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Discussion: RNA-Seq and Gene Expression

Examining genes that are differentially expressed between and among populations in both controlled experiments and in the field provide useful insights into how organisms shift in response to external changes and can help predict future shifts to populations. By relating gene expression of different groups to environmental variables and/or other phenotypic/physiological measurements, we are also able to infer whether or not organisms are exhibiting phenotypic plasticity or shifting towards genetic assimilation of certain traits. In this week’s discussion papers, Lohman et al. (2017) and Walworth et al. (2016) utilized transplantation experiments in natural and lab-settings (respectively), while Bernal et al. (2018) exposed two generations of a coral reef fish to heightened temperatures mirroring projected future climate change scenarios. In all of the above, there were significant differences among treatment groups, even if more standard (physical) measurements of fitness and phenotypic changes were not evident. All of the papers show how RNA sequence analysis can identify suites of genes simultaneously and robustly, though their applicability depends on how well reference transcriptomes are annotated and interpreted in the literature.

Lohman et al. (2017) utilized transplantation experiments of lake and stream stickleback to see if there were baseline differences between the two populations, and how those compared to individuals transplanted from one ecosystem to the other. They extracted RNA out of the head kidney (to detect immune responses) and combined their gene expression data with parasite counts and other morphological traits of the different fish populations. They confirmed that native lake and stream populations were different from each other at a morphological, parasitological, and transcriptomic level. When transplanted, lake populations exhibited more plasticity than stream populations, even though it was expected that stream populations would have more plasticity due to their more temporally dynamic native environment. However, the authors explained that lake populations harbor more diverse parasite communities, suggesting that lake populations require a more varied immune response. Overall, there were both heritable and plastic differences that jointly contributed to differential expression between the two native and transplanted populations. One correlation analysis Lohman et al. (2017) used that other papers did not was the Weighted Correlation Network Analysis (WGCNA), which unbiasedly finds clusters of highly correlated genes with similar expression patterns. The benefit of WGCNA is that candidate biomarkers can be identified (in this instance, the MEgreenyellow, MEred, and MEpurple expressions) and correlated with other indices, like infection rates and parasitism. In addition, their Linear Discriminant Analysis (LDA) provides a more robust way to visualize the response of treatment groups more than other related ordination methods alone (like PCA).

In the lab, Walworth et al. (2016) transplanted *Trichodesmium* cyanobacteria to/from low and high CO2 conditions to examine the relationship between short-term plastic responses and long-term adaptive responses to increased temperature. In comparison to Lohman et al. (2017)’s transplantation experiment, Walworth et al. (2016) used their transplantation experiment as a test for whether or not a plastic phenotype in low CO2 conditions would get fixed once in a higher CO2 treatment. They found that when high CO2 -reared individuals were put back into the low CO2 condition, there was a loss of sensitivity to CO2, evident by an evolutionary shift in reaction norms and fitness increase by the transplanted high CO2 individuals. When low CO2 individuals were put into the high CO2 treatment, they expressed the high CO2 phenotype (that later gets genetically assimilated after multiple generations), providing further evidence of the plasticity-to-fixation shift. Because Walworth et al. (2016) used the whole RNA profile using Illumina Hi-Seq (rather than specific organs like Lohman et al. 2017 and Bernal et al. 2018), they had less apriori information for which pathways would be enriched. They examined changes to functions including transcriptional shifts and metabolism as a result. Their conclusions are helpful for understanding the different ways in which natural selection may act on populations, particularly hypotheses about how phenotypic plasticity may affect the rate at which populations evolve.

Similar to Walworth et al. (2016), Bernal et al. (2018) tested future warming conditions in a step-wise fashion (along with other experimental treatments) to better understand the physiological and phenotypic changes of a coral reef fish. Bernal et al. (2018) sought to discover how conditions during the parental generation affected offspring generations, thereby using less generations and more experimental treatments than Walworth et al. (2016). Like Lohman et al. (2017), Bernal et al. (2018) targeted a particular body part (the liver) of the fish to sequence for RNA to measure metabolism. Bernal et al. (2018) also measured the oxygen consumption (routine and maximum) of the fish by putting them in chambers with standing or moving water. However, Bernal et al. (2018) did not do as complicated of analyses as Walworth et al. (2016), only utilizing PCA and Mann-Whitney-U tests of GO terms to visualize and test the significance of their treatment groups. Walworth et al. (2016) used LDA, WGCNA, as well as a variety of linear models in DESeq2 to explicitly test the origin-transplantation relationship. Overall, Bernal et al. (2018) found that the +3.0°C condition had the highest upregulation of genes related to metabolic rate in comparison to control and lower (+1.5 °C) groups. They also found that metabolic compensation is possible under warmer water conditions, though the particular response is dependent upon the conditions in which their parents were exposed to. Their data is more ecologically relevant since it is more likely that step-wise temperature and environmental changes will occur. The control, step-wise, developmental, and acute treatments they did helped eliminate alternate explanations for their results, though their sample sizes were relatively low for each of the treatments.

The three papers in general followed the same workflow, though each used a different sequencing technology (TagSeq for Lohman et al. 2017, Illumina Hi-Seq for Walworth et al. 2016, and TruSeq for Bernal et al. 2018) and gene expression techniques (DESeq2 for Lohman et al. 2017 and Bernal et al. 2018 & EdgeR for Walworth et al. 2016). Without a lot of background information on these different methods, it is challenging to determine which workflow is the most robust, though each of the papers had slightly different questions about the survivability of their species of interest in different environments and/or eco-evolutionary patterns more broadly. Overall, all three papers combined genomic data with physiological measurements to examine the hidden internal mechanisms behind environmental responses, showing the complementary nature of next-generation sequencing technologies.

Works Cited:

Bernal, Moisés A., et al. "Phenotypic and molecular consequences of stepwise temperature increase across generations in a coral reef fish." *Molecular Ecology* 27.22 (2018): 4516-4528.

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