Adaptive Evolution in the Mitochondrial Genome of the European Green Crab
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The European green crab (*Carcinus maenas*) is an invasive species that was first introduced to the United States in the early 19th century. By attaching itself to ship hulls, it was transported from southern Europe to Massachusetts. Since then, this "southern type" variant has spread across a large portion of the East Coast (Carlton and Cohen 2003). In 1980, a second introduction occurred from northern Europe to the Canadian maritimes, giving rise to a "northern type" variant (Roman J 2006). These two populations have since been shown to possess different levels of thermal tolerance specific to their native environments with the northern type crab exhibiting superior cold tolerance and the southern type exhibiting superior heat tolerance (Tepolt and Somero 2014).

Interestingly, thermal tolerance was later found to be correlated with mitochondrial haplotype, indicating that variation in the mitochondria is driving the disparity between the northern and southern crabs (Coyle et al. 2019). If true, this suggests that the mitochondria can influence phenotype—an idea that has had increasing evidence in recent years. The exact mechanism for this phenomenon in green crabs remains largely unknown, and my research aimed to expand on Coyle et al. 2019 by sequencing and comparing the complete mitochondrial genome of different variants to find potential explanations. This could be summarized with the research question, *what role does the mitochondrial genome play in thermal tolerance*?

For this project's methods, sequencing the mitochondrial genome began with collecting and identifying crabs of each variant. The northern and southern crabs are subdivided into haplogroups based on variation in the mitochondrial CO1 gene: A1 and A2 for the southern types, and B1, B2, and C for the northern types. 50-60 crabs were collected at each site from Harpswell, Downeast Institute (DEI), and Lubec. To sequence the CO1 gene, crabs were dissected, and DNA was extracted and amplified for this gene specifically. Once identified, two of each variant was selected for sequencing the entire mitochondrial genome. RNA was extracted from the heart, gill, and muscle tissue then sent to Novogene for a process known as RNA-Seq. This method sequences all RNA transcripts at the time of sampling to obtain the expressed genome. Once the raw data was collected, it was assembled into a genome using existing data from another crab, *Thalamita crenata*, as a reference for locating genes. Finally, the data was analyzed for differences among variants.

A total of 9 samples were successfully sequenced, representing two of each variant (except for B2, which only had one). During the initial CO1 screening, a new warm type variant was discovered (henceforth referred to as A3), but this was not sequenced. The RNA-Seq process yielded approximately 9000 nucleotides of the mitochondrial genome for each sample. Although this was not the complete genome, two-thirds of the genes were recovered for analysis. A total of 77 nucleotide substitutions were identified among variants—73 were synonymous (i.e. the resulting amino acid was unchanged), and 4 were nonsynonymous (i.e. the resulting amino acid was different). The two most notable nonsynonymous mutations were found in the ATP6 gene and Cox3 gene of both B1 replicates.

These are of significance as ATP6 and Cox3 can influence the amount of ATP available at different temperatures. In fact, variation in ATP6 has been shown to affect the thermal tolerance of mammals (Ballard and Whitlock 2004). However, this only accounts for B1, not B2 nor C. In the future, additional work should be done to recover the remaining mitochondrial genome to assess for nonsynonymous mutations in B2 and C. Nevertheless, the research conducted this summer marks a useful starting point for assessing the role of the mitochondria in thermal tolerance for green crabs.

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