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**Linking genotype to phenotype**

Understanding the genetic basis for natural selection, which acts on phenotypes, is a challenging undertaking involving a suite of genomic methods and crucial correlational experiments. The strength of inference behind the areas of genetic code responsible for visible changes in morphology or physiology are highly reliant upon the amount of reference material available for a species of interest, or related species. In this discussion, we have three papers that used independent confirmatory tests to support their genomic findings of adaptive phenotypic variance in geographically and/or morphologically distinct populations of organisms. Reid et al. (2016) used a paired and geographically vast sampling design paired with a controlled experiment where fish of varied pollution tolerance were exposed to toxicant PCB, whereas Nadeau et al. (2016) utilized in situ hybridizations of their identified gene *cortex* in in larvae to see how adult wing pattern would change. In contrast still, Bosse et al. (2017) utilized two geographically similar populations and an outgroup of great tits for their sample collection, including specimen from museum archives, and comparing those to fine-scale ecological data.

Reid et al. (2016) utilized whole-genome sequences and comparative transcriptomics of eight spatially-paired sensitive and tolerant killfish populations living in saltmarsh estuaries of varying amounts of toxicity and pollution to understand the mechanism of rapid adaptation to anthropogenic stress in wild populations. They found that tolerant populations have a desensitized aryl hydrocarbon receptor-based (AHR) signaling pathway (which when targeted by dominant pollutants, can lead to developmental issues and larval lethality) and were able to infer that this pathway was probably a repeated target of natural selection towards tolerant populations. They also found that tolerant populations had more AHR deletions and also generally contained more of the gene *CYP1A,* which is a transcriptional target of AHR and can help mediate for the impaired AHR signaling in fish living in more polluted sites. However, because the toxicity these fish are exposed to vary in their composition and complexity, they affect a more complex suite of genes, which means the signal is more challenging to detect. They conclude that there are multiple AHR signaling pathway elements and other genes that are affected by pollution, which has subtly differentiated tolerant and sensitive populations of killfish in more recent evolutionary history. One weakness of Reid et al. (2016) was that they did not discuss in detail the potential spatial autocorrelation (or shared demographic history) among sampling sites, though it is evident in their PCA (Fig.1) that adjacent populations were more similar to each other in terms of genetic variation than by levels of pollution exposure. They did account for this briefly by identifying outlier regions using one tolerant population against the two geographically closest sensitive populations (as discussed in their Supplementary Material). This may not have been an issue as they were only trying to identify the genetic mechanism behind pollution tolerance and were able to confirm their inferences by raising sensitive and tolerant populations in the same environment and exposing F2 populations to toxic pollutant PCB and measuring the amount of inducibility of AHR-regulated genes.

Unlike Reid et al. (2016), Bosse et al. (2017) used morphological differences to drive their inferences behind the wing patterns of three *Heliconus* butterfly species and additional subspecies, which made their identification of gene *cortex* within the locus *Yb* more convincing. Here, there was a clear speciation event across the different butterfly species, though we are not given the ecological context behind why the wing-color patterns are so different. They also attempt to discover the mechanism behind mimicry, which is another well-known ecological concept that is more challenging to disentangle genetically. Because *cortex* was shown to be differentially expressed in multiple samples of closely-related and distinct subspecies and species of varying morphological difference (from slight differences in yellow bands to completely different speckled patterns) and previous literature suggested that *cortex* was part of a lineage of cell-cycle regulators, they had strong support for their identification of *cortex* as a major gene contributing to wing pigmentation and pattern across Lepidoptera. In addition, they were able to hybridize *cortex* in the final instar larval hindwing of two *Heliconus* species with very distinct morphologies, only to find that wing development started to resemble that which corresponds strongly with *cortex* expression. They concluded that *cortex* might be expressed in varying amounts at varying time points in development in order for such distinct wing patterns to emerge. Their advantage, despite not incorporating ecological data, was the vast amount of genomic, morphological, and transcriptomic information available to them from other insects, collections, and previous publications.

Finally, and briefly, Nadeau et al. (2016) used fine-scale ecological data and data across longer time scales (due to the large collection of museum samples of great tits) to examine the adaptive genetic basis behind bill lengths. Because two populations (from Ireland) were geographically segregated across a body of water from the other population in the UK, they were able to utilize a synthetic environmental gradient using the first principal component of their PCA and test that against the bill lengths observed in these areas. They also conducted tests on supplemental feeding by humans and feeding propensity as well as utilized fledgling and fitness data collected from other monitoring studies to correlate with the target gene they identified, *COL4A5-C*, which is closer in line with traditional ecological studies determining the fitness of particular traits. They found that longer beaked individuals, with the *COL4A5-C* gene, were more likely to use feeders compared to those who were heterozygous for that gene, or homozygous for its corollary gene *4A.* Nadeau et al. (2016) was not only able to rigorously test for the genotype-phenotype relationship of this model bird species, but also provide the critical ecological context for why it’s important and makes a more convincing argument overall for its adaptive nature.