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Chapter

The Effects of Infection on Mosquito Rhythmic Behavior

Rafaela Vieira Bruno, Luana Cristina Farnesi and Luciana Ordunha Araripe

Abstract

Most organisms live in a rhythmic world, where daily environmental variation has a profound effect on their behavior and physiology. In addition to abiotic influence, interactions with other organisms that have their own particular cycles are also part of circadian rhythm formation. In this chapter, we present aspects of the biology of mosquito vectors, more precisely *Aedes aegypti*, which is a vector of arboviruses of great epidemiological importance, like dengue, Zika, and chikungunya. The successful transmission of the virus depends on the coordination of several behavioral and physiological traits involved in the virus-vector-host interaction. Thus, understanding the mechanisms of endogenous control of rhythmic traits of the mosquito vector and the impact that both environmental variation and virus infection can have on this regulation is key for a reliable estimate of the vectorial capacity. We discuss the infection-driven changes in traits used to calculate parameters of the vectorial capacity, and finally, we review the current knowledge on the molecular mechanisms underlying vector rhythmic behavior and the potential cellular targets of arbovirus infection.

Keywords: *Aedes aegypti*, arbovirus, behavior, vectorial capacity, physiology, neurotropism, Zika, dengue, chikungunya, circadian clocks

1. Introduction

1.1 The *Aedes aegypti* mosquito as a vector of arboviruses

Aedes aegypti (Diptera: Culicidae) is an insect belonging to the family Culicidae and subgenus *Stegomyia*. The species was originally found in Egypt, hence its specific name; but it is currently distributed worldwide, occurring mainly in tropical and subtropical regions [1, 2]. *Aedes aegypti* life cycle is composed of four phases: egg, four larval instars, pupa, and adult (**Figure 1**).

Since they spend most of their life cycle in water, mosquitoes are considered to be primarily aquatic; they gain the terrestrial environment only in adulthood, when they fly in order to seek for food and mates [3–5]. Easy to distinguish for taxonomists, *A. aegypti* is a dark-bodied mosquito in the adult phase, with white spots on the dorsal abdomen and legs and a white pattern composed of a lira-shaped drawing on its scutum (**Figure 2**) [1, 6].

The mosquito *A. aegypti* is considered a major disease vector in urban habitats, being able to host and transmit various arbovirus. Females of anautogenous

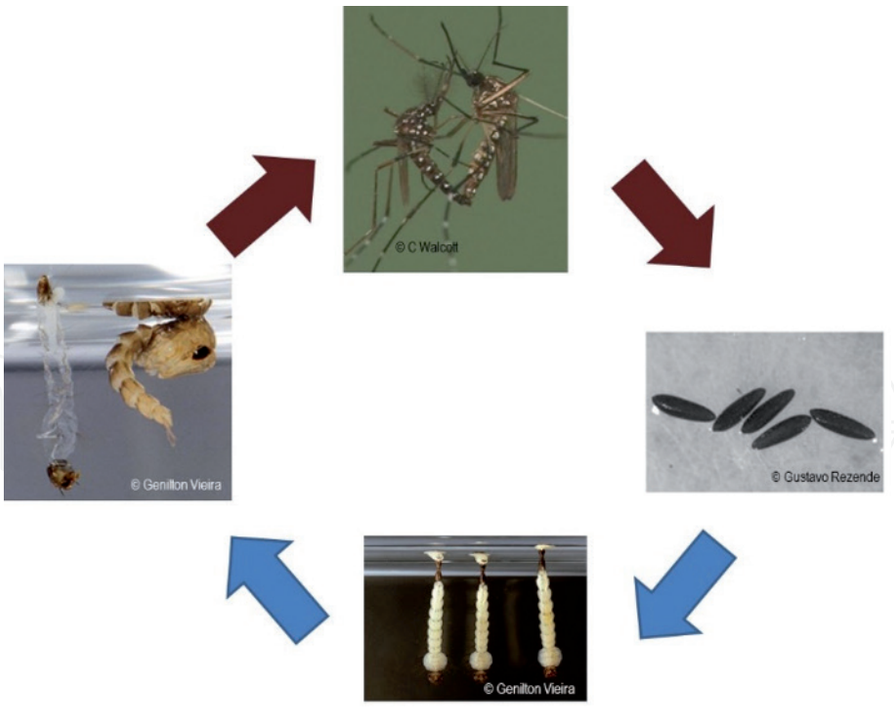

Figure 1.
Life cycle of the Aedes aegypti mosquito (credits of the photos on each image—out of ratio).

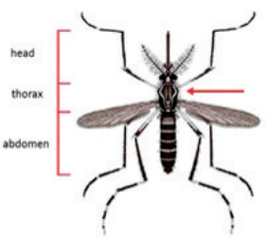

Figure 2.
Aedes aegypti mosquito. The arrow points to the scutum region that displays the lira-shaped (out of ratio). The three body parts, common to all insects, are indicated above. Adapted from [6].

mosquitoes, like *A. aegypti*, need a blood meal in order to mature their eggs and to perpetuate the species. Because of this, this mosquito is indubitably one of the most pathologically important arthropod vectors. One *A. aegypti* female is able to produce approximately 100 eggs after blood feeding on a vertebrate host, in each gonotrophic cycle (interval between blood meal and egg laying). Even being a diurnal mosquito, the female prefers to lay eggs in dark/shaded sites and in the dark phase of the day [7–10]. *Aedes aegypti* is an anthropophilic species, preferring to feed on human blood than the blood of other vertebrates [11, 12]. This feature is responsible for the role of *A. aegypti* as transmitter of many pathogens that cause important human diseases. The markedly anthropophilic and endophilic behaviors of *A. aegypti* make it a very efficient vector of yellow fever, dengue, chikungunya, and Zika viruses [3, 11, 13–16]. Many other factors related to behavior and physiology of vector and pathogens are significant for the success in arbovirus transmission, such as (i) the habit of laying eggs in multiple breeding sites; (ii) the diversity of posture sites; (iii) the gonotrophic discordance, that is, the ability to blood feed on more than one host for each batch of eggs produced; and (iv) the ability of the eggs in remaining viable, in quiescence state, for up to 1 year in dry conditions (called "egg resistance to desiccation") and in large temperature variation (i.e., 16–35°C) [1, 3, 17–20]. All these features can be associated to vector competence and vectorial capacity [7].

Both vector competence and vectorial capacity are critical for arbovirus transmission. Vector competence is the intrinsic ability of a vector to acquire, maintain, and transmit a pathogen to another host. In mosquitoes, a species is considered vector competent when females transmit the pathogen from one vertebrate to another during blood feeding [21]. This competence is related to intrinsic features of the vector, as well as the pathogen, such as pathogen genotype, pathogen strain, and vector strain. Specifically, for the viruses DENV and CHIKV, vector competence has been tested and confirmed in *A. aegypti* and *A. albopictus* laboratory strains. To DENV, environmental factors as daily temperature fluctuations have been demonstrated to impact vector-pathogen interactions, being able to modulate the *A. aegypti* competence to DENV transmission [15, 22, 23].

Although the number of studies on *A. aegypti* behavior and physiology, as well as arbovirus-mosquito interactions, has been growing, additional information is needed in order to promote the development of better mosquito control actions. Variation in the vector competence for different arboviruses highlights the existence of different virus-vector interactions. For example, both *A. aegypti* and *A. albopictus* show the competence to transmit the arboviruses DENV, ZIKV, YFV, and CHIKV; however these vectors do not exhibit the same transmission efficiency. Likewise, within-population genetic variation may explain the varied vector competence for different arboviruses and may also be related to the response of mosquitoes to control programs [24].

Other factors may be involved in the vector competence, for example, two different insecticide resistance mechanisms were described to enhance the vector competence of *Culex quinquefasciatus* for West Nile virus, which can impact on transmission dynamics of arboviruses for other mosquito vector species [25]. Measuring the vector competence of field mosquitoes for different arbovirus can help to assess the risk of arbovirus emergence [24].

Vectorial capacity, in turn, is the estimated value through a formula that takes into account a set of parameters of intraspecific physiology and behavior that, associated with environmental conditions, favor natural transmission of a given disease. The vectorial capacity is mainly influenced by population density, biting behavior (frequency of host contact for blood feeding), and mosquito vector survivorship [26]. The concept of vectorial capacity was initially established for the transmission of malaria by vectors of the genus *Anopheles* and calculated by the formula shown in **Figure 3**, where the total number of potentially infectious bites a day is one of the parameters. Many studies of mathematical models describing pathogen transmission by mosquitoes make similar assumptions [28, 29].

The World Health Organization emphasizes that mosquito vector control plays an important role in blocking the propagation of critical arboviruses. This is particularly relevant when no vaccines or specific drug treatments are available, as is the case for dengue, Zika, and chikungunya, which have the *A. aegypti* as the main vector [13, 14, 30]. Understanding vector competence and vector capacity mechanisms is important in designing safer vaccines and new strategies to prevent the transmission of pathogens. Specifically for the mosquito vector *A. aegypti*, many barriers hamper infection, dissemination, and transmission of arboviruses through mosquito vector tissues.

$$VC = ma^2\, bp^n / - \log_e p$$

Figure 3.
Vectorial capacity formula: here m is the number of female mosquitoes per host, a is the daily blood feeding rate, b is the transmission rate among exposed mosquitoes, p is the probability of daily survival, and n is the extrinsic incubation period (EIP). Adapted from Refs. [26, 27].

2. Aspects of mosquito behavior and their role on the vectorial capacity

In mosquitoes, locomotor activity [31–34], host-seeking and blood feeding [35], digestion, mate finding and reproduction, and site choice for oviposition [36–38] are examples of rhythmic patterns that are recognizably modulated by extrinsic factors [39]. While these patterns have been increasingly studied, ecological interactions between hematophagous females and their hosts and pathogens are not well understood [40]. Likewise, how female rhythms affect and are affected by males' biological aspects associated with courtship and mating is still obscure [41, 42]. An emerging field of study, namely, "the causes and consequences of daily rhythms in the interactions between vectors, their hosts and the pathogens they transmit," was reviewed in Rund et al. [40].

Cycles in behavior and physiology have coevolved so that the organism's fitness is optimized. A shift in the rhythm of these traits may disrupt important biological functions leading to impacts on fertility and viability. For instance, in *Drosophila*, a shift in the time of day that food is ingested leads to a reduction in fertility [43], whereas maintaining the expected time for food intake leads to the benefits of an improved cardiac function [44]. In mosquitoes, when females engage in foraging or seeking for hosts, a suite of enzymes responsible for blood digestion must be operating, their immune response must be on to avoid pathogen infection, and their detoxification against insecticides must be active [40]. Therefore, breaking the interlocked pathways for pathogen-vector-host interactions will affect the vectorial capacity and the epidemiology of arboviruses.

The vectorial capacity measures the chance of emergence of new cases of the disease departing from one infected human host. As such, the parameters of behavior and physiology used in the calculation assume that mosquitoes are infected. Other parameters include population density, frequency of bites [26, 40], and transmission competency, which are directly influenced by the vector's behavior and physiology, as well as by the pathogen's behavior and extrinsic incubation period (EIP) [26, 40, 45].

The magnitudes of most parameters of the vectorial capacity equation are highly dependent on the daily variation of locomotor/flight activity behavior. There are several ways of measuring the pattern of locomotor activity of insect species, varying from the traditional method of reporting the presence of one species in field traps, in different times of the day, to activity monitors and video imaging used in the laboratory. Data generated by all these methods are represented with similar graphics, where the amount of locomotor/flight activity registered at each time interval is plotted on a 24-h graph. Variation in activity is studied according to variation in a *Zeitgeber*, a term used for an environmental synchronizer such as light or temperature.

Field and laboratory studies show that *A. aegypti* is active during the day, with activity peaks at dawn and dusk and lack of activity at night [7, 9]. Because flight activity toward hosts is driven by olfactory signals in mosquitoes, one could expect that rhythms in the expression of odorant binding proteins should parallel the olfactory sensitivity to host odors in order to activate the behavioral output [40]. However, in *Aedes aegypti*, rhythms in olfactory sensitivity are not sufficient to explain the daily cycling in behavior toward hosts [39]. The authors performed electroantennography assays and Y-maze olfactometer experiments using five different volatiles (including plants and host odors) and found that the peak of olfactory behavior is decoupled from the variation in olfactory sensitivity. These results suggest that modulation of the behavior associated with olfactory cues happens in both the peripheral (antenna) and central (endogenous clock) levels.

Humans are the main hosts for *A. aegypti* females, and humans are most likely awake and active when these females are trying to land and blood feed. This imposes a risk for the mosquito. Body heat and carbon dioxide are the human factors that are the most attractive for mosquitoes [46]. The availability of these factors varies in a circadian way [40, 47, 48] and are subjected to changes in environmental conditions [40].

Light and temperature are the major environmental factors affecting the rhythmic behavior of most organisms. As such, variation in these factors has a profound effect on the vectorial capacity. For instance, the biting rate of *A. aegypti*, which is a fundamental parameter in the calculation of the vectorial capacity, is highly influenced by temperature and time of day. Since females need to be active in order to engage in blood seeking, temperatures below 15°C and above 36°C constrain locomotor activity and make the number and intensity of bites to cease [3, 49].

Mating interaction is another element influencing vectorial capacity. Significant alterations in females' physiology and behavior happen after copulation, when male accessory gland peptides are transferred along with sperm [50], though contrasting effects have been reported. Augmented host-seeking and blood-feeding activity [31, 51–53], as well as an increase in oviposition rates [54, 55] and egg development [56], were reported, suggesting that these alterations could boost up the vectorial capacity. On the other hand, Lima-Camara et al. [9] have found a significant decrease in the mean locomotor activity after insemination and after blood feeding in females of *A. aegypti*. Although this result was reported as the daily mean of locomotor activity, the occurrence of a significant increase in the dusk peak of activity, which is the peak associated with biological functions like host-seeking and oviposition, is remarkable.

3. The effects of infection on behavior and physiology of mosquito vectors

Since vectorial capacity suffers major influence of vector behavior, studying the degree of modulation that arbovirus exerts on *A. aegypti*'s behavior is a key factor for understanding infection dynamics and host pathogenesis. In a recent work, Gaburro et al. have shown that infection by Zika virus leads to neuro-excitation in *A. aegypti*'s brain, inducing changes in the mosquito's behavior. The increase in neuronal spikes in infected versus non-infected females reflected on an increase in flight activity when females were studied in pools [57]. The authors found replicating virus in ZIKV-infected female brains, characterizing the tropism for the central nervous system, as well as in sensory organs like antennas and eyes, potentially affecting neuronal communication. Likewise, dengue virus was also found to be neurotropic in mosquitoes [58].

A consequence of the neurotropic characteristic of these arboviruses is the alteration in the patterns of locomotor activity and feeding behavior. For instance, *A. triseriatus* becomes more avid for refeeding when infected by La Crosse virus [59, 60], while *Aedes aegypti* becomes more active when infected with serotype 2 of dengue virus [61] and with Zika virus when females are monitored in groups in cages (**Figure 4**) [57]. However, the assumption that virus infection would modulate behavior in a way to increase virus transmission and vectorial capacity is not always met. The example of West Nile virus indicates a possible decrease in virus transmission, where the mosquito vector *Culex pipiens* becomes less avid for host-seeking when infected with the virus [63]. Likewise, for individually monitored females of *A. aegypti*, Zika virus infection reduces flight activity, suggesting that infected mosquitoes may remain associated with closely distributed human hosts (**Figure 4**) [62].

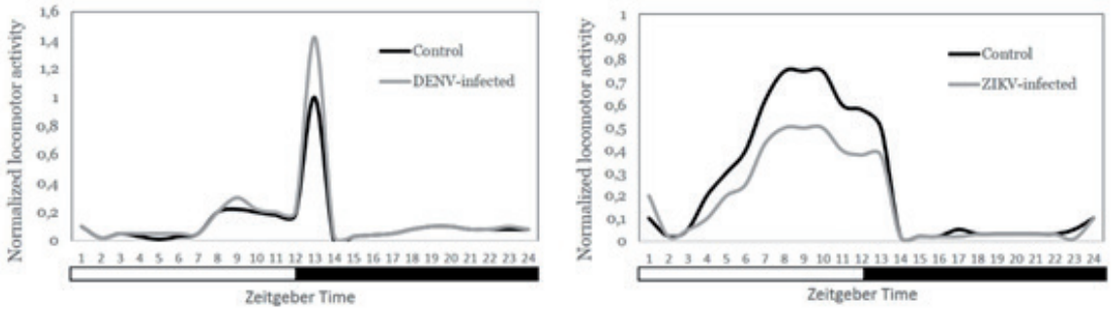

Figure 4.
Locomotor activity of virus-infected and not infected females. (A) Females infected with serotype 2 of dengue virus (modified from [61]). (B) Females infected with Zika virus (modified from [62]). The Zeitgeber time means the time passed, in hours, after light is turned on.

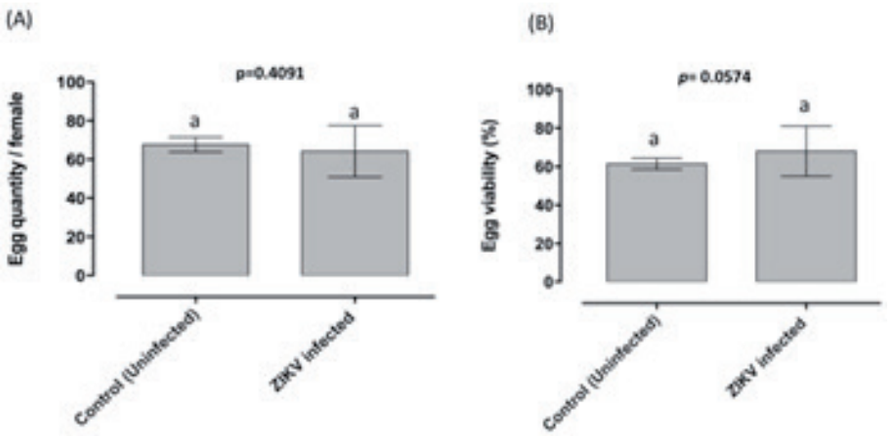

Figure 5.
Effect of Zika virus infection on the fecundity (A) and fertility (B) of Aedes aegypti females, on the third gonotrophic cycles. The lack of significance is represented by p values >0.05 obtained by using the nonparametric Mann-Whitney tests. Error bars represent mean ± s.d of three independent experiments (modified from [62]).

Arbovirus infection is also responsible for changes in physiological traits implicated in the estimate of the vectorial capacity. The number of female mosquitoes per host is one of the most important parameters of the vectorial capacity and is directly influenced by life history traits like the number of eggs laid by females (fecundity) and the number of viable offspring (fertility). These traits have been reported altered by arbovirus infection, although the effect varies depending on the virus. Dengue-infected females of *A. aegypti* produce a significantly lower number of eggs with a lower hatching rate [45], while ZIKV-infected and non-infected females do not show significant differences in fecundity and fertility (**Figure 5**) [62].

4. Human environmental impact and the effects on vector-host interaction and the risk of disease transmission

Human occupation may lead to profound alterations in the environment, such as global warming and light pollution. Some of these changes impose new selective pressures to all organisms involved with the infection, say pathogens, vectors, and hosts, but also their predators and the vegetation used as nutrition or habitat. The modeling of the effects of global warming on disease transmission indicates a shift in the global distribution of *Aedes*-borne virus with mild to severe effects on the risk of transmission [64, 65]. Concerning light pollution, the increase in domiciliary and peridomiciliary lighting may extend the phase of activity of *A. aegypti* by a couple

of hours, which may raise the biting rate and the chance of arbovirus transmission. A recent work showed that artificial lighting at night make house sparrows, the reservoirs of West Nile virus, to become infectious for a period of 2 days longer than house sparrows that get dark nights [66]. This leads to an increase of 41% in the potential of disease outbreak. Comparatively, in light-night areas, nocturnal mosquitoes like *Anopheles* species will begin their phase of activity when human hosts are still active and not under a bed net, leading to a higher chance of malaria transmission [46].

Altogether, both vectors and hosts undergo behavioral and physiological changes triggered by the virus infection, and in turn, the influence of environmental variation is behind all facets of this interaction. The next section will discuss the endogenous mechanisms regulating rhythmic behavior and physiology, as well as the role of environmental factors on synchronizing these rhythms.

5. Molecular control of the behavior

The different behaviors exhibited by mosquitoes are, in general, driven by internal biological clocks that generate circadian rhythms. These rhythms present a period of nearly, but not exactly, 24 h and are responsible for responses such as host-seeking, breeding site seeking, activity, and rest, among others [67].

These rhythms are directly influenced by natural cues from the environment, and the most important ones are the light/dark and the temperature cycles. These stimuli are received by specific receptors, like photoreceptors (in the eyes and head) and thermoreceptors (along the whole body) and are transmitted to the internal pacemaker or the biological clock itself. Thus, a rhythm or a physiological response is generated from the interaction of the stimuli with the pacemaker neurons [68].

The pacemaker neurons are so-called because they express the clock genes, which are the components of the circadian clock. These genes interact with each other and recruit kinases, phosphatases, and transcription factors to generate oscillating expression in a 24-h cycle [69]. They are also responsible for the regulation of many other genes, the clock-controlled genes (CCGs), that are directly associated with tissue-specific functions [70].

Drosophila melanogaster is the insect model for studying circadian rhythm, but it is already known that many features of the circadian clock of other insects differ considerably from the fly clock. The *Drosophila* clock is formed by three interconnected autoregulatory loops, in which the proteins coded by *Clock* (*Clk*) and *cycle* (*cyc*) genes play a central role. In the first loop, the heterodimer CLK-CYC binds to an E-box sequence in the promoter region of *period* (*per*) and *timeless* (*tim*) genes, activating their transcription. Once in the cytoplasm, the transcripts are translated into proteins that accumulate during the early night and later enter the nucleus to repress their own transcription. This cycle lasts 24 h due to the posttranslational modifications controlled by the activity of kinases such as DOUBLETIME (DBT), CASEIN KINASE 2A (CK2A), and SHAGGY (SGG), which together with phosphatases such as PP2A stabilize PER and TIM [71, 72]. In the second loop, two transcription factors, VRI and PDP1e, are involved, respectively, in the repression and activation of *Clk* and *cyc* genes. Finally, a third interconnected loop involves the activation of *clockwork orange* (*cwo*) gene and the repression exerted by its product, CWO, in PER targets [73]. **Figure 6** summarizes the *D. melanogaster* molecular circadian clock.

An interesting feature of this clock is its property of environmental synchronization, which adjusts the period to exactly 24 h. One of the most important synchronizers (or *Zeitgebers*) is light. The light-induced resetting mechanism is

dependent upon CRYPTOCHROME (CRY), which is a photoreceptor that induces TIM phosphorylation and leads it to degradation via proteossome [74, 75]. Other stimuli act as *Zeitgebers*, such as temperature and food, and their importance varies from species to species. In *A. aegypti*, temperature cycles are a very strong environmental cue, although the molecular mechanisms for entrainment are still unknown [76].

Molecular studies regarding the circadian clock in *A. aegypti* have been purely descriptive, because of the lack of genetic tools to unravel the function of clock genes in this species. Moreover, the phylogenetic distance between *Aedes aegypti* and *Drosophila melanogaster* implies significant differences between the two species in several biological aspects, beyond the circadian expression pattern. One significant difference is the presence, in *A. aegypti*, of a second cryptochrome gene, called *cryptochrome 2* (*cry2*), which does not exist in *D. melanogaster* [77]. This orthologue in mammals is a transcriptional repressor [78], and some studies done in *Danaus plexippus* confirmed this function in monarch butterflies [79]. Therefore, it is reasonable that this gene also plays this role in mosquitoes as well.

In a general manner, the circadian expression pattern of the main clock genes in *A. aegypti* under light dark cycles (LD12:12, which means 12 h of light followed by 12 h of dark) and constant temperature presents some similarities to what is observed in the *D. melanogaster* model. Genes *per* and *tim* present a cyclic mRNA expression with a peak in ZT 17, in the middle of the dark phase, and *vri* mRNA peak expression occurs some hours earlier than *Pdp1* mRNA peak expression (ZT 11 × ZT 17, respectively). However, two striking differences in relation to the *Drosophila* clock can be seen. The first one is related to the expression of the genes that encode the transcriptional activators, *Clk* and *cyc*. In Drosophila, *Clk* is the

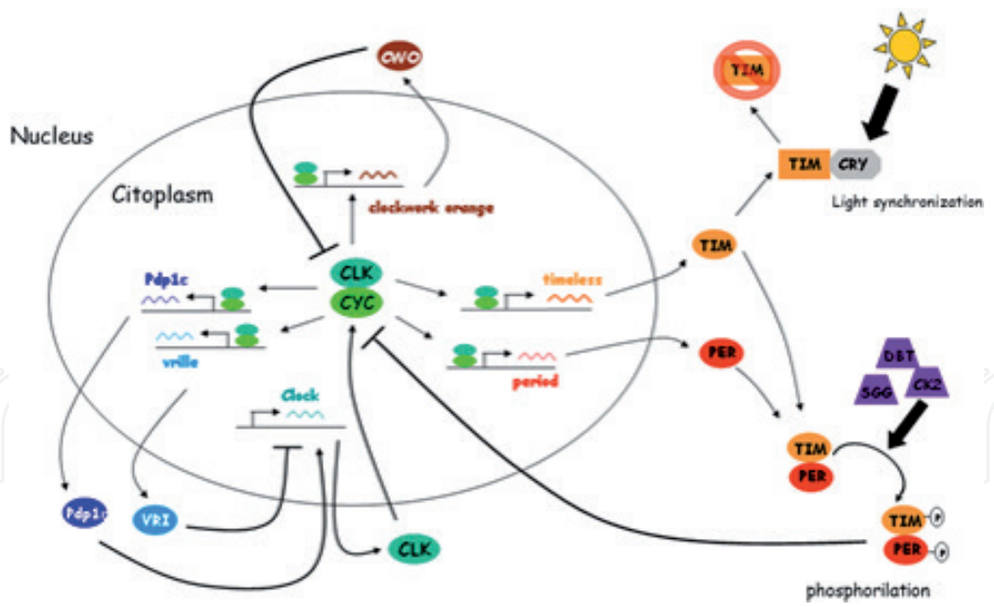

Figure 6.
The molecular circadian clock of Drosophila melanogaster. The heterodimer CLK-CYC plays a crucial role in the maintenance of the 24-h cycle, integrating the three regulatory feedback loops. In the first loop, CLK-CYC binds to the E-box regions in per and tim promoters and activates their transcription. Once in the cytoplasm, they are translated into proteins and suffer posttranslational modifications, caused by kinases and phosphatases. Once they can accumulate, the heterodimer PER-TIM enters in the nucleus and represses its own transcription. In the second loop, CLK-CYC heterodimer binds to E-boxes and activates vri *and* Pdp1ε *transcription. VRI binds to V/P boxes and inhibits* Clk *transcription, while PDP1ε accumulates a few hours later and shifts VRI from V/P box and activates Clk transcription. Finally, the third loop is related to the activation of* clockwork orange *gene and the inhibition of CLK-CYC by this gene product. The light synchronization is fired by a conformational change of CRY, which allows it to bind to TIM and leads to its degradation via proteasome, reinitiating the cycle. Straight arrows indicate following steps in the feedback loops; curved arrows indicate activation interactions; blocked line indicates inhibitory interaction. Colored forms represent proteins and wavy lines represent the mRNA. Based on [71, 72].*

main activator and presents a peak mRNA expression in antiphase to *per* and *tim*, whereas *cyc* presents a constitutive mRNA expression. On the contrary, *A. aegypti* Clk mRNA expression is arrhythmic, while *cyc* presents a very robust mRNA cycling pattern [8, 80]. Some years later, when *Clk* and *cyc* nucleotides and corresponding proteins were characterized, it was observed that the brain and muscle ARNT-like (BMAL) C-terminus region (BCTR) activation domain is missing in *Drosophila* CYC protein but is present in *A. aegypti* CYC protein [81]. These data can partially explain the variations in *Clk* and *cyc* expression pattern and suggest that there is a dissimilarity in circadian regulation between the two species. The second difference is related to the presence of the *cry2* gene and its bimodal mRNA expression pattern. As mentioned above, cry2 gene is present in *A. aegypti* and other *Diptera* but is absent in *D. melanogaster* [77]. According to putative clock models proposed by Yuan et al. in 2007, the presence or absence of *cryptochrome* genes may lead to crucial modifications in clock regulation.

5.1 The pacemaker neurons in insects

Clock genes are expressed in specific groups of neurons called pacemaker neurons, in the central nervous system of the organism, and are identified as pacemakers due to PER expression [82]. However, the distribution of these cells in the brain can vary from species to species; while in *Manduca sexta*, the pacemaker is located in the dorsal protocerebrum, in *Pachnoda marginata* it is located in the proximal optic lobe [82]. In *Drosophila*, around 150 clock neurons are located in the lateral protocerebrum, close to optic lobe and in the dorsal protocerebrum. The lateral neurons are subdivided in ventrolateral neurons (LNvs), dorsolateral neurons (LNds), lateral posterior neurons (LPNs), and dorsal neurons (DNs) [83]. Each group expresses the clock genes and communicates with each other according to different neurotransmitters, such as pigment-dispersing factor (PDF), which is the most well known [84]. Depending on the combination between neurotransmitters and hormone signaling among the neurons and clock genes' expression during the time of day, distinct neuronal clusters are responsible for different behaviors, such as feeding (which is regulated by the dorsolateral neurons) or temperature preference (regulated by dorsal neurons). On the other hand, the locomotor behavior, for instance, recruits all groups of neurons, which interact with each other to generate activity and rest along the 24-h day [72].

In *A. aegypti* there is not yet a map of the pacemaker neurons. However, it is described that some clustered neurons present a cycling expression of *cyc* and *per* mRNAs in antiphase, which strongly indicates that the areas where pacemaker neurons are found are similar to those observed in *D. melanogaster* [85]. Regarding the arbovirus infection, it was observed that both DENV and ZIKV are able to impact the neurons' spike activity in opposite manners. While ZIKV infection leads to hyperexcitation in a primary neuron culture, DENV2 infection does not seem to alter the spikes. Moreover, ZIKV reaches a plateau in replication around 2 dpi, whereas DENV2 initially decreases its replication and follows an increase in virus titers until 3 dpi [57]. It was already observed that ZIKV presents a strong neurotropism in mosquitoes [57], but it was not possible to associate the infected cells with clock neurons. Additional studies are necessary to establish the relationship between arbovirus infection and the pacemaker cells.

6. Conclusions

It is known that the virus-host interaction has a crucial importance in the spreading of a pathogen, since mutations in the viral genome or the genetic

background of a mosquito population can enhance or even inhibit the replication of the virus in the mosquito. Beyond this genetic interaction, behavior is also directly related to the vectorial capacity of *A. aegypti*. Reports about the influence of the viral infection on several mosquitoes' behaviors have been increasing along the years. However, we still do not know the way arboviruses modulate the expression of the core clock genes that control behavior. It is even possible that infection does not have a direct effect on the core clock genes themselves but possibly on the genes regulated by them, leading to alterations in behavior and, consequently, impacts on the vectorial capacity. Improving the knowledge on behavioral traits that are susceptible to infection-driven changes (e.g., time-of-day biting activity, time-of-day mating behavior, time-of-day oviposition behavior) can increase the efficacy of strategies of vector control.

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References

[1] Consoli R, Lourenço-de-Oliveira R. Principais Mosquitos de importância sanitária no Brasil. Rio de Janeiro: Fiocruz; 1994. 228 pp

[2] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. The global distribution and burden of dengue. Nature. 2013;**496**:504-507. DOI: 10.1038/nature12060

[3] Christophers S. *Aedes aegypti* (L.), the Yellow Fever Mosquito. Its Life History, Bionomics and Structure. Cambridge: The University Press; 1960. 739 pp

[4] Borror JD, Delong DM. Introdução ao estudo dos insetos. São Paulo: Edgar Blucher; 1988. 653 pp

[5] Forattini OP. Culicidologia Médica. Vol. 2. São Paulo: Editora da Universidade de São Paulo; 1996. 860 pp

[6] Rueda LM. Pictorial Keys for the Identification of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission. Zootaxa. Vol. 589. Auckland, New Zealand: Magnolia Press; 2004. ISBN 1-877354-46-5

[7] Clements AN. The Biology of Mosquitoes: Sensory Reception and Behaviour. London: Chapman and Hall; 1999. 740 pp

[8] Gentile C, Rivas GB, Meireles-Filho AC, Lima JB, Peixoto AA. Circadian expression of clock genes in two mosquito disease vectors: cry2 is different. Journal of Biological Rhythms. 2009;**24**(6):444-451. DOI: 10.1177/0748730409349169

[9] Lima-Camara TN, Lima JB, Bruno RV, Peixoto AA. Effects of insemination and blood-feeding on locomotor activity of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) females under laboratory conditions. Parasites & Vectors. 2014;**7**(1):304. DOI: 10.1186/1756-3305-7-304

[10] Farnesi LC, Barbosa CS, Araripe LO, Bruno RV. The influence of a light and dark cycle on the egg laying activity of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). Memórias do Instituto Oswaldo Cruz. 2018;**113**:e170362. DOI: 10.1590/0074-02760170362

[11] Clements A. The Biology of Mosquitoes: Development, Nutrition and Reproduction. London: Chapman and Hall; 1992. 509 pp

[12] McMeniman CJ, O'Neill SL. A virulent Wolbachia infection decreases the viability of the dengue vector *Aedes aegypti* during periods of embryonic quiescence. PLoS Neglected Tropical Diseases. 2010;**4**:e748

[13] Boorman JP, Porterfield JS. A simple technique for infection of mosquitoes with viruses; transmission of Zika virus. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1956;**50**(3):238-242

[14] Valle D, Pimenta DN, Cunha RV. Dengue: Teorias e Práticas. Rio de Janeiro: Fiocruz; 2015. 458 pp

[15] Costa-da-Silva AL, Ioshino RS, de Araújo HRC, Kojin BB, de Andrade Zanotto PM, Oliveira DBL, et al. Laboratory strains of *Aedes aegypti* are competent to brazilian Zika virus. PLoS One. 2017;**12**:e0171951

[16] Souza-Neto JA, Powell JR, Bonizzoni M. *Aedes aegypti* vector competence studies: A review. Infection, Genetics and Evolution. 2018;**67**:191-209

[17] Kliewer JW. Weight and hatchability of *Aedes aegypti* eggs (Diptera: Culicidae). Annals of the Entomological Society of America. 1961;**54**:912-917

[18] Rezende GL, Martins AJ, Gentile C, Farnesi LC, Pelajo-Machado M, et al. Embryonic desiccation resistance in *Aedes aegypti*: Presumptive role of the chitinized serosal cuticle. BMC Developmental Biology. 2008;**8**:82. DOI: 10.1186/1471-213X-8-82

[19] Farnesi LC, Martins AJ, Valle D, Rezende GL. Embryonic development of *Aedes aegypti* (Diptera:Culicidae): Influence of different constant temperatures. Memórias do Instituto Oswaldo Cruz. 2009;**104**:124-126. DOI: 10.1590/S0074-02762009000100020

[20] Farnesi LC, Vargas HCM, Valle D, Rezende GL. Darker eggs of mosquitoes resist more to dry conditions: Melanin enhances serosal cuticle contribution in egg resistance to desiccation in Aedes, Anopheles and Culex vectors. PLoS Neglected Tropical Diseases. 2017;**11**:e0006063. DOI: 10.1371/journal.pntd.0006063

[21] WHO. Technical Report Series No. 205. Malaria: Eighth Report of the Expert Committee. Geneva: WHO; 1961

[22] Lambrechts L, Paaijmans KP, Fansiri T, Carrington LB, Kramer LD, Thomas MB, et al. Impact of daily temperature fluctuations on dengue virus transmission by *Aedes aegypti*. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(18):7460-7465. DOI: 10.1073/pnas.1101377108

[23] Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D, et al. Differential susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika virus. PLoS Neglected Tropical Diseases. 2016;**10**:e0004543

[24] Amraoui F, Ben Ayed W, Madec Y, Faraj C, Himmi O, Btissam A, et al. Potential of *Aedes albopictus* to cause the emergence of arboviruses in Morocco. PLoS Neglected Tropical Diseases. 2019;**13**:e0006997

[25] Atyame Célestine M, Haoues A, Laurence M, Vazeille M, Diallo M, Mylène W, et al. Insecticide resistance genes affect *Culex quinquefasciatus* vector competence for West Nile virus. Proceedings of the Royal Society B. 2019;**286**(1894):20182273. DOI: 10.1098/rspb.2018.2273

[26] Kramer LD, Ciota AT. Dissecting vectorial capacity for mosquito-borne viruses. Current Opinion in Virology. 2015;**15**:112-118

[27] Macdonald G. Epidemiologic models in studies of vector borne diseases. Public Health Reports. 1961;**76**:753-764

[28] Garrett-Jones C. The human blood index of malaria vectors in relation to epidemiological assessment. Bulletin of the World Health Organization. 1964;**30**:241-261

[29] Smith DL, Dushoff J, McKenzie FE. The risk of a mosquito-borne Infection in a heterogeneous environment. PLoS Biology. 2004;**2**(11):e368

[30] McSweegan E, Weaver SC, Lecuit M, Frieman M, Morrison TE, Hrynkow S. The global virus network: Challenging chikungunya. Antiviral Research. 2015;**120**:147-152. DOI: 10.1016/j.antiviral.2015.06.003

[31] Taylor B, Jones MD. The circadian rhythm of flight activity in the mosquito *Aedes aegypti* (L.). The phase-setting effects of light-on and light-off. The Journal of Experimental Biology. 1969;**51**(1):59-70

[32] Jones MDR, Hill M, Hope AM. The circadian flight activity of the mosquito, Anopheles gambiae: Phase setting by the light regime. The Journal of Experimental Biology. 1967;**47**:503-511

[33] Rowland M. Changes in the circadian flight activity of the mosquito Anopheles stephensi associated with insemination, blood-feeding, oviposition and nocturnal light intensity. Physiological Entomology. 1989;**14**:77-84. DOI: 10.1111/j.1365-3032.1989.tb00939.x

[34] Peterson EL. Phase-resetting a mosquito circadian oscillator. Journal of Comparative Physiology. 1980;**138**(3): 201-211. DOI: 10.1007/BF00657038

[35] Fritz ML, Walker ED, Yunker AJ, Dworkin I. Daily blood feeding rhythms of laboratory-reared north American *Culex pipiens*. Journal of Circadian Rhythms. 2014;**12**:1. DOI: 10.1186/1740-3391-12-1

[36] Sumba LA, Okoth K, Deng AL, Githure J, Knols BGJ, Beier JC, et al. Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae* Giles (Diptera: Culicidae) on different types of aqueous substrates. Journal of Circadian Rhythms. 2004;**2**:6. DOI: 10.1186/1740-3391-2-6

[37] Fritz ML, Huang J, Walker ED, Bayoh MN, Vulule J, Miller JR. Ovipositional periodicity of caged *Anopheles gambiae* individuals. Journal of Circadian Rhythms. 2008;**6**:2. DOI: 10.1186/1740-3391-6-2

[38] Chadee DD, Ritchie SA. Oviposition behaviour and parity rates of *Aedes aegypti* collected in sticky traps in Trinidad, West Indies. Acta Tropica. 2010;**116**:212-216. DOI: 10.1016/j.actatropica.2010.08.008

[39] Eilerts DF, Giessen MV, Bose EA, Broxton K, Vinauger C. Odor-specific daily rhythms in the olfactory sensitivity and behavior of *Aedes aegypti* mosquitoes. Insects. 2018;**9**:147. DOI: 10.3390/insects9040147

[40] Rund SSC, O'Donnell AJ, Gentile JE, Reece SE. Daily rhythms in mosquitoes and their consequences for malaria transmission. Insects. 2016;**7**:14. DOI: 10.3390/insects7020014

[41] Alfonso-Parra C, Ahmed-Braimah YH, Degner EC, Avila FW, Villarreal SM, Pleiss JA, et al. Mating-induced transcriptome changes in the reproductive tract of female *Aedes aegypti*. PLoS Neglected Tropical Diseases. 2016;**10**(2):e0004451. DOI: 10.1371/journal.pntd.0004451

[42] Araripe LO, Bezerra JR, Rivas GBS, Bruno RV. Locomotor activity in males of *Aedes aegypti* can shift in response to females' presence. Parasites and Vectors. 2018;**11**(1):254. DOI: 10.1186/s13071-018-2635-9

[43] Xu K, Di Angelo JR, Hughes ME, Hogenesch JB, Sehgal A. The circadian clock interacts with metabolic physiology to influence reproductive fitness. Cell Metabolism. 2011;**13**:639-654. DOI: 10.1016/j.cmet.2011.05.001

[44] Gill S, Le HD, Melkani GC, Panda S. Time-restricted feeding attenuates age-related cardiac decline in Drosophila. Science. 2015;**347**:1265-1269. DOI: 10.1126/science.1256682

[45] Maciel-de-Freitas R, Koella JC, Lourenço-de-Oliveira R. Lower survival rate, longevity and fecundity of *Aedes aegypti* (Diptera: Culicidae) females orally challenged with dengue virus serotype 2. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2011;**105**:452-458

[46] Knols BG, Meuerink J. Odors influence mosquito behavior. Scientia Medica. 1997;**4**:56-63

[47] Rund SSC, Bonar NA, Champion MM, Ghazi JP, Houk CM, et al. Daily rhythms in antennal protein and olfactory sensitivity in the malaria mosquito *Anopheles gambiae*. Scientific Reports. 2013;**3**:2494

[48] Bailey SL, Heitkemper MM. Circadian rhythmicity of cortisol and body temperature: Morningness-eveningness effects. Chronobiology International. 2001;**18**:249-261

[49] Aragao BH. Mosquitoes and yellow fever virus. Memórias do Instituto Oswaldo Cruz. 1939;**34**:565-581. DOI: 10.1590/S0074-02761939000400007

[50] Jones MDR. The programming of circadian flight activity in relation to mating and the gonotrophic cycle in the mosquito *Aedes aegypti*. Physiological Entomology. 1981;**87**:511-521. DOI: 10.1111/j.1365-3032.1981.tb00275.x

[51] Adlakha V, Pillai MKK. Role of male accessory gland substance in the regulation of blood intake by mosquitoes. Journal of Insect Physiology. 1976;**22**:1441-1442

[52] Lee J-J, Klowden MJ. A male accessory gland protein that modulates female mosquito (Diptera: Culicidae) host-seeking behavior. Journal of the American Mosquito Control Association. 1999;**15**:4-7

[53] Klowden MJ. The check is in the male: Male mosquitoes affect female physiology and behaviour. Journal of the American Mosquito Control Association. 1999;**15**:213-220

[54] Leahy MG, Craig GB. Accessory gland substance as a stimulant for oviposition in *Aedes aegypti* and *Aedes albopictus*. Mosquito News. 1965;**25**:448-452

[55] Hiss EA, Fuchs MS. The effect of matrone on oviposition in the mosquito, *Aedes aegypti*. Journal of Insect Physiology. 1972;**18**:2217-2227. DOI: 10.1016/0022-1910(72)90250-8

[56] Wallis RC, Lang CA. Egg formation and oviposition in bloodfed *Aedes aegypti*. Mosquito News. 1956;**16**:283-286

[57] Gaburro J, Bhatti A, Harper J, Jeanne I, Dearnley M, Green D, et al. Neurotropism and behavioral changes associated with Zika infection in the vector *Aedes aegypti*. Emerging Microbes and Infections. 2018;7(1):68. DOI: 10.1038/s41426-018-0069-2

[58] Salazar MI, Richardson JH, Sánchez-Vargas I, Olson KE, Beaty BJ. Dengue virus type 2: Replication and tropisms in orally infected *Aedes aegypti* mosquitoes. BMC Microbiology. 2007;7:9. DOI: 10.1186/1471-2180-7-9

[59] Jackson BT, Brewster CC, Paulson SL. La Crosse virus infection alters blood feeding behavior in Aedes triseriatus and Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology. 2012;**49**:1424-1429

[60] Spengler CM, Czeisler CA, Shea SA. An endogenous circadian rhythm of respiratory control in humans. The Journal of Physiology. 2000;**526**:683-694

[61] Lima-Camara TN, Bruno RV, Luz PM, Castro MG, Lourenço-de-Oliveira R, Sorgine MH, et al. Dengue infection increases the locomotor activity of *Aedes aegypti* females. PLoS One. 2011;**6**:e17690. DOI: 10.1371/journal.pone.0017690.t001

[62] Padilha KP, Resck ME, Cunha OA, Teles-de-Freitas R, Campos SS, Sorgine MH, et al. Zika infection decreases *Aedes aegypti* locomotor activity but does not influence egg production or viability. Memórias do Instituto Oswaldo Cruz. 2018;**113**(10):e180290

[63] Vogels CBF, Fros JJ, Pijlman GP, van Loon JJA, Gort G, Koenraadt CJM. Virus interferes with host-seeking behaviour of mosquito. The Journal of Experimental Biology. 2017;**220**(19):3598-3603. DOI: 10.1242/jeb.164186

[64] Carlson CJ, Dougherty ER, Getz W. An ecological assessment of the pandemic threat of Zika virus. PLoS Neglected Tropical Diseases. 2016;**10**:e0004968. DOI: 10.1371/journal.pntd.0004968

[65] Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR. Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. PLoS Neglected Tropical Diseases. 2019;**13**(3):e0007213. DOI: 10.1371/journal.pntd.0007213

[66] Kernbach ME, Newhouse DJ, Miller JM, Hall RJ, Gibbons J, Oberstaller J, et al. Light pollution increases West Nile virus competence of a ubiquitous passerine reservoir species. Proceedings of the Royal Society B. 2019;**286**:20191051. DOI: 10.1098/rspb.2019.1051

[67] Saunders DS. Insect Clocks. 3rd ed. Amsterdam: Elsevier; 2002. 560 pp

[68] Moore-Ede MC, Sulzman FM, Fuller CA. The Clocks that Time Us. Cambridge: Harvard University Press; 1982

[69] Mendoza-Viveros L, Bouchard-Cannon P, Hegazi S, Cheng AH, Pastore S, Cheng HM. Molecular modulators of the circadian clock: Lessons from flies and mice. Cellular and Molecular Life Sciences. 2017;**74**(6): 1035-1059. DOI: 10.1007/s00018-016-2378-8

[70] Stanewsky R. Clock mechanisms in Drosophila. Cell and Tissue Research. 2002;**309**(1):11-26

[71] Hardin PE. Molecular genetic analysis of circadian timekeeping in Drosophila. Advances in Genetics. 2011;**74**:141-173. DOI: 10.1016/B978-0-12-387690-4.00005-2

[72] Franco DL, Frenkel L, Ceriani MF. The underlying genetics of Drosophila circadian behaviors. Physiology (Bethesda, Md.). 2018;**33**(1):50-62. DOI: 10.1152/physiol.00020.2017

[73] Kadener S, Stoleru D, McDonald M, Nawathean P, Rosbash M. Clockwork Orange is a transcriptional repressor and a new Drosophila circadian pacemaker component. Genes & Development. 2007;**21**(13):1675-1686

[74] Emery P, So WV, Kaneko M, Hall JC, Rosbash M. CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell. 1998;**95**:669-679

[75] Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA, et al. The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell. 1998;**95**:681-692

[76] Rivas GBS, Teles-de-Freitas R, Pavan MG, Lima JBP, Peixoto AA, Bruno RV. Effects of light and temperature on daily activity and clock gene expression in two mosquito disease vectors. Journal of Biological Rhythms. 2018;**33**(3):272-288. DOI: 10.1177/0748730418772175

[77] Yuan Q, Metterville D, Briscoe AD, Reppert SM. Insect cryptochromes: Gene duplication and loss define diverse ways to construct insect circadian clocks. Molecular Biology and Evolution. 2007;**24**(4):948-955

[78] Nangle SN, Rosensweig C, Koike N, Tei H, Takahashi JS, Green CB, et al. Molecular assembly of the period-cryptochrome circadian transcriptional repressor complex. eLife. 2014;**3**:e03674. DOI: 10.7554/eLife.03674

[79] Zhu H, Sauman I, Yuan Q, Casselman A, Emery-Le M, Emery P, et al. Cryptochromes define a novel circadian clock mechanism in monarch butterflies that may underlie sun

compass navigation. PLoS Biology. 2008;**6**(1):e4. DOI: 10.1371/journal.pbio.0060004

[80] Ptitsyn AA, Reyes-Solis G, Saavedra-Rodriguez K, Betz J, Suchman EL, Carlson JO, et al. Rhythms and synchronization patterns in gene expression in the *Aedes aegypti* mosquito. BMC Genomics. 2011;**12**:153. DOI: 10.1186/1471-2164-12-153

[81] Chahad-Ehlers S, Arthur LP, Lima ALA, Gesto JSM, Torres FR, Peixoto AA, et al. Expanding the view of clock and cycle gene evolution in Diptera. Insect Molecular Biology. 2017;**26**(3): 317-331. DOI: 10.1111/imb.12296

[82] Helfrich-Förster C. Organization of endogenous clocks in insects. Biochemical Society Transactions. 2005;**33**(Pt 5):957-961

[83] Helfrich-Förster C, Yoshii T, Wülbeck C, Grieshaber E, Rieger D, Bachleitner W, et al. The lateral and dorsal neurons of *Drosophila melanogaster*: New insights about their morphology and function. Cold Spring Harbor Symposia on Quantitative Biology. 2007;**72**:517-525. DOI: 10.1101/sqb.2007.72.063

[84] Peng Y, Stoleru D, Levine JD, Hall JC, Rosbash M. Drosophila free-running rhythms require intercellular communication. PLoS Biology. 2003;**1**(1):E13. Epub 2003 Sep 15

[85] Chahad-Ehlers S, Gentile C, Lima JB, Peixoto AA, Bruno RV. Analysis of cycle gene expression in *Aedes aegypti* brains by in situ hybridization. PLoS One. 2013;**8**(1):e52559. DOI: 10.1371/journal.pone.0052559