SPU Final Grant Report[1]

Academic Year & Type of Grant: 2015 - 2016 FRG

PI Name (and Co-PI's): Jennifer Tenlen (PI) and Eric Long (Co-PI)

Original Title of the Proposal: Are Blakely Island Deer Inbred? Assessing Genetic Diversity in an Isolated Population of Black-tailed Deer.

Island populations are physically isolated from maintain populations, leading to a decrease in gene flow and increase in inbreeding among island populations. We proposed to assess the degree of genetic variation present in black-tailed deer on Blakely Island, WA as a measure of inbreeding depression. Our study aimed to extract DNA, amplify selected genetic markers, and analyze resulting products. We expected to observe low levels of diversity (higher homogeneity) among selected genetic markers, given the isolation of this island population.

This project involved contributions from the following researchers, including four SPU students: Dr. Eric Long and SPU student Cynthia Saleh (BS Ecology, 2015) conceived the project, and Dr. Long provided blood samples he has collected from Blakely Island deer since 2007. Dr. Jenny Tenlen directed the isolation and analysis of genetic material, and trained and supervised all students involved in the project. Cynthia, who was supported by a summer research stipend through the FRG, performed all DNA extraction assays and validated the microsatellite markers used in this analysis. Stephanie Nguyen (BS Physiology, 2016) and Margie Soriano (BS Applied Human Biology, 2016) earned research credit (BIO 2979) to replicate Cynthia's analysis of microsatellite markers. Stephanie Null (BS Physiology, 2016) worked as a volunteer to validate another potential genetic marker. Both Cynthia Saleh and Stephanie Null aspire to research-related careers, and this experience provided transferable skills in core molecular biology techniques, data analysis and troubleshooting experimental challenges that will help them be successful in other positions. While Stephanie Nguyen and Margie Soriano plan to continue their education in health sciences professions, their research experience also provides transferable skills in data collection and analysis that will be applicable to their future studies. Furthermore, as future health care professionals, this experience provides them with valuable insight into the types of genetic analysis that underlie our understanding of human disease and therapies.

To collect genetic material, Cynthia catalogued the collected blood samples, and created a database that recorded available information on each sample. In all, 102 samples were collected from 63 unique deer. Cynthia tested the DNA extraction protocol on 22 deer blood

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samples for which there were duplicate tubes. The first challenge encountered in this project was obtaining DNA. DNA can be obtained from white blood cells, but not red blood cells (which lack nuclei). Upon collection, blood samples were centrifuged to separate white blood cells from red blood cells. During storage, the layer containing the white blood cells coagulated to the extent that it was difficult to recover DNA. Cynthia took the initiative to research this problem, and contacted several investigators (including Dr. Rick Ridgway in the SPU Biology Dept.) for advice on extracting DNA. Unfortunately, we were unable to recover DNA from the coagulated layer. We were able to recover DNA from the red blood cell layer (which contained some white blood cells) of 22 deer. However, our yields were much lower than ideal for these experiments. In the future, blood collection could be coordinated such that DNA is recovered from the white blood cells soon after collection. Alternatively, tissue samples (such as skin clippings) could be collected for DNA extraction.

From her literature search, Cynthia identified seven unique microsatellite markers that were previously developed for mainland black-tailed deer populations. Microsatellites are regions of DNA that contain repeating elements. Deer inherit two versions (alleles) of each microsatellite, one from each parent. Microsatellite alleles differ in the number of repeating elements, and hence their length. If a deer is homozygous (having two copies of the same allele), then a single product should be visible by gel electrophoresis. If a deer is heterozygous (having two different alleles, one from each parent), then there should be two distinct products visible by gel electrophoresis. For all microsatellite markers tested, Cynthia obtained a PCR product in the majority of deer samples. One marker ("Locus M") showed considerable homogeneity, with heterozygosity observed in only 9% of deer. Conversely, for the remaining six markers, heterozygosity ranged from 22% - 70%. Furthermore, at least three alleles appear to be present in the population for some of these markers. However, we often observed more than two bands for each products for a single deer, which is unexpected. While these results suggest that genetic diversity is maintained for at least some genetic regions, the presence of unexpected bands suggests that further optimization of PCR protocols is necessary before we will be able to assign genetic profiles to each deer.

Once experimental challenges are addressed we anticipate that we will be able to move the project toward completion by incorporating elements of the project into our Biology courses. For example, students in BIO 2103 (General Biology), BIO 3310 (Ecology) or BIO 3325 (Genetics) could work to complete PCR amplification and analysis of genetic markers. Once we have reproducible genetic data for each deer, this research will be incorporated into Dr. Cara Wall-Scheffler's Evolutionary Mechanisms course. In that course, students will learn how to use standard population genetics software to assess the degree of genetic variation in the Blakely Island deer population.

The preliminary results of this study were presented by Cynthia Saleh at the SPU Summer Research Symposium in August 2015. We anticipate further dissemination of results at other conferences in in publications once we resolve the analysis of the microsatellite markers. The timeline for completion of the project will depend in part on availability of students to continue the data analysis.