Influence of the mitochondrial genome on patterns of gene expression in response to temperature stress in the European green crab (*Carcinus maenas*)

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The European green crab (*Carcinus maenas*) is an invasive species that was introduced from Europe to the East Coast of North America on two separate occasions—first in 1817 from Portugal, then in the 1980s from Norway (Roman 2006). These brought about "warm-adapted" and "cold-adapted" ecotypes, respectively, with differing thermal tolerances that interestingly correspond to mitochondrial haplotype (that is, a specific set of mutations in the mitochondrial DNA) (Coyle et al. 2019). This holds true even in Maine, a hybrid zone where the two ecotypes interbreed and scramble their nuclear genomes. This begs the question of whether the mitochondria itself is driving the observed physiological differences (as opposed to the nucleus), setting in place my basic research question: *what role does the mitochondrial genome play in the thermal tolerance of Carcinus maenas?*

Previous research on the subject, conducted by myself, indicated that differential expression of mitochondrial genes may be a significant piece of the puzzle. To investigate this possibility, I designed an experiment that subjected representatives of each sex and haplotype to varying degrees of stress (5ºC, 13.5ºC, or 32ºC), followed by RNA extraction and sequencing (RNAseq) to quantify gene expression. This allowed me to identify any genes that were enriched between conditions. The Grua/O'Connell Award contributed significantly to the funding for RNAseq to accomplish this.

Ultimately, 27 samples were sequenced with a depth of 10 million reads each. To analyze this data, a reference transcriptome was first assembled which used the raw data to compile a list of all RNA transcripts belonging to the green crab. This was accomplished with a series of programs including Trimmomatic (raw data filtering), Trinity (basic assembly) and BlastN (gene annotation) to yield a set of 70,000 transcripts. Then, using this, expression levels for each sequence were quantified and compared between samples/conditions in a process known as differential expression analysis (DEA). In doing so, only 9 differentially expressed genes were observed with a false discovery rate (FDR) under 5%, but many of these provided novel insights. Most notably, cytochrome C oxidase 1 (COI) and NADH dehydrogenase 1 (ND1), genes encoded by the mitochondria, were upregulated in the cold haplotypes at all temperatures. This indicates that mitochondrial haplotype influences its own expression, potentially altering ATP production during oxidative phosphorylation. Another interesting gene was AGPAT4 (Acylglycerophosphate acyltransferase 4) which was upregulated in warm haplotypes at 13.5ºC as the most significant gene in any comparison (FDR < 0.0005%). It is involved with phospholipid biosynthesis and cell signaling, pointing towards a new potential pathway to explore.

The finding of constitutive upregulation of mitochondrial genes is especially notable, suggesting that thermal tolerance is not driven by stress response but instead a baseline expression profile. Furthermore, it indicates that other proteins must be regulating transcript persistence since transcription itself is equal across all mitochondrial genes (due to the genome's polycistronic nature) (Mercer et al. 2011). These results set the foundation for additional projects that can investigate post-transcriptional dynamics, the abundance of COI and NAD1 *protein*, and ATP output from the mitochondria for each haplotype. Once again, the mitochondria proves to be evolutionarily and physiologically relevant, and the context of the green crab invasion, it gives us a window to delve into the complex nature of the species' ubiquity.

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