Abundance and Ecotypes of the *Culex pipiens* Complex in Wooster, Ohio

Emilio Pac and Dr. Ferdinand Nanfack-Minkeu, Department of Biology

BACKGROUND

- *Culex pipiens* is a key vector of West Nile Virus and other arboviruses in the U.S. (West Nile Virus).
- Morphological similarities within the *Culex pipiens* complex make species identification difficult (*Culex pipiens* - Factsheet for Experts, 2020).
- Ecotypes like *C. pipiens f. pipiens* and *f. molestus* differ in habitat and feeding preferences
- (Vezzani & Albicócco, 2009).
- Molecular techniques (e.g., PCR, RFLP) can reveal hidden genetic and ecological diversity (Smith & Fonseca, 2004).
- Understanding local *Culex* populations helps improve disease risk prediction and vector control (Brugman et al., 2018).

FIGURE 1. Reference image of a *Culex* mosquito, showing key morphological features used for identification. Image sourced from the Walter Reed Biosystematics Unit (WRBU) via Lucid Player (Mosq_Gen_Nearctic_NORTHCOM_A, n.d.).

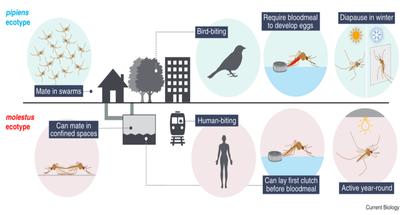

FIGURE 2. Illustration showing the two ecotypes of *Culex pipiens* found in colder regions. The pipiens type lives aboveground, while the *molestus* type is found in man-made underground places. The image highlights their main behavioral and physical differences (Haba & McBride, 2022).

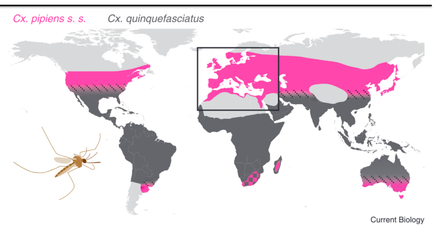

FIGURE 3. Global distribution of *Culex pipiens* sensu stricto (pink) and *Culex quinquefasciatus* (gray), adapted from Haba & McBride (2022). Areas with overlapping pink and gray dots represent hybrid zones where both species coexist and interbreed.

AIMS AND HYPOTHESIS

Aims

-Assess the abundance and distribution of *Culex pipiens* in suburban areas of Wooster, Ohio.

-Characterize phenotypic and ecotypic diversity within local *Culex pipiens* populations.

Hypothesis: Areas with less human disturbance will show higher genetic diversity due to greater environmental variability and reduced selective pressure.

METHODS

FIGURE 4. CDC gravid traps used for mosquito collection at Sites A, B, C, and D in Wooster, Ohio. Traps were placed in shaded, vegetated areas near stagnant water sources to attract gravid female mosquitoes.

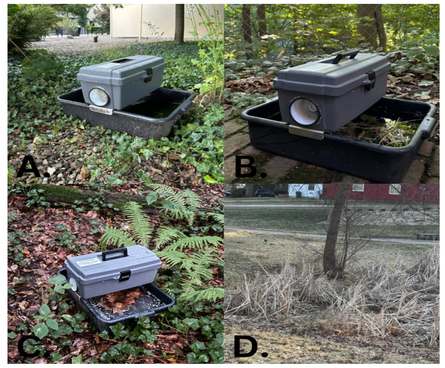

FIGURE 5. Summary of molecular workflow for identifying *Culex* species and ecotypes. Steps include DNA isolation, PCR amplification with species-specific primers, and RFLP-PCR using restriction enzymes. Key reagents and thermocycler settings are shown.

RESULTS

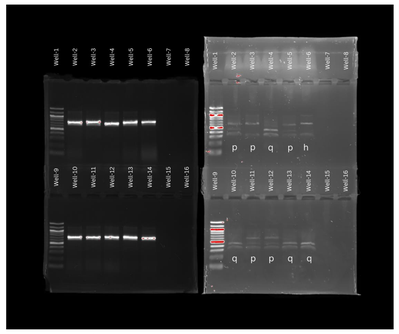

FIGURE 6. Gel Pair 1.
a. Left gel confirms all samples are *Culex* mosquitoes, showing consistent band sizes.
b. Right gel identifies species: *C. pipiens* (p) in wells 2, 3, 5, 11, 12; *C. quinquefasciatus* (q) in wells 4, 10, 13, 14; hybrid (h) in well 6. Water control (well 8) shows no contamination.

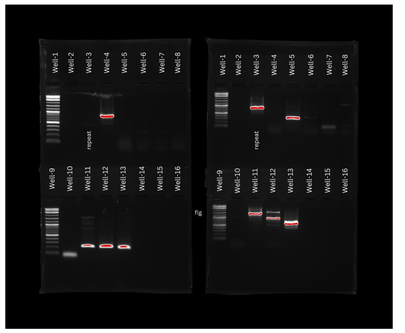

FIGURE 7. Ecotype gel.
Ladders in wells 1 and 9. *C. molestus* detected in well 5; *C. pipiens* in wells 7, 12–14. Wells 3–4 showed unclear high bands, and wells 6, 8, and 11 were not clearly visible or identifiable.

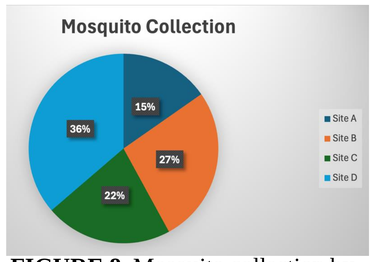

FIGURE 8. Mosquito collection by site in Wooster, Ohio. Site D had the highest catch (36%), followed by Site B (27%), Site C (22%), and Site A (15%).

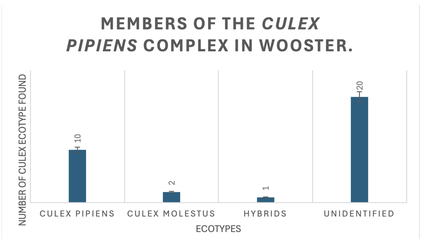

FIGURE 9. Ecotype identification of *Culex pipiens* complex via nested PCR. *C. pipiens* was most common (31.3%), with fewer *molestus* (6.3%) and hybrids (3.1%). Over half of the samples (59.4%) were inconclusive due to unclear PCR results.

DISCUSSION

- The near-equal presence of *C. pipiens*, *C. quinquefasciatus*, and hybrids mirrors patterns seen in overlap zones elsewhere, suggesting active gene flow in Wooster.
- Compared to studies like Bourguet et al. (1998), results support frequent hybridization where species coexist.
- Ecotype results, though limited, resemble findings from Algeria (Amara Korba et al., 2016), with *pipiens* dominant but *molestus* and hybrids also present.
- A high rate of inconclusive ecotype tests contrasts with clearer outcomes in similar studies, likely due to technical limitations such as low DNA purity or gel inconsistency.
- Despite these challenges, findings confirm Wooster's Culex population is genetically diverse, with public health implications similar to other hybrid zones.

Brugman, V. A., Hernández-Triana, L. M., Medlock, J. M., Fooks, A. R., Carpenter, S., & Johnson, N. (2018). The Role of Culex pipiens L. (Diptera: Culicidae) in Virus Transmission in Europe. *International Journal of Environmental Research and Public Health*, *15*(2), 389. https://doi.org/10.3390/ijerph15020389, *Culex pipiens—Factsheet for experts*. (2020, June 15). https://www.ecdc.europa.eu/en/infectious-disease-topics/related-public-health-topics/disease-vectors/facts/mosquito-factsheets/culex-pipiens, Haba, Y., & McBride, L. (2022). Origin and status of Culex pipiens mosquito ecotypes. *Current Biology: CB*, *32*(5), R237–R246. https://doi.org/10.1016/j.cub.2022.01.062, Smith, J. L., & Fonseca, D. M. (2004). Rapid assays for identification of members of the Culex (Culex) pipiens complex, their hybrids, and other sibling species (Diptera: Culicidae). *The American Journal of Tropical Medicine and Hygiene*, *70*(4), 339–345., Vezzani, D., & Albicócco, A. P. (2009). The effect of shade on the container index and pupal productivity of the mosquitoes Aedes aegypti and Culex pipiens breeding in artificial containers. *Medical and Veterinary Entomology*, *23*(1), 78–84. https://doi.org/10.1111/j.1365-2915.2008.00783.x, *West Nile Virus*. (n.d.). Retrieved April 29, 2024, from https://odh.ohio.gov/know-our-programs/zoonotic-disease-program/diseases/west-nile-virus