Mitochondrial adaptation in the green crab hybrid zone of the Gulf of Maine
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In eukaryotes, the mitochondrion is a key component of the cell's energy economy, responsible for generating stored energy in the form of ATP. The production of ATP requires the use of five protein complexes collectively referred to as the electron transport system (ETS), and alterations to these proteins can lead to significant physiological impacts along with the potential for adaptation. Moreover, the mitochondria contains its own genome that encodes subunits of ETS complexes, and recent studies suggest the potential for variation in mitochondrial DNA (mtDNA) to be under positive selection, particularly in response to environmental stressors such as thermal tolerance (Kong et al. 2020, Slimen et al. 2017). The invasive European green crab (*Carcinus maenas*) exemplifies this phenomenon, as previous studies at Bowdoin have found mitochondrial haplotype (i.e., a collection of mutations inherited together) to influence thermal tolerance in populations residing in the Gulf of Maine (Coyle et al. 2019). Yet, the underlying mechanism of this link between mtDNA variation and thermal tolerance is unknown.

To fill this gap in knowledge, the goal of my research was to explore the hypothesis that alterations in mitochondrial activity drive the observed changes in thermal tolerance across haplotypes. I predicted that, in cold-adapted haplotypes, the activity of specific ETS complexes would be higher than warm-adapted haplotypes to offset impaired catalytic capacity at low temperatures. At the same time, this increased activity would result in lower heat tolerance due to the tradeoff of excessive metabolic demand. To test these predictions, I employed high-resolution respirometry to measure the activity of specific ETS complexes (complex I, II, and IV) across different haplotypes. Mitochondria were isolated from frozen heart tissue for representatives of one warm-adapted and two cold-adapted haplotypes, then measured at 5ºC, 25ºC, and 37ºC to model temperature stressors and a control. Measurements from frozen extractions used a novel protocol that obviate the need for fresh heart tissue (Osto et al. 2020), but to ensure the data would not be significantly impacted by this, an additional, preliminary experiment was conducted to compare the two with the expectation of no difference.

Ultimately, we found frozen tissue to exhibit comparable activities for complex I, but for complex II and IV, activities were 50% and 35% lower, respectively. This was likely due to the sensitivity of crab hearts that the original protocol, optimized for mouse hearts, did not account for. Yet, frozen hearts continued to be used to compare haplotypes due to the ease of acquiring and storing them over fresh hearts, and any differences were still expected to emerge if present. Comparing haplogroups, however, revealed no difference in activity for complex I, II, and IV across any of the temperatures. The only differences to arise were based on complex (with complex IV having the highest activity) and temperature (with activities maximizing at 25ºC for complex I but 37ºC for complex II and IV).

These data indicate that, contrary to expectations, mitochondrial haplotype does not influence mitochondrial activity. Thus, activity does not appear to drive the observed differences in thermal tolerance across haplogroups in the Gulf of Maine. Yet, a multitude of alternative explanations exist to potentially explain this such as ATP production and the influence of alternative ETS pathways such as the electron transport flavoprotein (ETF). Future studies are especially encouraged to analyze heart rate across mitochondrial haplotypes as a broader physiological mechanism for thermal tolerance, driven by changes in the autonomic nervous system. The results of this study have added many more questions and complexities to consider, but the story of mtDNA continues to stand as an opportunity to study adaptive evolution, rich with possibility.

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References:

Coyle AE et al. (2019). Mitochondrial genotype influences the response to cold stress in the European green crab, *Carcinus maenas*. *J Exp Biol*, 222(17): jeb203521.

Kong M et al. (2020). Mitochondrial DNA Haplotypes Influence Energy Metabolism across Chicken Transmitochondrial Cybrids. *Genes (Basel)*, 11(1): 100.

Osto C et al. (2020). Measuring Mitochondrial Respiration in Previously Frozen Biological Samples. C*urr Protoc Cell Biol,* 89(1): e116.

Slimen SH et al. (2017). Selection on the mitochondrial ATP synthase 6 and the NADH dehydrogenase 2 genes in hares (*Lepus capensis* L., 1758) from a steep ecological gradient in North Africa. *BMC Evol Biol,* 17(1): 46.