My time as a student and as an instructor at Duke University, and now as a lab manager at Duke's Tung Lab have provided me with the rigorous training in methods and collaborative research experience necessary for me to carry out my proposed research project: Application of Cell Based Assays to Evaluate Gene Function in the North American Gray Wolf (*Canis lupus*).

Intellectual Merit: Research Methods

I graduated from Duke University in 2011 with a B.S. in Biology and Evolutionary Anthropology with a focus on behavioral ecology with distinction. After graduation, my areas of interests within Biology expanded as a laboratory instructor for Introduction to Molecular Biology (Biology 201) at Duke for five academic years. Since May 2016, I have been applying and honing my molecular skills as a lab manager in Dr. Jenny Tung's lab in Evolutionary Anthropology at Duke.

While an undergraduate at Duke, I received a strong background in behavioral ecology and research methods through the courses of Field Primate Biology, Methods of Primate Field Ecology, and Advanced Research in Evolutionary Anthropology. With this preparation, I completed a senior thesis of original research focused on a comparative study of the behavioral thermoregulation of ringtailed lemurs (*Lemur catta*) and ruffed lemurs (*Varecia variegata* and *V. rubra*) under the mentorship of my thesis advisor Dr. Leslie Digby. I successfully secured three sources of funding for my project: Duke Deans' Summer Fellowship, a Molly Glander Award, and a Duke Undergraduate Research Support Grant. I also applied for an addition to and worked under the IACUC protocol of Dr. Digby.

My research made use of both collaborative and individual work. Along with another undergraduate student, I developed an ethogram and common protocol for recording ten minute focal follows on a handheld Psion Workabout. Together, we produced more robust definitions and criteria for each behavior for our ethogram. Our joint work allowed us to share our individually collected data on *L. catta*, expanding the data available to both of us for our respective analyses. I concluded from my research that the three species observed display patterns of behavior that shift with changes in temperature. Comparative results support that these three lemur species undergo different levels of heat stress due to coat density and color as reflected in different behavioral patterns across temperature ranges. This work provides insight into how these species respond and cope with changes in temperature, which is of particular concern as we see the mounting impacts of climate change. Additionally, my findings are of interest to researchers across disciplines as lemurs represent a branch of the primate phylogeny that retains many primitive characteristics shared by an early, primate common-ancestor, potentially providing insights into human and other primate origins.

After my graduation in 2011, I began expanding my knowledge of molecular biology to better serve my undergraduate students as an instructor for Introduction to Molecular Biology at Duke. Through this work, I gained more experience with the course's project of creating a genomic library of the budding yeast (*Saccharomyces cerevisiae*). This semester-long project includes many essential techniques such as nucleic acid isolation, molecular cloning, genetic screening, polymerase chain reaction (PCR), Sanger sequencing, and basic local alignment search tool (BLAST). During the labs I also guided students to work in groups to answer challenge problems, many of which focused on troubleshooting odd or confusing experimental results to build students' abilities to think scientifically and tackle trials encountered in independent research. My duties also included writing questions for quizzes and exams that would challenge students to apply knowledge from lecture and lab to novel situations, requiring

me to think about biologically interesting problems and what scientific skills are necessary to address them.

In May 2016, I began work at the Tung lab, where I have honed my experience with molecular techniques. In the lab, I am trained and mentored by a group of strong, female colleagues, who have inspired my ambition to join them as a woman in STEM. When I first entered the lab, I often worked side by side with these Ph.D. candidates and another lab manager to learn not only how to execute commonly used techniques in the lab such as RNA sequencing (RNA-seq), quantitative PCR (qPCR), preparation of samples for high throughput sequencing, and transfection of mammalian cells, as well as the molecular principles governing them. Now I am reinforcing my practical skills with cell lines by using *E. coli* to molecularly clone plasmids. My work also requires the maintenance and use of two commonly used human cell lines (K562 and GM12878). In addition, I have assisted another lab manager in propagating macaque peripheral blood mononuclear cells (PBMC's) and discussed best practices in infecting owl monkey kidney cells (OMK 637-69) with herpesvirus saimiri and harvesting virus from the supernatant to produce a stock for further experimentation. I have also transfected cells with a plasmid containing GFP, assessed the transfection rate by using high-end fluorescence imaging, and prepared and submitted cells for flow cytometry. The skills I have built in the maintenance, growth, and experimental procedures using varied cell lines and types will be paramount in executing my proposed research.[1]

My work at the lab has also focused on DNA methylation, including the use of bisulfite sequencing and reduced representation bisulfite sequencing (RRBS). I have been helping construct and test a plasmid for a novel assay for DNA methylation of enhancers. As this project does not follow an established protocol, the procedure presents multiple opportunities for thinking about how best to test the proper construction and function of this plasmid. Our work in developing this assay could provide a new and streamlined protocol for studying DNA methylation of enhancers—adding to the tools available to the scientific community for addressing the role of these epigenetic modifications across biological fields. Meeting the inherent challenges involved in development of this assay will undoubtedly prepare me for developing and testing an innovative assay as proposed in my graduate research plan.

Broader Impacts

Mentoring and supporting underrepresented minorities in STEM is of particular, personal interest to me. My parents are both immigrants from Latin America, and my mother dropped out of community college to support my father while he completed his bachelors. I have been left to navigate my graduate school aspirations without my family's guidance. However, I have reached the current point in my career—including applying for this fellowship—with the assistance of multiple mentors I have actively searched out during my years as a student and employee at Duke, most of whom are other women in STEM fields. Through my persistent hard work and the aid of their mentorship, I have developed the skills to address a variety of biologically important questions. I wish to continue this cycle of mentorship as a graduate student and, eventually, as a professor.

As a graduate student, I will tap into my strong teaching and communication skills that I cultivated as an instructor for Biology 201, which taught me not only how to manage large groups in a lab, but also how to communicate scientific concepts to those with little scientific background. My goal within each individual lab was for students to not only learn how to

[1] Please see the methods section of my Proposed Research essay.

physically execute techniques used in the lab, but also to understand the underlying processes. Mentoring students in Biology 201 came with challenges due to the students' wide variety of exposure to biological concepts. Among my undergraduate students, those least prepared for college-level coursework were frequently underrepresented minorities, who are often discouraged from pursuing scientific careers. To mitigate the impact of prior disadvantages in their lives, I regularly scheduled one-on-one meetings with students in addition to regular open office hours.

I also have experience communicating scientific research to the broader, general public. Since October 2014, I have volunteered as a tour guide at the Duke Lemur Center (DLC). During my tours, I inform the public about prosimian primate physiology, ecology, and behavior; conservation efforts of the DLC; and information regarding current and past research projects. My goal in speaking to these typically non-scientific groups is to improve public knowledge of the work being done at the Lemur Center and why such efforts are beneficial to the public good. Many visitors come with the notion that this work is solely for the sake of preserving these charismatic fauna, and are thus surprised to learn of research such as efforts to better understand and potentially treat Alzheimer's disease using mouse lemurs. The continuation of such vital research is contingent on the science literacy and investment of the general public. For this reason, I have committed myself to continued engagement with the public and communicating the broader impacts of scientific research.

In addition to my work as an instructor at Duke, I have held varied instructional and administrative roles for the Duke Talent Identification Program (TIP). During the summers of 2015 and 2016 I assisted with the Adventures in STEM program, which is supported by a grant to allow low-income and underrepresented minority 4th through 6th grade students to attend a free one-day course. During these programs, I designed and taught a course on Evolution and Natural Selection and served as a teaching assistant in courses ranging from Primates to Emerging Infectious Disease. I am glad to work with students at such a young age and serve as a role model for them by encouraging them to engage in curiosity throughout their lives.

My extensive experience working with youth in the sciences and the general public perfectly situates me to act as an ambassador on behalf of the molecular sciences. My proposed research project not only expands our knowledge of a new, exciting molecular technique, but also contributes to efforts to preserve wolf populations and promote environmental conservation—two goals that must be communicated to the public for their financial and political support. The recognition and financial support of a Graduate Research Fellowship would allow me to continue my engagements with the public while I pursue my Ph.D. Additionally, it would mitigate the significant price of many molecular kits and reagents, including those necessary for RNA-seq, allowing me to potentially increase the scope and impact of my proposed research. My ultimate career goal is to become a professor, which lies at the intersection of my interests in research, education, and engagement with the public. My personal experiences working with mentors, primarily also underrepresented minorities in STEM, has been a crucial element in gaining the professional experience and skills necessary to carry out my proposed research, and has driven me to serve as a mentor for the next generation of scientists.