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# INSECT CLOCKS

BY

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## PREFACE

TIME is one of the three fundamental “quantities” in terms of which a physicist can describe the Universe; however, unlike the other two (mass and length), it is difficult to define. In this book I have looked at time from a biologist’s point of view, and in terms of the motion of the “heavenly bodies”, particularly the rotation of the Earth on its axis and around the Sun, and the revolution of the Moon around the Earth, movements which give rise to the familiar successions of day and night, months, years and tides. Organisms on this planet have been exposed to such rhythmic changes since life began, and this aspect of time must be the most meaningful one as far as they are concerned!

All aspects of physiology have a “time course”, and many phenomena — from heart beats and nerve impulses to the interactions between predator and prey — are rhythmic or oscillatory in nature. This book, however, is concerned only with those phenomena in which “environmental time” has a functional significance in the life of insects, enabling them to perform behavioural or physiological events at the “right time” of the day, month, year or tide. The majority of these rhythmic phenomena are endogenous, and when allowed to “free-run” in the absence of temporal cues, reveal a natural periodicity which is *close to* that of the solar day (or month, year or tidal cycle), accurate, and temperature compensated. They possess, in fact, the properties one normally attributes to man-made time measuring devices or clocks. It is the nature and functional significance of these insect clocks which is dealt with here: restriction of the examples to the Class Insecta merely reflects a life-long passion — or perhaps a prejudice — on my part.

The work has been organized so that the fundamental properties of circadian rhythms are presented first, followed by the longest sections on seasonal photoperiodism. This arrangement has been followed in order to present and discuss the problem of photoperiodism in terms of circadian rhythmicity. The alternative point of view that time measurement is accomplished by “hour glasses” has also been given full attention; my conclusion is that both forms of time measurement are to be seen in the insects, sometimes in the same species. Concluding chapters compare other insect clocks, rhythmic and non-rhythmic, with the circadian system, and describe what is known about the anatomical location of the circadian pacemakers and the photoreceptors which facilitate their entrainment to the environmental cycles of light and dark. Whilst much of the material and its interpretation has naturally been derived from the writings of others, I must accept all responsibility for reporting them faithfully, and for those opinions and suggestions which are mine.

The writing of this book has been greatly aided by stimulating discussions with my friends and colleagues, particularly C. S. Pittendrigh, J. Aschoff, A. D. Lees, J. N. Brady and W. Engelmann. These authors, together with many others, have given permission to reproduce copyrighted material, and in many cases provided me with original artwork or photographs of their published figures; these sources are individually acknowledged

in the respective figure legends. Much of my own research in the field of Insect Clocks has been carried out in the University of Edinburgh with the continued interest and encouragement of Professor J. M. Mitchison, and with the generous financial support of the Science Research Council. I also gratefully acknowledge the technical assistance provided by Mrs. Helen MacDonald and Mrs. Margo Downie, the help from Mr. D. F. Cremer in making photographic copies of most of the original figures, and from Mr. J. J. Holmes for redrawing Figs. 3.15 and 3.17. Lastly I would like to thank Miss A. Keegan for typing the manuscript.

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## CHAPTER 1

# INTRODUCTION: RHYTHMS AND CLOCKS

EVER since life first appeared on this planet it has been subjected to daily cycles of light and dark, and to seasonal cycles of climatic change, caused by the rotation of the earth around its axis and around the sun. Marine and intertidal organisms have in addition been subjected to tidal and lunar periodicities. Only those animals which have invaded the depths of the ocean, or underground caves and rivers, have avoided this fluctuating environment. Other species—especially those on the land, where daily and seasonal changes may include violent fluctuations in temperature and humidity—have developed strategies to counteract or to exploit this periodicity. The majority of insects, for example, show daily and annual cycles of activity and development. They may be nocturnal, diurnal or crepuscular. They may hibernate or aestivate. Plants may produce leaves or flowers only at certain seasons, and flowers may open and close at particular times of the day.

Some of these phenomena are direct responses to environmental change, but many more are overt manifestations of an endogenous periodicity. These innate rhythms must have astounded early workers such as the French astronomer De Mairan who discovered (in 1729) that the daily leaf movements of *Mimosa* would persist in constant darkness. The oscillations underlying such phenomena are now known to provide a temporal organization for physiological and behavioural activities in practically every group of organisms apart from the prokaryotes. Of particular interest are those endogenous oscillations which have evolved with a periodicity close to 24 hours (circadian rhythms), and are used by animals and plants to “time” daily events and thus allow the organism to perform functions at the “right time of the day”, or to attain synchrony with other individuals of the population. It is clear that these circadian oscillations in the cell and the organism have evolved to match almost exactly the oscillations in the physical environment. In *Drosophila melanogaster* the period of the pupal eclosion rhythm is inherited and the gene responsible has been located on the X chromosome (Konopka and Benzer, 1971). These rhythms, therefore, are not “imposed” on the organism by the environment, neither are they “learned”. The natural cycles of light and temperature, however, do serve to entrain and phase-control these endogenous oscillators so that under natural conditions their periods become exactly 24 hours. In the absence of temporal cues from the environment (i.e. in darkness and constant temperature) the rhythms “free-run” and reveal their own natural period ( $\tau$ ) which is close to, but significantly different from, that of the solar day. The observation that this period is temperature-compensated, and that the rhythms are used by the organisms to measure the passage of time (Pittendrigh, 1954, 1960), justifies the use of the term “biological clock”.

Apart from circadian rhythms which have evolved as a match to the 24-hour periodicity of the Earth's rotation around its axis, endogenous oscillations with tidal ( $\sim 12.4$  hour), semilunar ( $\sim 14.7$  day), lunar ( $\sim 29.4$  day) or annual ( $\sim$  a year) periods are also to be found in organisms, including the insects. In many cases the endogenous nature of these rhythms has been demonstrated by allowing them to "free-run" in the absence of the environmental cues (*Zeitgebers*) which normally entrain them.

The brief account of these biological oscillations given above—and the more extensive description of their properties given later in this book—amply demonstrate their endogeneity. They are, in fact, every bit as much a part of the organism as its morphological organization. Some investigators, however—principally Brown (1960, 1965)—have held an alternative view, namely that all of the observed periodicities are in some way exogenously controlled by "subtle geophysical forces" associated with the solar day (such as air pressure, periodic fluctuations in gravity associated with the Earth's rotation in relation to the Sun and the Moon, or cosmic ray intensity) which remain unaccounted for in laboratory experiments in which the obvious periodicities (light, temperature, etc.) have been eliminated. This view will receive no further attention in this book even though, theoretically, it must remain an open question until unequivocal experiments (perhaps involving organisms travelling away from the influence of the Earth) have been performed. As a partial answer to the endogenous-exogenous controversy, Hamner *et al.* (1962) maintained a number of organisms at the South Pole on a turntable arranged to rotate once every 24 hours counter to the Earth's own rotation, thereby eliminating most of the diurnal variables. Under these conditions several rhythmic systems, including the pupal eclosion rhythm in *D. pseudoobscura*, continued to show a circadian periodicity apparently unaffected by either their location at the South Pole or by their rotation on the turntable. Therefore, as far as these experiments or the results allow, the data support the endogenous hypothesis.

Although the clock analogy should not be pursued too closely it is a useful one, and there is an interesting parallel between the development of man-made "time-pieces" and those "clocks" found in nature. Early man was aware of the passage of time by watching the movement of the Sun, Moon, and stars, or by observing the movement of the Sun's shadow on the ground or on a dial. Such methods, of course, have nothing to do with clocks. Neither have the *direct* responses of animals and plants to daily periodicities. These exogenous effects are widespread in nature and in some animals the observed rhythm of activity is related to the immediate effects of the daily changes in light intensity. Under field conditions most daily rhythms of activity—although innate—are nearly always strongly modulated by the immediate character of the environment, particularly the rapid changes in light intensity at dawn and dusk. These effects will be discussed only where they modify an endogenous periodicity.

The first man-made time-measuring devices were probably sand-glasses, clepsydras (water clocks) and candles. These "clocks" did not oscillate and had to be reset or "turned over" once all the water or sand had run out, or the candle burnt to the bottom. This type of device finds its equivalent in some of the "hour-glass" timers performing night-length measurement in aphids which, after measuring the duration of the dark period, require to be "turned over" by light before they can function again (Lees, 1968).

Mechanical clocks introduced in the fourteenth and fifteenth centuries were either weight-driven or spring-driven and incorporated oscillatory devices which ran continuously so long as the weight was raised or the spring wound up. These find their counter-

part in the biological oscillations mentioned above. The escapement in these early clocks consisted of a crown wheel and a verge and foliot. The system was not isochronous and the clocks so constructed tended to lose or gain up to 15 minutes every day. In the seventeenth century the incorporation of a pendulum with an escapement to maintain a constant amplitude introduced isochrony to the clock, and brought the error down to about 10 seconds per day. Although the concrete nature of biological oscillations remains unknown, this pendulum analogy and sine-wave representation of the oscillation's time course are often instructive and useful in model building.

For really accurate time measurement temperature-compensation is required. In man-made clocks an uncompensated pendulum lengthens as the temperature rises and therefore swings more slowly. By the eighteenth century George Graham had compensated for such temperature changes by using a mercury-vial pendulum. When the quantity of mercury was correctly adjusted its thermal expansion raised the centre of oscillation to compensate for the lengthening of the pendulum rod. Graham's clock varied by as little as 1 second per day; Harrison's grid-iron pendulum, which operated on a similar principle, later cut this error down to less than 1 second. In biological systems most physiological processes more than double their rate with every  $10^\circ$  rise in temperature, and such temperature effects would render time measurement impossible. However, during evolution this challenge has been met: most biological oscillators with a "clock" function have a  $Q_{10}$  between 0.85 and 1.1. This property is an absolute functional prerequisite for a clock mechanism. It is also essential for effective entrainment by a natural (24-hour) *Zeitgeber* because if the oscillator had a  $Q_{10}$  of 2.0 or more it would, at some temperatures, fall outside the limits within which the light-cycle could hold it. The manner in which temperature-compensation is achieved in biological clocks, however, remains as obscure as the nature of the oscillations themselves.

The "clock" analogy begins to founder at this point. In man-made clocks hour-glasses clearly antedate oscillators, but the reverse would seem to be the case in biological systems. Pittendrigh (1966) suspects that circadian oscillations—which occur in all eukaryotes and possess the common but somewhat "improbable" properties of accuracy and temperature-compensation—are monophyletic in origin and therefore very ancient. Although it is far from clear what their original functional significance was, they are now widely used for the purposes of chronometry. In many species of animals and plants the circadian system is causally involved in the measurement of day- or night-length in "classical" photoperiodism. In some insects, on the other hand, this function is performed by means of an "hour-glass" rather than by circadian oscillations—which they surely must possess. Evidence of an evolutionary convergence such as this suggests that the adoption of an hour-glass for photoperiodic time measurement is a comparatively recent event.

Many aspects of insect physiology and behaviour are "clock-controlled". There are, for example, daily rhythms of general locomotion, feeding, mating, oviposition, pupation, and pupal eclosion, in which these activities are restricted to a particular part of the day or night. Photoperiodism also involves a clock which measures day- or night-length, the most frequent response being the seasonal appearance of a dormant stage in the life cycle. The adaptive significance of diapause is clear, but it is not always easy to see the adaptive significance of daily rhythms, and in the absence of concrete experimental evidence most conclusions must remain conjectural. However, adults of *Drosophila pseudoobscura* emerge from their puparia close to dawn when the relative humidity of

the air is at its highest, and it is known that success in the act of eclosion is greatest under these conditions (Pittendrigh, 1958). Cycles of feeding may be correlated with the supply of food: the classical example of this is probably the "time-memory" (*Zeitgedächtnis*) of bees. Bees can be "trained" to visit a food source at a particular time of the day (Beling, 1929), this mechanism ensuring that they visit nectar sources every day at the same time. The significance of this behaviour lies in the observation that not only do flowers open and close at particular hours, but that nectar production is also a circadian event (Kleber, 1935). In many cases the selective advantage of an event being clock-controlled lies in the synchrony attained between individuals of the population. Mating rhythms of certain Diptera, for example, ensure that all sexually active individuals in the population are looking for mates at the same time and thereby increase the likelihood of successful encounters between the sexes. Differences in mating times between different species are also known to provide effective mechanisms for genetic isolation (Tychsen and Fletcher, 1971).

Biological clocks have been classified in a number of ways. Pittendrigh (1958) differentiated (1) "Pure" rhythms, such as colour change in the crab *Uca pugnax* (Brown *et al.*, 1953), from (2) Interval timers, in which a particular event such as pupal eclosion occurs at a particular time of the day, and (3) Continuously consulted clocks such as the bees' *Zeitgedächtnis* and time-compensated sun orientation in which time may be "recognized" at any time of the day. Lees (1960a) has also used the term interval timer to describe some of the non-oscillatory timing devices in aphids.

Truman (1971d) has recently proposed that animal clocks fall into two well-defined groups. In Type I, such as the rhythm of pupal eclosion in *Drosophila* spp. (Pittendrigh, 1966) and *Antheraea pernyi* (Truman, 1971a), the compound eyes (or other "organized" photoreceptors) are not involved, the photoreceptors lying in the brain itself. These rhythms are also damped out by moderate intensities of continuous light, and the magnitude of the phase-shifts generated by quite short light perturbations may be in the order of 10 hours (for *D. pseudoobscura*). These clocks are generally associated with developmental rhythms such as hatching, moulting, eclosion or release of brain hormone. Truman also places photoperiodism in this category. In Type II clocks, such as those controlling locomotor activity rhythms, the compound eyes are the principal and sometimes the only photoreceptors involved and the brain itself is insensitive to light. These rhythms "free-run" in both DD and LL of quite high intensity and the phase-shifts generated by light perturbations are usually much smaller than in Type I. Truman includes *Zeitgedächtnis* and time-compensated sun orientation in this category solely because of their association with locomotor activity.

Although this scheme is attractive and points out several possibly fundamental differences between biological clocks, too few species have been examined in sufficient detail and it may be premature to adopt the classification. Nevertheless, the distinction between developmental rhythms which can only be appreciated in mixed-age *populations*, and those such as general locomotor activity which are performed repeatedly by *individual* insects often over quite long periods of time, is certainly a useful one. Consequently this distinction is used in the present book, and forms the basis for the first two chapters.

### **Annotated Summary**

1. Insects, like other organisms, have evolved in an environment dominated by daily, monthly, annual and, in some cases, tidal periodicities.

2. This environmental periodicity is frequently matched by an appropriate endogenous rhythmicity which is a constituent and characteristic physiological feature of living tissue. Organisms may possess circadian (~24 hours), circa-tidal (~12.4 hours), semi-lunar or circasyzygic (~14.7 days), circa-lunar (~29.4 days) or circannual (~a year) periodicities.

3. These oscillations have natural periods which are approximately equal to that in the environment, are accurate, and are temperature-compensated. They provide the organisms with a "temporal organization" allowing them to perform functions with a selective advantage at the "right time of the day", or to "measure time" as "biological clocks".

4. Biological clocks control a wide variety of behavioural and physiological activities in insects. These include daily rhythms of locomotion, feeding, mating, oviposition, pupation and eclosion. These rhythms may be operational either in individual insects or in populations which behave, in this respect, like "super-organisms". Clocks also control cuticle deposition, metabolism and the seasonal control of alternate developmental pathways (photoperiodism).

## CHAPTER 2

# CIRCADIAN RHYTHMS OF ACTIVITY IN INDIVIDUAL INSECTS

INSECTS, like other organisms, usually restrict their activity to certain times of the diel cycle. In natural conditions, or in the artificial light and temperature cycles provided in the laboratory, they may be—with respect to a particular activity—either night-active (nocturnal), day-active (diurnal) or twilight-active (crepuscular). The mechanisms controlling these activity rhythms may be exogenous (i.e. a direct response to environmental changes) or endogenous (i.e. controlled by an underlying circadian oscillation, or oscillations, which are a part of the physiological make-up of the organism). Most activity rhythms have proved to be a “mixture” of endogenous and exogenous components, the overt rhythm of activity, although controlled by an endogenous oscillation, being continuously modulated by the *direct* effects of the environmental cycles of light and temperature, particularly the abrupt changes in light intensity at dawn and dusk. Here we are mainly interested in the endogenous aspects of rhythmic phenomena because the intrinsic and self-sustained physiological oscillations controlling them function as “biological clocks”, and provide temporal organization for a wide array of behavioural activities.

This chapter is concerned with those aspects of activity such as general locomotion, flight, feeding, and oviposition which are performed repeatedly by *individual* insects and may persist as a daily rhythm for quite long periods of time. “Once-in-a-lifetime” events such as egg hatching, moulting, pupation and adult eclosion—which may also be governed by an on-going circadian oscillation—will be discussed in Chapter 3: questions of photoreception and the location of the “clock” will be dealt with in Chapter 10.

Activity rhythms have been studied in a wide range of insect types. Here we will illustrate the general properties of the circadian organization of these rhythms using some of the most intensively investigated systems. These include the general locomotor activity of cockroaches (Harker, 1956; Roberts, 1960), crickets (Lutz, 1932; Nowosielski and Patton, 1963), beetles (Lohmann, 1964; Birukow, 1964) and stick insects (Eidmann, 1956; Godden, 1973), and flight activity in mosquitoes (Jones *et al.*, 1967; Taylor and Jones, 1969) and other species of Diptera (Roberts, 1956; Brady, 1972). Emphasis is placed on the more recent or on the more fully investigated species, but a more comprehensive list of those insects exhibiting activity rhythms of this kind is presented in an appendix.

### A. Activity in Light/Dark-cycles

Cockroaches are almost entirely nocturnal in their habits. Under natural and laboratory conditions activity generally commences at or soon after dusk and continues more or less throughout the dark period; the insects become inactive during the day (Gunn, 1940; Mellanby, 1940; Harker, 1954; Roberts, 1960). In the laboratory their large size makes the recording of their activity a relatively easy task. Harker (1956, 1960a), for example, recorded the locomotor activity of *Periplaneta americana* in rocking actographs or in phototransistors using very dim red light, or by attaching a fine wire to the pronotum which wrote on a smoked drum when the insect moved. Roberts (1960) and a number of other authors have used running wheels.

In an artificial cycle of 12 hours light and 12 hours dark (*LD 12 : 12*) most cockroaches commence activity shortly after the onset of darkness. Considerable variation between individual insects, and between sex, age and physiological state is apparent, however. Roberts (1960) used males of *Leucophaea maderae*, *Byrsotria fumigata* and *P. americana* in preference to females because their activity was "less erratic". Leuthold (1966) found that the activity rhythm of female *L. maderae* varied with the insect's reproductive state, locomotion being suppressed when mature eggs were present in the lower reproductive tract. Working with mature adult females of *P. americana*, Lipton and Sutherland (1970) found no activity rhythm that was obviously related to the lighting regime, and similarly concluded that the reproductive cycle interfered with the normal expression of the rhythm. Virgin females, on the other hand, exhibited an entrained rhythm very similar to that shown by adult males. Amongst the males they also found considerable variability. The majority showed a clearly entrained rhythm of activity, or at least a weak nocturnal rhythm or "pattern", but about 4 per cent were apparently random in their activity. Most of those with a well-marked rhythm showed the typical onset of activity within the first few hours of dark, but over 30 per cent showed a secondary active phase in the first few hours of light. A similar variation in rhythmicity has been recorded by Nishiitsutsuji-Uwo *et al.* (1967); Ball (1972) has also described individuals of *Blaberus craniifer* with secondary activity after dawn.

Harker (1956) showed that most of the feeding took place during the active period. Nevertheless, when food was offered in the light period only, the insects became active at this time as well as during the night. When feeding was discontinued, however, the daytime feeding peak did not persist and she concluded that the activity rhythm in *P. americana* was not an expression of a hunger cycle.

The locomotor activity rhythm in the house cricket *Acheta domestica* is similar to that in cockroaches. Lutz (1932) showed that activity commenced soon after dark and continued for about 4 to 6 hours. As with cockroaches, however, the pattern of activity varied between individuals and with age. Nowosielski and Patton (1963), for example, showed that last instar larvae rarely showed a pronounced rhythm, and that some adults were biphasic with a second peak *prior* to the onset of dark. Cymborowski (1973) demonstrated three types of individual in *LD 12 : 12*: some commenced activity *at* the light/dark transition, some commenced activity up to 3 hours *after* dark, and some began their period of intensified activity as much as 1 hour *before* light-off.

The activity rhythms of several mosquito species have been recorded by automatic devices in which the flight noise is amplified (Jones, 1964; Nayar and Sauerman, 1971). In *LD 12 : 12* *Anopheles gambiae* is nocturnal but with an intense activity lasting 20 to

30 minutes following both light-off and light-on (Jones *et al.*, 1966; Jones *et al.*, 1967). *Aedes taeniorhynchus* is also a night-active insect with a similar bimodal pattern (Nayar and Sauerman, 1971). In this species the activity pattern originates in the adult instar and is not carried over from the developmental stages. The yellow-fever mosquito *A. aegypti*, however, is a diurnal insect with a main peak of activity about 1 to 2 hours before light-off and little or no activity in the dark portion of the cycle (Taylor and Jones, 1969). A bimodal pattern with a smaller peak following dawn is also apparent in this species.

The tsetse-fly *Glossina morsitans* is strictly diurnal. In a rocking actograph at LD 12 : 12 its activity occurs during the light in short bursts of about 1 minute duration separated by long intervals (Brady, 1970, 1972). Nevertheless, the mean hourly activity of groups of insects (teneral unfed males) reveals a clear V-shaped diurnal pattern with peaks in the morning and evening, similar to that observed in the field; activity during the dark is almost negligible. The question of bimodality in activity rhythms, especially in mosquitoes and tsetse-flies, will be re-examined in later sections, particularly with respect to its endogeneity.

## B. The Endogenous Nature of Activity Rhythms

### 1. "Free-running" behaviour in the absence of temporal cues

Rhythms of activity in a light/dark-cycle provide few clues as to the physiological nature of the controlling mechanism, which might have both endogenous and exogenous components. The endogenous nature of a rhythm, however, is usually revealed when the organism is transferred from a light/dark-cycle (LD) into continuous dark (DD) or continuous light (LL), provided that temperature and other possible *Zeitgebers* are also held constant (Aschoff, 1960). Under these conditions an endogenous oscillation controlling a rhythmic activity will "free-run" and reveal its natural periodicity ( $\tau$ ). In this state  $\tau$  often deviates slightly from 24 hours so that the onset or peak of activity appears either earlier or later by a few minutes every day. The fact that  $\tau$  is close to but rarely equal to 24 hours is powerful evidence for an endogenous oscillator which is uncoupled from the environment and not being "driven" by any uncontrolled *Zeitgeber* associated with the solar day.

Roberts (1960) studied the free-running rhythms of locomotor activity in the cockroaches *Leucophaea maderae*, *Byrsotria fumigata* and *Periplaneta americana*. When transferred to DD he found that the rhythms persisted for at least 3 months at a constant temperature of 25°C. In the three species studied  $\tau$  for individual cockroaches varied between about 23 and 25 hours. The value of  $\tau$  for an individual was not absolutely fixed, however, and in some instances was observed to change abruptly and spontaneously (Fig. 2.1). Nevertheless, the *range* of realizable  $\tau$  values in an individual insect is probably genotypic.

Earlier authors (Gunn, 1940; Harker, 1956) had reported a gradual loss of rhythmicity in cockroaches after a few days in constant light (LL). Roberts (1960), however, found no such loss for at least 20 days in *L. maderae* and for up to 7 weeks in *B. fumigata*. The difference between these results was attributed to the type of recorder used; in Harker's work, for example, tying the cockroach to a kymograph might have promoted a breakdown in activity not observed in a running wheel. Roberts (1960) also found