**Gene Expression and RNA-Seq: Discussion Summary**

*Introduction*

RNA-sequencing analysis has become an increasingly popular way to study gene expression in different organisms. Organisms respond to stress in a variety of ways, and RNA-seq and the associated bioinformatics tools can provide valuable insights on how they will respond to stress in the future. Gene Ontology tools, in particular, can provide information on what genes to focus on, and what purpose different genes play in an organism. Lohman et al. 2017, Bernal et al. 2018, and Walworth et al. 2016 all describe applications of RNA-seq to studying stress and plasticity.

*Comparisons*

Researchers use RNA-seq to study a variety of different stress events. In these examples, Bernal and Walworth studied the effects of climate change, while Lohman studied selection against migrants in two closely related populations. Bernal examined the effects of increasing temperatures on coral reef fish. They exposed the damselfish *Acanthachromis polyacanthus* to both gradual and sudden temperature changes, and analyzed molecular and metabolic traits. This was a multigenerational study (Bernal et al. Fig 1), allowing Bernal to test a variety of temperature treatments and also to touch on effects of heritability. They were able to draw the conclusion that the damselfish’s ability to adapt is dependent on what conditions their parents were raised in. Walworth cultured the cyanobacterium *Trichodesmium* at high and low CO2 levels over 4.5 years, also allowing for a variety of treatments and an examination of the effects of plasticity on fitness over time. Depending on the treatment, this resulted in ~570-850 generations, with growth rate as proxy for reproductive fitness. They found that while short-term stress was alleviated through plasticity, longer periods of stress caused plastic responses to become fixed, implying that genetic assimilation is a possible adaptive response to CO2 increase. Lohman, however, focused on one generation and performed reciprocal transplant experiments on lake and stream sticklebacks (*Gasterosteus aculeatus*) to test if phenotypic plasticity could help migrants adapt to new habitats. While they conclude that plasticity, while advantageous, will not fully homogenize populations, a further study of multiple generations over a longer time-scale would make this more compelling.

Researchers also had to extract RNA from a certain part of their organisms. It was relatively simple for Walworth to study the entire *Trichodesmium*, allowing them to draw whole-organism conclusions. Bernal and Lohman, however, had to decide what parts of the fish to sample. Bernal chose to assess liver gene expression, as the liver has previously been associated with fish’s responses to temperature stress. This assumption appeared to hold true, as they found that fish exposed to the +3.0 developmental treatment had significantly enlarged livers. Lohman extracted and sampled the head kidney. The head kidney is a major site of immune response and hematopoiesis. Immune response was important to this study because parasite infection was found to be much higher in transplanted fish. While the sample choices made by Bernal and Lohman make sense, it could be valuable in the future to perform similar experiments and sample different tissue, to see if other genes are upregulated or downregulated in response to stress.

During analysis, all studies chose to focus on certain genes. It can be useful to group similarly expressed genes into clusters. All three studies used classic RNA-seq procedures such as quality analysis, trimming, filtering, and mapping. They also all used Gene Ontology enrichment analysis to identify differentially expressed genes. Walworth found differing levels of regulation of transposition clusters, as well as “sigma switching,” both evidence of adaptive responses. Bernal identified upregulated gene clusters in the high temperature treatments associated with lipid transport, as well as inflammation, apoptosis, and tumor suppression. To investigate the importance of transposable element regulation, Walworth binned all TE with >70% identity into clusters. By doing this they were able to identify clusters associated with adaptive responses to CO2 stress.

Lohman was the only study to perform a weighted gene co-expression network analysis (WGCNA) on their RNA-seq data. Since Lohman looked at selection against migrants in two closely related populations, there was an increased possibility for researchers confounding of different factors. However, Lohman took great care to determine which gene clusters to focus on in as unbiased a way as possible. By conducting a WGCNA they were able to identify closely associated genes without any undue bias. They also ran WGCNA on lake and stream populations both separately and together, to test for any population bias in their clustering analysis.

*Conclusions*

As the natural environment changes and becomes more stressful to many organisms, RNA-sequencing analysis of gene expression can help us predict stress responses over time. It is important to judiciously choose your sample material, as well as choose which genes to focus on in an unbiased way. Longer-term studies are also vital in drawing any conclusions about fixed adaptations.

**Sources**

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