Characterizing mosquito blood feeding behavior using novel deep-learning based tools.

Mosquitoes are efficient vectors of a variety of human pathogens, including arboviruses (e.g. dengue virus) and parasites (e.g. *Plasmodium falciparum*) both of which are significant public health issues in South-East Asia. These pathogens are spread when an infected mosquito bites a human host to obtain a blood meal necessary for the production of eggs. Although biting behavior is essential for the spread of important human pathogens, it remains poorly understood. For instance, we lack a complete understanding of the sensory cues that drive blood feeding behavior, nor do we fully appreciate how physiological and environmental variables affect this behavior. A better understanding of the biological factors that govern the mosquito bite is not only of fundamental scientific interest, yet also has the potential to inspire novel strategies aimed at disrupting this behavior with the ultimate goal of reducing the transmission of mosquito-borne diseases.

Biting behavior of mosquitoes is typically studied through the direct observation (by a human) of mosquitoes feeding on a mammalian or artificial host. Due to its dependence on a human observer (or a human that annotates video data), this approach is very labor intensive, severely limiting the throughput at which blood feeding behavior can be studied. To overcome this limitation, we recently developed the biteOscope (Hol *et al*, eLife 2020, Murray *et al* CSHL Protocols 2023). The biteOscope consists of a host mimic that attracts mosquitoes and induces them to bite. Attracted mosquitoes blood feed on a transparent artificial blood meal enabling high-resolution imaging of the feeding mosquito. We have successfully implemented this technique to characterize the biting behavior of several arbovirus (*Aedes aegypti, Aedes albopictus*) and *Plasmodium* (*Anopheles gambiae, Anopheles stephensi*) vectors. To automate the analysis of the obtained video data, we have established a convolutional neural network to track the motion of 34 locations on the mosquito body (e.g. head, mouthparts, all leg joints) of multiple animals present in the same image. This tracking data can be used to describe the pose of a mosquito's body (i.e. the relative position of all body parts) which can be analyzed to provide a rich description of the behaviors a mosquito displays while feeding on the host mimic. This approach provides insights into the eagerness of mosquitoes to blood feed (e.g. how often do they land on the host mimic, how much time do they spend there), and the probing, biting, and engorging dynamics that lead to the uptake of a full blood meal.

In this LaMer project, we implemented the biteOscope experimental platform and deep-learning based analysis pipeline, at the CMES, Ehime University (see Fig. 1). In addition to the setup as previously published, we experimented with a new, hydrogel based bite substrate. This bite substrate has the advantageous

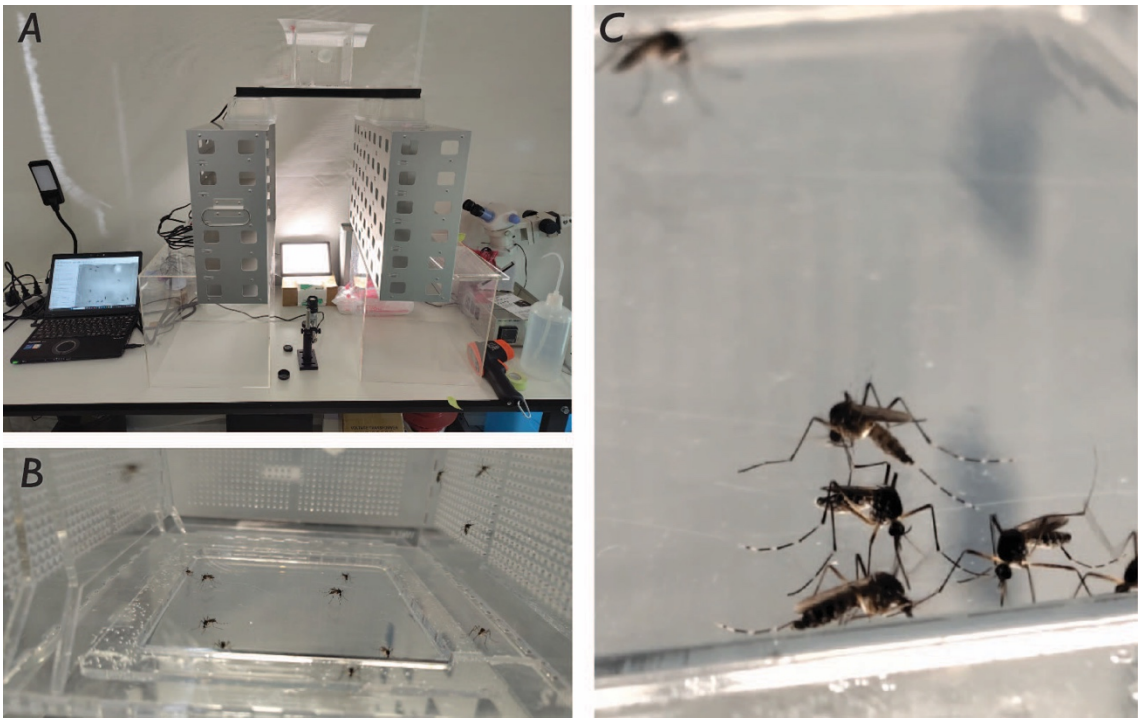

Figure 1. BiteOscope setup at Ehime university. *A)* A cage is placed on a heated window, a camera is placed below the cage. *B)* The bottom of the cage consists of a transparent bite substrate containing an artificial blood meal. *C)* Mosquitoes bite the artificial blood meal and engorge on it.

property that it can be coated with substances to determine the effects on biting behavior. We used the biteOscope setup to perform preliminary experiments to test whether components of cell culture medium can have mosquito repellant activity. Future experiments are necessary to accurately characterize this effect.

At Ehime, Associate Professor Yasutsugu Suzuki has established an *Ae. aegypti* colony that is naturally infected with an insect-specific virus (ISV) called Cell-Fusing Agent virus (CFAV). It has furthermore been shown that this virus can interfere with the transmission of arboviruses of medical importance. Using the biteOscope platform we can now investigate a potential mechanism through which insect-specific viruses could modulate arbovirus transmission: namely the impact ISVs may have on the biting behavior of mosquitoes. It is thought that arboviruses may modulate the biting behavior of infected mosquitoes, yet this possibility has never been explored for the case of ISVs. By implementing the biteOscope experimental platform at Ehime University, we will join forces and use it as a high-throughput behavioral assay to study the impact ISVs have on the biting behavior of *Ae. aegypti*.