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PROJECT & LEARNING PLAN

I) Intellectual Merit

The **overarching goal** of this research project is to identify the genetic changes underlying the evolution of learning behavior. To achieve this goal, my students and I investigate learning in threespine stickleback fish (*Gasterosteus aculeatus*), an important model system in the study of vertebrate evolution, behavior and developmental genetics[1]. We have identified differences among stickleback from different lakes with respect to how they come to recognize their predators: stickleback from some populations need to learn to identify predators (e.g., trout) as potentially dangerous, while stickleback from others innately recognize these threats without any prior exposure. Thus, there must exist an evolved, genetic difference between these types of populations. Our **primary question** is how learned anti-predator behaviors can evolve to become more or less reliant on experience (i.e., more "innate") and what underlying changes in gene expression accompany this transition. An "expressed" gene is a stretch of DNA that is being actively used to make a protein, which then carries out some essential function. In this case, we hope to identify genes that are expressed differently between fish that learn and fish that innately recognize their predators. Doing so can lead us to the genetic changes responsible for differences in gene expression, and ultimately for differences in learning behavior[2,3].

Work in some laboratory model systems (e.g., rats) has begun to assess the genetic underpinnings of learned versus innate behavior [4,5], but few, if any, studies have been carried out with natural populations where differences in learning exist, nor have they made the necessary comparisons among populations to determine how such differences evolve. The threespine stickleback fish provides an excellent opportunity to fill this gap in our knowledge, given that: 1) stickleback exhibit substantial variation in anti-predator behavior [6-11]; 2) stickleback from some populations rely more on learning about predators than stickleback from other populations (unpublished data and[11]); and 3) there is a growing number of tools for evaluating the genetic mechanisms underlying the development and evolution of adaptive behavior in stickleback [1,12]. Learning is known to alter gene expression in a number of model species [13-16]; and our preliminary experiments using RNA sequencing demonstrate that learning results in substantial changes in gene expression in at least on population of stickleback (unpublished data), a proof of concept that our approach is sound.

Our experiments are an important first step in identifying the genetic differences responsible for determining whether individuals rely on innate predator recognition, or instead must learn from experience. Our results will not only have implications for how stickleback fish cope with their predators, including exotic species introduced by humans[10,11,17], but more importantly for understanding the genetic basis for learning behavior in other animals, including humans. Investigations into the genetic basis of morphological variation in stickleback (e.g., bony armor, body pigmentation) have revealed similarities of genetic control with humans[18-21], so it is reasonable to expect that studying the genetic basis of behavioral differences will yield similar insights into the evolution of human learning.

II) Role of Students and Mentor

My role as a mentor will be to train my students in all relevant techniques, and to supervise the analysis and interpretation of the resulting data. The students will spend half of the MUSE session collecting data from videos of the behavior trials we are running this semester (Spring

2016). The behavior methodologies are well-established in my laboratory (unpublished data and[11]), and since both of my MUSE students are working on the project this semester, they will already have experience with many of the techniques involved. Once these videos are scored, my students and I will choose a representative subset of fish to be used for RNA sequencing, which is a procedure that will allow us to identify which genes are expressed in the brains of fish as a consequence of learning, and how those expression patterns differ in fish with innate recognition. Our collaborators at The University of Illinois will conduct the RNA sequencing experiment, which will be supported by supplementary funds from the National Science Foundation. Both of my MUSE students will be involved in the analysis and interpretation of the data when it becomes available in early Fall 2016.

My students should finish scoring videos by the middle of the MUSE session. Subsequently, they will pilot additional experiments investigating other modes of learning in these fish. We are currently considering how stickleback learn to recognize predators using odor cues, a common learning modality in fish[22-24]. Other possible mechanisms include learning from information obtained through social interactions, and/or learning from visual cues. Our goal will be to run one or more small-scale experiments to determine their feasibility and potential outcome, which would be then be expanded upon the following academic year.

III) Broader Impacts

Both students will continue to provide an essential contribution to my research program, which I expect to result in one or more publications that they will co-author. Our project addresses a question that is relevant to understanding the behavior of any animal that learns, including humans. My collaborator at the University of Illinois, Dr. Alison Bell, and I have secured $21,480 in supplemental funding from the National Science Foundation to conduct this work, and our hope is that it not only results in a quality publication(s), but that it provides a strong foundation for a full research proposal to substantially expand upon the project.

Both students have expressed interest in attending either graduate school or veterinary school, and their experiences in my lab will help them to further hone their interests and develop their professional skills. Meagan Rodriguez is a participant in the School of Science's "Gateway Scholars Program," which is a four-year research program designed to help prepare students from underrepresented groups for careers in scientific research. Meagan has been a member of my lab since her first semester at TCNJ, and has been doing a wonderful job. Her enthusiasm and curiosity are blossoming into intellectual sophistication, and participating in MUSE will greatly enhance her development. Liz Thoresen has been a member of my research lab for the past five months, and has shown impressive maturity, diligence, and motivation to conduct research. She intends to pursue a career in veterinary medicine, and/or veterinary medicine research, so the opportunity to study behavior in vertebrate animals will contribute to these goals.