Organization and molecular mechanism of insect circadian clocks

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ABSTRACT

Life could not exist without organisms' engineered ability to keep track of time on a 24-hour day-night cycle called a *circadian rhythm*. Insects display an impressive variety of daily rhythms, which are most evident in their behaviour. Circadian timekeeping systems that generate these daily rhythms of physiology and behaviour all involve three interacting elements: the timekeeper itself (i.e. the clock), inputs to the clock through which it entrains and otherwise responds to environmental cues such as light and temperature, and outputs from the clock through which it imposes daily rhythms on various physiological and behavioural parameters. The clocks control various behaviours, physiological functions, and developmental events, enabling adaptation to periodic environmental changes. Circadian clocks also function in time-compensation for celestial navigation and in the measurement of day or night length for photoperiodism. Molecular and physiological mechanisms are best understood for the optic-lobe and mid-brain circadian clocks, although there is no direct evidence that these clocks are involved in rhythmic phenomena other than circadian rhythms in daily events. Circadian clocks have also been localized in peripheral tissues. Circadian clocks are most well studied in *Drosophila melanogaster*. *Drosophila* flies spend their entire lives in small areas near the ground, and use their circadian brain clock to regulate daily rhythms of rest and activity, so as to organize their behaviour appropriately to the daily rhythms of their local environment. Migratory locusts and butterflies, on the other hand, spend substantial portions of their lives high up in the air migrating long distances and use their circadian brain clocks to provide time-compensation to their sun-compass navigational systems.

Introduction

Circadian rhythms

To cope with the daily environmental changes generated by the Earth's rotation, such as light and dark cycles, temperature fluctuations and changes in food availability, most organisms possess an intrinsic timing system to adapt their activities accordingly. The circadian (from Latin *circa*-around, and *diem* or *dies* is a day meaning "around a day"). Circadian rhythms are the base of circadian clock system. All individual cells have circadian rhythms.

Circadian clocks - General concept

Circadian clocks are some sort of biochemical oscillators that oscillates in every organism on 24 hours basis, and an organism can respond according to them. These biochemical oscillators are called it as "clock". These biochemical oscillating clocks sometimes called it as "Internal clock" or "Circadian clock." Circadian clocks are endogenous timekeepers that enable organisms to track daily changes of light and other environmental factors.

A circadian clock must possess three basic properties:

- A stable, near 24-hours period;
- Temperature compensation;
- Ability to be reset.

An intrinsic or free running period of approximately 24 hours of the circadian clock is required for measuring time and anticipating environmental changes. The rates of biochemical reactions are closely related to the temperature. To maintain a constant period, the circadian clock is required to possess a temperature compensation mechanism. A free-running clock, however, will eventually run out of phase without fine tuning and resetting. To avoid this, the clock must be able to incorporate various external feeds into the intrinsic self sustained cycle to synchronize day to day.

From (Fig. 1), the circadian system can be depicted as a cascade of processes leading from the circadian clock through molecular and cellular rhythms to the temporal organization of physiology and behaviour. The most evident insect rhythms are those associated with behaviour. They include daily rhythms of individual insects such as those affecting locomotion, flight, feeding, oviposition, and once in- a-lifetime rhythmic events such as hatching, molting, pupation, and adult eclosion.

Rhythms in physiological processes are not as obvious as behavioural cycles, but they also play an important role in temporal organization of body functions (Michel et al., 1993). Examples of insect physiological rhythms include the rhythm of pheromone synthesis, rhythm of spermatophore formation, rhythm of sperm release from testis and rhythmic secretion of ecdysteroids from prothoracic glands. Physiological rhythms can result from passive responses of tissues to a timing signal, e.g. daily fluctuations in circulating hormones. On the other hand, some physiological rhythms are tissue autonomous and thus likely to be underlined by cellular rhythms. Rhythms in cellular physiology are well documented in a wide range of organisms.
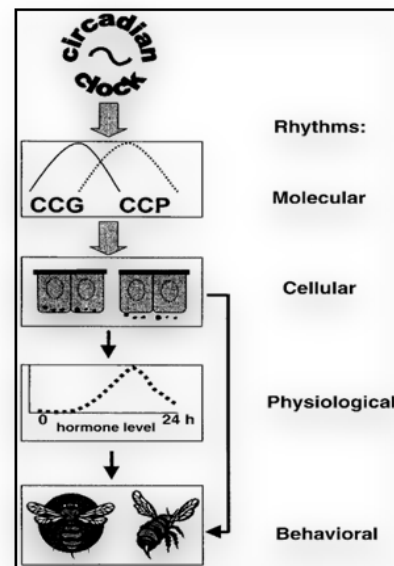

Fig. 1: Schematic illustrating levels at which the circadian clock may interact with biological processes. CCG – clock controlled genes, CCP- clock controlled expression of proteins (Giebultowicz, 2000).

In insects, only a few rhythms in cellular physiology are known. One prominent example is the rhythm of cuticle deposition which arises owing to changes in the orientation of secreted chitin by the underlying epidermal cells.

Composition of circadian clock

Circadian clock system can be theoretically divided into three integrated parts (Fig. 2). In the center is the circadian clock or pacemaker which drives the intrinsic rhythm with a period near 24 hours, even in the (temporary) absence of environmental cues. Upstream of the pacemaker is an input system which can receive multiple external cues, or Zeitgeber (German for time-givers), to entrain or reset the pacemaker. Downstream of the pacemaker are the output pathways directly governing the physiological and behavioural

rhythms. Zeitgeber includes light and temperature elements.
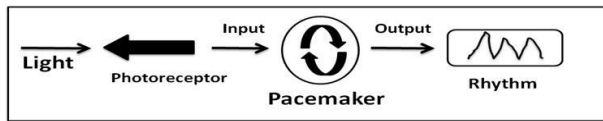
Fig. 2: Diagrammatic representation of circadian clock system.

An entrainment pathway that consists of a photoreceptor and coupling mechanism (input) synchronizes a self-sustaining oscillator (pacemaker) to the external light/dark cycle (Charlotte et al., 2011). The output of the pacemaker regulates the timing of various processes (e.g., activity) via coupling to the effectors mechanisms.

Clockwork network

Genes in the clock cell produce proteins at varying concentrations throughout the day. The rhythmic nature of the rise and fall of protein concentration enables the animal to know the time. Since only a few cells in the brain are responsible for time measurement, the animal needs a messenger to transmit the time to the rest of the body. There appears to be some direct communication between the clock cells and the nervous system, but more of the communication happens through hormones. This phenomenon not only in insects but also in mammals, which release corticosteroids into the bloodstream at varying concentrations depending on the time of day.
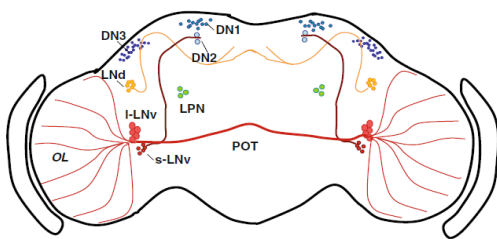
Fig. 3: Diagram showing *Drosophila* cerebral cells expressing PERIOD, Tomioka and Matsumoto (2010).

Cellular organization of the clock has been extensively studied in *Drosophila* and other insects, using molecular probes, such as antibodies against clock gene products and reporter genes driven by clock gene promoter, etc. In Drosophila, there are about 150 cells in the brain that express the clock genes, and they are divided into seven

subgroups (Helfrich-Forster, 2006) (Fig. 3). Three of them are located laterally between the optic lobe and the central brain, and are called lateral neurons (LNs). The ventrally located LNs consist of two subgroups of neurons with larger and smaller cell bodies and are called large-LNv (l-LNv) and small- LNv (s-LNv), respectively. The dorsally located LNs are called LNd. There are three groups of neurons in the dorsal region of the central brain called DN1, DN2, and DN3, respectively. The other group is located on the lateral posterior side of the central brain, and is called lateral posterior neurons (LPNs). Detailed analysis of the role of the cells revealed that s-LNvs drive the morning peak and a part of the evening activity, and the LNds regulate the evening peak (Rieger et al., 2006) Recently, l-LNv cells have been revealed to be involved in light-induced arousal and phase shifting in the late night.DNs, however, have some roles in locomotor rhythms, since the flies lacking LNs still show a circadian locomotor rhythm for at least a few days in constant conditions. In larval brain, DN2 cells have the principal role in temperature entrainment and might regulate the phase of LNs, while under LD, the larval DN2 s are controlled by LNs via PDF signalling (Picot et al., 2009).

Entrainment of the clock mechanism

Circadian rhythms are synchronized, or entrained, by environmental stimuli referred to as Zeitgeber (a German term for "time giver"). Predictable changes of light intensity associated with the solar day constitute a powerful Zeitgeber that entrains circadian clocks. Shifting the phase of light–dark (LD) cycles will result in a similar phase shift in rhythmic process, so that the characteristic phase relationship between the rhythm and Zeitgeber is maintained. Detailed analysis of entraining factors can be obtained by on strutting phase response curves (PRC), which plot changes in the phase of a given rhythm in response to entraining stimuli applied at different phase of free running circadian cycle.

Input pathways

Input pathways mainly include light and temperature entrainment. Light is the most important Zeitgeber for the circadian clock to synchronize to the environmental cycles.

Photoreceptors necessary for the entrainment, or resetting, of the clock have been localized in some insects. In hemimetabolous insects such as crickets and cockroaches, the compound eye is the most important photoreceptor, but ocelli also sometimes have minor roles in the photic entrainment. In *Drosophila*, in addition to these external eyes, H–B eyelet that is a remnant of larval eye, and the blue light receptor molecule CRY1, are known for circadian photoreceptors. In *Drosophila*, CRY1 is expressed in a circadian fashion in some of the cerebral clock neurons, and shifts the phase of the clock light-dependently. Besides CRY1, rhodopsins (Rhs) are also involved in photic entrainment. In Drosophila, Rh1 and Rh6 were implicated in entrainment to red light and Rh1, Rh5, and Rh6 to green and yellow light (Hanai, 2009). Histamine is suggested to be an important neurotransmitter for the Rh pathway. Cockroaches and crickets, in which the compound eyes appear to be the exclusive photoreceptors for entrainment because sectioning the optic nerves between the eyes and the optic lobe or painting over the compound eyes eliminated entrainment of the locomotors activity rhythm to light cycles. Newly identified light-responsive protein Quasimodo (Chen et al., 2011) deserves attention.

Temperature entrainment

Temperature is also an important time cue to synchronize the clock, especially in dark places, such as eggs laid underground or insects that pupate in darkness. Temperature cycle or temperature steps are known to cause phase shifts of the clock to adjust to the time of environmental cycles (Ikeda 1993). The molecular mechanism for the temperature synchronization is less understood. In Drosophila, the effects of temperature step-up and -down were examined at transcript levels. Under constant light, temperature step-up up-regulates the Clk (clock) gene, and step down down-regulates it. In contrast PER (period gene), TIM (timeless gene) and CYC (cycle gene) genes are down-regulated and up-regulated by step up and step-down, respectively. After temperature step down, all these clock genes showed an oscillation in constant conditions. These temperature responses are virtually eliminated in Clk mutant flies, suggesting that the Clk gene is the principal component for temperature entrainment. In addition, TIM mRNA

levels are increased earlier at low temperatures through photic stimulation of TIM expression Existence of temperature entrainable oscillators has been reported for other insects including cricket's, cockroaches and mosquitoes.

Output pathways

The pathways through which the clock regulates overt rhythms are not well understood. Pigment-dispersing factor (PDF) is the best characterized output molecule of the circadian clock system. Its involvement was first notified when immune histochemistry revealed that PDF co localized with PER in some clock neurons, s-LNv and l-LNv, in the *Drosophila* brain and is now thought to be an output neurotransmitter of the circadian clock in a wide variety of insects. It is a member of the pigment dispersing hormone (PDH), an octadeca peptide that was first found in crustacean as a hormone that disperses pigments in the epithelium. PDF gene expression shows no circadian rhythm in *Drosophila* while the dorsomedial termini of PDF-expressing neurons show daily morphological changes probably controlled by the circadian clock. *Drosophila,* PDF in cockroaches is both a phase regulator and a locomotors rhythm driver. In crickets, however, since the partial destruction of the optic lobe leads to arrhythmic locomotors activity without elimination of the PDF neurons, PDF may play only minor roles in the behavioural rhythm. Besides regulation of the activity rhythms; PDF plays an important role in the sensory system of crickets. PDF plays dual roles: 1) Synchronizing molecular oscillations of circadian clock neurons 2) Output pathway from the circadian clock. The vicinity of PDF fibres + mushroom bodies (*Apis mellifera)* famous for their marvelous time memory. So these PDF plays important role in output pathways for entrainment.
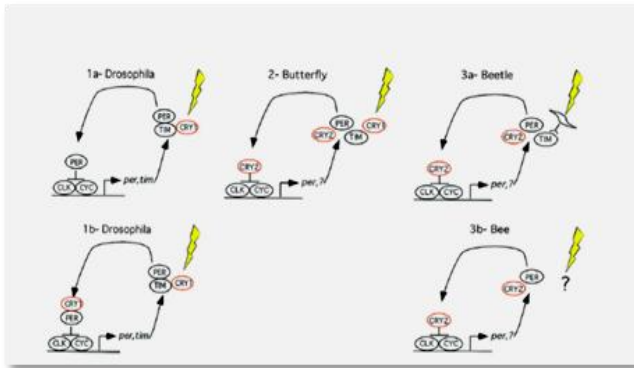
Insect clockwork models

With the existence of 2 functionally distinct CRYs in insects, 3 major types of clockwork models can be proposed as shown in Fig. 4 (Yuan et al., 2007).

Type 1: The Drosophila form) in which CRY1 only functions in the central brain clock as a circadian photoreceptor (panel 1a) or in peripheral clocks as both a photoreceptor and central clock component (panel 1b).

Type 2: (The ancestral form apparent in the monarch butterfly) in which both CRY1 and CRY2 exist and function differentially within the clockwork.

Type 3: In which only CRY2 exists and functions within the clockwork. In beetles, CRY2 acts as a transcriptional repressor of the clockwork and light input may be mediated through the degradation of TIM (panel 3a). In bees, which lack TIM, CRY2 acts as a transcriptional repressor and novel light input pathways (?) are used to entrain the clock (panel 3b).

Fig. 4: Clockwork network models
(Source: Yuan et al., 2007).

Phase response curve (PRC)

PRC is a graphical representation which depicts how organism can respond to given stimulus. A PRC is a plot of phase shifts as a function of the oscillator phase at which a stimulus is given. In circadian rhythms, PRCs are usually constructed by giving a light pulse on a free-running rhythm in constant darkness. In insects, a PRC in this form was first identified by Pittendrigh (1959) in the fruit fly *Drosophila pseudoobscura* and such PRCs have been reported in various species. PRCs not only explain the mode of entrainment but also prove the existence of an oscillator with a period of approximately a day.

Anatomical locations for circadian clocks

Circadian clocks have been localized either in the central nervous system or in peripheral tissues. The circadian clocks in the central nervous system, i.e., the central clocks, are localized in the optic lobe or midbrain. The optic lobe flanks the mid-brain, a medial part of the brain, and contains the neuropils involved in the pre processing of visual information from the compound eye. In cockroaches and crickets, the circadian clock in the optic lobes controls their activity rhythm. The optic lobe consists of lamina, medulla, and lobula complex.

In the cockroach *Rhyparobia maderae,* it was shown by transplantation experiments that the accessory medulla, a small neuropils situated at the ventromedial edge of the medulla, is the locus of the circadian clock. In the cricket *Gryllus bimaculatus*, the medulla and the obula are connected by the optic stalk. Removal of the lamina and medulla regions results in arrhythmic locomotion activity.

In Lepidoptera and Diptera, the circadian clock is localized in the mid-brain. In saturniid moths *Antheraea pernyi, Hyalophora cecropia* and *Samia cynthia,* the circadian clocks for the eclosion and flight rhythms are localized in the mid-brain. In *A. pernyi*, two pairs of neurosecretory cells in the dorsal part of the mid-brain have been shown to express the clock protein period (PER), and these cells are regarded as the site of the circadian clock.
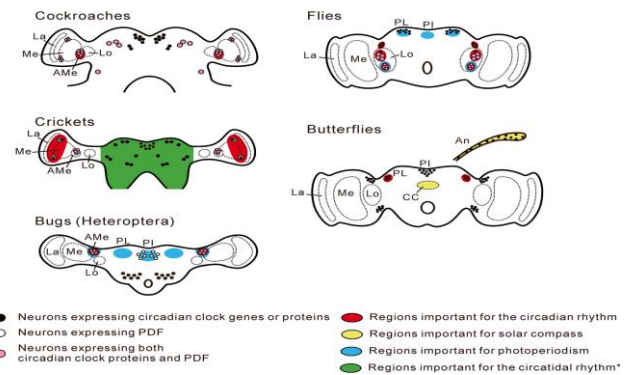

Fig. 5: Neurons or regions important for the circadian rhythm, solar compass, photoperiodism, and circatidal rhythm (Numata et al., 2015). (La- lamina; Me- medulla; A Me- accessory medulla; Lo- lobula; PI- Pars intercerebralis; PL-Pars lateralis; CC- central complex; An- antenna).

Neurons are important for the circadian rhythm, solar compass photoperiodism and circatidal rhythm. In cockroaches, the accessory medulla containing PDF-expressing neurons is the location of the circadian clock regulating activity rhythms. In crickets, the circadian clock is located in the lamina and medulla region. It is likely that the circatidal clock is not located in the optic lobe, but is probably located in the central brain,

although the possibility that the clock is located in an extra-brain region cannot be excluded. In *Rhodnius prolixus* (Heteroptera), lateral neurons, which co-express clock proteins and PDF, are considered to be the circadian clock that regulates the activity and hormone-release rhythms. The region containing these neurons is involved in the photoperiodic diapause in another heteropteran, *Riptortus pedestris*.

The PI and PL regions are also important for photoperiodic diapause in heteropterans. In flies, among neurons expressing clock proteins in the brain, several groups of lateral neurons control the circadian activity rhythm. One group of neurons that co-express clock proteins and PDF is important for photoperiodism. Neurosecretory cells in the PI and PL regulate photoperiodic diapause. In butterflies, the PL is the main location of the circadian clock in the brain. Neurons necessary for the solar compass are located in the central complex, and the circadian clock in the antennae is necessary for time compensation of the solar compass system. Note that the neuronal location may differ among species.

The molecular basis of the clock (*Drosophila melanogaster*)
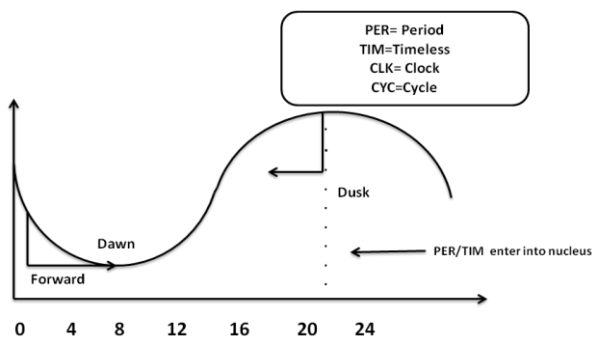

Fig. 6: Molecular basis of circadian clock in *Drosophila*.

The flies typically show a bimodel locomotor activity rhythm displaying two peaks associated with dawn and dusk. Timing of dawn and dusk peaks of activity is controlled by a dual-oscillator system consisting of physically separated evening oscillator (E) and morning oscillator (M). Four genes, the transcriptional regulators (PER), (TIM), (CYC) and (CLK), have been shown to be critical components for generating the basic circadian oscillation. Of the four, three, PER, TIM, CLK, are rhythmically expressed, and circadian oscillations

in both mRNA and protein levels are well documented (Fig. 6).

CLK-CYC heterodimers bind to E-boxes of nuclear DNA and activate transcription of PER and TIM. As PER protein is produced it is phosphorylated (e.g., by DBT) which leads to its degradation. However, if TIM is present it can bind to, and stabilize, phosphorylated PER, which remains bound to DBT. The TIM-PER-DBT complexes are further phosphorylated which promotes their transport into the nucleus. These complexes then bind to CLK-CYC, inhibiting PER and TIM transcription. As PER and TIM levels decline (degradation not shown), the inhibition is removed and the cycle begins again. Phase shifts and entrainment by light occur through a pathway in which activation of CRY by light leads to TIM degradation (Hardin, 2005). Both CLK and CYC proteins are transcription factors that utilize bHLH domains to bind to E boxes, hat mediate the association of the two proteins leading to the formation of heterodimers. The fundamental mechanism for generating the oscillation involves two, interlocked transcription/translation feedback loops—one that regulates rhythmic expression of PER and TIM and a second that regulates rhythmic expression of CLK. The mechanism of transcription and translation feedback loops in *Drosophila* is given in Fig. 7.
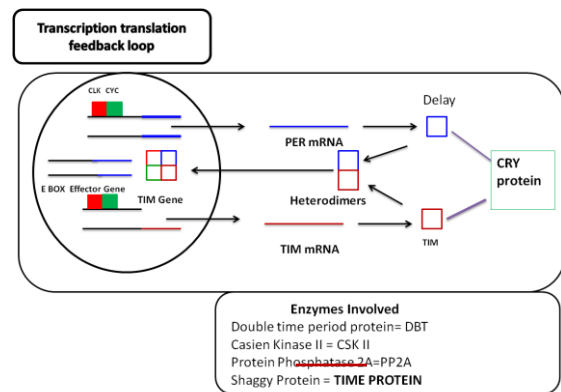
Fig. 7: Mechanism of transcription and translation feedback loops in *Drosophila*.

A heterodimers composed of CLK and CYC binds to promoters of PER and TIM leading to an increase in transcription of these two genes that continues throughout the day. Levels of mRNA for the two genes peak in the early night. Protein products of these two genes increase as well, but

peak levels of protein are delayed by several hours, peaking after the middle of the subjective night.

The heterodimers moves to the nucleus and functions as the negative element in the feedback loop, acting on the positive regulators CLK and CYC to suppress their activation of the PER/TIM promoters. This leads to a decline in the PER/TIM mRNA levels that continues throughout the night. The degradation of PER and TIM allows the cycle to start over. The time delay between mRNA synthesis and the accumulation of PER and TIM protein is likely to be critical element in the generation of the oscillation. PER is unstable in the absence of TIM whereas the dimerization with TIM stabilizes PER and promotes nuclear entry. Several kinases (e.g., double time—DBT) and phosphatases regulate the phosphorylation state of PER and TIM that, in turn, appears to be involved in regulation of PER degradation as well as nuclear entry of the PER-TIM heterodimers.

Photoperiodism, the seasonal clock

Photoperiodic clocks allow organisms to predict the coming season. The organism responds either to annual changes in day length, or directly to the length of day/night and uses this information to determine the timing of key events such as migration or the entry into a dormant state. Seasonal timing is just as important as daily timing and is essential for enabling animals to correctly anticipate seasonal environmental changes. Among insects, one of most prevalent photoperiodic responses is the onset of diapause. The ability to enter diapause has contributed to the evolutionary success of insects.

A photoperiodic response in insects comprises a sequence of several events**:** i) Photoreception; (ii) Measurement of day or night length by a photoperiodic time measurement system; (iii) Simultaneous counting of the number of photoperiodic conditions by a counter system; and (iv) Action of the endocrine effectors that determine seasonal events like diapause.

Other types of insect clocks:

Other types of insect clocks mainly includes,

Time memory (Zeitgeber): This clock-related phenomenon was shown in the honey-bee, *Apis mellifera* (Von Frisch 1948). Workers of this species inform other workers in the same hive about the direction and distance of a food resource by a "waggle dance". The direction of the waggle dance is closely correlated with the angle formed between the resource and the sun. The endogenous circadian clock which enables them to remembered the time of a day at which nectar sources are available. The oscillation free runs in LD (light and dark) with natural period slightly less than 24 hours.

Time compensated sun compass orientation: This orientation mechanism is called a "solar compass" or "sun compass". The monarch butterfly, *Danaus plexippus*, uses a similar mechanism in its long-distance migration (Perez et al., 1997). Monarch butterfly and sometimes locust can orient themselves to the sun, to pattern of the polarized light from sky or artificial light sources. A constant compass orientation maintains because the orientation mechanism also contain a circadian clocks component which compensate for sun's changing azimuth.

Lunar, semi lunar and tidal rhythm: There are endogenous rhythms with a period considerably different from a 24 hours day in organisms including insects. These rhythms have free-running periods close to the tidal (12.4 h), semi lunar (14.8 days), lunar (29.5 days) annual (365 days) (Saunders 1976).The varied carpet beetle, *Anthrenus verbasci,* show a circannual rhythm in pupation, and with this rhythm pupation occurs only in spring. A marine midge, *Clunio marinus*, lives in the lowest parts of the intertidal zone. Larvae of this species have a circa semi lunar or circalunar rhythm in pupation and, as a result, adults emerge in spring tides. The mangrove cricket, *Apteronemobius asahinai,* has a circatidal locomotor rhythm; it forages on the floor of mangrove forests during low tide and rests during high tide.

Circannual rhythm

There are hypotheses that a circadian clock produces a circannual, circa semi lunar, or circa tidal rhythm. Moreover, circa (semi)lunar rhythms

can be explained by beat reactions of circadian and circatidal clocks. Whether a circadian clock is involved in the generation of these non-circadian rhythms has been examined in the above three species, but no experimental evidence supporting such involvement has been found.

Circadian clocks for celestial navigation

Monarch butterfly

Monarch migration is miracle of nature, exactly how they navigate from Canada to Mexico is still a mystery and scientist have only some clues; 1) one theory is they fallow angle of sun in relation to earth 2) earth magnetic field provide subtle orientation guide, 3) some special cells in their brain which regulate their internal clocks and keep them on a course. Circadian clock for navigate to their overwintering sites during their seasonal long-distance migration. Use sun compass orientation and regulate output in the brain (Sauman et al., 2005) suggested that *D. plexippus*, cryptochrome (*Drosophila* type CRY I & CRY II mouse type) and core clock proteins (PER) and (TIM) circadian clock for time-compensated celestial navigation. Same circadian clock has been used for behavioural rhythms & time-compensated celestial navigation. The light-entrained antennal circadian clock is necessary for correct orientation. A peripheral clock also plays an important role in solar compass orientation of this species. Heinze and Reppert (2011) suggested *D. plexippus*; the solar compass mechanism involves two distinct circadian clocks, the azimuthal + elevation compensations (regulated by CR). Circadian clock for navigate to their overwintering sites during their seasonal long-distance migration.

Mosquito

Mosquitoes have an annoying tendency to come out around dusk to feed on human blood (Palmer 2013) there's a rather obvious cue for that behaviour: the setting of the sun. But researchers have shown that mosquitoes don't need that cue. If you place a mosquito in a light-free laboratory for several days, it will still begin searching for food at about the same time. On the first day, it may emerge a few minutes late, though, on account of the inaccuracy of its internal clock. On Day Two, it will become active a few minutes later, and so on.

In fact, one of the perplexing things about biological timing mechanisms is the consistency of their inaccuracy. All individuals belonging to the same species are mis-calibrated by almost exactly the same amount in each 24-hour cycle. When a mosquito perceives the sunset, it resets its clock, two 'CRY's clock system is present into the mosquito.

Cicada

One of the mysteries of cicadas is how they calibrate their internal clocks. They live long periods without the benefit of the sun, yet their life cycle still progresses like clockwork. Animals' biological clocks don't just assess time of day; they also perceive the time of year. When the animal's clock cells synchronize themselves to the rising and setting of the sun, they take note of the changing length of the day during the season. The rhythm of the seasons is thus recorded in the animal's brain. (Animals on the equator, revealingly, tend not to alter their behaviour based on time of year). Each of the life stages is controlled by a clock, and it can go into an overwintering stage at the end of each of these larval stages, according to the day length." It's possible that the cicada doesn't have to measure 17 years. All it needs to do is measure one year, which is the length of time it takes to go from one larval stage to the next. Perhaps the progression from birth to adult happens in 17 stages, until the perfected adult is ready to climb above ground, breed and die.

Conclusion

Although information is only available for quite limited orders of the Class Insecta, the error in internal clocks isn't a problem in nature, because all insects have methods for correcting their clocks. For day-to-day rhythms, they use the rising or setting of the sun. Insect's biological clocks don't just assess time of day; they also perceive the time of year. Future comparative studies should provide an insight for the general understanding of the insect clock mechanism as well as how the variety of clocks has diversified from an ancestral one. Insect Growth can be use as a natural Clock in forensic entomology. We can use of every experimental technique in our arsenal, from continued classical experiments up to recent RNAi and RNA sequence technology to determine the

exact role of circadian clocks in insects daily rhythm control and maintenance.

Conflict of interest statement

Authors declare that they have no conflict of interest.

References

Charlotte, H.F., Michael, N., Todd, C.H., 2011. Insect Circadian clock output. Biochem. Soc. Essays Biochem. 49, 87-101.

Chen, K.F., Peschel, N., Zavodska, R., Sehadova, H., Stanewsky, R., 2011. QUASIMODO, a novel GPI-anchored zona pellucida protein involved in light input to the *Drosophila* circadian clock. Curr. Biol. 21, 719-729.

Giebultowicz, J. M., 2000. Molecular mechanism and cellular distribution of insect circadian clocks. Annu. Rev. Entomol. 45, 769-793.

Hanai, S., Ishida, N., 2009. Entrainment of *Drosophila* circadian clock to green and yellow light by Rh1, Rh5, Rh6 and CRY. Neuroreport. 20(8), 755-758.

Hardin, P. E., 2005. The circadian time keeping system of *Drosophilla*. Curr. Biol. 15(17), 14-22.

Heinze, S., Reppert, S. M., 2011. Sun compass integration of skylight cues in migratory monarch butterflies. Neuron 69, 345-58.

Helfrich-Forster, C., 2006. The neural basis of *Drosophila*'s circadian clock. Sleep Biol. Rhythms 4, 224-234.

Ikeda, M., Tomioka, K., 1993. Temperature dependency of the circadian locomotor rhythm in the cricket *Gryllus bimaculatus*. Zool. Sci. 10, 597-604.

Michel, S., Geusz, M., Zarisky, J., Block, G., 1993. Circadian rhythm in membrane conductance expressed in isolated neurons. Science 259, 239-241.

Numata, H., Yasuke, M., Tomoko, I., 2015. Common features in diverse insect clocks. Zool. Lett. 1, 10.

Palmer, B., 2013. https://www.washingtonpost.com/national/healthscience/cicadas-are-going-but-theyll-be-back-like.

Perez, S. M., Taylor, O. R., Jander, R., 1997. A sun compass in monarch butterflies. Nature 387, 29.

Picot, M., Klarsfeld, A., Chelot, E., Malpel, S., Rouyer, F., 2009. A role for blind DN2 clock neurons in temperature entrainment of the *Drosophila* larval brain. J. Neurosci. 29, 8312-8320.

Pittendrigh, C. S., Bruce, V. G., 1959. Daily rhythms as coupled oscillator systems and their relation to thermoperiodism and photoperiodism (Ed.: Withrow, R. B.). American Association for the Advancement of Science, Washington DC. pp.475-505.

Rieger, D., Shafer, O. T., Tomioka, K., Helfrich-Förster, C., 2006. Functional analysis of circadian pacemaker neurons in *Drosophila melanogaster*. J. Neurosci. 26, 2531-2543.

Sauman, I., Briscoe, A. D., Zhu, H., 2005. Connecting the navigational clock to sun compass input in monarch butterfly brain. Neuron. 46, 457-467.

Saunders, D. S., 1976. Insect Clocks. Pergamon Press Ltd., Oxford, England.

Tomioka, K., Matsumoto, A., 2010. A comparative view of insect circadian clock systems Cell. Mol. Life Sci. 67, 1397-1406.

Von Frisch, K., 1948. Solved and unsolved problems of bee language. Bull. Anim. Behav. 9, 2-25.

Yuan, Q., Metterville, D., Briscoe, A.D., Reppert, S.M., 2007. Insect cryptochromes: Gene duplication and loss define diverse ways to construct insect circadian clocks. Mol. Biol. Evol. 24, 948-955.