Elucidating Chipmunk Parasite Evolutionary Histories

Introduction: Interactions between species vary greatly over space and time and are often are a driving force in evolutionary diversification[1]. Climate perturbations of the past have also been major determinants of ecological structure, faunal assembly, and diversification[2]. Yet, determining the roles of biotic and abiotic factors in diversification first requires a thorough understanding of the evolutionary and biogeographic history of species assemblages. Host-parasite systems, in particular, have great potential to explore coevolutionary interactions and diversification.

My dissertation research is broadly focused on a widespread clade of rodents, western North American chipmunks (genus *Tamias*), and their associated parasites. One chapter of my dissertation investigates ectoparasitic sucking lice (*Hoplopleura arboricola* and *Neohaematopinus pacificus*) that infest about 67% of individual western chipmunks[3]. Phylogenetic relationships among chipmunks suggest a complex and dynamic history produced their current diversity and distribution[4, 5, 6, 7], yet their demographic and geographic history is unknown. Chipmunks provide an excellent system to explore how abiotic factors impacted biotic distributions and range fluctuations, which in turn shape parasite associations and diversification. The groundwork for such investigations requires a thorough understanding of the evolutionary relationships among the parasite lineages and how those relationships compare to the relationships among the host lineages.

I will use genomic sequences to reconstruct phylogenetic relationships of each of the two species of louse to determine the roles of host association in genetic structuring of lice.

Hypothesis: Genetic relationships among lineages within each species of chipmunk louse reflect

long-term associations with hosts, but will also reveal past population expansion and range overlap through louse population demographic signals and host switching.

Methods: Chipmunk and parasite specimens have been collected with approved institutional animal care and use protocols (IACUC protocol # 2012-100764-MCC). All specimens are archived at the Denver Museum of Nature & Science and the Museum of Southwestern Biology. To obtain additional specimens, colleagues, mentees, and I have combed over 2000 chipmunk museum study skins. For this portion of my dissertation I have 48 *H. arboricola* and 48 *N. pacificus* on loan representing all 23 *Tamias* species in western North America.

DNA extractions, library preparation, and sequence runs will occur at the University of Illinois, Urbana-Champaign in the laboratory of Dr. Kevin Johnson. Dr. Johnson has extensive experience with DNA extractions and genomic sequencing of lice and I will use his proven lab and bioinformatics approaches. I will extract DNA from lice by grinding and digesting entire individuals and deposit high-quality images as a media voucher in each museum database for each specimen. I will use Kapa Biosystems Hyper Kits (Wilmington, MA, USA) to prepare DNA samples for sequencing on the Illumina Hi-Seq 2500. Lice will be indexed so that 12 individuals can be combined in one lane. The DNA data will be assembled against whole genomes for each species of louse. Once the genomic data are assembled, I will use a BLAST[8] search to select up to 1107 known loci for sucking lice (K. P. Johnson, unpublished) and 1510 ultra conserved element loci for arthropods[9]. The best model of DNA sequence evolution will be determined for each locus using jModeltest[10], and these models will be implemented to generate multilocus phylogenies for each species of louse in the program BEAST v.2[11]. I will compare phylogenetic relationships among louse lineages to host lineages using previously generated chipmunk phylogenies[7, 12]. This will reveal if parasite lineages are codivergeing with the hosts

and, potentially, if host switching has occurred in the past or is on-going. Within each louse species I will test for signals of stable and expanding populations (Tajima's D[13], Fu's Fs [14]) with the program Arlequin v3.5[15]. These demographic analyses provide insight into parasite population expansion and isolation and exploration of how parasites tracked hosts in the past.

Significance: Complete pictures of evolutionary histories require robust phylogenies and demographic histories, which are best obtained using markers from across the genome. While multi-locus phylogenies and genomic approaches are becoming common in many taxa, they are still costly and computationally intensive. Using a genomics approach for this project will greatly advance general knowledge of diversification of these parasites in western North America and will also expand methods for investigating intra-specific parasite diversification. Additionally, few parasite studies have sampled entire host-parasite systems across such a large geographic scale. This will permit an understanding of the evolutionary history of these lice and contribute to a greater understanding of the roles of climate and biogeographic history in driving parasite evolution. This project is one chapter of my dissertation and will also pave the way for at least two subsequent publications on chipmunk and parasite coevolution.

Finally, while working on this project I have had the opportunity to train eight UNM undergraduate students in biology field and lab methods. With funds provided by GRAC, I will be able to continue training UNM students, particularly in cutting-edge bioinformatics and phylogenetic analyses. In addition to a dissertation chapter, the results of this project will be published in a peer-reviewed journal and presented at professional society meetings.

Literature Cited

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Previous Use of GRAC Funds

I was previously awarded GRAC Research funds to generate DNA sequence data for pinworm specimens I am using for my dissertation research. I used the funds to purchase DNA extraction kits and lab consumables. With GRAC funding and other funds I procured, I was able to generate DNA sequences for 306 individual pinworms. Those data have formed the basis of my first chapter (in preparation now and will be submitted for publication this semester) and a large portion of my second chapter, which I will be presenting at the Evolution 2015 conference this summer. With those data I was able to determine that the pinworms have genetic signals of co-divergence with their chipmunk hosts but that they are also able to sometimes switch to infecting other species of chipmunks. The pinworm phylogenetic histories also revealed that the two species of pinworms have different histories associated with the chipmunks. GRAC funds enabled me to complete the data generation and analyses for a large portion of my dissertation. In addition to being very near the publication stage, these projects have been presented at five professional society conferences.

Budget

Item	Cost	Funding Source	Applied for/received
QIAamp DNA extraction kit	$162.50 each (X 2) **$325**	**BGSA Graduate Resource Allocation Committee**	**applying for here**
Bioo Scientific NEXTflex DNA Barcodes	$867	American Society of Mammalogists	received
Kapa Biosystems Hyper Library Preparation Kit	$1,800	Biology Department Gaudin Scholarship	received
Illumina Hi-Seq 2500 sequencing	$2,300 per run (X 7) $16,100	National Science Foundation Doctoral Dissertation Improvement Grant	received
		Biology Department Gaudin Scholarship	received
		American Society of Mammalogists	received
Shipping reagents and samples to Champaign, IL	**$75**	**BGSA Graduate Resource Allocation Committee**	**applying for here**

Total BGSA GRAC Request: $400.00

Budget Justification

I am requesting funds from the Biology Graduate Student Graduate Resource Allocation Committee to pay for the DNA extraction kits and the cost of shipping samples and reagents to the facility where the research will be conducted. Each Qiagen QIAamp Mini DNA Extraction kit has materials for 50 reactions and I will need two kits for all of my samples. The shipping costs are estimated from the FedEx rates for overnight shipping from Albuquerque, NM to Champaign, IL. The DNA Barcodes and Library Preparation kits are required for preparing samples to sequence on the Illumina HiSeq 2500 and the costs have been covered by funding awards from the UNM Biology Department Gaudin Scholarship and the American Society of Mammalogists Grant-in-Aid of Research. A National Science Foundation Doctoral Dissertation Improvement Grant that has already been awarded covers the majority of the costs for the sequencing on the Illumina HiSeq 2500. The funds from the BGSA Graduate Resource Allocation Committee will complete the funding needed for me to complete this project.