Research in Pathogen Biology (RIP BIO)
A Course-based Undergraduate Research Experience (CURE)

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Abstract

Course-based Undergraduate Research Experiences (CUREs) involve classes of students addressing real-world research questions without pre-defined outcomes. BIOL380/381, Research in Pathogen Biology, was designed as an advanced CURE for 300-level Biology students at ODU, and examines population genetics of two ticks, *Amblyomma maculatum* (Gulf Coast tick) and *Amblyomma americanum* (lone star tick) in Southeastern Virginia. This work will compare genetic connectedness of populations of these ticks within SE Virginia, and within their historical ranges in the US southeast. The work is relevant to the broader scientific community and to the public, as both of these ticks harbor human pathogens.

Figure 1a. Male Gulf Coast tick (*Amblyomma maculatum*)
Photo credit: CDC/ Dr. Christopher Paddock/ James Gathany - http://phil.cdc.gov/ ID#10878

Figure 1b. Female lone star tick (*Amblyomma americanum)*
Photo credit: CDC/Michael Levin
http://phil.cdc.gov/ #4407

Introduction

This study focuses on population genetics of *Amblyomma maculatum* (Gulf Coast tick; Fig. 1a) and *Amblyomma americanum* (lone star tick; Fig. 1b). Both ticks can carry pathogens that can affect humans, such as *Rickettsia parkeri*, the causative agent of Tidewater spotted fever. Understanding how these ticks move and spread from their historical ranges in the US Southeast is important to understanding of the pathogens they carry.

Amblyomma maculatum distribution and movement is thought to be heavily influenced by birds, which are used as hosts by juveniles of this species. *A. americanum,* in contrast uses large mammals as hosts at all life stages. We hypothesize that these life history differences will lead to differences in population structure between the two ticks.

Tick samples were collected in southeastern Virginia at several sites, including Eastern Shore barrier islands (Hog Island, Smith Is., Cobb Is.), and Mackay Island, Fort Pickett and near Tappahannock, VA (Fig. 2). The 16S mitochondrial ribosomal gene was sequenced to generate haplotypes. Students in BIOL380 performed DNA extractions, Polymerase Chain Reaction (PCR), and DNA sequencing. These data are currently being used for population genetic analysis in BIOL381.

What is a CURE?

- CUREs offer opportunities for students to make discoveries that are of interest to stakeholders outside the classroom (e.g., the broader scientific community)
- Students' work is iterative, meaning that students must trouble-shoot, problem-solve, and repeat aspects of their work for the research to progress
- CUREs offer opportunities for students to communicate their research results to those stakeholders
- Just like in a faculty member's research group, the research in a CURE progresses as students work. This means that new research questions and directions are generated each term and the CURE is unlikely to look the same from year to year
- Students may engage in a range of science practices such as collecting and analyzing data, building and defending arguments, and collaborating with one another and more experienced scientists. The work that students do in a CURE must build off and have the potential to contribute to a larger body of knowledge in the discipline

From: https://serc.carleton.edu/curenet/whatis.html

BIOL380/381: Research in Pathogen Biology

This course is a two-semester laboratory and analysis sequence that is designed to provide a genuine research experience for undergraduate students. Modern methods in molecular biology and data analysis will be used, including DNA sequencing, and statistical and bioinformatics techniques. Students participate in writing and data analyses during these courses with a goal to publish notable findings in scientific journals.

Students help to accomplish the project goals of a current $2.5M Ecology and Evolution of Infectious Diseases Grant (NIH/NIAID; H. Gaff PI, D. Gauthier and W. Hynes, co-PIs)

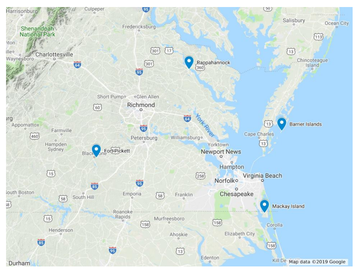


Figure 2. Map of collection sites for both *A. americanum* and *A. maculatum* in SE Virginia

Figure 3a. Range of the lone star tick (*Amblyomma americanum*), 2011
Image by the Centers for Disease Control and Prevention

Figure 3b. Range of the Gulf Coast tick (*Amblyomma maculatum*), 2014
Image by the Centers for Disease Control and Prevention

Materials and Methods

Tick Collection
- Ticks were collected via flagging vegetation at four sites (Fig. 2)

DNA Extraction
- Tick DNA was extracted and purified using silica columns (Thermo Fermentas)

PCR and Sequencing
- The 16S mitochondrial rRNA gene was amplified from tick DNA using PCR
- Amplified DNA was then sequenced using the Sanger method (Fig. 5)

Bioinformatics
- Consensus sequences were curated using the software Geneious.
- Successfully curated sequences were compared against the NCBI database to generate haplotype data (Fig. 4)

Fall 2018 Results

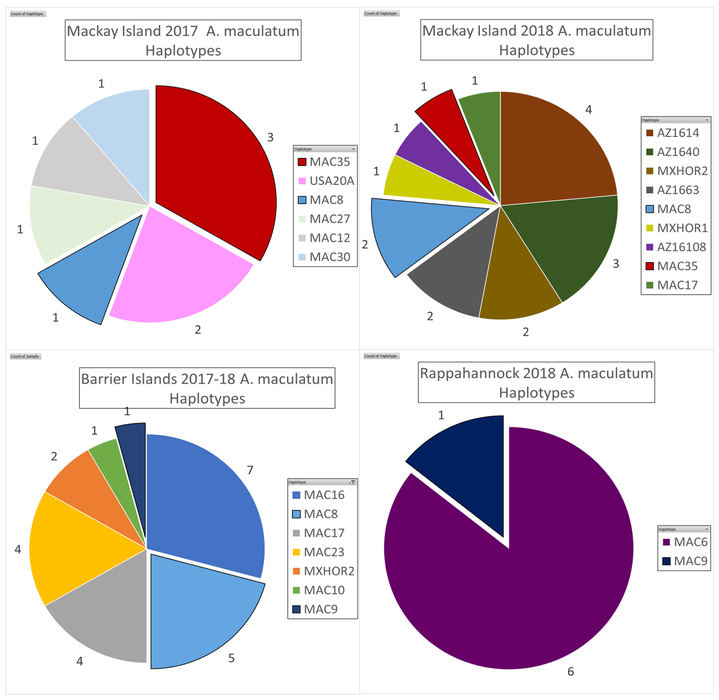


Figure 4. Final *A. maculatum* 16S haplotypes by site. Individual colors represent specific sequence haplotypes (e.g. MAC6). Exploded wedges represent haplotypes found among sites/years

Amblyomma maculatum
- 57/125 *A. maculatum* have been haplotyped. The remainder are currently being processed
- Initial results suggest significant differences in haplotype frequencies between sites, and between years at Mackay Island

Amblyomma americanum
- Most sequences obtained from *A. americanum* show ambiguous bases

Mitochondrial Heteroplasmy

- Some sequence variation observed in *A. americanum* appears to be heteroplasmy, or more than one mitochondrial genome (and haplotype) per individual
- Mitochondrial DNA is usually inherited maternally, but paternal DNA can "leak" into the zygote during fertilization (Holt et al. 1990)
- Heteroplasmy is displayed as mixtures of nucleotide bases when sequenced (Just et al. 2015). Many *A. americanum* specimens displayed an A/T heteroplasmy in the same location which is identified by the presence of identical peaks in the chromatograph (Fig. 5b)

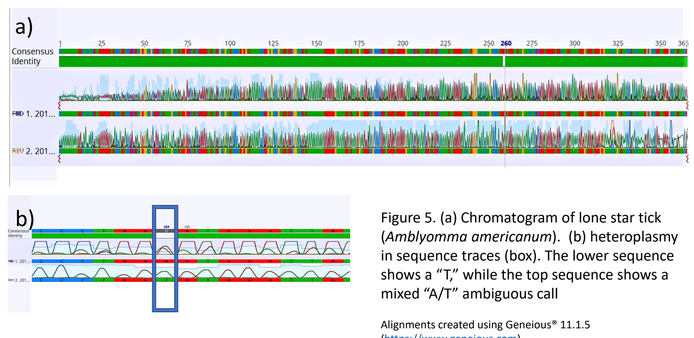


Figure 5. (a) Chromatogram of lone star tick (*Amblyomma americanum*). (b) heteroplasmy in sequence traces (box). The lower sequence shows a "T," while the top sequence shows a mixed "A/T" ambiguous call

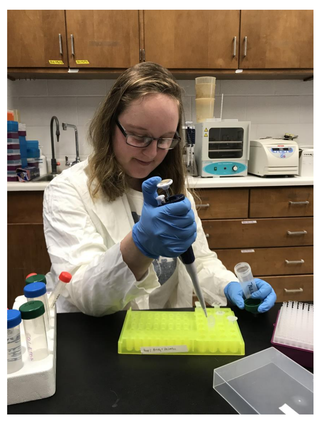
Alignments created using Geneious® 11.1.5 (https://www.geneious.com)

The remains of Tsar Nicholas II were confirmed by identifying shared heteroplasmic mtDNA in his brother, Georgij Romanov

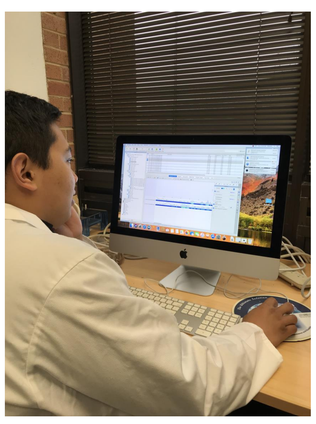
Figure 6. The Romanov family.
Image from the US Library of Congress.

Conclusions

- *Amblyomma maculatum* haplotype composition appears to vary between sampling sites, and between years within a sampling site. Unique compositions may indicate lack of connectivity between sites, and temporal instability
- Problems sequencing *A. americanum* may be due to the presence of heteroplasmy (Fig. 5) or nuclear copies of mitochondrial genes. Troubleshooting is in progress
- Ongoing work will increase the number of haplotypes characterized per site to investigate genetic differentiation between sites, and demographic stability within sites



Rebecca Ferrara performing DNA extraction



Josh Moreno using Geneious to edit and identify amplified sequences

Acknowledgments

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Literature Cited

Holt, I. J., A. E. Harding, R. K. Petty, and J. A. Morgan-Hughes. 1990. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. American Journal of Human Genetics 46: 428-433.

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