BRIDGING THE GAP: AN INVESTIGATION INTO THE GENETIC DIVERSITY AND LABORATORY COLONIZATION OF *AEDES JAPONICUS JAPONICUS* IN NORTHERN OHIO

THE COLLEGE OF WOOSTER

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Background

- Mosquitoes are the most dangerous disease vector in the world – malaria, dengue, West Nile virus, etc.
  - Lead to over a million deaths annually[1]
  - Continuous efforts to prevent/control spread of *Aedes* and *Culex* vectors and their arboviruses in U.S.
- Ongoing investigation into *Aedes japonicus japonicus*, the Asian bush/rock pool mosquito
- Introduced into U.S. in 1998 through used tire trade[2]
  - Outcompeting native mosquito species[2]
  - Potential disease vector[2]
- Sparked investigations into population-level genetic diversity
  - Increase in genetic diversity (haplotypes) over time[3]
  - Lack of statewide investigation in Ohio

Fig. 1 Image of *Ae. j. japonicus* from Walter Reed Biosystematics Unit, which highlight the subspecies' distinct morphological characteristics

Research Objectives

1. Laboratory colonization of of *Ae. j. japonicus*
2. Exploration of size phenotype heritability trends
3. Haplotype analysis of COI & NAD4 mitochondrial genes

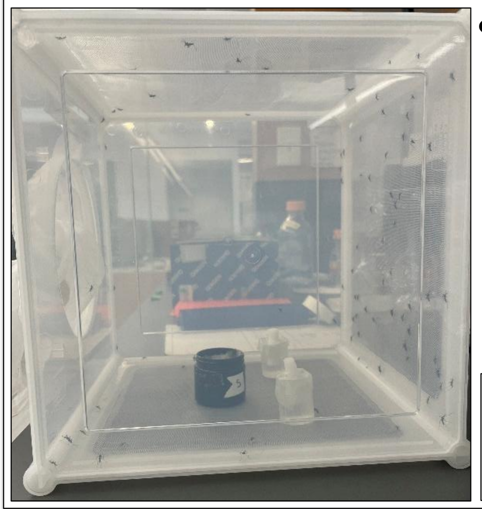
Methods

- Gravid trapping of six sites in three Ohio counties[4]

Table 1 Specific site names, locations, and trapping timeframes

Site Name	Location & County	Coordinates	Timeframe
WS	Wooster, Wayne	40.813314, -81.940379	5/24-6/14/2024
WF	Wooster, Wayne	40.819347, -81.923632	5/24-6/14/2024
CB	Pepper Pike, Cuyahoga	41.474209, -81.457545	6/16-7/7/2024
CG	Hunting Valley, Cuyahoga	41.463149, -81.436975	6/16-7/7/2024
NL	Hebron, Licking	39.933832, -82.447635	7/9-7/26/2024
NP	Granville, Licking	40.087384, -82.540485	7/9-7/26/2024

Fig. 2 Gravid trap placed at Site GG



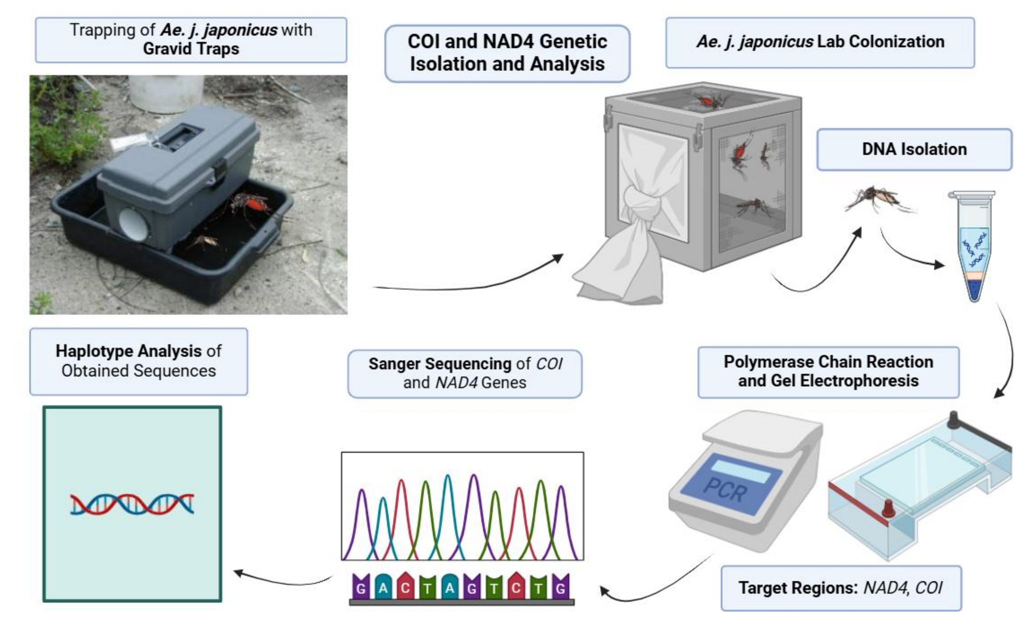
- Separation of mosquitoes into rearing cages - followed previous colonization protocols[5,6]
  - 16:8 hour photoperiod, 70-80% humidity
  - Females fed bovine blood

Fig. 3 Insect rearing cage with sugar water and oviposition (egg) cups

Methods (cont.)

- F_0 separated by size phenotype
- Larvae reared in separate basin
  - Fed TetraMin fish flakes every 2-3 days

Fig. 4 F_0 (bottom) and F_1 (top) generations inside incubator



Colonization and Size Phenotypes

- Significant difference between small and large abdomen size for both male and female mosquitoes
- Small mosquitoes appeared less phenotypically fit – died within a few days on avg.
- Increase in average bloodfeeding rate between F_0 and F_1 generation

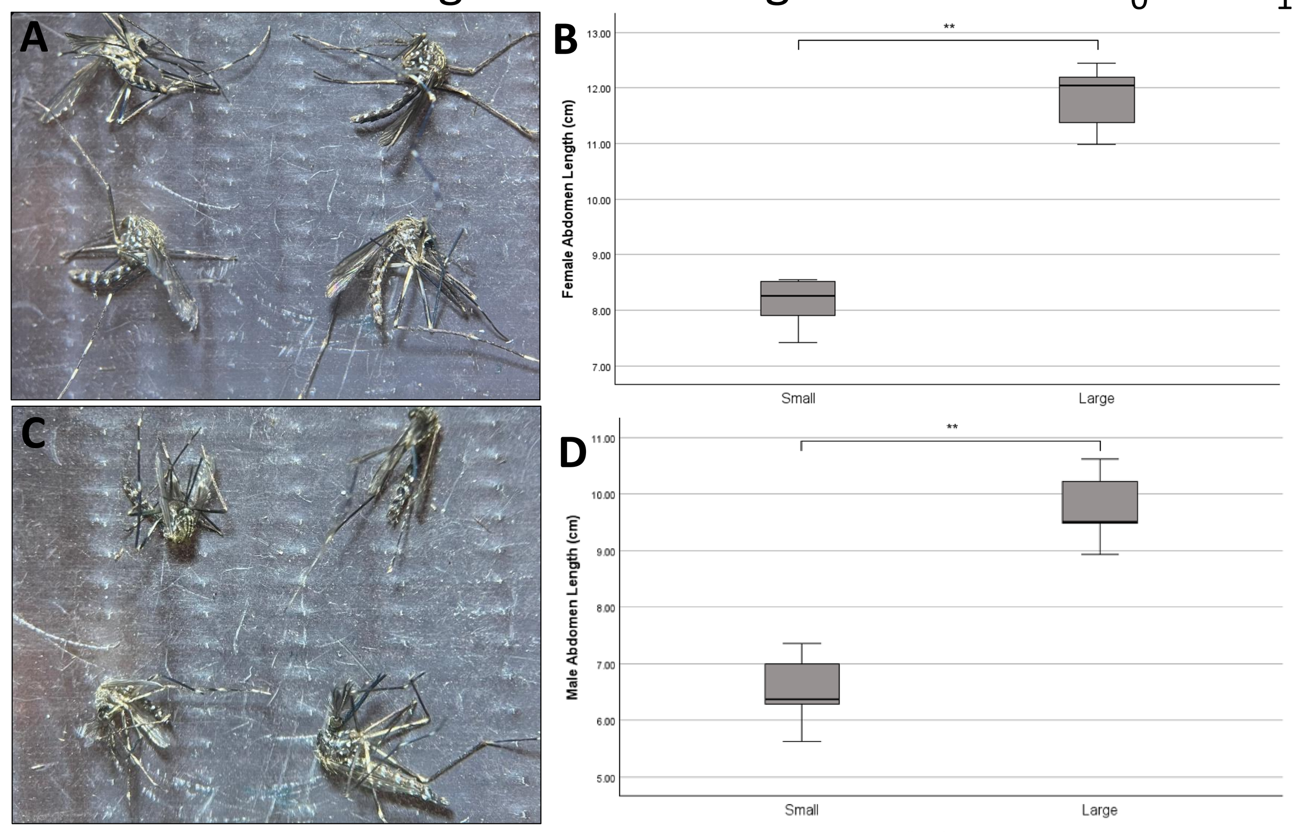


Fig. 5 Size differences between large and small mosquitoes **a)** Female visual **b)** Female abdomen length (cm) **c)** Male visual **d)** Male abdomen length (cm), ** = $p < 0.001$

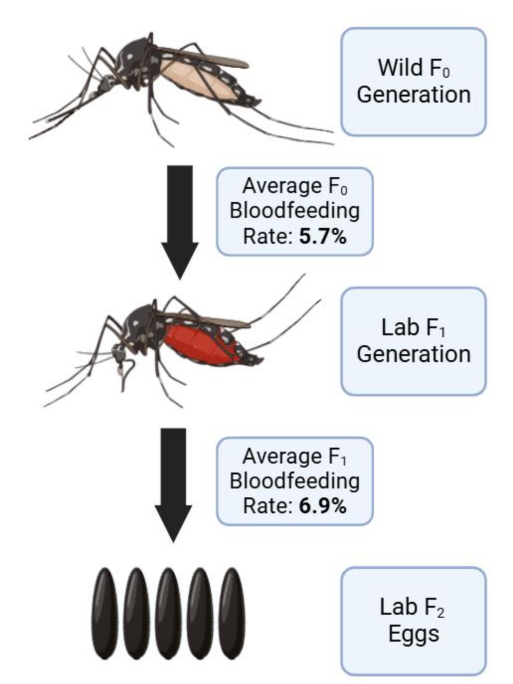


Fig. 6 *Ae. j. japonicus* generations achieved and their blood-feeding rates

- F_0 colony consisted entirely of large mosquitoes – gave rise to F_1 generation with both large and small mosquitoes
- F_2 eggs did not hatch, even after submerging in deoxygenated water

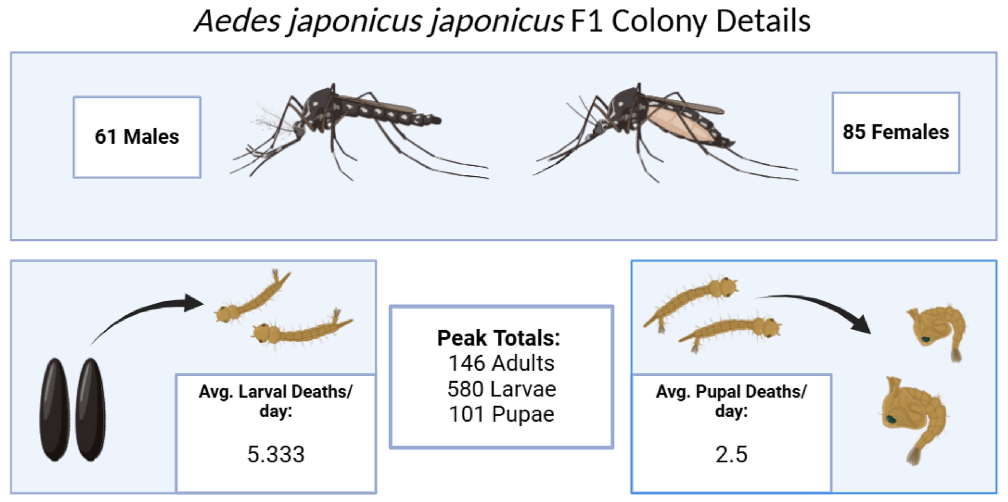


Fig. 7 F_1 generation peak totals and death rates

Mitochondrial Haplotype Analysis

- COI: six unique haplotypes
  - Hd = 0.7368, C5 matched consensus
  - 37/42 samples were high quality
  - Included 2022 COI sample
  - 4/6 present in both Cle. and Wooster
- NAD4: seven unique haplotypes
  - Hd = 0.8105, none matched consensus
  - All 18 samples were high quality
  - 4/7 present in both Cle. and Wooster

Mitochondrial Haplotype Analysis

Table 2 Haplotype distribution and frequencies compared to a consensus **a)** COI samples (n = 38) **b)** NAD4 samples (n = 18)

A

Haplotype	100	193	232	259	350	417	467	551	Freq.
Consensus	G	C	G	T	A	T	C	C	
C1	·	T	A	·	·	·	·	·	0.395
C2	·	·	·	C	G	·	·	T	0.316
C3	·	·	·	·	·	C	·	·	0.132
C4	·	·	·	·	·	·	T	·	0.079
C5	·	·	·	·	·	·	·	·	0.053
C6	A	T	A	·	A	·	·	·	0.026
									Hd: 0.7368

B

Haplotype	2	35	38	44	53	97	98	185	322	332	338	365	377	389	Freq.
Consensus	T	A	C	C	T	A	T	T	C	A	C	C	C	A	
N1	·	·	·	·	C	·	·	·	·	·	T	T	·	·	0.389
N2	·	·	·	·	·	·	·	·	·	·	·	·	·	G	0.222
N3	·	·	·	T	·	G	·	·	T	·	·	·	T	·	0.111
N4	·	·	T	·	·	·	·	·	·	G	·	·	·	·	0.111
N5	G	·	·	T	·	G	·	G	T	·	·	·	T	·	0.056
N6	·	G	·	·	·	·	C	·	·	·	·	·	·	·	0.056
N7	·	·	·	·	C	·	·	·	·	·	T	T	·	G	0.056
															Hd: 0.8105

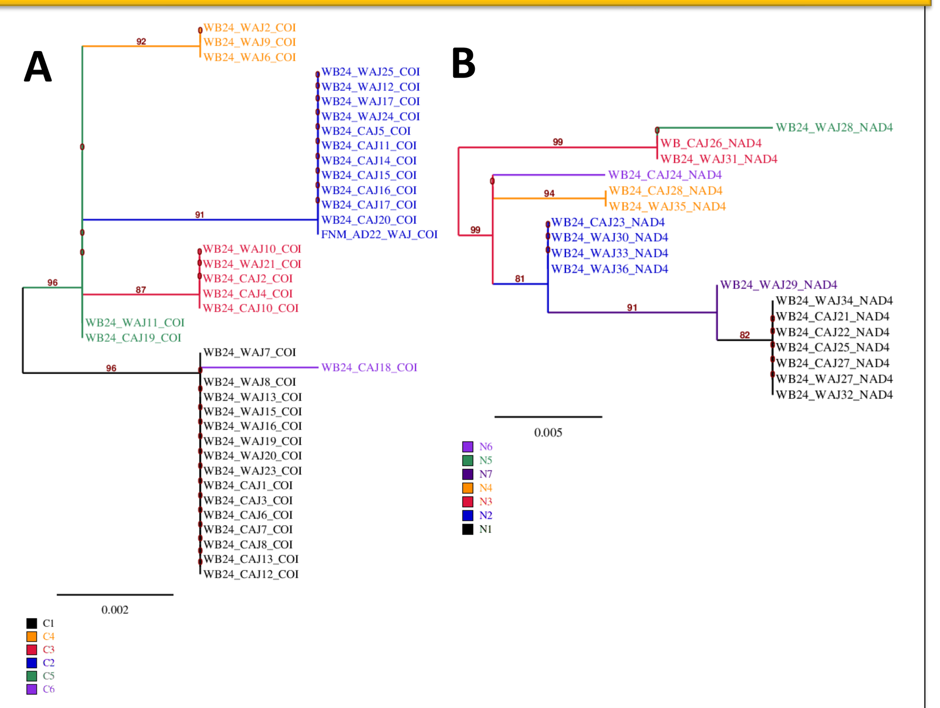


Fig. 8 Phylogenetic trees showing haplotype clusters between Wooster and Cleveland populations **a)** COI tree **b)** NAD4 tree

Trapping Data and Discussion

Table 3 Mosquito trapping data **a)** Grouped by male vs. female & size **b)** Grouped by site

A

County	Small Males	Large Males	Small Females	Large Females	*Culex spp.* (M & F)	*Ae. triseriatus*	*Ae. trivittatus*
Wooster	0	11	9	95	150	2	1
Cleveland	0	0	12	56	452	2	0
Newark	0	0	0	0	364	0	0
Total	0	11	21	151	966	4	1
							Total Mosquitoes: 1154

B

Site	*Ae. j. japonicus*	*Culex spp.*	*Ae. triseriatus*	*Ae. trivittatus*
WS	48	6	0	0
WF	22	139	2	1
CB	50	229	2	0
CG	18	223	0	0
NP	0	300	0	0
NL	0	64	0	0
Other	45	5	0	0
Total	183	966	4	1

- No *Ae. j. japonicus* collected in Newark – likely due to trapping sites
- Lower temperature (23 °C) and higher humidity (70-80%) than previous trapping protocols[5,6] – high mosquito mortality outside these conditions
- Size phenotype is likely determined through environmental factors such as diet
- Factors such as humidity swings[5,6] and larval pool microbiome[7] may have contributed to lower F_1 fitness and loss of F_2 eggs
- High genetic diversity present within genetic samples of both mitochondrial genes – NAD4 samples were more diverse despite smaller sample size

Future Works

- Sample more Ohio counties to generalize findings to statewide population
- Refine colonization protocols and achieve more generations
- Sample more mosquitoes and genes to gain a deeper understanding of haplotype diversity
- Perform more genetic analyses on samples (heterozygosity, fixation index, microsatellites)

References

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