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Do mosquitoes sleep?

Oluwaseun M. Ajayi^{1,*}, Diane F. Eilerts², Samuel T. Bailey¹, Clément Vinauger², Joshua B. Benoit^{1,*}

¹Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221, USA.

²Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA.

Abstract

Sleep is a phenomenon conserved across the animal kingdom, where studies on *Drosophila melanogaster* have revealed that sleep phenotypes and molecular underpinnings are similar to those in mammals. However, little is known about sleep in blood-feeding arthropods, which have a critical role in public health as disease vectors. Specifically, sleep studies in mosquitoes are lacking despite considerable focus on how circadian processes, which have a central role in regulating sleep/wake cycles, impact activity, feeding, and immunity. Here, we review observations that suggest sleep-like states likely occur in mosquitoes and discuss the potential role of sleep in relation to mosquito biology and their ability to function as disease vectors.

Keywords

Sleep; mosquitoes; circadian rhythms; mosquito biology; vectorial capacity

Mosquitoes, disease transmission, and circadian rhythms

Worldwide, mosquitoes are ubiquitous, present in nearly all geographical regions except the Antarctic [1]. Amongst the 3,000 species of mosquitoes, *Aedes*, *Anopheles*, and *Culex* are the principal genera associated with the transmission of human-associated vector-borne pathogens [1]. Infectious microorganisms vectored by these genera contribute to the most reported cases, highest mortality, and greatest economic burden of all known mosquito-borne diseases (MBDs) [1]. Over half, if not more, of the world's population is currently at the risk of exposure to and impairment by MBDs, highlighting their medical importance [2].

Several factors contribute to the prevalence of mosquitoes as disease vectors; these include mosquito population density, geographic overlap with humans, blood-feeding behavior, immunity, genetics, ecological factors, and longevity [3,4]. Specifically, mosquitoes' biting frequency and susceptibility to carrying transmissible pathogens are critical to their capabilities as etiological agents of diseases, and **circadian rhythms** (see Glossary) impact what time of day each mosquito species feeds [5,6]. Some species are commonly active and feed during the day (e.g., *Aedes aegypti*) and others at night (e.g., *Anopheles gambiae*) [6,7].

*Correspondence: ajayiom@mail.uc.edu (O. M. Ajayi) and joshua.benoit@uc.edu (J. B. Benoit).

Circadian rhythms influence other aspects of mosquito biology such as oviposition, locomotor flight activity, mating, metabolism, immune responses, and olfaction (reviewed in [8]). In other insect systems, sleep is influenced by and linked to circadian rhythms [9], but sleep-like state and mechanisms underlying this biological state remain to be studied in mosquitoes.

Sleep-like states among animals

Sleep has been observed in many lineages across the animal kingdom and is functionally critical in most systems [10]. In sleep-like states, animals are disconnected from the external environment, as a result of elevated **sensory thresholds** [11]. During the process of sleep, animals cannot feed, care for their young, or evade life-threatening situations [11]. Thus, sleep must provide a vital benefit since the trade-offs associated with sleep can be severe. Prolonged sleep deprivation among vertebrates results in a multitude of negative consequences, including hallucinations, communication difficulties, and even death [11,12]. As in vertebrates, sleep is critical for invertebrate systems to reach optimum biological functionality [13,14].

Sleep in insects has been primarily assessed in fruit flies [15–17], where relationships between sleep and specific biological and physiological parameters have been established [15,18,19]. Of interest, activation of **orthologous genes** occurs during sleep in mammals and fruit flies, indicating a conservation of this process [15,16]. Sleep-like states have been described in other insects such as wasps, cockroaches, and bees [20–22]. A single study on resting states of mosquitoes may be the entirety of sleep-based research on this system, but even this study did not consider this resting state as a potential period of sleep [23]. Circadian rhythms have been examined in mosquitoes [8,24,25] and the relationship between these rhythms and sleep [8] along with sleep-like states in many insect systems raises the question: “do mosquitoes sleep?” Here, we provide a synopsis of research that indicates sleep-like states likely occur in mosquitoes and highlight why these states are expected to be a critical component of mosquito biology and influence their ability as disease vectors.

Criteria to define sleep in insect systems

Behavioral correlates are one of the reliable markers of sleep in animals. In a sleep state; (i) animals assume a species-specific posture, (ii) there is a maintenance of prolonged **quiescence** which can be reversed when animals are stimulated, (iii) there is an increase in arousal threshold in response to stimuli, and (iv) observable sleep compensation occurs following sleep deprivation [21,26]. Hymenopterans, flies, and cockroaches choose obvious sleeping locations and adopt specific sleep postures that include positional changes in the body, appendages, and antennae [11,22]. Sleeping *Drosophila* exhibit sustained periods of immobility in the dark period (which are shorter during the light periods) characterized by increased arousal thresholds [15,16]. Reduced alertness and sleep rebound are experienced during recovery in honey bees, cockroaches, and fruit flies following sleep deprivation [16,21,27].

Electrophysiological correlates that involve the recording of electrical activity in the brain have been used to define sleep in animals beyond behavioral observations [28]. This is possible because brain activity, movement, and sleep in animals are intertwined [29], but these processes can be dissociated [26]. Although early studies have combined behavioral observations of sleep and wakefulness with brain activity recordings to improve the accuracy of sleep quantification [30], the sole use of electrophysiological measures is usually sufficient given the close relationship between brain activity and behavior [26]. In *Drosophila*, reduction in Ca^{2+} levels in many brain cells and decreased local field potentials (LFPs) in the medial **protocerebrum** of the brain correlate with sleep-state, while elevated Ca^{2+} levels and LFPs correspond with wakefulness [31,32].

As early as in the 1930s, studies in humans provided the first evidence that sleep amount and structure are under genetic control (reviewed in [33]). Mice, which share about 85% genetic similarities with humans, have also been utilized to understand the genetic regulation and other molecular aspects of sleep [34,35]. Other organisms including *Caenorhabditis elegans*, *Danio rerio* (zebra fish), and *Drosophila* have proven to be excellent models for understanding the genetic and molecular underpinnings of sleep [35,36]. These organisms are highly versatile as they are tractable and rely on relatively simple genomes to generate complex behaviors [37,38]. Identification of sleep-associated genes is easier in these organisms because of their lower genetic redundancy and increased ease of genetic manipulation [37].

In *Drosophila*, specific genes have been linked to sleep, as well as others whose transcript levels vary in conjunction with but might not directly control sleep (reviewed in [36,39]). Differentially expressed genes between sleep and awake states belong to different functional categories, which implies that these two states drive different molecular processes [11]. Wakefulness or short-term sleep deprivation are associated with the enrichment for gene ontology aspects of energy metabolism, **synaptic potentiation**, and response to cellular stress in specific brain regions of *Drosophila* [11]. Conversely, sleep yields increased expression for genes linked to protein synthesis, **synaptic downscaling**, and membrane maintenance (reviewed in [39]). Altogether, genetic and molecular studies in *Drosophila* have provided limited but helpful context to sleep in insect systems by establishing underlying functional mechanisms.

Putative sleep-like states in mosquitoes

Mosquito lineages are separated by 260 million years from fruit flies, thus mechanisms underlying sleep in *Drosophila* are likely shared between these two dipterans as sleep-like factors occur among distantly-related lineages across different phyla [36]. It is thus surprising that sleep in mosquitoes has garnered little attention since *Drosophila* studies can be used as an effective starting point for focused research on mosquitoes.

The first evidence of a potential sleep-like state in mosquitoes was the observation of unique postural differences between resting and active states in *Aedes aegypti* [23]. A key feature of the resting/sleep-like posture is the lowering of the hind legs, along with the abdomen closer to the resting surface and angled differently, which we observed as quantifiably different in

relation to the active state (Figure 1). Prominent circadian organization of rest and activity displayed among mosquito species provides additional support for sleep-like state during specific times of low activity each day [8,25,40]. Importantly, arousal and biting have distinct circadian profiles, which are usually increased during active periods compared to when inactive [7,41]; this suggests that arousal threshold differences are likely present in mosquitoes in relation to resting, which represents a second major hallmark of sleep-like states.

Identification of putative sleep-like genes in mosquitoes

Currently, specific genes associated with sleep in mosquitoes are unknown. To identify putative genes that underlie sleep-like state in mosquitoes, we used two approaches. First, we examined mosquito **proteomes** for orthologs that have been associated with sleep in *D. melanogaster* (supplemental Box S2). We identified 58 putative sleep-associated genes that are found in multiple mosquito species (Figure 2), which are functionally associated with sleep/wake cycles, neuronal signaling, stress responses, and catabolism of **dopamine** and **octopamine**. Second, we re-examined previous circadian rhythm gene expression studies from *An. gambiae* and *Ae. aegypti* [24,25]. In this analysis, we combined time points of low activity (sleep more likely) and high activity (sleep less likely). A general increase in metabolism is associated with “no sleep” (i.e. high activity) and aspects involving cuticle structure, protein repair, and sodium channel activity occur in the “sleep” grouping (i.e. low activity). These are comparable to those observed in *Drosophila* (reviewed in [11]). The increased expression of cuticle proteins in the low activity group could be to repair the cuticle structure or regenerate eyes [42]. Importantly, these expressional studies were not designed to examine sleep, so differentially expressed genes only represent potential sleep-associated factors. Targeted studies when mosquitoes are in their resting posture (Figure 1) and not impacted by the experimenter (who will be releasing specific cues signaling the presence of a host, see Box 1) will be necessary to fully elucidate molecular shifts during mosquito sleep-like state.

Sleep in mosquitoes, immune function, vector competence

A bidirectional relationship between sleep and the immune system has been established in *Drosophila* via genome-wide studies (reviewed in [43]). Similar to observations in mammals, an immune response in *Drosophila* promotes sleep, induced by signals from both relevant peripheral immune systems and the circadian clock [44]. Likewise, increased expression of **antimicrobial peptides** (AMPs) has been associated with changes in *Drosophila* sleep state [19,44,45], where increased sleep during sickness enhances fly survival [18]. Several signaling factors that influence stress and infection-induced sleep have been identified in *Drosophila* [43]. Notably, Toda *et al.* identified a conserved sleep-promoting AMP encoded by the gene *nemuri* [45]. NEMURI expression aids in bacterial immunity and induces sleep, suggesting a role in sleep homeostasis and confirming the link between sleep and immunity [45]. The **NF κ B** transcription factor Relish is central to the innate immune response in flies via the **IMD pathway** and is required for infection-induced sleep [18,19,43]. Similarly, Phe-Met-Arg-Phe-amide (FMRFa) neuropeptides are known to aid in sleep regulation and have been shown to be involved in stress- and infection-induced

sleep, suggesting they work to promote an adaptive sleep response to immune challenge [46].

While *Drosophila* is an excellent model for characterizing physiological processes such as sleep, immunity, and circadian rhythms, they are not likely to acquire blood-borne pathogens unlike those that can be obtained during blood feeding in mosquitoes. In general, it is assumed that mosquito sleep/wake cycles would coincide with their locomotor and activity patterns, which are maintained by the central circadian clock. Mosquitoes are also susceptible to different predators, and may optimize their chances of obtaining a blood-meal by seeking a host when it is available and the least defensive [8]. Locomotor/flight patterns and likely sleep/wake cycles in mosquitoes thus reflect both host availability and predation risks [8]. It is therefore likely that the relationship between sleep and immunity in mosquitoes will diverge from that of non-hematophagous flies. Given that immune processes and susceptibility to infection are both modulated by the circadian clock in mosquitoes [8], the interaction between the immune system, circadian clocks, and sleep/wake states is likely central to their adaptation to a **hematophagous** way of life. In addition, underestimations in many disease transmission models stem from the fact that not all factors are accounted for, such as periods of sleep [47,48]. Therefore, it will be interesting to confirm potential immunity-sleep dynamics and how these dynamics may influence indices of vectorial capacity.

Potential dynamics between ecological and physiological parameters and mosquito sleep

An evolutionarily conserved and experimentally tractable behavioral correlate of sleep in animals is a prolonged period of inactivity [49]. Based on this criterion, we hypothesize that there would be potential dynamic interactions between different ecological and physiological parameters and the putative mosquito sleep-like state. In unfavorable environmental conditions, one of the strategies associated with mosquito survival is aestivation (during hot and dry conditions in tropical regions) or diapause (during harsh winter in temperate climates) [50]. This mosquito survival strategy in adults is characterized by dormancy (lack of activity), low metabolic activity, and suppressed arousal thresholds – the hallmarks of sleep-like states – which in turn conserves energy to extend longevity [51]. Importantly, the severity of these unfavorable conditions influences the duration of this sleep-like survival strategy and differs among and within species [50]. Extended periods of inactivity and rest-seeking in shaded and moist places (putative sleep-like conditions) in nocturnal *Anopheles* mosquitoes during the light cycle confer significant ecological advantages; these include reduction in risk of desiccation and UV damage (especially during hot conditions), and protection from predators (e.g., *Anopheles freeborni* predation by dragonfly occurs during daylight since dragonflies locate their prey using visual cues, reviewed in [8]).

Host availability is also an ecological factor that is hypothesized to potentially impact sleep levels. In the absence of potential hosts, sleep-like duration is predicted to increase, and conversely putative sleep duration would likely decrease to maximize blood feeding when

hosts are available. Two anopheline species that predominantly bite indoors during the night have shifted to increased outdoor biting during the early evening and morning hours, coinciding with when human hosts are usually outdoors and not within bed nets [52]. Urbanization, which is often characterized by an increase in development due to population growth, can potentially modulate sleep cycles in mosquitoes due to an increase in host availability, temporal changes in host activity, or impacts of artificial light [53]. As a result of the replacement of natural vegetation by urbanized areas, local species of mosquitoes would also likely have fewer natural shelters that can accommodate their resting behavior and some species could be replaced by invasive species, such as *Aedes aegypti*, that thrive in urban areas [53]. Because increased host seeking is often the result of successful mating in female mosquitoes [50], we also hypothesize that mating behavior will likely influence sleep conditions in mosquitoes as energy obtained from blood meals are necessary for egg production and maturation. In *Drosophila*, a strong correlation between feeding status and sleep have been reported, giving credence to the highly conserved and interconnected nature of these two behaviors in animals. Fruit flies have been shown to have reduced sleep levels and increased activity during starvation periods [54], while increased sleep in previously starved flies occurred in the first few hours after feeding [55]. We predict the same scenario in mosquitoes, where levels of a sleep-like condition will be modulated by their feeding status. Confirming the potential dynamics between sleep and ecological factors in mosquitoes through robust laboratory studies will be necessary to determine the extent by which factors such as urbanization and feeding status could alter sleep and shift vectorial capacity.

Sleep-like state of other blood feeding arthropods

Along with mosquito systems, putative sleep-like states are likely to be critical for other blood feeding arthropods. Other dipterans, such as tsetse flies, have distinct circadian rhythms and show high activity during the dawn and dusk, and extended periods of inactivity and almost non-existent host seeking during the night [56]. Blood feeding hemipterans (e.g., bed bugs, kissing bugs) have high activity levels during dark periods with little to no movement under the **photophase** [57]. Interestingly, when off-host, ticks usually have long periods spent in quiescence – a sleep-like period of inactivity – that is critical for surviving extended starvation (months to years) between blood meals [58]. The occurrence of sleep-like states among a majority of animals suggests that most, if not all, hematophagous arthropods should be examined for sleep-like states, which could ultimately impact their ability as etiological agents of diseases.

Concluding remarks

As most research on mosquitoes requires intervention by the experimenter (who also represents a potential food source), a lack of focus on mosquito sleep may be a by-product that this state is not commonly observed and difficult to investigate (Box 1). Establishing a sleep-like state in mosquitoes will be transformative to the research field and set the stage for multiple subsequent lines of inquiry. Here, we provide a combination of observations that sleep-like states are likely among mosquito species and are likely distinct from the active, awake state (Figure 3). Most importantly, sleep-based studies will create a novel paradigm,

where specific aspects of mosquito biology should be measured under two independent periods, a non-resting (no sleep/awake) and sleep-like status (resting). There are intriguing questions (See Outstanding Questions) for future studies that will be particularly unique to mosquitoes and other blood feeding arthropods compared to their non-hematophagous counterparts. We hope these outstanding questions might provide additional perspective to understanding pathogen transmission dynamics in mosquitoes and other blood feeding arthropods. In conclusion, a lack of understanding of sleep-like states in mosquitoes and other arthropods that serve as disease vectors is likely a serious oversight. Sleep, or lack thereof, can have a significant impact on the biological performance of animals, which underlie changes in the transmission of pathogens by blood feeding arthropods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Antimicrobial peptides (AMPs)

diverse class of naturally occurring molecules that are produced as a first line of defense by all multicellular organisms to infections

Circadian rhythms

endogenous rhythms in behavior or physiology that reoccur approximately every 24 hours in most living organisms. These can be entrained by external environment cues (i.e. light and temperature) but persist in the absence of these cues

Dopamine

a chemical messenger involved in the transmission of signals in the brain and other vital areas. This chemical regulates sleep behavior at the circadian and homeostatic level in animal systems

Hematophagous

utilization of blood for nourishment

IMD pathway

mediates the humoral immune response to Gram-negative bacteria through the production of antimicrobial peptides (AMPs)

NF κ B

a major transcription factor that regulates genes responsible for both the innate and adaptive immune response

Octopamine

an organic chemical closely related to norepinephrine. Functions as a neurotransmitter for invertebrates

Orthologous genes

genes in different species that originated by vertical descent from a single gene of the last common ancestor

Photophase

the period of light during a day-night cycle

Proteomes

the entire complement of proteins that are or can be expressed by a cell, tissue, or organism

Protocerebrum

the first segment of the brain in an insect innervating the compound eyes

Quiescence

sleep-like state of inactivity or dormancy

Sensory thresholds

levels of strength stimuli must reach to be detected

Synaptic downscaling

negative feedback response to chronic elevated network activity to reduce the firing rate of neurons in the central nervous system

Synaptic potentiation

activity-driven lasting increase in the efficacy of excitatory synaptic transmission following the delivery of a brief, high-frequency train of electrical stimulation in the central nervous system

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Box 1. Limitations of sleep studies in blood feeding arthropods

Sleep offers a promising research area for disease vectors as this could improve existing disease transmission models. However, there are unique biological aspects of mosquitoes and other hematophagous arthropods that will pose obstacles to conducting robust sleep studies. First, the experimenter is a potential host (i.e. food source) and their presence near the mosquitoes can arouse them. Female mosquitoes detect diverse physical and chemical cues which include visual features, heat, moisture, body odor, and CO₂ when host seeking (reviewed in [59]). Thus, experimental manipulation that prevents mosquito exposure to host cues that impact sleep-like state will be nearly impossible. This means that experiments must be conducted under conditions that deprive the blood feeding arthropods of host cues (e.g., sensory deprivation cages where mosquitoes are only observed digitally or the experimenter wears materials that reduce release of host cues). Feeding, hydration status, and seasonality are likely to have significant impact on the duration and temporal periods of sleep-like states. However, sleep experiments are typically conducted in conditions that do not permit the easy replenishment of water and/or food sources. This could yield starvation or dehydration if not replenished and replacement will yield exposure to the experimenter, all of which may impact sleep [60]. Previous studies in *Drosophila* can serve as a guideline for experiments on mosquitoes and other blood feeding arthropods, but care needs to be taken as the physiology and behavior of disease vectors feature differences that make sleep experimentation much more difficult.

Figure 1. Resting (sleep-like) and active (awake) states are associated with stereotypical postures. (A) Illustration of posture changes associated with resting (Left) and active states (Right) in 5-day-old *Aedes aegypti* females. The angular differences between the main body axis (red line) and the main axis of the tarsomeres (blue line) were quantified in Fiji (NIH-NIAID). (B) Principal component analysis of posture metrics (elevation of the tip of the hind legs, hind leg - abdomen angle, distance between the thorax and the substrate), revealed a clear clustering of resting (blue) and active (orange) postures, classified according to [23] (Fuzzy clustering analysis, Dunn-coefficient = 0.85). Protocols for analyses are included in the supplemental Box S1.

Figure 2. Identification of putative resting/sleep-like state genes in mosquitoes.

(A) Orthology analysis between mosquito genes sets and those associated with sleep processed in *Drosophila*. (B) Re-analysis of previous circadian rhythm expressional studies in heads of *Aedes aegypti* [24] and *Anopheles gambiae* based correlation in expression profiles to periods of high and low activity [25]. Scale in red is high correlation and blue is low correlation. Full comparative analyses are found in supplemental Figure S1 and Figure S2. Details on the protocols used in analyses and complete module assignments are included in the supplemental Box S2.

Figure 3. Summary of factors indicative that the resting period is likely a sleep-like state in *Aedes aegypti* based on previous studies and our novel interpretation.

Front four legs are not included to emphasize positional changes in body and rear legs and details based on [23–25].