Transcriptomic differences between schistosome miracidia recovered from different host tissues

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Introduction

Schistosomiasis is a tropical disease caused by human-infective parasites of the genus *Schistosoma*. The larval stage, miracidia, develop in the snail intermediate host into cercariae that are infective to humans. Once a human is infected, the parasites will travel to the mesenteric vasculature connecting the liver and intestine. Here, the worms mature and lay eggs. Some eggs travel to the intestine and are excreted into an aquatic environment before Hatching to miracidia. However, other eggs travel to the liver, and these eggs do not get passed on and will not continue the cycle of infection. Liver eggs are typically used in rodent hosts for experimental research use. There are distinct functional differences in the eggs from the distinct tissues [1]. However, there is no understanding of the differences in miracidia from each tissue's eggs. We utilized bioinformatics techniques and performed RNA-seq analysis on miracidia from infected mouse livers and intestines. We sought to understand transcriptomic differences in these miracidia and potentially find specialized functions dependent on location of maturation.

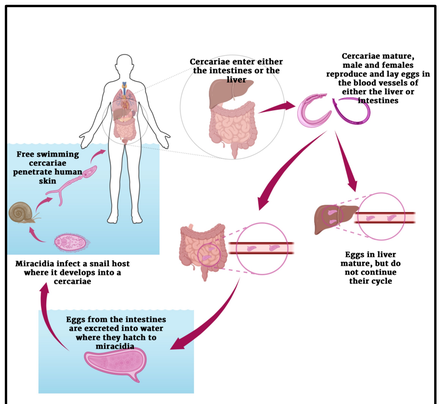

Figure 1. Life cycle of *Schistosoma mansoni*, including infection stage in humans.

Methods

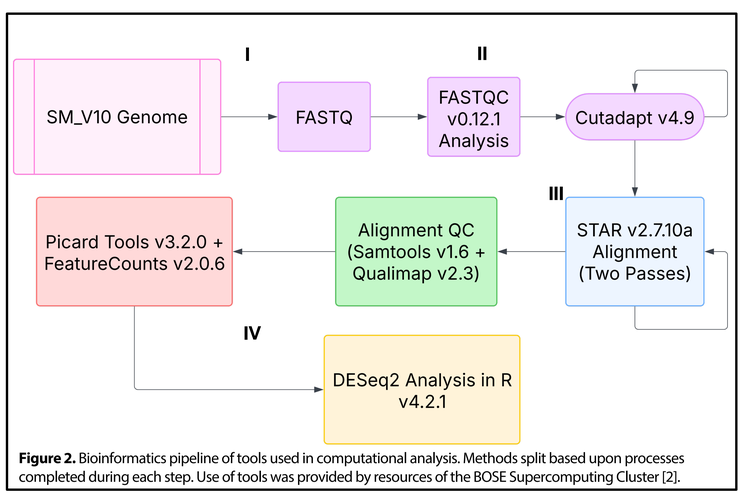

Figure 2. Bioinformatics pipeline of tools used in computational analysis. Methods split based upon processes completed during each step. Use of tools was provided by resources of the BOSE Supercomputing Cluster [2].

I. Downloaded the *S. mansoni* reference genome and annotations [3] for read mapping and feature counting.

II. Used FASTQs (compiled text-based files) to store the nucleotide sequences along with a quality score for each base. To achieve the best possible score, we trimmed the sequences using Cutadapt. QC was completed, and sequences were trimmed again if necessary.

III. Used the STAR aligner to create genome indices for the reference genome and annotations. Trimmed reads were then mapped to a location in the genome index. We performed this alignment twice to find novel splice junctions and improve the alignment quality.

IV. Used Samtools and Qualimap to analyze alignment QC. Used Picard to deduplicate and FeatureCounts to generate counts from the alignment. Used DESeq2 package to analyze for differential expression of transcripts between liver and intestine egg samples.

Results

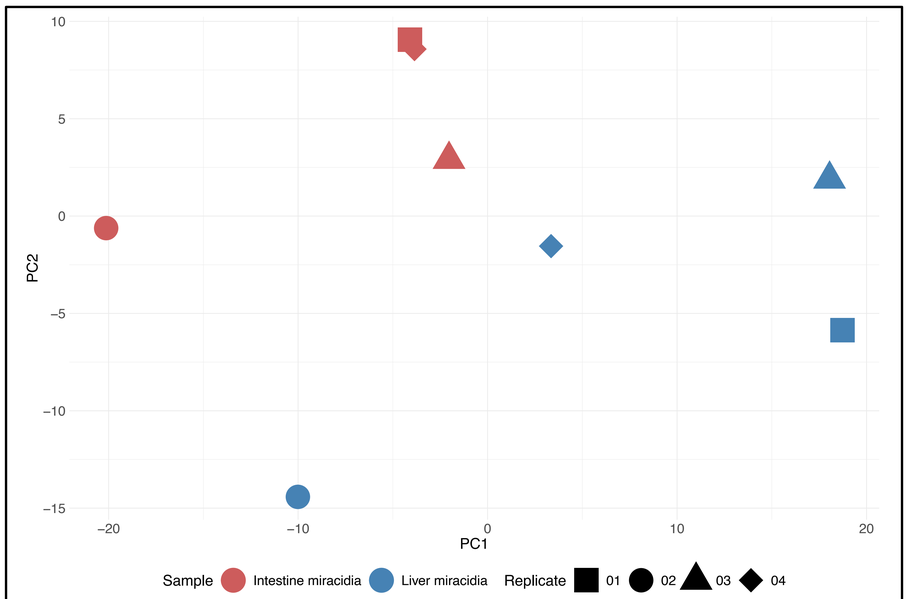

Figure 3. PCA plot created in R that depicts clusters of similar type. We see a clustering linked to tissue source. This result shows that from liver and intestine egg samples are transcriptomically distinct.

Key Findings

- Miracidia hatched from eggs harvested from mouse livers and intestines are transcriptomically different (Fig. 3)
- In the separate tissue samples, differentially expressed genes were found in the miracidia (Fig. 4-5)
- Biologically significant functions are differentially expressed (GO:0009116, GO:1901657 - Fig. 6)

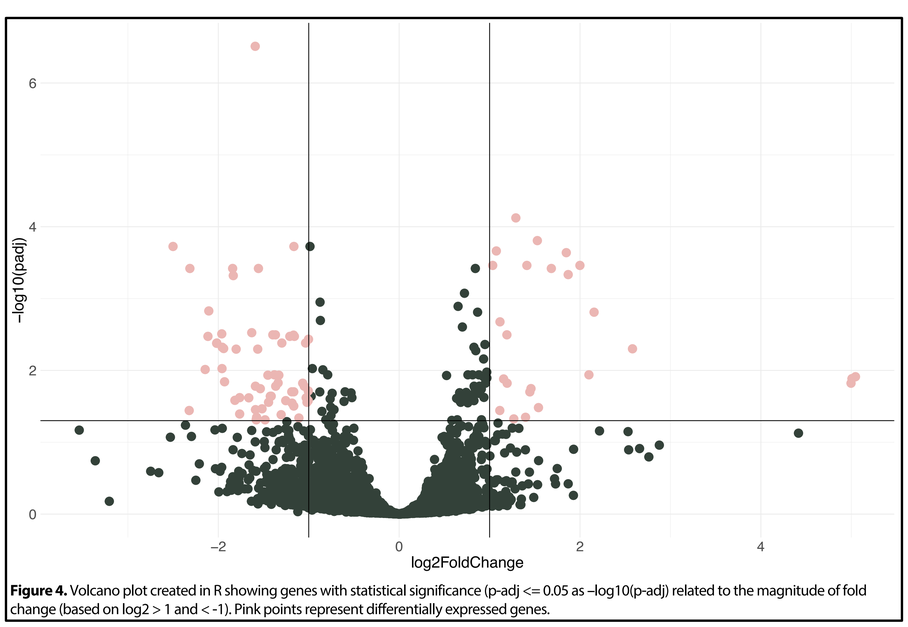

Figure 4. Volcano plot created in R showing genes with statistical significance (p-adj <= 0.05 as –log10(p-adj) related to the magnitude of fold change (based on log2 > 1 and < -1). Pink points represent differentially expressed genes.

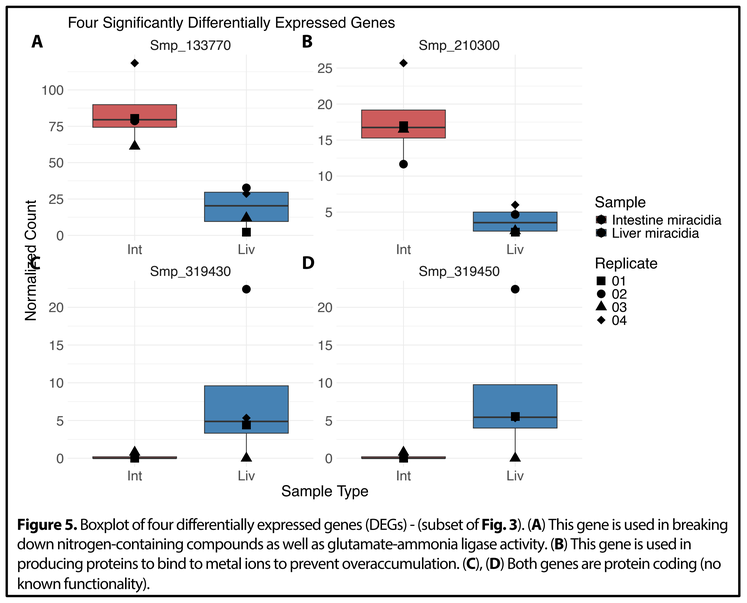

Figure 5. Boxplot of four differentially expressed genes (DEGs) - (subset of **Fig. 3**). (**A**) This gene is used in breaking down nitrogen-containing compounds as well as glutamate-ammonia ligase activity. (**B**) This gene is used in producing proteins to bind to metal ions to prevent overaccumulation. (**C**), (**D**) Both genes are protein coding (no known functionality).

Future Directions

- Testing the separate miracidia for differences in behavior and infection capacity.
- Utilizing the DEGs found in this study for gene "knockout" experiments to understand any functional dependencies that may be occurring.
- Exploring other DEGs found for other important biological functions.

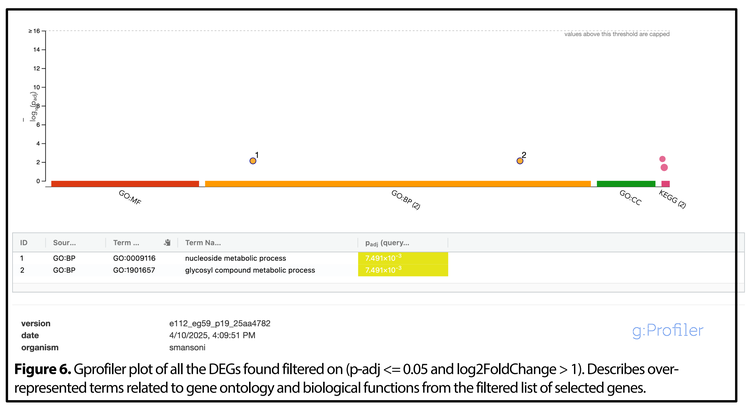

Figure 6. Gprofiler plot of all the DEGs found filtered on (p-adj <= 0.05 and log2FoldChange > 1). Describes over-represented terms related to gene ontology and biological functions from the filtered list of selected genes.

References

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2. Hudak et al., (2018). Open OnDemand: A web-based client portal for HPC centers. Journal of Open Source Software, 3(25), 622, https://doi.org/10.21105/joss.00622
3. Kevin L. Howe, Bruce J. Bolt, Myriam Shafie, Paul Kersey, and Matthew Berriman. WomBase ParaSite – a comprehensive resource for helminth genomics. *Molecular and Biochemical Parasitology* 2017 215 2-10 10.1016/j.molbiopara.2016.11.005

Acknowledgements

Infected tissues were provided by the Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD) through NIH-NIAID Contract HHSN272201700014I. The computational resources of the study were provided by the Blugold Center for High-Performance Computing under NSF grant CNS-1920220. Some schematics created on https://BioRender.com and https://lucid.app. The authors would like to thank the UWEC printing services for poster printing.