Thank you for submitting an application for the MU-MUSE Program. Below is your submission.

ptudent Name
Major Biology (BS)
Overall GPA 3.05
GPA in Major 3.38

Faculty Mentor]
Faculty Department
Faculty MU Email

Project Title Life Cycle Regulation in the Strongyloides Clade of Nematodes
Starting Date 05/20/2019
Ending Date 08/05/2019
Other Funding for this project
Neimeyer-Hodgson

Project Abstract:
Strongyloides stercoralis, a parasitic nematode that infects humans, causes strongyloidiasis—a potentially fatal disease. Although 30-100 million people worldwide are infected, the sole chemotherapeutic is ineffective for severe forms of strongyloidiasis. Disruption of the life cycle is a potential strategy for designing new chemotherapeutics. The lifecycle of S. stercoralis is regulated by the steroid hormone dafachronic acid (DA); however, the enzyme producing this hormone in the parasite is unknown. Since work in S. stercoralis has been unsuccessful in identifying this enzyme, I aim to use a closely-related free-living nematode, Rhabditophanes, to clone the gene daf-9, which I hypothesize encodes an enzyme that synthesizes DA. In parallel, I aim to use bioinformatics tools to identify genes in four signaling pathways in the Rhabditophanes genome that I hypothesize regulate the production of DA and thus nematode development.

Attached:
Grant Uploaded
Project Narrative
Faculty Letter of Endorsement

cc: Faculty Mentor
MUSE Committee

1. PERSONAL STATEMENT

I am a Biology Major with a concentration in Molecular and Biotechnology. I began my academic career as a Biochemistry major, who wanted to graduate and get a job. Throughout my academic career at Millersville, my career goals have changed dramatically. I took Genetics in my junior year, and I fell in love with the subject. When the class was over, I decided I needed to know more and changed my major. I began research in Spring of 2018 on molecular mechanisms that control nematode life cycles. I was amazed at how creative techniques in biotechnology allowed us to study things we cannot see. I also started working as a laboratory assistant for Genetics, the class that originally sparked my interest. As a laboratory assistant I improved my molecular biology skill set by making complex solutions, culturing bacteria, and learning to avoid contamination, which is essential in molecular biology. Over the summer I wanted to keep learning, so I did an internship at the Pennsylvania Department of Agriculture in a molecular plant pathology lab. As an intern, I learned how molecular biology can be used to detect pathogens that cause agricultural and economic harm. I also collected sequencing data for over 200 clinical samples of *Phytophthora spp.*, the pathogen responsible for both the Irish potato famine and sudden oak death, for a publication at Pennsylvania State University. At my internship, I enjoyed learning real world applications of biotechnology. When I returned to school, I continued my research with nematodes, and I took Molecular Biology. During this class I decided that I wanted to pursue a career in molecular biology research and go to graduate school. Making this decision during my senior year has not left me a lot of time to get the experience I need for graduate school. This fellowship would allow me to continue learning about my passion, help me to prepare for the next step in my career, and make a contribution to the field of molecular parasitology.

2. PROJECT PROPOSAL

Background: Parasitic nematodes infect approximately one in four people worldwide (Martin et al., 2011), and the human parasitic nematode *Strongyloides stercoralis* infects by direct skin penetration. The infectious form of *S. stercoralis* is a developmentally arrested third-stage larvae (iL3), which have a hardened cuticle and lipid stores in their gut—features allowing the iL3 to survive for weeks until it finds a suitable host. When the iL3 penetrates a host, the nematode will resume feeding and will progress into adulthood (Grove, 1996). iL3s are morphologically similar to a developmentally arrested larvae, called dauer larvae, in the distantly-related free-living nematode *Caenorhabditis elegans* (Viney and Lok, 2005). Dauer larvae also have a hardened cuticle and cease feeding. Dauer larvae form in poor conditions such as low food availability and high crowding. When conditions are improved, dauer larvae develop into adults (Cassada and Russell, 1975). A hypothesis in nematode evolution is the "dauer hypothesis" where formation of iL3 and dauer larvae are controlled by the same molecular mechanisms (Crook, 2014).

The genes that control dauer formation in *C. elegans* have been extensively studied. There are four signaling pathways that regulate dauer formation in *C. elegans*: the cyclic guanosine monophosphate (cGMP) signaling pathway, the insulin- like pathway (ILP), the transforming growth factor β (TGFβ) pathway, and DAF- 12/Dafachronic acid (DA) signaling. The cGMP, ILP, and TGFβ pathways are all upstream and converge on DAF-12/DA signaling (Fielenbach and Antenbi, 2008). The mechanisms in which these pathways converge on DAF-12/DA are not well understood. In *C. elegans*, DA precursors are derived from cholesterol, and the cytochrome P450 protein DAF-9 synthesizes DA from lanthosterone (Motola et al., 2006). High DA levels bind the DAF-12 nuclear hormone receptor and promote growth to reproductive adults, while low DA levels lead to dauer arrest (Hammell et al., 2009).

While molecular mechanisms that regulate dauer formation in *C. elegans* have been identified, iL3 formation in *S. stercoralis* may be regulated via different molecular mechanisms since the two species are in evolutionarily distinct lineages (Stoltzfus et al., 2017). In *S. stercoralis,* addition of purified DA favors development into reproductive adulthood, while lack of DA favors iL3 formation. DA also stimulates iL3s to resume feeding in host like conditions (Albarqi et al., 2016; Wang et al., 2009). An outstanding question regarding life cycle regulation is whether DA is synthesized in parasitic nematodes or nematode families other than *C. elegans.*

While *C. elegans* research has provided valuable insight on mechanisms controlling developmental arrest, *C. elegans* is in a different clade (clade V) of nematodes than *S. stercoralis* (clade IV) (Stoltzfus et al., 2017). Since *S. stercoralis* is an obligate parasite, a better model for studying *S. stercoralis* would be a free-living nematode in the same clade that also exhibits developmental arrest (Blaxter et al., 1998). *Rhabditophanes* is the closest free-living relative of *S. stercoralis* (also in clade IV), and the *Rhabditophanes* genome shares a high degree of conservation in *S. stercoralis* (Hunt et al., 2016). We are currently culturing *Rhabditophanes* in our lab and we have observed dauer-like larvae in poor environmental conditions. In addition, the free-living nature of *Rhabditophanes* allows easy maintenance in the laboratory. In order to study *S. stercoralis* directly, a human or canine host would be necessary for proliferation of the nematodes, which poses obvious health risks. Using this model, we can study clade-specific mechanisms of life cycle regulation. Therefore, in summer 2019, I aim to:

1) clone a *Rhabditophanes* homolog of *daf-9* that we hypothesize produces DA, and characterize the molecular architecture of this gene.

2) identify *Rhabditophanes* homologs of genes in the cGMP, ILP, and TGFβ pathways that may regulate DA production, and compare these to *S. stercoralis* and *C. elegans*.

Methods: The central dogma of biology is that DNA is transcribed into RNA, and RNA is then translated into a protein that has a specific role in the cell. When a gene is active, there are RNA transcripts in the cell. Primary RNA transcripts are modified in a process called splicing. Non-protein-coding fragments are removed from the primary transcript (introns), and the remaining fragments (exons) are spliced to form a mature messenger RNA (mRNA) transcript. In some cases, an RNA fragment from a different genomic location is connected to the beginning of a primary transcript, which is called a splice leader. Due to specific genomic sequences, we hypothesize that *Rhabditophanes daf-9* has a splice leader.

In Fall 2018, I purified RNA from *Rhabditophanes* and constructed DNA that is complementary to all of the mRNA in the cell (cDNA), but with specific known sequences appended to each end—allowing me to identify the beginning (5') and ending (3') sequences of any mRNA. Using synthetic single-stranded DNA oligos specific to *daf-9*, I used polymerase chain reaction (PCR) to amplify many copies of the 5' end. I am currently working to insert this fragment into a piece of circular DNA (plasmid) in a process called cloning. Once this is complete, I can sequence the target DNA fragment and use this to characterize the splice leader—work I intend to complete this semester.

This summer, I will work to amplify and clone the full-length cDNA of *daf-9*. I will compare this sequence to the genomic DNA to characterize the intron and exon structure as well as the untranslated regions. I will then clone the *daf-9* transcript into an expression vector, so I can send it to a partnering university to examine the function of this gene. If *Rhabditophanes daf-9* synthesizes DA, we can confirm the conservation of DAF-12/ DA signaling in the *Strongyloides* clade of nematodes. I have received a $500 Student Research Grant and a $700 Neimeyer-Hodgson grant to support this work.

In order to determine if other upstream pathways such as cGMP, ILP, and TGFβ are also playing a role in life cycle regulation, I will use the *Rhabditophanes* genome to identify possible homologs for ~80 genes across all 4 pathways. This summer, I will use a computer software program called Geneious™ (for which we already have a license) to BLAST search the *Rhabditophanes* genome to identify homologs of these genes. I will then align predicted protein sequences and construct phylogenetic trees in order to compare *Rhabditophanes* homologs to *S. stercoralis* and *C. elegans*. This will allow me to partially

reconstruct the evolution of these pathways and identify specific genetic changes that accompanied the transition to parasitism in the *Strongyloides* clade.

Contingent upon progress made in culturing techniques by another student in the lab, this summer I may also assist in the construction of cDNA libraries containing mRNAs from different *Rhabditophanes* developmental stages. I would work to quantify the concentrations of RNA from different developmental stages, assess the integrity of these transcripts, and then add DNA oligo adapters for sequencing. Together, this technique is known as RNAseq, which would be a valuable skill for me to learn.

Expected outcomes: This summer, I aim to identify the transcriptional start site, splice leader, and exon/intron structure of the *Rhabditophanes* homolog of *daf-9*, and subsequently clone the full-length cDNA into an expression vector. This would constitute a thorough characterization of the gene structure as well as identify key regulatory elements. Additionally, I aim to identify *Rhabditophanes* homologs of genes in the cGMP, ILP, and TGFβ pathways, which I hypothesize regulate DA signaling, and together regulate development through the life cycle of *Strongyloides* clade nematodes. Together, this work will increase our understanding of molecular mechanisms regulating development in *S. stercoralis*.

3. DISSEMINATION PLAN

I plan to present my progress and research plan at Made In Millersville. Depending on the due date for abstracts, I may present this work at a regional meeting. Ultimately, we hope to use this data to publish a peer-reviewed manuscript in a leading parasitology journal, where I would be a co-author.

4. TIMELINE

Weeks 1-2: PCR amplify and purify *daf-9* cDNA; familiarize myself with using Geneious ™.

Weeks 3-6: Clone purified *daf-9* PCR product into an expression vector; sequence recombinant plasmid; begin BLAST searches of *Rhabditophanes* cGMP, ILP, and TGFβ pathway homologs using Geneious ™.

Weeks 7-9: Complete gene characterization of *daf-9*; compete BLAST searches, perform protein sequence alignments, and construct phylogenetic trees. Construct *Rhabditophanes* RNAseq libraries time- and resource-permitting.

Week 10: Complete any remaining analyses. Write detailed methods and results for work completed.

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