A Simple Field Assay for Insect-Borne Diseases

Alex Flores,[1] LuAnn Scott,[1,2] Sanchita Bhadra,[3] Andrew D. Ellington,[3] Holly A. Wichman[1,2]

[1]Department of Biological Sciences, University of Idaho, Moscow, ID 83844
[2]Center for Modeling Complex Interactions, University of Idaho, Moscow, ID 83844
[3]University of Texas at Austin, Austin, TX 78712

Background

- Insect-borne diseases continue to expand across a variety of geographical locations.
- Expensive instrumentation for PCR makes monitoring the spread of these diseases difficult.

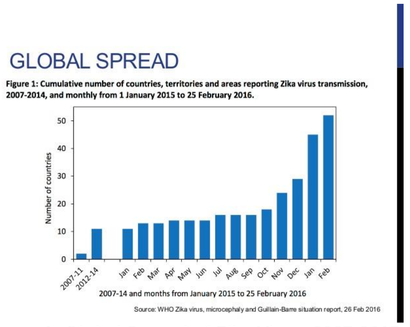

Figure 1: Global Spread of Zika Virus, 2007-2015.
Vectors: *Aedes aegypti* & *A. albopictus*

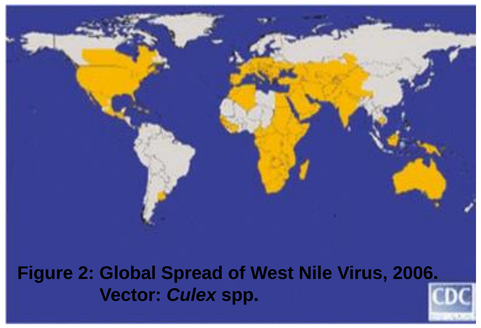

Figure 2: Global Spread of West Nile Virus, 2006.
Vector: *Culex* spp.

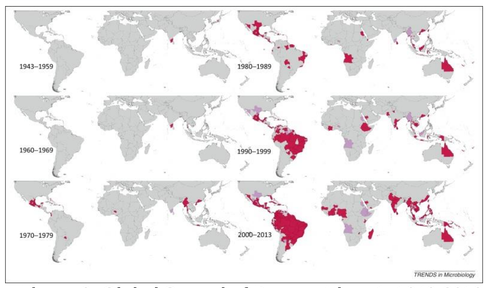

Figure 3: Global Spread of Dengue Virus 1, 1949-2013
Vector: *Aedes aegypti* & *A. albopictus*

Although *Aedes aegypti* is the primary vector of many disease-causing viruses (malaria, yellow fever, dengue, chikungunya, and zika), *A. albopictus* is providing a vector for new viral strains and has spread to all continents in the last 50 years.

Goals

1. Develop a genetic method that can type hundreds of insects quickly under field conditions.
2. Test the method in a *Drosophila* system to monitor infection and transmission in a controlled setting.
   - Develop smartphone-imaged LAMP-OSD assays to detect 2 *Drosophila* RNA viruses (DCV & DXV).
   - Determine limits of detection of fly viruses.
   - Test assay's capability to monitor a lab-based course of infection.

Acknowledgements

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Drosophila stocks are maintained by the Christine Parent lab.
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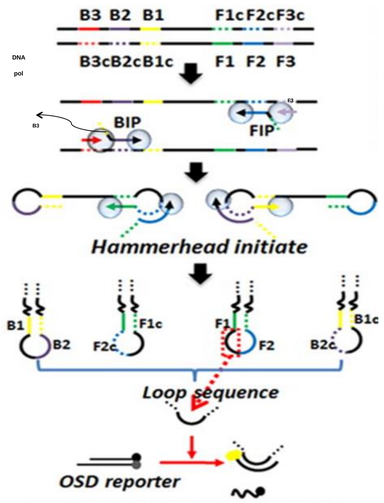
Insect images from Petey Mosquitey and MegaCatch.com.

Methods: *Drosophila* Model System

- Large populations
- Easy to grow
- Infected by injection (positive controls) or 'naturally' by oral ingestion (test flies).
- 2 RNA viruses grown in cell culture are used:
  Drosophila C Virus (DCV)
  Drosophila X Virus (DXV)
- 10 flies sampled each day over the course of infection and assayed individually.

Methods: LAMP-OSD Assay

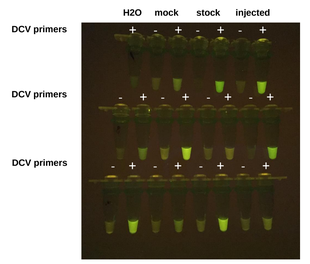
- Carefully design primers and probes.
- Amplify viral nucleic acids (loop-mediated <u>isothermal</u> amplification, LAMP).
- Transduce amplicons to visible fluorescence via unique toehold-mediated strand displacement reporters (OSD probes).



- Visualize with blue light and record readout with cell phone camera.

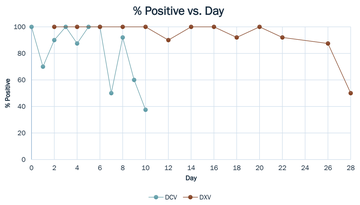
Results

- Both viruses were successfully amplified & visualized using ~10^2 copies of stock.
- Orally infected flies yield various densities of fluorescence.



<u>Raw Data – DCV day 2</u>

- All dead flies were assayed plus enough live flies to total 10 per day.
- Negative control without primers was run for each sampled fly.
- Fluorescent density of mock fly was used to determine cut-off for negative/positive result.



<u>Viral Dynamics Summary</u>

- DCV flies sampled over 10 days (blue).
  - Proportion of infected flies decreased after 6 days post-infection.
- DXV flies sampled over 30 days (red).
  - Proportion of infected flies decreased after 20 days post-infection.

Conclusions

- LAMP-OSD assays can identify the presence of virus in individual flies.
- Field studies of virus prevalence in arthropod populations can be carried out with minimal equipment: an incubator, a blue diode, and a cell phone.
- Course of infection data for both DCV and DXV is consistent with results from RT-PCR.

In Progress

- Experiments comparing LAMP-OSD and RT-PCR in detection of virus.
- Analysis of changes in viral dynamics in the presence of the *Drosophila* bacterial endosymbiont *Wolbachia*.