

Report of Activities
Sabbatical Leave Fall 2016
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Introduction

I applied for a sabbatical leave in Fall 2016 based on a NSF grant application that I submitted in Summer 2014. This application included funding to travel to Stockholm University to learn new techniques necessary to make progress on my collaborative research on climate change and leaf beetle population genomics. Fortunately, the application for a 3 yr research grant was funded. My sabbatical host, Dr. Christopher Wheat, and I received a supplemental grant to support my stay in Stockholm from the Wenner-Gren Foundation for 80,000 Swedish Kroner and this award supported my lodging and food costs. I was in Europe from September 3, 2016 until January 10, 2017.

A major stated goal of the NSF grant that funds this work was to 'genome-enable' our collaborative research program on this study system. New DNA sequencing technologies have revolutionized researchers' ability to study the relationship between variability across the genome and responses to environmental stress. Professor Wheat is a recognized leader in the field of genomics studies of non-model organisms (organisms that aren't already used extensively in research such as fruit flies, mice, and yeast). The purpose of my sabbatical was to learn how to interpret and analyze genomic data so that I could apply it towards answering our main research questions and train students more effectively in this area.

Activities

The main goal of the overall project is to uncover genetic mechanisms underlying adaptation to elevated temperature and hypoxia. The species we are utilizing for this is *Chrysomela aeneicollis*, the Sierra willow leaf beetle. Its range in the Eastern Sierra Nevada mountains includes variation in both latitude and elevation, providing populations experiencing differing and variable environmental conditions. We hypothesize that interactions between mitochondrial and nuclear genomes affect gene expression, which ultimately facilitates adaptation to changing environments.

Preparatory work

I worked with genomics data that were obtained using beetles were collected in the Eastern Sierra Nevada during the summer of 2013. Individuals of both sexes were sampled from four drainages: Taboose Pass, Big Pine Creek, Bishop Creek, and Rock Creek. DNA from each individual was extracted and quantified using a Qubit fluorometer. Samples were then combined at equimolar concentration for production of genetic libraries. Library preparation (200-300bp insertion size), barcoding, and full genome sequencing (Illumina HiSeq2000, 100PE) was performed by The Beijing Genomics Institute (Sacramento, CA). The resulting genomics files were cleaned and filtered for spurious sequence data and prepared for genomics analysis by Kevin Roberts and staff at Stockholm University.

Before I went to Stockholm, we had already analyzed variation in nuclear metabolic genes and along the mitochondrion of the beetles. We had also used a publically available script (METAPHLAN) to search for sequences associated with the microbiome of this species, which was previously unknown. These analyses lead my current graduate student Bo Zhang to investigate genetic variability in a bacterium that is transmitted from mother to offspring belonging to the genus *Wolbachia*. These bacteria, which infect many insects and some other invertebrates are especially interesting because they affect the reproductive biology of their hosts

and can interfere with other endosymbionts and pathogens. For example, *Wolbachia* bacteria are being used for biological control of dengue fever in Australia and considered for combatting the Zika virus in the Americas.

Techniques learned during my sabbatical leave

I learned basic commands in Unix to map DNA sequence fragments (400 bp long, with 100 bp paired end reads and a 200 bp unsequenced region in the middle) to a target DNA sequence 'contig.' I also learned how to construct a beetle genome from genomics data files and to assess the quality of the constructed genome. Further, I learned how to upload and download files to the Stockholm supercomputer and to examine sequence variability in the software programs IGV and Geneious. I then learned how to analyze population differences using the program Pooopulation and to use genomic data from databases to extract *Wolbachia* sequences from the beetle genome file using BLAST searches. Finally, I learned how to examine BLAST results to classify the sequence into different major lineages of *Wolbachia*. I am using these methods to prepare a manuscript on *Wolbachia* infection in *C. aeneicollis* and to mentor my graduate student. Since I returned from Stockholm, his progress has proceeded rapidly because I have the expertise to extract DNA sequences for use in his gene expression and genotyping analyses.

Invited seminars

I gave four invited seminars during my sabbatical leave.

1. Mitonuclear interactions influence cold and heat tolerance along elevation gradients in a montane insect, Stockholm University, Department of Zoology, Sweden.
2. Genetics and thermal physiology of a leaf beetle. Plenary presentation at annual 'Bloodbath' symposium of the Stockholm University Department of Zoology, held at the Torvetorp Research Station, Sweden.
3. □ Genetics and thermal physiology of a montane leaf beetle, Metapopulation Research Group at the University of Helsinki, Finland.
4. Mitonuclear interactions and the evolutionary ecology of a montane insect. Division of Environmental Biology, National Science Foundation.

I was a featured speaker at the 'Bloodbath' symposium. The Helsinki talk was part of a visit by Elizabeth Dahlhoff and me to the Metapopulation Research Group, where we also planned collaborative research with *Wolbachia* expert Dr. Anne Duploux. I gave the talk at NSF because I was being considered for a temporary NSF program officer position.

Publications

While I was in Stockholm, I worked on a publication for public outreach about our study system, which appeared February 2017..

Rank, N.E. and E.P. Dahlhoff. Exploring evolution by studying beetles living on the edge. *Scientia*, February 15, 2017.

<http://www.scientia.global/professor-nathan-e-rank-professor-elizabeth-p-dahlhoff-exploring-evolution-studying-beetles-living-edge/>

I also submitted a manuscript, which is still in review.

Wininger, K and N.E. Rank. Evolutionary dynamics of interactions between plants and their enemies: comparison of herbivorous insects and pathogens. *Submitted to the Annals of the New York Academy of the Sciences*.