BIO594 – Summary

Topic: Physiology and Gene Expression

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Kevin Wong

Differential phenotypic responses to new environments can be correlated to gene expression patterns. However, isolating true molecular responses to rapid environmental change poses many challenges, as biological pathways between the genome, transcriptome, metabalome, and phenome are complex and indirect. Additionally, experimental designs play a large role in identifying different types of plasticity and its relation to gene expression. The three studies discussed in the summary have similar approaches in relating phenotypic plasticity to transcriptomics, however their experimental designs allow them to answer different questions. This summay will outline their: (1) experimental designs and objectives, (2) their approaches to trancriptomics, and (3) how the visualizations reflect their goals and results.

*Experimental Design and Objectives:*

Bernal et al. 2018 (BE) focused on assessing the transcriptional responses of *Acanthochromis polyacanthus* to various scenarios of transgenerational warming and related it to physiological plasticity. Wild populations of *A. polyacanthus* were bred in the lab and their offspring (F1) were reared in either ambient, +1.5°C, or +3.0°C temperatures. The F2 generations from the ambient F1 parents were subjected to acute exposures of all three temperature treatments, and +3.0°C during the developmental stages. The F2 generation from the +1.5°C were exposed to the same +1.5°C or +3.0°C temperatures, while the F2 generation from the +3.0°C F1 parents were only exposed to +3.0°C. The physiological traits monitored in this experiment were: standard length, hepatosomatic index, and routine and maximum oxygen consumption. To assess differences in gene expression, tissue biopsies were taken from the liver of F2 individuals and RNA was extracted. Experimental factors such as tissue sampling only the liver would emphasize results related to metabolism, potentially missing other key factors relating to transgenerational plasticity in other organisms. Additionally, including natural selection factors by having unequal potential breeding pairs in F1 treatments could select for one or two strong genotypes that may not represent the whole population experiencing those conditions. Only 5 individuals were sampled from each treatment group, potentially leading to sampling bias error when not account for sex ratios or parental lineages.

The objective of the experiment in Lohman et al. 2017 (LO) was to determine if changes in gene expression patterns in lake and stream *Gastroerosteus aculeatus* could lead to phenotypic plasticity when transplanted to a new environment. An 8-week reciprocal transplant cage experiment was used on 240 *G. aculeatus* individuals from lake and stream environments. Wild individuals were also collected at the end of the experiment to control for cage effects. Tissues from the head kidneys were biopsied for trancriptomic analyses. Caveats in this experimental design is that they assume that all fish originating from the same environmental had the same experiences during development, which could be untrue. Additionally, they only tested for rapid plasiticity within the same generation, with no inferences about reproduction and fitness.

Walworth et al. 2016 (WA) used the cyanobacterium *Trichodesmium* reared under low and high CO­­2 conditions for 4.5 years to determine the physiological and transcriptomic patterns to a two-week reciprocal transplant. Growth rate and N2 fixation were the phenotypic traits used as proxies for fitness. Biological triplicates were sampled and used for RNA analyses. Due to the short life span of this model organism, WA were able to identify pathways for adaptation in a 4.5 year experiment. However, to understand how plasticity could shape evolution in this system, the authors should have taken multiple time points during the rearing and reciprocal transplant experiment to showcase the timeline of plasticity.

*RNA Preparation and Data Analysis:*

After quality controlling the raw reads with TRIMMOMATIC, BE mapped the trancriptomes to a fully annotated *A. polyacanthus* genome using HISAT2. DESeq2 was used to determine differential gene expression and significant differences between treatments.LO extracted the RNA and TagSeq libraries were prepared for 96 individuals, creating a sample size of 16 for each treatment. The raw reads were quality controlled and filtered with the iRNAseq pipeline, and mapped to the *G. aculeatus* genome using Bowtie2. Gene Ontology was used to annotate the transcriptome using UNIPROTKB. Differential gene expression was analysed using DESeq2.WA used Bowtie2 for reference mapping to the genome and used the JGI (Joint Genome Insitutue) genome annotation pipeline for gene annotation. EdgeR was used for differential gene expression analysis.

*Visualization and Results:*

BE utiluzed a principal component analysis to visualize the differential genes being expressed between groups. Additionally, the gene ontology terms were presented that were differentially expressed and related to the metabolism data they measured. In the F2 generation, all individuals exhibited increase in hepatosomatic index in the +3.0°C treatment, higher routine oxygen consumption, and an upregulation of genes relating to metabolic pathways (i.e. mitochondria and respiratory chains), DNA maintenance, and apoptosis. When comparing the Step +3.0°C and Transgenerational +3.0°C, there is evidence of higher routine oxygen consumption and re-allocating energy to compensate of increased metabolism under high temperatures by upregulating the LAMTOR4 gene. This suggests that step-wise warming across generations may facilitate rapid adaption through transgenerational plasticity.

Using a weighted gene coexpression network analysis (WGCNA), LO determined that the wild stream and lake populations differed in gene expression patterns relating to parasite defence, which has been documented in previous studies. In terms of the reciprocal transplantation between sites, the origin of the fish had a greater influence than the transplant destination in relation to gene expression patterns. Additionally, lake fish seem to be more plastic than stream fish under cross transplantation, suggesting that early life stages in different environments may be the driving cause behind plasticity to a new environment.

Venn diagrams were used to showcase differentially expressed upregulated and downregulated genes between treatment groups. Strong correlations between pathways involving metabolism, cell signalling, and transcriptional regulation were differentially expressed, suggesting that adaption may be the diver to cellular homeostasis under altered environmental conditions. However, *Trichodesmium’s* lifecycle is relatively short compared to the other systems discusses in this summary, therefore selection for cellular traits that can adjust to altered CO2 conditions could be more easily showcased.