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
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.

\[
VC = \frac{ma^2bp^n}{-\log_e p}
\]

**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].
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

![Image of the molecular circadian clock of Drosophila melanogaster](image-url)

**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 Pdp1 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 *Pachnodia 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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The Effects of Infection on Mosquito Rhythmic Behavior
DOI: http://dx.doi.org/10.5772/intechopen.89409

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