



Mosquito Wing Measurements Separate Potential West Nile Vectors: A Morphometric Study of Three *Culex* Species

By: Paige Robinson, Charlie Sither, and Brian Byrd

Abstract

Culex interrogator (Dyar and Knab), *Culex restuans* (Theobald), and *Culex quinquefasciatus* (Say) are three morphologically similar species with syntopic distributions. *Culex restuans* and *Cx. quinquefasciatus* are known West Nile vectors, while *Cx. interrogator* has not been reported to transmit this virus. Recent range expansions of *Cx. interrogator* has increased the need to identify morphological characters that can differentiate between these three similar mosquito species. Accurate identification is crucial to aid with West Nile virus surveillance efforts and potentially prevent misappropriation of resources or unnecessary interventions (e.g. pesticide application). The four morphological characteristics used in this study include the length and width of the whole wing, the length of the R2 cell, and the length of the R2+3 vein. We evaluated both intraindividual and interindividual differences in the three species. In conjunction with prior research (Shin et al., 2016) these characters are useful for accurate discrimination of *Cx. interrogator* from *Cx. restuans* and *Cx. quinquefasciatus*. Preliminary results suggest that wing length or an index can be used to distinguish *Cx. interrogator* from *Cx. restuans* and *Cx. quinquefasciatus*. An index comparing the ratios of wing measurements can separate 92% (n=25, 95% CI: 74.0-99.0%) of the *Cx. restuans* and *Cx. quinquefasciatus*. However, some wing character measurements overlap between species and investigators may need to rely on either different morphometric measurements or molecular methods to confirm results. When taken together, these measurements accurately identify 94.9% (n=39, 95% CI: 82.7%-99.4%) of the three species. The current species identification is based on morphology alone (non-wing characters), and will be confirmed by a species-specific rDNA PCR assay which produces amplicon size polymorphisms visible by gel electrophoresis (in progress).

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Mosquito Wing Measurements Separate Potential West Nile Vectors: A Morphometric Study of Three *Culex* species

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Mosquitoes and Approach (Fig. 1)

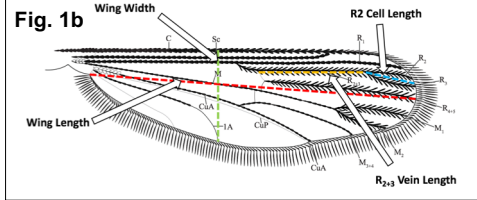


Fig. 1. Many members of the genus *Culex* remain difficult to morphologically identify, especially when damaged by collection devices such as fan-based traps. The three species we studied in this project include two known West Nile virus (WNV) vectors (*Cx. quinquefasciatus* (Fig. 1a) and *Cx. restuans*). The vector status of *Cx. interrogator* remains unknown. We used a combination of wing measurements (Fig. 1b) to evaluate potential indices that may be useful for accurately identifying the three species.

Intra-individual Variation (Fig. 2)

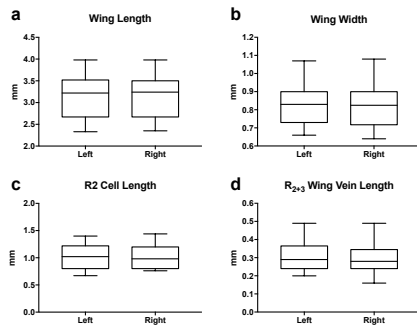


Fig. 2. To determine if there are intraindividual differences within a single specimen, we compared the wing length (a), wing width (b), R2 cell length (c), and R2+3 wing vein length between the left and right wings in 39 individual specimens. Paired comparisons (Mann-Whitney test) failed to reject the null hypothesis that there are differences between each wing ($P > 0.05$) for each series of measurements.

ABSTRACT: *Culex interrogator* (Dyar and Knab), *Culex restuans* (Theobald), and *Culex quinquefasciatus* (Say) are three morphologically similar species with syntopic distributions. *Culex restuans* and *Cx. quinquefasciatus* are known West Nile vectors (Fig 1a.), while *Cx. interrogator* has not been reported to transmit this virus. Recent range expansions of *Cx. interrogator* has increased the need to identify morphological characters that can differentiate between these three similar mosquito species. Accurate identification is crucial to aid with West Nile virus surveillance efforts and potentially prevent misappropriation of resources or unnecessary interventions (e.g. pesticide application). The four morphological characteristics (Fig. 1b) used in this study include the length and width of the whole wing, the length of the R₂ cell, and the length of the R₂₊₃ vein. We evaluated both intraindividual (Fig. 2) and interindividual differences in the three species. In conjunction with prior research (Shin et al., 2016) these characters are useful for accurate discrimination of *Cx. interrogator* from *Cx. restuans* and *Cx. quinquefasciatus* (Figs. 3 and 4). Preliminary results suggest that wing length (Fig. 3a) or an index (Fig. 4a) can be used to distinguish *Cx. interrogator* from *Cx. restuans* and *Cx. quinquefasciatus*. An index comparing the ratios of wing measurements (Fig. 4b) can separate 92% (n=25, 95% CI: 74.0-99.0%) of the *Cx. restuans* and *Cx. quinquefasciatus*. However, some wing character measurements overlap between species and investigators may need to rely on either different morphometric measurements or molecular methods to confirm results. When taken together, these measurements accurately identify 94.9% (n=39, 95% CI: 82.7%-99.4%) of the three species. The current species identification is based on morphology alone (non-wing characters), and will be confirmed by a species-specific rDNA PCR assay which produces amplicon size polymorphisms visible by gel electrophoresis (in progress).

Wing Measurements by Species (Fig. 3)

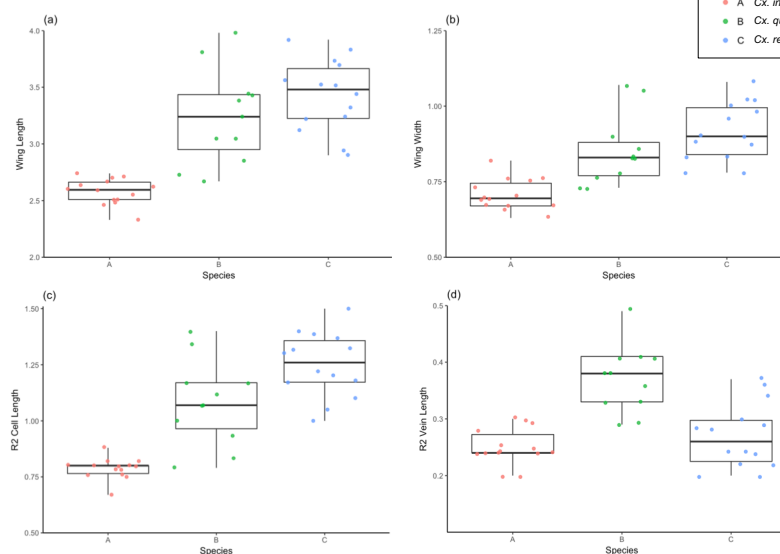


Fig. 3. Wing measurements were obtained using a stereomicroscope with a calibrated ocular reticle. Here we evaluated 39 specimens (14 *Cx. interrogator*; Species "A"; 14 *Cx. quinquefasciatus*; Species "B"; and 11 *Cx. restuans*; Species "C") and measured four morphological characteristics found on the wing. Wing lengths (Fig 3a), wing widths (Fig 3b), R2 cell lengths (Fig 3c) and the length of R2+3 vein (Fig 3d) were obtained. No single measurement accurately distinguished the four species.

Wing Indices (Fig. 4)

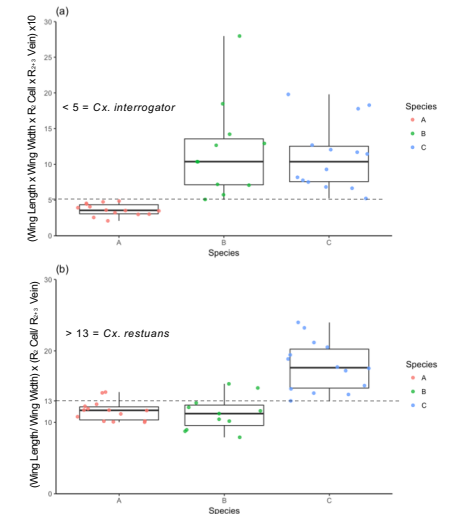


Fig. 4. Indices accurately distinguish *Cx. interrogator* (Species "A") from *Cx. quinquefasciatus* (Species "B") and *Cx. restuans* (Species "C"): a) The wing width, wing length, R2 cell and R2+3 lengths, when multiplied X 10, will distinguish *Culex interrogator* (Species "A") from the other two species. This index, when < 5 , distinguishes all *Cx. interrogator* from the other species. b) *Culex restuans* (Species "C") can commonly be distinguished (index ≥ 13) from *Cx. quinquefasciatus* (Species "B"), but not always (green dots above dashed line). This index [(Wing Length/Wing Width) X (R2 Cell Length/R2+3 Vein Length)] accurately distinguished 23 of 25 (92%) of the two species.

Conclusions/Future Directions

Conclusions: In this study we found: 1) No appreciable intraindividual variation (Fig. 2) in mosquito wing measurements, suggesting we can use either wing for measurements. 2) A single index (Fig. 4a) is useful to distinguish *Cx. interrogator* from *Cx. restuans* and *Cx. quinquefasciatus*, and 3) the two WNV vectors can be distinguished 92% (n=25, 95% CI: 74.0-99.0) of the time using a single index (Fig. 4b). Taken together, we can accurately identify 94.9% (n=39, 95% CI: 82.7%-99.4%) of the three species.

Future Directions: Our mosquitoes were originally identified using classical morphological characters often disturbed or removed during collection. Thus, our current species identification is based on morphology alone (non-wing characters), and will be confirmed by a species-specific rDNA PCR assay (in progress). We also plan to include additional specimens to increase our sample size and population diversity.

Selected References

- Harrison, B. A., Byrd, B. D., Sither, C. B., & Whitt, P. (2016). *Mosquitoes of the Mid-Atlantic Region: An Identification Guide* (Vol. 2016-01). Cullowhee, NC: WCU Mosquito and Vector-borne Disease Laboratory.
- Petersen, L. R., Braut, A. C., & Nasci, R. S. (2013). West Nile virus: review of the literature. *JAMA*, 310(3), 308-315.
- Shin, D., O'Neals, G. F., Civano, A., Shroyer, D. A., & Miqueli, E. (2016). *Culex interrogator* (Diptera: Culicidae), a mosquito species new to Florida. *J Vector Ecol*, 41(2), 316-319.

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