



COMPARATIVE HEAD TRANSCRIPTOME ANALYSIS IN TWO SPECIES OF THE *DROSOPHILA ELEGANS* SPECIES SUBGROUP

Fangning Luo, Department of Ecology and Evolutionary Biology
Laboratory of José M. Ranz



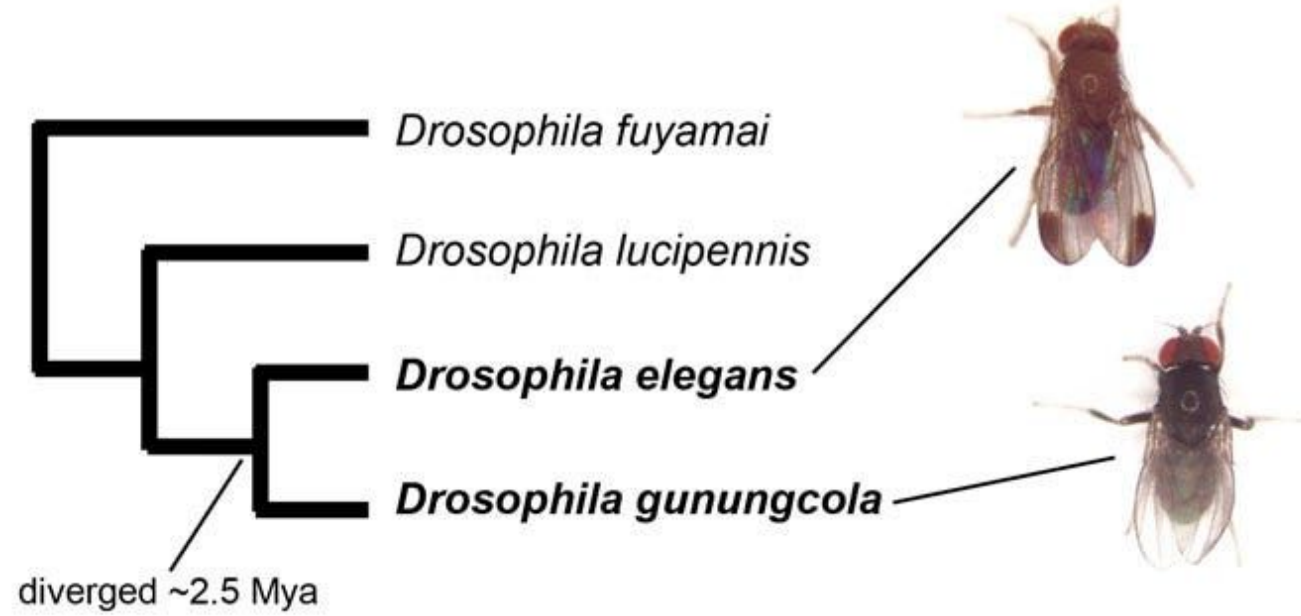
UCI BioSci

1. Abstract

The molecular basis of interspecific trait evolution remains poorly understood. The sister species *Drosophila elegans* and *D. gunungcola* have diverged in one component of their courtship behavior, the frontal wing display in males. This study investigates the genes and pathways that have diverged in expression between the two species in relation to frontal wing display, and more generally how their head transcriptome has become differentiated between sexes and developmental stages. The analysis of RNAseq data from males and females of both species at two different stages (0 days and 5 days) revealed that roughly 30% of the expressed genes were differentially expressed between the sexes, developmental stages, or both. The number of differentially expressed genes between stages within the same sex was substantially larger than the number of differences between the sexes for the same stage. Focusing on differentially expressed genes at Day 5 in males, the sex that features the interspecific phenotypic difference, the search for overrepresented functional terms uncovered functional signatures potentially related to wing display behavior and mating duration. Likewise, in male-biased genes in expression in Day 5, functional terms associated with synaptic transmission and signaling were significantly overrepresented. These findings provide new insights into the identity of genes and pathways potentially relevant for neural circuit functioning during courtship rituals while informing about candidate genes to be targeted in future functional studies.

2. Introduction

- Drosophila elegans* and *D. gunungcola* are sister species belonging to the *D. elegans* species subgroup within *D. melanogaster* species group.
- D. elegans* shows male-specific wing pigmentation and frontal wing display while *D. gunungcola* does not show either trait (Massey et al., 2020).
- Frontal wing display dance during courtship involves males extending their wings outward, positioning their dorsal wing surfaces towards the female, and moving them in an up-and-down motion (Massey et al., 2020)
- The genetic basis for courtship remains unknown in *D. elegans*.
- D. elegans-D. gunungcola* is an excellent system for studying the molecular evolution of trait divergence in the context of sexual selection.



3. Goal and Hypothesis

Goal: identify candidate genes and pathways that might underlie the divergence between two sister species in the frontal wing mating display which is exclusively present in *D. elegans* and absent in *D. gunungcola*.

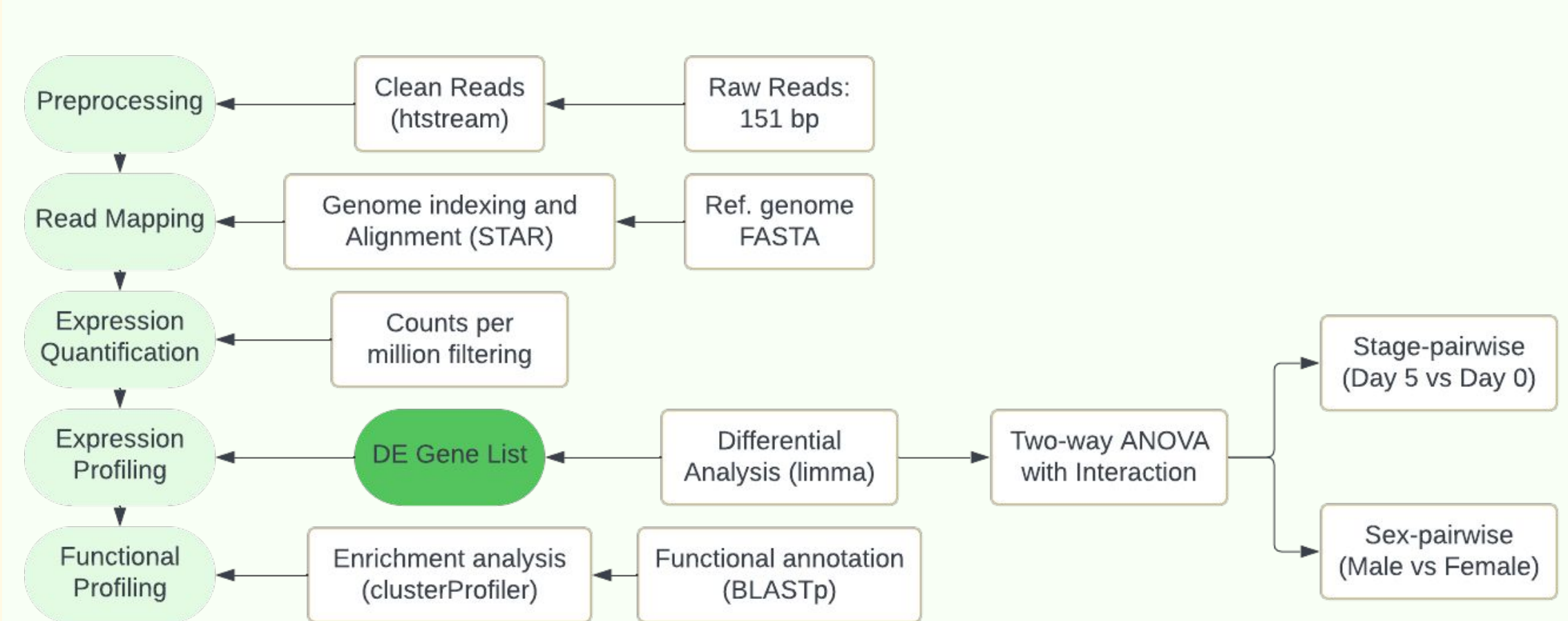
Hypothesis: *D. elegans* and *D. gunungcola* have diverged in gene expression in the brain area, with genes responsible for wing display being differentially expressed in relation to *D. elegans*. These differences should be sexually dependent.

4. Material and Methods

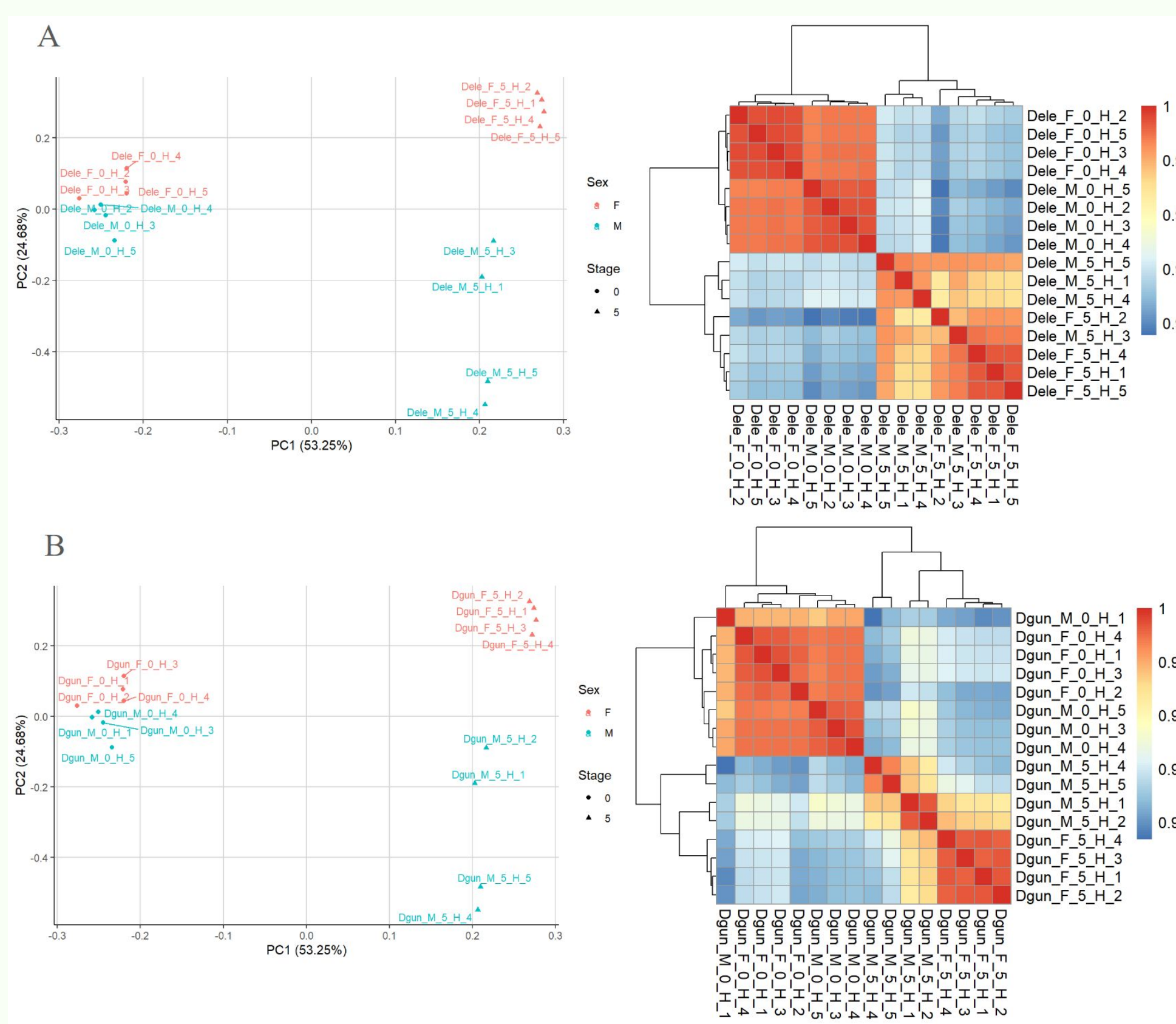
Material: Thirty-two *D. elegans* and *D. gunungcola* RNA-seq libraries from NCBI SRA archive under BioProject PRJNA837195.

- Paired-end 150 bp RNA-seq reads obtained from the head from two sexes
- Collection from two life stages, one from young adult phase (0-3 hr after pupal stage, Day 0), one from sexually matured adults (5 days after pupal stage, Day 5)

Methods:



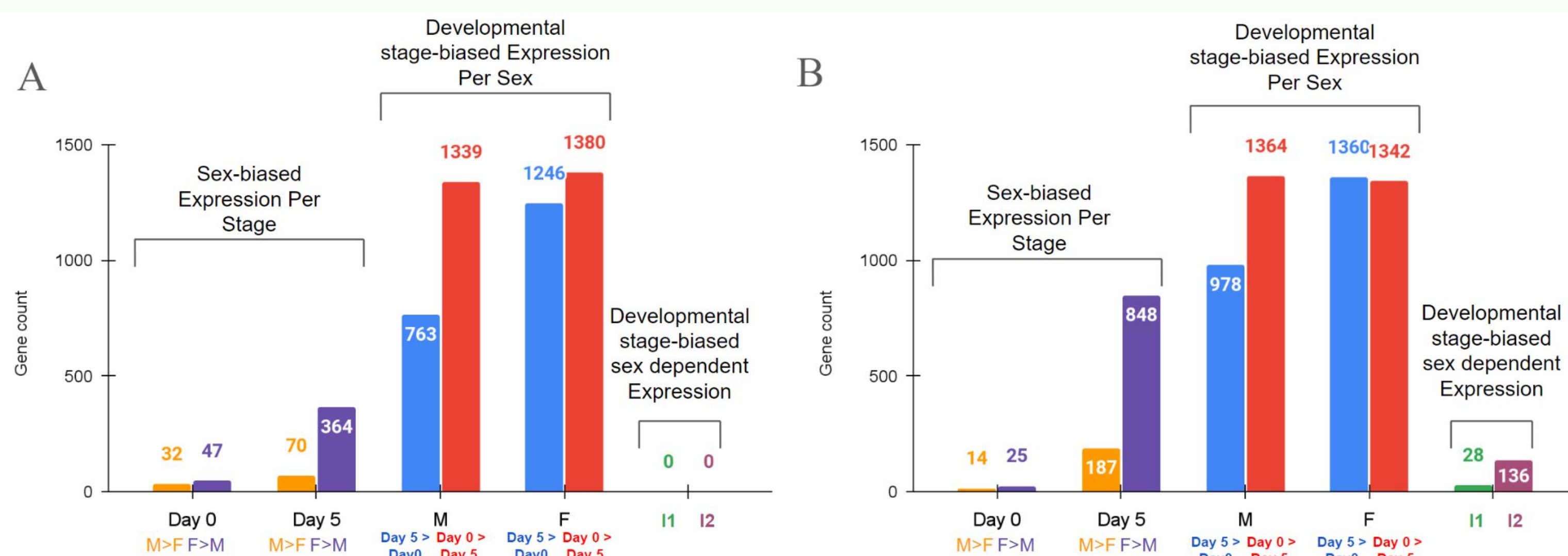
5. Transcriptome Quality Assessment



PCA and Pearson's correlation heatmap of RNA-seq head samples.

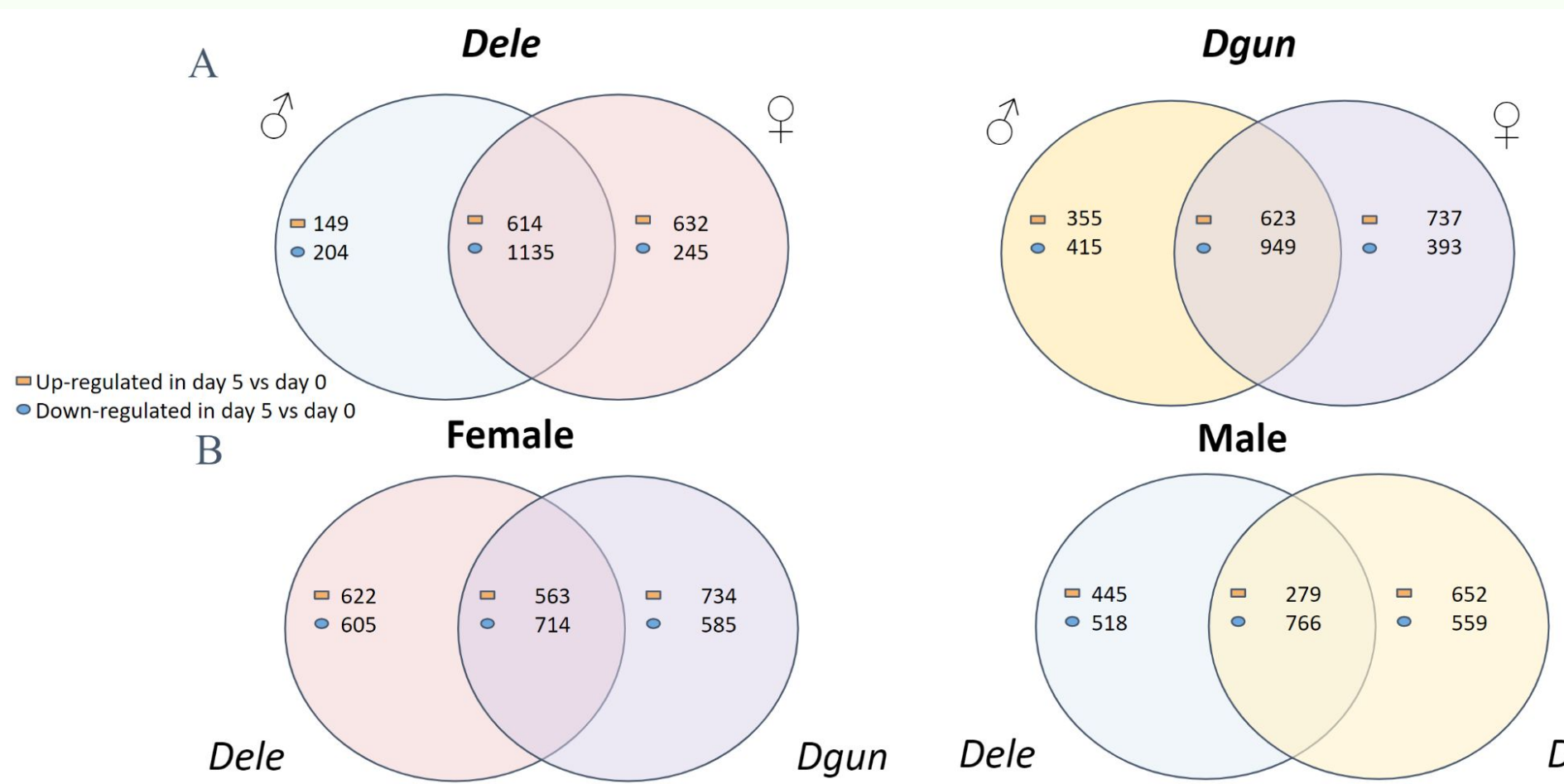
A *D. elegans* **B** *D. gunungcola*. **Left:** PCA of 16 head RNA-seq outputs corresponding to biological replicates of different types of samples. Male, blue; female, red; day 0, circles; day 5, triangles. **Right:** Pearson correlation heatmap. Dele, *D. elegans*; Dgun, *D. gunungcola*; F: Female; M: Male; 0: day 0; 5: day 5; H: Head; 1-5, replicate id number.

6. Stage- and Sex-Pairwise Expression Difference in Head



Differentially expressed genes by developmental stage and sex.

A *D. elegans* **B** *D. gunungcola*. The bar plot displays the results of two-way ANOVA with an interaction term in the analysis conducted to identify DE genes at a 5%FDR and FC>0.5 while controlling for stage differences between Day 5 and Day 0. M, male; F, female; I1, (M Day 5 > M Day 0, F Day 5 < F Day 0); I2, (M Day 5 < M Day 5, F Day 5 > F Day 0).

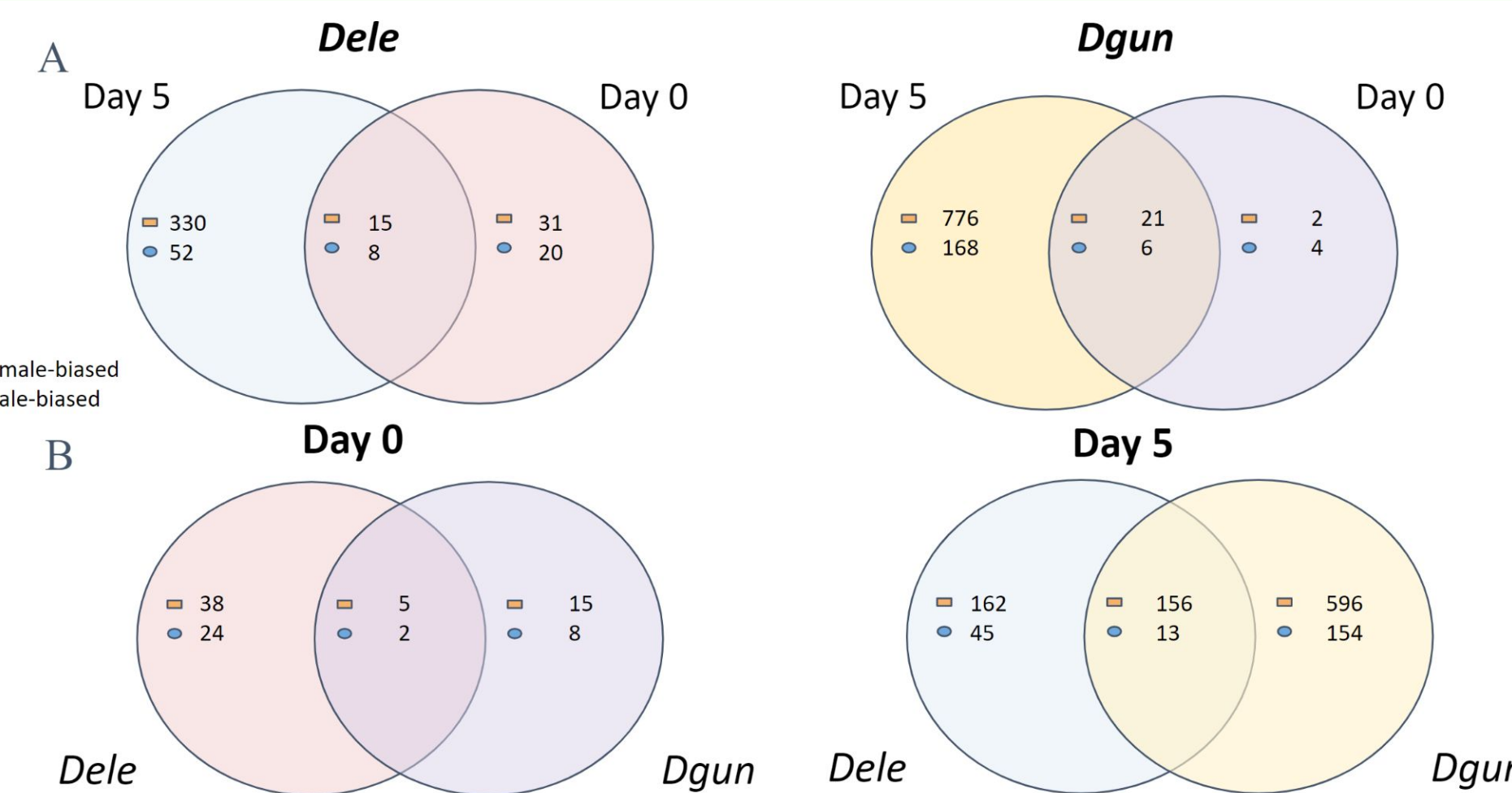


Venn diagrams for developmental-stage genes.

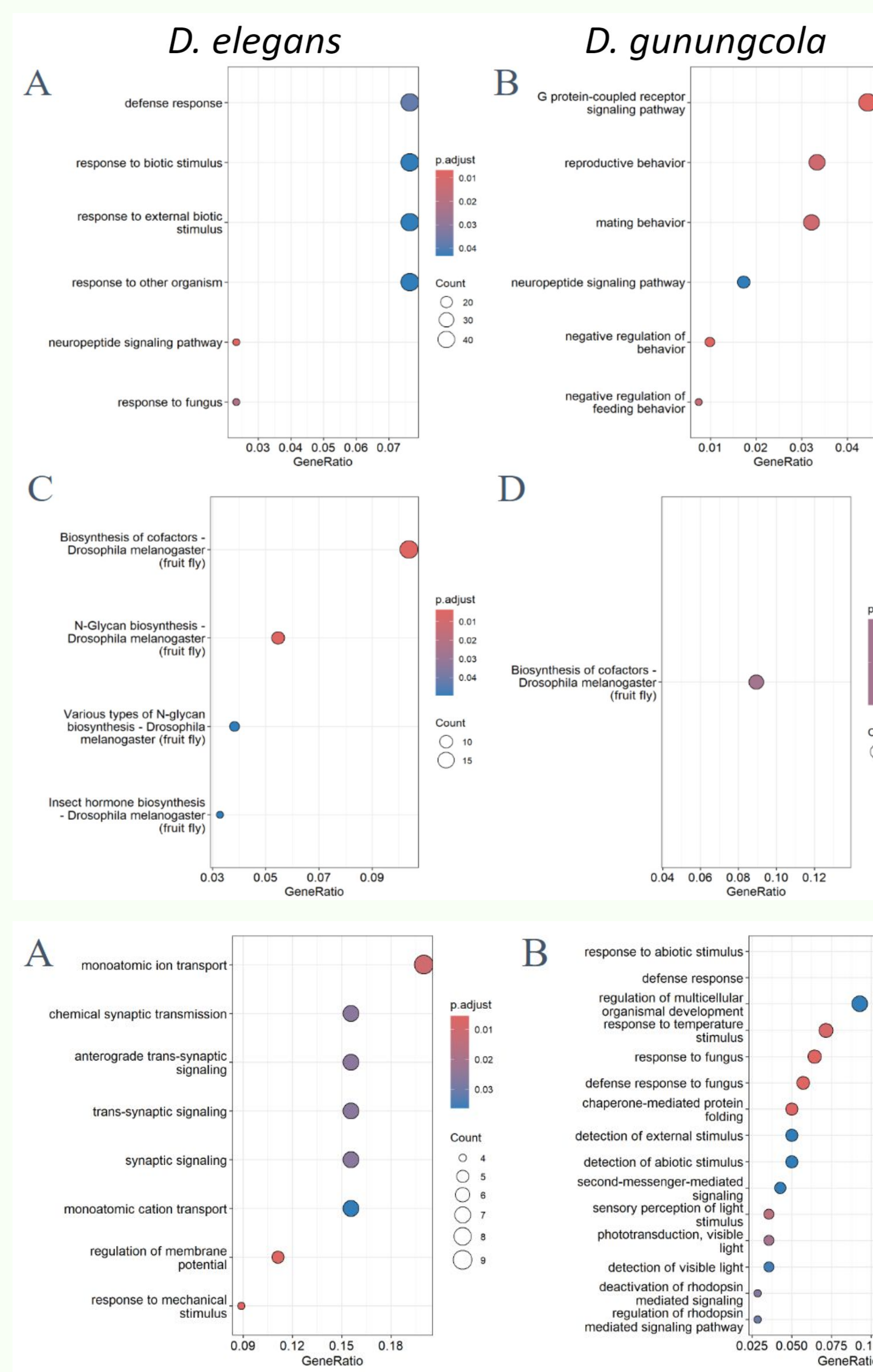
A Male- and female-biased genes in expression are shown in *D. elegans* (left) and *D. gunungcola* (right). Intersection, non-sex-biased genes. **B** Unique DE genes in females (left) and males (right) in the two species. Intersection, non-species specific genes. Dele, *D. elegans*; Dgun, *D. gunungcola*.

Venn diagrams for sex-biased genes in expression.

A Preferentially expressed genes in Day 5 or Day 0 in *D. elegans* (left) and *D. gunungcola* (right). Intersection, DE genes not unique to any of the developmental stages. **B** Sex-biased genes in expression unique to one of the species in Day 0 (left) and Day 5 (right). Intersection, DE genes not unique to any of the species. Dele, *D. elegans*; Dgun, *D. gunungcola*.



7. Enriched Functional Patterns



GO and KEGG analysis of up-regulated DE genes in Day 5 males relative to Day 0 males.

Bubble charts show significantly overrepresented GO:BP terms (A and B) and KEGG pathways (C and D) for genes up-regulated in Day 5 compared to Day 0 in males. **A** and **C**, *D. elegans*; **B** and **D**, *D. gunungcola*. Gene Ratio, proportion of DE genes relative to all genes known to be associated with a particular GO term or KEGG pathway. The level of statistical significance is indicated with a color-coded scale on the right.

Gene Ontology (GO) analysis of Day 5 male-biased DE genes.

Bubble charts show significantly overrepresented GO:BP terms (A and B) and KEGG pathways (C) for Day 5 male-biased genes. **A** *D. elegans*; **B** and **C**, *D. gunungcola*. Gene Ratio, proportion of DE genes relative to all genes known to be associated with a particular GO term or KEGG pathway. The level of statistical significance is indicated with a color-coded scale on the right.

8. Conclusions

- Developmental-stage expression differences within the same sex appear to be more prevalent than expression differences between the sexes within the same developmental stage.
- Two general patterns of differential expression:
 - As the individuals of both species grow older, downregulation prevails over upregulation.
 - there is an increase in the number of sex-biased expressed genes on Day 5, with a larger proportion of them being female-biased, both in *D. elegans* and *D. gunungcola*.
- Very limited number of genes showed significant developmental-stage sex-dependent differences.
- Overrepresentation of Day 5 upregulated genes involved in GO terms and KEGG pathways in *D. elegans*:
 - Biotic stimulus cluster
 - neuropeptide signaling pathway cluster
- Overrepresentation of male-biased genes in Day 5 involved in GO terms and KEGG pathways in *D. elegans*:
 - synaptic transmission and signaling cluster
- Future directions: investigate candidate genes affecting courtship behavior through silencing studies; as *D. elegans* males are also characterized by wing pigmentation, can conduct comparative expression analyses for wing between *D. elegans* and *D. gunungcola* to facilitate the study of the genetic basis of possible coevolution between both traits.

9. Acknowledgement and References

I would like to express my appreciation to Dr. Ranz and Ashlyn Kimura for their guidance and support in the completion of this study. I am also grateful to Hailin Liang and Tina Soroudi for their feedback on the project. Lastly, I acknowledge the contributions of my family and partner Junping Luo for their unwavering encouragement and emotional support.

1. Baker BS, Taylor BJ, Hall JC. 2001. Are complex behaviors specified by dedicated regulatory genes? reasoning from drosophila. *Cell* 105:13-24.
2. Benjamin Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 57:289-300.
3. Jacob F. 1977. Evolution and breeding. *Science* 196:1161-1195.
4. Kachmarczak M, Nicola SM. 2022. The arousal motor hypothesis of dopamine function: Evidence that dopamine facilitates reward seeking in part by maintaining arousal. *Neuroscience* 499:94-103.
5. Kim WJ, Jan LY, Jan YN. 2013. A PDF/NPF neuropeptide signaling circuitry of male drosophila melanogaster controls rival-induced prolonged mating. *Neuron* 80:1190-1205.
6. Koganezawa M, Kimura K, Tatematsu D. 2016. The neural circuitry that functions as a switch for courtship versus aggression in drosophila males. *Current Biology* 26:1395-1403.
7. Korkmaz S. 2020. Evolution of wing pigmentation in drosophila: Diversity, physiological regulation, and cis-regulatory evolution. *Development, Growth & Differentiation* 62:269-278.
8. Massey JH, Li J, Stern DL, Wilkoso P. 2021. Distinct genetic architectures underlie divergent thorax, leg, and wing pigmentation between drosophila elegans and D. Gunungcola. *Heredity* 127:467-474.
9. Massey JH, Rice GR, Firdaus AS, Chen C, Yeh S, Stern DL, Wilkoso P. 2020. Co-evolving wing spots and mating displays are genetically separable traits in drosophila. *Evolution* 74:1098-1111.
10. Meritz J, Shalizi BC. 2001. Asian quantitative genetics. *Current Ornithology* 16:179-255.
11. Raudvere U, Kolberg L, Kuzmin I, Adler T, Adler P, Peterson H, Vilo J. 2019. Gprofiler: A web server for functional enrichment analysis and conversion of gene lists (2018 update). *Nucleic Acids Research* 47.
12. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43.
13. Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: A bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:154-161.
14. Setlles M, Street D, Angel J, Hume S, & Hoogstraal Y. (2023). HTStream. GitHub repository. <https://github.com/htstream/HTStream>
15. Shen P, Wan X, Wu F, Shi K, Li J, Cao H, Zhou L, Zhou C. 2022. Neural circuit mechanisms linking courtship and reward in drosophila males. *Current Biology* 33.
16. Storey JD. 2002. A direct approach to false discovery rates. *Journal of the Royal Statistical Society Series B: Statistical Methodology* 64:479-498.
17. Stutzky D, Gable AL, Lyon O, Jung A, Wyder S, Huerta-Cappa J, Simonovic M, Doncheva NT, Morris JH, Bork P et al. 2019. Sling V1: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research* 47:D607-D613.
18. Tabataki A. 2013. Pigmentation and behavior: Potential association through pleiotropic genes in drosophila. *Genes & Genomics* 8:165-174.
19. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, et al. 2021. ClusterProfiler 4.0: A universal enrichment tool for interpreting OMICS data. *The Innovation* 2:100141.