**Plasticity and Epigenetics Discussion Summary**

*Goals and Experimental Design*

When studying the genomics of an organism, it is important to take into account how the genes in question are being expressed. This can be affected by epigenetics, specifically DNA methylation. Ryu et al. set out to determine if DNA methylation impacts transgenerational responses to climate stress in damselfish, specifically looking at responses to higher temperatures (2018). Similarly, Liew et al. studies the effects of DNA methylation on corals’ ability to acclimate to lower pH levels, another kind of climate stress (2018). Both focused on epigenetics as a possible mechanism for organisms to adjust to a rapidly changing environment, possibly through phenotypic acclimation. Liew especially focused on this. It can also be informative to examine phenotypic plasticity. Ghalambor et al. studied Trinidadian guppies to investigate how non-adaptive phenotypic plasticity can affect evolution (2015). They tested the theory that the direction of plasticity in gene expression is generally opposite to the direction of adaptive evolution.

All three studies began with practical experimental designs. Ryu tested both step-wise and developmental temperature increases (+1.5 and +3.0°C) on the damselfish ﻿*Acanthochromis polyacanthus*, continuing their study through the F2 generation and making it a true transgenerational analysis. This experimental design allowed them to draw strong conclusions on the acclimation effects of DNA methylation across multiple generations. Liew fragmented *Stylophora pistillata* corals and raised in a tank system under different pH levels of 7.2, 7.4, 7.8, and 8.0. This system has been maintained since 2010, but this specific experiment was carried out for two years. Ghalambor raised wild-caught Trinidadian guppies in enclosures connected to other tanks, some of which contained cichlids (predators of the guppies), some of which did not. They then split the offspring of these guppies between the two treatments, resulting in four different treatment groups. All three studies designed clear experiments, with good replication and controls.

*Analysis*

While Ryu and Ghalambor posed vastly different research questions, they both performed genomic analysis on fish. Since fish are complex organisms, it is difficult to perform whole-organism genomic analysis, and researchers must decide what part of the anatomy to focus on. Ryu chose to sample the liver, as they knew from previous research that metabolic functions related to stress response were found in that organ. Ghalambor sampled the brains of the fish, as they were looking at responses to the presence or absence of predators. These types of reactions are found in the brain, so it makes sense to focus on this area for sampling. Liew, on the other hand, studied corals: a clonal organism with a comparatively simple body plan. Tissue sampling was therefore more flexible, and they were able to get much closer to whole-organism genome analysis.

When it came time to analyze samples, the different research questions determined the different strategies used. Since they were studying phenotypic plasticity, Ghalambor focused on measuring patterns of transcription using RNA-sequencing techniques. However, these strategies will not help determine epigenetic changes. Both Ryu and Liew used whole genome bisulfite sequencing to analyze their samples. This method gives single base pair resolution on what is being methylated, leading to very accurate results. Ryu sequenced the genome, the methylome, and the transcriptome from the liver, and found unique DMRs for both CpG and CHH. CpG DMRs were found in introns, exons, and repeats, while CHH DMRs were found only in exons and repeats. In addition to WGBS, Liew also used generalized linear models to identify genes that underwent different methylation in response to the different pH treatments. To verify these results, they performed amplicon-specific bisulfite sequencing.

*Conclusions*

All three studies found significant results. Ghalambor supported their theory that plasticity is often non-adaptive and under strong selection to change, meaning that plasticity potentiates rapid adaptive evolution (Figure 2, Ghalambor et al. 2015). As stated previously, Ryu’s true transgenerational experiment makes their results especially compelling. Their results were significantly different in transgenerational fish than in other treatment groups. They concluded that intensive epigenetic control of insulin and mitochondrial function could be crucial in maintaining energy balance and metabolic function in a warmer environment. In short, they found that DNA methylation altered the epigenome after being exposed to higher than normal temperatures. Liew found that methylation reduces both spurious transcription and transcriptional noise, and that changes in methylation lead to a more porous skeletal structure. In sum, the propose that DNA methylation could help corals acclimate to climate change by fine-tuning gene expression. However, they admit that their results are merely a strong correlation, and not a strict causative relationship between methylation and phenotype.

Ryu and Liew both studies DNA methylation, while Ghalambor focused on phenotypic plasticity, but all three experiments investigated organisms’ responses to stress. They all set up practical stressor experiments over generations, and they all had to make decisions about what tissue to sample and how to analyze it. In the end, all made cases for the importance of examining levels of genomic analysis other than just the genome itself. Epigenetics and phenotypic plasticity can also have strong impacts on adaptive trends.

**Sources**

Ghalambor CK, Ruell EW, Hughes KA, Reznick DN, Hoke KL, Fischer EK. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature.* 525:372–375.

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Ryu T, Veilleux HD, Donelson JM, Munday PL, Ravasi T. 2018. The epigenetic landscape of transgenerational acclimation to ocean warming. *Nat Clim Chang*. 8:504–509.