Transworld Research Network
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Comparative Aspects of Circadian Rhythms, 2008: 000-000 ISBN: 978-81-7895-329-8
Editors: María Luisa Fanjul-Moles and Raúl Aguilar Roblero

4

Circadian rhythms in insects

Claudio R. Lazzari and Teresita C. Insausti
Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035. Université
François Rabelais, Tours, France

Abstract

The expression of rhythmicity in insects has been largely documented at the cellular level, in organs, as changes in the physiology and behaviour of individuals, as well as in population synchrony. Circadian rhythms are particularly ubiquitous in insects and, as in other organisms, have been the best analysed. Fruit flies, cockroaches, butterflies, honey bees, and other insects cast much light on the physiology and molecular basis of the circadian clock. Insect adaptation and success in the colonisation of the most diverse environments is also associated with the temporal organisation of their daily life. In this

---

Correspondence/Reprint request: Dr. Claudio R. Lazzari, Institut de Recherche sur la Biologie de l'Insecte Faculté des Sciences et Techniques, Université François Rabelais, Avenue Monge – Parc Grandmont, 37200 Tours, France. E-mail: claudio.lazzari@univ-tours.fr

chapter, we will analyse how circadian rhythms are expressed and controlled in insects. Classical and recent findings will be discussed from an adaptive and integrative perspective.

1. Introduction

Insects, like other organisms, evolved in an environment dominated by daily periodicity. Their presence on the earth for longer than, for example, vertebrates, allowed selective forces the time for fine tuning in time for several processes not only at the cell, organ, and individual levels but beyond—at the population and multitrophic interactions. Their ectothermic condition together with their small size render insects particularly sensitive to the environmental temperature, and their high surface-to-volume ratio facilitates water loss. Additionally, the design of their eyes does not permit, in many cases, sufficient sensitivity to see at night. These facts, along with others originated in their corporal and functional design, acted as selection forces to predict changes in their environment. Thus, it is not surprising that insect life is temporally strongly and finely organized, and that they express many physiological and behavioural daily rhythms at many different levels. Chronobiologists some time ago realized the suitability of insects for experiments and made them one of their favourite subjects of study. Indeed, insects are closely associated with the history of the study of biological rhythms. They allowed unravelling many fundamental questions to persons as Colin Pittendrigh, Jürgen Aschoff, Edwin Bünning, Tony Lees, and several other personalities in Chronobiology.

This chapter attempts to synthesise some aspects concerning circadian rhythms in insects which we found particularly relevant in order to provide readers with an idea regarding their importance in insect biology. For a deeper discussion of the different issues and to learn about other aspects not treated herein, we invite the reader to look at the excellent book by David Saunders, whose third revised edition was published a few years ago (1). It should be noted here that only true circadian rhythms, i.e. those for which endogenous control has been shown, will be presented. Other rhythms, among which endogeneity was not tested or that are under the direct control of external factors, will be eventually mentioned as functionally associated with endogenous rhythms.

2. Circadian rhythms in individual insects

2a. Physiological rhythms

Physiological circadian rhythms in insects have been well- documented in relation to hormone production, particularly hormones controlling post-embryonic development. The first evidence of rhythmicity obtained from cytological observations of daily cycles in the appearance of various cells or cell types. Nevertheless, whether a particular rhythm of cellular events was responsible for any other rhythm remained unknown in the majority of cases. This limitation became relevant when it was clear that cellular rhythmicity was the norm rather than the exception (2). Further analysis revealed that, in addition to their purely endocrine functions, certain hormones, such as the prothoracicotropic hormone (PTTH), ecdysteroids, and juvenile hormones (JHs), the main hormones responsible for insect moult and metamorphosis, form a key component of the circadian system and may represent the central timekeeping system (2).

Figure 1 represents the hormonal axis controlling moulting in insects, as well temporal control and synchronization by light.

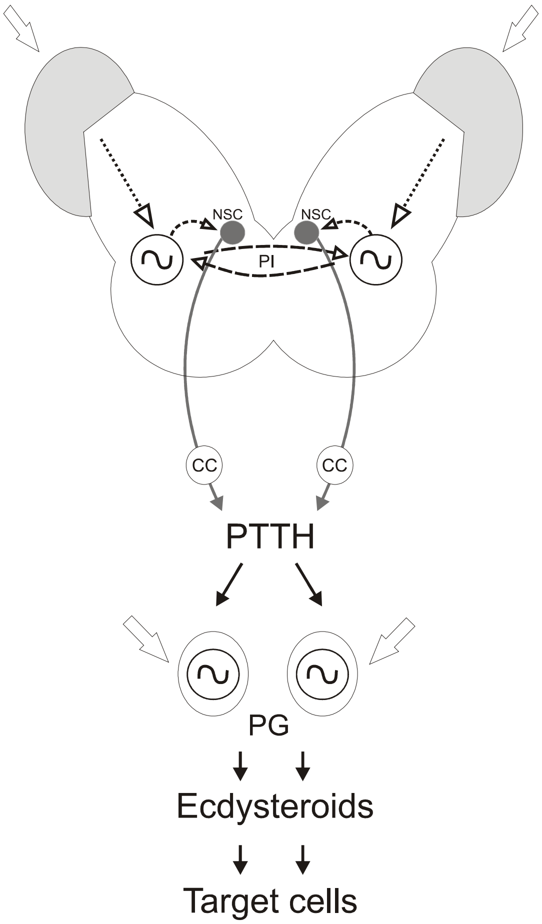

Figure 1. Control of the rhythmic release of hormones controlling moult in *Rhodnius prolixus*. In insect brain, cells possessing clock-gene cycling expression are entrained by light (open arrows). These cells regulate the rhythmic release of PTTH (prothoracicotropic hormone) through the corpora cardiaca (CC). PTTH stimulates and acts as a *Zeitgeber* for release of ecdysteroids by prothoracic glands (PG), which also possess light cycle-entrainable autonomous oscillators. Ecdysteroid action triggers the rhythmic expression of genes on different tissues. Modified from (2).

Insect moult involves replacement of the entire exoskeleton by a larger one. This process, under the control of ecdysteroids, implies deposition of proteins and chitin (n-acetyl-glucosamine) in the newly formed tegument, which continues between moults. This deposition takes place in a precise manner, with chitin fibres organized in layers with different orientations and thicknesses (3). These layers alternate regularly, giving rise to a lamellate cuticle, which is deposited during one part of each day, and non-lamellate cuticle that is formed during the remainder of each day, representing daily growth layers whose formation is controlled by a circadian clock (4–6).

Other hormones, such as the juvenile hormone (JH), also appear to be produced in a rhythmic fashion. JH has multiple targets and is concerned with different physiological processes, such as control of ovarian activity (7) and control of sensitivity to sexual pheromones in moths (8), eventually inducing rhythmicity in their expression.

True circadian rhythms in sensory sensitivity to odours have been revealed at both sensory and behavioural levels. First described in *Drosophila* (9) at the peripheral level, this rhythm expresses as a general variation of the entire antenna’s sensitivity to odours. This rhythm is expressed even in isolated antennae and appears to be controlled by an autonomous antennal oscillator (10). A variation in the sensitivity of antennal chemoreceptors was also verified in the cockroach *Leucophaea maderae* (11). As in *Drosophila*, this variation renders the insect more sensitive to several odours, probably to all of these. It means that the animal is more or less sensitive to chemical signals at given moments of the day. Paradoxically, in both cases maximal sensitivity occurs during resting periods and not when the animal is active making use of olfactory information. Different hypotheses have been proposed to explain this (9,11), but more experimental data are necessary to unravel this paradox.

Interestingly, when behavioural response to a host odour (carbon dioxide) has been evaluated in blood-sucking bugs (Hemiptera: Reduviidae), no such paradox occurred. The bugs evinced a narrow temporal window of responsiveness, but in contrast to the previously described experiences this window perfectly matched the activity period when the insect searches for food (12). Furthermore, these bugs evince a bimodal activity pattern. They search for food at the beginning of the night, and for refuge at dawn. Because both behaviours are guided by odours (i.e., volatiles released by the host and aggregation pheromones, respectively), this does not appear as adaptive for sensitivity to every odour during only a moment each day, as predicted by results in *Drosophila* and *Leucophaea*. Even when both host odours and pheromones are always present in their habitat, they should only respond to the odour that is relevant at a particular moment. Further experiments should shed light on this problem.

The example of blood-sucking bugs has interesting consequences beyond chronobiology. Actually, the chemical ecology of disease vectors is a field in which much effort is concentrated in order to seek novel tools to control pests. It is clear from the previously presented examples that the response of an insect to a given odour will be influenced by temporal matching between the moment of the experiment and the temporal context in which such an odour is relevant in nature. Nevertheless, a review (13) of the recent literature concerning the chemical ecology of insect vectors revealed that this matching has rarely been taken into account. Thus, chronobiological aspects should form an integral part of studies in insect olfaction.

An interesting example of both circadian control and integration of endogenous and exogenous rhythms comprises adaptation to light intensities of the haematophagous bug *Triatoma infestans*. The photonegative response of this species is modulated by a circadian oscillator; bugs trained to light/dark (LD) cycles and then exposed to light/dark LD, dark/dark, constant dark (DD), or light/light, constant light (LL) conditions, are always more sensitive to light during scotophase than during photophase, either real or subjective (14). This variation in responsiveness is associated with dynamic changes in insects' eyes. Screening pigments migrate to render the eye more or less sensitive to light during the night and during the day, respectively. Light/dark adaptation also falls under the control of a circadian clock in the case of compound eyes (15), but is completely exogenous in the case of the ocelli (the simple eye of the adult insect) (Fishbein et al., in preparation). It is noteworthy that both visual systems work in parallel in this species to mediate negative phototaxis (oriented light avoidance) (16). The fact that one system is controlled by a circadian clock and the other directly by external conditions provides the insect with the ability to predict changes and prepare itself for expected illumination conditions when these leave their shelters and to react directly to any change in the actual conditions, rendering the system plastic for adaptation to any unpredicted situation.

Only a few examples of physiological circadian rhythms have been presented in this section to illustrate the manner in which they are present in insects. Further examples can be found in the excellent review by Steel & Vafopoulou (2).

2b Behavioural rhythms

2bi. Activity patterns

The easiest behavioural rhythm observable in insects comprises the daily pattern of spontaneous locomotion as representative of activity and resting periods. It is relatively easy to measure and to test for its endogenous or exogenous nature. The majority of activity rhythms in individual insects possess a strong endogenous component, although they may be, to a variable extent, modulated by the direct effects of the environment. The pattern of rhythm varies across species, depending, as in other animals, on specific adaptation for exploiting particular resources. Therefore, unimodal and bimodal activity distributions can be observed in both diurnal and nocturnal insects.

It should be noted that the daily pattern of spontaneous locomotion represents the summation of all the different activities performed by the animals. Conversely, not every behavioural activity is carried out at every moment the insect is in motion (Figure 2). Temporal allocation of each behaviour represents an adaptation to exploitation of a particular resource, of the environmental conditions, or even the avoiding of deleterious conditions or predators. For instance, several haematophagous insects feed at the moment of the day when their vertebrate hosts are less active. If the host is diurnal, these insects' main biting activity is concentrated during the night, and vice-versa. This minimizes the risk of being detected by the host, which in many cases may become a predator when active (17,18).

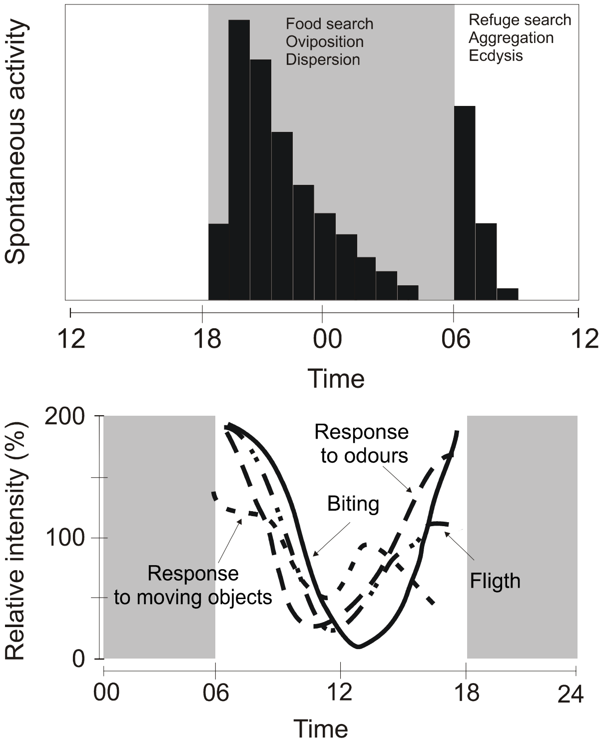

Figure 2. Two examples of temporal allocation of different activities in insects exhibiting bimodal patterns of spontaneous locomotion. Top panel: *Triatoma infestans* (Heteroptera: Reduviidae), a nocturnal insect. Bottom panel: the bimodal diurnal activity pattern of tse-tse flies. In both cases, different activities follow particular dynamics along entire activity periods or a part thereof. Data from (18).

Insect activity patterns also provide some interesting insights concerning the adaptive organisation of behavioural and physiological rhythms. For instance, the ant *Cataglyphis* habits in the Sahara desert and is one of the most thermo-tolerant land animals known (19). Their bodies reaches temperatures >50°C during foraging trips over the desert's sandy surface at midday. At this time, predators hide in the shadow to avoid excessive heating. Each day, foraging activity is preceded by the synthesis and accumulation of heat-shock proteins, which protect the animal from the excessive heat (19).

The crepuscular activity of certain insects appears to contain a strong exogenous component, because the period of day at which activity can occur is restricted by environmental factors, particularly light intensity. Mating in the fruit-fly *Dacus tryoni* occurs at an optimal light intensity of ca 0.8 lux. Under a constant low light of this intensity, mating followed a circadian rhythm that free-ran with a period ca 28 h for ca 4 days; nonetheless, under either DD) or LL conditions mating is strongly depressed (1, 20). Nevertheless, even under these conditions the endogenous rhythm of motivation to mate persists for two or three cycles prior to ceasing. Under natural conditions, given that both high light intensity and darkness suppress mating, mating activity is restricted to a remarkably short period of 30 min daily (Figure 3). The adaptive value of limiting mating to dusk is presumably because this synchronises the sexual behaviour of all individuals in the population, thereby increasing mating efficiency (1,20).

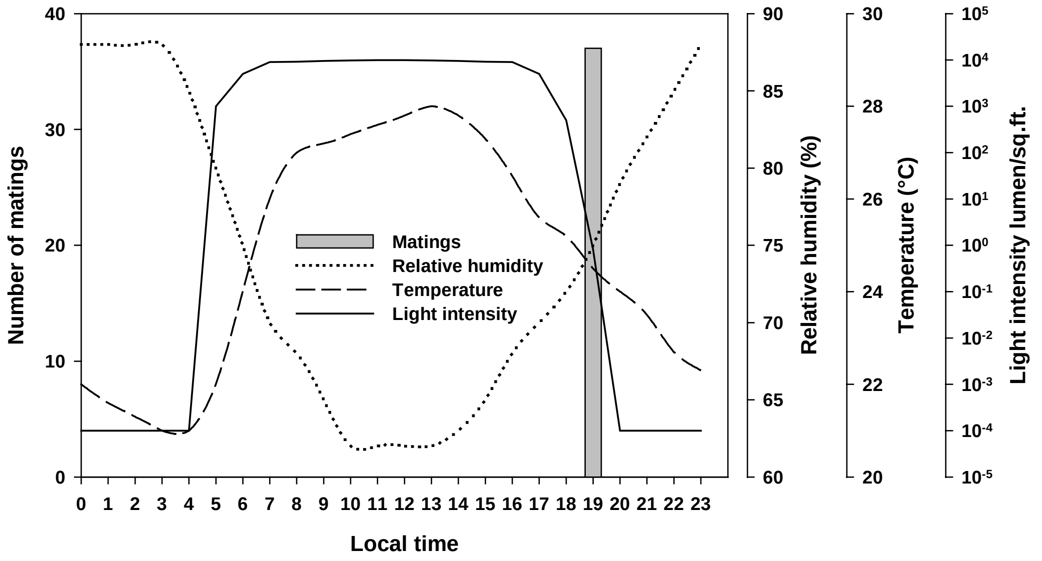

Figure 3. Mating activity in the fruit-fly *Dacus tryoni* throughout 1 day under natural conditions. Changes in relative humidity, temperature, and light intensity are also shown (modified from [20]).

2bii. Time-memory (Zeitgedächtnis)

The ability of honey-bees (*Apis mellifera*) to return to a food source at the same time each day was known nearly 100 years ago, when the Swiss naturalist August Forel observed that bees arrived at his breakfast table for food. Because the bees always came at the same time—even when food was not present—Forel (21) proposed that bees possess a memory for time (*Zeitgedächtnis*) (1). Later, Beling (22) trained bees by offering them a sugar solution in an artificial feeding set in a certain place at the same time each day. During subsequent days (the test period), the feeding place was without sugar, but each visiting bee and its time of arrival was recorded. Beling demonstrated that bees do indeed return at the same time each day (Figure 4). She also showed that bees could be trained to come at any time of the day and, moreover, that they could be trained to come at two or more separate periods during the day, provided that the interval between two successive training periods was >2 h (1). The adaptive value of this ability is related to the fact that different plant species bloom at different times throughout the day and this is another consequence of the co-evolution plant-pollinator.

The unequivocal test for the endogeneity of bee time-memory came during the 1950s. Renner (24,25), trained bees to a food source in Paris between 8.15 and 10.15 a.m. local time in a closed chamber under LL and constant temperature. The bees were then transported overnight to New York and tested the following day under identical conditions. In this now classical translocation experiment, the bees had been transported over 76° of longitude, or a difference

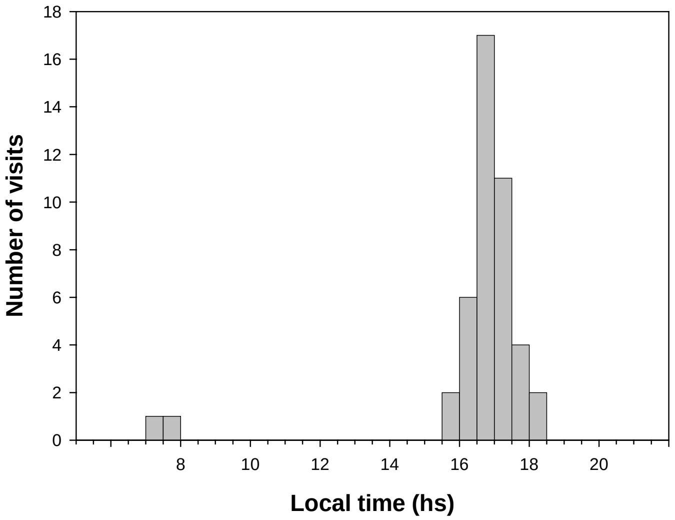

Figure 4. The time-memory (*Zeitgedächtnis*) of honey-bees. Bees were trained to collect sugar solution from a feeder at the same time (16–18 h) during several consecutive days. On the test day, sugar was omitted, but the bees continued to arrive at the dish at the same time of the day (modified from [22]).

of ca 5 h in real local time. If an endogenous circadian rhythm were involved, bees should have come to the test dish 24 h after the training period; if, on the other hand, the bees were responding to subtle local influences they should forage at the same local or sun time. The results showed that the former alternative was the case: bees came to the feeding dish at 3.00 a.m. Eastern daylight time, exactly 24 h after their last feeding period in Paris. The reciprocal experiment involving translocation from New York to Paris yielded an analogous result (1).

2biii. Learning ability rhythms

Decker and co-workers (25) have recently shown that olfactory learning in the cockroach *Leucophaea maderae* is regulated by the circadian system. Insects were trained and tested at different circadian phases for their performance in odour discrimination 30 min after training (short-term memory) or 48 h after training (long-term memory). After differential conditioning in which one odour was associated with a positive reward (sugar solution) and another different odour with a negative one (saline solution), cockroaches conditioned in the early subjective night exhibited a strong preference for the former and retained the memory for at least 2 days. Animals trained and tested at other circadian phases demonstrated significant deficits in performance for both short- and long-term memory. Performance depended on circadian time (CT) of training, not CT of testing, and results indicate that memory acquisition rather than retention or recall is modulated by the circadian system.

The question arises concerning the adaptive value of circadian learning and memory regulation. In the majority of instances in which circadian control of memory formation has been demonstrated, humans and other animals perform better with training during the active phase. Decker and co-workers proposed that memories are only profitable when formed within the environmental context in which they will be utilized. Because the environment is periodic and because cockroaches are active at night and spend the daytime hidden in dark shelters, little would be gained by forming memories during the daytime. Memories based on information obtained at a time and in an environment in which the animal is unlikely to be foraging could interfere with successful foraging during its normal foraging at night-time hours (25).

3. Circadian rhythms in insect populations

Some rhythms are not evident at the individual level, but as gated rhythms in the population. Events such as egg hatching or ecdysis (hatching from the old exoskeleton) do not occur each day in insects but rather once or a few times along the individual life. The timing of this process needs to be quite precise, because both processes render the animal vulnerable to predators and expose it to desiccation, in that the novel cuticle is not maintained rigid, nor fully impermeable. In addition, the novel cuticle must exit the old exoskeleton over a tiny layer of lubricant fluid. Thus, hatching at the moment when relative humidity is relatively high would allow individuals to free themselves from the old cuticle quicker, with water loss minimisation, and more importantly, avoiding the risk of remaining as a partial or totally prisoner of the old exoskeleton and eventually dying in the process. On the other hand, short-lived species such as mayflies are compelled to proceed toward adult life synchronically, in order to find a mature sexual partner and reproduce in solely a few hours.

Therefore, processes as early in insect life as egg-hatching express circadian rhythmicity. This was first demonstrated by Minis and Pittendrigh (26), who investigated the egg-hatching rhythm in the moth, *Pectinophora gossypiella*. At 20°C, egg development lasted ca 9–10 days. Eggs raised in either LL or DD hatch arythmically, but when raised in (12 h light/12 h dark (LD 12:12), they exhibited a distinct rhythm during which hatching occurs immediately after dawn. This rhythm could also be initiated by a single non-recurrent light or temperature pulse. The rhythm cannot, however, be initiated until after the midpoint of embryogenesis (132 h from deposition). Because both light and temperature pulses fail equally in inducing rhythmicity when applied prior to this period, it appears that the oscillator controlling egg hatching is not yet functional, rather than this being related to coupling between the photoreceptive system and the clock (1).

The relationship between rhythmic hatching and environmental conditions can be illustrated by the bug *Triatoma infestans*. Larvae and adults of this species are strongly xerophilic and can be found in dry areas of Argentina in refuges in which the microenvironment is even drier than that outside. Paradoxically, and as other insects, egg-hatching success is strongly affected by low relative humidity values (27). Given that females do not change their preference for dry places when they oviposite and that eggs are not able to move to another site, the question arises concerning how they avoid the deleterious effects of dryness at hatching. As shown in Figure 5, the majority of eggs hatch at dawn, i.e., during daytime when relative humidity is highest. Even in the driest areas, an increase in the airs water vapour content occurs and these insects utilise this moment to hatch. Thus, not only larvae and adults are able to express hygropreference, but also eggs. In the latter case, however, this hygropreference is not spatial, as in mobile instars, but temporal. If eggs are exposed to LD cycles during the first half of the development, egg-hatching gated rhythm in *T. infestans* persists under constant darkness, evincing its circadian nature (28).

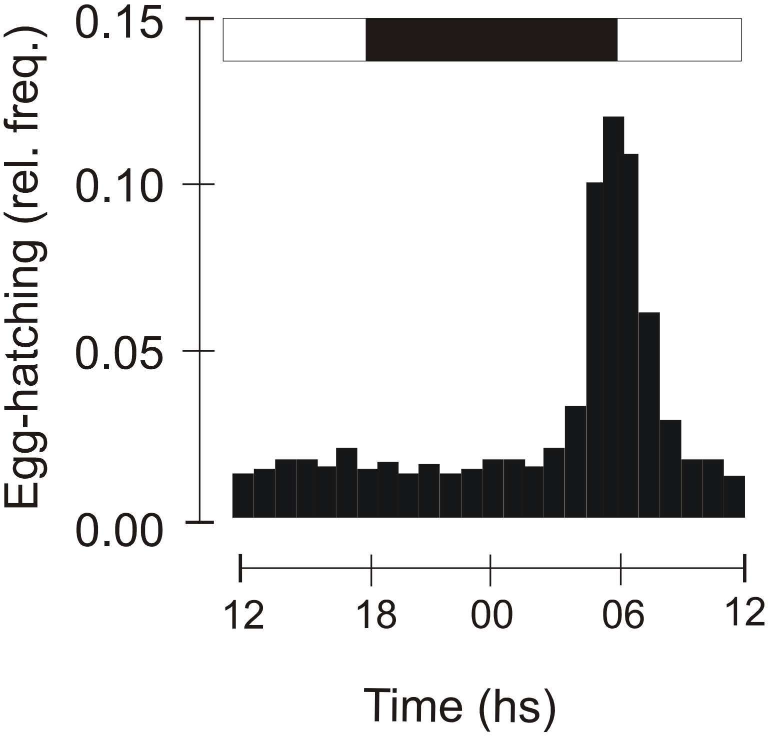

Figure 5. Rhythm of egg hatching in the haematophagous bug *Triatoma infestans*. The majority of eggs hatch at dawn, when the relative humidity of the environment is highest (modified from [28]).

In some insect species, population rhythms evince the coordinated expression of endogenous clocks possessing different rhythmicities. Midges of the genus *Clunio* (Chironomidae) are found in inter-tidal Atlantic- and Pacific-shore zones ranging from temperate areas to the Arctic. *Clunio marinus* lives on Western Europe’s Atlantic coast, occupying the lowest parts of the inter-tidal zone, which are exposed solely during times of spring low water. Only during these times are insects able to emerge (29). The females of this species are wingless. The males emerge before the females, assist the females in their own emergence, copulate with these, and subsequently carry them to the larval habitat. Both sexes are extremely short-lived (<2 h) and oviposition must occur before the tide rises to cover the larval site (1). Neumann (29–31) showed that the rhythm of adult emergence in *C. marinus* is governed by the superposition of a circadian rhythm that controls pupal eclosion and a semi-lunar rhythm that determines the beginning of pupation.

4. Interspecific synchrony

Synchronisation of activities among individuals belonging to different species is a usual phenomenon that allows animals to concentrate efforts on the search for food at the moment of the day when it is present. Predators seek for

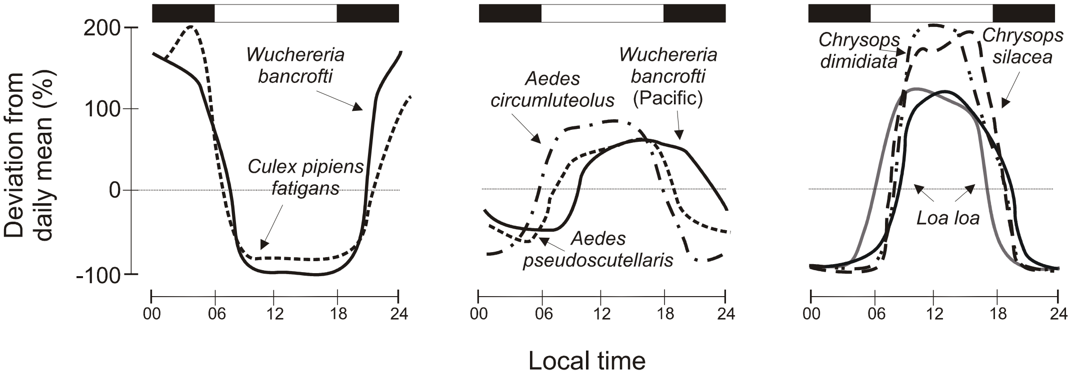

Figure 6. Synchronisation between the microfilaria migration into the host's blood stream and the biting activity of their vectors in different geographic areas. The number of parasites in the peripheral blood vessels is depicted as a function of time (redrawn from [34]).

their prey when these are outside their refuges, and honey-bees return to flowers to collect nectar immediately on their blooming (*Zeitgedächtnis*).

One of the most interesting cases of synchronisation across species is that of insect vector-transmitted parasites. C. Pittendrigh (32) emphasized that the functional significance of the entrainability of self-sustaining oscillators—and in particular their predictive value and ability of timing under conditions of absence of adequate sensory input—can be clearly illustrated with the migration of microfilaria into the circulatory system of their vertebrate hosts. This displacement of parasites toward peripheral blood vessels is timed to match the time when the local insect vector (a mosquito) is actively searching for a host to feed upon (Figure 6). The parasites would lack the capacity to perceive this by themselves in the presence of vectors. However, host internal cues could provide parasites with reliable temporal cues that, in turn, are phased to local time by the host's circadian system's entrainment to the light cycle. Migration would be coupled with the appropriate phase of the parasite's circadian oscillator, assuring its presence under the host's skin when the vector is actively seeking a blood meal (32). This time differs between both localities and vector species, but always corresponds with the vector's feeding time (32–34).

5. Photic and non-photic entrainment

The daily light-dark cycle is undoubtedly the major environmental synchroniser, or *Zeitgeber*, with the temperature cycle (a non-photic *Zeitgeber*) second in importance. Insects do not appear to be the exception, but several other non-photic *Zeitgeber* have been described as evincing their capacity to employ different kinds of information. Within a natural context, each species can be more or less exposed to light according to their habitat, and use of different sensory modalities permits obtaining more reliable temporal information.

The ability of insects to colonize very different environments brought them to habitats with little or no periodicity, such as deep caves, inside logs or tree trunks, of trees, or even high latitudes where during long periods of light intensity remain constant. Species living in these places range from arrhythmic to those possessing well-defined activity rhythms (1), depending on their contact with the periodic outside.

Certain insects, such as the haematophagous bug *T. infestans*, are characterized by spending the majority of their time hidden in dark places and having a highly developed thermal sense. Do they use light or thermal environmental cycles as *Zeitgeber*? Experimental work revealed that, as expected, they synchronize their activity very rapidly to high/low temperature cycles in both DD and LL. In addition, a sole transient cycle is necessary to resynchronize their activity after a 12-h temperature-cycle phase-shift. Given that a light/cycle requests 3–4 cycles to resynchronize post-inversion, one might think that temperature and not light is the main cue followed by these bugs. However, when confronted by the simultaneous presence of both cycles at different phase-relationships, a dominant light effect becomes evident. Insects can shift their activity to high- or low- temperature periods, but never to photophase (17). This demonstrates that it is reliability that makes a *Zeitgeber* occupy the highest rank and not the insect’s sensitivity for the different variables.

6. The circadian clock

The ubiquity of circadian rhythms in insects is expressed in different forms. One of these is the ability of some organs to continue to express self-sustained rhythmicity *in vitro* and isolated from the remainder of the insect. This is the case of the reproductive organs of certain butterfly species, in which the descent of spermatozoa from testicles through genital ducts occurs with daily periodicity (35). In addition, sensory organs, such as compound eyes and antennae, have revealed the presence of peripheral oscillators that are capable of maintaining the rhythm of sensitivity (10, 36).

As in mammals, local oscillators are submitted to the control of a central master clock that synchronises these to assure coordinated work. The paired master clock of insects is located in each optic lobes of the brain. In a series of very elegant experiments, Page (37) raised cockroaches in two different light cycles (LD 11:11, or LD 13:13) that resulted in two groups of insects: one with a short period (22.7 h) and the other with a long period (24.2 h). These differences were maintained for at least 5 months in DD. Ablation of both optic lobes, which resulted in arhythmicity, was then followed by reciprocal transplantation of optic lobes from short-to long-period insects, and vice-versa. One to two months later, rhythmicity was restored, revealing that the recipient

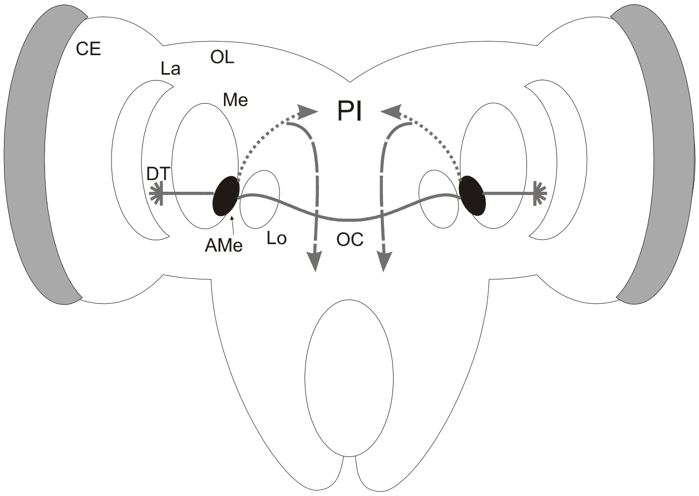

Figure 7. Location and main connectivity of the circadian master clocks in brain of cockroach, Leucophaea maderae (frontal view). Paired master clocks are located inside each optic lobe (OL) near medulla (Me) in accessory medullae (AMe). They receive indirect light input from compound eyes (CE) via lamina (La) and distal tract (DT). Projections leave the AMe and run to the pars intercerebralis (PI). Both aMe are connected via optic commissure (OC) fibres. Lo = lobula. Modified from (38).

adopted the donor phase and donor period. Histological examination revealed that transplanted optic lobes regenerated appropriate connections to brain. This experiment provided unequivocal evidence that optic lobes contained circadian pacemakers (1).

Figure 7 represents the master-clock organisation of the cockroach Leucaphaea maderae as well as main nervous tracts, which synchronize the two clocks with each other and convey information to different parts of central nervous system, including pars intercerebralis, in which neurosecretory cells are involved in hormonal and developmental rhythms, as indicated previously in this chapter.

7. Photoperiodism and the circadian clock

As discussed at the beginning of this chapter, insects are strongly affected by the conditions of their environments. Despite that their small size and ectothermic condition impose important constraints on them, they have succeeded in conquering many different habitats in nearly every region of the earth. In the majority of regions, at least one period of the year is too cold, too hot, or too dry for insects or their food supplies. To overcome this problem, many insect species have developed specific strategies: either they migrate, enter into diapause (programmed dormancy state), or change their bodies to better adapt to these conditions. All three processes take place on a seasonal basis during a period of the year that varies according to the region. A common characteristic of these processes comprises that these require need the insect to be able to anticipate the arrival of the deleterious season for preparing its body for survival during this period. For this, insects react to the variation of the relation between the length of the day and that of the night, i.e., the photoperiod. Winter is characterized by short days and long nights and the reverse for the summer, and by measuring the photoperiod and its variation along successive days, insects can predict their proximity. Processes controlled by the photoperiod rely on functional elements for measuring the day length (or night length), for counting short or long days (to avoid weather interference) and a third one analyses the direction of the change (shortening or lengthening of the days). We will discuss here just the first one, i.e. the measuring of the day length, because it is the only which could be associated to the circadian system.

Two types of mechanisms have been proposed for measuring the day/night ratio, either involving or not, a circadian clock. The first, independent of a circadian mechanism, would work similarly to an hour-glass. Although the exact mechanism remains elusive, it can be illustrated as the accumulation of a metabolite that is synthesized during the night and degraded by light. During long nights, product concentration would reach a given threshold and the day would be considered as short. Provided that measurement is conducted on a single-night basis, no circadian component is necessary. Aphids constitute the main group employing an hour-glass for measuring photoperiodic time and the model system utilized to uncover its properties.

The second way to measure day or night length is to employ a circadian clock. Suppose that you wake up at 7 a.m. on a summer day. If you look out the window, you see daylight outside. However, when winter approaches, natural light at this time becomes weaker and weaker. Thus, you are not required to measure all time, but only to observe the correspondence between external conditions and the inner clock. A mechanism based on this principle was first proposed by Erwin Bünning and is known as the external coincidence model (1).

The circadian system may also effect this measurement in a different manner. Suppose that there are two circadian oscillators, one locked at dusk and the other at dawn. Each oscillator will experience maximum and minimum at different moments throughout the day, but their relative phase will vary according to the day’s length. This model was proposed by Pittendrigh and differs from the previous one, in that know the coincidence of observing not between the clock and the outside, but between two internal oscillators: therefore, this is known as internal coincidence (1).

External and internal coincidence are not the only models proposed to explain the way different insect species measure day length employing a circadian clock. Other circadian system-based mechanisms have been proposed and are described in detail by Saunders et al. (1).

More recently, whether hour-glass and circadian mechanisms to measure photoperiodic time are mutually exclusive and non-related has been critically reviewed by D. Saunders (39). The main conclusion of his work comprises that there is sufficient evidence to consider the hour-glass of Aphids and other insects as a heavily damped circadian oscillator.

8. Concluding remarks

Insects have contributed much to unravelling many big questions in chronobiology and continue highlight many relevant aspects. They are excellent experimental models, as demonstrated in seminal works conducted by the pioneers of the study of biological rhythms. They can be easily reared in the laboratory, have short generation times, and are robust for experimental surgical manipulation (e.g., ligatures, decapitation, transplantation, parabiosis). Conversely, chronobiological studies on pest insects can greatly improve our knowledge concerning their biology, providing basic knowledge on the temporal organisation of pest life. This information possesses a large applied value, because it can aid in improving their control by applying control measures during the time of higher susceptibility, for example, when ecdysis or hatching take place and the body cuticle is most permeable. We hope that the information summarised here will encourage biologists to conduct work in this area.

References

1. Saunders, D.S., Steel, C.G.H., Vafopoulou, X. and Lewis, R.D. 2002, *Insect Clocks*, third ed. Elsevier Science, Amsterdam.
2. Steel, C.G.H. and Vafopoulou, X. 2002. Physiology of circadian systems. In: Saunders, Steel, Vafopoulou, & Lewis (Eds.) *Insect Clocks*, third ed. Elsevier Science, Amsterdam, pp. 115-118.
3. Chapman, R.F. 1998. *The Insects. Structure and Function*. University Press, Cambridge, UK.
4. Neville, A.C. 1963 Daily growth layers in locust rubber-like cuticle, influenced by an external rhythm. *J. Insect Physiol*. 9, 177-186.
5. Neville, A.C. 1965 Circadian organization of chitin in some insect skeletons. *Quart. J. Micro. Sci.* 106, 315-325.
6. Neville, A.C. 1967 A dermal light sense influencing skeletal structures in locusts. *J. Insect Physiol.* 13, 933-939.
7. Davey, K. 2007. The interaction of feeding and mating in the hormonal control of egg production in *Rhodnius prolixus. J. Insect Physiol.* 53, 208-215.
8. Anton, S. and Gadenne, C. 1999 Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *PNAS* 96, 5764-5767.

9. Krishnan, B., Dryer, S.E. and Hardin, P.E. 1999 Circadian rhythms in olfactory responses of *Drosophila melanogaster*. *Nature* 400, 375-378.
10. Tanoue, S., Krishnan, P., Krishnan, B., Dryer, S.E. and Hardin, P.R. 2004 Circadian clocks in antennal neurons are necessary and sufficient for olfaction rhythms in *Drosophila*. *Current Biology* 14, 638–649.
11. Page, T.L. and Koelling, E. 2003. Circadian rhythm in olfactory response in the antennae controlled by the optic lobe in the cockroach. *J. Insect Physiol.* 49, 697–707.
12. Barrozo, R.B., Minoli, S.A. and Lazzari, C.R. 2004 Circadian rhythm of behavioural responsiveness to carbon dioxide in the blood-sucking bug *Triatoma infestans* (Heteroptera, Reduviidae). *J. Insect Physiol*. 50, 249-254.
13. Lazzari, C.R., Minoli, S.A. and Barrozo, R.B. 2004 Chemical ecology of insect vectors: the neglected temporal dimension. *Trends Parasitol.* 20, 506-507.
14. Reisenman, C.E., Lazzari, C.R. and Giurfa., M. 1998 Circadian control of photonegative sensitivity in the haematophagous bug *Triatoma infestans*. *J. Comp. Physiol. A* 183, 533-541.
15. Reisenman, C.E., Insausti, T.C. and Lazzari, C.R. 2002 Light-induced and circadian changes in the compound eye of the haematophagous bug *Triatoma infestans* (Hemiptera: Reduviidae) *J. Exp. Biol*. 25, 201-210.
16. Lazzari, C.R., Reisenman, C.E. and Insausti, T.C. 1998 The role of the ocelli in the phototactic response of *Triatoma infestans* (Heteroptera: Reduviidae). *J. Insect Physiol*. 44, 1159-1162.
17. Lazzari, C.R. 1992 Circadian organization of locomotion activity in the haematophagous bug *Triatoma infestans*. *J. Insect Physiol*. 38, 895-903.
18. Barrozo, R.B., Schilman, P.E., Minoli, S.A. and Lazzari, C.R. 2004 Daily rhythms in disease-vector insects. *Biol. Rhythm Res.* 35, 79–92.
19. Gehring, W. and Wehner, R. 1995 Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. *PNAS* 92, 2994-2998.
20. Tychsen, P.H. and Fletcher, B.S. 1971 Studies on the rhythm of mating in the Queensland fruit fly, *Dacus tryoni*. *J. Insect Physiol.* 17, 2139-2156.
21. Forel, A. 1910 *Das Sinnesleben der Insekten*. Munich.
22. Beling, I. 1929 Über das Zeitgedächtnis der Bienen. *Z vergl Physiol*. 9, 259-338.
23. Renner, M. 1955 Ein Transozeanversuch zum Zeitsinn der Honigbiene. *Naturwissenschaften*, 42, 540-541.
24. Renner, M. 1957 Neue Versuche über den Zeitsinn der Honigbiene. *Z. vergl. Physiol*. 40, 85-118.
25. Decker, S., McConnaughey, S. and Page, T.L. 2007 Circadian regulation of insect olfactory learning. *PNAS* 104, 15905–15910.
26. Minis, D.H. and Pittendrigh, C. S. 1968 Circadian oscillation controlling hatching: its ontogeny during embryogenesis of a moth. *Science* 159, 534-536.
27. Roca, M. and Lazzari, C.R. 1994 Effects of the relative humidity on the haematophagous bug *Triatoma infestans*. Higropreference and eclosion success. *J. Insect Physiol*. 40, 901-907.
28. Lazzari, C.R. 1991 Circadian rhythm of egg hatching in *Triatoma infestans* (Hemiptera: Reduviidae). *J. Med. Entomol*. 24, 740-741.

29. Neuman, D. 1963 Über die Steuerung der lunaren Schwarmperiodik der Mücke *Clunio marinus*. *Verh. dt. Zool. Ges.* 1962, 275-285.
30. Neuman, D. 1966a Die intraspezifische Variabilität der lunaren und taglichen Schlüpfzeiten von *Clunio marinus* (Diptera: Chironomidae). *Verh. dt. Zool. Ges*. 1965, 223-233.
31. Neuman, D. 1966b Die lunare und tagliche Schlüpfperiodik der Mücke *Clunio*. Steuerung und Abstimmung auf die Gezeitenperiodik. *Z. vergl. Physiol.* 53, 1-61.
32. Pittendrigh, C.S. 1974 Circadian oscillations in cells and the circadian organization of multicellular systems. In: Schmitt and Worden (Eds.), *The Neurosciences: Third Study Program*, Cambridge, Mass., MIT Press, pp. 437-458.
33. Hawking, F. 1975 Circadian and other rhythms of parasites. *Adv. Parasitol.*, 13: 123-182.
34. Aschoff, J. 1989 Temporal orientation: circadian clocks in animals and humans. *Anim. Behav.* 37, 881-896.
35. Giebultowicz, J.M. 1999. Insect circadian clocks: is it all in their heads? . *J. Insect Physiol*. 45, 791-800.
36. Pyza, E. and Meinertzhagen, I.A. 1997 Circadian rhythms in screening pigment and invaginating organelles in photoreceptor terminals of the housefly's first optical neuropile. *J. Neurobiol.* 32, 517–529.
37. Page, T.L. 1982 Transplantation of the cockroach circadian pacemaker. *Science* 216, 73-75.
38. Helfrich-Förster, C. 2004 The circadian clock in the brain: a structural and functional comparison between mammals and insects. *J. Comp. Physiol. A*, 190, 601-613.
39. Saunders, D.S. 2005 Erwin Bünning and Tony Lees, two giants of chronobiology, and the problem of time measurement in insect photoperiodism. *J. Insect Physiol*. 51, 599–608.