Validity of morphological characters used to distinguish *Culex restuans* and *Culex pipiens*

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La Crosse virus is endemic in Western North Carolina and is the major focus of our research. In particular, we are interested in the local scale ecology of three mosquitoes: the principal vector (*Aedes triseriatus*) and other potential vectors (*Ae. albopictus* and *Ae. japonicus*). Photo Credits: CDC
Undergraduate Education (Environmental Health and Biology)
Background

*Culex pipiens* and *Culex restuans* are primary vectors of West Nile virus in the eastern US

- Enzootic (avifaunal) transmission and magnification
- Primary vectors in epidemic transmission of WNV in many regions, but roles in transmission are complex and variable (Apperson 2002; Apperson 2004)
- *Cx. pipiens* is a more important vector than *Cx. restuans*
Background

Difficult to differentiate morphologically
- (NY) Harrington and Poulson 2008

Molecular ID by PCR more accurate
- Requires molecular techniques
- Resource intensive
- Contamination an issue
Research Purpose

To determine the validity of 3 morphological characters used to identify *Cx. pipiens* and *Cx. restuans*
Top of head with erect forked scales on median, and lateral areas of vertex and occiput black (Fig. 232); scutum with fine dark brown scales and usually 2 small round white spots (Fig. 233). . . . . Cx. restuans

Top of head with several erect forked scales on median area of vertex and/or occiput light tan or pale, lateral erect forked scales dark brown (Fig. 234); scutum with coarse light tan scales, without small pale spots (Fig. 235). .............................................. Cx. pikiens
"Cx pikiens complex"

Cx. quinquefasciatus
(See Note 9)
Mosquitoes collected from gravid traps (PA, VA, NC) were identified by BB using the three morphological characters. DNA was extracted using the DNAzol procedure (MRC, Inc.). PCR was analyzed as described in Crabtree et al. 1995. Data were analyzed to determine the validity of morphological characters.

Conserved Primer: 28s (CP16)

**18S**  →  **5.8S**  →  **28S**

**PQ10-CP**  →  *Culex pipiens* (698 base pairs)

**R6-CR**  →  *Culex restuans* (506 base pairs)

**Western Carolina University**
Scutal Spots Character

<table>
<thead>
<tr>
<th></th>
<th>Culex restuans (PCR ID)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Scutal Spots Present</td>
<td>30</td>
</tr>
<tr>
<td>Scutal Spots Absent</td>
<td>28</td>
</tr>
</tbody>
</table>

Sensitivity: 100%  (95% CI: 88%-100%)
Specificity: 67%   (95% CI: 56%-77%)

Although the specificity is low (67%), the sensitivity is perfect at 100%.

30 of 58 (52%) of the PCR confirmed *Culex restuans* had the dorsal spots on the scutum.

No *Culex pipiens* had these spots.

n=116
**Abdominal Band Character**

<table>
<thead>
<tr>
<th>Culex restuans (PCR ID)</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Character Present (Straight Bands)</td>
<td>45</td>
<td>9</td>
</tr>
<tr>
<td>Character Missing (Centrally Enlarged)</td>
<td>21</td>
<td>70</td>
</tr>
</tbody>
</table>

Sensitivity: 83% (95% CI: 71%-92%)
Specificity: 77% (95% CI: 70%-85%)

45 of 66 (68%) of PCR confirmed *Culex restuans* were identified with straight bands on the abdomen.

However, 70 of 79 (89%) *Culex pipiens* were identified correctly by the presence of a centrally enlarged band on the abdomen.

n=145
Head (Vertex) Character

<table>
<thead>
<tr>
<th>Character Present (Dark, erect, forked scales)</th>
<th>Culex restuans (PCR ID)</th>
<th>Culex pipiens</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>52</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>3</td>
<td>55</td>
</tr>
</tbody>
</table>

Sensitivity: 96% (95% CI: 87%-99%)
Specificity: 95% (95% CI: 86%-99%)

52 of 55 (95%) of the *Culex restuans* had dark erect forked scales on the vertex. Only 2 of 57 (3.5%) of the *Culex pipiens* were misidentified by this character.

This character is highly sensitive and specific when present. However, of the 158 mosquitoes with PCR confirmed identities, this character was only present on 112 specimens (71%).
“Traditional” Characters

Scutum

Abdomen

- *Culex restuans* morphology (Character State)
- *Culex pipiens* morphology (Character State)
“Novel” Character

Vertex

- Culex restuans
- Culex pipiens

![Graph showing count of Culex restuans and Culex pipiens](image)

- Culex restuans morphology (Character State)
- Culex pipiens morphology (Character State)
Take Home Messages

• The novel vertex character (pale vs black scales) works very well
  – Sensitivity= 96% for Cx. restuans
  – Better than the abdominal character
  – However, not always present (missing ~30% of the time)

• Scutal spots, when present, are 100% sensitive for Cx. restuans
  – Of 58 Culex restuans, 28 (48%) were missing the pale scales.
  – Specificity is low (67%); absence does not identify Cx. pipiens

• The abdominal band character appears to be the least predictive
  – Perhaps does a better job at identifying Cx. pipiens than Cx. restuans

• Public health professionals would be best served by a hierarchy of multiple characters
Take Home Messages

Culex restuans

Y

WHITE SPOTS ON SCUTUM

N

Culex pipiens

Y

PALE, ERECT, FORKED SCALES ON VERTEX & OCCIPUT

N

Culex restuans

WHITE ABDOMINAL BANDS CONTIGUOUS

Culex pipiens

WHITE ABDOMINAL BANDS NOTCHED

Y: Character Present
N: Character Absent
I: Indeterminate
Selected References


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Questions?