# Differential targeting of the apical extracellular matrix is associated with extreme cell shapes accompanying morphological diversification in Drosophila genitalia



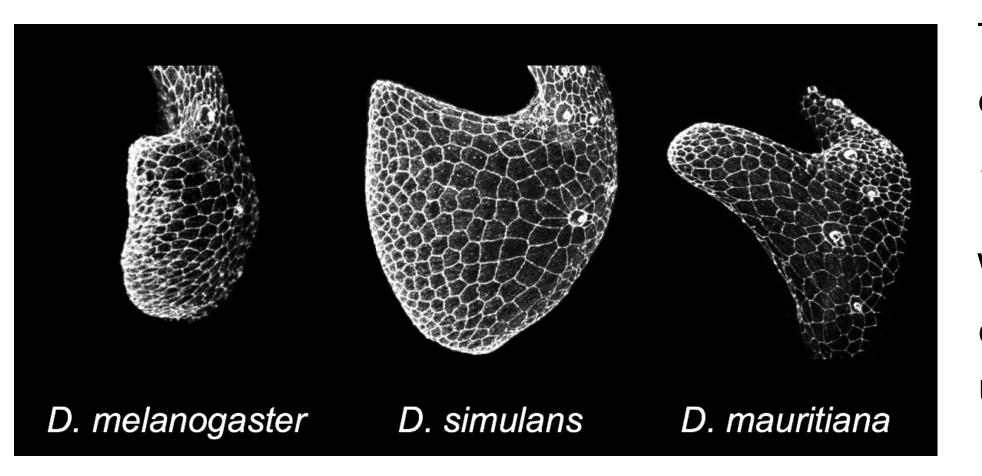
Ben J. Vincent<sup>1</sup> (he/him), Ana Pinharanda<sup>2</sup>, Eden McQueen<sup>1</sup>, Sarah J. Smith<sup>1</sup>, Peter Andolfatto<sup>2</sup> and Mark Rebeiz<sup>1</sup> <sup>1</sup>Department of Biological Sciences, University of Pittsburgh; <sup>2</sup>Department of Biological Sciences, Columbia University

\*Corresponding author: bjv22@pitt.edu; 🔰 @benjvincent @PinharandaA @ewmcqueen @RebeizLab

Our goal is to connect genetic changes between species with cellular phenotypes.



The posterior lobe – a model for quantitative studies in evolution and development.



The lobe is a genital structure used to distinguish closely related species in the Drosophila melanogaster clade.

We can measure gene expression and cellular morphology at high resolution using confocal microscopy.

**Figure 1: Developing posterior lobes in closely related Drosophilids.** Apical cell surfaces of pupal genitalia are labelled using E-Cadherin immunohistochemistry.

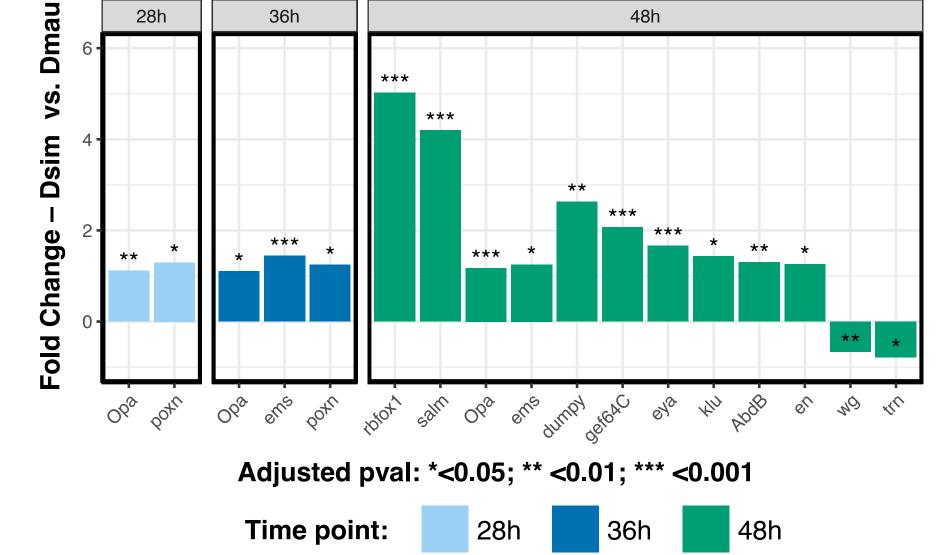
morphological change

genetic causes

cellular effects

### Finding genes that underlie lobe diversity with QTL mapping and RNA-seq

 Compare gene expression in **individual species** –
Changes could be due to differences in regulation of the gene itself, or any upstream regulators (*cis* changes or *trans* changes). Posterior lobe network genes



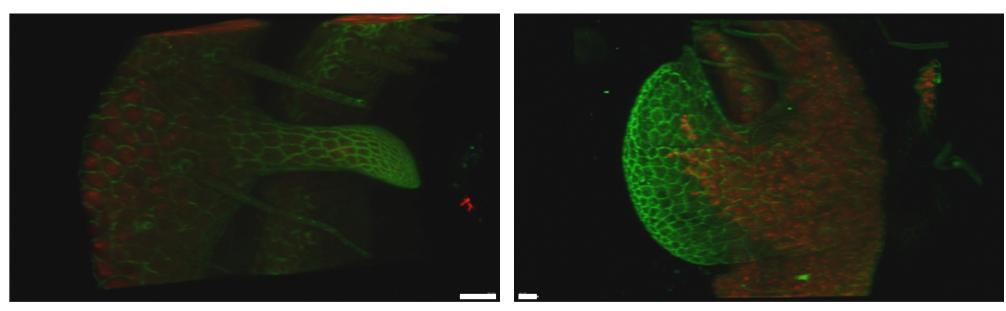
**Figure 2: Differentially expressed genes in the posterior lobe network.** RNA-seq data from pupal genitalia at 28h, 36h or 48h after puparium formation reveals differences in gene expression between *D. simulans* and *D. mauritiana.* Genes shown here have been implicated in genital development in *D. melanogaster.* 

#### Genes that colocalize with one QTL peak

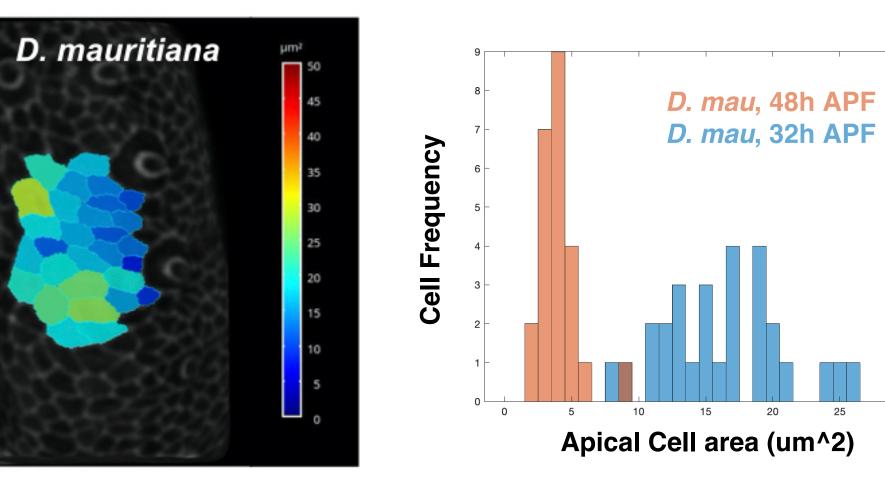
## Quantifying cell number, size and shape with immunofluorescence and image processing

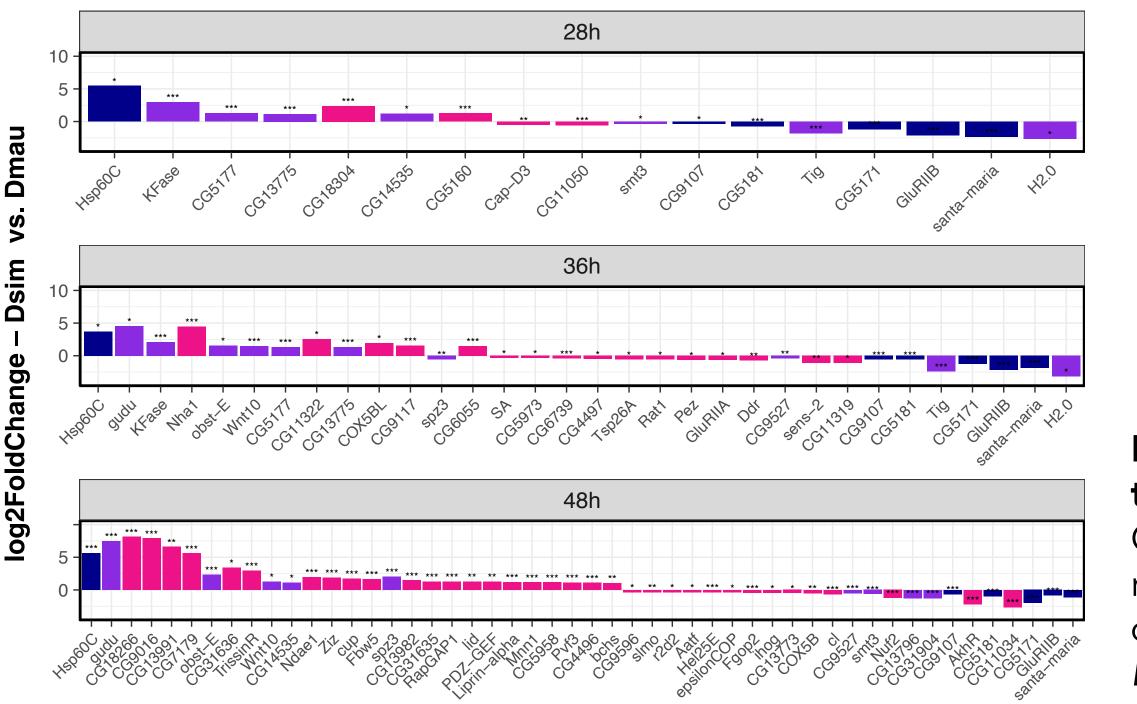
Figure 5: Cells adopt distinct morphological fates during posterior lobe development. Top: mature lobes in different species. Apical cell borders are labeled in green using E-cadhering immunofluorescence, and cell nuclei are labelled in red using SYTOX green. Samples collected 56 hours APF. Bottom left: We used MorphographX to segment individual cells and measure their apical area. Cells are labelled as a heat map with cooler colors indicating cells with smaller apical areas. Bottom right: We can quantify changes in apical cell area as the posterior lobe develops. In late states, the apical area of cells at the tip of the lobe become progressively smaller. This histogram plots the frequency of cells as a function of area in individual *D. mau* lobes at different stages of development.

D. mauritiana

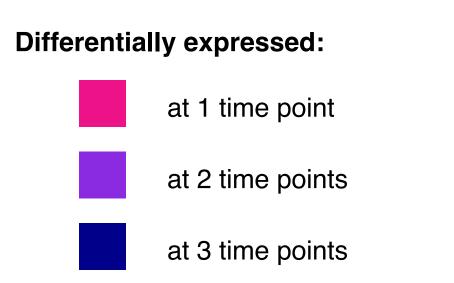


D. simulans





Adjusted pval: \*<0.05; \*\* <0.01; \*\*\* <0.001



**Figure 3: Differentially expressed genes that co-localize with a OTL peak on 2L.** Combining RNA-seq and QTL mapping reveals candidate genes that many underlie differences in lobe size and shape between *D. simulans* and *D. mauritiana*.

#### Posterior spiracle genes that colocalize with QTL peaks

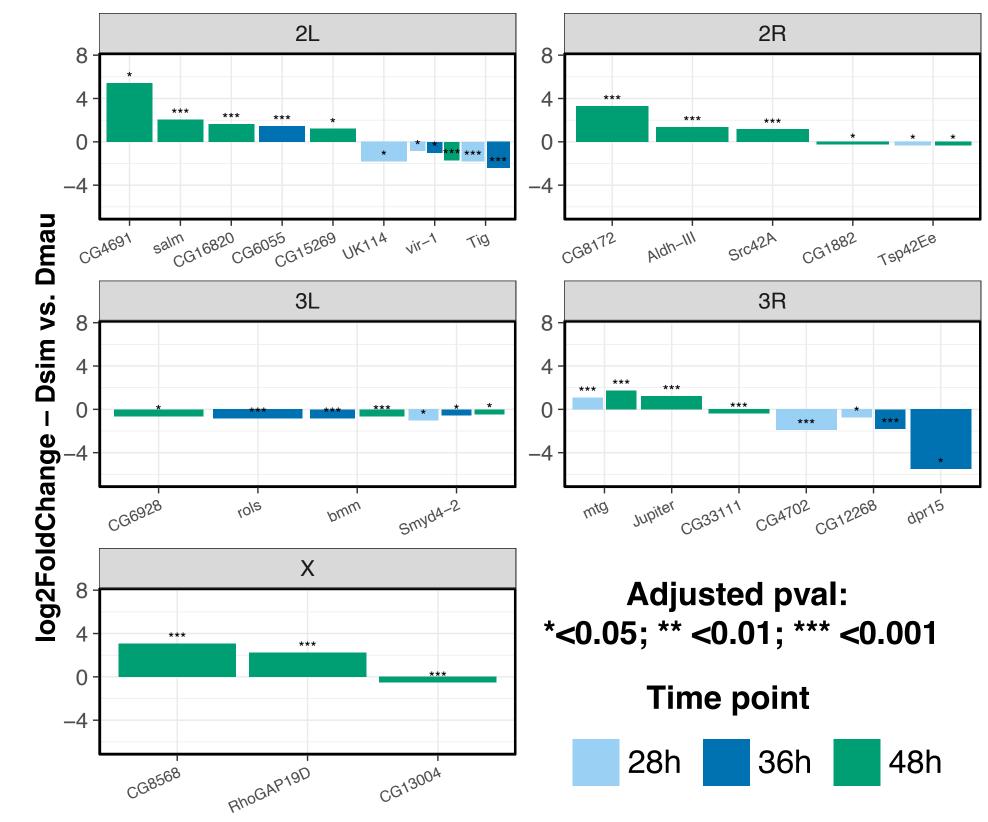


Figure 4: Differentially expressed genes in the posterior spiracle network that co-localize with QTL peaks. The gene regulatory network that governs lobe development was co-opted from a different structure – the well-studied posterior spiracle. RNA-seq reveals that genes governing spiracle development also lie within QTL associated with lobe diversification.

## We found differences in expression patters for genes associated with the apical extracellular matrix (aECM).

#### dumpy RNA piopio RNA

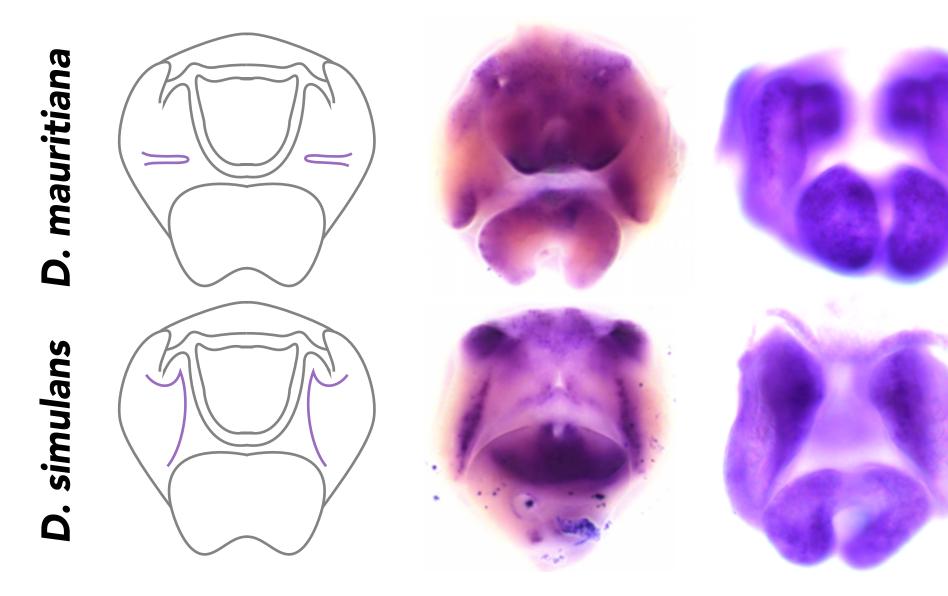
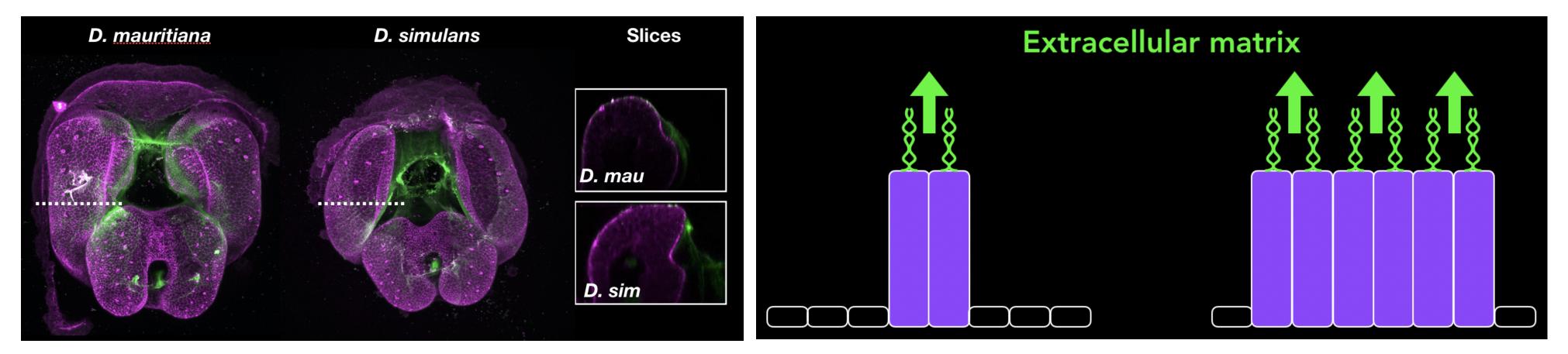


Figure 6: Expression patterns for genes associated with the aECM correlate with lobe size in different species. We used *in situ* hybridization to label RNA for aECM-associated genes in whole genital samples at 32h APF. *D. simulans* patterns were expanded in areas near the developing posterior lobe. We believe that these expression changes may contribute to structural differences in the aECM between species, which may exert different external forces on the cells during development.

### We can visualize differences in the aECM using fluorescent lectins.



Compare expression in **hybrids** –

changes in individual genes must be due to changes in regulatory sequences for those genes (*cis* changes, not *trans* changes).

**Coming soon - stay tuned!** 

In conclusion, Drosophila genitalia are completely rad and I think they're the future for quantitative studies of evolution and development.

**Figure 7: External forces during development may contribute to changes in genital morphology between species.** Left: We use fluorescent lectins (vicia villosa, green) to label the aECM in different species. We find a tight association between aECM and the edge of the developing lobe in *D. simulans,* which suggests that it may play a role in its shape. Right: a working model for how changes in the expression of aECM components may contribute to morphological diversification in the posterior lobe. Differences in *dumpy* expression may commit more cells to extreme morphological fates in *D. simulans* compared to *D. mauritiana*.

Thanks to the entire Rebeiz lab for constant support and advice.

This project is supported by NIH grants R01GM112758 (to PA and MR) and F32GM130034 (to BJV).

