Investigations of Morphological Differences Between *Aedes triseriatus* and *Ae. hendersoni*

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Undergraduate Program

Vector-Borne Infectious Disease Lab
La Crosse Encephalitis

- Most common human arboviral disease in NC
- Recognized in WNC since 1964
- Infections greatly under-recognized (1:150-300)
- Disease most prevalent in children (<15 yrs)
- Western NC counties have the largest burden of LACE
- Primary research focus of the WCU Vector-borne Infectious Disease Lab

**Aedes triseriatus**

LACV Primary “natural” vector:
Eastern Tree-hole Mosquito
Sister species: *Aedes hendersoni*

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- *Aedes triseriatus* is the primary vector of LACv
- *Aedes hendersoni* is mostly incompetent for LACv transmission
- Identification of *Aedes triseriatus* and *Aedes hendersoni* is difficult
- Accurate identification is required for proper surveillance and public health efforts
- Paradigm of vertical distribution
- These two species are known to hybridize!

*Photo Credit: CDC: J.Gathany (2002)*
Surveillance of LACv in Endemic Areas

- Ground-level oviposition surveillance efforts do not collect only for *Aedes triseriatus*
- Proper identification of vectors is crucial as not to inflate the risk of transmission
- Possible hybrids also may be observed

Other vectors are observed:

- *Aedes japonicus*
- *Aedes albopictus*
- Non-vectors observed occasionally:
  - *Toxorynchites rutilus*
  - *Aedes hendersoni*
**Vertical Distribution ‘11: A Pilot Study**

### Relative Abundance 2011

- **Ae. albopictus** (43%)
- **Ae. triseriatus** (24%)
- **Ae. hendersoni** (13%)
- **Ae. japonicus** (20%)

**2011 Native vs Invasive Vertical Distribution**

- **Aedes triseriatus**: 75% of the total eggs identified were oviposited at 3 or 6 meters
- **Aedes hendersoni**: 67% of the total eggs identified were oviposited at 6 or 9 meters
- **Aedes albopictus**: 74% of the total eggs were oviposited at 3 meters or below (ground level)
- **Aedes japonicus**: 61% of the total eggs were oviposited at 3 meters or below (ground level)

Pilot Study conducted in 2011 on WCU Campus

~6,500 Aedes Eggs Collected--41% hatch rate

2,686 reared larvae identified to species

63% of identified larvae were invasive species

21% of all **Ae. triseriatus** unable to confidently ID

Photo Credit: CDC: J.Gathany (2002)
A
Elev. 2674 feet
100+ years

B
Elev. 2227 feet
80-100 years

C
Elev. 2322 feet
30-60+ years
Vertical Distribution ‘12: A Comparative Approach

49% of all *Ae. triseriatus* identified found above 0 meters! 33% of all *Ae. hendersoni* identified found at 0 meters!!

Where’s the *Aedes hendersoni*?

Overall, 33% of *Aedes hendersoni* were collected at ground level in 2012 across three different sites.

**Native vs Invasive**

- **A**
  - Native: n = 349
  - Invasive: n = 37
  - Native: 10%
  - Invasive: 90%
  - 90% (95% CI)

- **B**
  - Native: n = 665
  - Invasive: n = 379
  - Native: 36%
  - Invasive: 64%
  - 89% (95% CI)

- **C**
  - Native: n = 482
  - Invasive: n = 58
  - Native: 11%
  - Invasive: 89%
  - 64% (95% CI)

**Site**

<table>
<thead>
<tr>
<th>Site</th>
<th>Ground Level Percent (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>36.4 (26.5-47.5)</td>
</tr>
<tr>
<td>B</td>
<td>26.5 (21.2-32.4)</td>
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<tr>
<td>C</td>
<td>61.2 (47.2-73.5)</td>
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**Overall hatch rate 2011: 41.0%**

**Overall hatch rate 2012: 22.7%***

*2482 larvae identified 2012 (*Aedes*)

There appears to be site specific differences!
Vertical Distribution ‘12: A Comparative Approach

Overall hatch rate 2011: 41.0%
Overall hatch rate 2012: 22.7%
*2482 larvae identified 2012 (Aedes)

Vertical Distribution by Species and Height (2012)

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<th>Aedes triseriatus</th>
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<th>Aedes albopictus</th>
<th>Aedes japonicus</th>
<th>Taxonychites rutilus</th>
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<tbody>
<tr>
<td>A</td>
<td>50.4</td>
<td>33.2</td>
<td>62.3</td>
<td>74.4</td>
<td>30</td>
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<td>B</td>
<td>36.4 (26.5-47.5)</td>
<td>26.5 (21.2-32.4)</td>
<td>74.4 (68.9-80.9)</td>
<td>26.5 (22.2-30.8)</td>
<td>30</td>
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Vertical Distribution ‘12: A Comparative Approach

Overall, 33% of Aedes hendersoni were collected at ground level in 2012 across three different sites.

There appears to be site specific differences!

Where’s the Aedes hendersoni?

49% of all Aedes triseriatus identified found above 0 meters!
33% of all Aedes hendersoni identified found at 0 meters!!
Vertical Distribution ‘12: A Comparative Approach

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*2482 larvae identified 2012 (Aedes)*

**Native vs Invasive**

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**Vertical Distribution by Species and Height (2012)**

- **Aedes triseriatus**: 50.4%
- **Aedes hendersoni**: 33.2%
- **Aedes albopictus**: 62.3%
- **Aedes japonicus**: 74.4%
- **Taxonychites rutilus**: 30%

49% of all Aedes triseriatus identified found above 0 meters!
33% of all Aedes hendersoni identified found at 0 meters!!
Vertical Distribution ‘12: A Comparative Approach

- Overall hatch rate 2011: 41.0%
- Overall hatch rate 2012: 22.7%*

*2482 larvae identified 2012 (Aedes)

Native: n= 349
Invasive: n= 37

Native: n= 482
Invasive: n= 58

90%
10%

Aedes hendersoni

There appears to be site specific differences!

Vertical Distribution by Species and Height (2012)

<table>
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<tr>
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<tr>
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<td>(21.2-32.4)</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Aedes japonicas</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
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49% of all Aedes triseriatus identified found above 0 meters!
33% of all Aedes hendersoni identified found at 0 meters!!

Overall, 33% of Aedes hendersoni were collected at ground level in 2012 across three different sites.
Purpose of Study

• To investigate morphological differences between the larvae of *Aedes triseriatus* and *Aedes hendersoni* and their hybrids
• To determine the validity (sensitivity and specificity) of 2° characters
• Correlate morphological data with DNA identities (In Progress)
• To determine the potential for verifying hybridization between the sister species
Traditional morphological differences (e.g., acus and anal papillae; Darsie and Ward, 2005) were used to identify *Ae. triseriatus* and *Ae. hendersoni*.

Secondary morphologic characters (setae 1-S, setae 4-X and setae 1-X; Lunt et al., 1977) were used to describe differences between *Ae. triseriatus* and *Ae. hendersoni*.

- **2011**: Unable to identify 21% (n= 209)
  - Issues with 1° characters not always present for use in ID
  - Observed ‘weird’ species/ questionable
  - Investigated the utility of previously described 2° characters (Lunt)
  - Investigation of potential novel 2° characters
  - 209 specimens. +14 character states = 2926 obs.

- **2012**: Unable to identify 11% (n= 74)
  - Issues with 1° characters not always present for use in ID
  - Observed ‘weird’ species/ questionable
  - Implementation utilizing previously described 2° characters plus potential novel 2° characters showed reduction in amount identified!
  - 74 specimens. + 14 character states= 1036 obs
14 Character States Investigated

- 1-S setae Branch #’s
- 1-S setae length ratio
- 4-X # of branch pairs (ANT: 1,2,3 of branches)
- 1-X Branch #, Branch Equality (noted insertion)
- 1-X Saddle Ratio (Saddle Length vs. Setae Length)
- 6-I Branch #’s
- 3,4M Length Ratio
- 8-P Branch #’s
- 7-C Branch #’s
- 6,4-C Length Ratio
# Distal Morphology

<table>
<thead>
<tr>
<th>Characters: $2^\circ$</th>
<th><em>Ae. triseriatus</em></th>
<th><em>Ae. hendersoni</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Setae 1-S</strong></td>
<td>2 branches</td>
<td>3 branches</td>
</tr>
<tr>
<td><strong>Setae 1-X</strong></td>
<td>4-5 branches</td>
<td>2-3 branches</td>
</tr>
<tr>
<td>Setae/Saddle Ratio</td>
<td>≤1</td>
<td>&gt;1</td>
</tr>
<tr>
<td><strong>Setae 4-X</strong></td>
<td>5 Setae (origin within grid)</td>
<td>4 Setae (origin within grid)</td>
</tr>
<tr>
<td>Setae with 3-4 branches</td>
<td></td>
<td>Setae with 2-3 branches</td>
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*Vector graphics by Charles Sither*
## Distal Morphology

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Vector graphics by Charles Sither
Setae 1-X (Unknowns)

Number of Branches

All unknowns have 4 or more branches on 1-X

Consistent with Lunt (1977).
Setae 1-X (Unknowns)

1-X Saddle Ratio

86% of unknowns have saddle ratio of less than 1.0
Suggestive for ID........
Novel Species Specific PCR

- Quicker (hours vs. days)
- Simpler (less steps)
- Easier to interpret
- Less expensive
- Fewer opportunities for operator error
- Less chance of contamination
- Potentially useful to determine hybrid species

Previously published molecular identification method (Reno and Novak, 2000) is more complicated and time consuming.
Species Specific Primer Design (rDNA)

Ae. hendersoni
(CACCGAAGAGAGGGGAAA)

Ae. triseriatus
(CATCAAGAGGTTAAGG)

Conserved
(CGCGCCTGACTATCTTCAAT)

Ae. hendersoni PCR Amplicon (550 bp)

Ae. triseriatus PCR Amplicon (691 bp)

141 bp difference
### Novel Assay

<table>
<thead>
<tr>
<th>LANE</th>
<th>SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>100 bp ladder (DNA Standard)</td>
</tr>
<tr>
<td>1-2</td>
<td><em>Ae. triseriatus</em> (691 bp) amplicon</td>
</tr>
<tr>
<td>3-4</td>
<td><em>Ae. hendersoni</em> (550 bp) amplicon</td>
</tr>
<tr>
<td>5-6</td>
<td>DNA from both</td>
</tr>
</tbody>
</table>
Lanes:
1: 100 bp Ladder
2: #34
3: #34
4: #37
5: #37
6: #39
7: #39
8: #45
9: #45
10: 100 bp Ladder
11: #48
12: #48
13: #50
14: #50
15: #64
16: #64
17: #69
18: #69
19: 100 bp Ladder
20: #70
21: #70
22: #71
23: #71
24: #72
25: #72
26: #73
27: #73
28: Negative Control

Notes: PCR Amplification for all samples except #s 34, 48, and 50. No primer-dimer. No evidence of contamination.

PCR Conditions: 40 Cycles, (95-54s, 54-30s, 72-45s)
Conclusions

- *Ae. hendersoni* collected NOT only in the canopy!

- *Ae. japonicus* oviposits mostly at ground level

- Use of secondary characters increases ID success
  - We like character setae 1-X!!

- Novel PCR assay is useful for verification
  - On going work


Questions?