Early transcriptome measurements in the lower reproductive tract of mated *Drosophila melanogaster* females reveal changes in RNAs involved in wound healing, neuronal remodeling, and transcripts derived from the male.

Delbare S.Y.N., Clark A.G., Wolfner M.F.
Department of Molecular Biology and Genetics, Cornell University, Ithaca NY, USA

INTRODUCTION

The female lower reproductive tract is exposed directly to the male's ejaculate during and after mating, making it a hotspot for mating-induced responses**. In *D. melanogaster*, mating induces changes in the reproductive tract's epithelia, muscles and innervation, to prepare the tract for fertilization events[1, 2, 3]. Currently, our knowledge of the molecular players that underlie these changes in the reproductive tract, is limited**. Here, we measure changes in the abundance of coding and noncoding RNAs within 35 min after the start of mating (ASM), to capture the earliest transcriptional responses to mating in the reproductive tract.

METHODS & DESIGN

- **Sample collection and strains used**: Oregon R females X *w*^{1118} males → collected 30-35 min ASM
  Oregon R virgin females → collected in parallel
  3 replicates of ~60 pooled females each.
- **Dissections**: female lower reproductive tract

→ Excludes ovaries, most of common oviduct.

→ Includes bursa, sperm storage organs, part of surrounding fat body, most distal part of common oviduct.

- **Library Prep**: Ovation ® RNA-seq System (TECAN) (No poly-A selection, but depletion of rRNAs).
- **Identification of differentially expressed (DE) genes**: edgeR[4]: comparison of virgin vs. mated females.
- **Identification of RNAs transferred by males**: SNP calling using GATK[5]; assignment of reads to "female" (Oregon R) or "male" (*w*^{1118}) genotype using SNPsplit[6].

RESULTS 1) MOST DIFFERENTIALLY EXPRESSED GENES ARE UPREGULATED AFTER MATING.

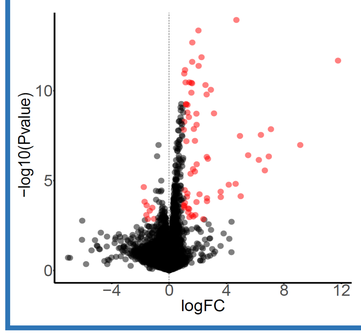


Fig. 1: Volcano plot showing results of DE analysis.
76 differentially expressed genes with q ≤ 0.05 and undergoing at least a 2-fold change in RNA abundance are shown in red.
An additional 223 genes undergo a significant change in expression (q ≤ 0.05), but with a smaller than 2-fold change. The majority of DE genes are upregulated post-mating (logFC > 0).

RESULTS 2) GO ANALYSIS IDENTIFIES SIGNIFICANT ENRICHMENT OF PROCESSES RELATED TO DEVELOPMENT, DEFENSE/ WOUNDING, TRANSCRIPTION AND REPRODUCTIVE BEHAVIOR.

Fig. 2: Upset plot listing enriched GO categories, the number of DE genes that fall into each category (Set Size) and how many DE genes are shared between GO categories (Intersection Size).

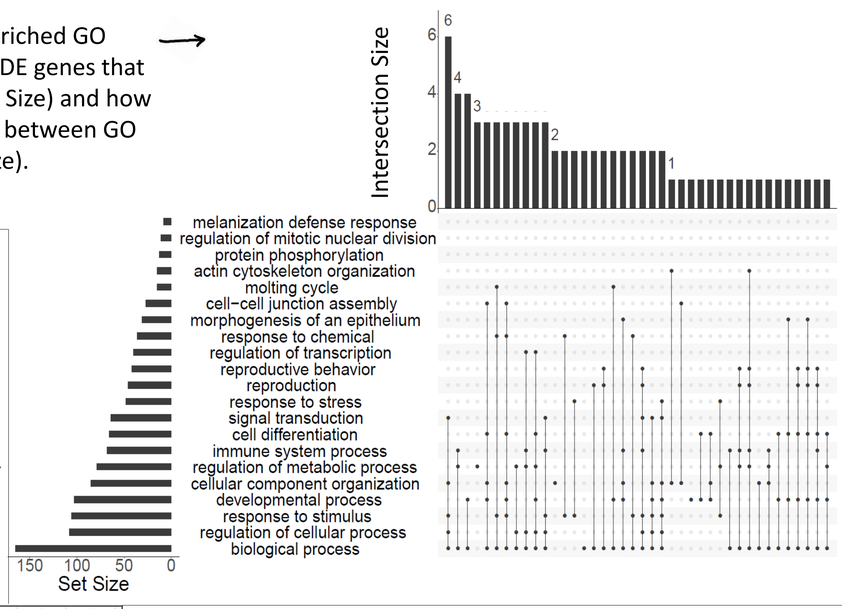
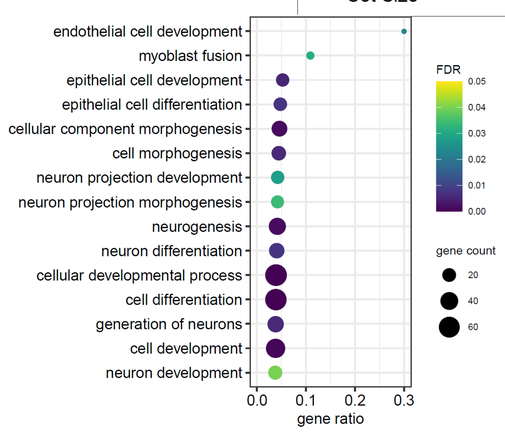


Fig. 3: Dotplot showing enriched GO categories that fall under the term "Developmental process". Dot size reflects gene number; dot color reflects p-value after correcting for multiple testing.



RESULTS 3) 18 NONCODING RNAs CHANGE IN ABUNDANCE AFTER MATING.

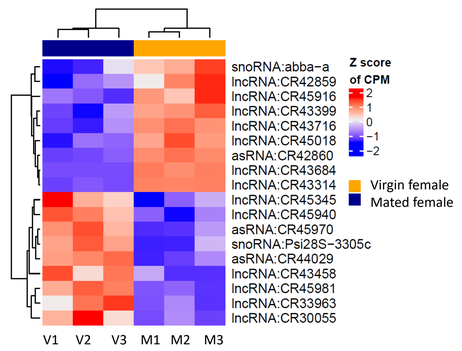


Fig. 3: Heatmap showing changes in the abundance of 18 noncoding RNAs. V = virgin female; M = mated female. CPM = counts per million

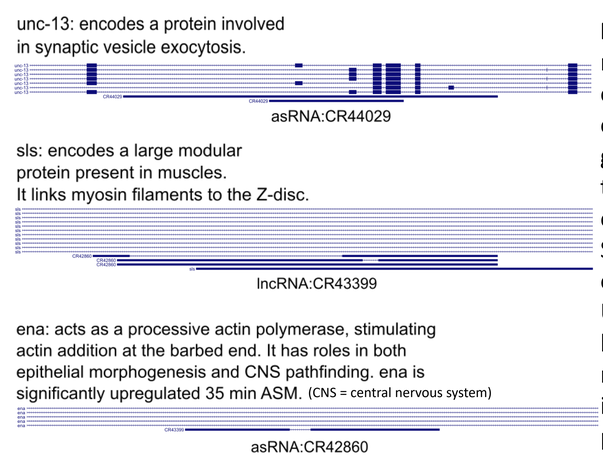


Fig. 4: Of 18 noncoding RNAs, 6 overlap with introns of protein-coding genes with roles in the nervous system or muscle function. Shown here are 3 examples, with UCSC genome browser tracks of 3 ncRNAs and introns/exons of protein-coding genes.

RESULTS 4) TRANSCRIPTION FACTOR BINDING SITES UPSTREAM OF DE GENES IDENTIFY 39 TRANSCRIPTION FACTORS THAT POTENTIALLY RESPOND TO MATING TO REGULATE DE GENES.

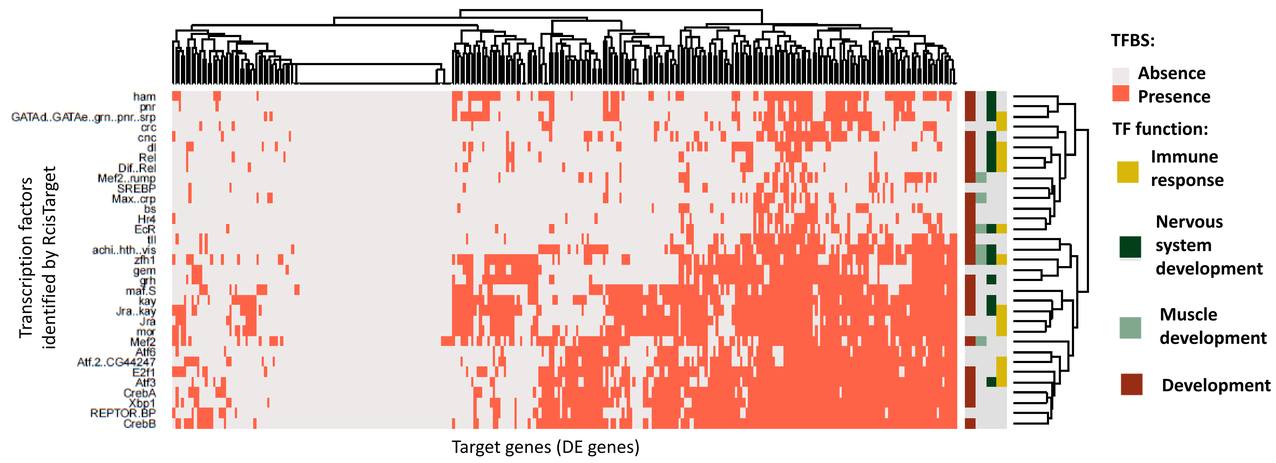


Fig. 5: Heatmap indicating the presence of putative transcription factor binding sites (TFBSs) as determined by analysis using the R package RcisTarget[7]. Many of the transcription factors identified by RcisTarget have known roles in development and the immune response.

RESULTS 5) MALES TRANSFER RNAs TO FEMALES DURING MATING.

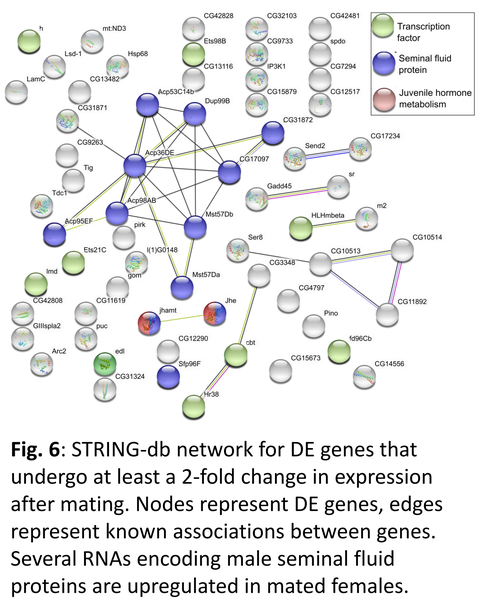


Fig. 6: STRING-db network for DE genes that undergo at least a 2-fold change in expression after mating. Nodes represent DE genes, edges represent known associations between genes. Several RNAs encoding male seminal fluid proteins are upregulated in mated females.

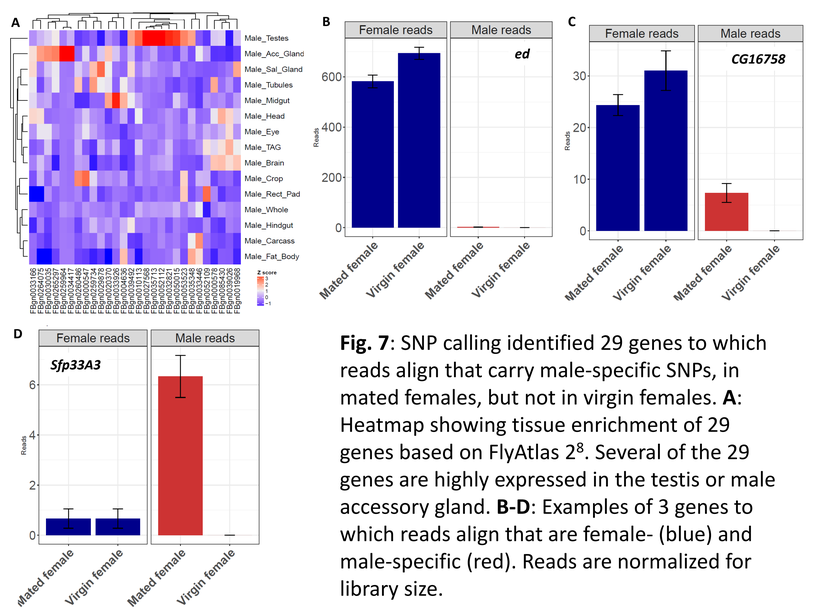


Fig. 7: SNP calling identified 29 genes to which reads align that carry male-specific SNPs, in mated females, but not in virgin females. **A**: Heatmap showing tissue enrichment of 29 genes based on FlyAtlas 2[8]. Several of the 29 genes are highly expressed in the testis or male accessory gland. **B-D**: Examples of 3 genes to which reads align that are female- (blue) and male-specific (red). Reads are normalized for library size.

CONCLUSIONS:

Our RNA-seq analysis identifies many genes that likely contribute to remodeling of epithelia and muscles of the female reproductive tract, and the nervous system surrounding the tract. Several DE genes encode transcription factors, indicating that mating activates transcriptional cascades to mediate these changes. Using RcisTarget[7], we identified transcription factors that could act upstream of the DE genes. Future studies could determine whether these transcription factors are present, as proteins, in virgin female reproductive tracts, and whether they activate transcription in response to mating. Additional changes in gene expression reflect a response to copulatory wounding[1] and indicate that male RNAs are transferred to the female during mating. Whether these male RNAs are functional in the female is unknown. Intriguingly, DE long noncoding and antisense RNAs are present in loci that also harbor genes involved in muscle or nervous system function, making it possible that these ncRNAs play a regulatory role in the remodeling of the tract.

ACKNOWLEDGEMENTS, FUNDING & REFERENCES:

We thank A. Jain for help with library prep, the Cornell Genomics Facility for sequencing, E. Cosgrove for help with assembling a SNP calling pipeline, and RO1 HD059060 to AGC and MFW for funding this project. **1.** Mattei et al. (2015). Integrated 3D view of postmating responses by the Drosophila melanogaster female reproductive tract, obtained by micro-computed tomography scanning.*PNAS, 112*(27), 8475–8480. **2.** Kapelnikov et al. (2008). Tissue remodeling: a mating-induced differentiation program for the Drosophila oviduct. *BMC Dev Bio*, *8*, 114. **3.** Heifetz et al. (2014). Mating regulates neuromodulator ensembles at nerve termini innervating the Drosophila reproductive tract. *Curr. Biol. 24*(7), 731–737. **4.** Robinson et al. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinform*, Vol. 26, pp. 139–140. **5.** https://software.broadinstitute.org/gatk/documentation/article.php?id=3891 **6.** Krueger et al. (2016). SNPsplit: Allele-specific splitting of alignments between genomes with known SNP genotypes. *F1000Res. 5*, 1479. **7.** Aibar et al. (2017). Single cell regulatory network inference and clustering. *Nat. Methods* doi:10.1038/nmeth.4463 **8.** Leader et al. (2018). FlyAtlas 2: a new version of the Drosophila melanogaster expression atlas with RNA-Seq, miRNA-Seq and sex-specific data. *NRA 46*(D1), D809–D815.