

EFFECTS OF ACCLIMATION POSITION ON DETACHMENT OF TETHERED *CULEX QUINQUEFASCIATUS*

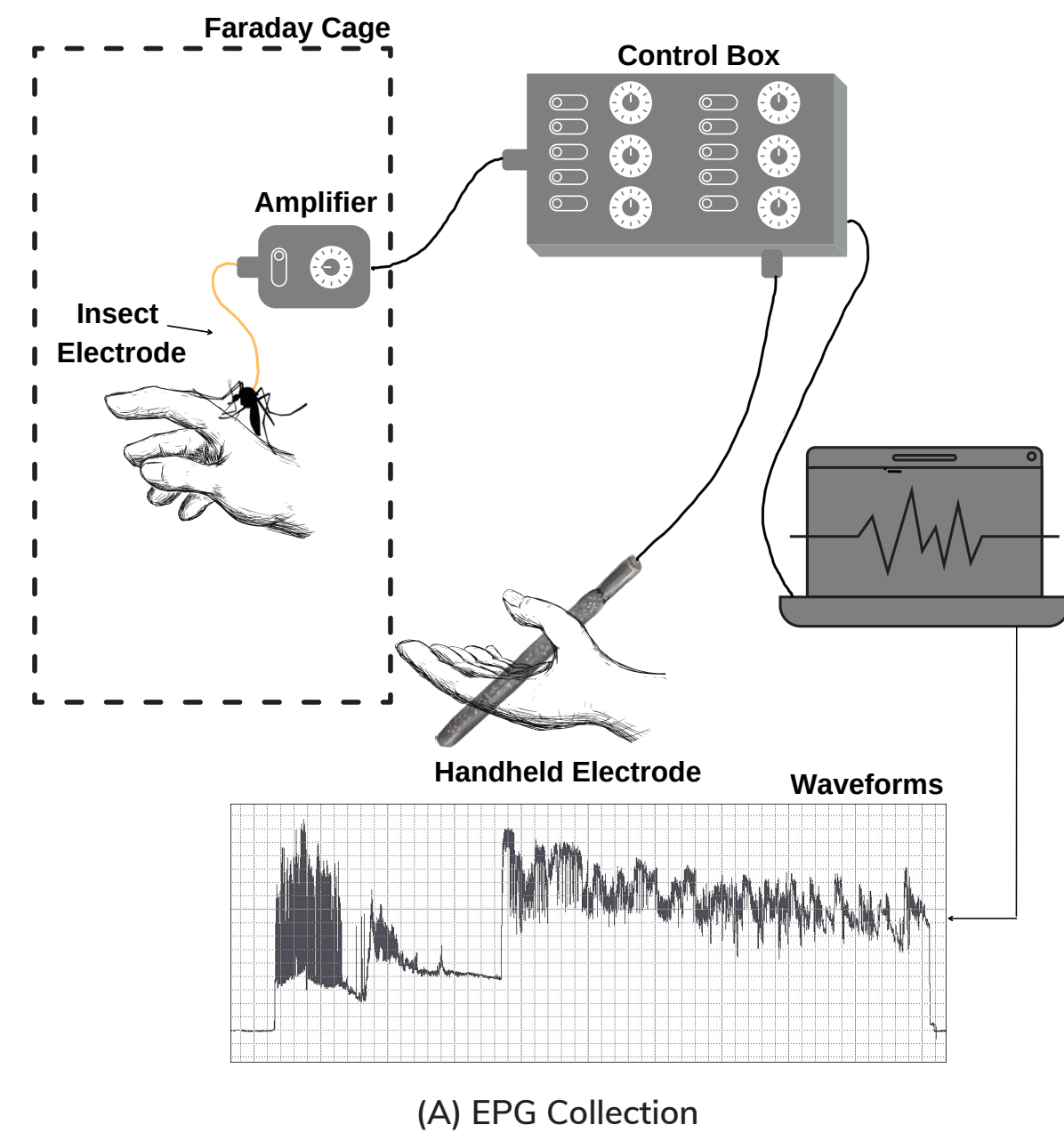
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Introduction

- Electropenetrography (EPG) allows researchers to observe, record, and quantify the feeding behaviors of arthropods. [2] EPG has been typically used to study the relationship between plants and plant-eating arthropods.
- EPG involves wiring insects into electrical circuits to visualize electrical signals associated with each insect feeding behavior. (A)
- By using EPG, researchers can understand how various factors affect arthropod feeding patterns. [1]
- *Culex quinquefasciatus* (*Cx. quinqs.*) are known vectors of viruses such as Rift Valley fever and Japanese encephalitis, which can significantly affect veterinary and human health.
- To safely study how viruses affect mosquito feeding behavior, detachment from the EPG wire must be zero. Since July 2021, the detachment rate in our studies has been 0.47%.
- Different wiring protocols have the mosquitoes either hanging or standing following the attachment of EPG wires, but it is not clear if using one method or the other results in more insect detachments.



Hypothesis

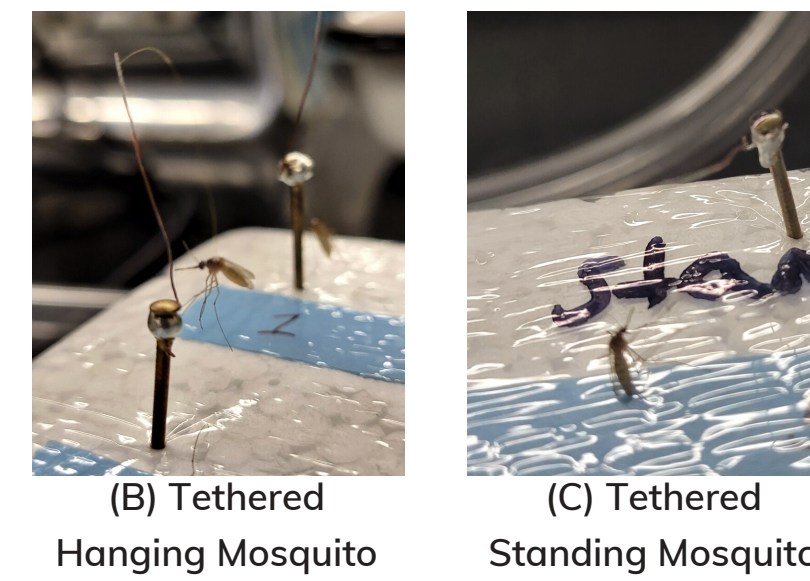
More mosquitoes will detach from their wires if they are left standing versus hanging in the storage container.

Objective

Determine if the detachment of mosquitoes from EPG wires is affected by how they are stored before and during EPG.

Methodology

- Adult female *Cx. quinqs* were used.
- Three replications with 12 mosquitos per treatment were performed. The replications were run on three consecutive days within the same time period.
- Following wiring, mosquitoes were permitted to hang from their wire (B) or stand (C).



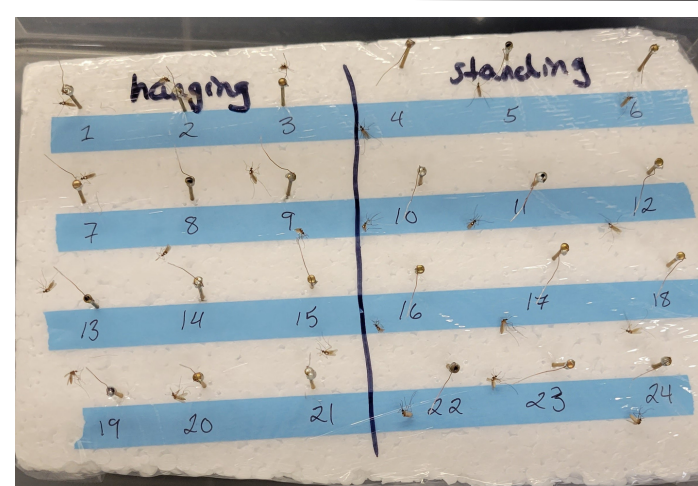
CO₂ was used to anesthetize the mosquitoes.

Gold wire was used to attach the thorax of each mosquito to a brass nail using silver glue. (D)



(D) Attaching EPG Wires

Then the *Cx. quinqs* were placed in the storage box, so 12 were hanging and 12 standing (E) and allowed to acclimate for one hour.



(E) Mosquitos in replication box

A student's t-test assuming equal variance ($\alpha=0.05$) was used to analyze the results to determine significance.

The number of insects detached from the wires was recorded after the mock EPG and the following day. Then escape rates were calculated.

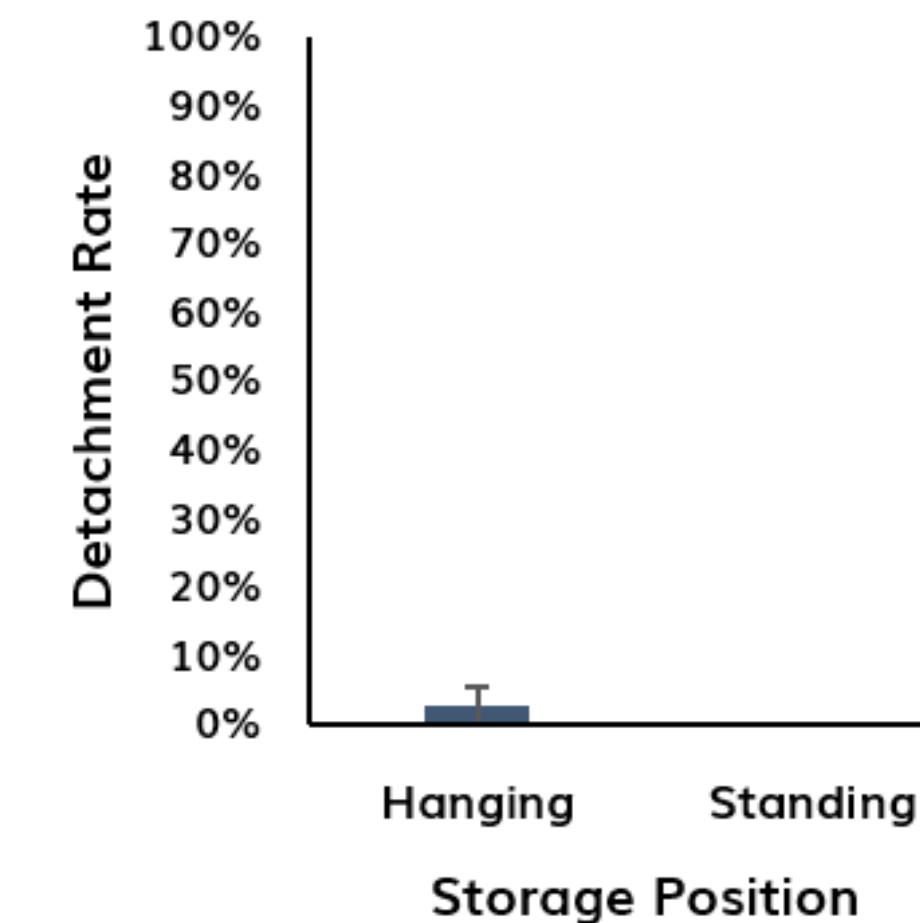
A mock EPG procedure was then performed where tethered mosquitoes were attached to the head stage amplifier using the insect stub and allowed to rest upon a human hand for 1 min before being returned to the storage box.

References

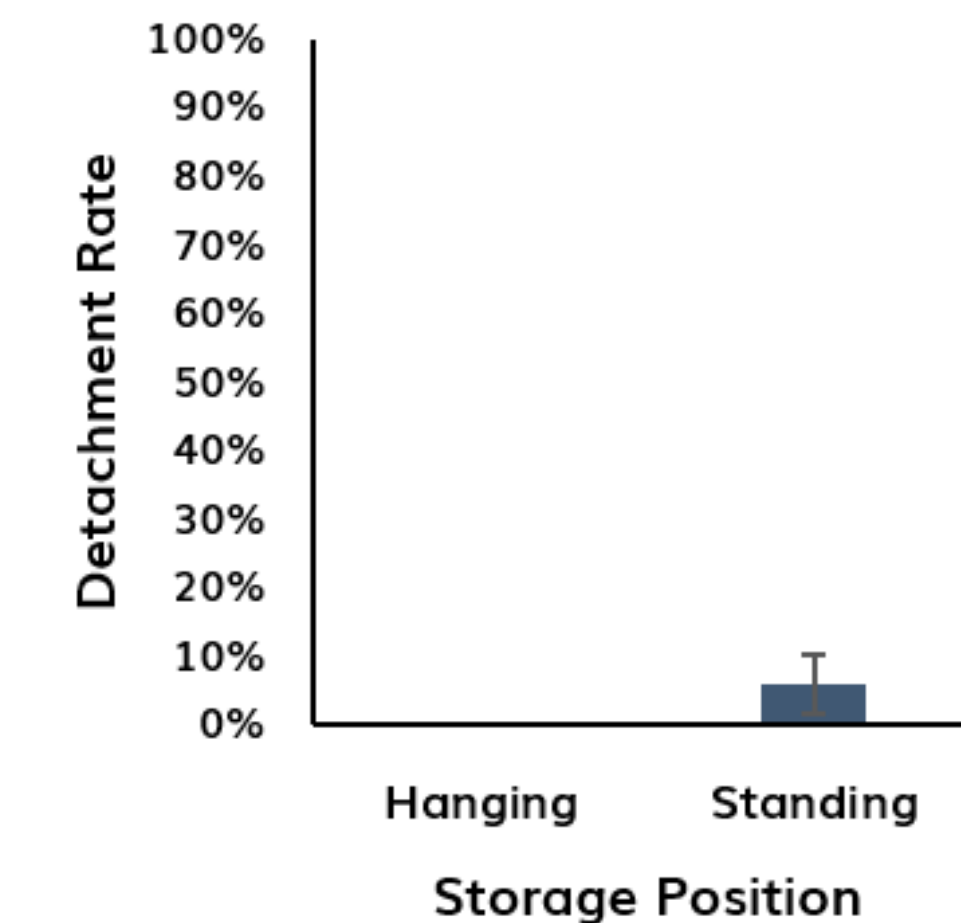
- [1] Backus, E. A., Guedes, R. N. & Reif, K. E. AC–DC electropenetrography: Fundamentals, controversies, and perspectives for arthropod pest management. *Pest Management Science* 77, 1132–1149 (2020).
- [2] Wayadande, A. C., Backus, E. A., Noden, B. H. & Ebert, T. Waveforms from stylet probing of the mosquito *Aedes aegypti* (Diptera: Culicidae) measured by AC–DC electropenetrography. *Journal of Medical Entomology* 57, 353–368 (2019).

Results

After Mock EPG



Next Day



Detachment of mosquitoes from the gold wires was not significantly different on the same [t(34)=1, p=0.32] or next day [t(31)=1.43, p=0.16] after wiring regardless of whether the mosquitoes were stored hanging or standing. However, it seemed like hanging mosquitoes tended to detach more on the first day, whereas standing mosquitoes tended to detach more on the second. Also, hanging mosquitoes appeared to be more active than their standing counterparts.

Discussion

- It was found that there is no significant difference in detachment rates based on storage, and different variables must be explored to reduce the observed detachment rates.
- Detachment rates in this study were higher than previously recorded in this lab due to a change in the wiring procedure from using a new pump.
- Strategies need to be explored to lower the detachment rate to zero, to conduct further studies using mosquitoes with viral infections safely.

Future Work

- Explore how storage position affects the biting rates.
- Investigate additional strategies to reduce the detachment rate to zero.
- Determine if hanging position affects detachment rates of other study organisms, such as *Cx. tarsalis* and *Culicoides* biting midges.

Aknowledgement

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