

Advances in Insect Physiology

Volume 10



Advances in Insect Physiology

edited by

J. E. TREHERNE

M. J. BERRIDGE

and V. B. WIGGLESWORTH

*Department of Zoology, The University
Cambridge, England*

Volume 10



1974

ACADEMIC PRESS
LONDON AND NEW YORK

A Subsidiary of Harcourt Brace Jovanovich, Publish

ACADEMIC PRESS INC. (LONDON) LTD
24-28 Oval Road
London NW1

US edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue,
New York, New York 10003

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Library of Congress Catalog Card Number: 63-14039
ISBN: 0-12-024210-9

PRINTED IN GREAT BRITAIN BY
THE WHITEFRIARS PRESS LTD., LONDON AND TONBRIDGE

Contributors

John Brady

*Department of Zoology and Applied Entomology
Imperial College of Science and Technology
London, England*

Bernt Linzen

Zoological Institute, University of Munich, Germany

Axel Michelsen

Biological Institute, University of Odense, Denmark

Harald Nocke

Zoological Institute, University of Cologne, Germany

Lynn M. Riddiford

*Department of Zoology, University of Washington
Seattle, Washington, USA*

James W. Truman

*Department of Zoology, University of Washington
Seattle, Washington, USA*

Contents

Contributors	v
The Physiology of Insect Circadian Rhythms JOHN BRADY	1
The Tryptophan → Ommochrome Pathway in Insects BERNT LINZEN	117
Biophysical Aspects of Sound Communication in Insects AXEL MICHELSEN and HARALD NOCKE	247
Hormonal Mechanisms Underlying Insect Behaviour JAMES W. TRUMAN and LYNN M. RIDDIFORD	297
Author Index	353
Subject Index	369
Cumulative List of Authors	399
Cumulative List of Chapter Titles	401

The Physiology of Insect Circadian Rhythms

John Brady

*Department of Zoology and Applied Entomology
Imperial College of Science and Technology London, England*

1	Introduction	1
2	Circadian principles	2
3	Types of insect circadian rhythms	6
3.1	Behavioural rhythms	6
3.2	Developmental rhythms	15
3.3	Physiological rhythms	22
4	Timing processes	43
4.1	Entrainment	43
4.2	Control of overt rhythms by driving oscillators	52
4.3	Temperature effects on insect clocks	72
4.4	Genetics of insect clocks	74
4.5	Truman's two clock types	76
4.6	Mechanisms of driving oscillators	81
5	Conclusions	91

1 Introduction

No full-length review devoted to the physiology of insect circadian rhythms has ever been published. Earlier surveys, having a wider scope (Harker, 1958a, 1961), provided the basis for the view of the control of insect rhythms incorporated into the textbooks of the period (e.g. Harker, 1964; Wigglesworth, 1965; Marler and Hamilton, 1966), but since then much new information has accrued which must alter many of those earlier assumptions. Not only does the resulting confusion require disentangling, but the 300 or so relevant papers published in the interim demand some kind of distillation.

Reviews related to the physiology of insect circadian rhythms have been written since Harker's (e.g. Corbet, 1966; Beck, 1968; Danilevsky *et al.*, 1970), and the present work will not duplicate their coverage, except where necessary for clarity. In particular, the relationship between photoperiodism and circadian rhythms will only be touched upon, since it has

been amply covered by Lees (1968, 1972), Beck (1968), and, with a more ecological flavour, by Danilevsky *et al.* (1970; this paper is particularly valuable for its Russian bibliography, but has few English language references beyond 1965). Because of their relevance to the subject, the contents of two briefer reviews (Brady, 1969; Truman, 1972a) will, on the other hand, be extensively repeated.

Otherwise, the present work will cover the advances in this subject since the early 1960s. It sets out to do two things: first, to outline those insect rhythms that have been described since Harker's reviews (1958a, 1961); and secondly, in greater detail, to attempt to synthesize the various aspects of the underlying timing mechanisms that have been revealed by recent publications. It is with the endogenicity of insect circadian rhythms and their coupling to the implied underlying physiological oscillators that it is primarily concerned. Numerous entrained diel rhythms (see p. 4) have been described in the last 10 years, but these will not be discussed unless it appears that they indicate a true circadian rhythm, or are illuminating for some other reason. To begin with, a brief summary of the principles of circadian rhythms is given in order to set the scene and to clarify the terminology.

The word "clock" is used throughout for brevity and convenience to describe the unknown biochemical systems which comprise the relevant driving oscillators; this does not imply that the author necessarily considers that rhythms are controlled in insects by a single physiological oscillator. Indeed, that the reverse is the case will become apparent in the later sections. Also for convenience, the words "dawn" and "dusk" are used in quotes to indicate the instantaneous transitions from dark to light, and light to dark in artificial light cycles; when used without quotes, they refer to natural sunrise and sunset.

Where a number appears after a colon in a text reference as, for example in (Robinson, 1973: 123), this refers to a page (i.e. p. 123) in that work. Cross-references to pages within the present review come after a semicolon and the word "see", e.g. in (Robinson, 1973: 123; see p. 456), the last number directs the reader to a relevant passage on p. 456 of this review, as would the interpolation (p. 456) by itself.

2 Circadian principles

Circadian rhythms, along with their related circa-tidal and circa-lunar rhythms, have characteristics which distinguish them from all other biological oscillations. The most obvious of these is their link with the environmental cycles of days or tides. Most other bio-rhythms (e.g. spontaneous spike discharge, heartbeat, spiracular opening, and even some life-cycles) have no such temporal relationship with external cycles. This

link with the environment, while suggesting the adaptive significance of circadian rhythms, has led to the almost certainly erroneous assumption that they are a direct response to, and consequence of, cyclical environmental changes.

A cockroach placed in an actograph and kept in a 12-h light: 12-h dark cycle (LD 12 : 12) performs the majority of its locomotor activity during the first hour or two of darkness. This activity recurs at the same time every day, indefinitely (Fig. 1). As long as the LD cycle is maintained, it is impossible to say whether this activity rhythm is a direct response to the environmental signal provided by the artificial sunset, or whether it is endogenously timed. That the latter may be the case is suggested when the activity starts to change before the light signal is given (as in the records shown by *Periplaneta*, Harker, 1960b; *Acheta*, Cymborowski, 1969; *Aedes*, Nayar and Sauerman, 1971; *Glossina*, Brady, 1972a).

The proof that these pre-signal changes indicate endogenous timing is provided by withdrawing the light cycle, i.e. leaving the animal in constant light (LL) or constant darkness (DD). Peak activity is then still found to

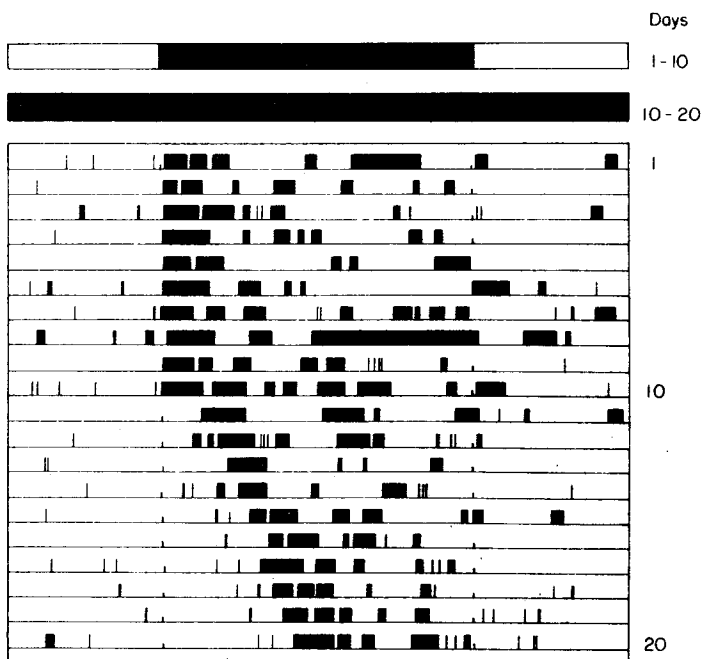


Fig. 1. Locomotor activity record of a cockroach, *Leucophaea maderae* (simplified from original record by Roberts, 1962). Each horizontal line represents 24 h of record; the black blocks indicate periods of activity. Successive days are arranged in order down the page. Upper bars represent the light : dark cycle for the days as indicated.

occur at approximately its normal time, in spite of the absence of time cues. Furthermore, the activity continues to be expressed rhythmically for days in the absence of any periodic input from light, temperature or humidity (Fig. 1).

The important feature of such LL or DD rhythms is that they typically continue at periods which differ slightly, but consistently, from 24 h. This is the strongest evidence that their timing is endogenous, and the result of a response to an underlying physiological "clock". If they continued at a period of exactly 24 h it would not be possible to rule out their being a response to any of the other environmental features that vary with the earth's rotation, such as magnetic field or cosmic radiation, which are not controlled in normal circadian experiments. But in practice such rhythms invariably drift relative to solar time (Fig. 1), and are therefore temporally independent of such external signals.

This *free-running* drift away from solar time is characteristic of all endogenously timed daily rhythms, and is the origin of the term *circadian* (from *circa diem*). Strictly, this word should therefore only be applied to free-running rhythms, or rhythms which are known to free run in constant conditions. It carries with it a clear implication of endogenous physiological timing, but is often incorrectly used to describe any rhythms in a LD cycle, even when there is no evidence for their endogenicity (Wurtman, 1967). Such entrained (see below) LD rhythms are conventionally termed *diel* (i.e. daily) to distinguish them from the implications of the words diurnal and nocturnal.

Circadian terminology borrows freely from physical theory (a full glossary is provided by Aschoff *et al.*, 1965). Thus a rhythm is said to *oscillate*, or to be the overt expression of an underlying *self-sustaining oscillator*. This oscillation has a natural free-running *period* (and therefore *frequency*) which is characteristic of the individual under the ambient conditions. It has a period of 24 h when it is *entrained* by (i.e. synchronized to) a 24-h LD cycle, which provides it with its time-cue, or *zeitgeber*. The term *photophase* is used to describe the light part of a LD cycle in order to avoid confusion with the specific connotation of the term "photoperiod", and the dark part of the cycle is therefore the *scotophase*. Any particular point in the oscillation, e.g. peak activity, is a given *phase* of the rhythm, and when the rhythm is entrained there is a characteristic time-lag, or *phase angle*, between the zeitgeber and the measured phase (one oscillation = 360°). In some rhythms it is also useful to consider the *amplitude* of the oscillation. The word *noise* is used in the electronic sense to imply irrelevant or extraneous interference in the recording of the oscillation.

Fitting these terms to the upper half of Fig. 1: the *zeitgeber* is "sunset"

(i.e. the LD transition), the measured *phase* of the rhythm is the onset of peak activity which, since it starts exactly at "sunset", bears a *phase angle* of 0° to the zeitgeber; the period is exactly 24 h and the *amplitude* might be described as the amount of locomotor movement performed during the activity peak. The lower half of Fig. 1 has the rhythm no longer *entrained*, but *free-running* at a period of c. 24.5 h.

There is a whole class of circadian rhythms which involve developmental events occurring once only in the life of each individual (e.g. eclosion), and which only appear as overt rhythms in synchronous populations. The term *gating* is applied to such events, implying that the event can only occur when the circadian clock opens the *gate* at the appointed time, and that if an individual misses a given day's gate it must wait 24 h for the next one, or 48 h for the one after that.

Circadian rhythms are fairly stable, and will not entrain to environmental cycles differing by more than a few hours from the 24; insects usually entrain only within the range of about 18 to 30 h, but even well within this range, their survival is reduced by prolonged entrainment to periods other than 24 h (Pittendrigh and Minis, 1972). Another consequence of this stability is that such rhythms generally do not adjust instantaneously to a new zeitgeber cycle, that is, they can only be *phase shifted* a few hours each cycle in response to a changed zeitgeber time so that they show *transient* cycles of less than or more than 24 h until they are re-entrained. Moreover, their ability to phase shift is not equal throughout the 24 h, and the amount of shift they perform depends on the phase difference between some sensitive phase of the animal's "clock" and the new zeitgeber. This results in a *phase-response curve* (see p. 48) relating the amount of phase-shift to the time of stimulus. Phase-response curves are characteristic for the species and conditions, and imply certain important features of the underlying *driving* oscillator or "clock" (see p. 48).

The use of these terms is due to the fact that the formal characteristics of circadian rhythms are closely analogous to those of physical oscillators. It is dangerous to follow such analogies too far, however, and the terms are in general used as convenient shorthand only.

One other feature of circadian rhythms that marks them off from other bio-rhythms is their stability at different temperatures. Unlike nearly all other physiological processes, circadian rhythms exhibit Q_{10} s which are typically close to unity. They are thus *temperature compensated* within normal biological limits (see p. 72).

To summarize, circadian rhythms have three prime characteristics: (1) they persist in the absence of external time cues; (2) they persist at a period which is, in principle, never exactly 24 h but always fairly close to it; (3) they have Q_{10} s of approximately 1.0.

3 Types of insect circadian rhythms

3.1 BEHAVIOURAL RHYTHMS

3.1.1 *Locomotor activity*

Locomotor activity has been a favourite parameter for the measurement of insect circadian rhythms, no doubt mainly because it is so simple to record (e.g. via a thread attaching the insect to a pen writing on a smoked drum, Harker, 1956). But it has a less pragmatic advantage, too, since it is performed as part of a number of behaviour patterns and may therefore be taken as the integrated response to several different endogenous stimuli modulated by the underlying clock.

A whole battery of techniques for recording insect movements have been developed: the archetypal rocking box (Szymanski, 1914), running wheel (Roberts, 1960), photocell/light-beam (Brown and Unwin, 1961), capacitance transducer (Schechter *et al.*, 1963), sound recording (Jones, 1964), flight mill (Chambers and O'Connell, 1969), temperature differentials (Macaulay, 1972), and their modifications, among many others. Some of these measure whole body movement (e.g. Brown and Unwin) and thereby simplify the recording of the behaviour involved in locomotion to a question of movement or no movement. Others (e.g. Roberts's running wheel) are more selective and record only walking (or flying) movements. This has the advantage of reducing noise in the record, but the disadvantage of missing parts of the activity, such as feeding, that may be an important component in the rhythmic expression of behaviour.

The assumption behind all these studies is that the daily rhythmicity of locomotor activity is the result of a response to the insect's internal clock. In so far as the activity is periodic and circadian, this is evidently true, but because locomotion is behaviour, it is liable to non-rhythmic interferences from many sources. Insects respond to the environment provided by their actograph in unpredictable ways. Thus, the cockroach, *Periplaneta americana*, placed in the small cage of a Brown and Unwin or rocking box recorder may show gradually declining activity peaks over a series of days until the rhythm is apparently lost, even in LD. Yet when transferred to a running wheel such an insect immediately reverts to showing a very clear rhythm (Brady, 1967b). The cricket, *Acheta domesticus*, on the other hand, continues to show a clear rhythm for weeks in small rocking boxes (Nowosielski and Patton, 1963). The submergence of the measured phase of the rhythm into background noise has led to confusion in the interpretation of some experiments (see Brady, 1967b: 159).

Single phase, sharp onset rhythms of the cockroach or cricket type are perhaps the most convenient for circadian research, but by no means the only form of expression of insect circadian locomotor activity. Bimodal

versions commonly occur, though these frequently appear only in entrained diel rhythms (e.g. Gillett *et al.*, 1962; Roberts, 1962; Jones *et al.*, 1967) with the second peak presumably forced by the light-on signal. The relationship between bimodal behaviour, "dawn" and "dusk" signals, duration of light phase, and total activity has been analysed for *Anopheles gambiae* by Jones *et al.* (1972b); the second peak in this rhythm is evidently only partly explicable as a kinetic response to light-on. In some cases, both peaks are truly endogenous and free run in constant conditions (Chiba, 1964; Nayar and Sauerman, 1971). In cockroaches, for some reason, this occurs more commonly in LL than in DD (Roberts, 1960; Lohmann, 1967). No cases have yet been reported in insects of such bimodal rhythms dissociating from each other as has been shown to occur in some mammals (Hoffmann, 1971).

A rather different form of circadian activity pattern is shown by some Diptera. The tsetse fly, *Glossina morsitans*, restricts its locomotion to brief 1-min flights separated by long intervals (Brady, 1970). Throughout a 12-h photophase less than eight of these flights may be performed, and yet they are distributed in a circadian manner in constant darkness (Brady, 1972a). This is only evident from a study of the mean activity of several tsetse flies. The frequency of flight falls along a U-shaped course through the light phase of a LD cycle (see p. 14), and in a weakly bimodal pattern in DD. The mean total flight duration of an individual fly in LD is 12.5 min in a 12-h photophase, performed in about 17 bursts spread through the day, though mostly occurring before noon. In DD the activity of an individual is barely recognizable as a rhythm, and in LL not at all (Brady, 1972a). Activity patterns similar to the tsetse type also occur in other species (Parker, 1962; Green, 1964a; Jones *et al.*, 1967).

A variant on the simpler locomotor activity rhythms is provided by the granary weevil, *Sitophilus granarius* (*Callandra granaria*). This shows a circadian rhythm in positive and negative geotaxis, but only does so in unfavourable culture conditions (Birukow, 1964). This implies some sort of migratory response to environmental stress. A circadian component in what is in effect the migratory activity of the milkweed bug, *Oncopeltus fasciatus*, has also been demonstrated and shown to be a response to photoperiod—as the days lengthen the bugs fly more and get carried north on the prevailing winds (Caldwell and Dingle, 1967; Dingle, 1968). Activity in that classically migratory species, the locust, *Schistocerca gregaria*, on the other hand, appears not to be circadian (Odhiambo, 1966), at least not in long-established laboratory cultures, although in *Locusta migratoria* it apparently is (Edney, 1937). The relationship between locomotor rhythms and insect migration has been discussed at length by Johnson (1969) and Dingle (1972).

No doubt some insects are not rhythmic in their locomotor activity, but

the monumental studies of Lewis and Taylor (1964) on 500 species of night flying insects in England, and Haddow *et al.* (1961) in Africa, suggest that enormous numbers of species are.

3.1.2 Feeding rhythms

Historically, the most obvious of all insect rhythms must have been the feeding activity of blood-sucking Diptera, and yet this rhythm has never been examined in the laboratory. The precision of the timing of feeding by some mosquitoes in the field, e.g. *Taeniorhynchus (Mansonia) fuscopennata* (Haddow *et al.*, 1961: 319), suggests that this might be a rewarding field for circadian study now that artificial feeding and recording techniques are available (e.g. Kashin, 1966; Galun and Margalit, 1969). The nearest approach has been through the observations of Gillett *et al.* (1962) on populations of *Aedes aegypti* in which the sugar-feeding rhythm persists for at least three days in DD.

The inseparability of spontaneous locomotor activity from feeding activity might have been inferred from the relation between the intensity of activity and the degree of starvation of an insect (e.g. Ellis and Hoyle, 1954; Green, 1964a, 1964b; Brady, 1972a). Only rarely have feeding and locomotion been examined independently, but simultaneously in a single insect. In *Periplaneta*, however, Lipton and Sutherland (1970) found that, with only one possible exception (their Fig. 10), locomotor activity and feeding were effectively synchronous in both LD and DD.

Somewhat surprisingly, the feeding rhythm of *Oncopeltus* only persists for 1 or 2 cycles in LL and not at all in DD, even though the oviposition rhythm continues unabated for at least 6 cycles in constant conditions (Caldwell and Dingle, 1967; though see p. 12). One might have expected feeding to be sufficiently modulated by oviposition activity to make it, too, appear rhythmic. In the mosquito, *Anopheles gambiae*, which is in a different situation from *Oncopeltus* because it normally has to fly some distance to oviposit, a feeding rhythm would appear to be inseparable from the oviposition rhythm (Haddow and Ssenkubuge, 1962). In this case, however, it looks as if it is oviposition which is the endogenously timed activity, since it is the oviposition, rather than the feeding, which coincides with the endogenous locomotor peak (Jones *et al.*, 1967).

An unusual form of feeding rhythm has been demonstrated in the ant-lion, *Myrmeleon obscurus*. This insect digs its pit traps with a clear circadian rhythm phased to dusk, when maximum feeding occurs (Youthed and Moran, 1969a). The unexplained feature of this rhythm is that it is apparently modulated into both lunar-monthly and, possibly, lunar-day rhythms even in constant conditions in DD (Youthed and Moran, 1969b). What advantage the ant-lion gains from this lunar periodicity is not clear, since prey availability apparently shows no obvious lunar periodicity.

What might be termed a post-feeding rhythm has been demonstrated in the tick, *Haemaphysalis leporispalustris*, by George (1964). This is a parasite of the rabbit, to which it is attached for 5-8 days each instar while it feeds. Having completed engorgement it drops off its host, but only does so during daylight when the rabbit, which is nocturnal, is resting in its "form". The tick thereby ensures that it is likely to find its host again once it has completed moulting. Detecting an endogenous rhythm in a parasite creates obvious difficulties, but by ringing the changes on LD, LL and DD light cycles independently on rabbit and tick George (1971) produced strong circumstantial evidence that the drop-off rhythm is primarily endogenous and circadian, probably entrained by a "dusk" zeitgeber, though modulated under some circumstances by the metabolic cycle of the host.

What are probably the only examples of learned feeding rhythms in insects occur in the Hymenoptera. Virtually all the research has been done on the honey bee, *Apis mellifera*, and related species, though ants, too, may possibly be able to learn feeding times (see Wilson, 1971). The adaptive value for bees in being able to feed at specific sites at specific times of day relates to the fact that flowers are rhythmic in their secretion of nectar and presentation of pollen (see Renner, 1960). Bees have an extremely refined ability to time their foraging, and can be taught (in the field) to come to artificial food sources at up to nine different times per day (Koltermann, 1971). They will even remember to come to the correct scent at the correct time, if trained to two scents at two different times.

This very precise timing ability is evidently a function of their underlying circadian clock. They ignore local time and continue to forage at their correct circadian time when translocated through several time zones (Renner, 1957), have a free-running rhythm of feeding in LL (with a period of c. 23.4 h), and cannot be trained to feed at cycles which differ by more than 2 h from the 24 (Beier, 1968; their rhythm of locomotor activity is also circadian, with a free-running period of c. 22 h in DD, Spangler, 1972). As might be expected, the clock mechanism which underlies this ability to feed at specific times is also involved in the time compensation of their sun-compass orientation (Beier and Lindauer, 1970). How bees manage to couple their foraging behaviour to this clock in a manner which permits them to divide the day into as many as nine segments is completely unknown, but clearly indicates a more sophisticated system than that possessed by any other organism.

3.1.3 Sexual rhythms

Sexual behaviour in insects is frequently observed in the field to be associated with certain times of day. These rhythms seem to have been investigated in the laboratory in the Diptera and Lepidoptera only. The

first, and still apparently the best evidence that nematoceros mating behaviour is endogenously timed is Bates's (1941) demonstration that *Anopheles superpictus* swarms at approximately 24-h intervals when kept under constant dim light. The fact that *Aedes triseriatus* start to swarm 2 h before light-out in the constant temperature and unchanging light intensities of a laboratory LD cycle (Wright *et al.*, 1966) also suggests endogenous timing. Although mosquito and midge swarming has been studied on other occasions, it has usually been in relation to the light intensities at which it occurs (e.g. Nielsen and Nielsen, 1962; Chiba, 1967). It is evident from Bates's work that these light intensities are simply the relevant zeitgebers which entrain a circadian rhythm of swarming.

In the Queensland fruit fly, *Dacus tryoni*, mating is restricted under natural daylight to about 30 min around dusk. In the laboratory, an instantaneous "dusk" from 10 000 lux to 10 lux (the optimum intensity for mating under natural conditions) elicited a higher mating response than occurred in field cages (Tychsen and Fletcher, 1971). On the face of it, this rhythm looks like a direct response to an environmental signal, but Tychsen and Fletcher found that underlying it is a relatively smooth-wave, endogenous, circadian rhythm of mating responsiveness. In LD, the percentage of both sexes "ready to mate" rises sharply over the last 4 h before "dusk" from zero during most of the photophase to a peak of 80 per cent at "dusk", then falls steadily through the night. In males, this rhythm free runs in constant dim light for at least 4 days, but in both sexes damps out rapidly in constant bright light. This loss of rhythm is probably the result of the individuals becoming aperiodic and not because of asynchrony in the population. The courtship rhythm of male *Drosophila* fruit flies is also extinguished by constant light (50 lux), though it persists in DD (Hardeland and Stange, 1971).

The most extensively documented circadian sexual rhythms in insects are those associated with the production of and response to pheromones (Fig. 2). The males of four species of noctuid moths have been shown to vary their responsiveness to a standard female pheromone stimulus along a marked circadian cycle (Shorey and Gaston, 1965). In the case of the cabbage looper moth, *Trichoplusia ni*, in LD, this varied from a near zero response during the day to a broad peak with 80 per cent of the males responding during the night; in DD the amplitude of the peak was only slightly less. A similar situation prevails in male flour moths, *Anagasta kuehniella*, though in this species the amplitude of the first peak in dim LL was scarcely significant statistically (Traynier, 1970).

Varying the light and dark components in a 24-h cycle suggested that the male *T. ni* response rhythm is not specifically phased to either "dawn" or "dusk". By contrast, the rhythm of the pheromone release by the female is phase set by the DL transition (Sower *et al.*, 1971). It has a much narrower

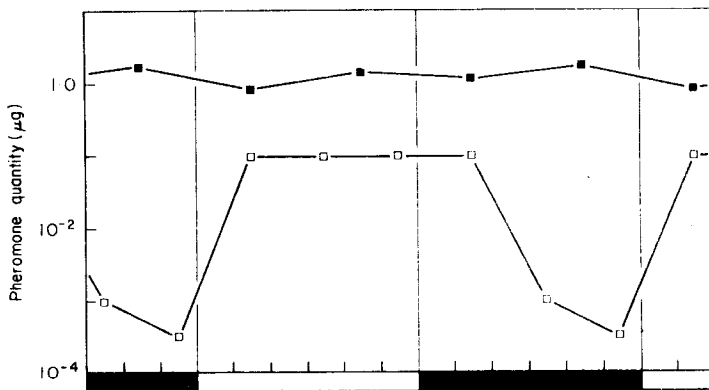


Fig. 2. Effect of circadian rhythm and light cycle on the electroantennogram, EAG (■) and behavioural responsiveness (□) of male *Trichoplusia ni* to female sex pheromone. Ordinate, amount of pheromone required to elicit a significant EAG or a 50 per cent behavioural response. Points from last 12 h plotted twice. (Redrawn from Payne *et al.*, 1970.)

peak than the male's response in a LD cycle with near zero "calling" from dawn to midnight and a sharp rise to 30 per cent of females "calling" between midnight and dawn. In a population of females, it free runs for at least three cycles in DD, with little loss in amplitude (Sower *et al.*, 1970). The only non-lepidopterous species from which a pheromone release rhythm has been reported (Marsh, 1972) seems to be the aphid, *Megoura viciae*.

From the physiological point of view, an interesting aspect of the *T. ni* male's response is the way it is affected by light (Fig. 2). The antennal response to female pheromone, as measured by electro-antennogram, is dose dependent! But, whereas the behavioural response is modulated by light intensity and time of day, the antennal response is quite unaffected (Payne *et al.*, 1970). Evidently, the response rhythm is a consequence of central and not peripheral modulation. Although spontaneous activity was not measured in the *T. ni* observations, closely similar work on the apple moth, *Epiphyas postvittana*, shows that locomotor activity and pheromone responsiveness are probably exactly synchronous (Bartell and Shorey, 1969).

The only investigation of the physiological control of rhythmic pheromone release has been on female silkmoths (Riddiford and Williams, 1971). In *Hyalophora cecropia* kept under LD conditions, calling begins shortly before "dawn" and continues for about the first 30 min of the light phase. Since calling starts before the lights go on, this rhythm may be presumed to be endogenous and circadian, as in other moths. Riddiford and Williams found that the percentage of females calling before "dawn" was unaffected by the removal of their corpora allata, whereas removal of the

corpora cardiaca reduced calling more than four-fold. Re-implantation of up to three pairs of corpora cardiaca, however, did not restore the calling response to the light cycle, nor did calling occur after the nervous connection had been cut between the brain and corpora cardiaca of otherwise intact moths. It appears, therefore, that a corpus cardiacum hormone may be involved in the release of calling behaviour, and that hormone secretion is under nervous control from the brain. (See Chapter 4 by Truman and Riddiford.)

Other rhythmic sexual activities that have been shown to be endogenously timed are singing in grasshoppers (Dumortier, 1968; Loher, 1972), and mating in *Oncopeltus* (Caldwell and Dingle, 1967), though this latter rhythm damps out very quickly in LL and DD. It seems likely that related diel sexual rhythms, such as spermatophore production in crickets (Mcfarlane, 1968) and glowing by female *Lampyris noctiluca* (Dreisig, 1971), will also prove to be endogenous and circadian, though they have not yet been tested for this.

Female reproductive behaviour after mating is also endogenously rhythmic. Thus the oviposition rhythm of the pink bollworm, *Pectinophora gossypiella* (Minis, 1965; see p. 28), the grasshopper, *Chorthippus curtipennis* (Loher and Chandrashekar, 1970), and the mosquito, *Aedes aegypti* (Gillett *et al.*, 1961), and the larviposition rhythm of the viviparous tsetse fly (Phelps and Jackson, 1971), are all clearly circadian. No doubt the diel oviposition rhythms of other species such as *Anopheles gambiae* (Haddow and Ssenkubuge, 1962), *Drosophila* (Rensing and Hardeland, 1967), and the spider mite, *Tetranychus urticae* (though not retained in LL, Polcik *et al.*, 1965), will also prove to be circadian. On the other hand, the oviposition rhythm of *Oncopeltus fasciatus* which is retained in LL, is apparently not circadian, since it is not temperature compensated (Rankin *et al.*, 1972; see p. 72).

A unique case of the extinction of an activity rhythm following mating has been reported by McCluskey and Carter (1969). Virgin females of the ant, *Pogonomyrmex californicus*, show a clear diel rhythm of activity in LD. When mated they shed their wings, become photonegative, start to lay eggs and cease to show any detectable periodicity. The switch is apparently the result of mating itself, and not the loss of their wings or the act of nuptial flight. It is not known whether this loss of behavioural rhythmicity indicates a stopping of the clock itself, or merely an uncoupling of the behaviour from it, but in either case it has interesting implications for circadian control.

3.1.4 Changes in responsiveness

The behavioural responses of insects to various stimuli have been studied in relation to endogenous input from the physiological state of the animal