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Gene Expression Summary

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Analysis of gene expression is key to understanding the underlying mechanisms associated with phenotypic changes in response to different environmental conditions. Lohman et al. (2017), Bernal et al. (2018), and Walworth et al. (2016) are three studies that observed changes in gene expression in response to changing environmental conditions. Lohman used data from transplant experiments of stickleback between adjacent but ecologically different lake and stream habitats to see if phenotypic plasticity in gene expression may help migrants adjust to unfamiliar habitats. The Bernal study focused on phenotypic and molecular changes that occur with temperature increases across generations in a coral reef fish. Walworth investigated the molecular progression underlying the transition from plasticity to adaptation under elevated CO2 in the cyanobacterium *Trichodesmium*.

*Methods*

Lohman reciprocally transplanted stickleback from a lake and stream habitat by caging both native and immigrant fish. After 8 weeks, they collected caged fish and uncaged wild fish to measure both physical traits and gene expression profiles via TagSeq and GO enrichment. They tested for differences in gene expression between wild fish from lake vs. stream habitats and found that wild fish from the lake and stream habitats differ in a range of morphological and parasitological traits. They also tested for changes in gene expression of the transplanted fish, which revealed significant effects of both origin and destination for many genes. The effect of origin represents genotype effects that persisted after transplantation, whereas the effect of destination represents plasticity that was independent of genotype. They found several genes whose expression depended on the interaction of origin and destination. The team then used weighted gene coexpression network analysis (WGCNA) to estimate correlations between coexpressed genes and traits. They found negative correlations between morphology and many different modules of coexpressed genes in wild fish and weak correlations between origin and phenotypes unique to transplanted fish. To determine how well immigrants converge on the expression profiles of natives, the team conducted a PCA of expression of all genes in transplanted fish and used the leading PC axes for linear discriminant analysis (LDA). They concluded that immigrant stickleback partially converge on native expression profiles after emigration to a new habitat. Their analysis also suggested that lake fish exhibit a more plastic response to being transplanted into the stream, compared to stream fish transplanted into the lake.

Bernal analyzed metabolic and molecular traits in the damselfish *Acanthochromis polyacanthus* that were exposed to +1.5°C in the first generation and +3.0°C in the second (Step +3.0°C). This treatment of stepwise warming was compared to fish reared at current-day temperatures (Control), second-generation fish of control parents reared at +3.0°C (Developmental +3.0°C) and fish exposed to elevated temperatures for two generations (Transgenerational +1.5°C and Transgenerational +3.0°C). Hepatosomatic index, oxygen consumption and liver gene expression were compared in second-generation fish of the different treatments. Analyses of gene expression used the genome of *A. polyacanthus* as a reference. Each gene was annotated with their corresponding Gene Ontology. A likelihood ratio test was used to identify differentially expressed genes between the thermal treatments of the experiment. They then assessed enrichment of GO categories for both upregulating and downregulating terms. They found significant upregulation of genes related to mitochondrial activity and energy production in Step +3.0°C fish, which could be associated with the increased metabolic rates.

Walworth cultured *Trichodesmium* at both low and high CO2 levels for 4.5 years (ancestral environments), followed by reciprocal transplantation experiments to test for adaptation. Growth (fitness) and N2 fixation were monitored. They found that when the high-CO2 adapted *Trichodesmium* in the ancestral environment were reciprocally transplanted, fitness increased. They then sequenced the triplicate transcriptomes from both the long-term treatments and the reciprocal transfers. They found strong evidence for genetic assimilation, which is supported by the identification of expression changes and pathway enrichments that rapidly occurred in response to elevated CO2 that were maintained upon adaptation. They also found evidence of down-regulation of genes involved in broad metabolic processes (sigma factor activity and carbon transport) that was only observed upon adaptation.

A major difference between the studies is the duration of the experiment. The Bernal and Walworth experiments spanned several years, resulting in multiple generations where transgenerational plasticity can play a role. In contrast, the Lohman transplant experiment lasted only 8 weeks, where they observed an initial plastic response, however, it is unclear how this response would transfer to offspring.

Expression analyses also differed between studies because of the organisms used. The Lohman and Bernal studies used fish, so they were limited in which tissue samples they could analyze. The choice of tissue to obtain RNA places some constraints on the trait space being studied. Because Walworth used cyanobacteria, the transcriptome of the entire organism was sequenced, revealing expression patterns of all genes related to increased CO2 levels.

*Conclusions*

Lohman found extensive gene expression differences between genetically divergent stickleback populations in stream and lake habitats. They also found that transcript abundance is highly plastic for many genes. Fish that immigrate into the nonnative habitat will partially converge on the gene expression profile typical of their new habitat, suggesting that expression plasticity can lessen the impact of immigration into a different habitat. They consistently found that lake and stream fish differed, which implies that sticklebacks’ transcriptional reaction norms may be evolving as they adapt to different habitats.

Bernal identified the consequences of temperature increase across generations, compared with an increase in temperature within a generation and maintenance of elevated temperatures across generations. Their results suggest that metabolic compensation is possible if fish experience warmer water than their parents. Tropical damselfish acclimation to warming oceans via plasticity will depend on the temperature at which the parents develop, as well as on the magnitude and rate of temperature increase across generations.

Walworth identified genetic assimilation as a potentially adaptive response of *Trichodesmium* and identified underlying metabolic pathways paralleling the fixation of the plastic phenotype upon adaptation. These results have broad implications for microbial evolution and biogeochemistry.

**Literature Cited**

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