INTRO

Outline:

* Gene expression important for phenotypic evolution

Gene regulation guides spatiotemporal gene expression through development, making it a likely mechanistic step to for mechanistic changes underlies developmental evolution. Gene regulation can be simplified to a few steps: 1) chromatin remodeling: making nucleosome accessible, 2) transcriptional activation: transcription factors binding and activating, and 3) post-transcriptional processing: miRNAs degrading nascent transcripts. Historically, changes at the level of transcriptional activation have been well studied and best-documented. However, our functional understanding, technological capacity, and comparative understanding, now allow us to interrogate other mechanistic levels, such as chromatin remodeling.

* Chromatin remodeling increasingly well studied and being integrated into our understanding of evolutionary mechanisms.

Chromatin remodeling functions to make chromatin accessible and to facilitate regulatory regions to activate transcription. Although there is more to clarify, chromatin remodeling seems to occur at multiple levels: large scale remodeling at the chromosome wide level as well as through more locus-specific mechanisms, which also may vary. For example, in eye-antennal discs, single cell sequencing and QTL analyses provided evidence for enhancers that are remodeled by either 1) a pioneer factor or 2) collective TF binding.

Comparative work has shown one common trend across organisms and divergence times: genome-wide chromatin accessibility divergence is largely due to changes in *cis* rather than *trans.* However, there are variable results on the extent to which changes in chromatin accessibility correlate with changes in gene expression. In yeast, multiple studies have found little correlation between changes to these two mechanistic layers, although more notable correlations were found with *Drosophila* population variation. Notably, a modelling approach between *Drosophila* species found that chromatin accessibility variation explained a non-overlapping amount of TF binding compared to sequence changes, suggesting that chromatin accessibility changes can play a causal role in downstream regulatory changes.

* Gap and we did this to fill it.

Much of chromatin remodeling remains unclear in both a functional and evolutionary context, and a more direct integration of the two is needed to advance both. More specifically, chromatin remodeling does not occur in the same way across the genome: how does this impact evolutionary patterns? Here, we collect chromatin accessibility and gene expression data from *Drosophila* species and their hybrids in a tissue with more extensive molecular understanding of chromatin remodeling mechanisms to better interrogate the evolutionary consequences of molecular differences in chromatin remodeling. We find that, consistent with previous results,

RESULTS

*Experimental schematic*

To interrogate the mode of chromatin accessibility and gene expression evolution, we collected ATAC- and RNA-seq data from the imaginal wing discs of D. melanogaster and D. simulans and the F1 hybrid. These strains were chosen because of 1) their capacity to interbreed and 2) the extensive previous work to catalog the genomic variation between these species. Following alignment and processing, accessibility was measured for four genomic categories: +/- 500bp transcriptions start sites (TSS), +/- 500bp transcriptions end sites (TES), +/- 500bp center of called peaks that fall within coding regions (intragenic), and +/- 500bp center of called peaks that fall between coding regions (intergenic). After filtering regions with low read counts, we had X, X, X, and X numbers per group. We then used an empirical Bayes method to estimate the difference between parental strains, hybrid alleles, and the parental vs hybrid difference. This provided us with divergence estimates as well as statistical metrics to determine cis and trans categories.

*High divergence due to cis changes is constant across genomic categories*