2021_SSP Faculty Projects

Row 10

Research Group	Pilger
Project Title	**Refining developmental toolkit gene expression in sipunculan larvae using whole mount in situ hybridization.**
Research Question, Hypothesis, or Conjecture	What is the parahox code for Themiste lageniformis (Sipuncula)?
Project Description	Have you ever visited natural site, observed the animals there and asked, how did these wondrous forms appear? How do animal forms become different, unique? How did their extraordinary adaptations arise? We know that it is through development that adult forms are produced and that changes in the developmental program can result in different adult forms. We have learned in the last 30 years that all animals use a set of developmental toolkit genes (hox and parahox genes) to build their bodies. Changes in the physical location where these genes are used or the timing of their use leads different adult forms, the successful forms add to the diversity of life on Earth. These genes are used to correctly build human bodies too. But when there are mutations in these genes or improper gene expression of these genes during development, a broad suite of developmental disorders may result. These are known as hox disorders. The aim of my research program is to understand the phylogenetic relationship of a particular unsegmented marine organism (a peanut worm) to the formally designated group of worms that have segmentation as a hallmark feature. I aim to understand how the deployment of hox and parahox genes in the peanut worm compares to that of the true worms. The spatial and temporal expression pattern of these genes is known as the hox code and parahox code. Previously I have identified the gene sequences for all fourteen toolkit genes, raised thousands of peanut worm larvae, fixed them at different developmental stages, and produced molecular probes that can be used to identify the timing and location of each gene's action. The experimental procedure used to visualize the expression of these important genes is called Whole Mount in Situ Hybridization (WMISH). For reference, each experiment may take 3-7 days to complete. Unlike experiments using vertebrate animals where complete and tested experimental protocols are well known, similar experiments on invertebrate animals typically require modifications that must be discovered through reasoning and troubleshooting. I have run initial experiments with the animals but the gene expression regions of the genes are not as crisp as we would like to have. The goal for this summer is to run several variations of the protocol to perfect WMISH experiments using the three known parahox genes. These experiments are tedious and require accuracy over a period of two to seven days. Thus, in order to achieve the goal, experiments may have to be run in parallel.
Introductory References	

Project Timeline (weekly), during June 1 - July 31	Wk. 1: Reading and discussion of key literature. Protocol training. Basic lab skill training/review. Solution preparation for experiments. Instruction to basic microtechnique. Wk. 2: Begin WMISH experiments. Evaluation of protocols based on results. Wk.: 3-4: Continue experiments, compile data records for developmental stages. Evaluation of intended success or unintended failure. Recommendations for the "next" experiments. Prepare for SSP presentation.
Expected Learning Outcomes	This research is the first to discover and document the parahox code for any peanut worm. The student who participates in this research will learn and employ research methods used to document gene expression patterns in the development of animals as well as how to interpret these data relative to the larval anatomy and the developmental process of the study organism. In addition, the student will learn to photo-document whole mount in situ hybridization (WMISH) evidence and how to create microscopic sections of preserved and embedded larvae in order to develop a morphological model against which the WMISH data may be interpreted. Pending my success in the first half of the summer, training in microtechnique may be included. The students will prepare and present the results of their work to others in the STEM Scholar Program contingent on the format specified by that program.
Research Team & Environment	This research will be done with up to two ASC undergraduate students who will work together under my supervision. I have been conducting this research in collaboration with a colleague at the Smithsonian Institution's Marine Station in Florida. I expect that we will interact with my research colleague from time to time to discuss the emerging results. Working together and with me, we will establish a dynamic learning environment wherein students are co-equals and asked to interpret results and troubleshoot problems.
Project Duration	4 weeks
Project Dates	July 1 - July 31, 2021
Institutional Approvals	None needed
Required Trainings_each student	None needed
# of full-time student positions requested (1-3)	2
Minimum Requirements (for research novices)	Students should have successfully completed Bio 110 and 111 with a grade no lower than B. The students should have knowledge of or be trainable in skills such as microscopy, pipetting and solution preparation. Availability for experiments that may extend beyond the normal workday hours. Attention to detail.
Requirements for Advanced students	Advanced skills include critical reading of scientific papers and accurate bench-skills of molecular biology and solution preparation.

Recommended Preparation (but not required)