UNIVERSITY *of* WISCONSIN
LA CROSSE

18 February, 2024

Dear members of the CSH Undergraduate Research Travel and Supply Grants Committee:

I am writing in enthusiastic support of Ben Graham's proposal for a spring 2024 CSH undergraduate supply grant. Ben is a talented and motivated student who has demonstrated maturity, technical capability, and dependability as a student researcher in my laboratory over the past 2.5 years. I have full confidence that he has the capacity to complete the proposed research with a high level of quality, and I am enthusiastic about working with him.

I first met Ben even before he began his first semester at UWL. Ben has a strong interest in the field of fungal biology (mycology) and chose to attend UWL due to our strong reputation in this field. Ben contacted me during the summer of 2021 to discuss his interests, and then I happened to be his advisor during START advising, giving us another opportunity to talk. Ben is an incredibly self-motivated, enthusiastic, and intelligent student who as a high school student learned to recognize many species of fungi in the field, culture fungi from the environment, and cultivate edible mushrooms. He reads voraciously and already has a strong background in the biology of fungi. When scheduling students for research opportunities, I send each student a list of my available times, and most students select one or two times per week to come to the lab; Ben chose every time that didn't conflict with one of his classes. In his time in the lab, Ben has learned a number of the fundamental techniques in molecular genetics that my lab uses to study fungal ecology, evolutionary biology, and conservation. His level of knowledge and his hunger to learn more have made him a great conversation partner with the graduate students working in my laboratory, and he motivates the other undergraduate students in the lab. He is a well-liked and well-respected member of our lab group.

In a previously-funded Undergraduate Research and Creativity project, Ben sought to expand his repertoire of genetic techniques to include mutagenesis, an essential technique for research on gene function. He wanted to combine this interest with his previous interest and experience in cultivating edible mushrooms by examining the ability of mutagenesis to enhance commercially valuable traits such as growth rate, yield, and resistance to contamination. Ben independently researched scientific journal articles on mutagenesis, and concluded that the King Oyster mushroom (*Pleurotus eryngii*) was a promising experimental system as well as a species for which little work of this type has been done compared to many other edible mushroom species. He then designed a mutagenesis study using ultraviolet light to mutagenize *P. eryngii* spore suspensions. Ben successfully carried out his protocols, and is currently screening mutants.

One drawback to using UV mutagenesis is that it is not straightforward to determine where mutations occurred – an essential element of understanding the genetic mechanisms of changes in phenotype. Therefore, Ben seeks to extend his research using *Agrobacterium*-mediated

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insertional mutagenesis to explore the genetic location and specific functions of the mutations that he generates. His previous grant was sufficient to include a purchase of *Agrobacterium* cells and hygromycin (an antifungal agent that is used to screen for successful mutants); therefore, his present proposal requests funding for only the additional supplies that are necessary for mutagenesis protocol. I have some additional funding that can cover DNA sequencing but not supplies, so I will cover expenses related to the DNA sequencing component of his project.

Ben will take the lead on all aspects of the research, with me acting as a consultant and research mentor and collaborator. I will provide general laboratory equipment and space as needed, and collaborate on dissemination of results as described in Ben's proposal. As Ben's project involves genetic manipulation of living organisms, I will also assure that he obtains Institutional Review Board approval prior to beginning the project and will ensure that proper protocols are followed to contain the organisms and destroy any mutated material at the end of the experiments. I estimate that Ben will require approximately 100 hours to complete the laboratory and analysis components of his project.

The proposed research will support Ben's educational goals of learning genetic laboratory and analytical skills in order to prepare for graduate studies and/or a career in applied mycology research and mushroom strain development. With the skills that he has already developed and the ones that he would develop in the proposed research, I believe that he would be a top candidate for employment in the edible mushroom industry and an excellent candidate for graduate studies by the time that he graduates from UWL. The research will advance my lab's research program in the genetic mechanisms of mushroom development, continuing upon my work with previous graduate student Thomas Roehl that used gene expression profiling to identify genes that are preferentially expressed during development in the edible enoki mushroom, *Flammulina velutipes*.

Ben is an experienced, talented, and motivated student that I believe is perfectly suited to conduct the proposed research. The CSH supply grant would provide this exceptional young student with an additional avenue to gain research and professional development skills necessary to support his educational and career goals. I endorse his proposal with complete enthusiasm and I thank you for giving his application your utmost consideration.

Sincerely,

Todd Osmundson, Ph.D.
Professor
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