Linda Gai
Miller Lab - University of California, Berkeley

EDEN Undergraduate Training Program Report - Visit Narrative

The objective of this project was to elucidate the genetic basis of naturally evolved tooth number differences. The threespine stickleback, *Gasterosteus aculeatus*, is well-suited for identifying the genetic architecture underlying this trait, as heritable tooth number differences exist between natural, reproductively-isolated populations. Previous work in the lab has identified a number of quantitative trait loci (QTL) associated with tooth gain in a large genetic cross between a high-toothed Paxton Lake freshwater benthic population and a low-toothed Japanese marine population. Our goal was to further elucidate the processes underlying evolved tooth gain with a combination of genetic and developmental approaches. Firstly, we hoped to determine which genomic regions are associated with tooth gain in lab-reared populations of Paxton Lake benthic stickleback. Secondly, we wished to characterize the expression patterns of candidate genes located near a peak QTL marker, as well as look for expression differences between high-toothed and low-toothed populations that could be responsible for tooth number divergence.

For the genetic portion of my project, my goal was to determine if tooth gain QTL were present in a subset of the lab's stocks of Paxton Lake benthic fish. Because the lab stocks are derived from wild individuals, these genetic crosses help estimate the allele frequencies of the QTL in the population. To do this, I examined 96 individuals from a single F2 clutch derived from Paxton benthic and Japanese marine grandparental fish. I first stained for bone and subsequently dissected out the branchial skeleton to determine pharyngeal tooth counts. I then determined the genotype of each fish by genotyping microsatellite markers near the QTL. By comparing the tooth counts of fish with different genotypes, we found that both of the Paxton benthic grandparental chromosome 21s in this cross possess the large-effect QTL previously identified. In addition, several other previously identified QTL replicated in this cross, and others were suggestive that they would reach significance with a larger sample size.

My goal for the developmental portion of my project was to use in situ hybridization to examine the expression patterns of two genes close to a peak QTL marker, *Bmp6* and *Tfap2a*. Both genes have homologs with established roles in vertebrate craniofacial development: *Bmp* genes regulate reciprocal induction events between the dental epithelia and odontogenic mesenchyme, while *Tfap2a* specifies the neural crest, a cell type that eventually gives rise to much of the head skeleton. Using in situ hybridization, I created a developmental time series comparing the expression patterns of these two genes between a lab-reared low-toothed marine population and a high-toothed freshwater population. I detected *Bmp6* expression in the thymus and oral jaws, and *Tfap2a* expression near joints of the branchial skeleton. However, expression in deeper tissue such as pharyngeal teeth was less robustly detected, suggesting technical improvements to the in situ hybridization would further optimize the protocol. Possible improvements include further dissecting out tissue of interest prior to staining, extending the probe incubation time, and/or changing the duration of tissue digestion in proteinase K.

My EDEN internship has provided me with an invaluable opportunity to work on a fascinating project and to hone my skills as a developing scientist in an immersive research environment. I have learned much about previous, current, and potential future research in the biology of threespine sticklebacks, as well as a diverse array of lab techniques, ranging from in

situ hybridization to quantitative genetics to PCR genotyping to programming in R. I look forward to returning to the Miller lab next year, and continuing to explore the genetic and developmental basis of evolved tooth number divergence.