadian system which allowed for immediate entrainment so that high activity occurred early on the following afternoon would be clearly disadvantageous. On the other hand it is very unlikely that light intensity would increase hours earlier than normal in the day, and therefore the possibility of immediate entrainment is immaterial.

The adaptive value of peak flight activity in the early hours of morning or evening may possibly be related to the lower temperature at

these times of day, at least in very hot climates, for although temperature itself is not the primary entraining factor Rowley and Graham [133, 134] have shown that flight in virgin female mosquitoes is more readily undertaken, and sustained, at temperatures below 27° C.

An experimental approach to biting cycles has proved to be more difficult, and virtually nothing is known about this aspect in experimental conditions.

The common occurrence of bimodal biting cycles in the field has led to the suggestion that there may be two sections of the population involved, each showing different behaviour patterns according to age [122, 135, 136]. Several early studies [137, 138, 139] revealed no significant differences in the biting pattern of young and old females, although there appears to be a significant drop in biting "drive" during ovarian development [140, 141]. Corbet [142] however, found that although the biting cycle of *M. fuscopennata* is not related to the age-composition of the population, there are significant differences in the average age of females biting at different *levels* at different times. At ground level the mosquitoes biting at night are younger than those biting during the day: above the canopy the average age increases from sunset to sunrise, with a corresponding drop below the canopy. Corbet suggests tentatively that these age changes may be related to the fact that nullipara may have just mated before biting, whereas parous females will have oviposited before biting and their flight from oviposition sites may affect the position at which they bite.

The pattern of oviposition appears to be related to the biting cycle in some species, as has already been seen (Fig. 5). On the other hand although *A. africanus* shows a single oviposition peak followed by a biting peak, it also shows another general activity peak related to neither biting or oviposition [143]. McClelland [144] studied the oviposition periodicity of *A. aegypti* at 12 different heights above ground level, and with different exposures to sunlight, and in this species found a pattern resembling that of biting and flight activity.

A number of workers have explored the oviposition rhythm of captive populations of *A. aegypti,* and find that it can be entrained by a light period of as short a length as 5 min. When 5 min light is given every 24 h two oviposition peaks occur, one taking place before the onset of light and the other 4 to 8 h after onset of darkness [145-148]. The authors concluded that no oviposition cycles occurred in either LL or DD. In a later study Gillett [149] points out that ovarian development takes about 55 h from the time of the blood meal, on which ovarian development depends. The problem therefore arises as to how this developmental cycle contributes to a circadian rhythm of oviposition.

Gillett has used an ingenious experimental procedure which enabled him to follow the ovisposition cycle of individual mosquitoes. He found

that in LD 12 : 12 a mosquito which has completed ovarian development withholds its eggs until the first "available" laying period, and should all the eggs not be laid during that period at least some are retained for a further 24 h. In LL however the eggs are laid as soon as development is completed, and oviposition continues until all the eggs are laid. If there is a change to DD during ovarian development oviposition becomes rhythmical, and any mosquito which has already completed egg development close to the time when the light is switched off delays laying until 24 h after the onset of darkness. Although Gillett does not specifically make this point these results must imply that the rhythm is indeed a truly circadian one which can be maintained in DD.

McClelland [144] points out that testing oviposition rhythms in the laboratory may have drawbacks, in that confined mosquitoes are continuously exposed to some of the stimuli which would be received in the field only after appetitive behaviour, and the act of oviposition might occur in the laboratory in conditions which might not stimulate the act in the field. However oviposition rhythmicity of *A. aegypti* appears to show the same general characteristics in both field studies and in the laboratory study.

Just what selective advantage there might be in the synchronization of oviposition is difficult to assess, but it should be kept in mind that the actual act of oviposition may not be the major factor on which selection acts, for closely related to oviposition must be the timing of the flight activity which precedes egg laying. Those mosquitoes which oviposit early in the evening, and have a peak of biting activity later in the night, are thus ensured of having time for another blood meal after egg-laying and so can begin the sequence again in the shortest possible time [150].

Taylor and Jones [131] have made the interesting suggestion that the geographical range of *A. aegypti* is not, as previously thought, limited by the range of summer temperature, but may be limited by a range of summer day-length which will permit a physiological balance between factors controlling flight activity and oviposition rhythms.

This brief survey of the rhythmical activities of just one group of insects indicates the complexity and diversity of the problems which still await solution. Even should the nature of the biological clock itself be finally resolved we shall still be very far from understanding the full range of the inter-relationship between the clock, the physiological and behavioural systems, and the biotic and physical environment.

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