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Dispatches

Circadian Clocks: Mosquitoes Master the Dark Side of the Room

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***Aedes aegypti* and *Anopheles coluzzii* mosquitoes exhibit diurnal and nocturnal behaviors, respectively. Baik *et al.* reveal the clock network architecture underlying each species' light preferences.**

Animals adapt not only to take advantage of specific resources, but within specific contexts. In particular, the time of day an animal is active affects what information their brains can rely upon to find food and mates, and to avoid predators. For instance, nocturnal species may need to rely heavily on auditory cues relative to diurnal or crepuscular species. Even the same stimulus can evoke different responses in species active at different times of day. Take mosquitoes, for example: in particular, the diurnal *Aedes aegypti* and the nocturnal *Anopheles coluzzii* (formerly '*An. gambiae* molecular form M' [1]). Both species are anthropophilic, vector-competent dipterans from sub-Saharan Africa that reproduce year-round and prefer to oviposit in man-made water sources [2,3]. *Ae. aegypti* are active throughout the day alongside their human hosts, whereas *An. coluzzii* is nocturnal, targeting sleeping hosts and generally only biting once [3,4]. What differences in neural circuitry might promote divergent stimulus responses between nocturnal and diurnal species? What differences in neural circuitry might promote an animal to be active throughout the day versus only at night? A new study by Baik and colleagues in this issue of *Current Biology* [5] explores the light preferences of diurnal *Ae. aegypti* and nocturnal *An. coluzzii*, and how the clock network within their brains may regulate these preferences.

Baik *et al.* developed a chamber in which a mosquito's preference for a shaded area, or an area lit with either ultraviolet (∼365 nm), blue (∼450 nm), or red (∼630 nm) light, could be measured throughout the day. Both species are capable of perceiving these wavelengths [6], but they display very different behavioral phenotypes. For instance, *Ae. aegypti* preferred unshaded areas (regardless of the wavelength of light), while *An. coluzzii* strongly avoided ultraviolet-lit areas during the day. Moreover, *An. coluzzii* would move to the ultraviolet-lit areas of the chamber in anticipation of presumptive nighttime. Interestingly, males of both species had an anticipatory shift in their preference for ultraviolet light at the onset of dusk. This sexual dimorphism may be a reflection of mating pressures wherein males must be the 'first to the party' so as to aggregate in swarms, which the females will then approach, enter, and exit *in copula* [7].

Daily changes in light preference in *Drosophila melanogaster* are known to be influenced by the circadian system [8,9], but relatively little is known about the mosquito clock network compared to *D. melanogaster*. Whereas clock-gene transcripts are known to cycle in *Ae. aegypti* and *An. coluzzii* [10–12], daily oscillations in clock-gene protein levels and the clock-network architecture of these mosquitoes have not been explored. In *D. melanogaster* there are ∼150 neurons that express the canonical clock gene *period* (PER); together these neurons support distinct aspects of the animal's rhythm [13,14]. As a first step towards characterizing the clock networks of these mosquitoes, Baik *et al.* characterized the expression of PER and the neuropeptide pigment dispersing factor (PDF), a canonical clock transmitter that is released by the 's-LN$_v$s' and the 'l-LN$_v$s' clock-neuron clusters in *D. melanogaster* [15]. Both mosquito species possess PER$^+$/PDF$^+$ neuron clusters with similar projection patterns to the *D. melanogaster* s-LN$_v$s and l-LN$_v$s, although with more cells per cluster. Additionally, both *Ae. aegypti* and *An. coluzzii* had species-specific clusters of PER$^+$ neurons, suggesting each had unique clock-network components. Strikingly, several clock-neuron clusters found in *D. melanogaster* are absent in both mosquito species, with the exception of 1–2 cells. Altogether, these results suggest that the clock network of mosquitoes and *D. melanogaster* share some commonalities, yet also possess some dramatic architectural differences.

Knowing the neurons likely involved, Baik and colleagues then explored if differences in the temporal expression patterns of PER and PDF could explain species-specific light preferences. First, the authors determined the temporal patterns of expression of PER and PDF under normal light conditions, by measuring immunofluorescence of each factor at different times of day. This analysis showed PER and PDF levels were highest near dusk in the nocturnal *An. coluzzii*, and near dawn in the diurnal *Ae. aegypti* (Figure 1). These results suggest that changes in PER and/or PDF concentrations could underlie the species-specific switches in light preference. To explore the relationship between PER expression and light preference, Baik *et al.* exposed mosquitoes to constant light to disrupt clock-protein oscillations and in turn disrupt any circadian regulation of light preference. Under constant light, PER and PDF expression were greatly reduced in both species, to an extent that, in some animals, PER expression was undetectable. Moreover, under constant light conditions, the normal shifts in light

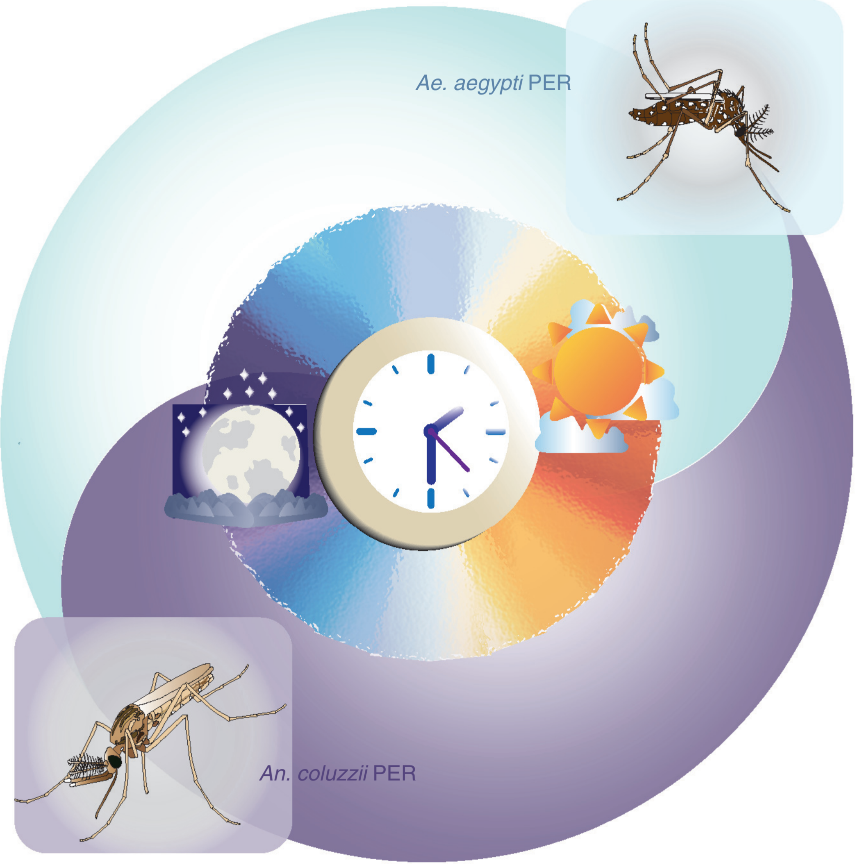

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Figure 1. Anti-phasic oscillation of the circadian gene *period* (PER) in diurnal *Ae. aegypti* vs. nocturnal *An. coluzzii* mosquitoes.
Cartoon schematic depicting the anti-phasic behavioral peaks of the diurnal *Ae. aegypti* and nocturnal *An. coluzzii* mosquitoes which are accompanied by anti-phasic expression of the clock protein PER (blue swath for *Ae. aegypti* and purple for *An. coluzzii*).

preferences of both species were disrupted. Together, these results suggest that the cycling of clock proteins supports the species-specific light preferences of *Ae. aegypti* and *An. coluzzii*.

This study raises several interesting questions about the diversity of clock-network architectures. The mosquito clock network is dominated by a large number of s-LN$_v$s and l-LN$_v$s, and appears to lack several circuit members found in *D. melanogaster*. Do the individual s-LN$_v$s in mosquitoes support different phases of activity throughout the day? Indeed, this is the case in *D. melanogaster*, where a single PDF$^-$ s-LN$_v$ contributes to the generation of the evening activity peak, thus playing a role that is distinct from the remaining PDF$^+$ s-LN$_v$s, which support the morning activity peak [16,17]. Alternatively, the 'missing' clusters of clock neurons in mosquitoes may simply not express PER, yet still contribute to the circadian regulation of behavior as downstream intermediaries of the s-LN$_v$s. Baik and colleagues also discovered species-specific clusters of PER$^+$ neurons within *Ae. aegypti* and *An. coluzzii*. Might these species-specific PER$^+$ clusters support the roles served by the clusters absent from the mosquito clock networks, but found in *D. melanogaster*? Furthermore, what molecular and/or circuit-based mechanisms might allow PER cycling to be anti-phasic between the two mosquito species? In *D. melanogaster*, clock neurons have asynchronous peaks of activity, wherein the s-LN$_v$s provide suppressive signals to delay the activity of other clock neurons [18]. It would be interesting to see if the activity of the s-LN$_v$s is anti-phasic across the two mosquito species, and whether they also suppress the activity of the species-specific clock neuron clusters described in this study. Now that transgenic approaches are available in multiple mosquito species [19,20], detailed circuit and molecular approaches will enable future exploration of the circadian regulation of many behaviors of one of the most dangerous creatures on Earth.

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Touch: Fluctuating Waves of Perception

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Does sensory input flow into the brain as a stream, or does it come in waves? New research shows that tactile information in the cortex rises and falls in phase with the forward and back motion of whiskers during surface exploration.

Sailing back from a neuroscience retreat on Catalina Island, the swell was up. Peering ahead through the mist, a pier, container ships, and staggered arrays of giant cranes at the Port of Long Beach came into view. But only for a moment. As we slid down the swell's surface, only the most prominent features, the cranes, remained observable above the next crest. The port had not changed; our ability to sense it had. While these cyclic shifts in our visual perception were imposed by nature's waves, a new study by Isett and Feldman [1], reported in this issue of *Current Biology*, shows how body and brain can interact to create similarly fluctuating waves of tactile perception.

Like all animals, mice gather information through senses evolved for their natural environments. Sniffing for the scent of food, friend, or foe, they scurry through dark tunnels, tracking along walls with an array of long facial whiskers. These whiskers are peculiar, their bases encircled with a dense set of nerves and each resting within a muscular sling. During exploration or running, these slings are in constant motion, swinging each whisker forward and back in synchrony, like frantic rows of Roman oarsman paddling towards battle. This 'whisking' is cyclic, and the relative position within the cycle of motion is called the phase, like phases of the moon. But instead of cycling once every 29½ days, whisking cycles about 15 times a second.

This phasic motion provides an attractive reference frame for models of how sensory input from the whiskers is transformed by the brain into knowledge about the world. For example, several models of object-location determination use phase as a reference signal for whisker position. During whisking in air, the activity of neurons in primary somatosensory cortex (S1) rises and falls with the whisking phase [2]. Contact with an object during whisking in air drives a sharp impulse of activity in S1 [3,4]. Phase modulation has been hypothesized to be combined with these touch signals in various ways to produce neural codes of object location [5–7]. However, recent behavioral experiments suggest other whisking features are more useful than phase during object localization with single whiskers [8]. Whether phase is, or is not, used for object localization in space, similar neural mechanisms could be used for identifying the location of object features on a surface, like a pattern of bumps on the tunnel wall that lets a mouse know it is finally home.

To investigate how S1 activity could identify the location of surface features, Isset and Feldman [1] created a controlled environment that mimicked mouse habitat in several important ways. Mice were allowed to run on a disc in a dark box while their head was held steady. A round drum of varying surface textures was placed adjacent to the face and rotated in closed-loop with mouse running, thus creating a virtual tactile reality of running down a tunnel. Neural activity from S1 was recorded with slender silicon probes [9]. Whisker position and acceleration resulting from the combination of whisking with whiskers sticking to and slipping past bumps on the surface were tracked at high speed. Comparing these two measurements allowed the experimenters to determine how neural activity in S1 was related to the phase of whisking and these stick-slip events.

As mice ran in place and swept their whiskers against the drum, S1 chattered