A Time-Course Analysis of Host DNA Degradation in the mosquito *Aedes triseriatus*

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Abstract: Native and invasive mosquitoes (**Fig. 1**) are important vectors of La Crosse virus in western NC. Assessing mosquito biting behaviors is a critical step in investigating arboviral disease ecology. Thus, host DNA identification is an important tool is disease ecology. Once a host blood meal is obtained, mosquitoes produce and secrete many digestive enzymes in the midgut which facilitate the decomposition (**Fig. 2**) of blood proteins into peptides and amino acids. Digestive enzymes also degrade host DNA subsequently reducing the success of PCR amplification (**Fig. 3**) which can impede efforts to identify hosts by molecular "barcoding" methods. In this experiment, we blood-fed >150 *Aedes triseriatus* (eastern tree hole mosquito – primary La Crosse virus vector:) females on a human volunteer and removed samples (15 mosquitoes) every 12 hours for 84 hours (**Fig. 4**). A commercially available human DNA quantification kit (Applied Biosystems™ Quantifiler™ Trio DNA Quantification Kit) was used to both quantitatively and qualitatively measure host DNA degradation over time. We PCR amplified small autosomal (80 bp- **Fig. 3A**) and large autosomal (214 bp – **Fig. 3B**) targets to calculate a degradation index (DI) – a parameter used to indicate the extent of DNA degradation in a sample (**Fig. 3 C**). Our results suggest that 1) Subsequent field collections should be qualitatively scored based on the visualization of blood meal (**Fig. 2**) in 24 hour groupings so that PCR and sequence analysis success is contextualized, 2) Mixed-blood meal experiments should be completed before 24 hours after feeding, and 3) More frequent field collections (every 24 hours) should occur to ensure host identification by PCR methods.

Background: La Crosse virus (LACV) is an important cause of pediatric arboviral neuroinvasive disease in the United States and is endemic within western North Carolina (WNC). The eastern tree hole mosquito (*Aedes triseriatus*), is the primary LACV vector and highly abundant in WNC. Two invasive species (*Ae. albopictus* and *Ae. japonicus*) are likely secondary vectors and are also commonly found in WNC. Despite the medical relevance, little is known about the blood-feeding habits of *Ae. triseriatus* or the two invasive species (**Fig. 1**) in the context of LACV transmission or host availability in WNC. Recent evidence suggests that peridomestic *Ae. triseriatus* abundance is likely a function of host availability and environmental conditions – we seek to learn more about this species host preferences.

Fig. 1 - Vectors

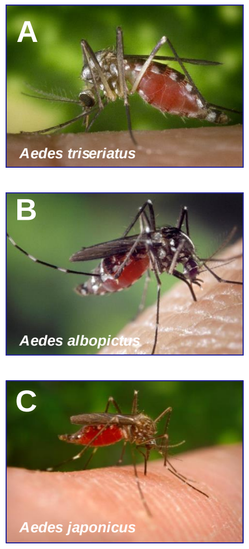

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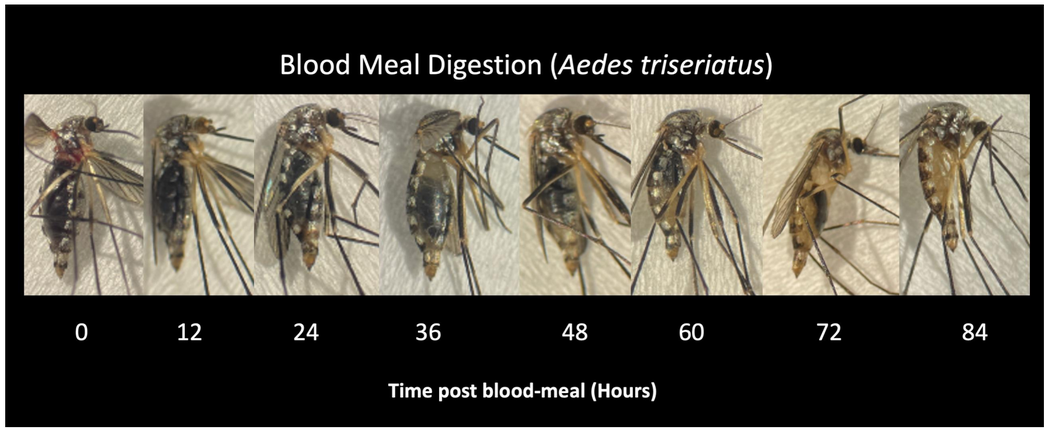
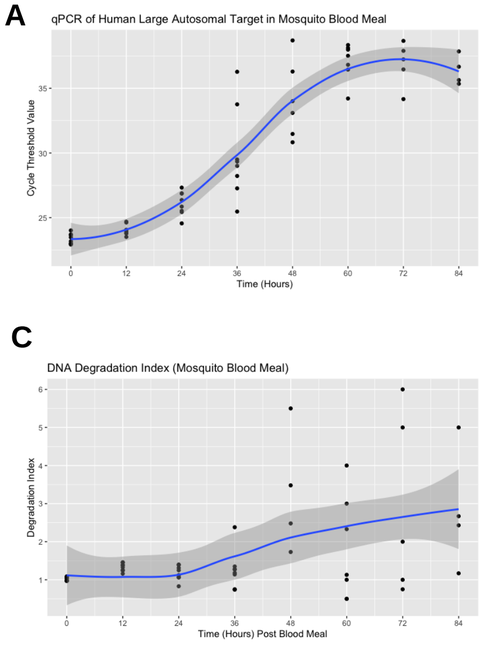
Fig. 2 - Blood meal digestion in *Aedes triseriatus* over 84 hours



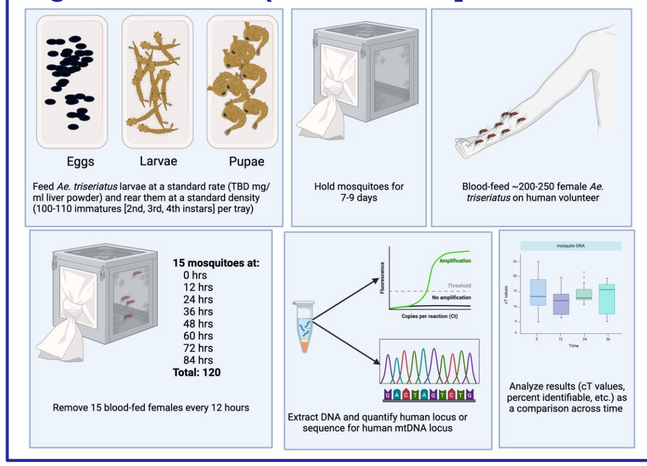
The primary La Crosse virus vector (*Aedes triseriatus*) blood meal digestion over an 84 hour time course. Mosquitoes with recent (time 0) blood meals have "fully blooded" abdomens. As the blood meal is digested, egg development becomes more pronounced. At 84 hours, the blood meal is absent (or difficult to visually detect) and the abdomen is full of eggs.

Fig. 3 - Human DNA detection (qPCR) and degradation during digestion



Both large and small human autosomal DNA targets (**3A, 3B**) were amplified from mosquitoes at time points from 0-84 hours post-blood meal. Significant variability in cycle threshold (ct) values was evident after 36 hours and as time progressed the rate of successful amplification decreased (50% at 84 hours). The degradation index (**3C**) is a ratio of small autosomal : large autosomal DNA concentration. A value of ≤ 1.0 suggests no degradation. DNA degradation was evident after 12 hours, but became highly variable after 48 hours. Plots are LOESS curves and grey shaded areas are 95% confidence intervals.

Fig. 4 – Methods (Abbreviated [Visualization])



Summary Results:

Findings: Cycle threshold values (inversely proportional to DNA template) increased after 36 hours of blood meal digestion for both the large and small autosomal human target (**Fig 3 A/B**). In addition, the rate of failed PCR attempts increased after 60 hours. We observed 50% PCR amplification failure for the large autosomal DNA target at 84 hours. A DNA degradation index (the ratio of small autosomal to large autosomal DNA concentration) was highly variable at 48 hours. Degradation was evident as early as 12 hours post blood meal.

Why does this matter? Field collected specimens contain host DNA from many different types of animals (birds, mammals, amphibians, reptiles, etc.). Field sampling may need to occur more frequently to ensure successful PCR amplification and sequencing success. In addition, a visual scoring approach (24-hour increments) based off known digestion patterns (**Fig. 2**) may help with problem solving of failed PCR amplification attempts. Finally, future experiments with mixed blood meals (two or more hosts) will need to be assayed within 24 hours to reduce the rate of amplification failures.

Next steps: Because mitochondrial DNA is more robust than nuclear DNA for blood meal analyses, we will repeat the approaches reported here with two mitochondrial loci. A series of experiments with mixed blood meal analyses and mtCOI barcoding primers will be conducted to understand the limitations of Sanger and next-generation sequencing techniques for host identification. The lessons learned from these studies will then be applied to field studies (Summer 2024).

Selected References

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