ANALYZING GENOTYPIC VARIATION IN DIFFERENTIALLY EXPRESSED "DARK GENES" OF *GALAXEA FASICULARIS* UNDER THERMAL STRESS

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Introduction

Corals are highly susceptible to environmental heat stress, as it can induce the expulsion of symbionts, referred to as coral bleaching.[1] Understanding how elevated temperatures are affecting the genotype-specific transcriptional changes in coral is important for evaluating the response of coral reefs to rising global temperatures.[2] However, many genes associated with the thermal stress response remain unannotated, termed "dark genes."[3] Reviewing gene expression networks can provide a framework for the annotation of dark genes that could enhance the understanding of the coral gene function.[4]

The purpose of this study is to investigate how genotypic variation influences the differential expression of "dark genes" exposed to thermal stress and identify candidate "dark genes" that warrant further functional analysis.

Questions:

1. How does "dark gene" expression change between genotypes of coral under thermal stress?
2. What proportion of the differentially expressed genes are "dark" and how many "dark genes" are unique to each genotype?

Methods

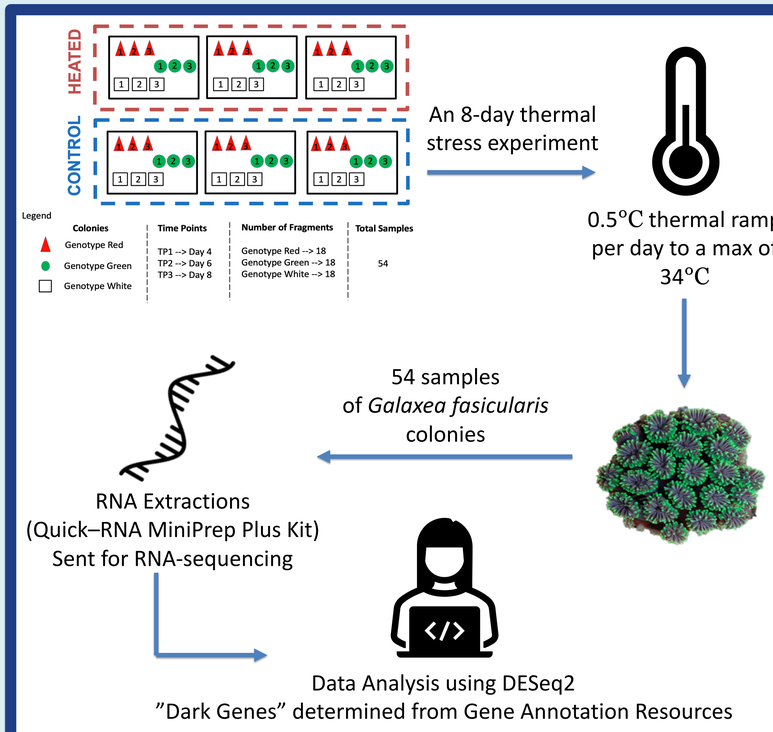

Fig. 1: Thermal stress experimental design and RNA extraction workflow.

Global Gene Expression Profile

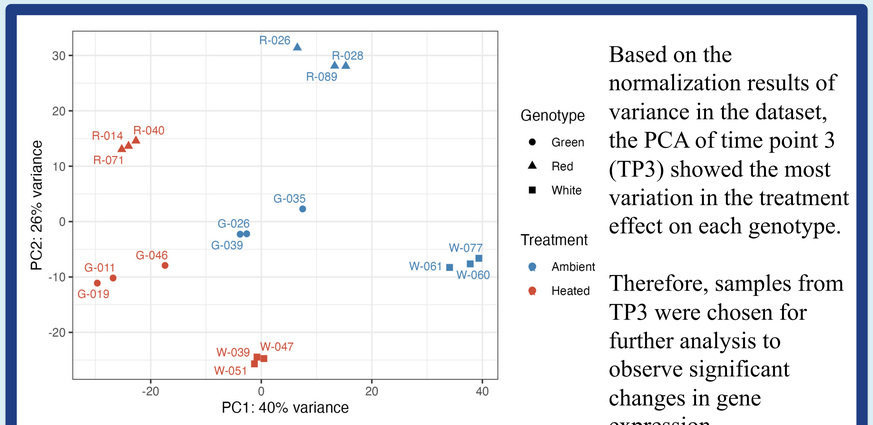

Fig. 2: Principal Component Analysis of the normalized time point 3 (TP3) counts.

Based on the normalization results of variance in the dataset, the PCA of time point 3 (TP3) showed the most variation in the treatment effect on each genotype.

Therefore, samples from TP3 were chosen for further analysis to observe significant changes in gene expression.

Genotype-Specific Comparisons

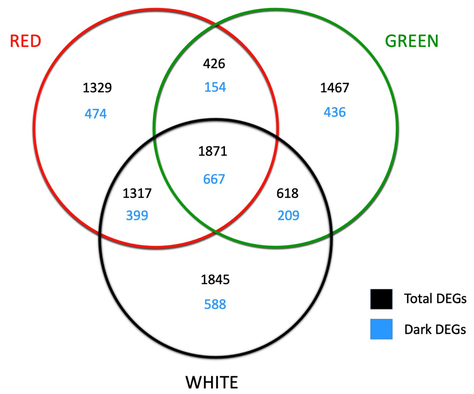

Fig. 3: Venn diagram of differentially expressed genes (DEGs) based on treatment effect at time point 3 (TP3).

- 8,873 total DEGs
- 2,927 (33%) were "dark genes"
- Genotype Green
  - least DEGs
  - least "dark genes" present
  - most fully shared " dark genes" across all
- Genotype White
  - most DEGs
  - most "dark genes" present
  - most unique "dark genes" among all
- Genotype Red
  - least unique "dark genes" among all

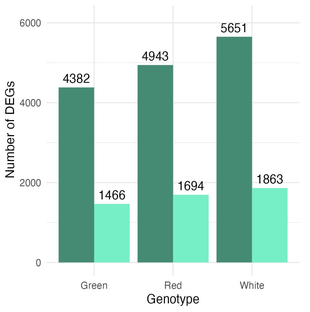

Fig. 4: Bar plot of total DEGs compared to "dark" DEGs for each genotype at TP3.

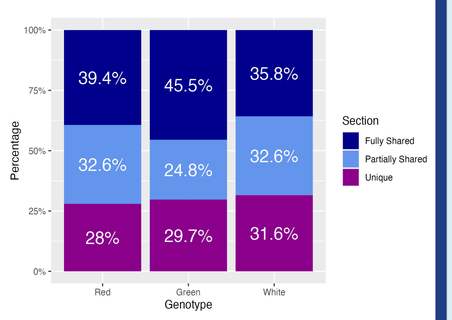

Fig. 5: Stacked bar plot of fully shared, partially shared, or unique "dark genes" for each genotype at TP3.

Candidate "Dark Gene" Identification

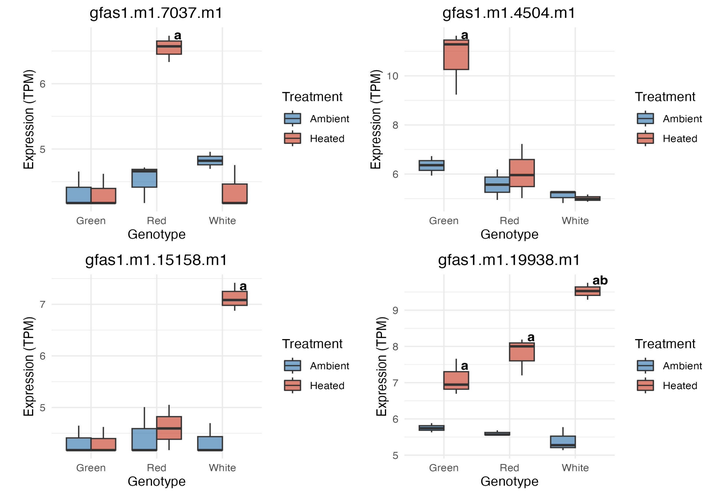

Fig. 6: Paired boxplot of "dark gene" expression (transcripts per million). Statistical significance between heated and ambient determined by a Tukey's test is denoted with an "a". The "ab" denotes statistical significance between heated and ambient as well as between the other heated genotypes.

Conclusions and Future Directions

1. "Dark gene" expression was significantly influenced by genotypic variation.
2. Four candidate "dark genes" with significant expression levels were identified that could be influential for the understanding of the bleaching response: gfas1.m1.7037.m1, gfas1.m1.4504.m1, gfas1.m1.15158.m1, and gfas1.m1.19938.m1.
3. Characterizing these identified "dark genes" could enhance our understanding of both the genotype-specific nature of the thermal stress response and the thermal response in general.
4. Now that we have specific gene candidates, future directions should further characterize these genes through correlations with metabolomics, proteomics, and reverse genetics.

Acknowledgments and References

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