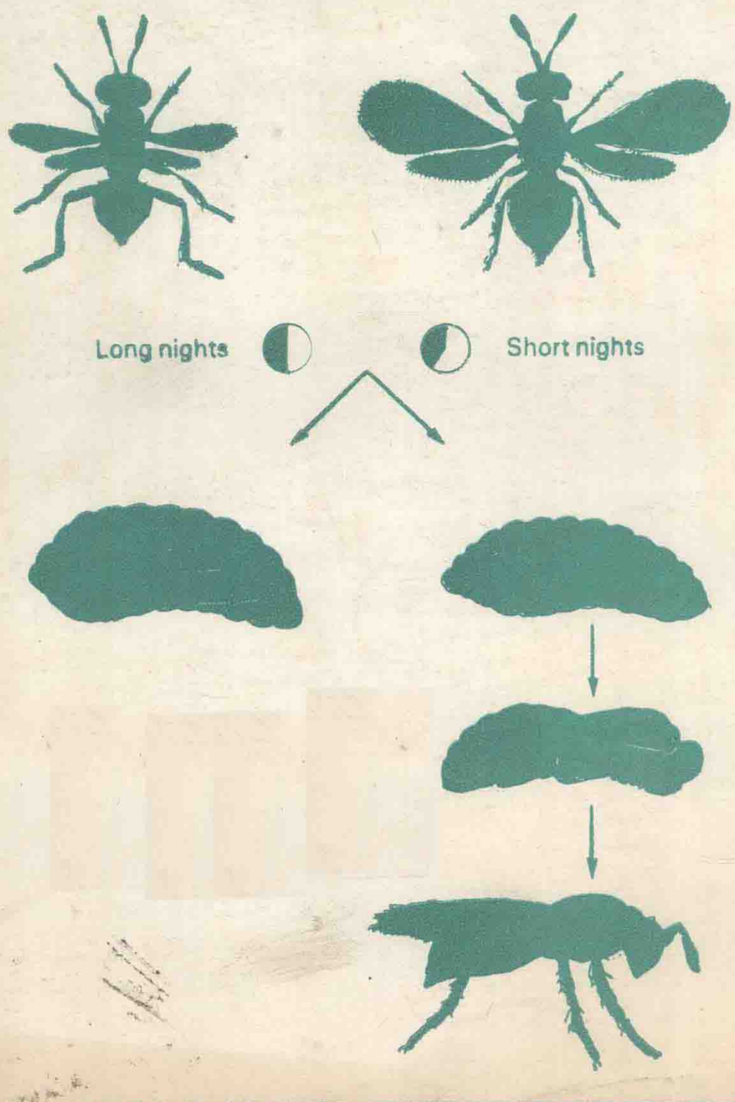
INSECT CLOCKS

Second Edition

D. S. SAUNDERS



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BY

D. S. SAUNDERS

Department of Zoology, University of Edinburgh

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PREFACE TO THE SECOND EDITION

IT is customary for authors to remark, or perhaps complain, that a second edition has become necessary because of the enormous increase in our knowledge as the years proceed. Nowhere is that more true than in the field of Insect Chronobiology, and in the seven years since the first edition was assembled, significant advances have been made in several areas. In 1976 the use of insects alone in illustrating biological clocks could be justified because of the huge size of the group, and because certain aspects of our knowledge were almost totally derived from a study of insect examples. Other areas, however, were admittedly better served by a consideration of other taxa such as unicellular algae, flowering plants, birds or mammals.

In the intervening years, however, many of these 'gaps' in insect clocks have been filled. We can now provide a much more complete account of circadian rhythmicity in individual insects, comparable to that afforded by the study of rodents, for example; and illustrating, among other things, the certainty that the circadian system is of a multioscillator 'construction'. Other major advances have been in the location and isolation of self-sustained circadian pacemakers, with those in the optic lobes of cockroaches, and brains of fruitflies, joining the silkmoth brain in the small group of known cases, the others being the sparrow pineal and the hamster's suprachiasmatic nucleus. These studies will undoubtedly open up an era of progress in neurobiology in which the physiology of the pacemakers, the manner of their entrainment to the light cycle via photoreceptors, the manner of their control of overt rhythmicity, and their mutual coupling within the central nervous system will be investigated by modern techniques of neurophysiology and endocrinology.

With the ever-increasing number of insects being systematically studied with respect to their photoperiodic responses, it is becoming possible to generalize about this aspect of time measurement. It now seems possible that all insect photoperiodic clocks, like those in other taxa, are circadian-based—even the 'hour glass' examples—although the way in which circadian rhythmicity is used to measure day- or night-length remains unclear and is clearly diverse. The 'working models' of 'external' and 'internal' coincidence (and others) have yet to be experimentally distinguished (perhaps revealing one of their inadequacies); on the other hand, the existence of multioscillator types of clock is becoming increasingly more attractive and plausible as more is learned about circadian systems in general. The central problems of time measurement in photoperiodism and the way in which the clock controls diapause and seasonal morphs remain unsolved, however.

In this edition an attempt has been to incorporate the significant advances made since the first, and to update the ever-growing list of species known to possess daily and seasonal clocks. A new chapter (5) on the Circadian System has been added, and the chapter on the Photoperiodic Clock (8) has been reorganized. As before, the task of writing this book has been made possible by the investigations and writings of numerous friends and colleagues, and by meeting and talking with them. Many of these authors have again given permission for me to use their material and illustrations. Here in Edinburgh my own work has enjoyed continued support from the University, the Science Research Council and the Nuffield Foundation, and able assistance from a number of colleagues of whom Kathleen Rothwell is the latest. Special thanks are due to Stephen Goldson who read and criticized the book during its preparation. Without this moral and practical support this second edition would not have appeared.

Edinburgh, October 1981 D. S. SAUNDERS

PREFACE TO THE FIRST EDITION

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. 3.24 and 3.30. Lastly I would like to thank Miss A. Keegan for typing the manuscript.

Edinburgh, 1974 D. S. SAUNDERS

GLOSSARY AND LIST OF SYMBOLS USED TO DESCRIBE CLOCK PHENOMENA

This terminology is based on the *Circadian Vocabulary* of Aschoff, Klotter and Wever (1965), with additions.

Activity time (α). Time in a 'sleep–wake' or activity cycle when the animal is active.

Advance phase-shift (+Δϕ). One or more periods shortened after perturbation by a light or temperature signal causing the overt phase to occur earlier in steady-state than in the control (unperturbed) oscillation.

Aestivation. A summer dormancy (diapause or quiescence). An aestivation diapause is frequently induced by long daylength.

Amplitude. The difference between the maximum (or minimum) value and the mean value in a sinusoidal oscillation. In 'population' rhythms it often also refers to the number of individuals emerging or eclosing, etc., through a particular gate.

Aschoff's rule ($\tau_{DD} < \tau_{LL}$ nocturnal; $\tau_{DD} > \tau_{LL}$ diurnal). Period of the free-running oscillation (τ) lengthens on transfer from DD to *LL*, or with an increase in light intensity, for dark-active animals, but shortens for light-active animals (Pittendrigh, 1960).

Asymmetrical skeleton photoperiod. A skeleton photoperiod comprising a 'main' photoperiod (e.g. 4 to 12 hours) and a short supplementary light pulse which systematically scans the accompanying 'night' (Pittendrigh and Minis, 1964).

Bistability phenomenon. Ability of an endogenous oscillation to adopt *either* of two distinct phase relationships to a symmetrical skeleton photoperiod (PP_s), depending on (1) the phase-point in the oscillation which is illuminated first, and (2) the value of the first interval. In *Drosophila pseudoobscura* the bistability phenomenon is observed between PP_s 10.3 and PP_s 13.7 (Pittendrigh, 1966).

Bivoltine. An insect life cycle with two generations per year.

Circadian (rhythm). An endogenous oscillation with a natural period (τ) *close to*, but not necessarily equal to, that of the solar day (24 hours).

Circadian rule. The relationship of the ratio of activity time (α) to rest time (ρ), and the total amount of activity, to the light intensity. In many vertebrate species α/ρ and activity increase with light intensity in day-active animals, but decrease in night-active animals (Aschoff, 1965).

Circadian system. The sum total of circadian oscillators (driving oscillators) and driven rhythms in an organism. (These may be independent, loosely coupled or coupled.)

Circadian time (Ct). Time scale (in hours, radians or degrees) covering one full circadian period of an oscillation. The zero point is defined arbitrarily. In *Drosophila pseudoobscura* the point at which the oscillation enters darkness from *LD 12*:12 or from *LL* is considered to be the beginning of the subjective night (Ct 12); the first eclosion peak occurs at Ct 03, 15 hours after the *LL*/DD transition (Pittendrigh, 1966).

Circadian topography. A three-dimensional plot of diapause incidence against the length of the photoperiod and the period of the driving light cycle (T). In species with a circadian photoperiodic clock the topography shows 'mountains' of diapause at 24-hour intervals.

Circannual. An endogenous oscillation with a natural period (τ) close to, but not equal to, a year. In the beetle *Anthrenus verbasci* (Blake, 1958) τ is 41 to 44 weeks.

Circasyzygic. An endogenous oscillation with a natural period which approximates to the interval between successive spring or neap tides (14.7 days) (= semilunar rhythm).

Cophase (θ). The interval between the end of the light perturbation and the centroid of the eclosion peaks (Winfree, 1970).

Crepuscular. Twilight active.

Critical daylength (or nightlength). The length of the light (or dark) fraction of the light/dark cycle which separates the 'strong' long daylengths from the 'strong' short daylengths in the photoperiodic response curve, i.e. a 50 per cent response.

Day-neutral (species, response, etc.). Apparently with no reaction to photoperiodic influences.

Delay phase-shift ($-\Delta\phi$). One or more periods lengthened after perturbation by a light or temperature signal causing the overt phase to occur later in steady state than in the control (unperturbed) oscillation.

Diapause. A period of arrest of growth and development which enables the species to overwinter (hibernate) or aestivate, or to synchronize its development cycle to that of the seasons. In most cases diapause involves the cessation of neuro-endocrine activity, and is most frequently induced by photoperiod.

Diurnal. Occurring during the day or light period of the cycle, day-active (cf. *Nocturnal*). An older usage of this term denoting a daily cycle is not used in this book.

Endogenous rhythm (or oscillation). A periodic system which is part of the temporal organization of the organism. It is self-sustaining, i.e. it 'free-runs' in the absence of temporal cues such as the daily cycles of light and temperature.

Entrainment. The coupling of a self-sustained oscillation to a *Zeitgeber* (or forcing oscillation) so that both have the same frequency ($\tau = T$) (synchronization) or that the frequencies are integral multiples (frequency demultiplication). Entrainment is possible only within a limited range of frequencies.

Eudiapause. A facultative cessation of development with species-specific sensitive periods and diapausing instars. In favourable conditions development proceeds unchecked; as unfavourable periods approach diapause supervenes. This type of diapause is usually induced by photoperiod and terminated by a period of chilling or by a change in the level of the temperature (Müller, 1970).

Exogenous rhythm. A rhythm of activity which is a direct response to the environmental cycle of light and temperature. In the absence of these variables the rhythm does not persist.

External coincidence. A model for the photoperiodic clock in which light has a dual role: (1) it entrains and hence phase-sets the photoperiodic oscillation, and (2) it controls photoperiodic induction by a temporal coincidence with a photoperiodically-inducible phase (ϕ_i) (Pittendrigh and Minis, 1964).

Fixed point. A point in the circadian oscillation at which a light pulse leaves the oscillation at the *same* circadian time it was when the pulse started, regardless of the duration of the pulse. In *Drosophila pseudoobscura* the theoretical position of the fixed point is at Ct 10.75 (Johnsson and Karlsson, 1972b).

Forcing oscillation (see also *Zeitgeber*). An oscillation or periodic environmental factor capable of synchronizing or entraining another oscillation.

Free-running period (τ). The period of an endogenous oscillator, revealed in the absence of a forcing oscillation or *Zeitgeber* (i.e. in constant temperature and DD, or *LL*).

Free-running rhythm. A biological rhythm or oscillation in its 'free-running' condition (unentrained).

Frequency. The reciprocal of period.

Frequency demultiplication. The entrainment of an endogenous oscillation to a *Zeitgeber* when the frequencies are integral multiples, e.g. the entrainment of a circadian oscillation ($\tau \sim 24$ hours) to environmental light cycles with $T = 4$, 6, 8 or 12 hours, resulting in an entrained steady state with a period of exactly 24 hours.

Gate. The 'allowed zone' of the cycle, dictated by the circadian clock, through which flies may emerge, hatch, etc. If a particular insect is not at the 'correct' morphogenetic stage to utilize one gate it must wait a full 24 hours for the next (Pittendrigh, 1966).

Hibernation. The state of dormancy (diapause or quiescence) which occurs during the winter months.

Hour-glass. A non-repetitive (i.e. non-oscillatory) timer which is set in motion at, say, dawn or dusk and then runs its allotted time. Such timing devices are to be found in aphid photoperiodism (Lees, 1965) and in certain other clock phenomena.

Intermediate photoperiodic response. The photoperiodic response in some 'univoltine' species in which non-diapause development is restricted to a very narrow range of daylengths (18 to 20 hours); with both longer and shorter daylengths diapause occurs (Danilevskii, 1965).

Internal coincidence. A model for the photoperiodic clock in which two or more oscillators are independently phase-set by dawn and dusk, and photoperiodic induction depends on the phase-angle between the two (Pittendrigh, 1972; Tyshchenko, 1966).

Interval timer. (a) A non-repetitive timer or hour-glass (Lees, 1965). (b) A type of oscillatory clock which dictates the times of the day at which a particular event (e.g. eclosion) can occur—in contrast to 'pure rhythms' or 'continuously consulted' clocks (Pittendrigh, 1958).

Isoinduction surface, see *Circadian topography*.

Long-day (species, response, etc.). The photoperiodic response in which the insects grow and develop during the summer months at long daylength, but enter diapause in the autumn as the days shorten.

Long-day–short-day response. The photoperiodic response which requires short days following long days for its operation.

Multivoltine. An insect life-cycle with many generations per year.

Night-interruption experiment. An experiment in which an insect's photoperiodic response is investigated by asymmetric skeleton photoperiods.

Nocturnal. Occurring during the night or dark period of the cycle, night-active (cf. *Diurnal*).

Oligopause. A facultative arrest of development often with the induction and termination of diapause under photoperiodic control (Müller, 1970).

Oscillator. (A-oscillator). In Pittendrigh's terminology (1960, 1967) the A-oscillator is the self-sustained and light-sensitive oscillator whose period is temperature-compensated and which drives the temperature-sensitive B-oscillator (rhythm) which more immediately controls the overt rhythm of activity (e.g. eclosion). The circadian pacemaker.

Parapause. An obligatory diapause observed in univoltine species. Clearly defined inductive periods and diapause supervenes in every generation in a species-specific instar. Onset appears to be independent of the environment (Müller, 1970).

Period. The time after which a definite phase of the oscillation reoccurs. In biological systems it should be stated what overt phase reference point (ϕ_r) has been used to determine the period, e.g. onset of activity, or median of eclosion peak, etc.

Phase. Instantaneous state of an oscillation within a period.

Phase-angle (ψ). Value on the abscissa corresponding to a point of the curve (phase) given either in radians, in degrees, or in other fractions of the whole period. It can be given in time units if the length of the period is stated.

Phase angle difference. Difference between two corresponding phase angles in two coupled oscillators, given either in degrees of angle or in time units.

Phase response curve (PRC). Plot of phase shift ($\Delta\phi$) (magnitude and sign) caused by a single perturbation at different phases (circadian times) of an oscillator in free-run.

Phase shift ($\Delta\phi$). A single displacement of an oscillation along the time axis may involve either an advance ($+\Delta\phi$) or a delay ($-\Delta\phi$).

Photonon. The kinetics of a clock after the onset of the light (Truman, 1971b).

Photoperiod. The period of light in the daily cycle (daylength), measured in hours.

Photoperiodic counter. That aspect of the photoperiodic response which consists of a temperature-compensated mechanism which accumulates 'information' from successive photoperiodic cycles.

Photoperiodically inducible phase (ϕ_i). A hypothetical phase-point in an oscillator (or perhaps a driven rhythm) which is light-sensitive, and an integral part of the external coincidence model for the photoperiodic clock (Pittendrigh, 1966).

Photoperiodic response curve (PPRC). The response of a population of a particular insect to a range of stationary photoperiods (DD to *LL*) usually including the critical daylength.

Photophase = photoperiod, daylength (Beck, 1968).

Quiescence. A state of dormancy directly imposed by adverse factors in the environment (e.g. cold torpor, dehydration) (cf. *Diapause*).

Range of entrainment. Range of frequencies within which a self-sustained oscillation can be entrained with a *Zeitgeber*. For most organisms the range is from about 18 to 30 hours.

Rest time (ρ). Time in a 'sleep–wake' cycle in which the organism is inactive (Aschoff, 1965).

Rhythm. A periodically reoccurring event. In Pittendrigh's (1967) sense the word is restricted to the driven elements (i.e. the temperature-dependent B-oscillations) directly coupled to the light sensitive driver (A-oscillation).

Required day number (RDN). The temperature-compensated number of inductive photoperiods in the photoperiodic counter, required to raise the incidence of diapause in a particular day's batch to 50 per cent. Equivalent to the 'criticial day number' of Tyshchenko *et al.* (1972).

Resonance experiment (= *T*-experiment). An experimental design in which the photoperiod is held constant but the period of the driving light cycle (*T*) varied (e.g. 12 to 72 hours).

Scotonon. The kinetics of a clock after the onset of the dark (Truman, 1971b).

Scotophase. The dark period, or night-time, of the diel-cycle (Beck, 1968).

Semivoltine. An insect life cycle which occupies a 2-year period, i.e. half a generation per year.

Sensitive period (SP). The period of an insect's life-cycle when it is sensitive to photoperiodic control of diapause induction or termination.
Short-day. (Species, response, etc.) The photoperiodic response in which the insects grow and develop at short daylength, but enter diapause (aestivation) during the summer months when days lengthen.
Short-day–long-day response. A photoperiodic response which requires long days following short days for its operation.
Skeleton photoperiod. A light regime using two shorter periods of light to simulate dawn and dusk effects of a longer, complete, photoperiod (PP_c). See *Symmetrical* and *Asymmetrical skeleton*.
Singularity (T^*S^*). A critical annihilating light pulse of a particular duration (S^*) or intensity placed a certain time (T^*) after the *LL*/DD transition (i.e. at a particular circadian time) which puts the clock in a non-oscillatory state (i.e. stops the clock). In *Drosophila pseudoobscura* T^*S^* is a 50-second pulse of dim blue light (10 $\mu W/cm^2$) placed 6.8 hours after the *LL*/DD transition (i.e. at about Ct 18–19) (Winfree, 1970).
Subjective day. The first half of the circadian cycle (Ct 0 to 12) of an oscillation, and that half in which 'day' normally occurs.
Subjective night. The second half of the circadian cycle (Ct 12 to 24) of an oscillation, and that half in which 'night' normally occurs.
Symmetrical skeleton photoperiod (PP_s). A skeleton photoperiod comprised of two short pulses of equal duration which may simulate a complete photoperiod (PP_C) (Pittendrigh and Minis, 1964).
Synchronization. State in which two or more oscillations have the same frequency due to mutual or unilateral influences. See also *Entrainment*.

T-experiment, see *Resonance experiment*.
Thermoperiod. A daily temperature cycle which may be sinusoidal, 'square-wave', etc., and may act as a *Zeitgeber*.
Token stimulus. A seasonal signal which serves to indicate the approach of adverse conditions (e.g. winter) but is itself *not* adverse (e.g. short photoperiod) (Lees, 1955).
Transformation curve. A plot of the circadian time of an oscillation at the end of a light-pulse as a function of the circadian time at the beginning of the pulse (Johnsson and Karlsson, 1972b).
Transients. One or more temporary oscillatory states between two steady states caused, for instance, by light or temperature perturbations.

Univoltine. An insect life-cycle with one generation per year.

Voltinism. Referring to the number of generations per year.

Zeitgeber. That forcing oscillation which entrains a biological oscillation, e.g. the environmental cycles of light and temperature.
Zeitgeber time (*Zt*). Time of environmental cycle measured in hours, usually, in the case of light, after the lights-on signal or 'dawn'.
Zeitgedächtnis. The 'time-memory' of bees.
Zeitsinn. The 'time-sense' (= time memory) of bees.

Symbols

L	Light fraction of the cycle (intensity may be specified).
D	Dark fraction of the cycle.
LD	Light/dark cycle. *LD 4*:20 represents 4 hours of light and 20 hours of darkness in each 24-hour cycle.
LL	Continuous light.
DD	Continuous dark.
τ	Natural period of a biological oscillation as revealed in 'free-running' conditions.
T	Period of *Zeitgeber*.
ϕ	Phase point.
ϕ_r	Phase reference point. ϕ_r for environmental light cycle may be beginning of light-pulse; ϕ_r for the oscillator in *Drosophila pseudoobscura* is the easily assayed point in the phase response curve where a 360° phase-jump occurs (Ct 18.5); ϕ_r for the rhythm (in *D. pseudoobscura*) is the median of pupal eclosion. In other systems it may be, for instance, the onset of locomotor activity.
ϕ_i	The photoperiodically inducible phase.
ψ	Phase relation.
$\psi_{R,L}$	Phase angle difference between phase reference point of rhythm and light-cycle.
$\psi_{R,O}$	Phase angle difference between phase reference point of rhythm and oscillation.
$\psi_{O,L}$	Phase angle difference between phase reference point of oscillation and light.
$\Delta\phi$	Phase shift.

$+\Delta\phi$	Advance phase shift.
$-\Delta\phi$	Delay phase shift.
α	Activity time.
ρ	Rest time.
θ	Cophase. The time interval between the end of the light perturbation and the centroid of the eclosion peaks.
Ct	Circadian time.
Zt	*Zeitgeber* time.

CONTENTS

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 changes, 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 and the overt events achieve a particular phase relationship to the environmental periodicity. 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 (τ) 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 (~12.4 hours), semilunar (~14.7 days), lunar (~29.4 days) or annual (~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—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