

Read the suggested paper and the paper guidelines. Copy and paste a quote from the suggested paper that is an example of each of the numbered descriptions below. If there is not an example of one of the following numbered points in the paper fill that section with NA.

## Abstract

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### 1. Broad introduction to the field

*A cue for long-range vision allows mosquitoes to identify hosts and differentiate the ecological niches (e.g., habitats). However, the visual factors involved in attracting mosquitoes to a host are complex and have not been fully understood.*

### 2. Purpose of the study, including the hypotheses and predictions.

*"Mosquitoes recognize cues from hosts and environments, modulating direct important behaviors (e.g., host-seeking and blood-feeding) associated with their overall vectorial capacity. The purpose of this study was to investigate how color brightness and intensity affect host attraction in both diurnal (*Ae. albopictus*) and nocturnal (*Cx. pipiens*) species. Specifically, we asked two questions: 1) How do color brightness and intensity affect host attraction? and 2) How do these species differ in their color perception mediating host-seeking behavior?"*

### 3. General description of main methods. What was measured and how?

*"We used a binary behavioral assay using the Mosquito Preference Index (MPI) as a preference ratio with a range of 0–1. Seven fundamental colors (black, white, red, yellow, green, blue, and purple) were used, and each color was tested under controlled conditions. The MPI data were analyzed by two-way ANOVA followed by Tukey's HSD test to determine mosquito preferences for each color"*

### 4. Main results (clear patterns and relationships) in support of or refuting the hypothesis

*"Our analyses showed that *Ae. albopictus* had a greater response to black (MPIs, 0.7), followed closely by red, blue, and purple (MPIs, 0.6). We also found that red, blue, and purple were significantly higher than green (MPI, 0.5), white (MPI, 0.3), and yellow (MPI, 0.2). Similarly, *Cx. pipiens* exhibited a strong preference for black and red (MPIs, 0.7), with much lower preferences for white and yellow"*

### 5. Concluding statement putting the results into the general context of the field

*"Our analyses provide a greater understanding of how color plays a role in visual sensory stimuli and how this could potentially affect mosquito host-seeking behavior. These findings may inform the development of more effective mosquito control strategies, especially in vector-borne disease prevention"*

### 6. Broad impact statement(s). Why is this study important?

*"This study could lead to significant implications for effective trapping devices, precise estimates of the vector population, and reduced costs for mosquito surveillance programs. Understanding mosquito preferences for specific visual cues can help in developing improved methods for controlling mosquito populations and minimizing the transmission of mosquito-borne diseases"*

# Introduction

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1. The overarching problem that is addressed and the broad biological context

*"Mosquitoes (Diptera: Culicidae) are a ubiquitous group of insects that pose imminent threats to both humans and animals. Climate change has expanded the potential distribution of mosquitoes, accelerating incidents of mosquito-borne diseases worldwide. More recently, the introduction of vectors, pathogens, and reservoir hosts via international trade and travel has further increased the impact on public health"*

2. A description of the specific dataset or type of methods used

*"We used a Biogents Sentinel (BGS) trap (BioGents, Regensburg, Germany), which is designed to mimic convection currents by human body heat with a fan. The trap was covered with polyethylene sheets in seven different colors (black, white, red, yellow, green, blue, and purple), and mosquito responses were measured in a controlled laboratory setting"*

3. Describe the gap in knowledge this experiment addresses

*"While much of the research on mosquito visual preferences has focused on anthropophilic species such as *Anopheles gambiae* and *Aedes aegypti*, the visual factors involved in attracting *Aedes albopictus* and *Culex pipiens* remain understudied. This experiment addresses the gap in knowledge regarding how these species respond to color and how their visual preferences could influence their host-seeking behavior"*

4. Describe the question and hypothesis this experiment is designed to address

*"We sought to answer two key questions: 1) How do color brightness and intensity affect mosquito host attraction? and 2) How do diurnal and nocturnal species differ in their color perception when mediating host-seeking behavior? Our hypothesis was that *Ae. albopictus*, a diurnal species, would have a greater capacity for color distinction than *Cx. pipiens*, a nocturnal species, due to differences in their visual systems"*

*"In a controlled laboratory setting, we exposed *Ae. albopictus* and *Cx. pipiens* to seven different colors using a binary choice behavioral assay. Our results showed that both species were significantly more attracted to darker colors (black, red, blue, and purple) compared to lighter colors (yellow and white). These findings support the hypothesis that color plays an important role in mosquito host-seeking behavior, and they provide insights into the visual preferences of these species"*

# Materials and Methods

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1. Brief, yet descriptive explanation of the experimental steps

*"We assessed the color preferences of *Ae. albopictus* and *Cx. pipiens* by conducting a binary choice behavioral assay in a flight-screened enclosure. Two BGS traps covered in different colors were placed 60 cm apart, and 25 mated female mosquitoes were released into the enclosure. The number of mosquitoes captured in each trap was recorded at various time intervals, and the Mosquito Preference Index (MPI) was calculated for each color combination"*

## 2. Enough detail to repeat the experiments

*"The mosquitoes were kept in aluminum screened wire mesh cages and maintained in an environmental chamber at  $27.0 \pm 1.0^{\circ}\text{C}$ ,  $65.0 \pm 5.0\%$  RH, with a 14:10 (L) photo regime. Traps were rotated to prevent positional bias, and four replicates were performed for each color combination. The MPI data were analyzed by two-way ANOVA followed by Tukey's HSD test"*

## 3. Statistical analyses with rationale

*"The MPI data were analyzed using a two-way ANOVA to determine whether the level of variance in mosquito responses differed significantly across colors and time. Tukey's HSD test was used to assess pairwise differences in mosquito preferences, and linear regression was used to evaluate the relationships between color brightness (L-value) and intensity (b-value)"*

# Results

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## 1. Written results section of graphical and statistical results without biological or physiological interpretation

*"Both Ae. albopictus and Cx. pipiens showed significant preferences for darker colors, particularly black and red, over lighter colors such as yellow and white. For Ae. albopictus, black had the highest MPI (0.7), followed closely by red, blue, and purple (MPIs, 0.6), while Cx. pipiens displayed a similar pattern with a strong preference for black and red"*

## 2. Statistics reported appropriately

*"Color ( $F_6 = 20.4213$ ;  $P \leq 0.0001$ ) significantly impacted mosquito response, and there was no significant color by time interaction ( $F_{18} = 0.7420$ ;  $P \leq 0.7683$ ). Linear regression analysis showed a negative relationship between mosquito preference and color brightness (L-value), with darker colors being more attractive to both species"*

## 3. Graphical results with proper labels and figure captions

*"Figures 1 and 2 illustrate the pairwise comparisons of color preferences for Ae. albopictus and Cx. pipiens, respectively. Figure 1 shows that Ae. albopictus had a significantly greater preference for black, red, blue, and purple over lighter colors. Figure 2 shows similar results for Cx. pipiens"*

## 4. Appropriate referencing of figures in the written section

*"As shown in Figure 1, the response of Ae. albopictus was significantly greater on black than on white, yellow, and green. In Figure 2, Cx. pipiens displayed a preference for black and red over white and yellow"*

## Discussion

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1. Brief summary of the experiment, in one or two sentences

*"This study assessed the color preferences of Ae. albopictus and Cx. pipiens in a laboratory setting using a binary choice behavioral assay. Our results showed that both species were more attracted to darker colors, particularly black and red, than to lighter colors"*

2. Is the hypothesis supported?

*"Yes, our hypothesis that color brightness and intensity influence mosquito host-seeking behavior was supported. Both Ae. albopictus and Cx. pipiens showed a strong preference for darker colors, with black and red being the most attractive"*

3. Provide biological and physiological rationale for the results observed.

*"The preference for darker colors observed in this study may be related to the visual systems of mosquitoes, which are adapted to detect specific wavelengths of light. Darker colors, such as black and red, may provide stronger visual stimuli for mosquitoes seeking hosts, as these colors saturate the spectral sensitivity of their vision"*

4. Relate your results to previous results from other researchers. Are they different? Are they the same?

*"Our results are consistent with previous studies that have shown mosquitoes are more attracted to darker colors. For example, Kennedy (1940) and Bidlingmayer (1994) found that Ae. aegypti preferred black and red over lighter colors. Similarly, studies have shown that darker skin tones are more attractive to mosquitoes than lighter skin tones"*

5. Experimental limitations or errors (not personnel errors, but biological ones)

*"One limitation of this study is that it was conducted in a laboratory setting using artificial lighting conditions, which may not fully replicate natural environments. Additionally, only two mosquito species were tested, and their responses may vary in the field"*

6. Future directions; what is a new experiment one would do next based on these findings?

*"Future studies should explore the interaction between color and other sensory cues, such as odor and heat, in mosquito host-seeking behavior. Additionally, testing a wider range of mosquito species and conducting field experiments would provide more comprehensive insights into mosquito color preferences"*

7. Broader impacts and implications; what is the take home message and why is it important?

*"The key takeaway from this study is that color plays an important role in mosquito host-seeking behavior, with darker colors being more attractive. This finding has practical implications for public health, as wearing lighter-colored clothing may help reduce the likelihood of mosquito bites and lower the risk of mosquito-borne diseases"*