

GENDER SPECIFIC VISION IN BED BUGS (HEMIPTERA: CIMICIDAE)

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Abstract Eye morphology, spectral sensitivity, and harborage color preferences all have significant differences between male and female bed bugs. Bed bug eye automontage images revealed that females had wider eyes than males. Electroretinogram studies gave evidence that bed bugs have at least one photoreceptor with a spectral sensitivity curve peak in the green ($\lambda_{max} = 520$ nm) region of the spectrum. This broad peak potentially indicates another photoreceptor in the yellow-green ($\lambda_{max} = 550$ nm) portion of the spectrum, or the presence of additional screening pigments. Electroretinogram studies also showed that males were significantly more sensitive to 520 nm wavelengths compared to females, whereas females were more sensitive to 580nm wavelengths compared to males. Bed bug bioassays indicated that, overall, bed bugs preferred red and black harborages, whereas green harborages were least attractive. Additionally, female bed bugs preferred colored harborages with shorter wavelengths whereas males preferred colored harborages with longer wavelengths. Collectively, these advancements in understanding bed bug vision are vital for improving bed bug monitoring tools.

Key words Spectral sensitivity, *Cimex lectularius*, eye morphology, colored harborages.

INTRODUCTION

The common bed bug, *Cimex lectularius*, is a nest parasite, which implies that, when it is not feeding, it is searching for shelter in a crack or crevice, and spends the majority of time within that harborage or looking for a new harborage (Pinto et al., 2007). Insects use visual cues, which are often based on color perception, to recognize hosts, find mates, oviposit, and also seek shelter. Color is one of the important visual cues that hematophagous insects use to seek shelter (Steverding and Troscianko, 2004). Spectral sensitivity is how sensitive an organism is to various wavelengths within the light spectrum, and can be influenced by 1) a single visual pigment present within the photoreceptor cells of the eye, 2) numerous types of photoreceptors, or 3) by the presence of other factors such as screening pigments (Kelber et al., 2003). The size and development of compound eyes are also important for understanding how insects respond to visual stimuli (Weiss, 1943). Early studies by Aboul-Nasr and Erakey (1969) documented that the common bed bug is able to distinguish between different wavelengths of light. Short-wavelengths such as violet (haematoxylin 0.005% solution) and bluish-green (fast green 0.001%) were preferred compared with other colors tested. Red (eosin 5%) had attractive qualities, while yellow (Bouin's solution) appeared to be the least attractive. More recent studies by Singh et al., (2015) have also shown that aggregations of adult male bed bugs combined with 3rd-5th instars prefer black and red harborages compared with other tested colors. To gain a better understanding of bed bug vision, this study focuses on the differences found between male and female bed bugs in harborage eye morphology, spectral sensitivity and harborage color preference.

MATERIALS AND METHODS

Equipment and techniques used to capture images of the bed bug eye were modified from Obenauer et al., (2009). A JVC KY-F70B 3-CCD digital camera (Cypress, CA), integrated with a dissecting Leica MZ12.5 stereomicroscope (Leica Microsystems GmbH, Munich, Germany) was used to capture images of the compound eyes of bed bugs. Bed bugs were killed using ethyl acetate and then mounted on a rotating microscope stage. Five bed bugs from each life stage were then used to determine the number of ommatidia as well as the length and width of the compound eye. Electroretinograms (ERG) were used to determine bed bug spectral sensitivity. All tests were conducted on insects that had been dark-adapted for 30 minutes. A pulse of light (200 milliseconds) was provided by a manual shutter, and eyes were allowed to recover for one minute before presentation of another light pulse. Light intensity through all interference and neutral density filters was measured with a concave grating spectroradiometer (Black comet CXR-SR, StellarNet Inc, Tampa, FL). Bed bugs were exposed to a maximum intensity of 5.2×10^{15} photons/cm²/sec (520 nm). All experiments were comprised of 10 replicates (i.e. five males, five females). Following adaptation, each bed bug was exposed to light passed through each of the 15 filters representing wavelengths between 340 and 700 nm to produce electroretinogram responses of the designated criterion response. Spectral sensitivity curves were calculated from the mean of all ten replicates. To remove differences in absolute sensitivity between individual insects, mean spectral curves were full-scale normalized and then averaged (Allan et al., 1991).

The bed bugs used in these experiments were the Harlan strain of the common bed bug (Harold Harlan, Armed Forces Pest Management Board, U.S. Department of Defense, Washington, D.C.) reared at the Urban Entomology Laboratory at the University of Florida (Gainesville, FL) using methods similar to Pfiester et al., (2009). Visual bioassays were conducted in a Lab Tek, extra deep, Petri dish (150 by 20 mm; ThermoFisher Scientific, Pittsburgh, PA). To prevent positional biases within each experiment, a clean Petri dish arena was randomly selected and placed within a large Pyrex dish (25 by 33 cm; Anchor Hocking, Co. Lancaster, OH), to further prevent bed bugs from escaping. Each Petri-dish arena contained seven color choices that were arranged as small tent-like harborage. The colored tent-harborage (2 cm long by 1 cm wide) were made from various colored cardstock paper (Michaels Stores Inc., Irving, TX). All bed bugs were placed in the experimental room for acclimatization 24 hours prior to bioassays. Gloves were used in all situations to keep human odor from arenas and harborage. Colored harborage were arranged in a semicircular manner in the arena with each end harborage ~2.5 cm from the edge of the Petri dish to control for edge effects. Bed bugs were tested individually and one replicate consisted of all seven color choices randomly arranged (based on an Excel randomization algorithm) with a bed bug in the center of the arena. Bed bugs were given 10 minutes to make a choice for a particular colored harborage. At the end of the 10 minute period, the number of bed bugs under each harborage was counted and recorded.

DATA ANALYSIS

Nominal logistic regression was used to determine whether gender influenced harborage color choice and un-paired t-tests were used to analyze differences between genders. Seven-choice preference data were analyzed using the χ^2 analysis (JMP 9.0.2 SAS Institute Inc. 2010), and adult bed bug data were pooled to determine overall bed bug color preference. Mean separation was determined by comparing upper and lower 95% confidence interval (CI) limits for overlap. Means with confidence interval limits that did not overlap were considered significantly different. Data on the number of ommatidia and the size (length and width) of the eye for each bed bug life stage were analyzed by a one-way analysis of variance (ANOVA) to determine if differences existed between life stages. Tukey's Honest Significant Difference was used as the post-hoc test for comparison of means for eye morphology. Differences between males and females under dark adaptation were compared using unpaired t-tests (JMP software, version 9.0.2 SAS Institute Inc. 2010).

RESULTS AND DISCUSSION

Automontage imaging of the common bed bug eye, for nymphs and adults, revealed the presence of a red-pigmented area surrounding dark-pigmented ommatidia, whose number ($F = 114.61$; $df = 6$; $P < 0.0001$) and size (length: $F = 72.89$; $df = 6$; $P < 0.0001$; width: $F = 110.54$; $df = 6$; $P < 0.0001$) varied with life stage. (Table 1). No ocelli were present for any life stage. The number of ommatidia found in adult male and female eyes were not significantly different. However, females had significantly wider eyes compared with males ($F = 72.89$; $df = 6$; $P < 0.0001$). This is in contrast to a general trend of eyes of male insects being larger than females (Land, 1989) and may reflect the lower role of vision in the mating behavior of bed bugs. These eye morphology data can also be used to help differentiate the common bed bug from the tropical bed bug (McNeill et al., 2016a).

Table 1. Anatomical characteristics of the compound eye of *Cimex lectularius* across various life stages.

| Life Stage | Number of Ommatidia | Length of Eye (μm) | Width of Eye (μm) |
|------------------------|---------------------|---------------------------------|--------------------------------|
| 1 st Instar | 5.2 \pm 0.2c | 74 \pm 5.1d | 62 \pm 3.7d |
| 2 nd Instar | 7.8 \pm 0.4c | 84 \pm 2.4d | 64 \pm 2.5d |
| 3 rd Instar | 9.0 \pm 0.0c | 90 \pm 3.2d | 72 \pm 2.0d |
| 4 th Instar | 21.2 \pm 1.6b | 154 \pm 5.1c | 112 \pm 3.7c |
| 5 th Instar | 27.0 \pm 1.8a | 182 \pm 3.7bc | 130 \pm 3.2b |
| Adult Male | 30.0 \pm 0.9a | 192 \pm 14.7ab | 146 \pm 7.5b |
| Adult Female | 30.4 \pm 0.7a | 214 \pm 4.0a | 164 \pm 2.5a |

The common bed bug has a primary photopigment in the green region (λ_{max} 520 nm) with a characteristic large α -peak in the long wavelength region and a smaller β -peak in the UV region (Figure 1) (McNeill et al., 2016a). This was determined by using a rhodopsin adsorption template from Stavenga (2010). While the theoretical pigment absorption curve for λ_{max} at 520 nm provided a close fit to the spectral sensitivity curve in the 430 to 500 nm range, there was poor fit in the UV region and the spectral sensitivity curve was broader from 500-600 nm than the single photopigment curve (McNeill et al., 2016a). A possible explanation for the broader curve could be the presence of another longer wavelength photopigment. The eye color of bed bug eyes, which was dark red, is presumably due to the presence of screening pigments, which often have the effect of broadening the spectral sensitivity curves (Stavenga, 2002; Carlson and Chi, 1979). Males were significantly more sensitive at 520 nm in the green region ($t = 2.33$, $df = 8$, $P = 0.024$) whereas females were significantly more sensitive at 580 nm in the yellow region ($t = 1.97$, $df = 8$, $P = 0.004$). These data may provide some understanding on how bed bugs perceive different colors and use colors for colonization, survival and reproduction.

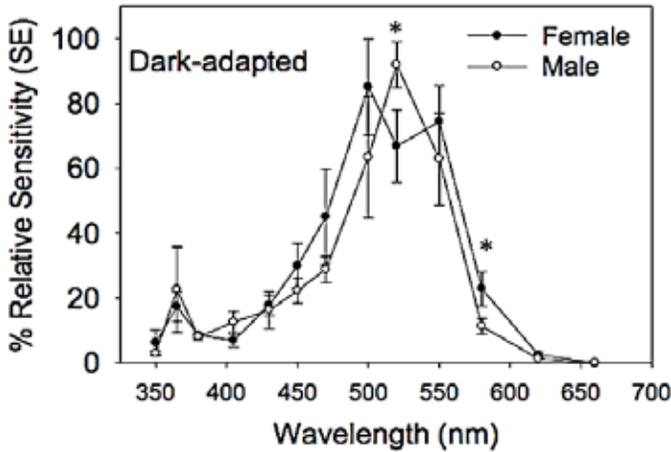


Figure 1. Spectral sensitivity curves with means \pm SE of dark adapted male and female bed bugs. $N = 5$ bed bugs per gender.

Bed bugs are able to preferentially select harborages based on color-specific visual cues. In behavioral studies with bed bugs, green harborages were the most avoided by adult bed bugs and red harborages were preferred to all other harborages except black ($F = 10.23$, $df = 6$, $P < 0.0001$) (McNeill et al., 2016b). Singh et al. (2015) speculated that red and black colors may only appear as general dark colors to bed bugs based on their photonegative reactions, and it is not known whether bed bugs can differentiate between red or black colors. Bed bugs could however be attracted to red harborages because it is similar to the red color reflected by their own integument and may represent the chance for aggregation with other bed bugs (McNeill et al., 2016b). Although there is no evidence based on the spectral sensitivity data that bed bugs possess red visual pigments, they may be using their green photoreceptors to detect red wavelengths similar to the potato aphid (Döring and Chittka 2007). While the common bed bug is nocturnal and considered endophilic, the presence of the green photosensitive pigment may be derived from an ancestral state and provides visual background contrast. For most insects, enhanced sensitivity to green as the predominate color of foliage, provides heightened contrast for detection of objects and movement (Briscoe and Chittka, 2010). Although there is a general harorage color preference for adult bed bugs, gender significantly influenced harorage color choice ($\chi^2 = 48.7$, $df = 6$, $P < 0.0001$). Female bed bugs preferred shorter wavelength (lilac, violet and blue) harborages to hide under compared with males, while males preferred longer wavelength (red and black) harborages (Figure 3). These color preferences can be used to understand how bed bugs seek out and colonize new harborages (McNeill et al., 2016b). Females bed bugs prefer female-female aggregations rather than female-male aggregations (due to excessive mating) (Pfiester et al., 2009) and so females may use color cues to find safer harborages away from males (McNeill et al., 2016b).

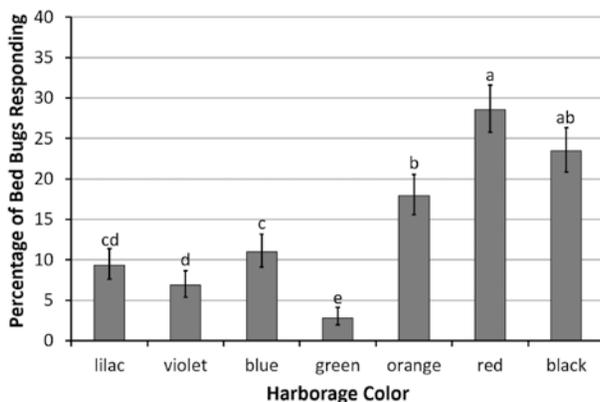


Figure 2. Harorage color preference for adult bed bugs. Different letters are significantly different means ($P < 0.031$; 96.9% confidence intervals based on Bonferroni corrections).

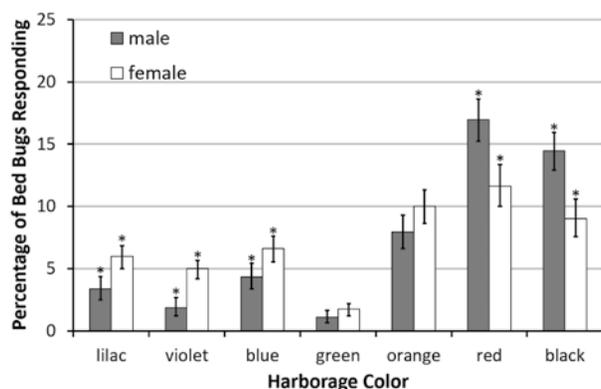


Figure 3. Harborage color preference for male and female bed bugs. Asterisks (*) indicate significantly different means ($P < 0.031$; 95% confidence intervals).

CONCLUSIONS

Bed bug eye morphology, spectral sensitivity, and harborage color preferences show significant differences with respect to gender. This information on bed bug visual biology is important for developing future monitoring and control strategies within bed bug IPM.

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