REVISTA
DO
INSTITUTO
DE
MEDICINA
TROPICAL
DE
SÃO PAULO

JOURNAL OF THE SÃO PAULO
INSTITUTE OF TROPICAL MEDICINE

ORIGINAL ARTICLE

http://doi.org/10.1590/S1678-9946202365025

Electrophysiological detection of visible wavelengths of artificial lights inducing take-off in adults of *Rhodnius prolixus* (Hemiptera: Triatominae)

Mario Iván Ortiz[1], Eduwin Hincapié-Peñaloza[1], Jorge Molina[1]

ABSTRACT

Rhodnius prolixus is the most important vector of *Trypanosoma cruzi* in the northern part of South America. The compound eyes in adults of *R. prolixus* are involved in the nocturnal flight dispersion from sylvatic environments into human dwellings. During this behavior, the artificial lights play an important role in attracting *R. prolixus;* however, it is still not clear whether the compound eyes of this species use different visible wavelengths as a cue during active dispersion. We applied electrophysiological (electroretinography or ERG) and behavioral (take-off) experiments in a controlled laboratory setting to determine the spectral sensitivity of the compound eyes and the attraction of *R. prolixus* adults to discrete visible wavelengths. For the ERG experiments, flashes of 300 ms at wavelengths ranging between 350 and 700 nm at a constant intensity of 3.4 µW/cm^2 were tested after adaptation to darkness and to blue and yellow lights. For the behavioral experiments, the adults were exposed to nine visible wavelengths at three different intensities, and their direction of take-off in an experimental arena was established with circular statistics. The ERG results showed peaks of spectral sensitivity at 470–490 nm and 520–550 nm in adults, while behavioral experiments showed attractions to blue, green and red lights, depending on the intensity of the light stimuli. The electrophysiological and behavioral results confirm that *R. prolixus* adults can detect certain wavelengths in the visible spectrum of light and be attracted to them during take-off.

KEYWORDS: Compound eyes. Electroretinography. Active dispersion. Nocturnal flights. Spectral sensitivity.

INTRODUCTION

Rhodnius prolixus (Hemiptera: Triatominae) is an obligate hematophagous insect with a wide geographical distribution that includes countries such as Colombia, Venezuela, Bolivia, Brazil, Costa Rica, El Salvador, Ecuador, Guatemala, Guiana, French Guiana, Honduras, Mexico, Nicaragua, Panama, Suriname, and Trinidad and Tobago[1]. Within the Triatominae subfamily, *R. prolixus* is considered an efficient vector of *Trypanosoma cruzi* due to its wide distribution[2], its fast defecation during the blood-sucking process[3], the presence of populations in sylvatic conditions[4], and the presence of insects inside and around human dwellings[5].

The arrival of sylvatic triatomine insects into human dwellings has been extensively reported[1,6]. Likewise, the association of this behavior with the presence of artificial lights has been reported both in rural[6,7] and urban[8] areas of different countries, and also in laboratory conditions[9,10]. These experiments have shown that *R. prolixus* adults take-off towards white light sources and exhibit an attraction/

[1]Universidad de los Andes, Centro de Investigaciones en Microbiología y Parasitología Tropical, Bogotá, Colombia

Correspondence to: Jorge Molina
Universidad de los Andes, Centro de Investigaciones en Microbiología y Parasitología Tropical, Cra 1 #18A-12, Bloque A, Bogotá 111711, Colombia

E-mail: jmolina@uniandes.edu.co

Received: 13 September 2022

Accepted: 7 November 2022

repellence behavior towards UV light, confirming that this insect does not arrive randomly into dwellings and that light is a very important factor influencing its dispersion[10,11].

R. prolixus possesses two simple eyes or ocelli, used to detect light stimuli only in the adult stage, and two compound eyes, which are present in all development stages[12]. From a morphological point of view, *R. prolixus* possesses apposition compound eyes with open rhabdomes[13]. The constraints inherent to nocturnal insects with apposition eyes are physiologically corrected by the movements of screening pigments within cells and rhabdome movements toward the cornea in accordance with circadian rhythms[13]. These rhythmic changes are under endogenous control, providing protection for photoreceptors during daytime hours and taking maximum advantage of the few photons available at night[13].

The sensitivity of the *R. prolixus* visual system to different wavelengths is unknown; nonetheless, behavioral experiments with *Triatoma infestans* (Hemiptera: Triatominae) have shown sensitivity to UV, blue and green wavelengths[14,15].

Genomic evidence has identified UV and long-wavelength photoreceptors in *R. prolixus*[16]. However, electrophysiological experiments are required to explain the behavioral responses observed in *T. infestans* and to confirm the genomic data in *R. prolixus*.

One common tool used to determine the sensitivity and number of photoreceptors in the visual system of arthropods is electroretinography (ERG)[17,18]. This technique consists of recording the summed extracellular electrophysiological activity of the facets of the compound eyes and/or ocelli when exposed to light stimuli[18,19]. The change in amplitude is observed as a response toward light stimuli of different intensities and/or wavelengths[18]. Moreover, if the insect is selectively adapted to environments with particular wavelengths, then the sensitivity of those photoreceptors displaying response peaks close to the adaptation range can be decreased, revealing the presence of other visual pigments[20]. This methodology has allowed the determination of the sensitivity and number of photoreceptors in different insect orders[17]. Within the Heteroptera group, the ERG technique has only been applied to *Myzus persicae*[20] and *Notonecta*[21,22]. In these cases, three photoreceptors showing maximum responses in the UV, blue and green wavelengths have been found[17,20-22]. However, *Notonecta* and *Myzus* are known as predator and phytophagous diurnal insects, respectively[20-22], in contrast with *R. prolixus*, which is characterized as a nocturnal and blood-sucking insect[1].

To better understand the light-induced behavior of *R. prolixus*, we decided to carry out electrophysiological and behavioral experiments with discrete wavelengths in the visible range and test the epidemiological value of individual wavelengths in the attraction of *R. prolixus* to human dwellings. Therefore, the objectives of this work were as follows: first, to determine the spectral sensitivity of the compound eyes in *R. prolixus,* in laboratory-reared individuals; and second, with the information obtained, to evaluate the effect of discrete visible wavelengths on the take-off behavior of laboratory-reared *R. prolixus*.

MATERIALS AND METHODS

The research was developed under the approval of the ethics committee of the Universidad de los Andes, Act Nº 005, June 19, 2008.

Insects

The laboratory-reared *R. prolixus* were obtained from colonies established since 1979 in the Universidad de los Andes from individuals collected in San Juan de Arama city, Meta Department, Colombia.

The insects were fed monthly *in vivo* with *Gallus gallus* to ensure molting. Adults were separated by sex, and blood intake was avoided 15 days before the experiments. The rearing conditions were: 27 ± 2 °C, 75 ± 10% relative humidity and 6:00 am/6:00 pm light/dark photoperiods. Before testing the insects in the take-off experiments, we measured both male and female individual body length and abdomen and pronotum widths with a Vernier (Caliper Discover, New York, USA), and we measured the body weight with an analytical balance (Galaxy 160 OHAUS, New Jersey, USA).

Electroretinography experiments (ERG)

Adults were fastened dorsally with plasticine inside a metal Faraday box. For the experiments, one eye and both ocelli were covered with black paint. A micromanipulator ensured contact between the experimental compound eye and the recording electrode. The glass-recording electrode was filled with saline solution for triatomines[23], and inside was a tungsten recording wire. The tungsten wire that served as a reference electrode was inserted into the dorsal portion of the pronotum[18].

A digitizer (PowerLab/8SP ADInstruments, Colorado Springs, Colorado, USA) and the LabChart 7® software for Windows® (ADInstruments Inc.) were used to record and analyze insect responses and stimulus signals. The sampling rate used for recordings was 20 KHz. The recording electrode signal was registered at a voltage range of 2 mV

(AC coupled) using a low-pass filter of 10 Hz. The stimulus channel was registered at a voltage range of 5 V.

Stimuli and adaptation environments

A tungsten bromine lamp, (LLC-1A Tungsten-Bromine Lamp Lambda Scientific Systems, Florida, USA) using a 30 W halogen light bulb and variable voltage source, emitted a ray displaying wavelengths ranging from ultraviolet to infrared. The constant light beam produced by the tungsten lamp was controlled with a shutter (LS443, Cannon, Tokyo, Japan) in order to establish the 300 ms duration of the light stimulus.

A monochromator (Miniature Monochromator LEOI-92 f100 Lambda Scientific Systems, Florida, USA) decomposed the light beam into almost monochromatic rays (± 3 nm). Thirty-six wavelengths between 350 and 700 nm in 10 nm steps were produced. The emission spectra of each stimulus were measured with a spectrophotometer (USB4000-UV-VIS Ocean Optics, Ostfildern, Germany), directed at the location of the insect eye during the experiments.

The lowest intensity tested was 0.06 µW/cm^2, which was increased with the lamp power source up to 21.5 µW/cm^2. Calibration of each of the intensities tested was carried out for all wavelengths as well, using a laser photodiode sensor (PD300 Ophir, Massachusetts, USA) directed at the focal distance where the insect eye was located.

The setup had two LEDs generating selective adaptation environments either in the yellow (590 ± 30 nm) or the blue (450 ± 30 nm) range. Both LEDs were located inside the Faraday box and above the insect at a distance of 5.0 cm. The selective adaptation environment was only turned off during the 300 ms corresponding to each of the monochromatic light stimuli. Selective adaptation LED measurements and calibrations regarding intensity and wavelength were carried out using the laser photodiode sensor (PD300 Ophir, Massachusetts, USA) and the spectrophotometer (USB4000-UV-VIS Ocean Optics, Ostfildern, Germany), respectively, on the exact point where the insect compound eye was placed.

Controls and experimental design

Each ERG experiment was carried out with a different adult insect, testing each individual only once. Before initiating the experiments, two types of negative controls for the setup were carried out. The first one consisted of preventing the passage of the light toward the setup, and the other one consisted of keeping the tungsten lamp off. Both controls did not produce any voltage change. However, voltage differences were registered between both electrodes when the shutter was opened, the lamp was on and without obstructing the light passage toward the insect eye. The voltage changes obtained in this part allowed the typical ERG components to be observed (Figure 1).

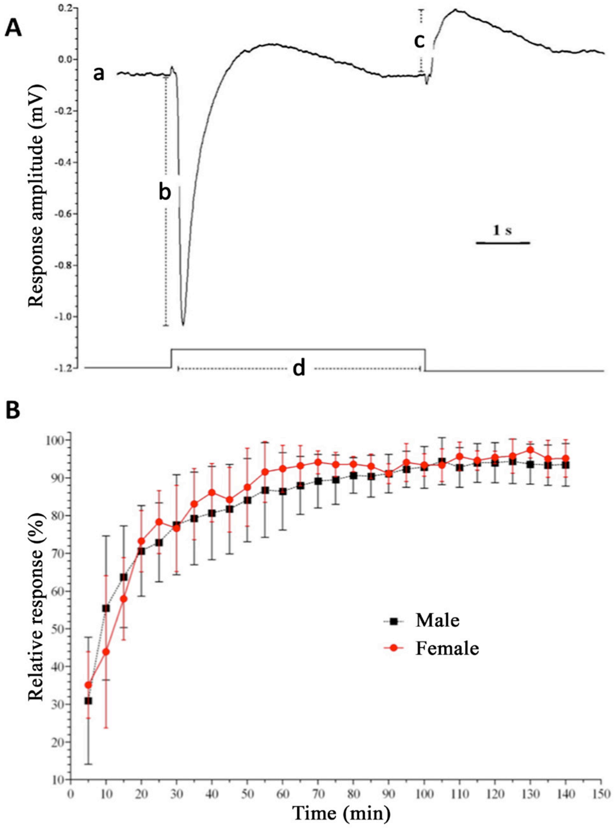

Figure 1 - Electroretinogram (ERG) responses from compound eyes in *Rhodnius prolixus*. A. Typical ERG response of compound eyes adapted to darkness and stimulated with white light. a) Reference potential; b) ERG receptor component; c) 'Off' response; d) Stimulus duration. B. Relative responses in ERG from laboratory-reared males (black squares) and females (red dots) exposed to different adaptation times in darkness (Stimulus: 530 ± 3 nm, 3.4 µW/cm^2, 300 ms, every 5 min). Vertical bars represent the 95% confidence interval. N = 5 females and 5 males (Mann–Whitney's U test $p < 0.001$).

The variable value assumed in all experiments was the ERG receptor component (Figure 1B). Each voltage difference obtained after stimulation (DVi) was multiplied by 100 and divided by the greatest voltage difference obtained from the total sampling time or sampling spectrum (DVmax) for each insect. Thus, the response variable is shown as a 'relative response' and corresponds to values ranging from 0 to 100[20]. The experiments were carried out with the following conditions: 20 ± 3°C and 55 ± 10% relative humidity measured with a datalogger (U10-003 HOBO Onset, Massachusetts USA).

To determine the effect of gender on the electrophysiological response, ERGs were carried out with *R. prolixus* males and females (five individuals of each sex) under selective adaptation to a dark environment (0 µW/cm^2), from 06:00 pm to 08:25 pm. Light pulses with the following characteristics were presented to the compound eye of each insect in five-minute intervals: wavelength of 530 ± 3 nm, intensity of 3.4 µW /cm^2 and duration of 300 ms. Normalized voltage differences were plotted with a 95% confidence interval using GraphPad Prism software (GraphPad, San Diego, California, USA). Each adaptation interval was analyzed using the non-parametric Mann–Whitney's U test and a Bonferroni correction in order to adjust the probability, using SPSS Statistics 17.0 software (IBM, Armonk, New York, USA). The probability value after applying the Bonferroni correction was 0.001.

ERGs were carried out with nine colonial individuals selectively adapted to darkness (0 µW /cm^2) in order to choose the intensity to be used for spectral sensitivity experiments. Each insect was stimulated with four different wavelengths (350, 450, 550 and 650 nm), starting from the shortest to the longest wavelength and using an intensity range from 0.06 to 20.35 µW/cm^2. This study includes light intensity values usually found on cloudy days (6 µW/cm^2), which are above the intensities detected in a moonlit night (0.03-0.05 µW/cm^2), beneath the trees (0.003–0.009 µW/cm^2) and inside a crack in a house wall without artificial light (0.002–0.004 µW/cm^2)[15,24]. Normalized voltage differences were plotted with a 95% confidence interval using GraphPad Prism software (Dotmatics, Massachusetts, USA). A one-factor ANOVA was used to determine the intensities at which the differences between relative responses were the highest among all wavelengths tested.

Finally, ERGs were carried out for 25 laboratory-reared insects exposed to three selective adaptation environments: darkness, blue and yellow lights. The intensity chosen as a standard was 3.4 µW/cm^2. Stimuli between 350 nm and 700 nm were applied increasingly in 10 nm steps until the longest wavelength was reached, and then went back to the shortest one. The time between stimuli was 50 s. Both responses were averaged and used in the analysis as a sampling unit for each insect. Normalized voltage differences were plotted with a 95% confidence interval using GraphPad Prism software.

Take-off experiments

Take-off experiments were carried out with some modifications following the methodology suggested by Minoli and Lazzari[10]. Afterwards, the experimental arena was a 2 m^3 tent made of white cloth and supported by a PVC pipe frame (1.27 cm in diameter). Inside the cubic tent, a take-off platform located 1 m above the ground (Plexiglass dish 15.0 cm in diameter and 5.0 cm high with walls covered in Vaseline) was used to test the nightly take-off directions of ten *R. prolixus* that were marked individually with white body paint. Triatomine adults were only able to leave the take-off platform by flying from a circular piece of cardboard (3.0 cm in diameter) marked with eight 45° sectors and elevated with a 15.0 cm long wooden stick.

A source of light located at 1.0 m above the ground was randomly rotated each night outside one of the four sides of the cubic tent. Lights were produced with an LED-Lite (World Precision Instruments, Florida, USA) by using six different LED lights (ELS World Precision Instruments, Florida USA) with the following wavelengths: 370 ± 40, 450 ± 40, 525 ± 40, 590 ± 40, 623 ± 40 and 660 ± 40 nm. The light intensity for each wavelength was adjusted before the experiments to 6.0 µW/cm^2, 0.6 µW/cm^2 or 0.06 µW/cm^2 with a radiometer (ILT 1400-A International Light Technologies, Massachusetts, USA).

Each wavelength and intensity was tested on twelve consecutive nights, and, in each test, five males and five females were released on the take-off platform and tested from 6:00 pm to 6:00 am. The behavior of the adults was recorded with an infrared sensitive video camera (DCR-SR200 Sony, Tokyo, Japan) located 50 cm above the take-off platform. Videos allowed us to record the number, time and direction of the take-offs. For each wavelength and intensity, we evaluated 120 insects (60 males and 60 females).

The behavioral experiments were performed in a dark room with an ambient temperature ranging between 21 and 27 °C and a relative humidity between 33 and 50%. Temperature and relative humidity were recorded every 5 min with a data logger (U23-001 HOBO Onset, Massachusetts, USA). Control experiments were performed without LED lights and at two different room temperatures, 15 to 21 °C (12 tests, 120 insects) or 21 to 27 °C (19 tests, 190 insects). Statistical differences in take-off between sexes were tested with a Chi-square test. Logistic regressions were used to evaluate the body measurements and take-off of the insects. The proportion of triatomines taking off per h was compared with Fisher's exact test for proportions. Take-off directions during control experiments were analyzed by the Kuiper's test to evaluate the von Mises distribution (normal circular distribution)[25] using MATLAB v.8.3 R2014a software. All data from control experiments showed $p > 0.05$, establishing a von Mises distribution. Since the data from control experiments followed a normal circular distribution, we used a Rayleigh test to analyze their randomness using circular statistic software (Oriana Kovach Computing Services, Anglesey Wales)[25].

To determine the effect of the different visible wavelengths and intensities on the orientation of take-offs, we used a V test with 0° (light source position), specified a priori as the expected mean direction, together with the average vector, average length of the vector and concentration, using circular statistic software (Oriana Kovach Computing Services, Anglesey, Wales)[25].

RESULTS

Electroretinography experiments (ERG)

ERGs with typical wave forms were obtained from laboratory-reared adult insects (males and females) (Figure 1A). We found that an increase in the time of selective adaptation to darkness also increases the voltage differences in laboratory-reared males and females (data not shown). Nevertheless, the voltage changes reached an asymptote after approximately 70 min of adaptation to darkness. Therefore, the experiments were carried out with this adaptation time. No significant differences were found in the relative voltage responses obtained for females and males adapted to darkness (Mann–Whitney's U tests for each adaptation time. P values > 0.001 in all cases after Bonferroni's correction) (Figure 1B). Therefore, further analyses were carried out by pooling together the results obtained from both sexes.

Rhodnius prolixus compound eye sensitivity

Intensity-response curves in *R. prolixus* compound eyes of laboratory-reared individuals showed that all four wavelengths tested produced significantly different responses, both at low and high intensities. However, the F-value of the 0.06 μW/cm^2 intensity treatments was lower (F = 290.9) than those of the other tested intensities (Supplementary Figure S1). At the intensity of 0.6 μW/cm^2, the F-value was 831.6 between wavelengths, but the confidence intervals at 350, 450 and 650 nm showed responses approaching each other when plotted. The intensity of 3.4 μW/cm^2 showed a greater differentiation between relative responses of different wavelengths (F = 2,364.6) (Supplementary Figure S1). Therefore, the standard intensity for further spectral sensitivity experiments was set at 3.4 μW/cm^2. This intensity showed the greatest differentiation and was also the highest intensity at which our setup could generate stimuli in the spectral range.

ERG responses in *Rhodnius prolixus* to selective light adaptation

Laboratory-reared insects selectively adapted to darkness have shown a single peak of maximum response between 520 and 550 nm, corresponding to the green region (Figure 2). During the selective adaptation of insects to blue light, there was again a single maximum response peak between 520 and 550 nm (Figure 2). Finally, when the insects were adapted to yellow light, a second maximum

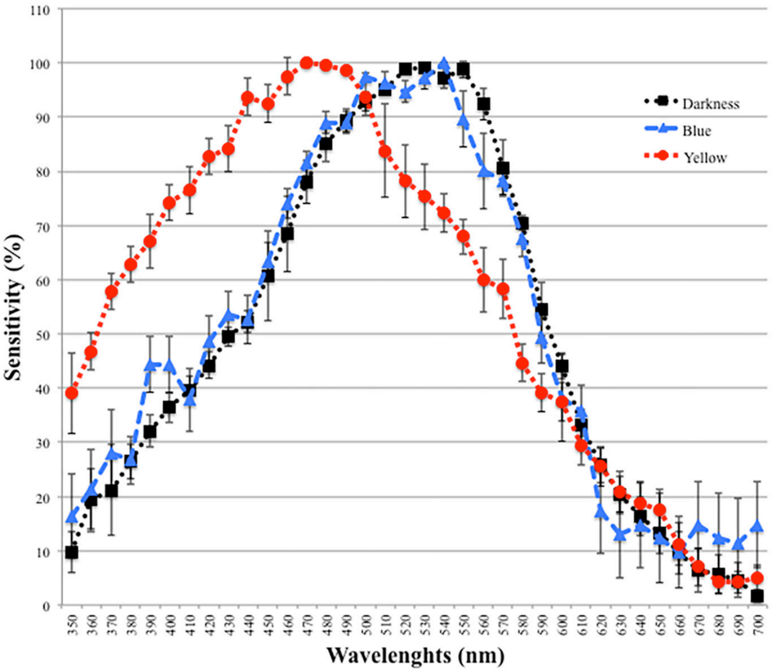

Figure 2 - Mean spectral sensitivity in compound eyes of laboratory-reared *R. prolixus*. Laboratory-reared insects were selectively adapted to darkness (solid squares, N = 9 individuals), yellow light (590 ± 30 nm, solid circles, N = 9 individuals), and blue light (450 ± 30 nm, solid triangles, N = 9 individuals), using a light intensity of 3.4 μW/cm^2. In all cases, vertical bars represent the standard deviation.

peak in the relative response was found between 470 and 490 nm (blue region) (Figure 2).

Take-off experiments

Controls

Statistical differences in the number of *R. prolixus* taking off were found between controls carried out at different temperatures ($p = 6.4 \times 10^{-8}$, $p < 0.05$). At lower temperatures, only 2 (1.6%) insects took off out of the 120 tested, while at higher temperatures, 68 (35.7%) insects took off out of the 190 tested. However, the insects that took off at higher temperatures did not show any directionality (Rayleigh test $p = 0.12$, $p < 0.05$) (Supplementary Figure S2). Therefore, further behavioral experiments with LED lights were only carried out at temperatures higher than 21 °C.

Behavioral experiments

No significant differences were found in take-off behavior between males and females ($\chi^2_{(1)} = 2.19$, $p > 0.1$); therefore, all results will be presented without making a differentiation between sexes. No relationships were found between take-off behavior and body length ($p = 0.190$, $p < 0.05$), abdomen width ($p = 0.27$, $p < 0.05$), pronotum width ($p = 0.16$, $p < 0.05$) or weight ($p = 0.730$, $p < 0.05$) of the tested insects. We found that a large number of insects (≥ 69%) started to fly during the first h (06:00 pm to 07:00 pm) of the behavioral experiments (Fisher's exact test, $p < 0.05$, with $\alpha = 0.05$).

Finally, the results showed an interaction between wavelengths and light intensities in the observed behavioral responses. Using blue wavelengths (450 nm), take-off toward the light source was only observed at the highest intensity of tested lights (6.0 μW/cm^2) (V test $p = 3.4 \times 10^{-11}$), while green and yellow wavelengths (525 nm and 590 nm) induced oriented take-off at both the highest and the lowest intensities tested here (6.0 μW/cm^2 and 0.06 μW/cm^2) (V test $p < 0.05$). Red wavelengths (660 nm) induced directional take-off only at the lowest intensities tested (0.6 μW/cm^2 and 0.06 μW/cm^2) (V test $p = 5.8 \times 10^{-7}$ and V test $p = 5.6 \times 10^{-6}$) (Table 1 and Figures 3, 4 and 5).

DISCUSSION

Our electrophysiological experiments showed that *R. prolixus* has compound eyes that are able to detect blue and green wavelengths. In concordance with the electrophysiological results, the behavioral experiments also showed that adults of *R. prolixus* are highly attracted to blue and green lights during nocturnal dispersal flights.

Electroretinography experiments (ERG)

ERG experiments have been widely used to characterize the spectral sensitivity of insects and spiders[17,20,26]. The ERG shape obtained for *R. prolixus* (Figure 1A) showed similarities to those obtained for different insects, including other Hemiptera such as *Notonecta* and *Myzus*[20-22]. In insects, the only major exception regarding the shape of the ERG is found in dipterans, where a biphasic voltage response has been reported[27].

Darkness adaptation time

The time required by the compound eyes of *R. prolixus* to reach the highest values in electrophysiological responses with darkness adaptation was approximately 70 min. This phenomenon can be related to two processes previously observed in this species and in *Triatoma infestans*: First, accessory and retinular cells of each ommatidium have pigments that move depending on light conditions, forming a type of 'pupil' that controls the arrival of photons to the rhabdom[13,28]. In *R. prolixus,* the maximum opening size of this 'pupil' has been shown to be achieved after 30 min of selective adaptation to darkness[28]. Second, there is a 'retinomotor' process that shows a distal movement of rhabdomes

Table 1 - Summary results of the take-off behavioral experiments obtained with *Rhodnius prolixus* after exposure to different visible wavelengths and intensities of light. p values are for the V test with an α of 0.05. In parenthesis, the percentage of insects taking off are shown (N = 120).

Wavelengths	Intensities		
	0.06 μW/cm^2	0.6 μW/cm^2	6.0 μW/cm^2
370 nm	$p = 0.002$ (57.5%)	$p = 0.129$ (50.8%)	$p = 0.072$ (63.3%)
450 nm	$p = 0.25$ (44.1%)	$p = 0.093$ (55%)	$p = 3.4 \times 10^{-11}$ (66.6%)
525 nm	$p = 0.026$ (42.5%)	$p = 0.341$ (55%)	$p = 3.6 \times 10^{-8}$ (44.1%)
590 nm	$p = 0.007$ (30%)	$p = 0.49$ (43.3%)	$p = 0.012$ (72.5%)
623 nm	$p = 0.025$ (44.1%)	$p = 5.4 \times 10^{-10}$ (43.3%)	$p = 0.067$ (75.8%)
660 nm	$p = 5.6 \times 10^{-6}$ (44.1%)	$p = 5.8 \times 10^{-7}$ (50.8%)	$p = 0.179$ (75.8%)

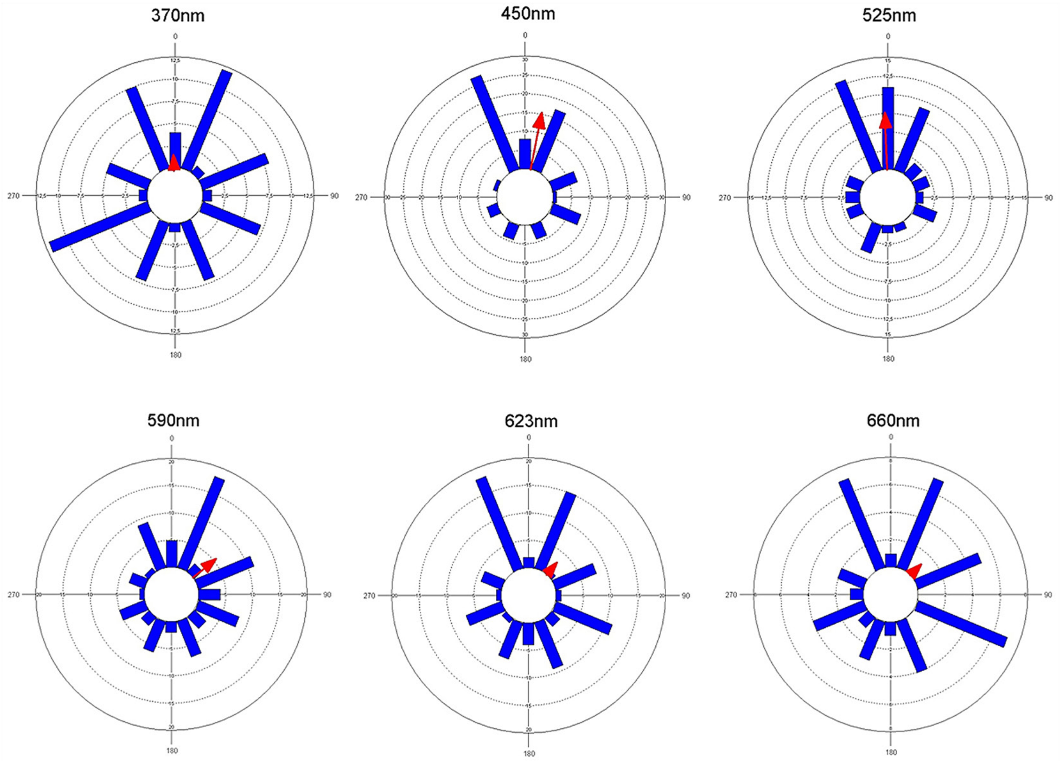

Figure 3 - Take-off directions of *Rhodnius prolixus* with a light intensity of 6.0 µw/cm^2. Circular histograms showing take-off directions of *Rhodnius prolixus* at different wavelengths. Reference angle V test = 0°, Vector r is shown as a red arrow.

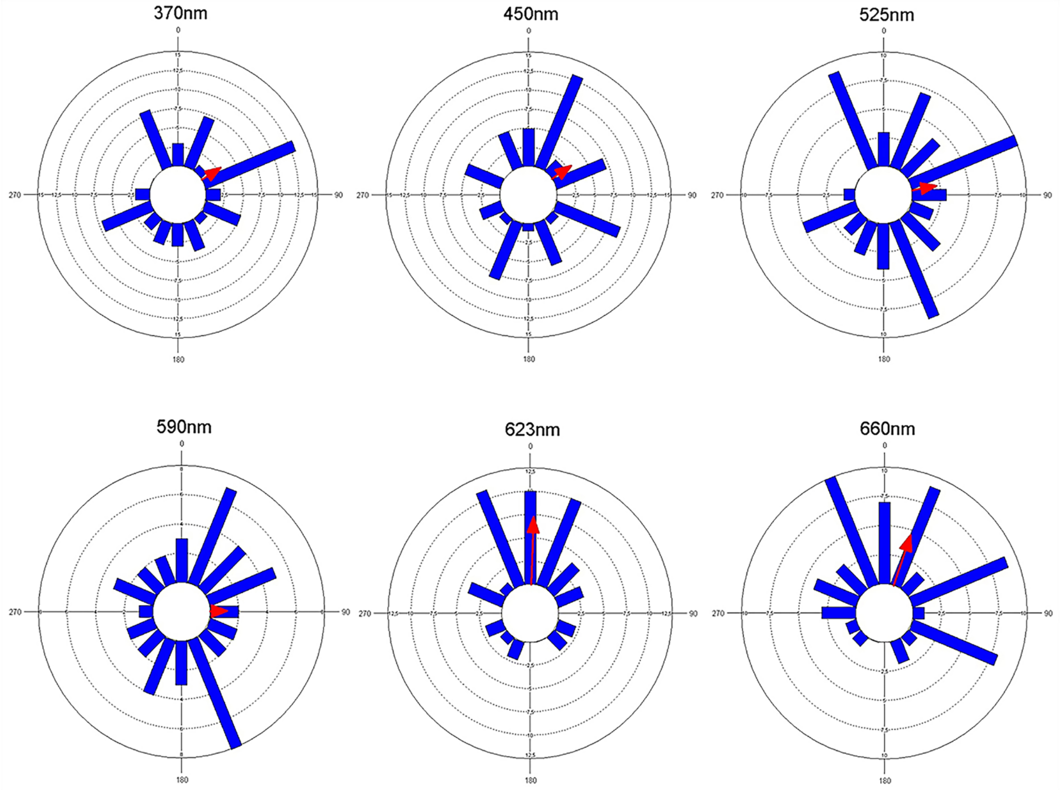

Figure 4 - Take-off directions of *Rhodnius prolixus* with a light intensity of 0.6 µw/cm^2. Circular histograms showing take-off directions of *Rhodnius prolixus* at different wavelengths. Reference angle V test = 0°, Vector r is shown as a red arrow.

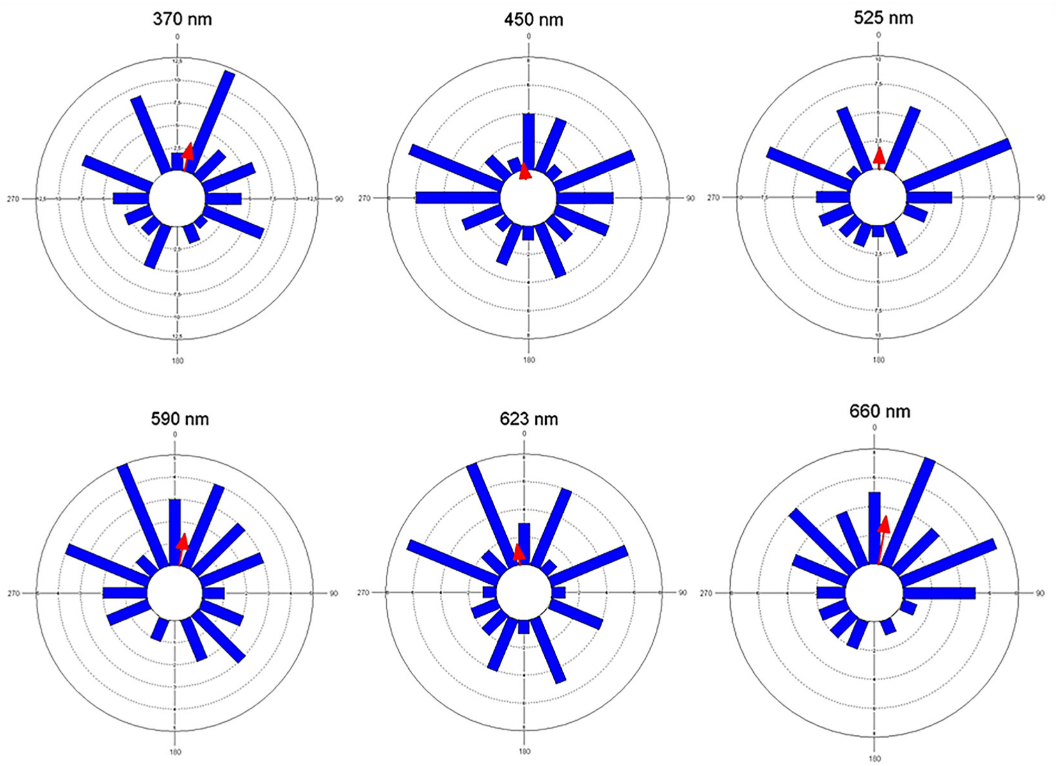

Figure 5 - Take-off directions of *Rhodnius prolixus* with a light intensity of 0.06 µw/cm^2. Circular histograms showing take-off directions of *Rhodnius prolixus* at different wavelengths. Reference angle V test = 0°, Vector r is shown as a red arrow.

toward the crystalline cone to maximize the few photons available in poorly lit environments. For *R. prolixus,* this movement is fully achieved after 60 min of selective adaptation to darkness[28].

Considering both previous results together, it is expected that the electrophysiological response of the compound eyes of *R. prolixus* adapted to darkness depends both on the 'pupil' opening and on rhabdome movements. Furthermore, the anatomical and physiological observations reported by Müller[28] confirm our results.

No differences between sexes

No significant differences were observed in ERG relative responses displayed by males and females of *R. prolixus*. Although there are few works describing electrophysiological responses on Hemiptera, differences between sexes have not been reported in the species so far[21]. However, studies in diurnal Lepidoptera have shown differences at electrophysiological[29], genetic[30] and behavioral[31] levels between sexes. The most plausible explanation for these variations is based on behavioral perspectives; first, in oviposition behaviors associated with females, and second, in mate-searching behaviors associated with males[29]. Regarding *R. prolixus,* it is clear that both sexes show very similar behavioral patterns, and that oviposition and mate searching are not strongly affected by visual information. Considering all this information, similar responses in ERG were expected and observed in males and females of *R. prolixus* (Figure 1B).

Chromatic hypothesis and its relationship with behavioral experiments

Only two spectral sensitivity curves were found in the ERGs obtained in *R. prolixus* reared under laboratory conditions (Figure 2). One response was found in the blue range (470–490 nm) and the other in the green range (520–550 nm). The three light-sensitive peaks in *R. prolixus* (two well-differentiated sensitivity peaks within the visible range (Figure 2) and one in the UV range[16]) have also been reported in other species within the Hemiptera order: *Notonecta* and *Myzus persicae*[17,20]. The existence of three maximum peaks in the compound eyes of *R. prolixus* and the subtractive interaction between them could be used to justify a possible chromatic mechanism, explaining the different aggregation and negative phototaxis behaviors reported in other triatomines such as *T. infestans* in the visible range of light[14,15] and in the UV range for *R. prolixus*[10].

The number and size of facets in the compound eyes change morphologically during the life cycle of triatomines[32]. In *Notonecta*, the posterior ommatidia responsible for color detection are the first ones to appear during development, and it seems that the addition of new facets at the anterior region does not affect color vision[21]. Based on these results with Hemiptera, it could be inferred that the addition of new facets in *R. prolixus* should not affect its ability for the detection of wavelengths; however, electrophysiological experiments conducted during different developmental stages are required to confirm the absence of inter-stage variations in light spectral detection in triatomines.

Even though different internal factors in insects contribute to flying behavior, our results with visible lights (Supplementary Figure S1) could be used to explain why adults of *R. prolixus* orientate their take-off toward short wavelengths only at high intensities (> 6.0 µW/cm^2), whereas for long wavelengths, the intensity should be decreased (< 6.0 µW/cm^2) to stimulate *R. prolixus* take-off under laboratory conditions. In a similar vein, studies with *T. infestans* nymphs have shown negative phototactic behavior in response to long wavelengths (> 600 nm) at relatively high intensities[15]. In Supplementary Figure S1, blue (450 nm) and green (550 nm) wavelengths had a greater effect on ERG relative responses of eyes in *R. prolixus* at intensities higher than 0.6 µW/cm^2, but reached an asymptote after 6.0 µW/cm^2. These differences in the slopes observed in both curves could explain the behavioral results obtained, with *R. prolixus* taking off toward blue and green lights at high (6.0 µW/cm^2) but not at low (< 0.6 µW/cm^2) intensities.

Behavioral experiments

Attraction of Rhodnius prolixus to artificial lights

Nocturnal flights for dispersion and attraction to light traps have been shown in triatomines[8,9]. Take-off experiments showed that artificial white light attracts *R. prolixus*, *Triatoma dimidiata*, *T. rubida* and *T. infestans*[8-10]. This means that triatomines do not randomly invade human dwellings[11]; in fact, they are attracted to incandescent lights[1,6,8,10].

We found no differences in take-off behavior between males and females. A similar observation has also been reported by other authors[6,10]. Our results show that any *R. prolixus*, male or female, with a total body length between 1.6 and 1.95 cm and with a body weight between 0.1 and 0.02 g, has the same probability to take off.

As previously shown for *R. prolixus* and other triatomines[6], we also found a higher number of take-offs during the first hours after sunset. Our experiments with two different temperatures suggest that under natural conditions, the presence of artificial light sources and higher temperatures after sunset could be factors contributing to flight dispersion.

Interaction of visible wavelengths and intensities in the attraction to artificial lights

The results obtained with different wavelengths and intensities showed a clear effect on the directionality of take-offs (Table 1 and Figures 3, 4 and 5). The light intensities used here (0.06 µW/cm^2, 0.6 µW/cm^2 and 6.0 µW/cm^2) are similar to the amount of light on a cloudy day (6.0 µW/cm^2) but above the amount of light on a clear night with a moon (0.03–0.05 µW/cm^2), inside a forest (0.003–0.009 µW/cm^2) and inside a crack in a wall without artificial lights (0.002–0.004 µW/cm^2)[15,24].

Our electrophysiological experiments, supported by genomic data[16], showed the presence of three light-sensitive peaks in *R. prolixus*, but not in the red range (Figure 2). However, an attraction to red lights, especially at low light intensities, was shown (Table 1 and Figures 3, 4 and 5). Behavioral responses to red lights have been reported in *T. dimidiata*, *T. rubida* and *T. infestans*[8,9,15]. In *Anopheles* and *Lutzomyia*, no photoreceptors with sensitivity to red lights have been detected, and only low spectral sensitivities at 630–670 nm have been reported[17,33]. However, similar to in our case, behavioral experiments have shown attraction to these wavelengths[34,35].

Interestingly, behavioral responses to red lights in *Anopheles gambiae* have been observed only at very low intensities (0.1 µW/cm^2)[34]. This result suggests that the wavelength-intensity interaction plays a role in both *Anopheles* and *Rhodnius* (Figures 3, 4 and 5). The differences between electrophysiological and behavioral experiments could be explained by the fact that a spectral peak in a compound eye can have some sensitivity in the red range, at a level of several units lower than what is observed for the maximum of the peak[34]. In this case, a spectral peak can have a maximum in the green range while there is still some lower sensitivity in the red range.

With regards to the change in behavioral responses of *R. prolixus* to visible wavelengths at very low light intensities (Table 1 and Figures 3, 4 and 5), this change has been also reported by other authors in *Anopheles gambiae*[34] and *Lutzomyia longipalpis*[36]. Therefore, behavioral experiments with *R. prolixus*, *An. gambiae* and *L. longipalpis* confirm that the responses to different wavelengths can change according to the intensity of the source.

CONCLUSION

The results obtained here show that take-off in *R. prolixus* is directed and affected by the particular wavelength and intensity of artificial white lights (Table 1 and Figures 3, 4 and 5). These results suggest that future experiments with light sources to capture or reduce the attraction of *Rhodnius prolixus* to human dwellings should consider the interaction between wavelength and intensity in order to maximize their results.

ACKNOWLEDGMENTS

We would like to thank the Department of Biological Sciences of the Universidad de los Andes for funding part of this work, through the project 'Determinacion electrofisiologica de las propiedades espectrales en la fotorecepcion del ojo compuesto de *Rhodnius prolixus* (Reduviidae, Triatominae)'. We would also like to thank the Departamento Administrativo de Ciencia Tecnologia e Innovacion (Colciencias) for funding this work, through the project Nº 1204-459-21479, 'Aspectos de ecologia sensorial que contribuyen a la transmision de la enfermedad de Chagas en Colombia'.

AUTHORS' CONTRIBUTIONS

MIO and JM: behavioral experiments; JM and EHP: electrophysiological experiments; MIO, EHP and JM: project conception, data analysis, manuscript preparation and writing.

FUNDING

Authors want to thanks the Faculty of Sciences-Universidad de los Andes for funding through the research Program INV-2021-128-2303, and also its fund to support publications.

REFERENCES

1. Galvão C, Carcavallo R, Rocha D, Jurberg J. A checklist of the current valid species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their geographical distribution, with nomenclatural and taxonomic notes. Zootaxa. 2003;202:1-36.
2. Molina J, Gualdrón LE, Brochero HL, Olano VA, Barrios D, Guhl F. Distribución actual e importancia epidemiológica de las especies de triatominos (Reduviidae: Triatominae) en Colombia. Biomedica. 2000;20:344-60.
3. Zeledón R, Alvarado R, Jirón LF. Observations on the feeding and defecation patterns of three triatomine species (Hemiptera: Reduviidae). Acta Trop. 1977;34:65-77.
4. D'Alessandro A, Barreto P, Saravia N, Barreto M. Epidemiology of Trypanosoma cruzi in the Oriental plains of Colombia. Am J Trop Med Hyg. 1984;33:1084-95.
5. Sandoval CM, Gutiérrez R, Luna S, Amaya M, Esteban L, Ariza H, et al. High density of Rhodnius prolixus in a rural house in Colombia. Trans R Soc Trop Med Hyg. 2000;94:372-3.
6. Jacome-Pinilla D, Hincapie-Peñaloza E, Ortiz MI, Ramirez JD, Guhl F, Molina J. Risks associated with dispersive nocturnal flights of sylvatic Triatominae to artificial lights in a model house in the northeastern plains of Colombia. Parasit Vectors 2015;8:600.
7. Angulo VM, Esteban L, Luna K. Attalea butyracea próximas a las viviendas como posible fuente de infestación domiciliaria por Rhodnius prolixus (Hemiptera:Reduviidae) en los Llanos Orientales de Colombia. Biomedica. 2012;32:277-85.
8. Pacheco-Turuch FS, Ramirez-Sierra MJ, Gourbière S, Dumonteil E. Public street ligths increase house infestation by the Chagas disease vector Triatoma dimidiata. PLoS One. 2012;7:e36207.
9. Indacochea A, Gard CC, Hansen IA, Pierce J, Romero A. Short-range responses of the kissing bug Triatoma rubida (Hemiptera: Reduviidae) to carbon dioxide, moisture, and artificial light. Insects. 2017;8:90.
10. Minoli SA, Lazzari CR. Take-off activity and orientation of triatomines (Heteroptera: Reduviidae) in relation to the presence of artificial lights. Acta Trop. 2006;97:324-30.
11. Lazzari CR, Lorenzo MG. Exploiting triatomine behaviour: alternative perspectives for their control. Mem Inst Oswaldo Cruz. 2009;104 Suppl 1:65-70.
12. Insausti TC, Lazzari CR, Campanucci VA. Morphology of the nervous system and sense organs. In: Carcavallo RU, Giron IG, Jurberg J, Lent H. Atlas of Chagas disease vectors in the Americas. Rio de Janeiro: Fiocruz; 1998-1999. p.1017-51.
13. Reisenman CE, Insausti TC, Lazzari CR. Light-induced and circadian changes in the compound eye of the haematophagous bug Triatoma infestans (Hemiptera: Reduviidae). J Exp Biol. 2002;205:201-10.
14. Reisenman CE, Lorenzo Figueiras AN, Giurfa M, Lazzari CR. Interaction of visual and olfactory cues in the aggregation behaviour of the haematophagous bug Triatoma infestans. J Comp Physiol A. 2000;186:961-8.
15. Reisenman CE, Lazzari C. Spectral sensitivity of the photonegative reaction of the blood-sucking bug Triatoma infestans (Heteroptera: Reduviidae). J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2006;192:39-44.
16. Feuda R, Marlétaz F, Bentley MA, Holland PW. Conservation, duplication, and divergence of five opsin genes in insect evolution. Genome Biol Evol. 2016;8:579-87.
17. Briscoe AD, Chittka L. The evolution of color vision in insects. Annu Rev Entomol. 2001;46:471-510.
18. Stowasser A, Mohr S, Buschbeck E, Vilinsky I. Electrophysiology meets ecology: investigating how vision is tuned to the life style of an animal using electroretinography. J Undergrad Neurosci Educ. 2015;13:A234-43.

19. Crescitelli F, Jahn T. The electrical response of the dark adapted grasshopper eye to various intensities of illumination and to different qualities of light. J Cell Comp Physiol. 1939;13:105-12.
20. Kirchner SM, Döring TF, Saucke H. Evidence for trichromacy in the green peach aphid, Myzus persicae (Sulz.) (Hemiptera: Aphididae). J Insect Physiol. 2005;51:1255-60.
21. Bennett RR, Ruck P. Spectral sensitivities of dark and light-adapted Notonecta compound eyes. J Insect Physiol. 1970;16:83-8.
22. Bruckmoser P. Die spektrale Empfindlichkeit einzelner Sehzellen des Ruckenschwimmers Notonecta glauca L. (Heteroptera). Z Vergl Physiol. 1968;59:187-204.
23. Taneja J, Guerin PM. Ammonia attracts the haematophagous bug Triatoma infestans: behavioural and neurophysiological data on nymphs. J Comp Physiol A. 1997;181:21-34.
24. Leuterer G, Gothe R. On the reaction of adult Rhipicephalus evertsi mimeticus and Hyalomma truncatum to horizontally incidenting optical radiation of various wavelength ranges and different irradiances and to optical radiation of a sun-simulating wavelength spectrum. Parasitol Res. 1991;77:353-8.
25. Baschelet E. Circular statistics in biology. London: Academic Press; 1981.
26. Skorupski P, Chittka L. Photoreceptor spectral sensitivity in the Bumblebee, Bombus impatiens (Hymenoptera: Apidae). PLoS One. 2010;5:e12049.
27. Yinon U. Similarity of the electroretinogram in insects. J Insect Physiol. 1970;16:221-5.
28. Müller VJ. Feinbau und Dunkelanpassung der Komplexaugen von Rhodnius prolixus (Stal). Zool Jb Physiol Bd. 1970;75:111-33.
29. Imafuku M, Shimizu I, Imai H, Shichida Y. Sexual difference in color sense in a Lycaenid butterfly, Narathura japonica. Zoolog Sci. 2007;24:611-3.
30. Arikawa K, Wakakuwa M, Qiu X, Kurasawa M, Stavenga DG. Sexual dimorphism of short-wavelength photoreceptors in the small white butterfly, Pieris rapae crucivora. J Neurosci. 2005;25:5935-42.
31. Silberglied RE. Visual communication and sexual selection among butterflies. In: Vane-Wright RI, Ackery PR, editors. The biology of butterflies. London: Academic Press; 1984. p.207-23.
32. Settembrini B. The compompound eyes of Triatoma infestans and Rhodnius prolixus (Hemitera: Reduviidae). J Med Entomol. 1984;21:477-9.
33. Mellor H, Hamilton JG, Anderson M. Spectral sensitivity in the eyes of the male and female Lutzomyia longipalpis sand flies. Med Vet Entomol. 1996;10:371-4.
34. Gibson GA. A behavioral test of the sensitivity of a nocturnal mosquito, Anopheles gambiae, to dim white, red and infra-red light. Physiol Entomol. 1995;20:224-8.
35. Hoel DF, Butler JF, Fawaz EY, Watany N, El-Hossary SS, Villinski J. Response of phlebotomine sand flies to light-emitting diode-modified light traps in southern Egypt. J Vector Ecol. 2007;32:302-8.
36. Mellor HE, Hamilton JG. Navigation of Lutzomyia longipalpis (Diptera: Psychodidae) under dusk or starlight conditions. Bull Entomol Res. 2003;93:315-22.

Supplementary Material available from:
https://doi.org/10.48331/scielodata.QMAPBK