

## Introduction - Supplemental Figure Legends

**Supplemental Figure 1:** Total genomic coverage of Transposable Element clusters across the *Glossina* genomes in order of abundance and colored by conservation across species. A. Conservation of LINE/CR1 type clusters across the genomes of the six tsetse species. B. Conservation of the DNA/mariner type clusters across the genomes of the six tsetse species. Abbreviations: G. aus (*Glossina austeni*), G. bre (*Glossina brevipalpis*), G. fus (*Glossina fuscipes*), G. mor (*Glossina morsitans*), G pal (*Glossina pallidipes*), G. pap (*Glossina palpalis*)

**Supplemental Figure 2:** Maximum Likelihood, Bayesian and Astral based phylogenetic analysis of a concatenated single gene ortholog alignment. A: Maximum likelihood tree inferred by Raxml on a concatenated alignment of 117,783 amino acids from 286 genes and employing the LG+G+F model of replacement. Value at nodes are bootstrap supports from the consensus of 100 pseudoreplicates in Raxml. B: Bayesian phylogenetic tree inferred with Phylobayes 3 using CAT+G replacement model, supports at nodes are posterior probabilities; C: Astral analysis based on 286 genes with bootstrap support.

**Supplemental Figure 3:** Tsetse variable mitochondrial DNA sequence and species delineation by high resolution melt curve analysis

**Supplemental Figure 4:** Application of high-resolution melt curve analysis to distinguish Tsetse haplotypes/populations

**Supplemental Figure 5:** The percent of female-, male-, and un-biased genes that are on an X chromosome scaffold (Muller elements A, D, or F) is plotted for each species. Sex-biased expression was measured between males and either lactating (L) or non-lactating (NL) females. Asterisks indicate a significant difference between the percent of sex-biased genes that are X-linked when compared to unbiased genes (\* $P < 0.05$  in Fisher's exact test).

**Supplemental Figure 6:** The distribution of the log<sub>2</sub> (fold-change between females and males) is plotted for autosomal and X-linked genes in each species. Female-male gene expression comparisons are between males and either lactating or non-lactating females.

**Supplemental Figure 7:** Rates of non-synonymous to synonymous substitution (dN/dS) along different evolutionary lineages within the *Glossina* genus.

**Supplemental Figure 8:** Rates of non-synonymous to synonymous substitution (dN/dS) rates of female, male and non-sex biased genes across the X and autosomal muller elements.

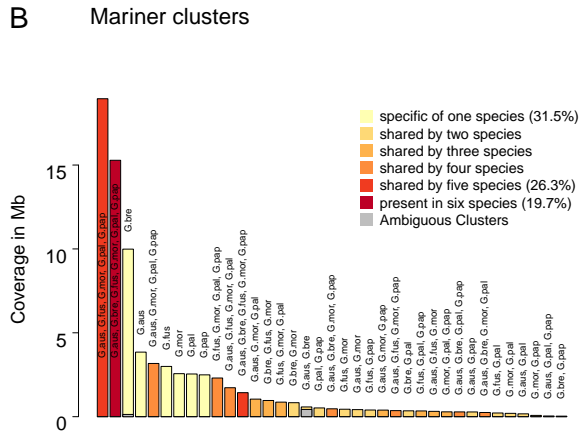
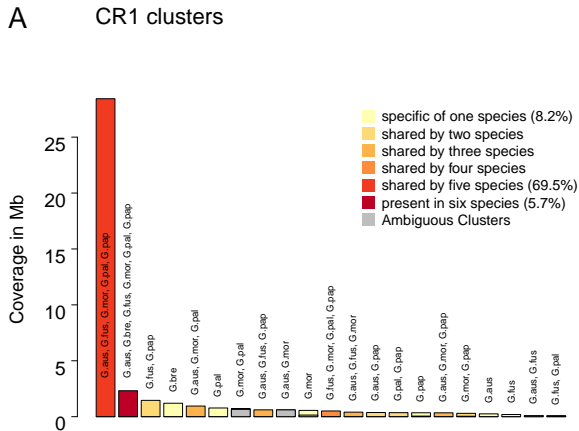
**Supplemental Figure 9:** The distribution of male biased gene expression across the predicted *Glossina* Muller Elements. Bar heights represent the percentage of genes per element with male biased gene expression.

**Supplemental Figure 10:** Rates of non-synonymous to synonymous substitution (dN/dS) rates across the predicted muller elements.

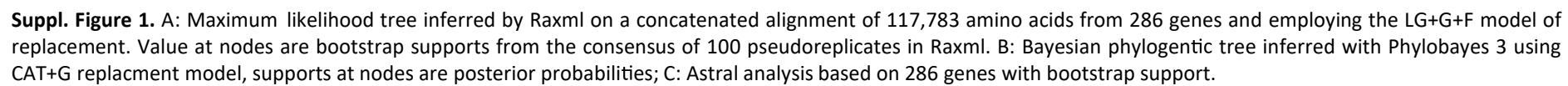
**Supplemental Figure 11: Sub-genus specific gene family expansions/retractions (with orthology group number annotations).** Principal component analysis-based clustering of gene orthology groups showing significant differences in the number of representative sequences between the six *Glossina* species.

**Supplemental Figure 12: Distribution of transcription factor families across insect genomes.** Heatmap depicting the abundance of transcription factor (TF) families across a collection of insect genomes. Each entry indicates the number of TF genes for the given family in the given genome, based on presence of DNA binding domains. Color key is depicted at the top (light blue means the TF family is completely absent) – note log (base 2) scale.

Supplemental Figure 1 : Total genomic coverage of TE clusters across the Glossina genomes in order of abundance and colored by conservation across species

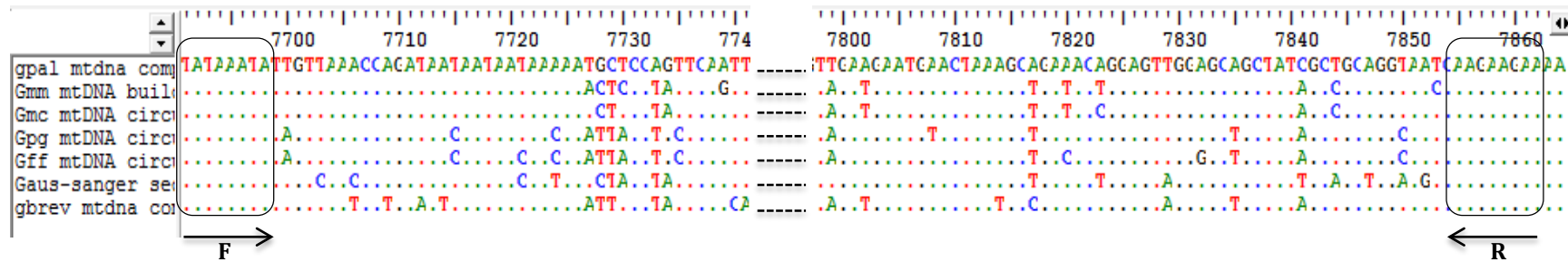


**A**

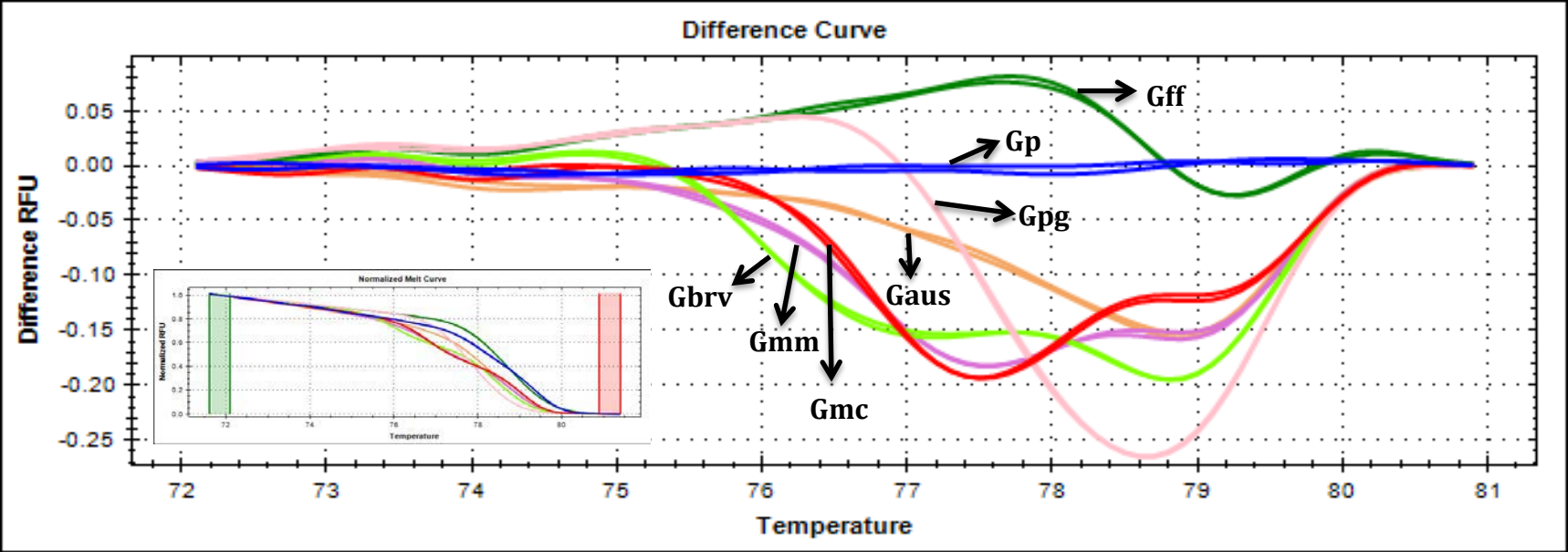


Supplemental Figure 3

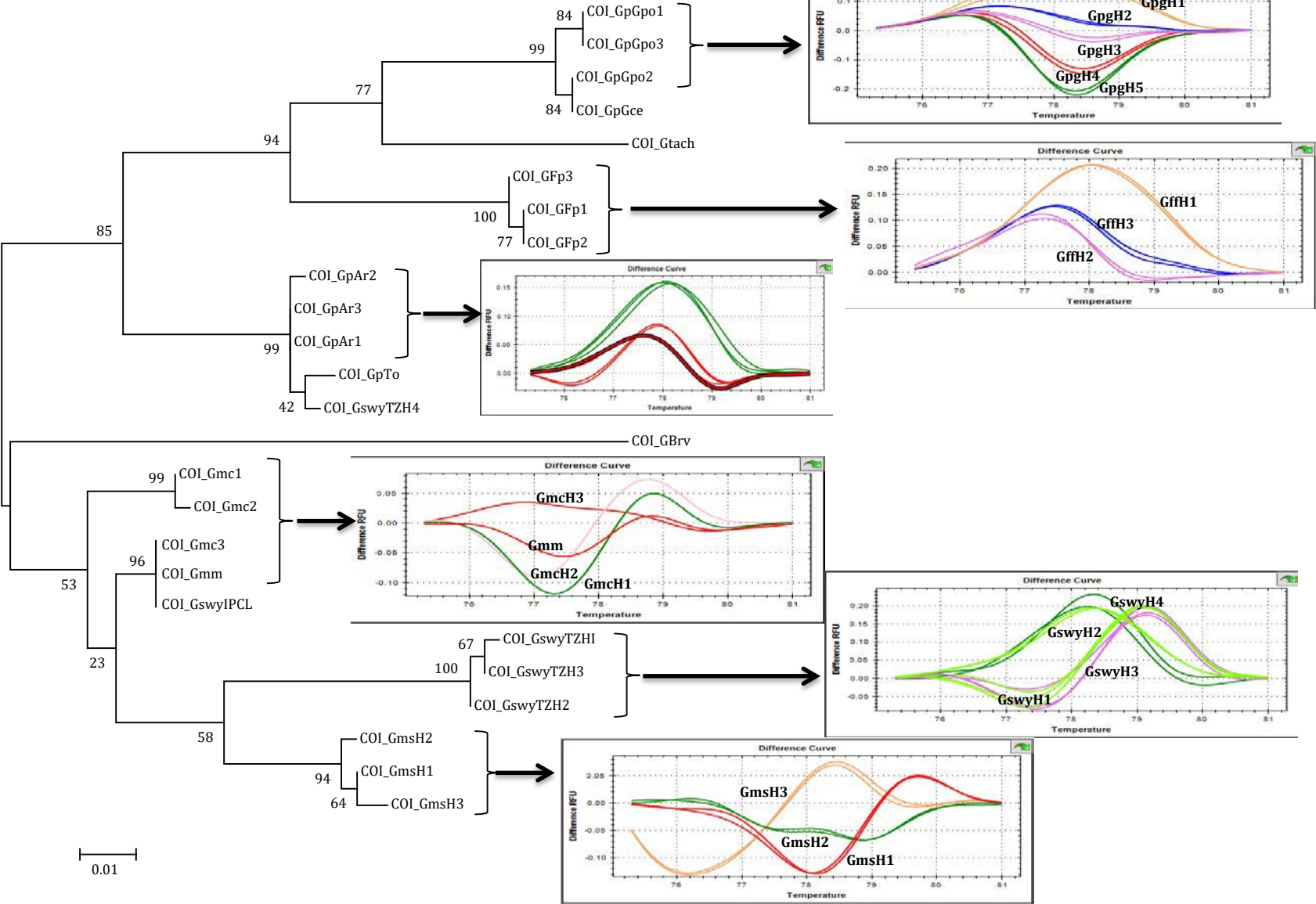
A



B

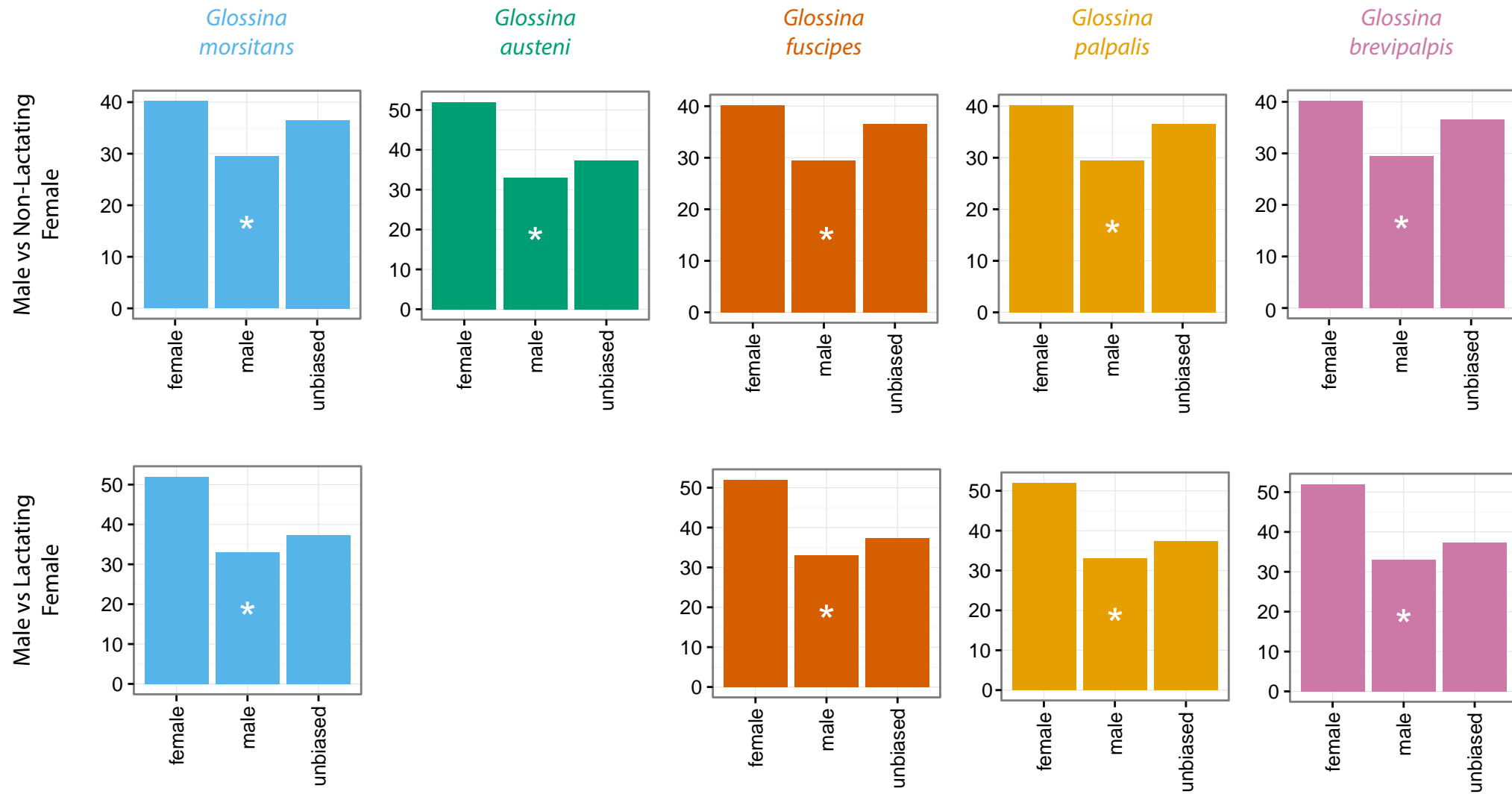


Supplemental Figure 4

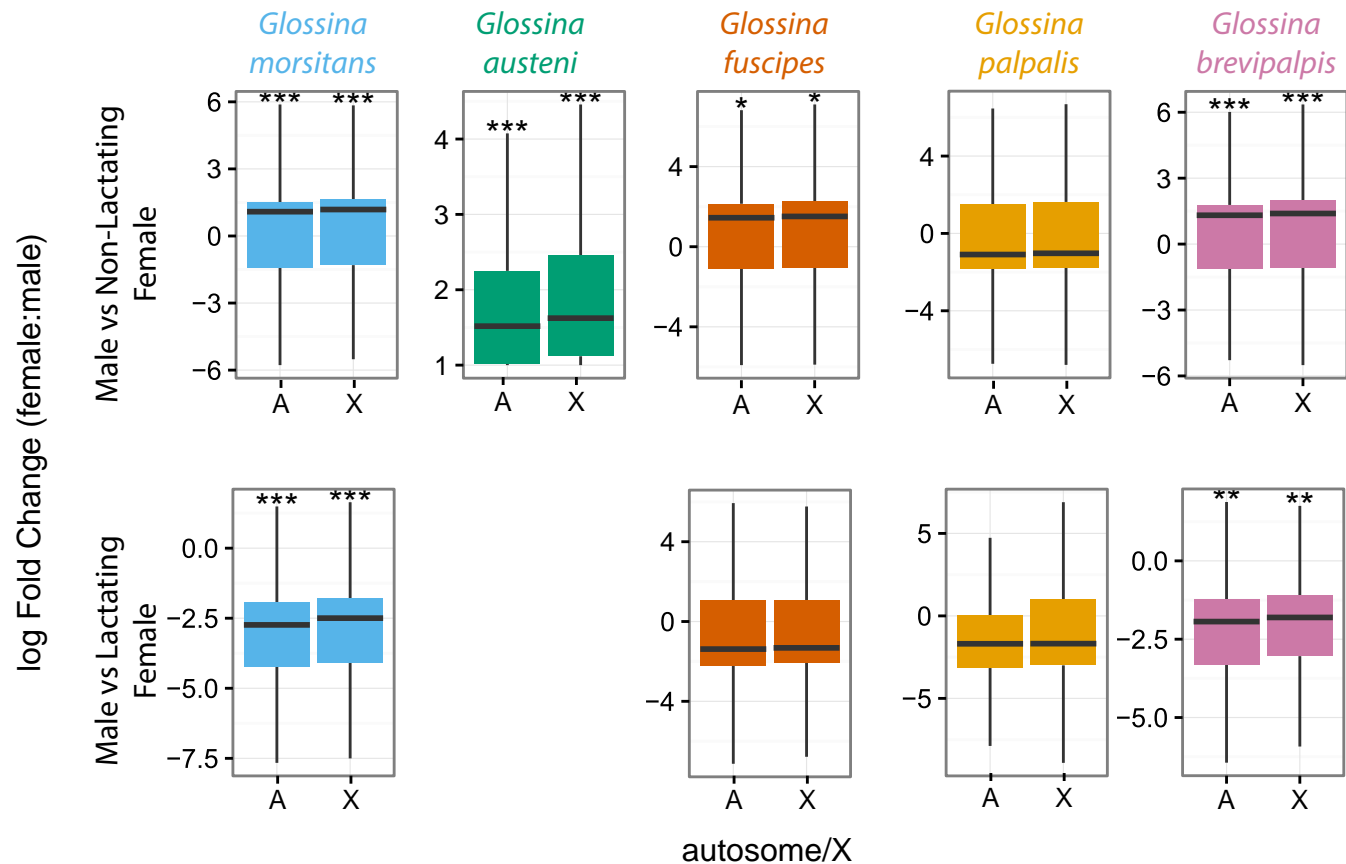


Supplemental Figure 5

% X-linked

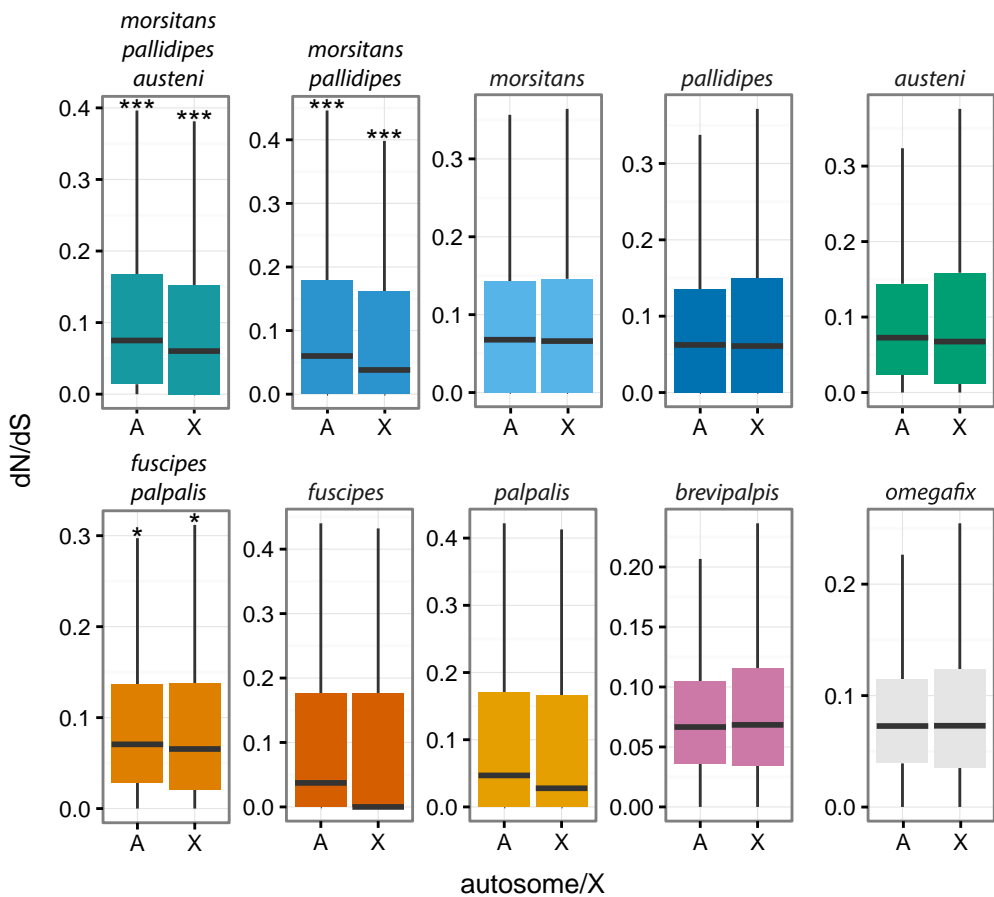


Supplemental Figure 6

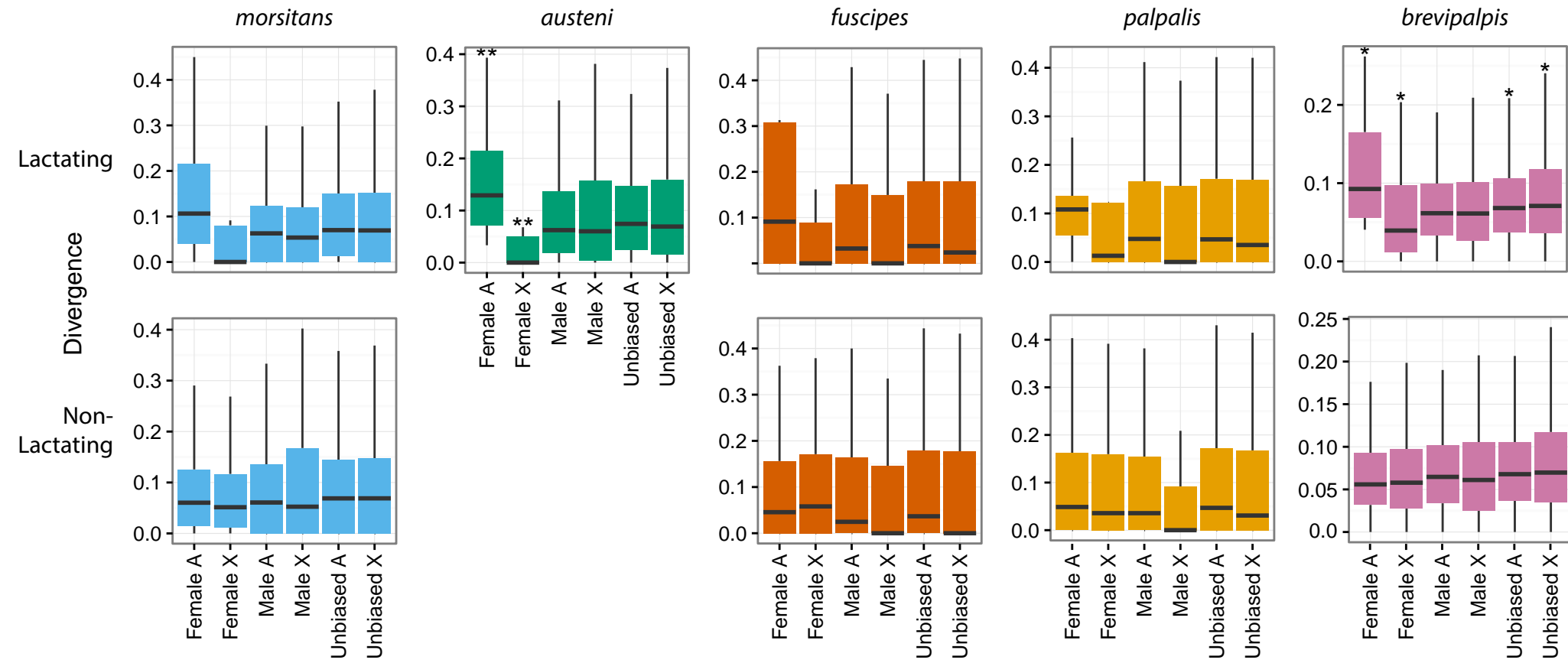




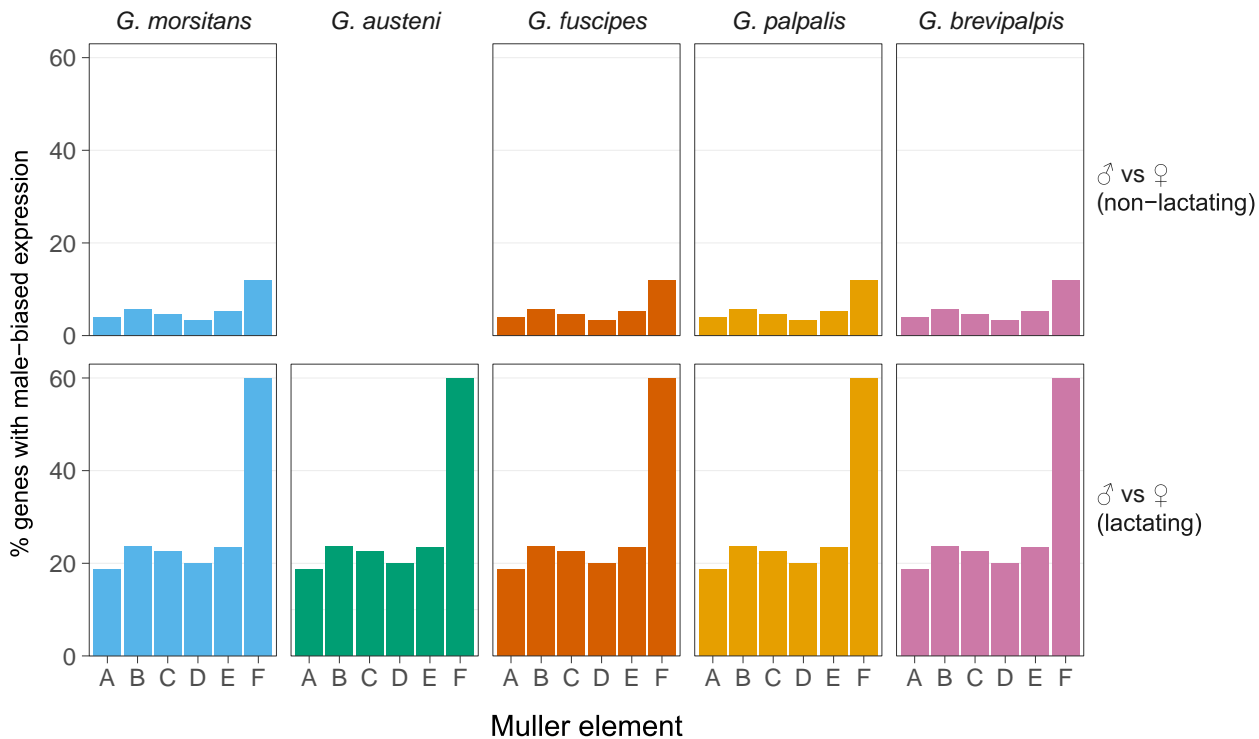
Supplemental Figure 7



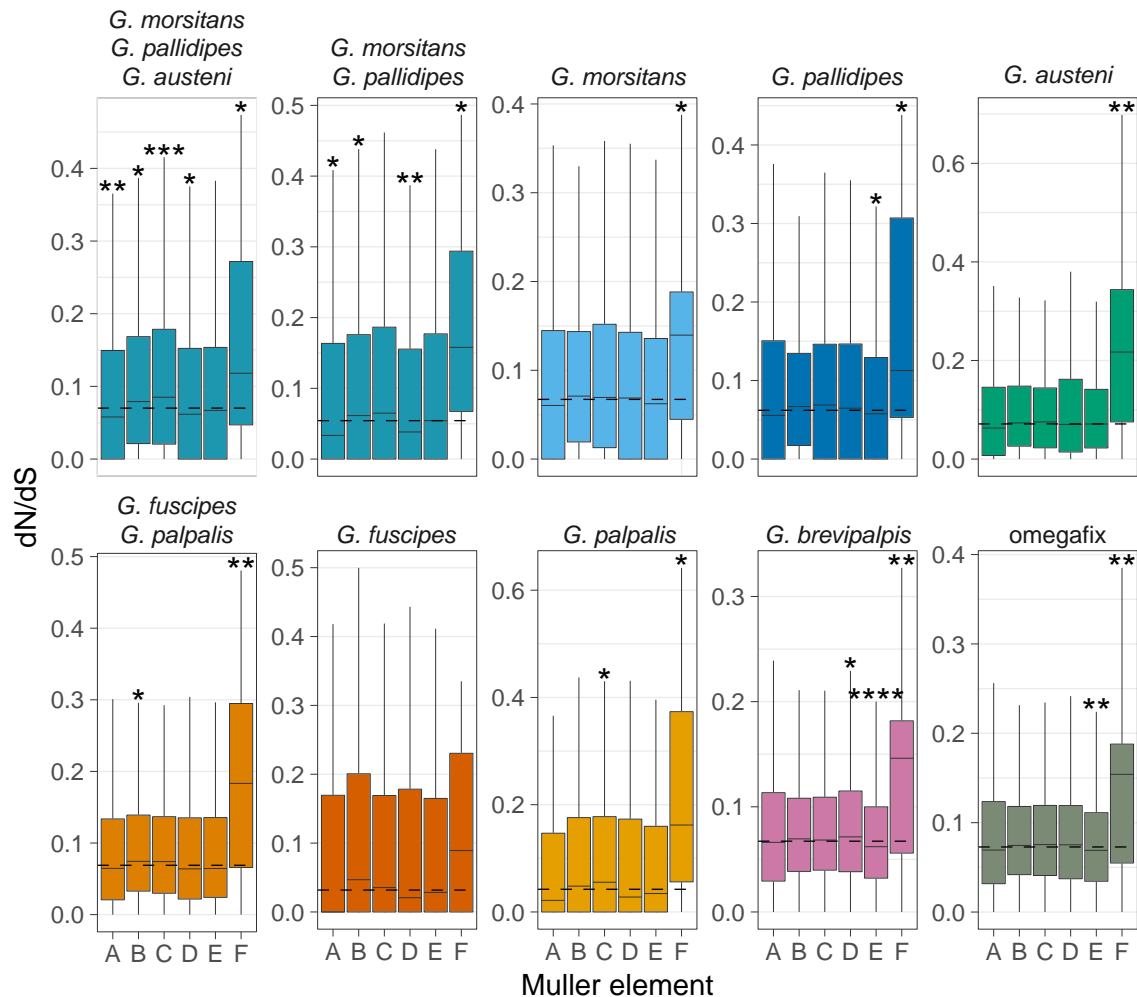
Supplemental Figure 8



# Supplemental Figure 9



Supplemental Figure 10



**Groups**

- Fusca Expanded
- Fusca Reduced
- Morsitans Group Expanded
- Morsitans Group Reduced
- Palpalis Group Expanded
- Palpalis Group Reduced

**Dim1 (77.4%)**

**Dim2 (13.2%)**

**Palpalis Group Reduced**

**brevipalpis Expanded**

**brevipalpis Reduced**

**Morsitans Group Expanded**

**Morsitans Group Reduced**

**Palpalis Group Expanded**

**Fusca Expanded**

**Fusca Reduced**

VBGT0082000045940

VBGT00770000031214

VBGT00820000046004

VBGT00820000045950

VBGT00840000047885

VBGT00840000047886

VBGT00840000047828

VBGT00820000045989

VBGT00190000009725

VBGT00190000009711

VBGT001900000011642

VBGT001900000009808

VBGT001900000009793

VBGT001900000009849

VBGT001900000013689

VBGT001900000010265

VBGT001900000012451

VBGT001900000012301

VBGT001900000010375

VBGT001900000009945

VBGT001900000010218

VBGT001900000010512

VBGT001900000010664

VBGT001900000010980

VBGT001900000009926

VBGT001900000010913

VBGT001900000009892

VBGT001900000009794

VBGT001900000016440

VBGT001900000012881

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VBGT00190000001176

VBGT00770000031191

VBGT00190000001176

VBGT00780000038281

VBGT001900000014373

VBGT001900000016667

VBGT001900000016741

VBGT001900000015811

VBGT001900000014894

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VBGT00750000029331

VBGT001900000012418

VBGT001900000012100

VBGT00770000031281

VBGT001900000014659

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VBGT001900000014141

VBGT001900000011125

VBGT001900000010757

