Evolution of cell types and gene expression patterns in a *Drosophila* sex organ

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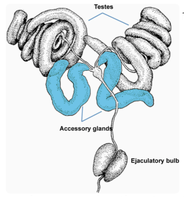
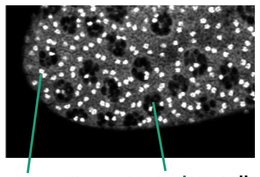
Tempo and mode of evolutionary change varies across both cell type and lineage

Background

Sexual selection often drives rapid adaptation & diversification in reproductive traits. Recent work has linked tissue-specific gene expression evolution to sexually selected traits, but little attention has been focused on cell-type specific expression evolution. As cell-types are the basic functional units of multicellular organisms, we expect the tempo and mode of evolutionary change to vary across cell-types within a tissue. We are using the Drosophila accessory gland (AG) as a model of cell-type evolution. The AG produces seminal fluid proteins (SFPs), which induce a set of behavioral and physological changes in the female known as the post-mating response (PMR). The AG and its products evolve rapidly and experience frequent directional selection. The AG is comprised of two distinct, binucleate secretory cell types, main cells and secondary cells. While main cells are essential for inducing the PMR[2], secondary cells appear to have a smaller impact, modulating the timing of PMR phenotypes, especially remating latency[3]. Given these differences, **we hypothesize that secondary cells will A) show greater divergence in gene expression phenotypes, and B) be more likely to evolve under directional selection than main cells.**



male reproductive tract with accessory glands highlighted



main cells
- smaller
- abundant (96%)
- essential for inducing PMR

secondary cells
- larger
- rare (4%)
- vacuoles modify SFPs
- modulate PMR timing

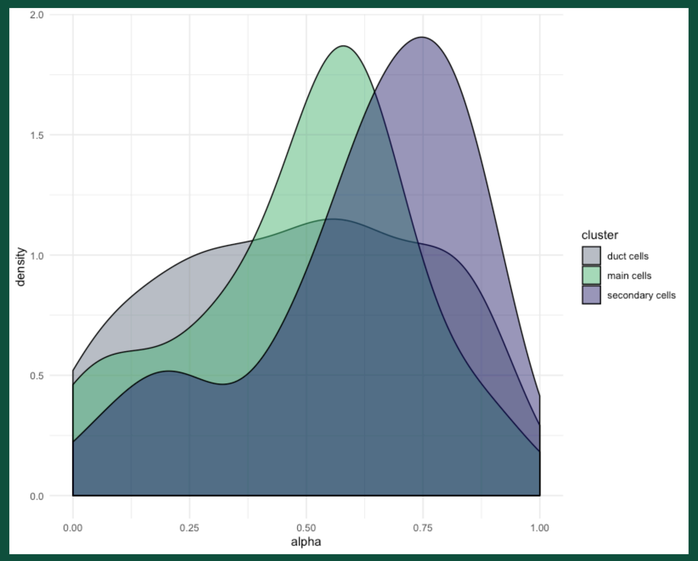
Objectives

Cell-type specific inference of adaptive evolution: To characterize recent evolution of AG cells, we are using the melanogaster subgroup. *Drosophila melanogaster* diverged from *D. yakuba* 3.4 million years ago[4], a much shorter amount of time than in previous studies of cell-type evolution.

A. Characterize among-species variation in main and secondary cell transcriptomes in three focal species.
B: Detect signatures of adaptive protein substitutions in genes differentially expressed across cell-types

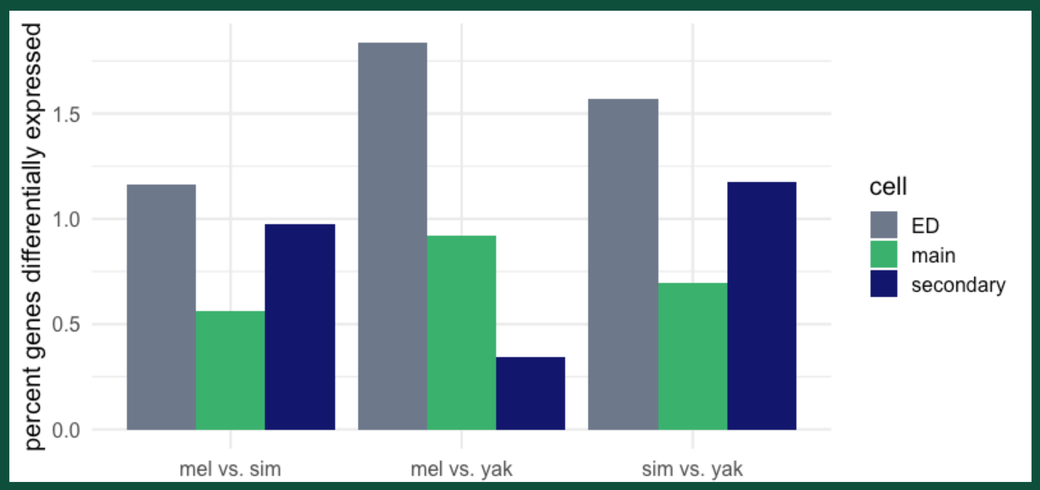
Methods

We dissected the accessory gland and ejaculatory duct and performed single-nucleus RNA-Seq using the 10X Genomics platform. We analyzed the data jointly across three species using Seurat. k-nearest neighbor was used to identify clusters, and UMAP was used to visualize clusters. We estimated differentially expressed genes using Limma. We calculated alpha (proportion of sites with adaptive substitution) from unpolarized MK tests between *D. melanogaster* and *D. simulans*.



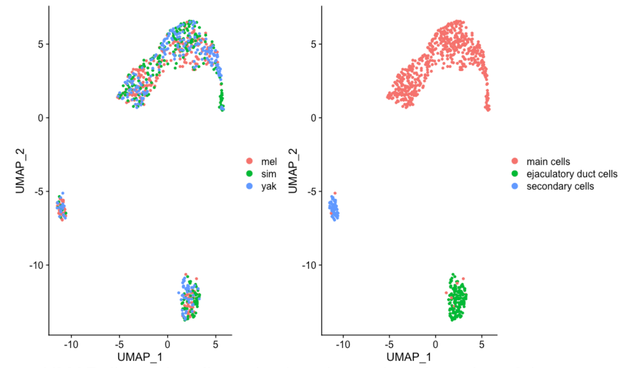
Among significant marker genes across three cell types, we find significant differences in the distribution of the level of adaptive substitutions. Secondary cell-expressed genes are significantly more enriched for adaptive substitutions, evidence of directional selection acting on these genes.

Kruskal-Wallis rank sum test
chi-squared = 9.8309, df = 2,
p-value = 0.007333

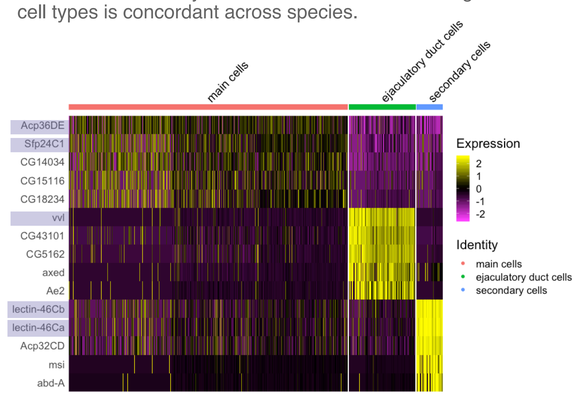


The percentage of expressed genes showing differential expression has both a cell-type and lineage-specific effect. In ejaculatory duct (ED) and main cells, we find that transcriptome divergence increases in the comparisons with the more distantly related species (*D. yak*). Interestingly, in secondary cells, there appears to be greater divergence in *D. sim* relative to the other species.

Results



UMAP dimensionality reduction shows that clustering of three cell types is concordant across species.



Relative expression of the top five most significant marker genes across cells in each of the three cell-type clusters. Gene names that are highlighted indicate marker genes already known from the literature. Interestingly, the hox gene abd-B is essential to development of secondary cells[7] and is well-studied in this context. Potential functions of abd-A in secondary cells are unknown.

Discussion

We observe heterogeneity in both the rate of transcriptome divergence and relative levels of adaptive substitutions across cell-types. While we see higher levels of adaptive protein divergence for secondary cell expressed genes, supporting our hypothesis, we unexpectedly observed the highest levels of transcriptome divergence in ejaculatory duct cells. We also observe a lineage effect suggesting higher divergence in secondary cell transcriptomes of *D. simulans*, but we cannot rule out potential technical impacts of background gene expression biasing these preliminary results. While the field of evolutionary development has contributed much to our understanding of cell type evolution over long evolutionary time scales, our study is among the first to explore the evolutionary dynamics of cell types among closely related species, making this a promising model for understanding how functional context influences evolution across cell types.

Future Aims

- apply background reduction techniques to strengthen inferences of cell-type markers and remove potential bias from differential expression analysis
- expand to other clades of Drosophila to capture the diversity of mating systems within the genus

Citations

[1]Patterson and Stone, 1952. Evolution in the genus Drosophila. *Macmillan*
[2]Kalb et al., 1993. Probing the function of *Drosophila melanogaster* accessory glands by directed cell ablation. *Proc Natl Acad Sci*
[3]Hopkins et al., 2019. BMP-signalling inhibition in Drosophila secondary cells remodels the seminal proteome, and self and rival ejaculate functions. *BioRxiv*
[4]Obbard et al., 2012. Estimating Divergence Dates and Substitution Rates in the Drosophila Phylogeny. *Mol Bio Evol*
[5]Stuart et al., 2018. Comprehensive integration of single-cell data. *Cell*
[6]Ritchie et al, 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nuc Ac Res*
[7]Gilvogov et al., 2013. A novel function for the hox gene abd-B in the male accessory gland regulates the long-term female post-mating response in Drosophila. *PLoS Genet*

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