Comparative genomics of eelpouts to understand the evolution of polar extremophiles

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Project overview:

Resolving links between phenotypes, environmental variation, and underlying genomic architecture is a central goal of modern evolutionary genetics. Realizing this goal can be difficult, however, as unclear or varying selective pressures or a lack of genome-scale data can undermine conclusions and lessen impact. At high latitudes, the clarity of selective pressures simplifies expectations with obvious pressures (e.g., extreme cold) driving evolution. Perhaps nowhere is the evidence of this strong selection more apparent than polar seas, where many organismal groups have evolved under a regime of chronic cold stress. In fishes, for instance, polar conditions have driven the evolution of antifreeze proteins (AFPs) which function to reduce cellular freezing points.

Our existing research project focuses on the evolution and cold adaptation of eelpouts (family Zoarcidae), a globally distributed group of fishes. We are comparing the genomes of eelpout species from three major geographic regions–Arctic, Temperate-Tropical, Antarctic–to better understand their evolution in response to cooling of the Arctic and Southern Oceans. Broadly, we are interested in the rates at which different genes evolved in Arctic versus temperate-tropical species. Such inquiry is likely to uncover changes in genes and pathways expected to be under selection (e.g., AFP genes) as well as other, unexpected evolutionary targets. We are interested in three questions: (1) What genes have been subjected to the strongest selection between polar and non-polar eelpouts? (2) Is there evidence for AFP-associated genes in the genomes of eelpouts from lower latitudes that rarely, if ever, experience freezing stress? (3) Is there evidence of a "dosage effect" where the number of AFP (or similar) gene copies scales with increasing cold stress (i.e., few or no AFP genes in low-latitude eelpout genomes with many AFP copies in the genomes of high-latitude eelpouts)? For each question, we are using Arctic and Antarctic species as naturally evolutionary replicates of adaptation to extremely polar seas.

Progress:

Previously, our project was largely focused on Arctic/non-Arctic comparisons, largely overlooking the Southern Ocean--the most extreme marine environment on Earth. Using funding from the Antarctic Bursary, we extended our project to include an Antarctic comparison. Specifically, we sequenced the genome of *Ophthalmolycus amberensis,* a deepwater eelpout commonly found south of the Antarctic Circle in subfreezing waters. We used high-coverage (~100X) Pacific Bioscience long-read sequencing to generate the most contiguous eelpout genome ever produced by more than an order of magnitude. After annotating the genome with existing transcriptomic data, we first tested an existing hypothesis regarding the evolution of AFPs across all three eelpout groups (Figure 1). The results were unequivocal in their support of a single origin of AFPs from a duplication, translocation, and subsequent modification of the sialic acid synthase B gene (SAS-B). This evolutionary event likely occurred at the base of the family (or perhaps even at the base of the suborder Zoarcoidei).

Next steps:

Now, we are taking a more in-depth comparative genomic approach to understand how selection in polar seas has shaped eelpout genomes. Using our existing Arctic and Temperate-Tropical eelpout genome assemblies, our new high-quality Antarctic eelpout genome, and published genome assemblies for other polar fish groups (e.g., icefish, family Channichthyidae), we will identify the genes and pathways that have been under selection in polar fish broadly, as well as in Arctic/Antarctic groups alone. We will also use our *new O. amberensis* genome to better understand how genomic architecture, and specifically copy number, shapes adaptive evolution in these extreme fishes. For instance, we have already identified a difference in the

number of AFP gene copies in *O. amberensis* versus another Antarctic eelpout, *Lycodicthys dearborni* (Figure 2).

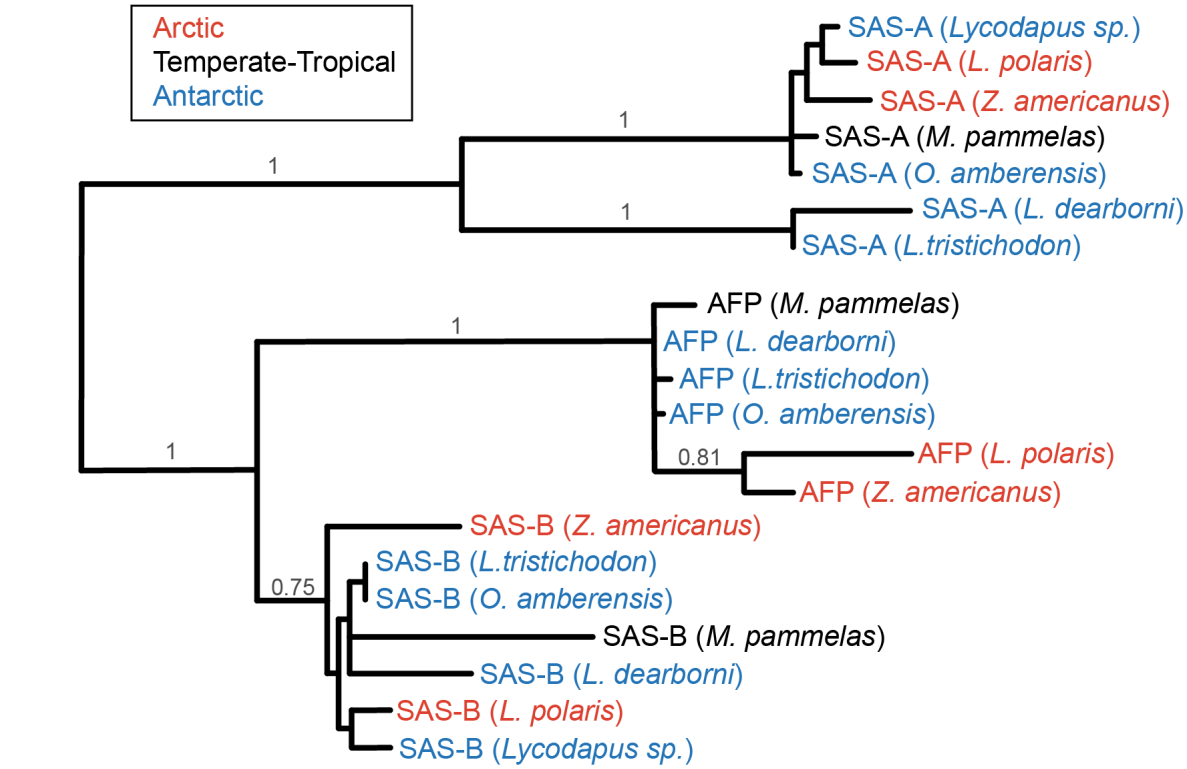

Figure 1. A maximum-likelihood phylogeny of the AFP exon 1 and the 5' flanking region for sialic acid synthase (SAS) and AFPs genes in seven eelpouts. The relationships show that AFPs likely have a single ancestral origin in eelpouts, evolving from a duplication, translocation, and modification of SAS-B.

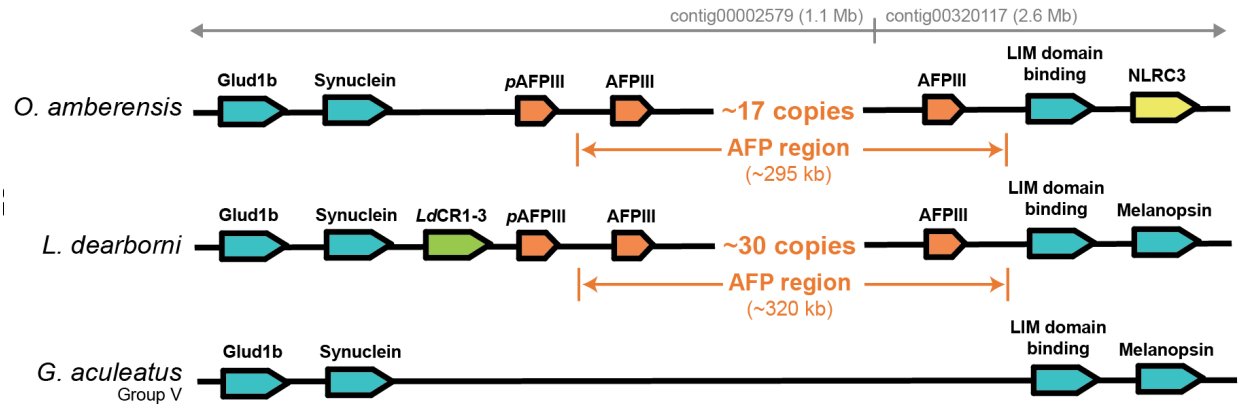

Figure 2. Synteny of the AFP locus in two Antarctic eelpouts (*O. amberensis* and *L. dearborni*) and a non-polar species, stickleback (*G. aculeatus*).