Neural Manipulation of Host Behavior by a Parasite

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SEMESTER REQUESTED: Spring 2021

PROPOSAL ABSTRACT:
Parasites manipulate their host's behavior to benefit themselves, which has detrimental effects on the host's ability to survive or reproduce[1]. For example, parasites inhibit behaviors that could increase host mortality, thereby minimizing the risk of the host dying before the parasite enters its next stage of development[2]. Alternatively, parasites may initiate behaviors otherwise absent from the host's usual repertoire, such as behaviors that facilitate the transport of the parasite to the next environment required to complete its lifecycle[3]. Despite its relevance to the effective biocontrol of major agricultural pests[4] and the application of how pathogens cause significant behavioral changes in animals[5] and humans[6], the mechanisms behind parasite modification of host behavior are not well-understood. Here, I propose examining whether the direct manipulation of the host's hormonal signaling[7] by the parasitic horsehair worm alters its host cricket's behaviors, specifically those that minimize predation risk and guarantee the parasite's transfer into an aquatic environment. I propose investigating two important neural signaling molecules, octopamine and serotonin, as the causative effectors of the modulation of behavior. Together with my collaborator [redacted], we will: 1) use High Performance Liquid Chromatography (HPLC) to quantify octopamine and serotonin levels in the brains of healthy and infected crickets at sexual maturity and parasite emergence, and 2) determine behavioral causality by experimentally altering the activity levels of octopamine and serotonin receptors in cricket brains via injection of pharmacological agents[13] and quantifying subsequent changes in cricket locomotor activity, courtship calling, aggression, and water-seeking behavior.

SPECIFIC AIMS & OUTCOMES FOR SPRING 2021 SABBATICAL:

Research:
- *Complete analysis of cricket brain samples at the* [redacted]
- *Complete experimental serotonin and octopamine manipulations on crickets*

Publications to submit:
- *Manuscript on insect immune priming with students* [redacted]
- *Manuscript on fluctuating asymmetry with* [redacted] *and student* [redacted]
- *Manuscript on reproductive trade-offs of parasitism with student* [redacted]

Presentations:
- *Regional Southwestern Association of Parasitology spring 2021 meeting*
- *National Animal Behavior Society summer 2021 meeting*

Grants and proposals to submit:
- *Preliminary data collection for and writing of NSF-Career or NSF-IOS*

BACKGROUND & SIGNIFICANCE
I propose examining the chemical mechanisms of how the horsehair worm, *Paragordius varius*, alters its terrestrial host cricket's behavior to minimize predation and guarantee its transfer back into an aquatic environment when it is ready to reproduce. The horsehair worm is a long-lived parasite that infects terrestrial invertebrates, including the sand field cricket *Gryllus firmus*. Previous studies have demonstrated that *P. varius* drastically reduces the amount of time male crickets engage in courtship activity, a behavior which attracts not only females but also deadly predators. Additionally, *P. varius* induces water-seeking behavior in its host when it is ready to emerge for reproduction to lay eggs in streams, resulting in *G. firmus* jumping into open water[10], a behavior that is otherwise absent in crickets and often results in drowning. Although this host-parasite interaction has received significant media attention in recent years, in part due to pet owners' concerns that their dogs will become infected if they ingest infected crickets (answer – they

won't), we still have little understanding of how host behavior is manipulated to benefit the parasite. Further, host manipulation is not unique to this system, and in fact has significant implications on human health because the parasites responsible for well-known disease such as toxoplasmosis, rabies, and sleeping sickness also alter the behaviors of infected individuals in detrimental and deadly ways. Therefore, understanding how parasites hijack host physiology and alter host behavior has important ecological and clinical ramifications on the global scale.

Here, I propose investigating two important neural signaling molecules, octopamine and serotonin, as the causative effectors of the modulation of behavior in the cricket-horsehair worm system. Octopamine plays a strong role in mediating the 'fight or flight' response and courtship behavior[11], with aggressive and courting individuals exhibiting higher levels of octopamine. Alternatively, serotonin modulates the daily circadian rhythm[12], where increases of it during daylight suppress locomotor and courtship activity at a time when predators are abundant. If horsehair worms indeed manipulate either of the chemicals in the brains of their hosts, they would be able to alter an entire suite of host behaviors to assist in their own growth, development, and emergence.

My lab has investigated the behavioral modification of the sand field cricket by the horsehair worm for the past two years, and this project will be a significant expansion of my research focus by allowing us to investigate the more proximate mechanisms responsible. We have determined from previous and current projects that infected crickets have fewer energy stores, take longer to start courtship calling when a female is present, call for less time, and have significantly lower mating success compared to healthy crickets. They also produce fewer spermatophores (i.e. sperm-containing packets that are transferred to females). This upcoming summer we intend to collect preliminary behavioral data on infected vs. healthy crickets to identify differences in aggression, locomotion, and water-seeking behavior, which will lead directly into the proposed project below.

RESEARCH OBJECTIVES, PREDICTIONS, AND METHODS

Objective 1: Identify a) whether crickets infected with parasitic horsehair worms exhibit altered levels of signaling molecules in their brains, and b) whether these altered levels align with the specific times during parasite development when the host behaviors are modified.

Prediction 1: If decreased octopamine levels mediate the reduction in aggression and courtship behaviors of infected crickets, then infected crickets will have lower levels of octopamine in their brains upon reaching sexual maturity relative to healthy crickets.

Prediction 2: If increased serotonin levels suppress locomotor and courtship behaviors in infected crickets, then infected crickets will have higher levels of serotonin in their brains upon reaching sexual maturity relative to healthy crickets, but these levels will fall below those exhibited by healthy crickets once the parasite is ready to emerge from its host.

Summary of Methods: Juvenile crickets will be infected with horsehair worms approximately two weeks prior to reaching adulthood by feeding the crickets several milligrams of snail tissue harboring infective horsehair worm cysts. When hosts approach either sexual maturity (~21 days post-infection) or parasite emergence (~28-days post-infection), cricket brains will be dissected and stored at -80 °C. Once all tissues have been collected, my student [redacted] and I, along with our collaborator [redacted], will quantify the levels of octopamine and serotonin in each sample using HPLC. [redacted] is an expert at analyzing biogenic amine levels in the preserved neural tissue of various insects and mammals, and will provide the necessary expertise to accurately quantify the levels of octopamine and serotonin in our samples.

Objective 2: Determine behavioral causality of altered neural signaling molecule levels by experimentally increasing or decreasing the activity levels of octopamine and serotonin receptors in cricket brains via injection[13] and then quantifying changes in cricket a) locomotor activity, b) courtship calling, c) aggression, and d) water-seeking behavior.

Prediction 1: If decreased octopamine levels are responsible for the reduction in aggression and courtship behaviors in infected crickets, then crickets injected with an octopamine-receptor agonist (i.e. mimics the effect of high octopamine levels) will exhibit higher rates of courtship calling and be more aggressive than control individuals. Alternatively, crickets injected with an octopamine-receptor antagonist (i.e. mimics the effect of low octopamine levels) will exhibit lower rates of courtship calling and be less aggressive than control individuals.

Prediction 2: If increased serotonin levels are responsible for the suppression of locomotor activity and courtship behaviors in infected crickets, then crickets injected with a serotonin-receptor agonist (i.e. mimics the effect of high serotonin levels) will have lower activity levels and lower rates of courtship calling. Alternatively, crickets injected with a serotonin-receptor antagonist (i.e. mimics the effect of low serotonin levels) will have higher activity levels, higher rates of courtship calling, and potentially also exhibit greater water-seeking behavior compared to control crickets.

Summary of Methods: Upon reaching sexual maturity (~10 days post-adult molt), crickets will be injected with one of the following substances directly into their head capsule: 1) chlordimeform hydrochloride (octopamine-receptor agonist, dose = 10 μL of 1 mM); 2) epinastine hydrocholoride (octopamine-receptor antagonist, dose = 10 μL of 20 mM); 3) quipzaine (serotonin-receptor agonist, dose = 10 μL of 1 mM); 4) metergoline (serotonin-receptor antagonist, dose = 10 μL of 1 mM); or 5) DMSO in insect saline (the vehicle in which the other pharmacological agents are dissolved in for injection, dose = 10 μL of 1% DMSO in insect saline). These pharmacological agents have been proven effective in altering cricket neural activity upon injection[13,14], and I have mastered microinjection of crickets using glass microcapillary needles[15]. Crickets will be tested for behavioral responses 1 hour after injection, which has proven to be enough time for the crickets to recover from handling and also allow sufficient times for the drugs to become effective. The order of the four behavioral assays will be randomized with 20 minutes of uninterrupted rest between to allow crickets to acclimate to each behavioral arena. We will use assays of locomotor activity[16], courtship calling[9], aggression[17], and water-seeking behaviors[18] that have been proven effective in previous work– detailed methods for each can be found in the references.

IMPACT ON STUDENTS, THE DEPARTMENT/COLLEGE, AND THE APPLICANT

Department/College: During my first three years at Creighton, I have set up a successful research program that expanded the repertoire of expertise our Biology program has to offer to our students and have greatly increased the number of Creighton students that are able to gain quality, first-hand research experience. I have also committed to excellence in teaching and apply innovative practices of teaching and learning in my classrooms. As such, I consistently have among the highest student evaluations of faculty in my department. This dedication to teaching excellence has slowed my research progress in some respects, and this sabbatical would allow me to wrap up several research projects, publish the findings in high-quality journals, and increase the broader impact of research being conducted in my department by increasing our visibility within the field.

Students: One of the most rewarding parts of my job includes the long-term mentorship of my research students. It provides me the opportunity to foster meaningful, personal relationships with a subset of students and guide them in their academic and professional development. In my three years at Creighton, I have mentored 13 students in research (all for a minimum of one year, some for as many as 3.5 years) and I have helped five write proposals for summer research funding (all were successful). Further, I have taken five high school students into my lab as either members of the Haddix Stem Corridor Summer Research Program or the CURAS Central HS Outreach program. I help all my students experience research from the conception of a project all the way through to publication. I encourage them to use creativity to design projects that suit their own interests, then help them seek funding, learn laboratory techniques, analyze their own data, and disseminate their results through presentations and publication. I encourage my students to attend meetings and co-present with me, prior to which I specifically mentor them on how to give effective poster

and oral presentations. These opportunities allow my students to network with other experts in the field and connect with potential future mentors for graduate work. Collectively, my students have given nine off-campus & 26 on-campus presentations and have won six competitive awards for their posters and talks. In regard to publications, I take an approach of mentoring my students to write up their research as first authors. This takes more time, but the result is that students end up with a greater sense of ownership over their project, and I ensure they have received one-on-one training on how to effectively write in a scientific voice. Beyond activities directly related to research, we hold weekly lab meetings to share project updates, read/discuss relevant articles, and provide constructive criticisms on upcoming presentations or publications. This facilitates a strong sense of community in my lab that I take great pride in maintaining. Most importantly, I foster a positive lab environment where all of my students feel welcome and respected by everyone. I encourage this by coordinating social lab events with my research students throughout the year to celebrate their progress and hard work. Two important take-aways from my lab are: 1) although there may be a hierarchy of experience in a given workplace, everyone deserves to be treated with respect and dignity at all times, and 2) fostering an inclusive supportive environment with strong personal relationships increases overall productivity and students should seek out labs, programs, schools, and/or jobs that prioritize this as a foundation of their mission.

Applicant: My lab has investigated host physiological and behavioral modifications by parasites for the past two years, which is a significant expansion of my previous research interests. Therefore, support for this project will not only help to prepare me for an advancement in rank and tenure, but it will have a significant impact on my competitiveness to obtain external research grants. Specifically, this opportunity will facilitate the collection of vital preliminary data, allow me to learn new research techniques, provide me time to mentor undergraduates in publishing their research as first authors in high-quality peer-reviewed journals, and afford me time to train a new cohort of undergraduate research students that will be able to further expand my research program. In addition to this opportunity, I am concurrently applying for two grants that would have significant synergistic effects with this sabbatical. Funding from Creighton's 2020 Summer Faculty Research Grant would guarantee summer support for one of my research students, and The Woodrow Wilson Course Hero Fellowship for Excellence in Teaching would support a full-time research assistant in my lab for the year of my proposed sabbatical. Both opportunities would increase my research productivity in the time directly leading up to or overlapping with my sabbatical.

PLANS FOR DISSEMINATION

I regularly attend and present my research at local, regional, and national conferences, as evidenced by my attendance at four national conferences and numerous local/regional meetings in the three years that I have been at Creighton. As mentioned above, I encourage my students to attend and co-present with me at these meetings, and I help them seek funding to fully cover their travel expenses. In addition to attending conferences, I have been invited to three different universities to present seminars on my research since joining Creighton and continue to strive to publish my research in high-quality peer-reviewed journals. Publication of my work at Creighton has been slow due to my strategy of mentoring my students to write up their research as first authors. While this takes considerably more time than writing the manuscripts myself, I feel it is imperative for their development to gain these skills. If awarded this sabbatical, I would not only be able pursue a novel project that my students and I will publish upon its completion, but I will also have the time to provide quality mentorship to my other students for the publication of three additional projects.

EVIDENCE OF SERVICE TO THE DEPARTMENT, COLLEGE, AND UNIVERSITY

As both a Biology and EVS Program core faculty member, I have been advising an average of 12 students each semester. In addition to this form of mentoring, I take pride in writing high-quality, personalized letters of recommendation for my students applying to post-graduate programs, and collectively I have written 120 letters since Spring of 2017. I have also written three letters of

affirmation for students attending [redacted] retreats in which they listed me as their favorite professor, and this past spring I volunteered to draft our department's nomination for the CAS senior award. Additional departmental service includes conducting peer faculty teaching evaluations, acting as co-faculty mentor for the Phi Sigma Biological Honors Society, being the executive editor of the Phi Sigma Research Newsletter, organizing an annual Elevator Talk Competition, acting as the faculty administrator of the Dr. John F. Sheehan Scholarship (and subsequently reviewing applicants for the award), and meeting with prospective students during on-campus visits. Further, the Department of Biology has held two faculty searches since 2016 that I have been extensively involved with, and this summer I volunteered to attend a day-long PULSE workshop with two other faculty to gather information on how Biology would like to assess student learning in our program in order to inform our future curriculum development. At the level of the college/university, I have recently been elected to the Faculty Senate for the next three years and am also a member of the Bylaws Committee. Last spring I participated in the Magis Core Assessment Day for the written designation, conducted Dean's Fellows Interviews for Special Programs Day, and volunteered to give tours of my research lab for Admitted Students Day – all are activities I intend to participate in annually from now on. I try to support high school and undergraduate researchers in as many ways as possible, which includes volunteering my time to the programs that engage with them. As such, I volunteer annually to judge student presentations at St. Albert's Day and review CURAS summer research fellowship applications. In regard to the local community, I volunteer annually to engage with local underprivileged high school students through the Nebraska Wildlife Rehabilitation Inc. "High School Academy", and professionally I continue to review grants and manuscripts in my broader field, as well as judge student presentations and volunteer at networking & mentoring opportunities at national conferences.

TIMETABLE OF GOALS

Proposed studies and scholarly activities *(Requested sabbatical for Spring 2021)*	**Fall 2021**	**Spring 2021**	**Summer 2021**	**Fall 2021**
Rear crickets and dissect/freeze cricket brains	X			
Analyze cricket brain samples at USD		X		
Manipulation experiments on crickets		X		
Complete manuscripts from previous projects		X		
Present findings at regional/national meetings		X	X	
Write manuscript for publication from sabbatical data				X
Prepare NSF-Career or NSF-IOS grant			X	X

PROPOSED BUDGET

If no other research funding has been acquired to help support this work, the stipend will be used to support travel to [redacted] for myself and student [redacted] to work with our collaborator. Travel will include mileage, gas, food, and lodging expenses. Funds may also be used to purchase disposable supplies for the proposed study and defray publication costs incurred from the resulting manuscript. Excess funds will be received as a stipend to reduce summer teaching obligations.

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