Opsin1 regulates light-evoked avoidance behavior in Aedes albopictus

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Abstract
Background: Mosquitoes locate a human host by integrating various sensory cues including odor, thermo, and vision. However, their innate light preference and its genetic basis that may predict the spatial distribution of mosquitoes, a prerequisite to encounter a potential host and initiate host-seeking behaviors, remains elusive.

Results: Here, we first studied mosquito visual features and surprisingly uncovered that both diurnal (Aedes aegypti and Aedes albopictus) and nocturnal (Culex quinquefasciatus) mosquitoes significantly avoided stronger light when given choices. With consistent results from multiple assays, we found that such negative phototaxis maintained throughout development to adult stages. Notably, female mosquitoes significantly preferred to bite hosts in a shaded versus illuminated area. Furthermore, silencing Opsin1, a G protein-coupled receptor that is most enriched in compound eyes, abolished light-evoked avoidance behavior of Aedes albopictus and attenuated photonegative behavior in Aedes aegypti. Finally, we found that field-collected Aedes albopictus also prefers darker area in an Opsin1-dependent manner.

Conclusions: This study reveals that mosquitoes consistently prefer darker environment and identifies the first example of a visual molecule that modulates mosquito photobehavior.

Keywords: Opsin1, Aedes albopictus, Aedes aegypti, Culex quinquefasciatus, Photobehavior, Vision, Light preference, Biting behavior, Blood feeding, Compound eyes

Background
Mosquitoes bite for animal blood to sustain their reproduction, but concomitantly transmit various human diseases including dengue fever, yellow fever, and malaria, thus posing global threat to humans and public health. They integrate various sensory systems to combine olfactory, thermoreceptive, and visual stimuli in order to detect, identify, and locate a specific host and acquire a bloodmeal [1–3]. Studies have elucidated how carbon dioxide, thermo, and host body odor activate host-seeking behavior of mosquitoes, with the corresponding chemical receptors and downstream signaling molecules identified [3–10]. However, visual features controlling mosquito light preference and target-seeking behavior have only begun to be addressed very recently [3, 11, 12]. Many insects display strong spectral sensitivity for short-wavelength light. Such light response mediated by insect compound eyes is critical to shape physiological adaption and specific behavioral features underlying insect survival and reproduction. The Aedes aegypti compound eyes is composed of ommatidia each containing eight photoreceptors (R1-8), with R1-7 forming a circular structure and R8 localized in the center [13]. Each photoreceptor cell possesses a microvillar rhabdomere, the place where photoreception and phototransduction take place [14]. While microvillar rhabdomeres of R1-6 and R8 cells are attached to neighboring rhabdomeres to create a fused rhabdom, presumably leading to enhanced...
light sensitivity, R7 cell has a small rhabdome located at the distal surface of the fused rhabdom [13]. Embedded in rhabdome membranes are Opsins, a class of seven transmembrane-containing GPCR proteins coupled to a light-sensitive chromophore that initiate phototransduction [15]. Although *Ae. aegypti* is diurnal and *Anopheles gambiae* is nocturnal, the two species share similar photoreceptor organization [13].

Bioinformatic analysis followed by manual annotation has identified thirteen Opsins in *Culex quinquefasciatus* and eleven Opsins in *An. gambiae* [16], while the genome of *Ae. aegypti* encodes ten potential *opsin* genes [17]. Different *opsin* genes show a unique expression pattern. In *Ae. aegypti* larvae, *Opsin3* is expressed in the majority of photoreceptors while *Opsin7* is expressed in two small clusters of photoreceptors located within the satellite and central stemmata [18]. In the compound eyes of adult *Ae. aegypti*, *Opsin1* is expressed in R1-6 and R8 cells, and interestingly, *Opsin2* and *Opsin8* are expressed exclusively in R7 cells that separate from the fused rhabdom [13, 19]. Additionally, *Opsin9* is expressed in all R7 cells and a subset of R8 cells located in the dorsal region of adult *Ae. aegypti* [14]. Although studies have revealed the expression pattern and light-triggered movement of Opsins in photoreceptors of both larval and adult mosquito retina [13, 14, 18–21], there is limited functional insight into the roles of mosquito *opsin* genes.

In this study, we characterized the photobehavior of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* at different developmental stages and confirmed our conclusion that adult mosquitoes are photonegative across the length of a day.

**Results**

**Adult female mosquitoes are photonegative**

While previous studies reported behavioral attraction to ultraviolet (UV) light for *Aedes*, *Anopheles*, and *Culex* in semi-field setting and in field conditions at night [24, 25], we extended our Y-maze assay to study mosquito photobehavior to UV light versus darkness (Fig. 1g). *Cx. quinquefasciatus* and *Ae. albopictus* were found to exhibit consistent phototactic attraction to UV light of both 395 nm and 345 nm (Fig. 1h, i). However, *Ae. aegypti* exhibited phototactic avoidance to UV light of both 395 nm and 345 nm (Fig. 1h, i). Taken together, our Y-maze assay argues that the phototaxis of *Aedes*, *Anopheles*, and *Culex* to UV light is more complex than originally believed, in sharp contrast to a consistent and robust avoidance of visible light by all the three species.

Since mosquito behaviors are guided by circadian rhythms [26], we investigated the photobehavior of the *Aedes* and *Culex* mosquitoes across zeitgeber time (ZT). Mosquitoes were entrained under 12h:12h light:dark cycles and subsequently presented with a choice of illuminated versus shaded environments in a Y-maze assay at indicated ZT time points. Our data indicated that *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* maintained robust photonegative behavior across all ZTs (Fig. 1j). Collectively, our results show that both daytime-active and nighttime-active mosquitoes are innately photonegative across the length of a day.

We cannot so far rule out the possibility that mosquitoes prefer light of certain intensities. To better reveal their phototaxis features, we measured light preference with different levels of illumination intensity available ranging from 0 lux, 15 lux, 150 lux, and 1500 lux, which correspond to darkness, dim light, home lighting, and extremely bright conditions, respectively. In binary choice assays (Additional file 2: Fig. S2a–c), mosquitoes consistently preferred environment with illumination of lower intensities, choosing 0 lux over 15 lux, 15 lux over 150 lux, and 150 lux over 1500 lux (Fig. 2a–c). Trinary
Fig. 1 Photobehavior of adult female mosquitoes. a, b Y-maze assay. a Assay schematic. b Preference index between illuminated and shaded environment (n = 400, 350, 250 females). c, d Tube assay. c Assay schematic and d preference index between illuminated and shaded environment (n = 250, 250, 150 females). e, f Shading assay. e Assay schematic. f Photobehavior of Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus upon shading (n = 209, 325, 246 females). g–i Photobehavior assay with blacklight. g Assay schematic. h Photobehavior assay with UV light of 345 nm performed at ZT17-ZT23 (n = 250 females per species). i Photobehavior assay with UV light of 395 nm performed at ZT17-ZT23 (n = 250 females per species). j Photobehavior of adult female mosquitoes across zeitgeber time. Photobehavior of Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus across zeitgeber time (n = 450 females per species). Mosquitoes were maintained on 12 h light/12 h dark cycles, with dark periods highlighted in black. Kruskal-Wallis test, followed by Dunn’s multiple comparisons test was performed for testing among different groups for Ae. aegypti. One-way ANOVA, followed by Tukey’s multiple comparisons test was performed for testing among different groups for Ae. albopictus and Cx. quinquefasciatus. Data labelled with different lowercase letters are significantly different from each other. Experimental groups denoted by “ab” are not significantly different from either “a” or “b” groups. Data includes over three biological repeats, each with four technical replicates. b, d, h–i Data represent PI of individual repeats, which includes over three biological repeats, each with two technical replicates. b, d, f, h–j Data are presented as mean ± SEM. Total number of mosquitoes released are indicated. Photobehavior was analyzed using one sample t test or Wilcoxon signed-rank test for tests against chance. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns: not significant.
preference assay (Additional file 2: Fig. S2d-e) revealed that over 65% female mosquitoes of all the three species preferred the darkest environment available (Fig. 2d, e). In quaternary choice assay (Additional file 2: Fig. S2f), over 60% adult female mosquitoes preferred complete darkness and around 20% mosquitoes preferred dim light (Fig. 2f). Our data collectively suggests that adult female mosquitoes exhibited light-evoked avoidance behavior and consistently exploited darker environment.

**Mosquitoes are photonegative throughout all developmental stages**

The fruitfly _Drosophila melanogaster_ changes light preference during development [22, 27, 28]. We also investigated mosquito innate response to light during larval and pupal stages using a previously described plate assay [29] with modifications (Fig. 3a). Similar to adults, larvae and pupae migrated towards the darker quadrants (Fig. 3b, Additional file 4: Video S3, Additional file 5: Video S4). Additionally, the negative phototaxis of larvae and pupae was confirmed with a tray assay (Fig. 3c, d, Additional file 6: Video S5, Additional file 7: Video S6). In these assays, larvae and pupae showed swarming behavior and tended to gather stochastically in one of the two dark areas in each independent test; however, the overall distribution among the two dark quadrants was similar in the plate assay (Additional file 2: Fig. S3a-c). Interestingly, in the tray assay, larvae and pupae significantly preferred dark region and larvae significantly swarmed at the edge (Additional file 2: Fig. S3d-f).

As females were photonegative at adult stage (Fig. 1b, d, and f), we further studied the photobehavior of males with the abovementioned assays. Male mosquitoes were found to exhibit significant aversion to light in both Y-maze assay (Fig. 3e) and tube assay (Fig. 3f). Therefore, mosquitoes are photonegative independent of their stages and sexes. Hereafter, we characterized the light-evoked avoidance behaviors only in female adults, which transmit human pathogens via bloodmeal and possess threat to public health.
Biting behaviors of adult mosquitoes are instructed by light

To investigate whether light perception modulates biting behaviors of mosquitoes, we adopted a biting assay in which female mosquitoes are exposed directly to live hosts (Fig. 4a). Significantly higher ratio of *Ae. aegypti* (Fig. 4b), *Ae. albopictus* (Fig. 4c), and *Cx. quinquefasciatus* (Fig. 4d) preferred biting mice in dark over illuminated environment. Since mosquitoes in the field likely make choices between multiple illumination intensities, we further applied a multi-chamber biting assay. Mosquitoes were allowed to navigate between four chambers of different illumination intensities each housing a host (Fig. 4e-h). In this assay, significantly higher ratio of *Ae. aegypti* and *Cx. quinquefasciatus* preferred blood feeding in complete darkness as compared with illumination intensities of both 150 lux and 1500 lux (Fig. 4f, h). In contrast, significantly higher ratio of *Ae. albopictus* preferred both 0 lux (darkness) and 15 lux (dim light) as compared with the other two illumination intensities (Fig. 4g). Interestingly, blood feeding ratio was not significantly different among mosquitoes under different illumination intensity under condition that mosquitoes were not given alternative choices (Additional file 2: Fig. S4). Collectively, these results support that mosquitoes preferred hosts in dark environment.

Photobehavior of *Aedes albopictus* was associated with molecules in the compound eyes

To uncover the mechanisms that underlie the photonegative behavior, we first blindfolded mosquitoes to test if the compound eyes are mediating the photonegative behavior, using methods previously described for *Drosophila melanogaster* [30–32]. As expected, ocularly painted *Ae. albopictus* were unable to differentiate between illuminated and shaded area, showing that eye occlusion completely abolished the photonegative behavior of *Ae. albopictus* (Fig. 5a). Surprisingly, painting the eyes, which attenuated approximately 99.14% light flux tested using glass coverslips painted in the same way, failed to abolish the photonegative behavior of female *Ae. aegypti* and *Cx. quinquefasciatus* (Fig. 5a). We further investigated the biting behavior of blindfolded *Ae. albopictus* with a binary choice of host in illuminated versus shaded chamber (Fig. 4a). While *Ae. albopictus* with normal vision preferred hosts in dark environment, similar amount of
ocularly occluded \textit{Ae. albopictus} bit hosts from a dark and illuminated environment (Fig. 5b), indicating that biting behavior of \textit{Ae. albopictus} was modulated by light flux via compound eyes.

**Knocking down \textbf{AalbOpsin1} abolished negative phototaxis of \textit{Aedes albopictus}**

Opsin, the light receptor in photoreceptors of the retina, plays a decisive role in visual perception [33]. Genome sequencing and bioinformatic analysis revealed ten \textit{opsin} genes in \textit{Ae. aegypti} and thirteen \textit{opsin} genes in \textit{Cx. quinquefasciatus} [16, 17]. Ten \textit{opsin} genes were manually annotated in the genome of \textit{Ae. albopictus} via homologous comparison with those of \textit{Ae. aegypti} and denoted as \textit{AalbOpsin1-10} (Additional file 8: Table S1). Phylogenetic analyses were performed with Opsins from \textit{Ae. albopictus}, \textit{Ae. aegypti}, \textit{Cx. quinquefasciatus}, and \textit{D. melanogaster} to discern their evolutionary relationship (Additional file 2: Fig. S5). The results suggest that Opsins of \textit{Ae. albopictus} and \textit{Ae. aegypti} are phylogenetically close, while Opsins are quite divergent among different genera. Furthermore, we characterized the expression of all the rhabdomeric \textit{opsin} genes present in \textit{Ae. albopictus}. While \textit{AalbOpsin3}, \textit{AalbOpsin4}, and \textit{AalbOpsin5} were highly expressed in larvae, their expression decreased along with development (Fig. 5c). By contrast, \textit{AalbOpsin1}, \textit{AalbOpsin2}, \textit{AalbOpsin8}, and \textit{AalbOpsin9} had a relatively low level of expression at the larval and pupal stage, but were highly expressed in adults of \textit{Ae. albopictus} (Fig. 5c). We further characterized the expression of Opsins that specifically enriched in adults of \textit{Ae. albopictus} and found that the Opsins were abundant at 1 day post emergence and the expression of \textit{AalbOpsin1} and \textit{AalbOpsin2} were further upregulated at 7 days post emergence (Fig. 5d).

To reveal the functional aspects of Opsins, we initially performed a screen of Opsins with high expression level in females of \textit{Ae. albopictus} by silencing each gene...
with double-stranded RNA (dsRNA) via thoracic injection. Knockdown of AaalOpsin1 with dsAalbOpsin1#1 completely abolished the photonegative behavior of Ae. albopictus, while silencing GFP (as negative control), AalbOpsin2, AaalOpsin8, and AaalOpsin9 had no discernable effect (Fig. 5e, Additional file 2: Fig. S6a-j). Of note, knocking down AalbOpsin2, which shares 87.94% similarity with AalbOpsin1 (Additional file 2: Fig. S6k), was unable to change the photobehavior of Ae. albopictus. We then checked if AaegOpsin1, which shares 93.85% similarity with AalbOpsin1 (Additional file 2: Fig. S6l), plays a role in the photobehavior of Ae. aegypti. Silencing of AaegOpsin1 led to a decrease of the photonegative behavior of Ae. aegypti, although an overall but weak preference for dark regions was still maintained (Fig. 5f, Additional file 2: Fig. S6m).

To further validate the role of AalbOpsin1 in photonegative behavior of Ae. albopictus, two additional dsRNA designated as dsAalbOpsin1#2 and dsAalbOpsin1#3 were synthesized (Fig. 6a). Injection of both dsRNA significantly downregulated AalbOpsin1 expression (Fig. 6b) and further abolished photonegative behavior of Ae. albopictus (Fig. 6c). To check the duration of RNAi effect, Ae. albopictus injected with AalbOpsin1 dsRNA were subjected to Y-maze assay consecutively for 3 days. While mosquitoes injected with dsGFP remained photophobic from third to fifth day post dsRNA delivery, the mosquitoes injected with dsAalbOpsin1 became photoneutral, both in the present and absence of host cues (Fig. 6d-f). The finding that knocking down AalbOpsin1 failed to abolish the preference of Ae. albopictus to bite host in shaded environment (Additional file 2: Fig. S6n), suggests additional molecules might be needed for preferring to bite hosts in shaded environment. Collectively, these results showed that AalbOpsin1 knockdown alone is sufficient to abolish the photonegative behavior of Ae. albopictus.
**AalbOpsin1 mediates light-evoked avoidance behaviors of field-collected *Ae. albopictus***

The aforementioned results indicate that *AalbOpsin1* is a critical protein in the compound eyes of *Ae. albopictus* for photophobic behavior. As mosquito physiology varies dramatically among strains and species [2, 34, 35], we next assessed the photobehavior of field-caught *Ae. albopictus*. Larvae, pupae, and adults of *Ae. albopictus* were collected from three different districts, including indoor and outdoor sites in villages, cities, and nearby natural environments (Additional file 2: Fig. S7a-b). Consistent with results obtained from laboratory strains, female adults of *Ae. albopictus* caught in different districts all exhibited photophobic behavior (Fig. 7a). Field-collected larvae and pupae also preferred shaded over illuminated environment (Fig. 7b, Additional file 2: Fig. S7c). To validate the role of *AalbOpsin1* in photophobic behavior of *Ae. albopictus*, we silenced *AalbOpsin1* in field-collected mosquitoes using RNAi. Adult *Ae. albopictus* emerged from field-collected larvae and pupae were injected with dsRNA and subjected to photobehavior assay for a consecutive measurement of 3 days. From third to fifth day post dsRNA delivery, silencing *AalbOpsin1* was always found to accompany with a consistent decrease of photonegative behavior in field-collected *Ae. albopictus* (Fig. 7c, d). Collectively, these results indicated a crucial role of *AalbOpsin1* in the innate light preference of field-caught *Ae. albopictus*.

**Discussion**

Insect physiology and behaviors are guided by the sunlight. However, mechanistic insights into insect innate light preference are still lacking. With multiple experimental apparatuses, we found that *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* mosquitoes consistently
avoid visible light and prefer to take a bloodmeal from hosts in the shaded environment.

Insect innate photobehavior can be exploited for vector control. While incandescent light bulb is being used to catch *Anopheles* in outdoor mosquito surveillance [36], laboratory experiments have shown that *An. gambiae* significantly preferred resting in darkness at both dusk and dawn [37]. *An. stephensi*, however, responds differently to light depending on sex and crepuscular period [37]. A recent study reported behavioral attraction to UV light for daytime-biting *Ae. aegypti* but behavioral avoidance of UV light by the nighttime-biting *Anopheles coluzzii* [23], contrasted to the finding in the field surveillance study whereby UV light attracted both *Anopheles* and *Culex* mosquitoes [24]. The discrepancy of behavioral response to visible and UV light may
result from different source of light, circadian timing, and combined effect of sensory perception by mosquitoes in various studies. Our study critically conducted in the laboratory under well-controlled conditions has thus provided comprehensive data to shape our understanding of mosquito innate light preference. While our study shows that adult mosquitoes are generally photonegative, we also extended the photobehavior study to *Aedes* and Culex larvae and pupae using both plate assay and tray assay, and uncovered the same photophobic behavior, in line with a previous study that only recorded the light avoidance behavior of *Ae. aegypti* larvae [38]. We also noticed that larvae and pupae tend to swarm, which has been previously noticed and interpreted as gathering near preferred cues through random aggregation [39]. Interestingly, larvae tended to swarm at the edge of shaded area, an interesting phenomenon and warrants further study.

Previous studies have characterized the role of heat, odor, and CO₂ levels in host-seeking and biting behavior of various mosquito species [8, 40]. However, it was not clear if light perception also influences biting behavior. Notably, our study showed that *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* consistently preferred to bite host in a dark area over host in an illuminated area, representing the first evidence of light-instructed host preference and biting behavior. To investigate whether the light preference and biting behavior are interrelated, we performed blood feeding assay under indicated illumination intensities in which mosquitoes were not given alternative choices for host. Unexpectedly, mosquito blood feeding rate was not significantly different under a gradient of illumination intensities. Additionally, painting the eyes of *Ae. albopictus*, which is known to abolish the photonegative behavior, led to equivalent blood feeding in dark and illuminated environment. Along the same line, knocking down *AalbOpsin1* to render *Ae. albopictus* photoneutral, did not affect preference to bite host in the dark region. Thus, the preference to bite in the darker environment when mosquitoes are provided with choices of different light intensities is serendipitously driven by the photonegative behavior.

An intriguing question raised by our study is the different sensitivity of the compound eye photoreceptors in closely related mosquito species. Firstly, the same blindfolding assay was only able to completely abolish the photonegative behavior of *Ae. albopictus* but not that of *Ae. aegypti* and *Cx. quinquefasciatus*. The different outcomes of this blindfolding manipulation suggest that the compound eyes of *Ae. aegypti* and *Cx. quinquefasciatus* are highly sensitive and can still respond to the remaining light flux. Secondly, silencing of *Opsin1*, one of the major light receptors expressed in the adult mosquito eyes, completely abolished the light avoidance behavior of *Ae. albopictus*, while only lead to weak preference for dark regions of *Ae. aegypti*. The result, together with previous blindfolding results, suggest that *Ae. aegypti* might be highly sensitive to the light flux.

*Opsin1* and *Opsin2*, which are the most abundant Opsins in the *Aedes* with over 80% similarity, were recently found to play a redundant role in CO₂-induced, vision-guided target attraction to black objects in *Ae. aegypti* [41]. Interestingly, our data showed that knocking down *Opsin1* rather than *Opsin2* abolished photonegative behavior of *Ae. albopictus*. Previous study revealed that *Opsin1* is expressed in all R1-6 photoreceptors and most R8 cells of the adult eye, while *Opsin2* is only expressed in R7 cells [13, 19, 21]. The different expression pattern may partially explain the discrepant requirement for *Opsin1* and *Opsin2* in the two *Aedes* species, suggesting functional divergence in different aspect of mosquito vision. Abolishing Opsin expression via CRISPR would be of importance to validate the results, especially for *Opsin2*, because RNAi was unable to completely abolish gene expression.

Photophobic behavior, which is regarded as an innate behavior that drives animals to choose an environment to maximize their survival fitness, has been widely observed in species of rodents and insects [28, 42, 43]. Sighted mice innately avoid illuminated areas, while *rd1* mice with photoreceptor degeneration lost the light avoidance behavior [44]. Photonegative behavior of *D. melanogaster* serves to keep larvae close to food source, to avoid desiccation and predation [29], and to further safeguard themselves by pupariating in the dark [28]. Thus, it is possible that light-evoked avoidance behavior allows mosquitoes to avoid desiccation under sunlight and reduce potential attack from host during biting, contributing to their adaption and survival. Remarkably, the three mosquito species tested showed consistent photophobic behavior throughout all developmental stages; in contrast, *D. melanogaster* reverses photobehavior during development [22, 27–29]. Further understanding of the genetic basis and the neurocircuit underlying the mosquito innate photonegative behavior should help design novel strategy of vector control and arbovirus disease prevention.

**Conclusions**

In this work, we have quantified the response to light by both laboratory-reared and field-collected mosquitoes with multiple experimental settings (Y-maze assay, tube assay, Y-maze assay with host scent, and biting
assortment throughout the circadian cycle. For all the three species tested, adult females which transmit arbovirus via blood feeding significantly preferred host in a darker environment, which underlies a notable trait in both vector control and arbovirus prevention. In addition to adult females, negative phototaxis was validated in adult males and throughout developmental stages. Mechanistically, light receptor Opsin1 expressed in the insect compound eyes is essential for the photonegative behavior of *Ae. albopictus*. Collectively, this study presented novel insights into mosquito innate light preference and biting behavior and identifies the first example of a visual molecule that modulates mosquito innate photobehavior.

**Methods**

**Mosquito rearing**

* Ae. aegypti, * Ae. albopictus*, and *Cx. quinquefasciatus* were obtained from Guangzhou Wolbaki Biotechnology Co., Ltd. Mosquito larvae were reared in dechlorinated tap water in plastic containers and raised with standard diet, which consisted of a 0.1% solution of three parts liver broth (CM0077, Oxoid) and two parts yeast extract (LP0021, Oxoid). Adult mosquitoes were maintained in a cage with unlimited access to water and sugar (raisin for standard diet). Mosquitoes were maintained in incubators (HWS-1000, Ningbojiangnan) with 12h:12h light:dark cycle at 27 °C with 75% humidity. Female mosquitoes aged 6–10 days post-eclosion were subjected for further investigation unless otherwise mentioned.

**Collection of field mosquitoes**

We collected larvae, pupae, and adults from three different districts (Additional file 2: Fig. S7a) from June to September, 2021. Collections were made in villages, cities, and nearby natural environments by visually scanning artificial containers left outdoors and recovering larvae and pupae with a metal sieve. Adults were either collected directly in the field by aspirating females approaching the experimenter or emerged from field-collected larvae and pupae. For gene silencing experiments, adults emerged from field-collected larvae and pupae were used.

**Mosquito dissection and RNA extraction**

For RNA expression profile of *Ae. albopictus* across developmental stages, pupa and larvae of the indicated instar (without differentiating sex) were homogenized for RNA extraction. For RNA expression profile of *Ae. albopictus* across developmental stages, adult mosquitoes aged 7 days after eclosion were subjected for total RNA extraction unless otherwise indicated. Female adult mosquitoes 3 days post injection of dsRNA were homogenized for RNA extraction and quantification of gene expression unless otherwise indicated. The mosquitoes and their tissues were homogenized and RNA was isolated using the MultiSource RNA miniprep Kit (AP-MN-MS-RNA-250G, Axygen). cDNA was synthesized with Primerscript RT reagent kit (RR037A, Takara) following the manufacturer's instruction.

**Quantification of gene expression**

Gene expression was quantified via real-time PCRs (qPCRs) using the LightCycler 480 SYBR Green I Master (04887352001, Roche) in the Quantagene q225 thermal cycler system (q225, Kubo technology). The primers used for this analysis are listed in Additional file 8: Table S2. The indicated gene expression was normalized against *Ae. albopictus* (AALF010408) or *Ae. aegypti* actin (AAEL011197).

**Gene silencing in mosquitoes**

Double-strand RNA (dsRNA) for gene silencing was synthetized using MEGAscript T7 transcription kit (AM1334, Invitrogen). Mosquitoes used for gene silencing were adult females 0–5 days post-eclosion that had previously mated but not taken a bloodmeal. Female mosquitoes were anaesthetized with carbon dioxide (CO₂) and was intro-thoracic microinjected with 1 μg per 300 nl of dsRNA using the Nanoject (3-000-207, Drummond). The injected mosquitoes were allowed to recover for 3 days under standard rearing conditions before subsequent behavior experiments. The gene silencing efficiency was evaluated via qPCR. The primers used for dsRNA synthesis and gene expression analysis are shown in Additional file 8: Table S2.

**Generation of ocularly blindfolded mosquitoes**

Ocularly blindfolded mosquitoes were generated using methods previously described for *Drosophila melanogaster* [30–32]. Both eyes of female mosquitoes were painted with an opaque, black, and water-soluble acrylic paint (793, Marie’s). Specifically, mosquitoes were anesthetized with CO₂ and a painting brush was used to carefully apply one coat of water diluted acrylic paint. Applying the paint into coverslips in the same way reduced the light flux down to 0.86% measured with a luminometer (AS-V10, Aicevoos). Mosquitoes with the pigment layer painted over eyes were double checked.
before the photobehavior with Y-maze assay with overhead LED illumination at 120 lux.

**Photobehavior assay of adult mosquitoes**

Photobehavior assay of adult mosquitoes includes three assays tested mosquito preference over dark environment and visible light (Y-maze photobehavior assay, tube photobehavior assay, and shading assay) and one assay that tested mosquito preference over dark environment and UV light (photobehavior assay with blacklight). For photobehavior assay of adult mosquitoes without pre-treatment, including Y-maze photobehavior assay, tube photobehavior assay, and shading assay, fifty mosquitoes were released in each individual repetition. For experiments with dsRNA injected or blindfolded mosquitoes, approximately twenty to forty mosquitoes were released in each individual repetition. One day prior to the behavior assays, mosquitoes were anaesthetized at 4 °C and the number of mosquitoes was counted before being transferred into new containers and maintained under standard conditions. The number of mosquitoes in dark environment was calculated as the total number of mosquitoes released subtracting the number of mosquitoes in illuminated environment and the number of mosquitoes that did not make a choice.

Except for testing the photobehavior of the *Aedes* and *Culex* mosquitoes across zeitgeber time, the photobehavior experiments with visible light were conducted during ZT1 to ZT5. The photobehavior of oocularly painted mosquitoes was tested simultaneously with control group, and the photobehavior of mosquitoes with *Opsin* knock down was tested simultaneously with the mock group injected dsRNA against *GFP*.

**Y-maze photobehavior assay**

This assay was used to test mosquito preference for illuminated versus dark environment (Fig. 1a). The custom-designed experiment apparatus comprised three chambers of quartz glass, including two arms of choice (5 cm in diameter and 25 cm in length) and an entrance (5 cm in diameter and 10 cm in length). One chamber of the two arms was wrapped with black tape, providing the mosquitoes with a choice of shaded (black box) versus light-exposed environment (overhead white light LED illumination at 120 lux). Light intensities across the Y-maze apparatus were measured with a luminometer (AS-V10, Aicevoos). Mosquitoes acclimated in the meshed release cage were released into the Y-maze, with most mosquito halt activity at 2 min post release (Additional file 2: Fig. S1c). Thus, distribution of mosquitoes within the two arms of the Y-maze was determined at 5 min post release. In order to prevent bias within the Y-maze, the two arms were routinely interchanged after each individual experiment. In each individual test, mosquitoes (approximately 55%) that did not make a choice by staying either in the release cage or in the central entrance chamber were excluded when calculating the preference index.

**Tube photobehavior assay**

This assay was modified from previously described assays [22, 37]. To investigate mosquito preference between illuminated and dark environment, the quartz glass tube (8 cm in diameter and 60 cm in length) was subdivided into four sections (two shaded areas and two light-exposed areas) with black tape (Fig. 1c). All areas were 15 cm in length. Overhead white light LED provided the light-exposed areas illumination at 120 lux. Light intensities across the tube apparatus were measured with a luminometer (AS-V10, Aicevoos). Mosquitoes were introduced to the end of the shaded or light-exposed end alternatively. Mosquitoes acclimated in the meshed cage were released, and most mosquitoes halt activity at 2 min post release (Additional file 2: Fig. S1d). Thus, distribution of mosquitoes of the tube was determined at 5 min post release. Mosquitoes (approximately 10%) that did not make a choice and stayed in the release cage were excluded.

To investigate mosquito preference over multiple illumination intensities, the quartz glass tube (8 cm in diameter and 60 cm in length) was subdivided into indicated equal sections, which were set in chambers with indicated illumination intensities (Additional file 2: Fig. S2). For each technical replicate, chambers were rearranged and mosquitoes were introduced to the indicated end. Light intensities across the tube apparatus of representative chamber arrangement were measured with a luminometer (AS-V10, Aicevoos). Mosquitoes were allowed to acclimate for 5 min in the meshed release cage and were given 5 min to make a choice.

**Shading assay**

To investigate mosquito preference between illuminated and dark environment, mosquitoes were release into illuminated quartz glass tube (8 cm in diameter and 60 cm in length) and were allowed to acclimated for 5 min. Then, the glass tube was carefully half-shaded without agitating the mosquitoes rested in. Mosquito preference index was calculated at 0 h, 0.5 h, 2 h, and 4 h post shading.

**Photobehavior assay with blacklight**

Tunable light source (CME-TLSX300F, Microenerg Technology) with narrow peak wavelength ranging from 300 to 2000 nm was used for UV light. Experiment apparatus of Y-maze assay was illuminated by UV light located 90 cm away of the end of one arm, with another arm
wrapped in black tape (Fig. 1g). Light intensities around experiment apparatus were 400 μW/cm² for 395 nm and 150 μW/cm² for 345 nm UV light, as determined by a power meter (LS125+UVALED-X3, Shenzhen Linshang Technology).

**Larvae and pupae photobehavior assay**

Measurements of larval and pupal photobehavior were made via plate assay and tray assay modified from a previous study [29]. Mixed-gender larvae and pupae of the indicated instar were used for photobehavior study. For plate assay, the plastic Petri plates (150 mm × 15 mm) were filled with water and placed upon a template which divided the plate into four equal quadrants to get two opposed light quadrants and two opposed shaded quadrants (Fig. 3a). LED light below the plate and template provided illumination at 120 lux. Fifty larvae were placed in the middle of the petri dish at the onset of testing and were given 5 min to make a choice. For tray assay, the transparent quartz glasses (200 mm × 50 mm × 50 mm) were filled with water and divided into four equal sections (two shaded areas and two light-exposed areas) with black paper (Fig. 3c). LED light below the tray provided illumination at 120 lux. Fifty larvae or pupae were placed in the middle of the petri dish at the onset of testing and were given 5 min to make a choice.

**Biting assay for host preference**

This assay was used to test mosquito preference for host in illuminated versus host in dark environment (Fig. 4a) or hosts in environment with multiple illumination intensities (Fig. 4e). For host preference in illuminated versus host in dark environment, the experiment apparatus comprised three chambers (20 × 20 × 20 cm each) of acrylic board, including a chamber for mosquito releasing, a transparent chamber on one side, and a lightproof chamber on the other side (Fig. 4a). White LED lights provided overhead illumination. Female mosquitoes 5 days post microinjection of dsRNA were starved for 24 h before releasing into the middle of the chamber. For host preference over multiple illumination intensities, the experiment apparatus comprised five chambers (20 × 20 × 20 cm each) of acrylic board, including a chamber for mosquito releasing, one lightproof chamber, and three transparent chamber set in area with indicated illumination intensities (Fig. 4e).

Biting assay for *Aedes* was performed during ZT1 to ZT8, while biting assay for *Culex* was performed during ZT13 to ZT24 to maximize percentage of blood feeding. Mice (BALB/c strain, males, 6–8 weeks old) were anaesthetized and randomly assigned into chambers of indicated light intensity. Mosquitoes were allowed to fly through holes (10 cm diameter) between chambers and took a bloodmeal. The holes between chambers were sealed after 30 min, and mosquitoes were anaesthetized at 4 °C. The number of mosquitoes present at each chamber and the number of blood-fed mosquitoes were counted. Percentage of blood-fed mosquitoes was calculated as number of mosquitoes that blood-fed on the mice in the chamber with indicated illumination intensity divided by the total number of mosquitoes tested.

**Biting assay without host preference**

This assay was used to test mosquito biting rate under indicated illumination. One day prior to the assay, mosquitoes were anaesthetized at 4 °C, and 20–25 female mosquitoes were transferred into transparent containers. After 24 h starvation, mosquitoes were subjected to biting assay under environment with indicated illumination. Anaesthetized mice were randomly assigned to the mesh top of the transparent containers (Additional file 2: Fig. S4a). Biting assay with four different illumination was performed simultaneously. After 30 min, mosquitoes were anaesthetized at 4 °C and the number of blood-fed mosquitoes were counted. Percentage of blood-fed mosquitoes was calculated as number of mosquitoes that fully engorged divided by the total number of mosquitoes tested.

**Statistical analysis**

All data is presented as mean ± SEM. Statistical analysis and graphing were performed using GraphPad Prism 8 (Prism, La Jolla, CA, USA). Preference index was calculated as \((S - I)/(S + I)\), where \(S\) denotes the number of mosquitoes that preferred the shaded chamber and \(I\) denotes the number of mosquitoes that preferred the illuminated chamber. The sum of \(S\) and \(I\) denotes the total number of mosquitoes that made a choice.

To test whether the preference indices represent photobehavior that statistically differs from chance, one sample \(t\) test was performed for data that met the assumptions of normality and Wilcoxon signed-rank test was performed for data with skewed distribution. Unpaired \(t\) test or Mann-Whitney test was performed to determine the significance of photobehavior between two groups. For unpaired \(t\) test, data were tested and met the assumptions of normality and homogeneity of variance. One-way ANOVA, followed by Tukey’s multiple comparisons test was performed to determine the significance of photobehavior among different groups that met the assumptions of normality. Kruskal-Wallis test, followed by Dunn’s multiple comparisons test, was performed for testing among different groups that does not met the assumptions of normality. Two-way ANOVA, followed by Sidak’s
multiple comparisons test, was performed to determine the significance of photobehavior between two groups for a consecutive of 3 days. Two-way ANOVA, followed by Tukey’s multiple comparisons test, was performed to determine the significance of biting behavior between control and blindfolded or dsRNA treatment group. Different lowercase letters indicate significant difference. Experimental groups denoted by “ab” are not significantly different from either “a” or “b” groups. Sample size (n) of each experiment was indicated in the figure legends, and we typically performed no less than three independent biological replicates (N). Confidence level to reject the null hypothesis that the mean of the data set is chance was typically performed no less than three independent biological replicates (N). Confidence level to reject the null hypothesis that the mean of the data set is chance is 0.05, specifically ns: not significant, *P < 0.05, **P < 0.01, ***P < 0.001, **** P < 0.0001.

Abbreviations
PI: Preference index; GPCR: G protein-coupled receptor; ZT: Zeitgeber time; RNA: RNA interference; UV: Ultraviolet; LED: Light-emitting diode; GFP: Green fluorescent protein; qPCR: Quantitative polymerase chain reaction.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12915-022-01308-0.

Additional file 1: Video S1. Photopreference of Ae. aegypti adults with Y-maze assay (related to Fig. 1b). This video shows the photopreference of female mosquitoes tested with Y-maze assay which was slightly modified to facilitate video shooting. In the manuscript, choosing arm of the dark environment was completely wrapped in black tape to minimize light transmittance, and overhead LED provided illumination. In this video, screen light below the Y-maze provided illumination and cardboard paper shaded light for the choosing arm of the dark environment. Two cameras (canon 80D) recorded video simultaneously under different exposure settings. The video was sped up 4x times.

Additional file 2: Fig. S1. Photonegative behavior of adult mosquitoes, related to Fig. 1. (a-b) Y-maze assay with host cues. (a) Assay schematic. (b) Preference between illuminated and shaded environment. n = 150 females per species. The data are presented as mean ± SEM. Photobehavior were analyzed using one sample t test. Rejection of the null hypothesis that the mean of the data set is chance is 0.05, specifically ns: not significant, *P < 0.05, **P < 0.01, ***P < 0.001, **** P < 0.0001.

Expression levels of AalbOpsin1 were assayed by qPCR. Expression three days post microinjection of dsRNA. AalbOpsin1 (a), AalbOpsin2 (b), AalbOpsin8 (c) and AalbOpsin9 (d) expression three days post microinjection of AalbOpsin1 dsRNA. n = 10 mosquitoes per group. (e-h) Expression of AalbOpsins upon microinjection of dsRNA. AalbOpsin1 (e), AalbOpsin2 (f), AalbOpsin8 (g) and AalbOpsin9 (h) expression three days post microinjection of AalbOpsin1 dsRNA. n = 10 mosquitoes per group. (i) Expression of Opsins upon microinjection of dsRNA. AalbOpsin8 expression three days post microinjection of AalbOpsin1 dsRNA. n = 10 mosquitoes per group. (j) Expression of AalbOpsin1 and AalbOpsin8 expression three days post injection of AalbOpsin1 dsRNA. n = 10 mosquitoes per group. (k, l) Sequence identities between AalbOpsins in percentage. Pairwise comparison of amino acid sequences of opsins of Ae. albopictus and Ae. aegypti. Sequence similarity was presented with heatmap. (m) AalbOpsin1 expression three days post microinjection of dsRNA. Expression levels of AalbOpsin1 were normalized against Ae. aegypti actin (AAEL011197). n = 10 females per group. (n) Photobehavior of opsins1 silenced Ae. albopictus. Three days post thoracic inoculation of dsRNA, biting preference between hosts in illuminated and shaded environment was assessed (dsGFP), n = 111 females. The data are presented as mean ± SEM. Two-way ANOVA, followed by Tukey’s multiple comparisons test was performed for testing among different groups. Different lowercase letters indicate significantly different. (a-j, m) The data are presented as mean ± SEM, with each dot represents one mosquito. (a-j) Expression levels of AalbOpsin1 were normalized against Ae. aegypti actin (AAEL011197). (a, c, f, h, j, m) Mann-Whitney test was performed for testing between two groups. (d, e, g, i) Unpaired t test was performed for testing among different groups. Rejection of the null hypothesis that two groups have the same mean: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns: not significant.

Fig. S7. Field collection of Ae. albopictus in three districts and photobehavior of field-collected larval and pupa. (a) Ae. albopictus were collected from three sites in three districts. One site in district A (112°56'E, 28°11'N), one site in district B (112°11'E, 27°43'N) and one site in district C (111°56'E, 28°53'N). (b) Pictures of typical collection sites. (c) Tray assay to test photobehavior of field-collected larvae and pupae. Preference index of field-collected Ae. albopictus between illuminated and shaded environment (n = 150 larvae or pupae per group).
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Authors’ contributions
P.W. and X.L. designed experiments. X.L., S.Y., YY., and S.W. performed experiments. X.L., S.Y., YY., and S.W. performed data analysis. P.W. drafted the manuscript. P.W. and Z.Z. revised the manuscript. P.W. and Z.Z. provided resources and supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials
The exact number of mosquitoes that chose illumination or dark environment in each individual experiment, light intensities across the experiment apparatus, and a table summary of Shapiro-Wilk test checking whether the data meet normality assumptions are available in the Mendeley Data repository [45]. Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Pa Wu (wupa@hunnu.edu.cn).

Declarations

Ethics approval and consent to participate
The study has been approved by the Hunan Normal University Ethics Committee (Protocol 188/2022).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Wu P, Yu X, Wang P, Cheng G. Arbovirus lifecycle in mosquito: acquisition, propagation and transmission. Expert Rev Mol Med. 2019;21:e1.
2. Wu P, Sun P, Nie K, Zhu Y, Shi M, Xiao C, et al. A gut commensal bacterium promotes mosquito permissiveness to arboviruses. Cell Host Microbe. 2019;25(1):101–12.e5.
3. Wolff GH, Riffell JA. Olfaction, experience and neuronal mechanisms underlying mosquito host preference. J Exp Biol. 2018;221(Pt 4):jeb157131.
4. Raji JJ, DeGennaro M. Genetic analysis of mosquito detection of humans. Curr Opin Insect Sci. 2017;20:34–8.
5. DeGennaro M, McBride CS, Seeholzer L, Nakagawa T, Dennis EJ, Goldman C, et al. Orco mutant mosquitoes lose strong preference for humans and are not repelled by volatile DEET. Nature. 2013;498(7455):487–91.
6. McNemnian CI, Corfas RA, Matthews BJ, Ritchie SA, Voishall LB. Multimodal integration of carbon dioxide and other sensory cues drives mosquito attraction to humans. Cell. 2014;156(5):1060–70.
7. Corfas RA, Voishall LB. The cation channel TRPA1 tunes mosquito thermo-taxis to host temperatures. eLife. 2015;4:e11750.
8. McBride CS, Baier F, Omondi AB, Spitzer SA, Lutomiah J, Lani AC, et al. Evolution of mosquito preference for humans linked to an odorant receptor. Nature. 2014;515(7526):222–7.
9. Hallem EA, Nicolle Fox A, Zwiebel LJ, Carlson JR. Olfaction: mosquito receptor for human-sweat odorant. Nature. 2004;427(6971):212–3.
10. Carey AF, Wang G, Su CY, Zwiebel LJ, Carlson JR. Odorant reception in the malaria mosquito Anopheles gambiae. Nature. 2010;464(7285):65–71.
11. Vinauger C, Van Breugel F, Locke LT, Tobin KK, Dickinson MH, Fairhall AL, et al. Visual-olfactory integration in the human disease vector mosquito Aedes aegypti. Curr Biol. 2019;29(15):2509–16.e5.
12. Murt LE, Thorne MJ, Kay BH. Aedes aegypti (Diptera: Culicidae) vision: spectral sensitivity and other perceptual parameters of the female eye. J Med Entomol. 1992;29(2):278–81.
13. Hu X, England JH, Lani AC, Tung JJ, Ward NJ, Adams SM, et al. Patterned rhodopsin expression in R7 photoreceptors of mosquito retina: implications for species-specific behavior. J Comp Neurol. 2009;516(4):334–42.
14. Hu X, Whaley MA, Stein MM, Mitchell BE, O'Tousa JE. Coexpression of spectrally distinct rhodopsins in Aedes aegypti R7 photoreceptors. PLoS One. 2011;6(8):e23121.
15. Neal S, de Jong DM, Seaver EC. CRISPR/CAS9 mutagenesis of a single r-opsin gene blocks phototaxis in a marine larva. Proc Biol Sci. 2019;286(1904):20182491.
16. Giraldo-Calderón GI, Zanis MU, Hill CA. Retention of duplicated long-wavelength opsins in mosquito lineages by positive selection and differential expression. BMC Evol Biol. 2017;17(1):84.
17. Nene V, Wortman JR, Lawson D, Haas B, Kodira C, Tu ZJ, et al. Genome sequence of Aedes aegypti, a major arbovirus vector. Science (New York, NY). 2007;316(5832):1718–23.
18. Rocha M, Kimler K, Leming MT, Hu X, Whaley MA, O’Tousa JE. Expression and light-triggered movement of rhodopsins in the larval visual system of mosquitoes. J Exp Biol. 2015;218(Pt 9):1386–92.
19. Hu X, Leming MT, Metoxen AJ, Whaley MA, O’Toosa JE. Light-mediated control of rhodopsin movement in mosquito photoreceptors. J Neurosci. 2012;32(40):13661–7.

20. Moon YM, Metoxen AJ, Leming MT, Whaley MA, O’Toosa JE. Rhodopsin management during the light-dark cycle of Anopheles gambiae mosquitoes. J Insect Physiol. 2014;70:88–93.

21. Metoxen AJ, Leming MT, Hu X, Whaley MA, O’Toosa JE. Light-driven processes control both rhodopsin maturation and recycling in mosquito photoreceptors. J Neurosci. 2016;36(43):11051–8.

22. Humberg TH, Sprecher SG. Age- and wavelength-dependency of Drosophila larval phototaxis and behavioral responses to natural lighting conditions. Front Behav Neurosci. 2017;11:66.

23. Baik LS, Nave C, Au DD, Guda T, Chevez JA, Ray A, et al. Circadian regulation of light-evoked attraction and avoidance behaviors in daytime- versus nighttime-biting mosquitoes. Curr Biol. 2020;30(16):3252–9.e3.

24. Mwanga EP, Ngowe HS, Mapua SA, Mmbando AS, Kaindoa EW, Kifungo K, et al. Evaluation of an ultraviolet LED trap for catching Anopheles and Culex mosquitoes in south-eastern Tanzania. Parasit Vectors. 2019;12(1):418.

25. Huang R, Song H, Fang Q, Qian J, Zhang Y, Jiang H. Laboratory and greenhouse performance of five commercial light traps for capturing mosquitoes in China. J Am Mosq Control Assoc. 2021;37(4):250–5.

26. Wang G, Vega-Rodriguez J, Diabate A, Liu J, Cui C, Nigman C, et al. Clock genes and environmental cues coordinate Anopheles phenotype synthesis, swarming, and mating. Science (New York, NY). 2021;371(6527):411–5.

27. Riemensperger T, Isabel G, Coulom H, Neuser K, Seugnet L, Kume K, et al. Behavioral consequences of dopamine deficiency in the Drosophila central nervous system. Proc Natl Acad Sci U S A. 2011;108(2):834–9.

28. Yamansaka N, Romero NM, Martin FA, Rewitz KF, Sun M, O’Connor MB, et al. Neuroendocrine control of Drosophila larval light preference. Science (New York, NY). 2013;341(6150):1113–6.

29. Sawin-Mccormack EP, Sokolowski MB, Campos AR. Characterization and genetic analysis of Drosophila melanogaster photobeaviour during larval development. J Neurogenet. 1995;10(2):119–35.

30. Barth M, Hirsch HV, Meinertzhagen IA, Heisenberg M. Experience-dependent developmental plasticity in the optic lobe of Drosophila melanogaster. J Neurosci. 1997;17(4):1493–504.

31. Velez MM, Wernet MF, Clark DA, Clandinin TR. Walking Drosophila align with the e-vector of linearly polarized light through directed modulation of angular acceleration. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2014;200(6):603–14.

32. Aptekar JW, Keles MF, Mongeau JM, Lu PM, Frye MA, Shoemaker PA. Method and software for using m-sequences to characterize parallel components of higher-order visual tracking behavior in Drosophila. Front Neural Circuits. 2014;8:130.

33. Montell C. Drosophila visual transduction. Trends Neurosci. 2012;35(6):356–63.

34. Duffield GE, Acra DJ, George GF, Sheppard AD, Beeve NW, Ritchie SA, et al. Diel flight activity of wild-caught Anopheles f. and An. hinesorum malaria mosquitoes from northern Queensland, Australia. Parasit Vectors. 2019;12(1):48.

35. Wiwatanaratnanabut I, Allan S, Linthicum K, Kittayapong P. Strain-specific differences in mating, oviposition, and host-seeking behavior between Wolbachia-infected and uninfected Aedes albopictus. J Am Mosq Control Assoc. 2010;26(3):265–73.

36. Mathenge EM, Misiagi GO, Oulo DO, Irungu LW, Ndegwa PN, Smith TA, et al. Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of Anopheles arabiensis, An. funestus and culicine species in a rice irrigation in western Kenya. Malaria J. 2005;4:7.

37. Jenkins AM, Muskatchev MA. Crepuscular behavioral variation and profiling of opsin genes in Anopheles gambiae and Anopheles stephensi (Diptera: Culicidae). J Med Entomol. 2015;52(3):296–307.

38. Mysore K, Flannery E, Leming MT, Tomchaney M, Shi L, Sun L, et al. Role of semaphorin-1a in the developing visual system of the disease vector mosquito Aedes aegypti. Dev Dyn. 2014;243(11):1457–69.

39. Lutz EK, Ha KT, Riffell JA. Distinct navigation behaviors in Aedes, Anopheles and Culex mosquito larvae. J Exp Biol. 2020;223(Pt 7):jeb221218.

40. Reinhold JM, Chandrasegaran K, Oker H, Crespo JE, Vinauger C, Lahondère C. Species-specificity in thermopreference and CO(2)-gated heat-seeking in Culex mosquitoes. Insects. 2022;13(1):92.

41. Zhan Y, Alonso San Alberto D, Rusch C, Riffell JA, Montell C. Elimination of vision-guided target attraction in Aedes aegypti using CRISPR. Curr Biol. 2021;31(18):4180–7.

42. Bourin M, Hascoët M. The mouse light/dark box test. Eur J Pharmacol. 2003;463(1-3):55–65.

43. Owens ACS, Lewis SM. The impact of artificial light at night on nocturnal insects: a review and synthesis. Ecol Evol. 2018;8(22):11337–58.

44. Berry MH, Holt A, Salari A, Veit J, Visel M, Levitz J, et al. Restoration of high-sensitivity and adapting vision with a cone opsin. Nat Commun. 2019;10(1):1221.

45. Liu X, Yang S, Yao Y, Wu S, Wu P, Zhai Z. Opsi1 regulates light-evoked avoidance behavior in Aedes albopictus. Mendeley Data; 2022. https://doi.org/10.17632/7dtmb22jgj.1.