THE CELLULAR AND GENETIC MECHANISMS OF STRESSS RESPONSE PATHWAYS IN CARIBBEAN CORAL

by

BRADFORD AUSTIN DIMOS

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy at
The University of Texas at Arlington
December 2021

Arlington, Texas

Supervising Committee:

Laura Mydlarz (Supervising Professor)
Mark Pellegrino
Jeffery Demuth
Todd Castoe
Clay Clark

Abstract

THE CELLULAR AND GENETIC MECHANISMS OF STRESSS RESPONSE PATHWAYS IN CARIBBEAN CORAL.

Bradford Austin Dimos Ph.D.

The University of Texas at Arlington, 2021

Supervising professor: Laura Mydlarz

Reef-building corals are in decline around the globe due to both the effects of increased ocean temperatures and the emergence of marine diseases which affect corals. These emerging issues threaten the continued persistence of reefs and the ecosystem which they form as the mechanisms which corals use to respond to stress remain poorly characterized. To address these issues my dissertation focused on uncovering the cellular and genetic mechanism utilized by Caribbean corals during periods of stress. Towards this aim my second chapter identified and characterized a mitochondrial stress response pathway in the coral *Orbicella faveolata* and demonstrated that this pathway is activating during both exposure to increased temperature as well as immune stimulation. In chapter three I use the model system *Cassiopea xamachana* to show that the breakdown of symbiosis during temperature stress is associated with declining mitochondrial function. In chapter four I utilize comparative genomic data to characterize the expansion and domain shuffling of a key class of immune genes known as NOD-like receptors in *O. faveolata*. Finally, in chapter five I expanded upon these results to show that immune related genes demonstrate substantial variation in copy number leading to divergent expression. Overall, these research chapters advance the understanding of both the cellular and genetic mechanism utilized by Caribbean corals in response to stressful environmental conditions.

Acknowledgments

First, I would like to acknowledge my loving and supportive parents Duane and Cathy Dimos for their continued support, encouragement and advice throughout the course of my time in graduate school. They have fostered my scientific curiosity from an early age and have equipped me with the opportunities and skillsets needed to be a successful researcher for which I am eternally grateful. I am likewise thankful for my amazing grandmother Donna Dimos who has always been and continues to be a constant source of love and guidance throughout my life. I would also like to thank my best friends Spencer Hunter and Contessa Ricci for providing balance to my life and always making sure I stay on the right path, I wouldn't have gotten very far without them. I would also like to acknowledge my sweet dog Rennie for helping me to get over writers block on our morning walks. I am truly grateful to the many friends I have made in the biology department who have helped me to develop both scientifically and personally. In particular I would like to thank both Zach Nikolakis and Nicky Hales for being kind and faithful friends. Finally, I would like to acknowledge my dissertation committee, all of whom have always been very encouraging and willing to aid my development as a scientist. In particular, I am grateful for the tremendous amount of time which my advisor Laura Mydlarz as well as Mark Pellegrino have spent helping me to become a well-rounded researcher.

Figure List

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Table of Contents:

Chapter 1: Introductory Material

Anthropogenic impacts on the environment have altered ecosystems and caused widespread biodiversity loss. One ecosystem which has been particularly hard hit is coral reefs, as fifty percent all of corals globally have been lost within the last thirty years (Hughes et al., 2017). Coral reefs are biodiversity hotspots and a major food source for coastal communities, highlighting the severity of this loss (Bellwood, Hughes, Folke, & Nyström, 2004). Reefs are formed through the growth and calcification of reef-building corals within phyla cnidaria. The ability of corals to act as ecosystem engineers comes from their ability to host symbiotic algae of the genus Symbiodiniaceae within their gastrodermal tissues (Muscatine, Pool, & Trench, 1975). The symbiotic algae photosynthesize and transfer carbohydrates to the coral which provides the animal with a constant source of energy, which the coral uses for calcification. However, this symbiosis leaves coral vulnerable to environmental changes as increased ocean temperatures (a hallmark of climate change) can cause corals to lose their symbionts in a process termed coral bleaching (Ove Hoegh-Guldberg, 1999). Additionally, the symbiotic nature of corals makes these animals susceptible to a range of marine diseases as this symbiotic relationship leaves the coral in an immune-suppressed state (Weis, Davy, Hoegh-Guldberg, Rodriguez-Lanetty, & Pringle, 2008). Together bleaching and coral diseases are two of the biggest contributors to global coral loss and threaten the contained existence of these ecosystems and the services they provide (Bruno et al., 2007; O. Hoegh-Guldberg & Bruno, 2010).

Both coral bleaching and coral disease can reshape ecosystems by altering species composition and reducing coral cover, however the impacts of these two conditions differ. First, the relative importance of these two phenomena differ by ocean basin where coral bleaching is considered the primary issue in the Indo-Pacific Ocean basin, while coral diseases pose a greater

threat in the Caribbean (Gardner, 2003; Hughes et al., 2017). Several theories have been put forward to explain this geographic discrepancy including prevailing current patterns, nutrient load and species diversity, however the exact reason is still unknown (P. J. Edmunds & Carpenter, 2001). This uncertainty arises from a lack of knowledge about exactly why corals bleach and as well as the dynamics of marine epizootics. Together these knowledge gaps make it difficult to understand the observed declines of these ecosystems as well as forecast how they will change in the future and what interventions if any are possible.

Coral bleaching can occur in all species studied to date and happens when ocean temperatures exceed a critical threshold for an extended period of time (Gates, Baghdasarian, & Muscatine, 1992). Foundational work on bleaching described it as a dysfunction of the symbiont's ability to photosynthesize during elevated temperatures leading the generation of reactive oxygen species (ROS) which ultimately culminate in the breakdown of symbiosis (M. P. Lesser, 1997). This traditional view however has been challenged as recent work indicates that numerous host processes are likewise associated with bleaching leading to heterogeneity in both interspecies and intraspecies bleaching thresholds (Barshis et al., 2013; Claar et al., 2020; Simon R. Dunn, Pernice, Green, Hoegh-Guldberg, & Dove, 2012). Together these observations support that bleaching is a complex process of which the cellular mechanisms are not fully resolved. However, there are some commonalities among studies which routinely show that elevated temperatures cause increased production of ROS from both host and symbiont, disruptions to the protein folding environment of the cell (Oakley et al., 2017) and perturbations to the metabolic exchange between corals and their symbionts (Ainsworth, Hoegh-Guldberg, Heron, Skirving, & Leggat, 2008). The enigmatic nature of the bleaching response is due in part to the intractability of working with corals in the lab, which has to some extent been addressed by the development

of several potential model systems, however these models are still in their infancy (Neff, 2020). Overall, this lack of resolution at the cellular level precludes our ability to understand the basis of bleaching susceptibility and highlights the urgent need for more mechanistic work.

Coral diseases can be severe and long lasting with the power to reshape ecosystems (C. D. Harvell, 1999; D. Harvell et al., 2007). Marine diseases are difficult to predict, track and identify owing to the enigmatic nature of the disease itself (Vega Thurber et al., 2020). Despite over 20 forms of coral disease being described in the Caribbean (Weil & Rogers, 2011) causative agents have only been confirmed for a single disease and putative causative agents have only been suggested for a few others (Shore & Caldwell, 2019). Additionally, many coral diseases are multi-host pathogens where even species who are highly divergent from each other can contract the disease (Meiling et al., 2021). Together these factors make disease a problematic factor for predicting how reefs will change into the future. To combat this, substantial effort has been focused on how disease severity differs by coral species which has demonstrated difference on an order of magnitude (MacKnight et al., 2021a; Meiling et al., 2021). This variation in disease resilience is in large part due to differences in the activity of the coral innate immune system (Pinzón C., Beach-Letendre, Weil, & Mydlarz, 2014). Corals have a complex innate immune system centered around pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) which function to sense threats and initiate immune responses (Emery, Dimos, & Mydlarz, 2021). However, the mechanistic understanding how immune responses are activated and how these responses differ by species remain a significant knowledge gap, limiting researcher's ability to determine how a reef will be reshaped by disease.

To address these issues, I investigated both the cellular and genetic mechanisms of coral stress responses and how this influences corals ability to response to increased temperatures and

disease stress. In chapter one I demonstrate that the reef building coral *Orbicella faveolata* utilizes a conserved stress-response pathway termed the mitochondrial unfolded protein response (UPR^{mt}) to respond to both immune challenge and temperature stress. In chapter two I leverage an emerging model system of cnidarian symbiosis *Cassiopea xamachana* to show that temperature stress leads to both reductions in symbiont colonization as well as damage to the mitochondria, which is reflected at the transcriptional level. In chapter three I describe the expansion of a class of immune receptors which form part of the UPR^{mt}, known as NOD-like receptors in *O. faveolata* which function as a direct mechanism to initiate terminal immune responses. In chapter four I expand upon these results by investigating how the evolution of immune related homologous transcript families influences expression divergence in five species of reef-building corals, which demonstrates that adaptive changes in the number of paralogous immune transcripts corresponds to species-level difference in disease resilience. Overall, these chapters illustrate that the mitochondria is a key component of corals responses to stressful conditions through regulation of the immune system, which also demonstrates substantial variation across the primary reef-building corals of the Caribbean. Together these chapters advance our understanding of both the cellular and genetic mechanisms through which corals can adapt to their rapidly changing environments.

Chapter 2: Uncovering a Mitochondrial Unfolded Protein Response in Corals and its Role in Adapting to a Changing World

Authors: Bradford A. Dimos[1], Sirage A. Mahmud[1], Lauren E. Fuess[1], Laura D. Mydlarz[1], Mark W. Pellegrino[1]*

Affiliations: [1]Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA

Citation: Dimos, B. A., Mahmud, S. A., Fuess, L. E., Mydlarz, L. D., & Pellegrino, M. W. (2019). Uncovering a mitochondrial unfolded protein response in corals and its role in adapting to a changing world. Proceedings of the Royal Society B: Biological Sciences, 286(1905), 20190470. doi: 10.1098/rspb.2019.0470.

Abstract:

The Anthropocene will be characterized by increased environmental disturbances, leading to the survival of stress tolerant organisms particularly in the oceans where novel marine diseases and elevated temperatures are re-shaping ecosystems. These environmental changes underscore the importance of identifying mechanisms which promote stress tolerance in ecologically important non-model species such as reef-building corals. Mitochondria are central regulators of cellular stress and have dedicated recovery pathways including the mitochondrial unfolded protein response, which increases the transcription of protective genes promoting protein homeostasis, free radical detoxification, and innate immunity. In this investigation we identify a mitochondrial unfolded protein response in the endangered Caribbean coral *Orbicella faveolata*, by preforming what we believe to be the first instance of *in vivo* functional replacement using a transcription factor (Of-ATF5) originating from a coral in the model organism *Caenorhabditis elegans*. In addition, we use RNA-seq network analysis and transcription factor binding predictions to identify a transcriptional network of genes likely regulated by Of-ATF5 which is induced during immune challenge and temperature stress. Overall, our findings uncover a conserved cellular pathway which may will likely promote the ability of reef-building corals to survive increasing levels of environmental stress.

Introduction

Coral reefs have recently experienced massive declines (De'ath, Fabricius, Sweatman, & Puotinen, 2012; Hughes et al., 2017) primarily driven by marine diseases (Bourne et al., 2009; D. Harvell et al., 2007) and thermally-induced mass coral bleaching (Bruno et al., 2007; Hughes et al., 2018). As a result, many studies have investigated the mechanisms with which corals respond to disease (Gochfeld & Aeby, 2008; Libro, Kaluziak, & Vollmer, 2013; L. Mydlarz,

Couch, Weil, Smith, & Harvell, 2009) as well as factors associated with bleaching (Ainsworth et al., 2008; M. P. Lesser, 1997; Warner, Fitt, & Schmidt, 1999). Interestingly, these pathways show considerable overlap (Caroline V. Palmer, 2018) and involve both antioxidants (R. M. Wright et al., 2017; Yudowski, Roberson, & Marty-Rivera, 2018) and molecular chaperones, which have been suggested to mediate a protective response (Barshis et al., 2013) however additional cellular pathways promoting these protective responses are not yet known.

Both bacterial toxins and thermal stress lead to mitochondrial dysfunction (Simon R. Dunn et al., 2012; Kuwabara & Imajoh-Ohmi, 2004), suggesting a possible common mechanism worth further investigation. Cells use a variety of means to mitigate dysfunction to mitochondria including the mitochondrial unfolded protein response (UPR^{mt}), a retrograde pathway which functions to recuperate homeostasis to the organelle (Shpilka & Haynes, 2017). The UPR^{mt} is induced upon impairments in mitochondrial function arising from sub-optimal mitochondrial protein folding (Haynes & Ron, 2010; A. M. Nargund, Pellegrino, Fiorese, Baker, & Haynes, 2012), mitochondrial reactive oxygen species (ROS) production, or pathogenic infection (Pellegrino et al., 2014). This pathway induces a potent cell-survival response by promoting detoxification of ROS, mitochondrial protein homeostasis (by increasing the transcription of mitochondrial chaperones and proteases), and immune competence (A. M. Nargund et al., 2012; Pellegrino et al., 2014). The UPR^{mt} is regulated by the basic leucine zipper (bZIP) transcription factor ATFS-1 in the model organism and nematode *Caenorhabditis elegans* (A. M. Nargund et al., 2012), with the bZIP transcription factor ATF5 from *Homo sapiens* (Hs-ATF5) mediating a mammalian UPR^{mt} (Fiorese et al., 2016). Regulation of the UPR^{mt} occurs via organelle partitioning where ATFS-1/Hs-ATF5 are imported into healthy mitochondria and proteolytically degraded (Fiorese et al., 2016; A. M. Nargund et al., 2012), which is dependent on the

mitochondrial targeting sequence (MTS) of these proteins. Mitochondrial protein import efficiency is impaired in compromised mitochondria (G. Wright, Terada, Yano, Sergeev, & Mori, 2001), allowing ATFS-1/Hs-ATF5 to localize to the nucleus through its nuclear localization signal whereupon it regulates a diverse set of genes promoting mitochondrial recovery (Fiorese et al., 2016; A. M. Nargund et al., 2012).

As the UPR^{mt} regulates many of the elements thought to be important in coral stress responses including production of heat shock proteins (HSP) and antioxidants, and that the regulatory pathways in coral remain largely obscured, we sought to characterize a possible UPR^{mt} in the reef-building coral *Orbicella faveolata*. In this investigation we demonstrate the existence of a pathway in *O. faveolata* which bears high similarity to the described UPR^{mt}. By utilizing transgenesis of a genetic reporter line of UPR^{mt} activity in *C. elegans* we show that *O. faveolata* possesses a gene which is able to rescue a loss of function mutation of the UPR^{mt} mediator ATFS-1 *in vivo*. We also demonstrate that due to its increased expression, the importance of Of-ATF5 may function in coral during both immune challenge and heat stress. In addition, by using bioinformatic methods we determine that this transcription factor is associated with a mitochondrial protective pathway that contains well-known stress resistance genes previously identified in reef building corals (Downs, Mueller, Phillips, Fauth, & Woodley, 2000; C. D. Kenkel et al., 2014; Voolstra et al., 2009). Overall, our data suggest that the UPR^{mt} could play a key role in mediating the ability of corals to adapt to a changing world.

Materials and Methods

UPR^{mt} homology

Of-ATF5 was found with the tblastn algorithm available from NCBI, using *C. elegans* ATFS-1 or Hs-ATF5 as the query sequence with an e-value cutoff of 1 x 10-5. Protein sequence alignments were preformed through the tCoffee online alignment tool (Notredame, Higgins, & Heringa, 2000), and predictions of mitochondrial targeting sequences were performed with the online tool Mitoprot 2 (Claros & Vincens, 1996). Gene-tree analysis was performed in Mega7 (Kumar, Stecher, & Tamura, 2016) by creating a consensus maximum likelihood tree over 100 iterations. Species sequences: (*Acropora digitifera, Stylophora pistillata*, *Orbicella faveolata*, *Exaiptasia pallida*, *Danio rerio*, *Homo Sapiens*, *Mus musculus*) were downloaded from NCBI. Protein sequences were found through the blastp algorithm using either Of-ATF5 or Hs-ATF5 for cnidarian or vertebrate species respectively.

Transgenesis

The reporter worm strains *hsp-60pr::GFP* (SJ4058) and *atfs-1(tm4525) hsp-60pr::GFP* used have been previously described (Baker, Nargund, Sun, & Haynes, 2012; Yoneda, 2004). Hermaphrodite worms were raised on the OP50 strain of *Escherichia coli* unless they were treated with RNAi, in which case the HT115 *E. coli* strain expressing the described RNAi plasmid was used. *C. elegans* strains were raised on nematode growth media plates (NGM) at either 16°C, 20°C, or 25°C while the Of-ATF5 transgenic worms were maintained at 16°C, unless stated otherwise.

Plasmid construction

Total RNA was isolated from adult polyps of *O. faveolata* using RNAqueous Total RNA Isolation kit (ThermoFisher scientific, USA AM1912) according to the manufacturer's instructions. cDNA was obtained from total RNA using iScript cDNA synthesis kit (BioRad, USA 1708890) following manufacturer's instructions. Of-ATF5 cDNA was amplified using

primer pair Of-ATF5F (5'-TTTGGATCCATGGCCAGAACTTATCACAA-3') and Of-ATF5R (5'-TTTGATATCTTATGAAGCAAGAAACACT-3') and cloned into BamHI and EcoRV sites of the *C. elegans* expression vector pPD49.78, resulting in *hsp-16pr::Of-ATF5* (A. M. Nargund et al., 2012). The sequence of the cloned cDNA was confirmed by Sanger sequencing. The plasmid pPD49.78 includes the heat shock inducible promoter *hsp-16.2* which we used to conditionally express Of-ATF5. Transgenic *C. elegans* was generated by co-injecting *hsp-16pr::Of-ATF5* (10 ng/µl) with a *myo-2pr::mCherry* (5 ng/µl) marker plasmid and pBluescript (120 ng/µl) carrier plasmid into *hsp-60pr::GFP;atfs-1 (tm4525),* generating extra-chromosomal arrays.

RNAi

RNAi was performed as previously described (Kamath & Ahringer, 2003). Briefly, worms were fed *E. coli* bacteria harboring plasmids expressing double-stranded RNA for the mitochondrial quality control protease *spg-7* or the ATP synthase subunit *atp-2*, both of which are capable of activating the UPR^{mt} (Figure 1E).

Microscopy

C. elegans were imaged using a Zeiss AxioCam MRm mounted on a Zeiss Imager Z2 microscope. Exposure times were the same in each experiment. Fluorescence was quantified using the program ImageJ (Rueden et al., 2017) and the relative intensity between worm strains raised on each RNAi clone were compared using a one-way anova using the tm4525 strain as a reference.

Bioinformatic Analysis

Transcriptome analysis comes from a previous study where detailed methods can be found (Fuess, Pinzón C, Weil, Grinshpon, & Mydlarz, 2017). Briefly 10 colonies of *O. faveolata*

were collected near La Parguera, Puerto Rico. Samples were exposed to 1mL of 7.57 mg/ml lipopolysaccharide (LPS) from *E. coli* 0127:B8 (Sigma-Aldrich L3129-100MG) or vehicle control (filtered sea water) and incubated for four hours upon which time all colonies were flash frozen. RNA was extracted with the RNAaqueous kit with DNAase step (Life Technologies: AM1914) according to manufacturer's instructions and quality assessed using the Agilent Bioanalyzer 2100. Samples with a RIN greater than 8 were used to create cDNA libraries with Illumina TruSeq RNA with Poly-A selection library kit. Libraries were sequenced by the University of Texas Southwestern Medical Center Genomics Core facility. Reads were filtered for adaptors and low-quality reads using Trimmomatic software with default parameters (Bolger, Lohse, & Usadel, 2014) and mapped to an existing *O. faveolata* reference transcriptome (Pinzon et al., 2015) with the cufflinks package using default parameters (Trapnell et al., 2010), and read counts were generated by HTseq (Anders, Pyl, & Huber, 2015). Read normalization was performed in the R package DESeq2 (Love, Huber, & Anders, 2014) to generate rlog transformed normalized reads. Of-ATF5 expression level from the rlog normalization was used in an unpaired t-test (n=4 per group). The reference transcriptome was annotated using blastx algorithm against the Uniprot ensemble database with an evalue cut off of 1 x 10-5. All raw sequences used in this project are available on the NCBI GenBank database (SRA Accession #SRP094633).

Weighted gene co-expression network analysis

Rlog transformed counts were subject to weighted gene co-expression network analysis (Langfelder & Horvath, 2008) which creates groups of co-expressed transcripts (modules), based on expression similarity (Langfelder & Horvath, 2008). Network construction was performed with an unsigned Pearson's correlation to generate modules with a power of 15, minimum

module size of 30 and merge cut height of 0.25. The behavior of the identified co-expression network modules was investigated with respect to three traits: treatment condition, genotype, and expression of Of-ATF5.

Transcription Factor Binding Predictions

DNA binding sequence preference provided as a positional weight matrix (PWM) for Hs-ATF5 (Figure S2) was obtained from the online resource Cis-Bp (Kulakovskiy et al., 2013; Weirauch et al., 2014) as the sequence preference of DNA-binding domains have a deep homology and is often extremely well-conserved (Carroll, 2008; Weirauch et al., 2014). To investigate if the genes identified by our WGCNA module possessed Hs-ATF5 binding motifs within their regulatory regions, we extracted 1000bp upstream of the start codon for all annotated genes in the *O. faveolata* genome (ofav_dov_v1, GenBank: MZGG00000000.1) using a custom python script and bedtools (Quinlan & Hall, 2010). To identify motifs which match the PWM of Hs-ATF5 we used the program find individual motif occurrence (FIMO) (Grant, Bailey, & Noble, 2011), to scan the regulatory region of the genes within the WGCNA module which was correlated to Of-ATF5. To investigate for enrichment of Hs-ATF5 binding motifs, we additionally scanned the regulatory region of a set of "random genes", which were selected from a WGCNA module which had a minimal correlation to Of-ATF5 (R=-0.147, p=0.727) and contained a large number of transcripts (1676). To test for enrichment the percentage of genes with motifs which matched the PWM of Hs-ATF5 between the two gene sets were compared using a Fisher's exact test. To limit our search to regions of interest we scanned the promoter regions of selected target genes, identified as all nucleotides within five kilo-bases upstream of the start codon. The sequences of the promoter regions were extracted from the *O. faveolata*

genome and annotation files available on NCBI, with scanning of each target gene promoter preformed independently.

Gene Ontology Enrichment Analysis

Genes which appeared in the highly correlated module and possessed annotations were used in the R script Gene Ontology enrichment analysis with Mann-Whitney U test (GOMWU) (R. M. Wright, Aglyamova, Meyer, & Matz, 2015). Module membership scores acquired from WGCNA were used as a continuous trait for the genes in the highly correlated module, while all other genes in the transcriptome were given a significance measure of zero. Tests were performed to generate GO enrichment terms for biological process with parameters: cluster cut height = 0.25, largest = 0.1, smallest = 25, and cellular compartment with parameters: cluster cut height = 0.25, largest = 0.1, smallest = 25, with option for modules analysis from WGCNA.

Temperature stress experiment

In June 2017, six colonies of *O. faveolata* were collected from Brewer's Bay St. Thomas under the Indigenous Species Research and Export Permit number: CZM17010T and split into two corresponding fragments with a diamond bladed saw and housed at the University of the Virgin Islands flow-through sea water facility to acclimate for two weeks. For the experiment, each coral fragment was placed into separate containers and either held at either ambient temperature (27.5°C, STD =0.36), or subject to thermal stress (29°C, STD = 0.844) which reached 30°C over the course of 6 hours upon which time all colonies were flash frozen.

qPCR

RNA was extracted using Ambion RNeasy kit (ThermoFischer, USA: AM1920), and converted into cDNA using the iScript cDNA synthesis kit (BioRad, USA: 1708890). For qPCR reactions 500ng of cDNA was used in each well and samples were run in triplicate for each gene

with Universal SYBR Green mix (BioRad, USA: 1725271). Target genes were selected due to known involvement in the UPR^{mt} of *C. elegans*, heat shock protein 60 (*HSP-60*) and mitochondrial superoxide dismutase (*mtSOD*), the homolog of mammalian mitochondrial heat shock protein 70 (*mtHSP-70*), translocase of inner mitochondrial membrane 23 (*TIMM-23*). Additionally, we pursued mitochondrial inner membrane protease 2 (*IMP-2*) as this gene was present in our highly significant WGCNA module. Expression was normalized to coral housekeeping gene eukaryotic initiation factor 3 (*EIF3*) (Carly D. Kenkel et al., 2011) and fold-induction values were calculated with the Ct ΔΔ Ct method. Statistical analysis for Of-ATF5 expression was calculated with an unpaired t-test n=5 per group. Correlation analysis of UPR^{mt} genes was performed with Pearson's correlation between Of-ATF5 and target gene expression for each target gene separately. Primer design was accomplished through use of Primer3 online tool (Untergasser et al., 2012) and primers are listed in table S3.

Statistics

All statistical analysis including DESeq2, WGCNA, and GOMWU were performed in the R programming environment (*R Core Team (2020)*).

Results

We were unable to locate an obvious ATFS-1 homolog in the genome of *O. faveolata* (Figure S1), however a subsequent search revealed a putative homolog of Hs-ATF5 in the *O. faveolata* genome that contains a bZIP domain and a weakly predicted MTS termed Of-ATF5 (Figure 1A, B). Both Of-ATF5 and Hs-ATF5 have weak MTS predictions, reflecting the resemblance between Hs-ATF5 and Of-ATF5 but not to ATFS-1. We therefore performed an additional analysis to investigate the presence of homologous ATF5/ATFS-1-like proteins across

eight species: four symbiotic cnidarians, three vertebrate species and *C. elegans*. The created gene tree indicates that *C. elegans* ATFS-1 is an outgroup to all other sequences (Figure 1C) reflecting our ability to locate a Hs-ATF5, but not ATFS-1, homolog in *O. faveolata*.

We conducted a transgenesis experiment to investigate if Of-ATF5 is functionally orthologous to ATFS-1 by determining if Of-ATF5 can functionally replace a loss-of-function atfs-1 mutant in a UPR^{mt} genetic reporter line of *C. elegans*. Here, we used the transgenic C. *elegans* strain SJ4058 that contains a transcriptional green fluorescent protein (GFP) reporter for the mitochondrial chaperone gene hsp-60 (*hsp-60pr::GFP*), the promoter of which is directly bound by ATFS-1 during the UPR^{mt} (A. M. Nargund et al., 2012; Amrita M. Nargund, Fiorese, Pellegrino, Deng, & Haynes, 2015; Yoneda, 2004). We used two different sources of mitochondrial stress to induce the expression of *hsp-60pr::GFP*; RNAi knockdown of the mitochondrial quality control protease *spg-7* or the ATP synthase subunit *atp-2*. As expected, *hsp-60pr::GFP* was induced in wild-type animals but not in the atfs-1 loss-of-function mutant in the presence of these mitochondrial stress conditions (Figure 1E,F). Conditional overexpression of Of-ATF5 in the *atfs-1* mutant background could restore *hsp-60pr::GFP* expression in the presence of mitochondrial stress (Figure 1E,F), suggesting that Of-ATF5 may constitute a *bona fide* homolog of ATFS-1/Hs-ATF5.

Induction of a UPR^{mt} During Immune Stress

Of-ATF5 expression is increased during immune challenge (Figure 2A) ($p < 0.0001$) consistent with expectations. To computationally identify networks of genes (Rose, Seneca, & Palumbi, 2016) associated with Of-ATF5 expression we employed weighted gene co-expression network analysis (WGCNA) (Langfelder & Horvath, 2008). We identified one WGCNA module which was significantly correlated to expression level of Of-ATF5 ($R = 0.94$, $p < 0.001$)

containing 941 transcripts with gene annotations (Figure 2B), of which 91 are mitochondrially localized. Of-ATF5 is present as one of the core module genes ($p < 0.001$) indicating that this transcriptional network is robustly connected to Of-ATF5. Of the 941 genes identified by our WGCNA analysis we were able to identify and extract the regulatory region of 818 (87%) from the *O. faveolata* genome. By utilizing transcription factor binding predictions, we identified Hs-ATF5 binding motifs within the regulatory region of 213 of these genes (26%), which is a significant enrichment over the number of Hs-ATF5 binding motifs within the regulatory region of "random" genes (3.34%, p<2.2x10-16, Fisher's exact test). Gene Ontology analysis of our significantly correlated WGCNA module revealed enrichment of several mitochondrial cellular compartment terms (Figure 2C), and biological processes including mitochondrial transmembrane transport, proteolysis, regulation of cell death and oxidation-reduction process (Figure 2D).

Induction of the coral UPR^{mt} during temperature stress

Elevated temperatures are able to activate the two UPR^{mt} reporter lines *hsp-60pr::GFP* and *hsp-6pr::GFP* in *C. elegans*. We therefore tested if Of-ATF5 expression is increased in colonies of *O. faveolata* during pre-bleaching thermal stress of 2°C above ambient and found it to be increased relative to the control (Figure 3A) ($p < 0.05$). The expression of all our five investigated mitochondrial protective target genes had positively correlated expression with Of-ATF5 (Figure 3B-F) ($p < 0.05$) consistent with the expected relationship between a transcription factor and a target gene (Matys et al., 2003). For one of the samples we were unable to get amplification for either our mtSOD or TIMM-23 primers and for these genes only four of our five samples were used for this calculation.

Discussion

Uncovering a UPR^{mt} in *O. faveolata*

Identifying conserved cellular pathways such as the UPR^{mt} in environmentally important basal metazoans like coral has implications in understanding both the evolutionary roots of stress response pathways as well as molecular mechanisms promoting adaptability to a rapidly changing environment. By using multiple complementary approaches (transgenesis, bioinformatics, and qPCR) we uncovered members of a putative UPR^{mt} pathway in *O. faveolata* which is induced during both immune challenge and temperature stress. Overall, our data demonstrates that the UPR^{mt} appears to be a well-conserved stress-response pathway (Jovaisaite, Mouchiroud, & Auwerx, 2014) at the base of animal evolution, which likely has significant implications in a coral's capacity to respond to environmental stressors.

We were able to demonstrate functional conservation of Of-ATF5 as it can rescue UPR^{mt} activity in a *C. elegans* atfs-1 loss-of-function mutant, demonstrating *in vivo* functional replacement using a coral gene. Furthermore, because Of-ATF5 could only restore UPR^{mt} activity under conditions that perturb mitochondrial function, it suggests that Of-ATF5 may be regulated by mitochondrial import efficiency in a manner akin to ATFS-1 or Hs-ATF5 (Fiorese et al., 2016; A. M. Nargund et al., 2012).

The promising results of our transgenesis experiment led us to investigate if Of-ATF5 mediates a UPR^{mt} -like pathway in *O. faveolata*. We conclude that Of-ATF5 likely mediates a UPR^{mt} in *O. faveolata* which is similar to the response mediated by ATFS-1 in *C. elegans*, by regulating the expression of genes that are involved in protein homeostasis (Pellegrino, Nargund, & Haynes, 2013) the detoxification of damaging free radicals (A. M. Nargund et al., 2012), and innate immunity (Pellegrino et al., 2013). Furthermore, the UPR^{mt} likely has significant

implications in a coral's capacity to respond to environmental conditions that perturb mitochondrial function, since it is induced during two conditions affecting corals on a global scale, immune challenge and temperature stress.

The role of the UPR^{mt} in coral disease

Marine diseases are changing the face of reefs world-wide (Bourne et al., 2009; Bruno et al., 2007; D. Harvell et al., 2007; A. W. Miller & Richardson, 2015), and the mechanisms which corals use to overcome pathogens remain poorly characterized. The UPR^{mt} of *C. elegans* is induced during bacterial infection and serves to promote immune competence by improving both pathogen clearance and tolerance during infection (Y. Liu, Samuel, Breen, & Ruvkun, 2014; Pellegrino et al., 2014). We found support that Of-ATF5 likewise functions during immune challenge with LPS (Fuess et al., 2017), an endotoxin found in the outer membrane of bacteria that is a potent inducer of the immune response in both invertebrate and vertebrate model systems. Using gene network analysis and transcription factor binding site predictions we found support that Of-ATF5 possibly functions to directly regulate a broad transcriptional stress response involving both immunity, and cell death during immune challenge. Based upon the high level of similarity between the responses mediated by Of-ATF5 and ATFS-1 we generated a hypothetical model where the UPR^{mt} of *O. faveolata* might function similarly to the UPR^{mt} of *C. elegans* by promoting immune competence and mitochondrial recovery during immune challenge (Figure 4). Mitochondria are purveyors of innate immunity (Kroemer, Dallaporta, & Resche-Rigon, 1998; Mills, Kelly, & O'Neill, 2017) and mediators of cell death, and future investigations should explore if the immune promoting abilities of the UPR^{mt} are conserved in corals.

The role of the UPR^{mt} in a warming climate

Coral bleaching induced by elevated temperature disrupts the physiology of coral-dinoflagellate symbiosis (Gates et al., 1992; Nielsen, Petrou, & Gates, 2018), which involves excessive ROS production (M. P. Lesser, 1997). Our findings support previous work by Voolstra et al. 2009 who identified Of-ATF5 as part of the response to elevated temperature in *O. faveolata*. The UPR^{mt} functions to concomitantly promote the detoxification of ROS while simultaneously minimizing its production (A. M. Nargund et al., 2012; Amrita M. Nargund et al., 2015) this pathway may act as an antagonist of the coral bleaching process. Interestingly, of the mitochondrial genes which had correlated expression with Of-ATF5 during temperature stress both mtHSP-70 and TIMM-23 are known genes involved in the *C. elegans* UPR^{mt} and were also likewise identified by our WGCNA analysis. Thermal stress studies in symbiotic cnidarians have demonstrated dysfunctions in cellular processes consistent with target genes that form part of the UPR^{mt}, including protein misfolding (Oakley et al., 2017; Ruiz-Jones & Palumbi, 2017), ROS production and mitochondrial damage (Simon R. Dunn et al., 2012; Lutz, Raina, Motti, Miller, & van Oppen, 2015). We therefore developed a hypothetical model of a mitochondrial stress-based mechanism of dysfunction (Figure 4) as a parsimonious explanation for the involvement of the mitochondria in contributing to the physiology of cnidarians during temperature anomalies. In support of our model, increased expression of mitochondrial chaperones and antioxidants which are likely mediated by the UPR^{mt} have been shown to be bleaching protective and associated with improved thermal tolerance in corals (Barshis et al., 2013; Desalvo et al., 2008; C. D. Kenkel et al., 2014; Yudowski et al., 2018) indicating that the UPR^{mt} may be a key contributing pathway mediating corals ability to adapt to a warming ocean.

Conclusion

Despite world-wide declines, the cellular mechanisms which promote the ability of corals to survive and adapt to a changing ocean are not yet fully characterized. If these pathways can be uncovered then active interventions to restore and protect the world's reefs including coral restoration, assisted evolution and or gene flow (Oppen et al., 2017; Riegl, Purkis, Al-Cibahy, Abdel-Moati, & Hoegh-Guldberg, 2011) can become more effective. In lieu of our data, Of-ATF5 may show promise as a target gene for further investigation as it mediates a mitochondrial-protective gene network during exposure to two prominent environmental stressors: disease and hyperthermic-temperature. Overall our results point towards the potential of the UPR^{mt} to allow reef-building corals to persist under mounting environmental stress in a rapidly changing ocean.

Chapter 2 Figures

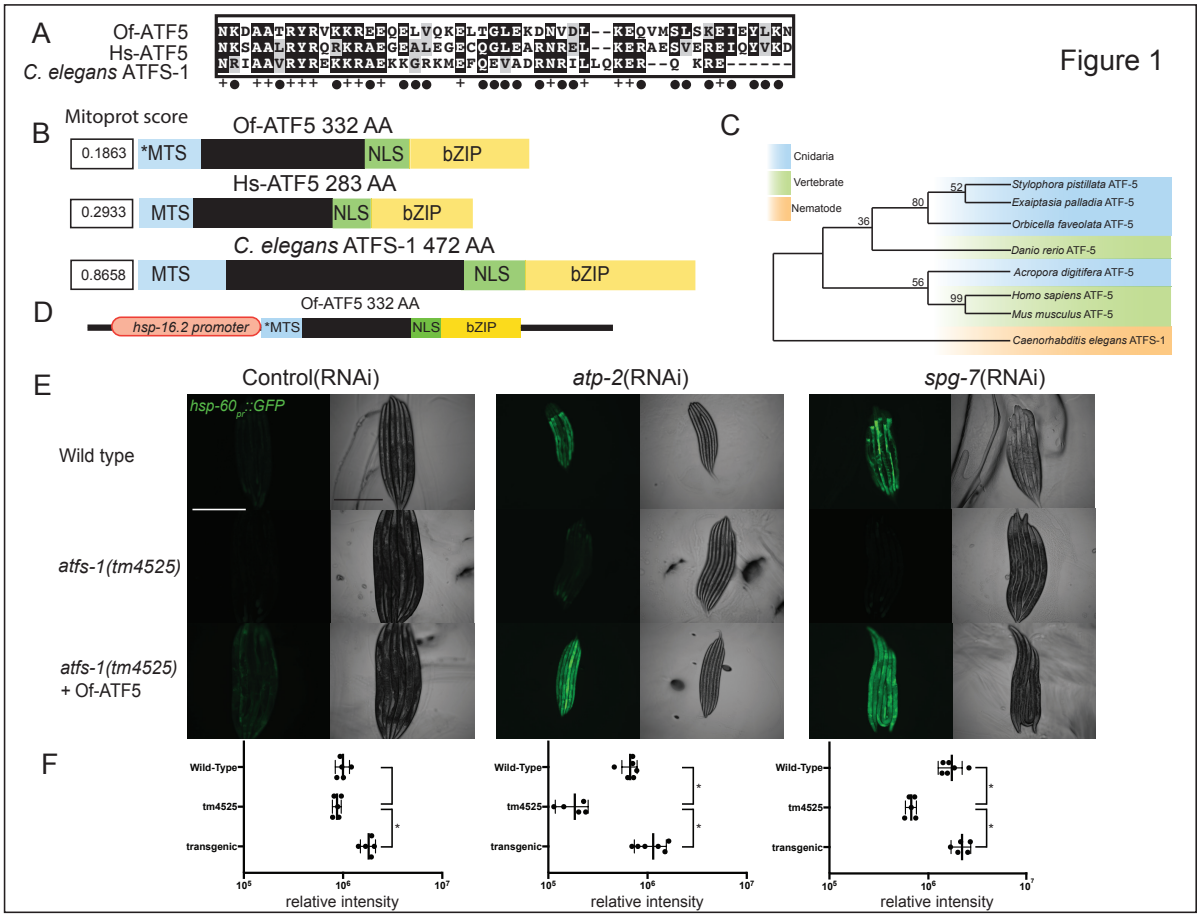

Figure 1: Expression of Of-ATF5 rescues UPR^{mt} activity in worms lacking ATFS-1: (A) Alignment of amino acid sequence of bZIP domains in the homologous transcription factors *O. faveolata* (Of-ATF5), *Homo sapiens* (Hs-ATF5), *C. elegans* (ATFS-1). (+) represents consensus between all three species, (•) represents amino acid similarity between two species. (B) Schematic comparing the homologous bZIP transcription factors: Of-ATF5, Hs-ATF5, *C. elegans* ATFS-1. MTS-mitochondrial targeting sequence with Mitoprot scores (*MTS denotes unconfirmed MTS), NLS-nuclear localization sequence, bZIP-basic leucine zipper, AA-amino acid number. (C) Gene tree of ATF5/ATFS-1 homologs across multiple species demonstrating *C. elegans* ATFS-1 as outgroup to all other sequences, with numbers at nodes representing support of each association. (D) Transgene construct pPD49.78 expression plasmid with Of-ATF5

coding sequence insert downstream of the *C. elegans* hsp-16.2 promoter. (E) Photomicrograph of wild type (*hsp-60pr::GFP), atfs-1(tm4525)* expressing Of-ATF5 raised on control (*HT115 RNAi*), *atp-2* or *spg-7* RNAi. Scale bar, 0.5 mm. (F) Relative intensity of the fluorescent signal (from Figure 1E) shown on a log scale (*, $p < 0.01$).

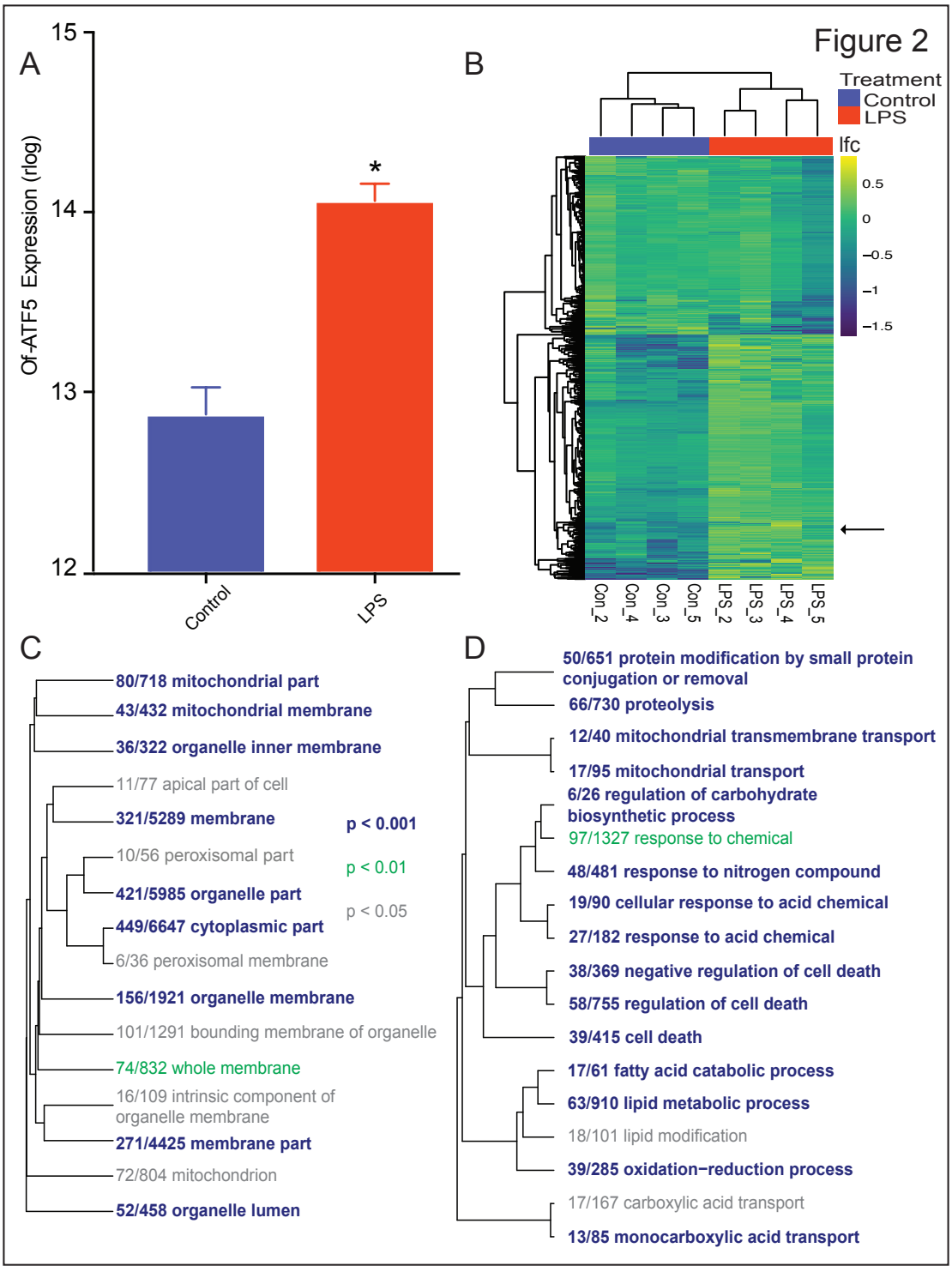
Figure 2
A
15
14
13
12
Of-ATF5 Expression (rlog)
*
Control
LPS
B
Treatment
Control
LPS
lfc
0.5
0
−0.5
−1
−1.5
Con_2
Con_4
Con_3
Con_5
LPS_2
LPS_3
LPS_4
LPS_5
C
80/718 mitochondrial part
43/432 mitochondrial membrane
36/322 organelle inner membrane
11/77 apical part of cell
321/5289 membrane
10/56 peroxisomal part
421/5985 organelle part
449/6647 cytoplasmic part
6/36 peroxisomal membrane
156/1921 organelle membrane
101/1291 bounding membrane of organelle
74/832 whole membrane
16/109 intrinsic component of organelle membrane
271/4425 membrane part
72/804 mitochondrion
52/458 organelle lumen
p < 0.001
p < 0.01
p < 0.05
D
50/651 protein modification by small protein conjugation or removal
66/730 proteolysis
12/40 mitochondrial transmembrane transport
17/95 mitochondrial transport
6/26 regulation of carbohydrate biosynthetic process
97/1327 response to chemical
48/481 response to nitrogen compound
19/90 cellular response to acid chemical
27/182 response to acid chemical
38/369 negative regulation of cell death
58/755 regulation of cell death
39/415 cell death
17/61 fatty acid catabolic process
63/910 lipid metabolic process
18/101 lipid modification
39/285 oxidation−reduction process
17/167 carboxylic acid transport
13/85 monocarboxylic acid transport

Figure 2: Induction of a coral UPR^{mt} during immune challenge. (A) Expression of Of-ATF5 during control or immune challenge (LPS) $p < 0.0001$, bar represents standard deviation, N= 4 per group. (B) Heatmap depicting the log2 fold change of annotated contigs in the highly correlated WGCNA module during LPS treatment, Of-ATF5 is denoted with an arrow. (C-D) Gene ontology category enrichment analysis. Dendrogram's depict the sharing of genes between categories, colors indicate significance of each term (Mann-Whitney U test) as indicated by the inserted key. Fraction indicates number of genes assigned to a specific GO term contained in the WGCNA module over the total number of genes possessing that GO term in the transcriptome for (C) Cellular compartment (D) Biological process.

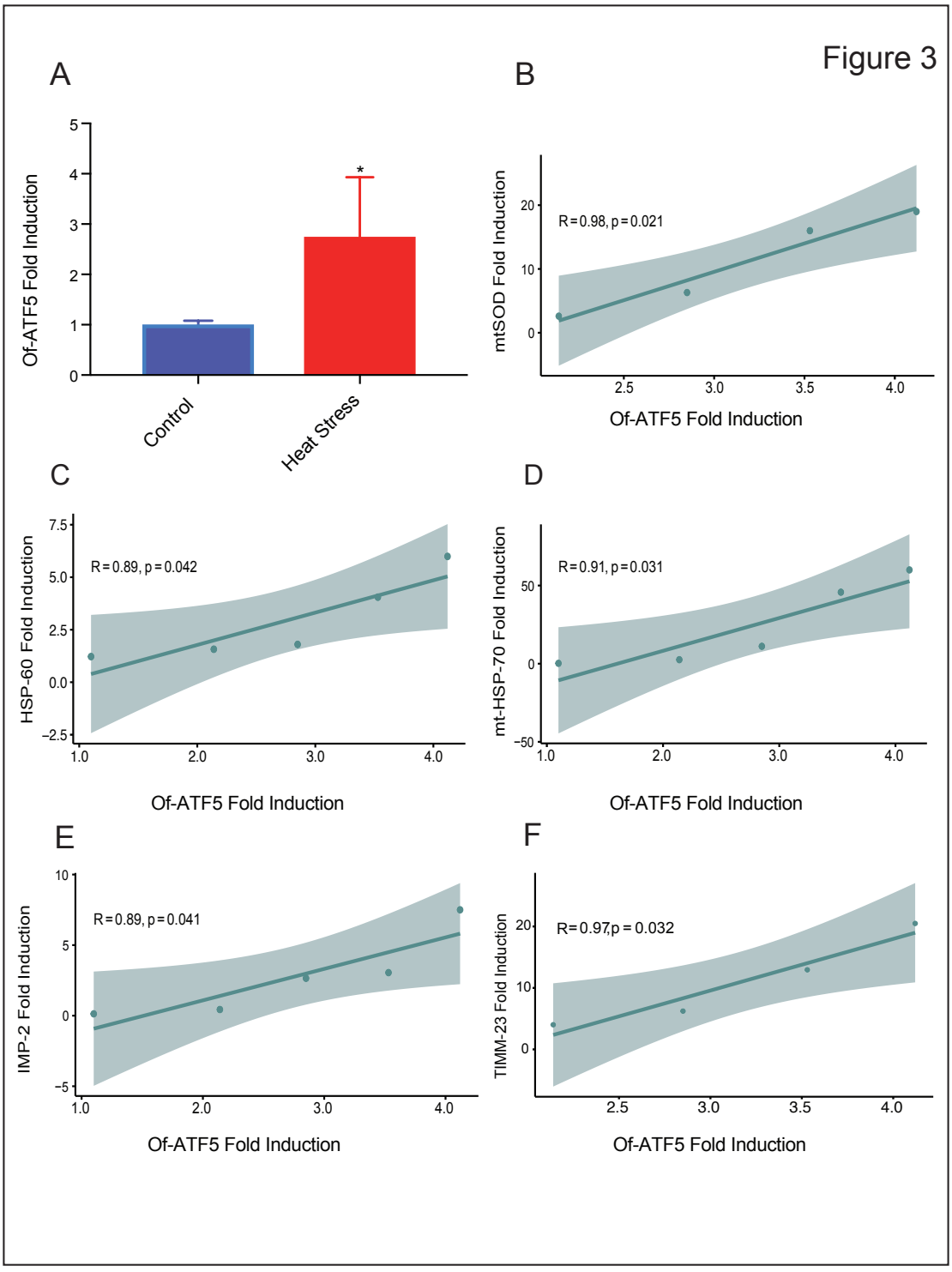
Figure 3
A
Of-ATF5 Fold Induction
Control
Heat Stress
*
B
mtSOD Fold Induction
R=0.98, p=0.021
Of-ATF5 Fold Induction
C
HSP-60 Fold Induction
R=0.89, p=0.042
Of-ATF5 Fold Induction
D
mt-HSP-70 Fold Induction
R=0.91, p=0.031
Of-ATF5 Fold Induction
E
IMP-2 Fold Induction
R=0.89, p=0.041
Of-ATF5 Fold Induction
F
TIMM-23 Fold Induction
R=0.97,p=0.032
Of-ATF5 Fold Induction

Figure 3: Induction of the UPR^{mt} during heat stress. (A) Expression of Of-ATF5 during control or heat stress, $p < 0.05$, bar represents standard deviation, N=5 per group. Correlation between Of-ATF5 and mitochondrial superoxide dismutase (*mtSOD*) (B), heat shock protein 60 (*HSP-60*) (C), mitochondrial heat shock protein 70 (*mtHSP-70*) (D), mitochondrial inner membrane protease-2 (*IMP2*) (E), and translocase of inner mitochondrial membrane-23 (*TIMM-23*) (F). Correlation coefficient (R) and p-values are shown on in-graph insert, dots represent individual samples and shaded area represents 95% confidence interval.

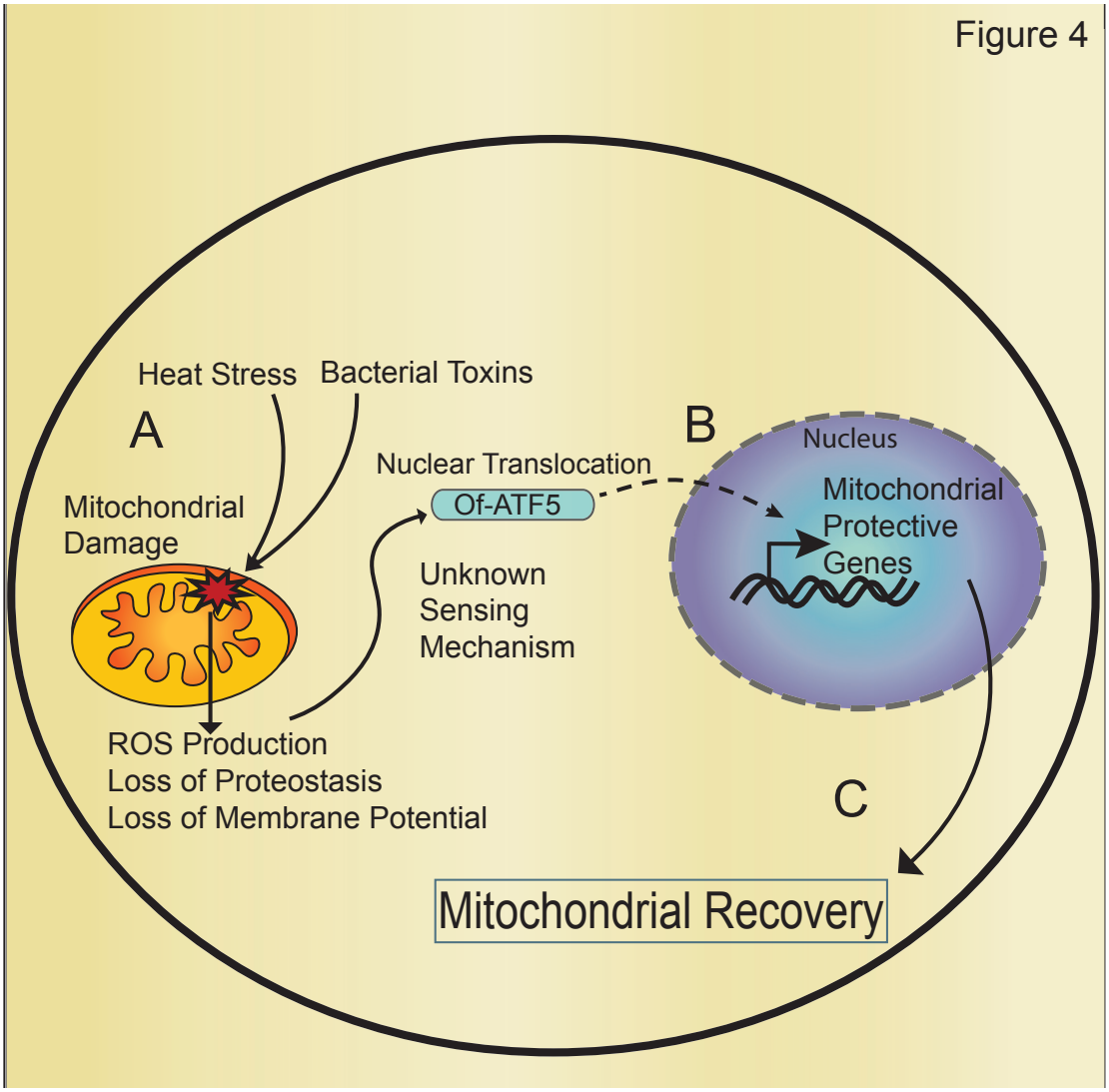

Figure 4: Hypothetical model of UPR^{mt} induction during periods of environmental stress in *O. faveolata*. (A) Mitochondrial damage occurs through the effects of LPS and/or other mitochondrial targeted bacterial toxins, or elevated temperatures. Mitochondrial damage results in increased production of ROS, loss of mitochondrial proteostasis and membrane potential. (B) Mitochondrial damage is sensed through an unknown mechanism, which causes the increased activity of Of-ATF5 and subsequent UPRmt induction. (C) Upon activation of the UPR^{mt} the expression of protective chaperones, antioxidants, and immune components is increased which likely leads to mitochondrial recovery.

Chapter 3: Temperature Stress Causes the Breakdown of Symbiosis and Loss of Mitochondrial Membrane Potential in *Cassiopea xamachana*

Authors: Bradford Dimos[1], Madison Emery[1], Mark Pellegrino[1], Laura Mydlarz[1]

Affiliations: [1]Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA

Intro

As corals reefs continue to decline around the world as a result of coral bleaching (Hughes et al., 2017), substantial efforts have been made to identify the cellular underpinnings of the bleaching response (Ainsworth et al., 2008; Barott et al., 2021). These efforts have uncovered numerous processes which contribute to bleaching including photosynthetic dysfunction of the algal symbiont (M. P. Lesser, 1997), the accumulation of Reactive Oxygen Species (ROS) (Nielsen et al., 2018) and disruptions to the protein folding environment of the cell (Oakley et al., 2017). Despite these findings there is still no clear consensus as to the mechanisms which underly the bleaching process. The lack of a clear bleaching mechanism is due in part to the lack of tractable model organisms (Cleves, Shumaker, Lee, Putnam, & Bhattacharya, 2020) to investigate specific hypothesis regarding the mechanisms which initiate bleaching. Adding to these issues, reef building corals are impractical to culture, and are difficult to maintain given the endangered status of many species.

To illustrate the difficultly of uncovering genetic mechanisms, the mitochondrial unfolded protein response (UPR^{mt}) was recently characterized in the reef-building coral *Orbicella faveolata* where this pathway was shown to be initiated during exposure to temperature stress using a combination in transgenic techniques and functional genomics (Dimos, Mahmud, Fuess, Mydlarz, & Pellegrino, 2019). Despite extensive characterization it is still unknown how activation of this pathway relates to the bleaching response and what the order of events in thermally induced breakdown of symbiosis are. For example the UPR^{mt} functions to increase the expression of antioxidant genes (A. M. Nargund et al., 2012) and decrease metabolic activity during periods of stress, conditions which occur when corals are exposed to elevated temperature (Cleves, Krediet, Lehnert, Onishi, & Pringle, 2020; Diaz et al.,

2016). However, the protective role of even characterized pathways such as the UPR^{mt} during temperature stress remain unknown. To address issues such as these several model systems have been developed including *Exaptasia*, *Astrangia* (Neff, 2020) and more recently the upside-down jellyfish (*Cassiopea xamachana*)(A. H. Ohdera et al., 2018), each with its own benefits and limitations.

C. xamachana in particular has several aspects of its biology which make it an attractive model for the study of Cnidarian symbiosis. *C. xamachana* is small, reproduces clonally, has a life cycle which can be completed in lab (A. H. Ohdera et al., 2018) and it can form stable symbiotic association with the same genera of *Symbiodiniaceae* of reef-building corals (Newkirk, Frazer, & Martindale, 2018). Despite *C. xamachana* falling under the classification scyphozoan within the clade medusozoan, while corals are anthozoans (Kayal et al., 2018), it is currently the only cnidarian model which has a closed life cycle in lab, opening up the future possibility of developing genetic techniques. As a medusozoan *C. xamachana* has three district stages of its life cycle beginning life as a planulae, which settles and metamorphoses into a benthic polyp which reproduces clonally and eventually produces pelagic medusa (Hofmann, Fitt, & Fleck, 1996). The life cycle of C. *xamachana* can also be precisely controlled as the transition from polyp to medusa is dependent on the establishment of symbiosis (Hofmann, Neumann, & Henne, 1978). *C. xamachana* has long been studied in the context of symbiosis with early reports on the mechanisms of symbiosis formation including symbionts residing within an arrested phagosome termed the symbiosome (Trench, Robert, 1983). However, unlike corals whose symbionts reside within gastrodermal cells, the symbiont containing cells of *C. xamachana* are derived from immune ameobocytes and reside in the mesoderm (Lyndby et al.,

2020). These traits coupled with the small size and translucent body of the polyp, which can be propagated indefinitely make it an attractive model for work on Cnidarian symbiosis.

In this investigation I used a clonal line of *C. xamachana* to test the role of the UPR^{mt} in the bleaching response due to increased temperatures by quantifying symbiont loss, mitochondrial membrane potential and gene expression over multiple time points. These data demonstrate that the breakdown of symbiosis is accompanied by a robust transcriptional response including differential expression of the master regulator of the UPR^{mt} ATF5. The reductions in symbiont colonization occur prior to reductions in mitochondrial membrane potential, which coupled with the transcriptional response to temperature stress indicates a role for starvation of the polyp and the increased transcription of antioxidant and immune genes as a likely initiator for the bleaching response. Coupled together these results indicate that the breakdown of symbiosis involves the generation of reactive oxygen species and reduced activity of mitochondria in the host.

Methods

Culture conditions

Clonal lines of *C. xamachana* polyps (T1-A genome line) were continuously cultured at a temperature of 27°C under coral culture lights which produce light wavelengths and intensities designed to facilitate photosynthetic capacity of the algae. *C. xamachana* polyps were infected with *Symbiodinium microadriacticum* (CasKB8) and maintained in stable symbiosis.

Temperature stress experiment

C. xamachana polyps were moved into falcon tubes and placed into one of two water baths held at either 27°C or 32°C representing control and temperature stress respectively and samples

were taken after two days then again at five days. For the physiology experiments polyps were either placed in individual tubes, while for RNAseq polyps were pooled (20 polyps) into tubes where each pooled tube was considered a biological replicate. Polyps were visually inspected prior to the experiment under a dissection microscope to ensure that polyps were uniform in size and symbiont density.

To quantify change in symbiont colonization individual polyps were imaged over multiple days on a Ziess imager Z2 compound fluorescent microscope. Polyps were imaged over multiple days by placing one polyp one a microscope slide with the coverslip overhanging the edge which allows the polyp to be recovered after imaging. Using this technique allows the same polyp to be imaged over multiple days. Change in symbiont density was measured by taking advantage of the background fluorescence of the symbiont by imaging with an enhanced GFP filter for 25msec per sample. Change in symbiont density for individual polyps was calculated as the change in fluorescent intensity in individual polyps (Tortorelli, Belderok, Davy, McFadden, & van Oppen, 2020) using ImageJ (Rueden et al., 2017) software . Significant changes in symbiont density were assessed through a t-test.

Mitochondrial membrane potential was measured using the fluorescent stain TRME. The uptake of this stain is dependent on mitochondrial transmembrane potential and fluoresces under a different range of wavelengths than the symbiont. To facilitate uptake of TMRE polyps were placed in the 1.5mL Eppendorf tubes with 1mL of artificial sea water and 9µL of TMRE. Tubes were kept in the dark and placed in a 25°C incubator for 2 hours. After incubation polyps were washed five times with artificial sea water and then were tumbled for ten minutes to remove excess stain from the surface of the polyp. Polyps were then placed on microscope slides and

were imaged under the RFP filter for 50msec. Membrane potential was quantified in ImageJ software.

RNAseq and Analysis

Polyps were flash frozen in liquid Nitrogen immediately prior to extraction. RNA was extracted using the Direct-zol RNA Microprep RNA kit (Zymo) in an RNA-free hood according to manufactures instructions except that all centrifugation stages were performed at 4°C. RNA libraries were prepared for sequencing using the Nexterra RNA prep kit at Novogene company and samples were sequenced on a Novaseq6000. Reads were trimmed for adaptors and low-quality reads using Trimmomatic (Bolger et al., 2014) with default parameters. Filtered reads were then mapped to the *C. xamachana* polyp transcriptome (A. Ohdera et al., 2019) using Salmon (Patro, Duggal, Love, Irizarry, & Kingsford, 2017) with an index k-mer size of 31. *C. xamachana* transcripts were annotated with the eggNOG mapper (Huerta-Cepas et al., 2019) against the metazoan database. The effect of temperature and day was assessed in DEseq2 (Love et al., 2014) using the model ~treatment+day. Differential gene expression was assessed at day2 and day5. Gene Ontology (GO) enrichments were conducted using the R script GO_MWU (R. M. Wright et al., 2017) based on gene log fold change.

Results

Temperature Stress Reduces Symbiont Colonization and Mitochondrial Membrane Potential

Exposure to 32°C water caused a mild but significant reduction in symbiont density ~25% (t.test 0.07 vs. -0.19, p=0.007) at two days (Figure 1a). After five days of exposure the magnitude of symbiont loss increased to ~75% (t.test -0.08 vs. -0.73, 2.4 e-8) (Figure 1b). Mitochondrial membrane potential increased slightly ~5%, though not significantly after two

days (t.test, p= 0.55) (Figure 1c), however after five days of exposure to 32°C water mitochondrial membrane potential was significantly reduced 31% (t.test, p=0.004) (Figure 1D).

Temperature Stress Causes Transcriptional Changes

Exposure to elevated water temperatures caused substantial transcriptional responses at both day two and day five. The number of differentially expressed genes was lower on day two (1791) (Figure 2a) than on day five (2725) (Figure 2b). On day two the directionality of the differentially expressed genes was fairly even with 870 upregulated and 921 down regulated genes respectively. On day five there were 967 up regulated genes and 1758 down regulated genes. There was high overlap in the differentially expressed genes between the two days as there were 1476 genes which were differentially expressed at both time points. Of these 594 were upregulated at both time points while 773 were down regulated at both time points, with 109 genes being differentially expressed in opposing directions (Figure 2C). Included in the genes down-regulated at both time points is the master regulator of the UPR^{mt} ATF5 (Day two -1.38 lfc , padj = 2.9e-6; Day five -1.54 lfc, padj 7.2e-5). On day two there were 29 enriched CC terms, 41 enriched BP terms. On day five there 70 enriched CC terms and 153 enriched BP terms (Figure 3A-D).

Discussion

Through leveraging the emerging model system of *C. xamachana* I was able to gain additionally insights regarding the interaction of mitochondrial function and loss of symbiosis during the bleaching process. By coupling physiologic and gene expression data across two time points this investigation demonstrates that the loss of symbionts occurs prior to observable

declines in mitochondrial physiology, however this loss of symbiosis is accompanied by down regulation of gene involved in mitochondrial metabolism. Additionally, as the duration of the stress increases so does the magnitude of the response at the level of both physiology and gene expression. Together these data indicate that the observed declines in mitochondrial function may be the result of reduced nutrient availability following the increase in temperature, and that the magnitude of the bleaching response and the survival of the animal during periods of temperature stress likely depend on the duration of exposure.

Reductions of symbiont colonization precede mitochondrial dysfunction

While temperature stress leads to both reductions in symbiont density and mitochondrial membrane potential, these occur at different time points. Symbiont density is reduced earlier in the experiment (day two) compared to mitochondrial membrane potential which is not reduced until the end of the experiment (day five). This indicates that temperature-induced dysfunction of the symbiont likely occurs first in the chain of events. This observation may be due to the thermal tolerance of the strain of symbiont used in this experiment (CasKB8) as this strain has been shown to have reduced photosynthetic capacity at 32C° *ex hospite* (Díaz-Almeyda et al., 2017). The observation of mitochondrial dysfunction at the end of the experiment may reflect the magnitude of symbiont loss as during day two only about 25% of symbionts have been lost, while by day five around 75% of have been lost. As symbiont-derived carbohydrates were the only source of nutrients available to the polyps the increasingly severe loss of symbionts may have limited the amount of available carbohydrates eventually leading to the observed decline in mitochondrial membrane potential. These data support findings from coral bleaching studies which typically observe both reductions in photosynthetic activity and disruptions to host nutrient state (Claar et al., 2020; Nielsen et al., 2018), however, host mitochondrial activity and

symbiont density have not previously been measured simultaneously. Worth noting is that modest symbiont reductions seen on day two would generally not constitute coral bleaching as the bleaching phenotype is generally not visually observable until a greater than 50% decline in symbiont density. Thus, the increased resolution of tracking symbiont colonization in *C. xamachana* polyps over multiple time points may yield insights into the early stage of bleaching which would not be observable in reef-building corals.

The gene expression magnitude increases over time

Temperature stress causes substantial changes to gene expression patterns, which increase in magnitude over the duration of the experiment. Notably, there are more genes differentially expressed by day five than at day two, indicating that the duration of exposure to temperature stress causes an increasingly pronounced transcriptional response. The general patterns of gene expression follow similar trends between the two days as most of the genes differentially expressed on day two are still differentially expressed on day five, with most genes displaying the same directionality in expression. This further supports that the difference between the two time points is likely a reflection of the duration of the temperature stress, rather than different transcriptional responses being activated at the respective time points. There are numerous GO term enrichments at both sampling time points, with similar patterns between the two days, with more enriched terms at the later time point. The enriched terms present on both days include general down-regulation of mitochondrial genes which includes genes involved in OX-PHOS, and up-regulation of genes involved in detoxification of ROS and response to starvation. These terms correspond to commonly observed responses to temperature stress and bleaching in reef-building corals (Putnam, Barott, Ainsworth, & Gates, 2017). Notably ROS accumulation is considered a hallmark of thermal stress and a potential initiator for the bleaching

response (M. P. Lesser, 1997; Nielsen et al., 2018). The increased expression of genes involved in ROS detoxification indicates that *C. xamachana* is likely increasing the expression to mitigate damage from ROS.

One transcriptional pattern which has different activities between the two days is that of immune related genes, where no differences are observed at day two but these genes are highly upregulated by day five. Interestingly, one current theory on the biology of bleaching is that temperature stress disrupts the symbionts ability to evade the host immune system leading the expulsion of the symbiont (Caroline V. Palmer & Traylor-Knowles, 2018). As the increased expression of immune genes is only observed during day five at which symbiont loss is severe this indicates that the increased expulsion of symbionts could be due to activity of the immune system. In support of this, we also observe the enrichment of "vesicle-mediated transport" after five days of temperature stress, potentially indicating that the activation of the immune system works to expel the symbiont containing vesicles. Together these results indicate a link between the magnitude of symbiont loss as a results of temperature stress and transcriptional activation of the immune system. The upregulation of immune genes may also be a consequence of the nature of the *C. xamachana*-*Symbiodiniaceae* symbiosis as the symbiont containing cells are repurposed immune ameobocytes (Lyndby et al., 2020). Thus, the increased expression of immune genes could be indicative of these cells returning to their original immune state as symbionts are expelled.

Links between gene expression and physiology

Our results demonstrate links between the transcriptional and physiologic response of *C. xamachana* exposed to temperature stress. As symbiont density is reduced and the food source of the polyps is depleted these organisms increase the expression of starvation associated genes, and

down-regulate metabolic genes in the OX-PHOS cascade. The magnitude of this transcriptional response increases from day two, to day five as symbiont loss becomes more pronounced. This pattern is likewise reflected by mitochondrial membrane potential which is maintained through two days of temperature stress but becomes reduced by day five. During periods of nutritional stress mitochondria are able to temporarily maintain organelle health through the activation of UPR^{mt} (Pellegrino et al., 2013) which serves to reduce demand on the organelle during periods of intermittent stress to maintain membrane potential of the organelle (Amrita M. Nargund et al., 2015). This response is initiated at the onset of stressful conditions, with activity receding if the stress persist leading to loss of organelle function. Thus, this pattern may explain why membrane potential was maintained until day five. The expression of the master regulator of the UPR^{mt} ATF5 is reduced at both time points, which is likely a reflection of the time course over which this experiment occurred. Specially, these data indicate that by two days of temperature stress there is sufficient cellular dysfunction to begin the process of symbiont expulsion, and thus the initiation event for bleaching is likely activated over shorter time scales. In support of this, the coral *O. faveolata* activates the UPR^{mt} (Dimos, Mahmud, et al., 2019) over a timescale of hours, whereas *C. xamachana* has reduced expression of mitochondrial genes and ATF5 over a timescale of days. Taken together these data indicate that regulation of mitochondrial function via the UPR^{mt} is involved in the response to increased temperatures, but that the time which this response is protective is measured in hours rather than days. This finding is line with previous work in coral as in general corals are able to handle high temperatures over fairly short periods of time such as during low tidal cycles, and that bleaching only occurs after an accumulation of temperature stress. These results highlight that model systems such as *C. xamachana* can provide

insight at resolutions difficult to obtain in reef-building corals which may have important implications in the process of bleaching.

Conclusions

I leveraged an emerging model of cnidarian symbiosis (*C. xamachana*) to demonstrate a link between the breakdown of symbiosis and alterations to mitochondrial functional at the level of both physiology and gene expression. These findings provide further support that these processes are linked as well demonstrate the utility of this *C. xamachana* for further investigations of the cellular pathways involved in thermally induced bleaching, such as the UPR^{mt}.

Chapter 3 Figures

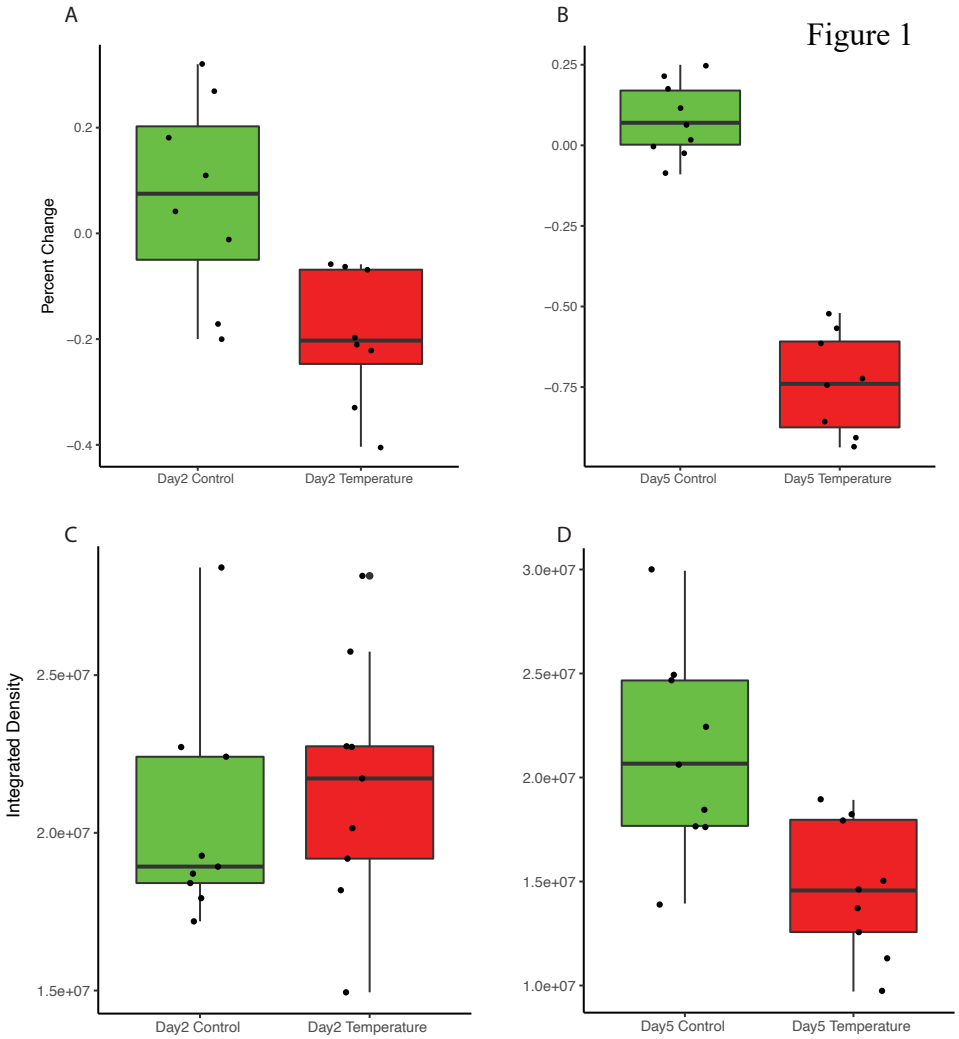

Figure 1

Figure1: Physiological response of *C. xamaxhana* to temperature stress. A) The percent change in symbiont density after two days of exposure to temperature stress ($p < 0.01$, n=9). B) The percent change in symbiont density after five days of temperature stress ($p < 0.01$, n=9). C) Fluorescent intensity measured as integrated density after two days of exposure to temperature stress ($p > 0.01$, n=9). D) Fluorescent intensity measured as integrated density after five days of exposure to temperature stress ($p < 0.01$, n=9).

Figure 2

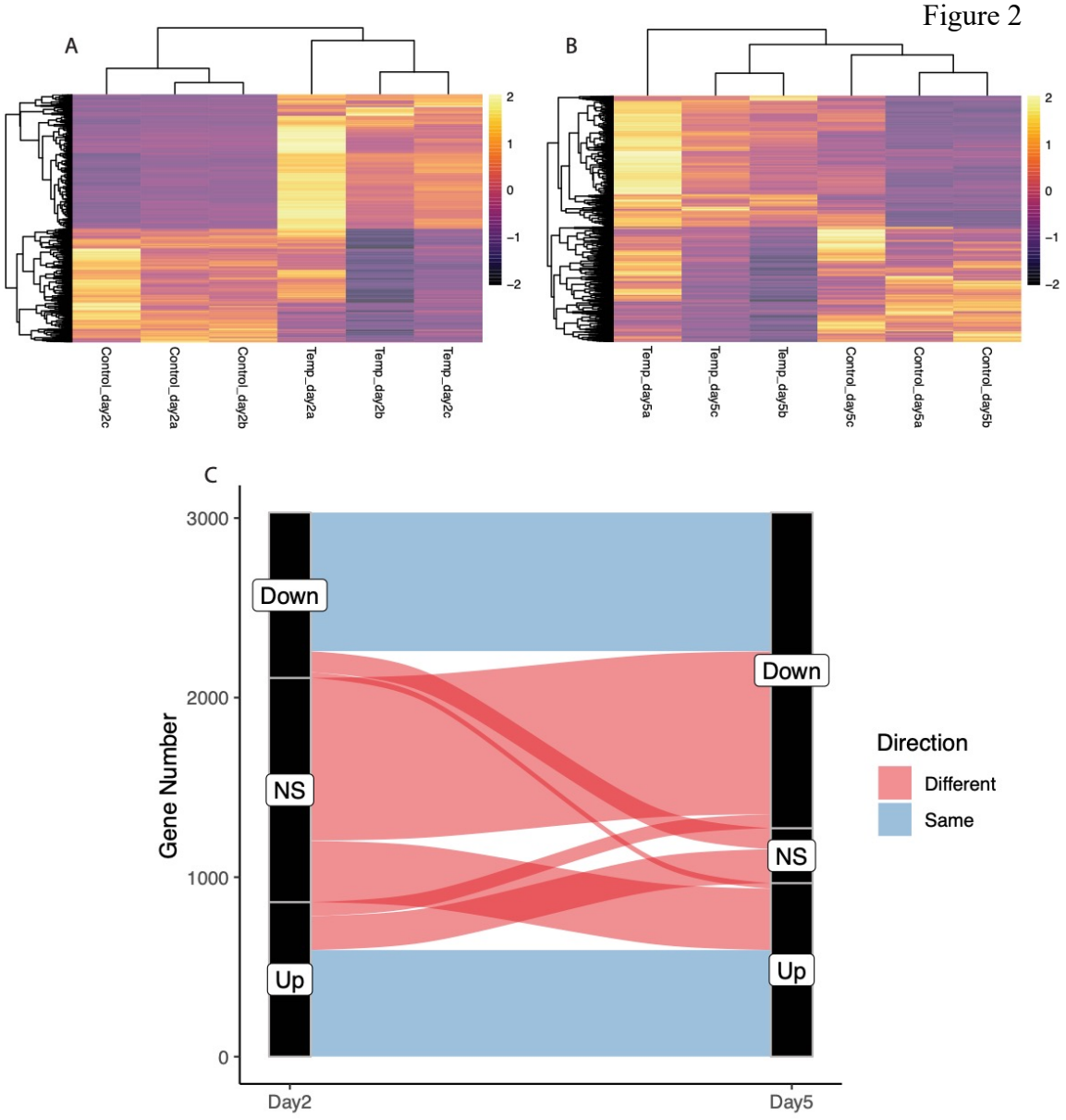

Figure 2: Gene expression changes of *C. xamachana* in response to temperature stress. A) Shown is the Z-score normalized expression of differentially expressed genes after two days of temperature stress. B) Shown is the Z-score normalized expression of differentially expressed genes after five days of temperature stress. C) Shown is an alluvial diagram comparing the differential gene expression profiles between day two and day five.

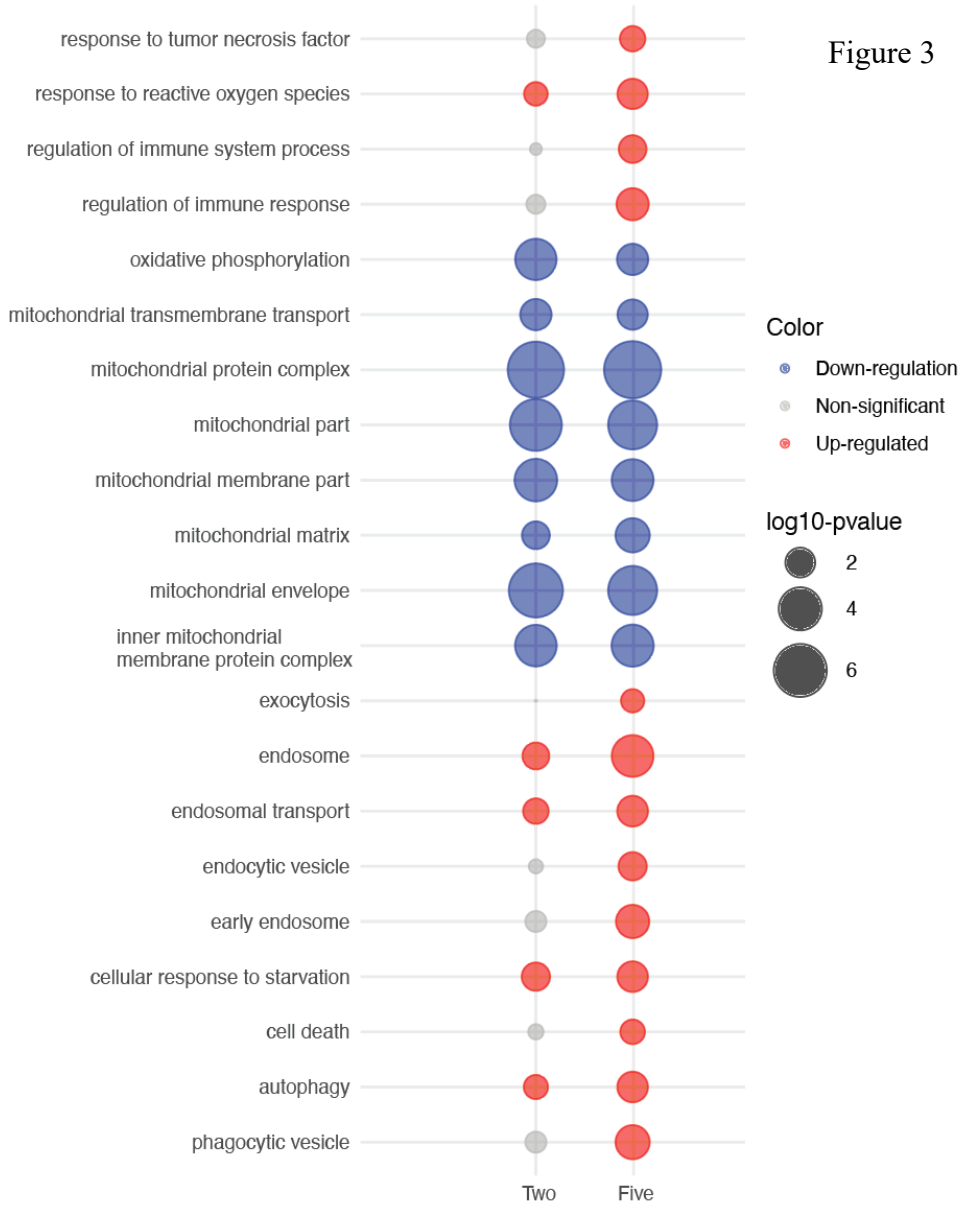

Figure 3

Figure 3: Gene Ontology Enrichment of *C. xamachana* polyps during exposure to temperature stress after two and five days. Shown is a subset of enriched terms which are commonly observed in temperature stress experiments where terms upregulated during temperature stress are shown in red and those down-regulated during temperature stress are show in blue while terms which non-significant differences in expression are shown in grey. Size of the circle

corresponds to significance level measured as the -log10 pvalues where larger circles have lower pvalues.

Chapter 4: Responding to Threats both Foreign and Domestic: NOD-like Receptors in Corals.

Authors: Bradford A. Dimos[1], Caleb C. Butler[1], Contessa A. Ricci[1], Nicholas J. MacKnight[1]

Laura D. Mydlarz[1*],

Affiliations: [1] Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA

*Corresponding Author: Laura Mydlarz

mydlarz@uta.edu

Citation: Dimos, B. A., Butler, C. C., Ricci, C. A., MacKnight, N. J., & Mydlarz, L. D. (2019). Responding to Threats Both Foreign and Domestic: NOD-Like Receptors in Corals. Integrative and Comparative Biology, 59(4), 819–829. doi: 10.1093/icb/icz111

Abstract

Historically mechanisms with which basal animals such as reef-building corals use to respond to changing and increasingly stressful environments have remained elusive. However, the increasing availability of genomic and transcriptomic data from these organisms has provided fundamental insights into the biology of these critically important ecosystem engineers. Notably, insights into cnidarians gained in the post-genomics age have revealed a surprisingly complex immune system which bears a surprisingly level of similarity with the vertebrate innate immune system. This system has been critically linked to how corals respond to the two most prominent threats on a global scale, emerging coral diseases and increasing water temperature, which are recognized cellularly as either foreign or domestic threats respectively. These threats can arise from pathogenic microbes or internal cellular dysfunction, underscoring the need to further understand mechanisms corals use to sense and respond to threats to their cellular integrity. In this investigation, we utilize resources only recently available in the post-genomic era to identify and characterize members of an underexplored class of molecules known as NOD-like receptors (NLRs) in the endangered Caribbean coral *Orbicella faveolata*. We then leverage these data to identify pathways possibly mediated by NLRs in both *O. faveolata* and the ecologically important branching coral *Acropora digitifera*. Overall, we find support that this class of proteins may provide a mechanistic link to how reef-building corals respond to threats both foreign and domestic.

Introduction

Coral immunity in disease and bleaching

Coral reefs are some of the most biodiverse ecosystems on the planet however, coral reefs are currently in decline as a result of increasing disease prevalence and water temperature (Hughes et al., 2017). Coral diseases involve colonization by potentially pathogenic microbes (Beurmann et al., 2018; Frias-Lopez, Bonheyo, Jin, & Fouke, 2003) and subsequent host pathology, whereas increased water temperature causes cellular dysfunction that can cause the coral to lose its algal symbiont and main source of nutrition in a process termed coral bleaching (Gates et al., 1992; Nielsen et al., 2018). These two stressors therefore represent different points of origin where pathogenic microbes represent an external or foreign threat (Ben-Haim, 2003, p.), while elevated temperature causes dysfunctions inside the coral perceived as a domestic threat (Tchernov et al., 2011). Pathogenic bacteria lead to upregulation of components of the coral immune system in an attempt to fight off the foreign invader (R. M. Wright et al., 2017) and either regain homeostasis or induce cell death (Fuess et al., 2017). A similar insult to cellular integrity can occur during temperature stress which leads to accumulation of reactive oxygen species (ROS) (M. P. Lesser, 1997; Michael P Lesser, 2004) and disruption in calcium homeostasis (Desalvo et al., 2008; Fang, Huang, & Lin, 1997) that can lead to activation of stress-signaling pathways including apoptosis (S. R Dunn, Schnitzler, & Weis, 2007). While our understanding of coral immunity and stress responses has progressed (see reviews by Mydlarz et al. 2016; Palmer and Traylor-Knowles 2018), there are certainly many opportunities to expand our knowledge about specific pathways and how they are affected by pathogen pressure and temperature stress.

In order to properly defend itself against a pathogen, the coral host must be able to recognize a pathogen, signal for a response and execute effector responses (Caroline V. Palmer & Traylor-Knowles, 2018). Pattern recognition receptors (PRRs) are essential for pathogen

recognition and responding to cellular stress (Pasquier, 2006; Tetreau et al., 2017) by recognizing and binding conserved molecular motifs, and inducing subsequent immune responses (Akira, Uematsu, & Takeuchi, 2006). The two primary classes of molecules sensed by PRRs are microbial associated molecular patterns (MAMPs) (Hargreaves & Medzhitov, 2005), and danger associated molecular patterns (DAMPs) (Newton & Dixit, 2012; Rosin & Okusa, 2011), which can be viewed as foreign and domestic danger signals to the host organism respectively. PRR signaling pathways consist of a number of intermediate molecules which promote the necessary changes in gene expression and protein activity to generate an effective defense response against potential pathogens (L. M. Williams et al., 2018) including production of antimicrobial compounds and the activation of phagocytic cells (Underhill & Ozinsky, 2002).

Recently, advances in our understanding of different immune PRRs including the membrane bound Toll-like receptors (TLRs) have shown remarkable homology between coral TLRs (D. J. Miller et al., 2007; Poole & Weis, 2014; Shinzato et al., 2011; Wolenski et al., 2011) and vertebrate TLRs in their ability to detect threats to cellular integrity in the form of MAMPs (L. M. Williams et al., 2018). While TLRs are readily known to be important in corals' responses to extracellular microbes, the cytoplasmic NOD-like receptors (NLRs) have received considerably less attention. NLRs are PRRs which serve as intra-cellular sentinels (Fritz, Ferrero, Philpott, & Girardin, 2006; Philip Rosenstiel, Philipp, Schreiber, & Bosch, 2009) that are capable of detecting both MAMPs such as bacteria and virulence factors (Hsu et al., 2008; Inohara & Nuñez, 2001), as well as DAMPs including accumulation of reactive oxygen species (ROS) (F. Bauernfeind et al., 2011; Q. Liu, Zhang, Hu, Zhou, & Zhou, 2018; Sutterwala, Haasken, & Cassel, 2014) and detection of cytoplasmic calcium (Lee et al., 2012), allowing them to respond to threats arising from both foreign and domestic sources. The mechanisms of how

biotic and abiotic stress signals are translated into effector responses such as apoptosis, or phenotypes such as symbiont loss and bleaching are still elusive, making NLRs great targets for investigation.

Advancements of next generation technology have led to rapid increases in our understanding of coral genomics and transcriptomics. The numerous datasets available to researchers have allowed for novel analyses and development of cellular models. We are now able to take advantage of numerous bioinformatic resources to characterize an underexplored class of receptors in cnidarians known as NLRs as well their possible protein interactions. Our goals of this inestigation is to investigate the composition of the NLR repertoire of the endangered Caribbean reef-building coral *Orbicella faveolata* and the potential NLR interactome of both our identified *O. faveolata* and previously identified *Acropora digitifera* NLRs (Hamada et al., 2013). Here, we show that the NLR repertoire of both *O. faveolata* and *A. digitifera* possess the molecular machinery to respond to cellular threats both foreign and domestic and may potentially mediate the response of these organisms to both biotic and abiotic stressors.

NLR Biology

The intracellular-localized NLRs are a critical portion of the innate immune system (Elinav, Strowig, Henao-Mejia, & Flavell, 2011) and therefore likely evolved as a way to protect an organism from threats both foreign and domestic, because of the ability of these proteins to sense a wide range of both externally derived (MAMPs), and endogenously produced threats (DAMPS) within the cytoplasm (Latz, Xiao, & Stutz, 2013). Initially NLRs were thought to have arisen in teleost fish as model invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster* do not possess homologs of NLRs (P. Rosenstiel, Jacobs, Till, & Schreiber, 2008; J. Ting & Williams, 2005), this idea was challenged when the purple sea urchin

(*Strongylocentrotus purpuratus*) genome revealed a large number of NOD-like proteins (Rast, Smith, Loza-Coll, Hibino, & Litman, 2006). The evolutionarily basal state of NLRs was further confirmed as NLR homologs were found in early diverging animals like cnidarians (Hamada et al., 2013; Lange et al., 2011). Surprisingly NLRs are not just present in early animals but may actually be expanded compared to their mammalian counterparts, as *A. digitiferia* possesses roughly 500 NLR like proteins (Hamada et al., 2013), and the purple sea urchin possesses more than 200 (Rast et al., 2006) compared to the 22 in humans (Sutterwala et al., 2014).

NLRs typically have a tripartite architecture containing: an amino terminus effector domain, a central NACHT/Nucleotide binding domain (NBD), and a carboxy terminal repeat region comprised of leucine rich repeats (J. P.-Y. Ting et al., 2008). The amino effector terminus of NLRs usually contains a death domain fold (Koonin & Aravind, 2000) which allows these molecules to directly translate ligand recognition into effector responses through their ability recruit and activate caspases (Duncan & Canna, 2018; Martindale & Holbrook, 2002). In mammals, the caspase activating effector domains usually comprise either a caspase recruitment domain (CARD), or a Pyrin domain (Inohara & Nuñez, 2003), with homotypic interactions (Kersse, Verspurten, Berghe, & Vandenabeele, 2011) occurring between the CARD domain of caspases and CARD containing NLRs directly, or via an adaptor molecule for Pyrin-containing NLR proteins (Vajjhala, Mirams, & Hill, 2012). The domain which facilities the ligand-detection ability of NLRs is not fully elucidated (Wilmanski, Petnicki-Ocwieja, & Kobayashi, 2008), however ligand detection is thought to induce a conformation change, which relieves the auto-inhibitory effects of the C-terminal repeats (Latz et al., 2013). This class of proteins is able to detect a diverse array of signals (Horvath, Schrum, De Nardo, & Latz, 2011), including: Lipopolysaccharide (LPS) (Mariathasan et al., 2006), reactive oxygen species (ROS) (Martinon,

Mayor, & Tschopp, 2009), and organelle calcium efflux (Lee et al., 2012) in addition to other signatures of cellular stress (Dostert et al., 2008; Masters et al., 2010; Zhou, Tardivel, Thorens, Choi, & Tschopp, 2010). The activation of NLRs can be a complex process which can involve both direct as well as indirect mechanisms (Sutterwala et al., 2014). As an example, the detection of gram-negative LPS by NLRs can occur via recognition through the C-terminal LRRs, though this response can be amplified if other signaling pathways such as TLRs are also concurrently activated (He, Hara, & Núñez, 2016). Likewise, the ability of these proteins to be activated by ROS can be accomplished directly, as ROS accumulation is essential to increased transcription of some NLRs (F. Bauernfeind et al., 2011), or indirectly via the redox-sensitive thioredoxin-interacting protein which binds and activates some NLRs (Zhou et al., 2010). These mechanisms demonstrate that in addition to direct ligand binding, indirect sensing mechanisms including protein-protein interactions between NLRs as well as other proteins are (Ferwerda et al., 2008; Horvath et al., 2011; Hsu et al., 2008) important in activation. Thus, NLRs act as cellular funnels to activate stress responses, in response to a diversity of cellular insults. Interestingly, compared to the well-characterized vertebrate NLRs, cnidarian NLR repertoires are expanded and contain novel domain combinations, both at the amino and carboxy terminus (Hamada et al., 2013; van der Burg, Prentis, Surm, & Pavasovic, 2016).

Regulation of NLR activity occurs at two levels: priming (F. G. Bauernfeind et al., 2009) and activation (Miao, Rajan, & Aderem, 2011), which consists of increasing transcription of NLR genes and ligand binding of NLRs respectively. The relative levels of priming and activation have important phenotypic effects (Lech, Avila-Ferrufino, Skuginna, Susanti, & Anders, 2010) as this can dictate whether immune activation and resolution, or cell death occurs (Latz et al., 2013). As the activating agents of NLRs are important in the response of reef-

building corals to environmental stress this class of molecules is worth further investigation into their possible role to facilitate or impeded adaptation to increasingly stressful environmental conditions experienced by reef-building corals.

Coral NLR repertoire

We investigated the proteome of *O. faveolata* for proteins which contain NACHT/NBD domains, and identified 46 putative NLR-like proteins (Figure 1) with the protein domain identification program HMMR (Eddy, 2011) using the NBD/NACHT consensus sequence obtained from the protein family (PFAM) database (El-Gebali et al., 2019). Canonical NLR's possess a central NBD domain with C-terminal LRRs (J. P.-Y. Ting et al., 2008) however, as previous reports indicate that NLRs in cnidarians have novel domain combinations (van der Burg et al., 2016) we considered all NBD containing proteins as putative NLRs for this analysis. As our results revealed significantly less NLRs than identified in the reef-building coral *A. digitifera* (Hamada et al., 2013), we performed a subsequent analysis on the *A. digitifera* proteome which yielded similar results as Hamada et al. 2013 confirming our analysis approach. We then investigated the domain composition of the identified O. faveolata NLRs by analyzing the peptide sequences with the online PFAM database (El-Gebali et al., 2019). This analysis identified novel domain combinations in the *O. faveolata* NLR repertoire at both termini which reflect the findings in other cnidarians (Hamada et al., 2013; van der Burg et al., 2016). Among the novel domain combinations, we identified NLR-like proteins with N-terminus death effector domains (DED), glycosyl-transferase domains, and HEPN domains (Fig. 1). DEDs belong to the death domain fold protein super family as do CARD and pyrin domains seen on the amino-terminus of mammalian NLRs. Interestingly, in contrast to *A. digitifera* we were unable to

identify any *O. faveolata* NLRs with N-terminal death domains (DD)s, which belong to the same protein superfamily as DEDs. Additionally, several of the effector domains in the *O. faveolata* NLR repertoire are associated with immunity, including glycosyl-transferase (Ohtsubo & Marth, 2006) and HEPN domains which have been implicated in anti-viral responses (Anantharaman, Makarova, Burroughs, Koonin, & Aravind, 2013). In addition, we also see novel C-terminal repeat domains in *O. faveolata*, including ankyrin, WD40, and Tetrocorticopeptide repeats (Fig. 1).

To provide support for the role of the above proteins during cellular stress conditions we investigated the expression patterns of NLRs in two existing *O. faveolata* transcriptomic datasets, one during treatment with the bacterial MAMP LPS (Fuess et al., 2017) and one during a natural bleaching event (Pinzon et al., 2015). We found that 6 NLRs were significantly upregulated in LPS treated colonies of *O. faveolata* and 3 NLRs were upregulated in bleached versus unbleached O. faveolata colonies. Overall, it appears that *O. faveolata* contains NLRs which appear to function in immunity and cellular stress consistent with previous reports of the function of NLRs in cnidarians (Hamada et al., 2013; Lange et al., 2011; van der Burg et al., 2016). These conclusions corroborate a finding by Libro et al. 2016 who determined that increased expression of NLRs confer protection from White Band Disease in *A. cervicornis*. As NLR effector responses are predicated upon interactions with other proteins and members of the Death Domain-fold superfamily function through homotypic interactions (Valmiki & Ramos, 2009), we investigated the potential interaction partners for the DED-containing NLRs of O. faveolata as well as the possible interaction partners of the previously identified DED, DD, and CARD-containing NLRs of *A. digitifera* (Hamada et al., 2013).

Coral NLR Reactomes

By using the protein domain identification program hmmr (Eddy, 2011), we identified 43 proteins in the *O. faveolata* proteome which match the DED consensus sequence obtained from PFAM (El-Gebali et al., 2019) which may serve as potential NLR interaction partners (Figure 2). The identified proteins are involved in several biological processes including: extrinsic apoptosis, immune signaling, and inhibition of intrinsic apoptosis (Figure 2) indicating that *O. faveolata* NLRs appear capable of playing a role in modulation of both apoptosis and immunity. The *A. digitifera* NLR repertoire is expanded compared to that of *O. faveolata* and likewise has additional N-terminal effector domains including: DED, DD and CARD containing NLRs (Hamada et al., 2013). We used the same methodology to find interaction partners for the expanded domains composition found on *A. digitifera* NLRs. Similar to *O. faveolata* the *A. digitifera* DED-interaction partners involve components of extrinsic apoptosis (Figure 3). The predicted DD-containing *A. digitifera* NLR interaction partners involve known components of the coral immune system, and apoptotic machinery including the APAF-1 apoptosome which mediates intrinsic apoptosis through activation of executioner caspases. *A. digitifera* CARD containing-NLR appear able to interact with additional apoptotic-activating proteins based upon homotypic interactions (Figure 3). Overall, it appears that both *A. digitifera* and *O. faveolata* NLRs are predicted to have the ability recruit and activate caspases as well as induce immunity, reflecting work in vertebrate systems (Ogura et al., 2001), and highlighting their probable role in mediating coral stress responses.

Inflammasomes

Mammalian NLRs possess the ability to oligermize (Inohara & Nuñez, 2003) into large multi-protein complexes called inflammasomes which provide a scaffold to activate inflammatory caspases, potentiate immune cascades as well as lead to apoptosis. The formation

of these complexes are dependent on the ability of NLRs to interact with caspases either directly or through adaptor proteins (Stutz, Horvath, Monks, & Latz, 2013; Van Opdenbosch et al., 2014). To investigate the possibility of coral NLRs to have an analogous function we searched for proteins which may function as NLR-Caspase adaptors in both species. We identified a previously undescribed *O. faveolata* adaptor protein which contains a DED and a DD which may allow *O. faveolata's* NLRs to interact with additional caspases (Figure 2). The *A. digitifera* proteome likewise contains an adaptor protein featuring both a DD and CARD domain which may also facilitate additional caspase interactions. These adaptor proteins could potentially expand the caspase repertoire upon which NLRs may interact in both *O. faveolata* and *A. digitifera* by forming inflammasome-like complexes. There is precedent for inflammasome-like complexes in basal animals as the cnidarian *Hydra magnipapillata* contains an NLR adaptor protein termed DODE which has been suggested to function like mammalian inflammasome adaptor proteins (Lange et al., 2011), and our analysis may have identified the coral versions of this protein. Overall, we show that both species of coral investigated may have the required molecular machinery to form inflammasome like complexes through the action of their NLRs, which would allow corals to potentiate immune and stress signaling when responding to threats both foreign and domestic.

Conclusions

Our investigation revealed the presence of an NLR repertoire in the coral *O. faveolata* which may be important in the regulation of both immunity and apoptosis. Cnidarian immune systems demonstrate remarkable similarity to vertebrate innate immunity given the high level of divergence between these clades (Mansfield et al., 2017), which we see reflected in NLRs. Given

the key role of immune proteins in allowing organisms to survive, conservation and convergence of key elements of immune pathways is not surprising (Meunier & Broz, 2017). In support of this our results are in agreement with other investigations which have highlighted a conserved function of NLRs to activate immunity as well as modulate apoptosis near the base of animal evolution (Lange et al., 2011; van der Burg et al., 2016).

NLRs have been suggested to functionally converge (Q. Zhang, Zmasek, & Godzik, 2010) and our findings indicate that both coral species have the necessary machinery to be able to form inflammasome-like complexes, similar to NLRs from more well-studied systems (Latz et al., 2013; Martinon et al., 2009). While direct detection of inflammasomes in cnidarians is lacking, our results corroborate the study by Lange et al. 2011 which demonstrated formation of an inflammasome like complex in the cnidarian Hydra. In addition, our data indicate that complex apoptotic mechanisms may be related to NLR activity in corals. As apoptosis has been demonstrated to be critically-linked to coral disease and bleaching (Fuess et al., 2017; Richier et al., 2006; Tchernov et al., 2011), NLRs may act as cellular mediators to translate both biotic and abiotic threats into apoptosis activation in corals.

Studies into the mechanisms which corals use to respond to both immune challenge and elevated temperature have highlighted overlapping pathways indicating that these stressors may not act via entirely distinct mechanisms (Caroline V. Palmer, 2018). Given the known battery of NLR-activating compounds this class of molecules may play a role in the observed overlap, as these proteins are capable of sensing both externally derived danger signals (MAMPs) and endogenously derived danger signals (DAMPS) (Mariathasan et al., 2006). Furthermore, when disease and temperature interact there is a synergistic effect on coral mortality (Bruno et al., 2007) indicating common mechanisms may be at play. NLR activity exists on a continuum

where lower levels of activation can potentiate an immune cascade leading to immune promotion and resolution (Broz et al., 2012; Kayagaki et al., 2011), whereas higher levels of activation can lead to inflammatory cell death (Latz et al., 2013). If both temperature and disease stress converge on NLRs, this could tip the balance towards cell death and be a cellular mechanism underling the synergistic interaction of biotic and abiotic stress in leading to coral declines. Overall, NLRs are a mechanism to sense and overcome threats both foreign and domestic, and functional characterization of the NLR repertoire of corals may shed light on mechanisms corals use to cope with increasingly stressful environments.

BOX – ROLE OF NLRs IN CORAL-ALGAL SYMBIOSIS

As a holobiont, corals must maintain balance between the presence of symbiotic microbes, pathogens and immunity. In particular, the intracellular symbiosis formed with dinoflagellate algae of the family *Symbiodiniaceae* is important as these algae provide the coral host with up to 90% of their energy requirements (Mies, Sumida, Rädecker, & Voolstra, 2017). These symbiotic dinoflagellates reside within the gastrodermal layer of coral host tissue in an arrested phagosome (termed the symbiosome) (Barott, Venn, Perez, Tambutté, & Tresguerres, 2015). Immune evasion or suppression by *Symbiodiniaceae* is a required component of the establishment and maintenance of symbiosis. Due to the intimate association between corals and their microbial symbionts, mechanisms such as NLRs which can both sense molecular patterns and regulate immunity may in be key in facilitating this delicate relationship.

The onset of symbiosis begins with the phagocytosis of the *Symbiodiniaceae* cell. A critical step is the arrest of phagosome-lysosome fusion (Davy, Allemand, & Weis, 2012), which prevents digestion of the dinoflagellate cell and allows the phagosome to mature into a symbiosome.

Currently, the molecules mediating this arrest remain unknown. However, in mice the interaction of two NLRs (NLRC4 and NAIP5) is necessary for phago-lysosomal fusion, and activation of NLRC4 occurs in response to flagellin (Lage et al., 2014). Interestingly, *Symbiodiniaceae* undergo a morphological shift when entering a symbiotic state, losing its heterokont flagella and transforming into a coccoid cell (Muscatine et al., 1975). Additionally, in-situ hybridization has shown that NLR expression in *Hydra* is limited to the gastrodermal layer (Lange et al., 2011) and future investigations should explore if this expression pattern is conserved as well in symbiotic cnidarians, which may highlight a possible mechanism through which PRRs may play a role in facilitating symbiosis.

NLRs are intracellular monitors for the presence of MAMPS and DAMPs, mediating the detection of cellular perturbations that occur during biotic and abiotic stress. If NLRs function in symbiosis as well as damage detection, they may play an integral role in the breakdown of symbiosis under environmental stress. Cellular stress presenting directly as DAMPs like ROS can activate NLRs. In addition, indirect mechanisms of damage can also activate NLRs, where NLRs are bound to resident proteins and activated upon the detection of a conformational change (Jones, Vance, & Dangl, 2016). Such conformational changes in NLR-bound proteins can occur during changes in cellular redox state as well as alterations in metabolism (Zhou et al., 2010). Well-known roles of *Symbiodiniaceae* include translocating photosynthetically-derived nutrients and suppressing immunity to ‘colonize’ hosts, supporting the fact that these indirect NLR activation mechanisms may be at play in coral. Further, during stress the cellular redox state is altered as is the flux of photosynthetically derived nutrients; both of which can induce direct and/or off-target effects. In this way NLR activation may be a mechanism that triggers immune activation and symbiont expulsion during stress conditions.

Chapter 4 Figures

Figure 1

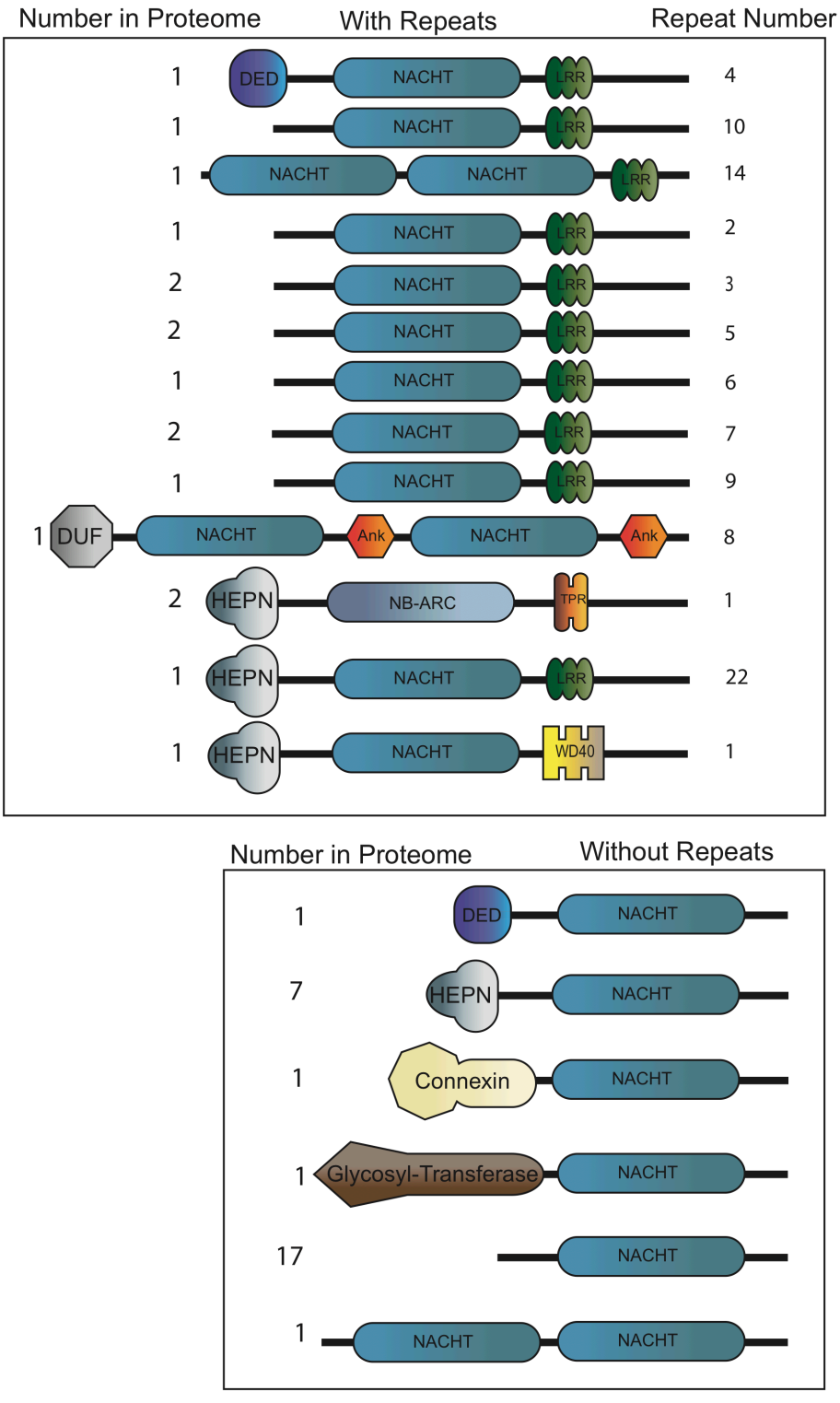

Figure 1: *O. faveolata's* NLR repertoire. 46 NLR containing proteins identified in the proteome of *O. faveolata*, 18 of which contain variable numbers of C-terminal repeats, and 28 of which do not contain repeats. The N-terminal domain composition of these proteins is variable containing: DED (death effector domains), DUF (domain of unknown function), HEPN (higher eukaryotes and prokaryotes nucleotide-binding domain), Connexin, and Glycosyl-transferase domains.

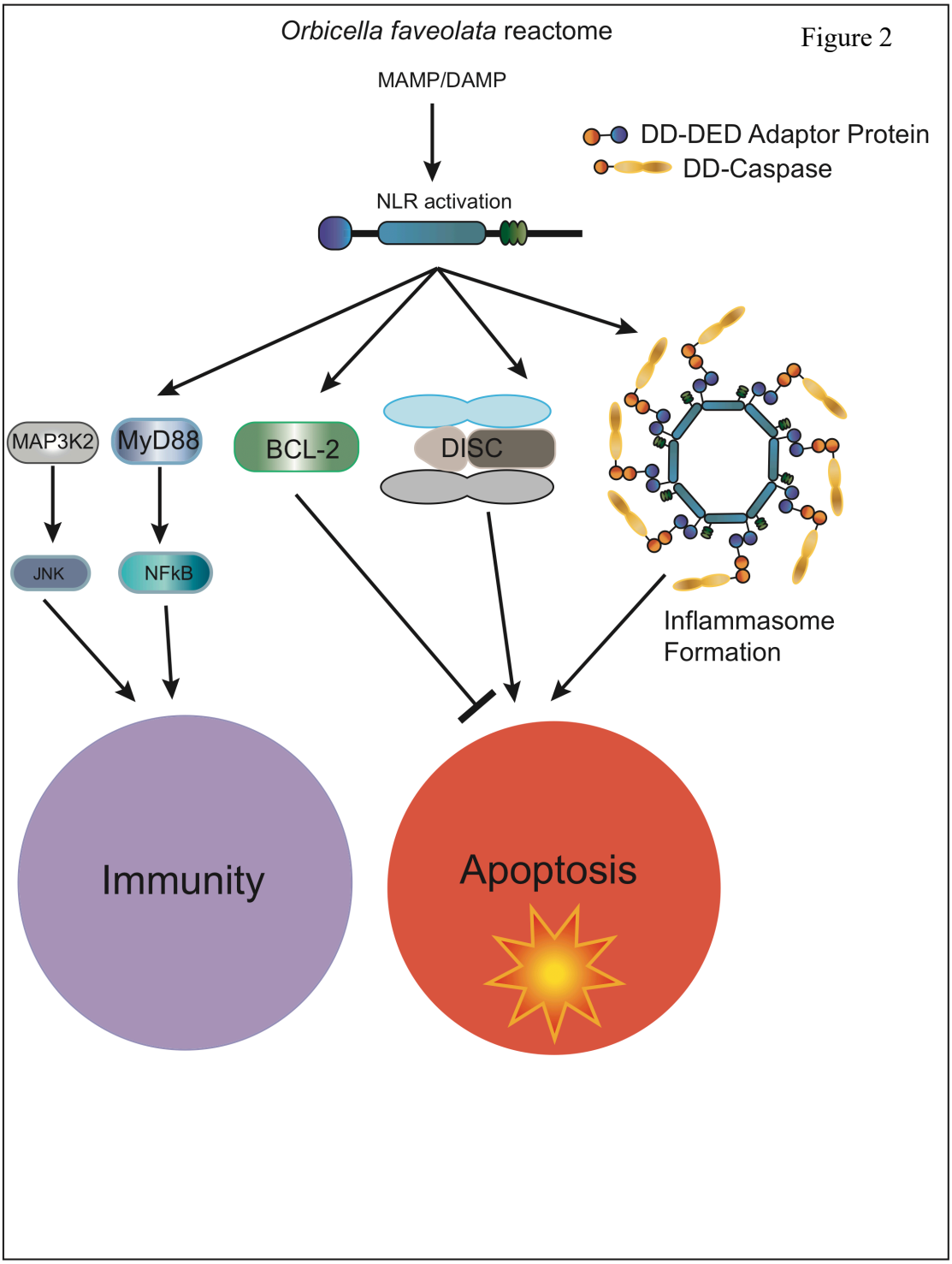
Orbicella faveolata reactome
Figure 2
MAMP/DAMP
DD-DED Adaptor Protein
DD-Caspase
NLR activation
MAP3K2
MyD88
BCL-2
DISC
JNK
NFkB
Inflammasome
Formation
Immunity
Apoptosis

Figure 2: The reactome of *O. faveolata* NLR proteins: *O. faveolata* contains potential NLR interaction partners involved in apoptosis, and immunity. Four key components of the death-induced signaling complex (DISC) were identified as NLR interaction partners including: Caspase-8, FADD, CFLAR and Caspase 10. The DISC which serves to induce apoptosis and has previously been suggested to involve interactions with NLRs. Two of the identified NLR interaction partners involved in immune signaling include mitogen activated kinase kinase kinase 2 (MAP3K2) and myeloid differentiation primary response protein (MyD88). MAP3K2 interacts with c-Jun kinase (JNK) which is involved in activation of various immune responses. MyD88 acts via a signaling pathway leading to the dissociation of the inhibitory Ikb complex from NFkb llowing the 2 subunits of NFkb to translocate into the nucleus and exert their transcriptional activities. Additionally, B-cell lymphoma 2 (BCL-2) was identified as a possible NLR binding partner which can inhibit intrinsic apoptosis through preventing Bak-Bax dependent cytochrome C release, in support of this interaction other systems have shown BCL-2 to inhibit NLRs. An *O. faveolata* DD-DED adaptor protein was identified which may allow DED-containing NLRs to interaction with *O. faveolata's* DD-containing caspases 2 and 3 and possibly function to form an inflammasome.

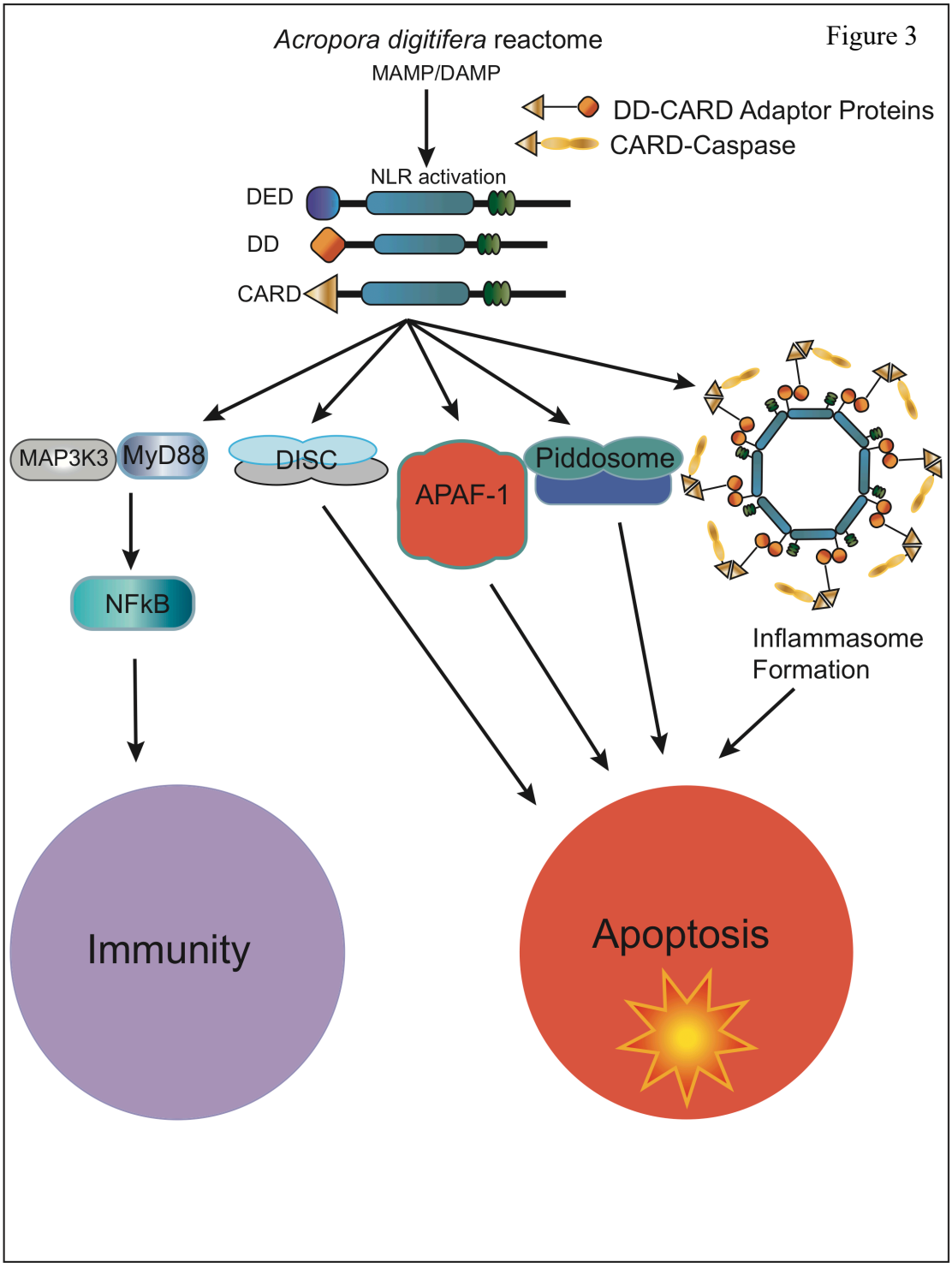
Acropora digitifera reactome
Figure 3
MAMP/DAMP
DD-CARD Adaptor Proteins
CARD-Caspase
NLR activation
DED
DD
CARD
MAP3K3
MyD88
DISC
APAF-1
Piddosome
NFkB
Inflammasome
Formation
Immunity
Apoptosis

Figure 3: The reactome of *A. digitifera* NLR proteins: *A. digitifera* DED-interaction partners involve components of extrinsic apoptosis DISC including Caspase-8 and Caspase-10. However, unlike *O. faveolata* other components of the DISC such as FADD and CFLAR do not appear. DD containing proteins in the *A. digitifera* proteome also involve components of apoptosome formation, and immune activation. The apoptosome is a large multi-meric protein complex which activates executioner caspases and relies upon the scaffolding protein APAF-1. Homotypic interactions between NLRs and the NFkb p100 subunit as well as the NFkb activating proteins MAP3K3 and MyD88 may cause immune activation reflecting work in vertebrate systems which demonstrate that NLRs can activate NFkb. CARD containing-NLRs appear able to interact with additional apoptotic proteins, including Caspase-2 and CRADD which are both components of the apoptosis-inducing PIDDomse protein complex, as well as APAF-1 and capase-8. An adaptor protein which contains a DD and a CARD was identified which may expand the caspase repertoire upon which *A. digitifera* NLRs can activate through formation of an inflammasome.

Chapter 5: Adaptive Variation in Homolog Number Within Transcript Families Promotes Expression Divergence in Reef-Building Coral

Bradford Dimos[1], Madison Emery[1], Kelsey Beavers[1], Nicholas MacKnight[1], Marilyn Brandt[2], Jeffery Demuth[1], Laura Mydlarz[1]*.

1) Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA

2) Center for Marine and Environmental Studies, University of the Virgin Islands, St. Thomas, US Virgin Islands 00802, USA

* Corresponding author, email: mydlarz@uta.edu

Abstract

Gene expression is commonly used to gain insight into the genetic basis of how organisms adapt and respond to changing environments, with multi-species gene expression experiments becoming increasingly popular. However, evolutionary processes which can influence gene expression patterns between species such as the presence of paralogs which arise from gene duplication events are rarely accounted for. Paralogous transcripts can alter the transcriptional output of a gene and thus exclusion of these transcripts can obscure important biological differences between species. To address this issue, we investigated how differences in transcript family size is associated with divergent gene expression patterns in five species of reef-building corals. Overall, we demonstrate that transcript families which are rapidly evolving in terms of size have increased levels of expression divergence. Additionally, these rapidly evolving transcript families are enriched for multiple biological processes, with genes involved in the coral innate immune system demonstrating pronounced variation in homolog number between species. Overall, this investigation demonstrates the importance of incorporating paralogous transcripts when comparing gene expression across species by influencing both transcriptional output and the number of transcripts within biological processes.

Intro

Quantifying gene expression patterns in order to understand how an organism interacts with its environment has become common place in ecological research. This practice is particularly popular to understand the mechanisms organisms use to respond to the types of stress which are associated with climate change (Rivera et al., 2021). It is also clear that investigating gene expression patterns across multiple species exposed to the same type of stress

is important to determine how the community will be reshaped by the said stressor (Bernal et al., 2020; Dixon, Abbott, & Matz, 2020; Fuess et al., 2017; Traylor-Knowles et al., 2021). However, multi-species gene expression experiments present a suite of challenges, the foremost of which is obtaining a comparable list of homologous transcripts for use in downstream analysis (Rey, Veber, Boussau, & Sémon, 2019). Most commonly, this barrier is resolved through the identification of one-to-one orthologs across species (Bernal et al., 2020; Foissac et al., 2019; Hao et al., 2019; Huerlimann et al., 2020; Khan et al., 2013). However, this framework necessitates removing all transcripts that do not display this type of one-to-one conservation, consequently excluding paralogs (Tekaia, 2016) and limiting the biological inferences that can be drawn. Additionally, as the number of species investigated or the phylogenetic distance between species increases, the number of one-to-one homologous transcripts declines. One solution to this problem is to group transcripts into homologous transcript families (HTFs) which include both orthologs and paralogs (David M. Emms & Kelly, 2019) removing the requirement of the one-to-one relationship.

Incorporating paralogs through the identification of HTFs can be especially important in studies of adaptation since gene gains and losses, which dictate the number of paralogs within an HTF, can become fixed in populations due to both neutral and adaptive processes (Force et al., 1999; Lynch, 2000; Ohno, Susumu, 1970). While expansion and contraction of HTF size may occur due to neutral processes, there is mounting evidence that some gene families gain and lose genes more rapidly than can be explained by a neutral model (Demuth & Hahn, 2009; Kondrashov, 2012). The most widely accepted explanations for gene family expansion are that it promotes neofunctionalization and subfunctionalization by reducing pleiotropic constraints (Des Marais & Rausher, 2008; Stearns, 2010), or simply increases the gene dose, thereby increasing

the potential transcriptional output (Force et al., 1999; Ohno, Susumu, 1970). Gene losses can also be adaptive through promoting genetic efficiency or removing genes whose costs are not outweighed by their benefits (Albalat & Cañestro, 2016). Numerous recent studies have described how changes in gene family size can promote adaptation and the diversification of organismal traits (Chain et al., 2014; Ronco et al., 2021; Tejada-Martinez, de Magalhães, & Opazo, 2021).Thus, the exclusion of paralogs from comparative gene expression studies may obscure important biological differences.

In homologous gene families, adaptive changes in copy number versus non-adaptive changes which arise over evolutionary time can be identified through first describing the background rate of gene family turnover, followed by the identification of gene families which have significant deviations from this rate (De Bie, Cristianini, Demuth, & Hahn, 2006; M. W. Hahn, 2005). As the background rate of gene turnover represents a neutral scenario, changes that have adaptive significance will demonstrate increased rates of change and can be described as rapidly evolving. This type of rapid gene evolution is particularly common in gene families which are involved in arms races such as immune genes (Machado & Ottolini, 2015; Malmstrøm et al., 2016). However, other types of ecologically-relevant adaptation have been shown to follow this pattern of rapid changes in copy number such as expansions in hypoxia-related genes in high altitude yaks (Qiu et al., 2012) and chemoreception and vision genes for predator responses in Daphnia (X. Zhang et al., 2021). While the rapid evolution of gene families is generally investigated from genome-derived gene models, a similar approach can be applied to transcriptome assemblies, as the paralogs which result from gene duplication events will generate novel transcripts within HTFs.

The number of transcripts within HTFs is an important consideration when comparing expression across species, since in principle larger transcript family size results in more spatially or temporally specialized expression (Duarte et al., 2006; Guschanski, Warnefors, & Kaessmann, 2017). The increased transcriptional output associated with increased gene dose are often deleterious and will be quickly resolved through mechanisms such as epigenetic silencing (K. M. Huang & Chain, 2021) or functional sub-division of the two gene copies (De Smet, Sabaghian, Li, Saeys, & Van de Peer, 2017). The transcriptional activity of paralogs can be assessed through quantifying global gene expression (Song, Potter, Doyle, & Coate, 2020) which shows that expression levels are subject to selection (Veitia & Birchler, 2021), and can result in the generation of new expression patterns across species (Gillard et al., 2021). These types of species-level changes can be identified by comparing how much the expression of a gene varies between versus within species (Rohlfs & Nielsen, 2015). This approach has proved successful in identifying divergent transcriptional responses to a heatwave in five species of coral reef fish (Bernal et al., 2020) and the unique expression of detoxification genes in Poecilia Mexicana which inhabit sulfide springs (Greenway et al., 2020). Importantly, this framework can be used to investigate if the rapid evolution of transcript families is associated with expression divergence.

Reef-building corals are an intriguing system to study how variation in the number of paralogs within HTFs influences expression divergence as there are numerous published gene expression studies across of a variety of species and stress conditions. Despite the importance and significance of gene expression data, the number of studies which investigate expression across multiple species either do so in a qualitative way (Fuess et al., 2017; Traylor-Knowles et al., 2021) or employ closely related species allowing reads to be mapped to a common reference

assembly (Dixon et al., 2020). To date only one published study in coral has quantitatively compared expression across species from different genera (Avila-Magaña et al., 2021), however this study excluded paralogs from their analysis. Whole tissue homogenates provides a good sampling of coral cell types (Levy et al., 2021) due to their relatively simply body plan. Demographically, corals have historically possessed large effective population sizes (Matz, Treml, Aglyamova, & Bay, 2018; Prada et al., 2016) and high genetic connectivity (S. W. Davies, Treml, Kenkel, & Matz, 2015), conditions which favor efficient selection. Further, adult corals are sessile meaning that they cannot track favorable environmental conditions and instead must be able to dynamically adjust gene expression profiles in order to persist during unfavorable conditions. Adaptive changes in gene copy number have been demonstrated to accommodate this sessile life-history strategy in plants (Prunier, Caron, & MacKay, 2017) and thus there may be substantial variation in HTFs within corals. In support of this, some investigations have reported variation in multi-copy gene families between coral species (Dimos, Butler, Ricci, MacKnight, & Mydlarz, 2019; Hamada et al., 2013; Shinzato et al., 2020), however a systematic evaluation of this process between species from different genera and how this influences expression patterns has not been performed.

Anthropogenic impacts on the environment have caused widespread coral loss across the globe (Maynard et al., 2015; Stuart-Smith, Brown, Ceccarelli, & Edgar, 2018) prompting numerous investigations which seek to understand the stress reposes and adaptive capacity of reef building corals. In particular, Caribbean coral reefs have been severely depleted (Gardner, 2003), owing to the combined effects of thermally induced coral bleaching and the emergence of diseases effecting reef-building corals (Smith et al., 2013a; van Woesik & Randall, 2017). The coral loss in the Caribbean has changed these communities by reducing coral cover and

reshaping existing species assemblages (McWilliam, Pratchett, Hoogenboom, & Hughes, 2020). As an example, the once dominant branching corals in the genus *Acropora* have been functionally extirpated from most of their historic range due to white band disease (Aronson & Precht, 2001; J. Miller et al., 2009), and the emergence of white plague disease has led to substantial reductions in the abundance of corals in the genus *Orbicella* (J. Miller et al., 2009). The loss of these once dominant species has led to the increased relative abundance of species in other genera such as *Porites* and *Siderastrea* (Green, Edmunds, & Carpenter, 2008; McWilliam et al., 2020). Studies focusing on comparable aspects of species biology, such as growth rate, immune activity and *Symbiodiniaceae* communities have sought to draw links between species differences and the heterogenous responses these species show during environmental stress (Baumann, Davies, Aichelman, & Castillo, 2018; Bove et al., 2019; MacKnight et al., 2021a; Pinzón C. et al., 2014). However, the genetic component and interspecies differences in expression have not been investigated in such as quantitative way.

In this investigation we use gene expression data to investigate the evolution of HTFs, in five ecologically important species of Caribbean corals: *Orbicella faveolata*, *Montastraea cavernosa*, *Colpophyllia natans*, *Porites astreoides*, and *Siderastrea siderea*. Our analysis demonstrates that HTFs which are rapidly evolving are enriched in numerous biological processes in each species and these transcript families have higher levels of expression divergence. These results highlight variation in transcript families which are linked with important immune mechanisms. Overall our study provides support for the association between rapid evolution of the number of paralogs within HTFs and its influence on gene expression patterns by providing a framework which allows quantitative comparison of gene expression patterns even among highly divergent species.

Methods

Coral Collection

Fragments of five colonies of each species, *O. faveolata*, *M. cavernosa*, *C. natans*, *P. astreoides*, and *S. siderea* were collected via SCUBA from Brewer's Bay St. Thomas (18.34403, -64.98435) in June 2017 under the Indigenous Species Research and Export Permit number CZM17010T. Colonies were collected from approximately 35 feet of depth and then transported to the Center for Marine and Environmental Studies at the University of the Virgin Islands. Coral samples were then haphazardly placed into one of two large flow-through holding tanks fed with filtered sea water from the bay which received natural sunlight under a neutral density shade cloth. The corals acclimated in this environment for two weeks. The temperature in the acclimation tanks approximates the temperature in the bay reaching a daily high of approximately 28°C. After the two-week acclimation period all samples were placed into individual mesocosms made from 5 L plastic multi-purpose mixing buckets and each mesocosm was aerated with an airstone attached to the central air line. The containers were randomly placed in the same large holding tank where the water line was below the top of the container to ensure that temperature remained constant among all samples, but that no water was exchanged between containers. All coral fragments from each species were randomized in the water table and held in these individual containers for six hours before being flash frozen in liquid nitrogen for transport back to the University of Texas at Arlington in a dry shipper. Samples were flash frozen in random order and the entire collection period lasted approximately an hour and a half.

RNA Extraction and Sequencing

Coral tissue was removed from the frozen samples by excising approximately one square centimeter of tissue using bone cutters and homogenized in lysis buffer. Total RNA was

extracted from coral fragments using the Ambion RNAeasy kit with DNase according to manufactures protocol and eluted in 100μl of elution buffer. RNA integrity and concentration were checked with an Agilent Bioanalyzer 2100 using the RNA 6000 Nano kit, and all samples with a RIN greater than 7.0 and above 1 μg of total RNA were used for sequencing. Samples were sequenced through the Novagene company and mRNA libraries were prepared with the Illumina TruSeq RNA library prep kit which uses Poly-A tail enrichment to purify mRNA. After library prep samples were sequenced on an Illumina HiSeqX at Novagene company. Five colonies of each *M. cavernosa* and *C. natans* were sequenced, while four colonies of *O. faveolata, P. astreoides* and *S. siderea* were sequenced. Raw reads from this experiment can be found on the NCBI short read archive (PRJNA723585).

Transcriptome Assembly and Cleaning

Transcriptome assemblies were generated with Trinity-v2.5.1 (Grabherr et al., 2011; Haas et al., 2013) with parameters (--normalize_reads --seqType fq --SS_lib_type FR) after removing adapters and low-quality reads with trimmomatic-v0.32 (Bolger et al., 2014) using default parameters. Since transcriptome assemblies derived from adult corals contain transcripts originating from their symbiotic algae we used a previously described in-silico approach to remove symbiont transcripts (Sarah W. Davies, Marchetti, Ries, & Castillo, 2016). In short, multiple coral assemblies consisting of both genome-derived predicted gene models and transcriptomes covering a diversity of coral families (Sarah W. Davies et al., 2016; Kirk, Howells, Abrego, Burt, & Meyer, 2017; Moya et al., 2012; Water et al., 2018) were combined to create a coral database. A symbiont database was constructed from the genome-derived predicted gene models from multiple genera of symbionts (Aranda et al., 2016; H. Liu et al., 2018; Parkinson et al., 2016; Shoguchi et al., 2021). Transcripts from each of our coral assemblies were

then queried using blastn-2.2.27 against each database and a transcript was retained only if it had greater than 80% identity over at least 100bp to the coral database and that transcript did not have a lower evalue when blasted against the symbiont database.

To confidently identify homologous transcript families, it is important to remove transcripts which are likely derived from alternative splice variants and isoforms to approximate a one-to-one correspondence between transcripts and genes. We took several steps to ensure these relationships held before conducting subsequent analyses. First, each assembly was filtered for longest isoform to attempt to remove extraneous transcripts in our assemblies which derive from splice-variants of a gene using the get longest isoform script available through Trinity-v-2.5.1 (Grabherr et al., 2011). Next the program TransDecoder-v5.5.0 (Haas et al., 2013) was used to extract the longest open reading frame of each transcript and this reading frame was utilized to generate a predicted peptide sequence. The predicted proteomes for all species were then collapsed for sequence similarity using the program cd-hit-v4.8.1 (Y. Huang, Niu, Gao, Fu, & Li, 2010) at a similarity level of 0.9 to further exclude transcripts which are likely derived from alternative splice-variants of a gene. To ascertain the quality of these assemblies after transcript filtration we employed several metrics of transcriptome completeness including Benchmarking Universal Single Copy Orthologs (BUSCO)-v.5.2.2-0 (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015) against the core metazoan database, DOGMA-v3.6 against the eukaryote protein domain database, N50 and total transcriptome assembly length. To generate Gene Ontology (GO) annotations from our assemblies we utilized the annotation software EggNog-mapper-v2 (Huerta-Cepas et al., 2017) through the online web portal against the eukaryote database.

HTF Assignment

To identify homologous transcripts across our species and group them into HTFs we utilized the program Orthofinder-v2.5.4 (David M. Emms & Kelly, 2015, 2019). This software groups protein sequences predicted from the transcripts into groups containing both orthologs and paralogs which we refer to as HTFs. A species tree was generated through the program species tree from all genes (STAG)-v1.0.0 (D.M. Emms & Kelly, 2018) as implemented within Orthofinder where branch lengths are measured in amino acid substitutions. We then converted the calculated branch lengths into time based on the estimated divergence times between these coral species (Pinzón C. et al., 2014).

Transcript Family Evolution

To quantify the rate of gene family evolution we employed the program Café-v4.2.1 (Han, Thomas, Lugo-Martinez, & Hahn, 2013). This program utilizes an ultrametric species tree with a set of gene families to determine the background rate of gene gain and loss per gene per unit time across the provided species tree. This background rate of gene turnover is then used as a null model to test for gene families which are experiencing significant deviations from this rate. For our analysis the species tree generated through STAG was converted into an ultrametric tree using phytools-v 0.7.70 (Revell, 2012) with the option “extend”. We ran this analysis on the HTFs which contained at least one transcript in all species which encompassed 4904 HTFs. To account for the role that errors in our assemblies may have played in over-inflating our estimate of gene turnover we utilized the error model script included in the Café package (Han et al., 2013). This script iteratively searches a priori defined error distributions to identify which distribution maximizes the probability of observing the data. The error term with the highest probability of observation 0.1 (which represents a predicted error in 10% of HTFs) was then used to run Café. To account for the possibility that assembly quality may have influenced the

estimates of transcript family evolution we constructed a multiple linear model to regress transcript number, number of complete and single-copy BUSCOs and number of identified protein domains against the number of rapidly evolving HTFs in each species. We also performed an additional regression using these same metrics against the number of transcripts within rapidly evolving HTFs in each species.

Gene Ontology Enrichment

To understand the processes which are undergoing rapid evolution in these species we utilized the R script Gene Ontology with Mann-Whitney U test (GO_MWU) (R. M. Wright et al., 2015), to perform a fisher's exact test where contigs in rapidly evolving HTFs were assigned a value of 1 while all other contigs were assigned a value of 0. The enrichments were run against species-specific backgrounds. This method was employed separately for the transcripts in expanded versus contracted HTFs separately. For each species identical parameters were used for biological process with the category smallest set to 50, largest to 0.1 and clusterCutHeight at 0.25.

Expression Divergence

In order to determine which HTFs demonstrate species-specific expression shifts we mapped reads from each coral colony to its respective species transcriptome with the read mapping program Salmon-v1.5.2 (Patro et al., 2017) using a k-mer index size of 31 in each species. Read counts were then extracted and summarized to transcript family level with the R package TXimport-v1.16.1 (Soneson, Love, & Robinson, 2015) using the "salmon" option. These expression profiles were then combined across species and filtered for low abundance expression with less than an average of 10 counts. The HTFs above this cutoff were rlog normalized by species in the R package DESeq2-v1.28.1 (Love et al., 2014). Species-level

expression shifts were quantified using the R package evemodel-v0.0.0.9005 using a β shared test (Gillard et al., 2021; Rohlfs & Nielsen, 2015). The rlog normalized counts of each HTF as well our generated phylogenetic tree were used as input files. This program compares expression variance within and across species to generate β values, where low β values represent HTFs with high levels of between species variance and high β values represent HTFs with high within species variance. β values were -log10 transformed to generate our expression divergence metric. All statistical analysis were carried out in the R programing language (R Core Team (2020)).

Comparison to Genome-based Approach

As both *O. faveolata* and *M. cavernosa* have available genome resources we performed the previously described analysis again, incorporating the genome resources to determine if our results were robust to the increased completeness which genomes provide. Genome-derived predicted gene models were obtained from the *O. faveolata* assembly on NCBI (assembly ofav_dov_v1) (Prada et al., 2016), while for *M. cavernosa* a draft genome assembly is available (Rippe, Dixon, Fuller, Liao, & Matz, 2021) and we chose to use this genome assembly to generate a genome-guided transcriptome assembly for *M. cavernosa*. Reads from our five *M. cavernosa* colonies were mapped to the draft genome with the read mapping program tophat2 (Kim et al., 2013) using default parameters. The merged bam files were then used in the Trinity genome-guided approach to generate our *M. cavernosa* transcriptome. We performed all the computational protocols described (isoform filtering, Transdecoder, cd-hit, Orthofinder, Café, Salmon, Evemodel) above with the exception of the *in-silico* symbiont filtration as the genome assemblies are already free of symbiont sequences.

Results

HTF Assignment

Assembly metrics including transcript number, N50 values, BUSCO scores and DOGMA results can be found in table1. Collectively we identified 47170 HTFs, of which 4904 were represented by at least one transcript in all species, and 1629 are single copy in all species. The frequency of size class distribution was similar across all species (Figure 1a); most transcripts are in HTFs with a single transcript and as the number of transcripts in a HTF increases the frequency of that size class decreases. HTFs with a single transcript are the most common size class in each species followed by HTFs with two transcripts, while HTFs with more than five transcripts are uncommon. The phylogenetic tree produced from these HTFs recapitulates the known phylogenetic relationship between these species of coral (Figure 1b).

HTF Evolution

By evaluating the evolution of the number of homologs within a transcript family we identified a background of turnover of 0.000854 homolog gains/losses per transcript family per million years (MYA) (Figure 1b) and 236 HTFs which are evolving significantly faster than this background rate of turnover. The number of HTFs with adaptive variations in size differed by species: 104 in *O. faveolata*, 105 in *M. cavernosa*, 50 in *C. natans*, 62 in *P. astreoides* and 59 in *S. siderea* (Figure 1b). The number of rapidly evolving HTFs in a species was not significantly associated with standard metrics of transcriptome assembly completeness including the percentage of complete single copy BUSCOs, number of transcripts in the assembly or the number of inferred protein domains in a multiple linear regression ($p=0.357$). Additionally, the number of transcripts within these rapidly evolving HTFs was also not associated with these same metrics ($p=0.379$) according to the multiple linear regression. While neither of these relationships were significant it should be noted that with only five species our power to detect

such associations is low and therefore it is not completely possible to rule out assembly quality as influencing our results. The number of transcripts within these rapidly evolving HTFs as well as the number of GO enrichments broken down by expansions versus contractions of the number of homologs within a transcript family can be found in table 2. A subset of select GO term enrichments are visualized (Figure 2) based on terms commonly observed in coral gene expression studies including terms related to stress responses, metabolism and tissue formation.

Expression Divergence

To test if the rapidly evolving HTFs demonstrate species-specific expression shifts, we quantified gene expression patterns and tested for expression divergence across the 4277 HTFs which were expressed across all species. The rapidly evolving HTFs had higher average expression divergence than the single-copy HTFs (0.830 vs. 0.447, p-value = 2.2e-16 two-sided t-test)(Figure 3a,b). This elevated expression divergence among the rapidly evolving HTFs was also higher than a size-matched random down-sampling of genes (0.830 vs. 0.623, p-value = 5.11e-06 two-sided t-test).

Genome Comparison

By performing the same analysis with the inclusions of the genomic data from *O. faveolata* and *M. cavernosa* our results differed slightly, however our main conclusions were still supported. We identified more single copy HTFs as well as more HTFs with at least one transcript present in all species with the genome-based assemblies 2009 and 6324 respectively. This difference resulted in a slightly higher rate of background transcript family turnover (0.00088 transcript gain/loss per transcript family per million years) and a greater number of rapidly evolving transcript families (354). The patterns of transcript family evolution were similar between the two analysis as *O. faveolata* possessed the most rapidly contracting

transcript families and *M. cavernosa* possessed the most rapidly expanding transcript families. Our observation of higher expression divergence within rapidly evolving transcript families compared to either single copy transcript families (0.543 single copy vs. 0.718 rapidly evolving, p-value = 7.687e-07, t-test) or a down sampled number of transcript families was likewise supported with the inclusion of genomic data (0.599 down sampled vs. 0.718 rapidly evolving, p-value = 7.77e-03, t-test). Overall, this additional analysis demonstrates that while the inclusion of genome data may influence our results, the primary findings of this study are robust even when different methods were used to generate the assemblies.

Discussion

Our investigation utilizes a framework to integrate paralogous transcripts into multi-species gene expression studies which demonstrates that rapidly evolving HTFs affect numerous biological processes and are associated with divergent gene expression patterns in five species of Caribbean coral. These findings provide support that the evolution of the number of transcripts within an HTF, a proxy for gene family size, is associated with divergent expression patterns (Taylor & Raes, 2004) and these processes are active in Caribbean corals. These findings highlight both the evolution of HTFs and their resulting expression patterns as an understudied aspect of coral biology which has the potential to influence these species responses to a changing climate.

Feasibility

Our study focused on variation in the number of homologous transcripts within transcript families, which ideally should represent gene family size and we employed extensive efforts to establish a one-to-one correspondence between transcripts and genes; however, some

considerations need to be addressed. First, without genome assemblies available for all species it is not possible to confidently say that each unique transcript arises from a unique gene. However, using the genomes available for *O. faveolata* and *M. cavernosa* did not substantially change our main findings. Additionally, as the corals were only sampled under a single condition there are likely genes which were not expressed and thus not sampled by our approach, leading to incomplete gene sampling, which has the potential to result in inflated estimates of gene family turnover. However, incomplete sampling should only be problematic for our interpretations if it is biased among species, which we have no reason to suspect. Indeed, if genes are missed stochastically across species, it should only serve to inflate the background (i.e. neutral) rate of gene family turnover, making detection of rapidly (i.e. adaptively) expanding or contracting families more difficult. Additionally, our calculated rate of gene family turnover approximates the observed rate among species of *Drosophila* (Han et al., 2013) and is lower than a study which utilized whole genomes from six species of mammals (Matthew W. Hahn, Demuth, & Han, 2007). This demonstrates that by employing stringent filtering approaches and including an error term in the Café model, these issues common to transcriptome assemblies can be reduced or accounted for to a level which allows investigation of the evolution of HTFs. Thus, by following the computational framework outlined here other investigations can incorporate the evolution of HTFs in species currently lacking genome assemblies, as long as whole organism homogenates can be acquired.

The Benefit of Incorporating Paralogs

By incorporating paralogs into our analysis we were able to increase the number of HTFs used for multi-species comparisons by more than three-fold compared to only using only single copy HTFs. This includes the additional 254 rapidly evolving HTFs, indicating an adaptive role

of this variation (De Bie et al., 2006; M. W. Hahn, 2005). While it is not possible to ascertain from these data the selective benefit of this rapid evolution in particular HTFs, these transcript families were enriched for GO terms in all five species, consistent with a role in species-level adaptation. Population level data coupled to fitness effects would be required to conclusively determine the adaptive significance of this variation and associated GO enrichments. Despite this limitation some of the enriched terms do qualitatively correspond to host biology. For example, *O. faveolata* the species which can attain the largest colony diameter (Madin et al., 2016), is enriched for "regulation of anatomical structure size" among the rapidly contracting HTFs, which may represent a loss of negative regulators of growth and could potentially underly the large colony size this species can attain. Similarly, *S. siderea* is enriched for "response to pH" among the rapidly expanding HTFs, which may be linked with this species exceptional ability to handle highly acidified water (Castillo, Ries, Bruno, & Westfield, 2014). While such connections between species biology and the rapid evolution of HTFs are speculative these examples underline how variation of HTFs could influence each species biology. These results also highlight potential new avenues of study such as the rapid evolution of HTFs involved in carbohydrate and lipid metabolism. These changes in metabolic processes may have important consequences for a coral's ability to efficiently assimilate symbiont-derived carbohydrates or potentially store nutrients both of which have been associated with corals ability to withstand periods of temperature stress (Roach et al., 2021). Thus, the observed differences in the number of homologs within these transcript families may play an important role in each species biology.

Rapidly Evolving HTFs Have Increased Expression Divergence

Rapidly evolving HTFs have an overall higher level of expression divergence demonstrating an association between the processes of gene duplication and gene expression.

This finding is in line with previous work showing that gene copy number influences transcriptional output, however, this may be modified by dosage compensation mechanisms (Jiang & Assis, 2019). While our findings are based on transcripts, rather than genome-derived gene models, the association between the rapid change in HTF size and expression divergence provides support for the idea that gene expression is often modified among paralogs in response to gene duplication or loss (Des Marais & Rausher, 2008; Stearns, 2010). It should be noted that gene expression divergence can arise from other processes which are not dependent on paralogs. However, the substantially higher levels of expression divergence within the rapidly evolving HTFs suggests that variation in the number paralogs may be an important contributor to the development of divergent expression patterns. To our knowledge this is the first investigation to link the rate of transcript family evolution with divergent expression patterns.

Importantly, the association between the variation in the number of HTFs and expression divergence may be an important mechanism of adaptation. If the new paralogs arise from a gene duplication event this can relax pleiotropic constraint, promoting the processes of neofunctionalization and subfunctionalization (J. Zhang, 2003). Gene neofunctionalization in newly arisen paralogs has been demonstrated to promote evolutionary innovation and adaptation in several systems (Deng, Cheng, Ye, He, & Chen, 2010; Zimmer et al., 2018). Gene subfunctionalization can also function as an adaptive mechanism (Abascal et al., 2013; Spady et al., 2006) though there are fewer examples of this processes. While our data doesn't explicitly demonstrate either of these processes per se, it does highlight that rapidly evolving HTFs whose paralogs are candidates for neofunctionalization and subfunctionalization have acquired new expression patterns consistent with changing function. These findings thus highlight that as more coral genome assemblies are produced and sequencing technology capable of detecting gene

copy number variation becomes more accessible investigating the processes of gene neofunctionalization and subfunctionalization may yield important insights.

Rapid Evolution of Immunity

We find that HTFs which have immune related annotations are common among the rapidly evolving transcript families leading to immune related enrichments in several species. This reflects findings from other systems which have characterized the rapid evolution and subsequent change in copy number of immune genes (Evans et al., 2006; Sackton, Lazzaro, & Clark, 2017; Sackton et al., 2007; Waterhouse et al., 2007). This common pattern is thought to be due to the propensity of immune genes to engage in evolutionary arms races through their ability to mediate interactions with other organisms (Lazzaro & Clark, 2012). This process is likely important in reef-building corals that live in a microbe rich environment (van Oppen & Blackall, 2019). The immune system of corals functions to both regulate commensal microbes (beneficial bacteria and the algal *Symbiodiniaceae*) as well as defend against pathogenic ones (Kvennefors et al., 2010; Mansfield & Gilmore, 2019; L. D. Mydlarz, Jones, & Harvell, 2006; Wu et al., 2019). Thus, the rapid evolution of immune transcript families indicates that these interactions may have played an important role in shaping the evolutionary history of these coral species. Other investigations have found similar trends including phylosymbiosis between coral species and bacterial communities on the great barrier reef (Pollock et al., 2018), as well as highly stable host microbe associations in the Red sea (Ziegler et al., 2019). Overall, this indicates that interactions with microbes may broadly play a role in shaping the evolution of corals.

Of particular interest is the interaction formed with *Symbiodiniaceae* as this relationship requires the modulation of host immune processes (Mansfield & Gilmore, 2019; Matthews et al., 2017; E.-F. Neubauer et al., 2017) in order to receive a supply of translocated carbohydrates

from their symbionts. Different species of coral are known to preferentially associate with one or a few species of *Symbiodiniaceae* (Thornhill, LaJeunesse, Kemp, Fitt, & Schmidt, 2006), although the mechanism by which hosts distinguish between these different symbionts is unknown. The rapid evolution of immune transcripts by the host may be one mechanism to facilitate this relationship through neofunctionalization of immune genes, however this hypothesis would need to be functionally confirmed. One interesting trend in our data is that *O. faveolata* which demonstrates flexibility in its symbiont associations (P. Edmunds et al., 2014) has reduced numbers of homologs in rapidly evolving transcript families which are involved in immunity while *P. astreoides* who transmits its symbionts vertically and demonstrates tight control of its symbiotic association (Chornesky & Peters, 1987; C. D. Kenkel et al., 2013) has an expanded number of homologs in rapidly evolving immune transcript families. The cellular mechanisms corals use to detect different strains of symbionts is still an ongoing area of research (Jacobovitz et al., 2021), though immune specificity is thought to play a critical role. In particular Scavenger Receptors and Thrombospondin (E. F. Neubauer, Poole, Weis, & Davy, 2016; E.-F. Neubauer et al., 2017) have been demonstrated to influence symbiont recognition and density, and we observe rapid evolution of transcripts matching these annotations. Thus, the changes observed between species within these and other immune-related rapidly evolving HTFs could potentially be an evolved mechanism to distinguish between different strains of Symbiodiniaceae.

In addition to modulating the relationship with beneficial microorganisms, corals must also defend themselves against disease-causing microbes. This is particularly pressing in the Caribbean as disease outbreaks affecting reef-building corals have re-shaped communities (Aronson & Precht, 2001; Gardner, 2003; J. Miller et al., 2009; L. D. Mydlarz et al., 2006) and

continue to be among the most pressing selective forces these organisms face (Vega Thurber et al., 2020). In response to disease, corals utilize a complex innate immune system involving pattern recognition receptors including Toll-like receptors (TLRs) (Poole & Weis, 2014; L. M. Williams et al., 2018) and C-type lectins (Emery et al., 2021; Kvennefors, Leggat, Hoegh-Guldberg, Degnan, & Barnes, 2008) to sense threats and initiate immune responses including the complement cascade (Poole, Kitchen, & Weis, 2016). Interestingly, we observe that *M. cavernosa, C. natans* and *P. astreoides* have an expanded number of homologs within rapidly evolving transcript families matching these annotations, while *O. faveolata* has a contracted number of homologs within these transcript families. This variation may influence disease-susceptibility as *O. faveolata* is highly susceptible to several coral diseases whereas *M. cavernosa* and *P. astreoides*, are considered more resilient to disease (Aeby et al., 2019; Smith et al., 2013b; S. D. Williams & Patterson, 2020). In support of this, species-level variation in immune activity is widespread in Caribbean corals (C. V. Palmer et al., 2011; Pinzón C. et al., 2014; Rosales, Clark, Huebner, Ruzicka, & Muller, 2020), which may be the result of the variation of immune transcript repertoires we observe. However, *C. natans* is also considered to be susceptible to disease (Aeby et al., 2019; MacKnight et al., 2021b) and demonstrates expansions in immune-related transcript families. Thus, the relationship between an expanded immune transcript repertoire and resistance to disease may not always lead to real-world immunocompetence.

Together these data indicate that the immune system is a major target of evolution in Caribbean coral. As coral diseases are currently among the strongest selective forces acting on Caribbean reefs (Vega Thurber et al., 2020) understanding the differing immune repertoires each species possesses and how this relates to disease resilience may be an important determinant of

which corals will persist on the reef. Indeed, in the Caribbean, coral species which have historically dominated stable forereef environments such as *O. faveolata* have been declining in abundance (Gardner, 2003) while corals such as *P. astreoides* that have traditionally been found in the variable environment of the reef edge are rising in abundance (Green et al., 2008; McWilliam et al., 2020) with disease playing a major role in these shifts.

Conclusion

We found that by expanding our analysis of multi-species gene expression through the identification of HTFs we were able to identify adaptive variation in homolog number within transcript families which is associated with divergent expression patterns. Utilizing this approach, we identified numerous biological processes influenced by this variation which notably includes several families of immune genes previously linked to both symbiont recognition and pathogen defense. These findings highlight the role that interactions with microbes have played in the evolution of these coral species and how this may influence species ability to persist as coral diseases becoming an increasingly pressing threat in the Caribbean. Therefore, further work towards linking variation within HTFs that arise through changes in gene copy number to adaptive capacity may help predict which coral species will be able to adapt and which are likely to face extirpation.

Chapter 5 Figures

Figure 1

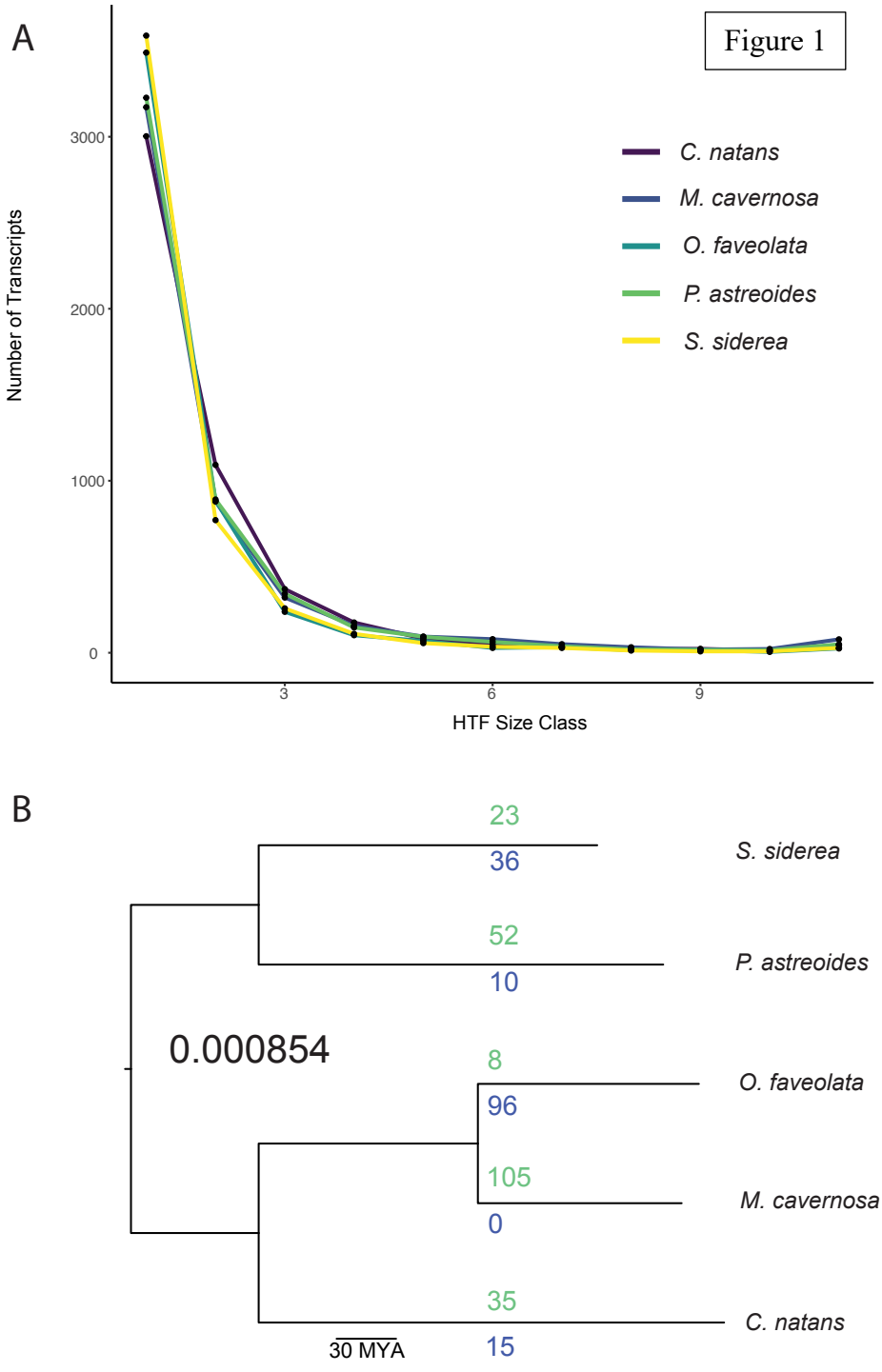
A
3000
2000
1000
0
Number of Transcripts
3
6
9
HTF Size Class
C. natans
M. cavernosa
O. faveolata
P. astreoides
S. siderea
B
23
36
52
10
0.000854
8
96
105
0
35
15
30 MYA
S. siderea
P. astreoides
O. faveolata
M. cavernosa
C. natans

Figure 1: Quantifying HTF evolution. A) Shown is the distribution of the number of transcripts contained in each transcript family size class separated by species. B) Species tree generated from STAG for the five species of coral with the number of expanded (green) and contracted (blue) transcript families as well as the background rate of transcript family turnover (8.54 e-4) superimposed on the tree. The number of expanded/contracted transcript families in a particular species are on the branch leading to that species.

Figure 2

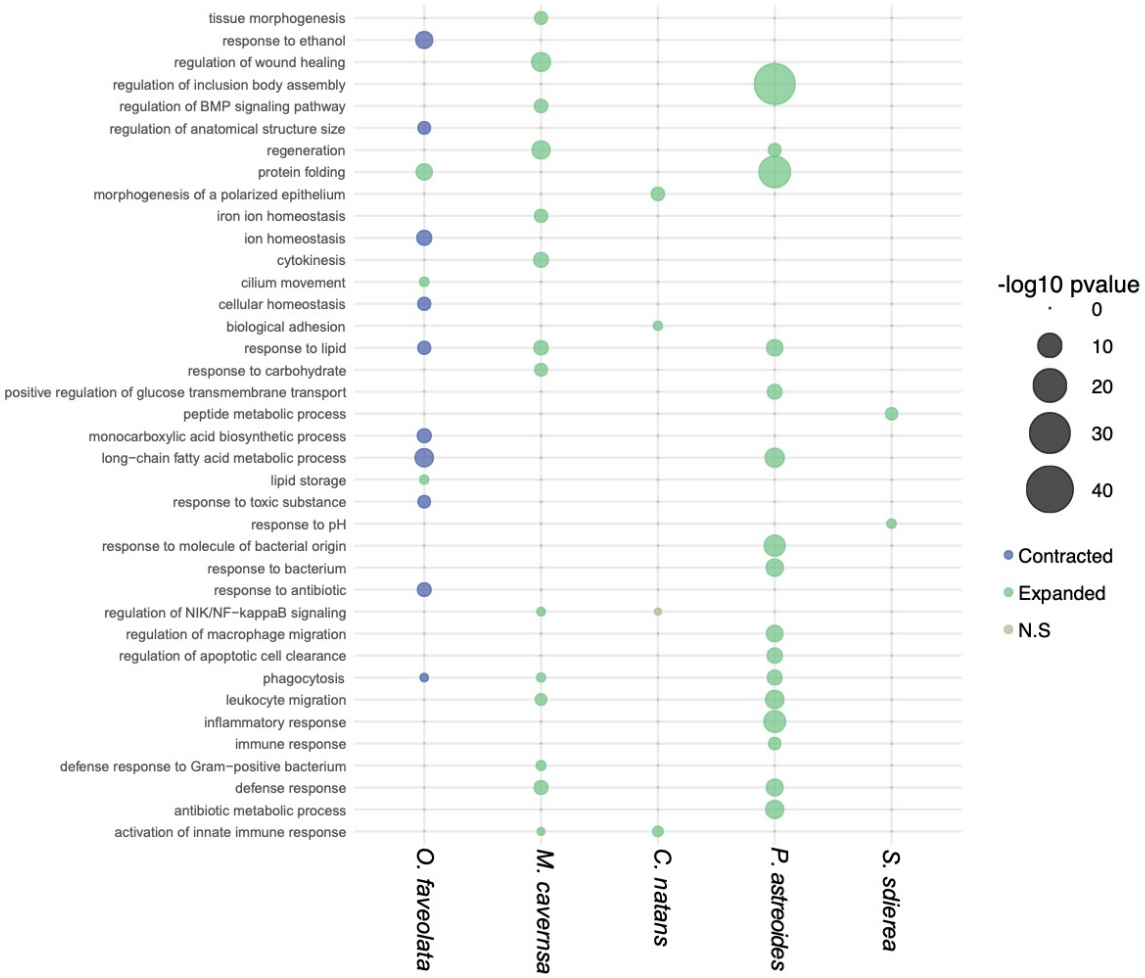

Figure 2: Selected subset of GO enrichments among the expanded and contracted transcript families. Shown is a bubble plot visualizing selected GO term enrichments in each species. Size

of the circle corresponds to the significance (-log10 transformed p-value) after false discovery rate correction, and color denotes if the term enrichment is in expanded (green) or contracted (blue) transcript families, or grey if the term is non-significant after false discovery rate correction. GO enrichments were selected for visualization based on their potential involvement either stress responses, metabolism or tissue formation. A table contained all of the GO enrichments can be found in Table S.1.

Figure 3

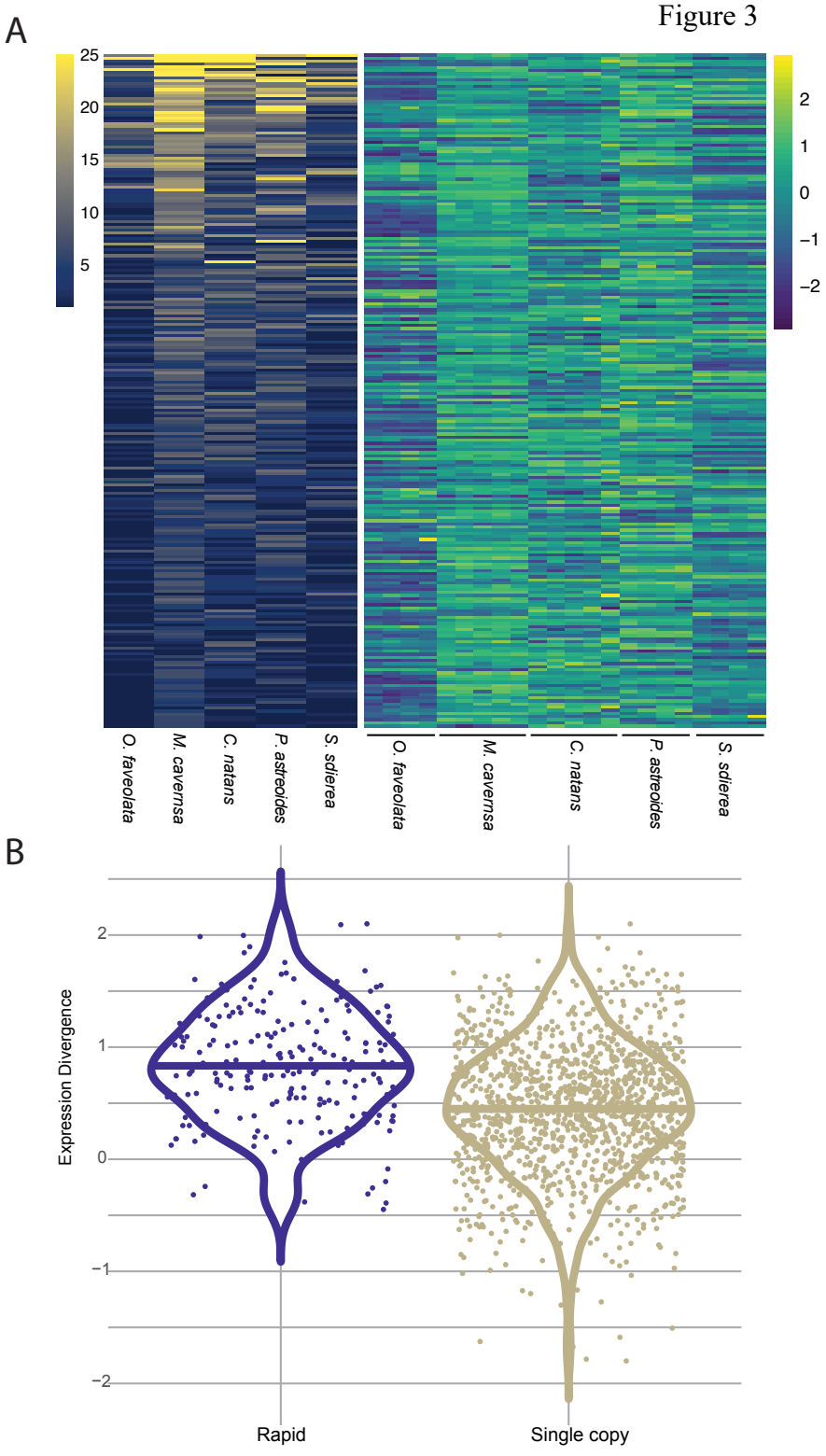
A
25
20
15
10
5
2
1
0
−1
−2
O. faveolata
M. cavernsa
C. natans
P. astreoides
S. sdierea
O. faveolata
M. cavernsa
C. natans
P. astreoides
S. sdierea
B
Expression Divergence
2
1
0
−1
−2
Rapid
Single copy

Figure 3: Rapidly evolving transcript families have increased expression divergence. A) Shown is both the number of transcripts contained within the rapidly evolving transcript families as well as the expression of those transcript families. The left heatmap visualizes transcript number where transcript families with more than 25 transcripts were reduced to 25 for visualization purposes. The right heatmap shown the corresponding z-score normalized expression value of each transcript family in the left heatmap. B) Violin plot showing the expression divergence of the rapidly evolving transcript families compared to the single copy transcript families. The thick line shows the mean of each group. The difference in expression divergence is significant between the two groups (0.830 vs. 0.447, p-value = 2.2e-16 two-sided t-test).

Figure 4

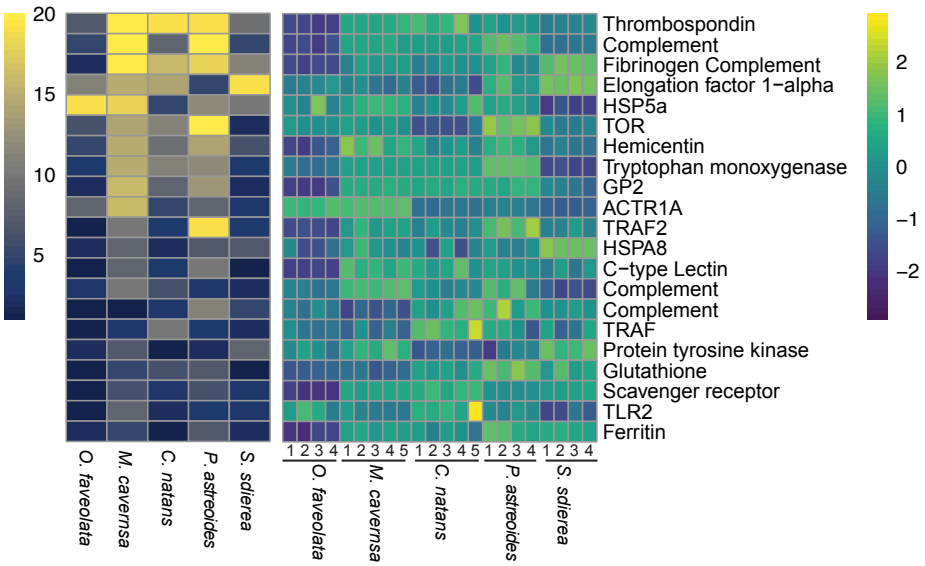

Figure 4: Rapid evolution of immune genes. Shown is both the number of transcripts contained within the immune related rapidly evolving transcript families as well as the expression of those transcript families. Immune transcript families were selected based on the transcripts within that

family possessing either the GO annotation “Immune system process, GO: 0002376” or “Innate immune response GO: 0002226”. The left heatmap visualizes transcript number where, while the right heatmap shows the corresponding z-score normalized expression value of each transcript family in the left heatmap. The transcript family names were assigned based upon the lowest seed ortholog evalue from eggNOG-mapper of all transcripts contained within the given transcript family.

Table 1

Species	**source**	**Predicted peptides**	**Number of transcripts used in Café**	**Complete single copy**	**Fragmented**	**Complete and duplicated**	**Missing**	**N50**	**Protein domains**
O. faveolata	de novo	47678	7986	698	39	84	157	11934	1091
M. cavernosa	de novo	57740	9978	757	34	78	109	13783	1186
C. natans	de novo	50582	9266	652	134	57	135	12255	952
P. astreoides	de novo	37167	9048	787	73	49	69	6457	1197
S. siderea	de novo	16635	7707	761	74	23	120	4921	1126

Table 2

Species	**expanding HTFs**	**transcripts in expanding HTFs**	**BP enrichments expanded**	**contracting HTFs**	**transcripts in contracting HTFs**	**BP enrichments contracted**

O. faveolata	8	265	16	95	239	33
M. cavernosa	110	1550	229	0	0	0
C. natans	35	606	13	15	31	0
P. astreoides	52	721	199	10	29	0
S. siderea	22	291	16	35	99	2

Chapter 6: Conclusion

In this dissertation I present four distinct research chapters regarding the cellular and genetic mechanism through which reef-building corals have the potential to adapt to a changing climate. In chapter two I characterize a stress response pathway known as the mitochondrial unfolded protein response which mediates numerous adaptive coral biomarkers during both immune-stimulation and temperature stress. In chapter three I utilize an emerging model system of Cnidarian symbiosis to demonstrate a link between the transcriptional and physiologic response to thermally induced bleaching. This work highlights the importance of the mitochondrial unfolded protein response as a mechanism during temperature stress as both changes in mitochondrial physiology and the transcription of immune genes are associated with the breakdown of symbiosis. In chapter four I describe the expansion and domain shuffling of an important class of innate immune receptors known as NOD-like receptors and demonstrate these proteins possess the required domain architecture to potentiate immune responses including the formation of inflammasome-like molecules. Finally, in chapter five I demonstrate how gene family evolution has likely shaped the evolutionary trajectory of five ecologically-important species of Caribbean coral by promoting genetic novelty and gene expression divergence which affects important regulators of the coral immune system. Overall, these studies provide new insights into the cellular and genetic mechanisms corals use to response to a changing climate by uncovering the regulatory basis of well-known stress responses such as the heat shock response and providing new avenues of study including the role of neofunctionalized immune genes in determining disease resilience. Specifically, this body of work demonstrates mechanisms both cellular and genetic which may allow reef-building corals to persist in a rapidly changing environment characterized by both increased temperature and prevalence of marine diseases.

References

Abascal, F., Corpet, A., Gurard-Levin, Z. A., Juan, D., Ochsenbein, F., Rico, D., … Almouzni, G. (2013). Subfunctionalization via Adaptive Evolution Influenced by Genomic Context: The Case of Histone Chaperones ASF1a and ASF1b. *Molecular Biology and Evolution*, *30*(8), 1853–1866. doi: 10.1093/molbev/mst086

Aeby, G. S., Ushijima, B., Campbell, J. E., Jones, S., Williams, G. J., Meyer, J. L., … Paul, V. J. (2019). Pathogenesis of a Tissue Loss Disease Affecting Multiple Species of Corals Along the Florida Reef Tract. *Frontiers in Marine Science*, *6*, 678. doi: 10.3389/fmars.2019.00678

Ainsworth, T. D., Hoegh-Guldberg, O., Heron, S. F., Skirving, W. J., & Leggat, W. (2008). Early cellular changes are indicators of pre-bleaching thermal stress in the coral host. *Journal of Experimental Marine Biology and Ecology*, *364*(2), 63–71. doi: 10.1016/j.jembe.2008.06.032

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen Recognition and Innate Immunity. *Cell*, *124*(4), 783–801. doi: 10.1016/j.cell.2006.02.015

Albalat, R., & Cañestro, C. (2016). Evolution by gene loss. *Nature Reviews Genetics*, *17*(7), 379–391. doi: 10.1038/nrg.2016.39

Anantharaman, V., Makarova, K. S., Burroughs, A. M., Koonin, E. V., & Aravind, L. (2013). Comprehensive analysis of the HEPN superfamily: Identification of novel roles in intra-genomic conflicts, defense, pathogenesis and RNA processing. *Biology Direct*, *8*(1). doi: 10.1186/1745-6150-8-15

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169. doi: 10.1093/bioinformatics/btu638

Aranda, M., Li, Y., Liew, Y. J., Baumgarten, S., Simakov, O., Wilson, M. C., … Voolstra, C. R. (2016). Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. *Scientific Reports*, *6*(1), 39734. doi: 10.1038/srep39734

Aronson, R. B., & Precht, W. F. (2001). White-band disease and the changing face of Caribbean coral reefs. In J. W. Porter (Ed.), *The Ecology and Etiology of Newly Emerging Marine Diseases* (pp. 25–38). Dordrecht: Springer Netherlands. doi: 10.1007/978-94-017-3284-0_2

Avila-Magaña, V., Kamel, B., DeSalvo, M., Gómez-Campo, K., Enríquez, S., Kitano, H., … Medina, M. (2021). Elucidating gene expression adaptation of phylogenetically divergent coral holobionts under heat stress. *Nature Communications*, *12*(1), 5731. doi: 10.1038/s41467-021-25950-4

Baker, B. M., Nargund, A. M., Sun, T., & Haynes, C. M. (2012). Protective Coupling of Mitochondrial Function and Protein Synthesis via the eIF2α Kinase GCN-2. *PLoS Genetics*, *8*(6), e1002760. doi: 10.1371/journal.pgen.1002760

Barott, K. L., Huffmyer, A. S., Davidson, J. M., Lenz, E. A., Matsuda, S. B., Hancock, J. R., … Gates, R. D. (2021). Coral bleaching response is unaltered following acclimatization to reefs with distinct environmental conditions. *Proceedings of the National Academy of Sciences*, *118*(22), e2025435118. doi: 10.1073/pnas.2025435118

Barott, K. L., Venn, A. A., Perez, S. O., Tambutté, S., & Tresguerres, M. (2015). Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. *Proceedings of the National Academy of Sciences*, *112*(2), 607–612. doi: 10.1073/pnas.1413483112

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences*, *110*(4), 1387–1392. doi: 10.1073/pnas.1210224110

Bauernfeind, F., Bartok, E., Rieger, A., Franchi, L., Núñez, G., & Hornung, V. (2011). Cutting edge: Reactive oxygen species inhibitors block priming, but not activation, of the NLRP3 inflammasome. *Journal of Immunology (Baltimore, Md.: 1950)*, *187*(2), 613–617. doi: 10.4049/jimmunol.1100613

Bauernfeind, F. G., Horvath, G., Stutz, A., Alnemri, E. S., MacDonald, K., Speert, D., … Latz, E. (2009). Cutting Edge: NF- B Activating Pattern Recognition and Cytokine Receptors License NLRP3 Inflammasome Activation by Regulating NLRP3 Expression. *The Journal of Immunology*, *183*(2), 787–791. doi: 10.4049/jimmunol.0901363

Baumann, J. H., Davies, S. W., Aichelman, H. E., & Castillo, K. D. (2018). Coral Symbiodinium Community Composition Across the Belize Mesoamerican Barrier Reef System is Influenced by Host Species and Thermal Variability. *Microbial Ecology*, *75*(4), 903–915. doi: 10.1007/s00248-017-1096-6

Bellwood, D. R., Hughes, T. P., Folke, C., & Nyström, M. (2004). Confronting the coral reef crisis. *Nature*, *429*(6994), 827–833. doi: 10.1038/nature02691

Ben-Haim, Y. (2003). Vibrio coralliilyticus sp. Nov., a temperature-dependent pathogen of the coral Pocillopora damicornis. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *53*(1), 309–315. doi: 10.1099/ijs.0.02402-0

Bernal, M. A., Schunter, C., Lehmann, R., Lightfoot, D. J., Allan, B. J. M., Veilleux, H. D., … Ravasi, T. (2020). Species-specific molecular responses of wild coral reef fishes during a marine heatwave. *Science Advances*, *6*(12), eaay3423. doi: 10.1126/sciadv.aay3423

Beurmann, S., Ushijima, B., Videau, P., Svoboda, C. M., Chatterjee, A., Aeby, G. S., & Callahan, S. M. (2018). Dynamics of acute Montipora white syndrome: Bacterial communities of healthy and diseased M. capitata colonies during and after a disease outbreak. *Microbiology*, *164*(10), 1240–1253. doi: 10.1099/mic.0.000699

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. doi: 10.1093/bioinformatics/btu170

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C. D. (2009). Microbial disease and the coral holobiont. *Trends in Microbiology*, *17*(12), 554–562. doi: 10.1016/j.tim.2009.09.004

Bove, C. B., Ries, J. B., Davies, S. W., Westfield, I. T., Umbanhowar, J., & Castillo, K. D. (2019). Common Caribbean corals exhibit highly variable responses to future acidification and warming. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1900), 20182840. doi: 10.1098/rspb.2018.2840

Broz, P., Ruby, T., Belhocine, K., Bouley, D. M., Kayagaki, N., Dixit, V. M., & Monack, D. M. (2012). Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1. *Nature*, *490*(7419), 288–291. doi: 10.1038/nature11419

Bruno, J. F., Selig, E. R., Casey, K. S., Page, C. A., Willis, B. L., Harvell, C. D., … Melendy, A. M. (2007). Thermal Stress and Coral Cover as Drivers of Coral Disease Outbreaks. *PLoS Biology*, *5*(6), e124. doi: 10.1371/journal.pbio.0050124

Carroll, S. B. (2008). Evo-Devo and an Expanding Evolutionary Synthesis: A Genetic Theory of Morphological Evolution. *Cell*, *134*(1), 25–36. doi: 10.1016/j.cell.2008.06.030

Castillo, K. D., Ries, J. B., Bruno, J. F., & Westfield, I. T. (2014). The reef-building coral Siderastrea siderea exhibits parabolic responses to ocean acidification and warming. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1797), 20141856–20141856. doi: 10.1098/rspb.2014.1856

Chain, F. J. J., Feulner, P. G. D., Panchal, M., Eizaguirre, C., Samonte, I. E., Kalbe, M., … Reusch, T. B. H. (2014). Extensive Copy-Number Variation of Young Genes across Stickleback Populations. *PLoS Genetics*, *10*(12), e1004830. doi: 10.1371/journal.pgen.1004830

Chornesky, E. A., & Peters, E. C. (1987). SEXUAL REPRODUCTION AND COLONY GROWTH IN THE SCLERACTINIAN CORAL *PORITES ASTREOIDES*. *The Biological Bulletin*, *172*(2), 161–177. doi: 10.2307/1541790

Claar, D. C., Starko, S., Tietjen, K. L., Epstein, H. E., Cunning, R., Cobb, K. M., … Baum, J. K. (2020). Dynamic symbioses reveal pathways to coral survival through prolonged heatwaves. *Nature Communications*, *11*(1), 6097. doi: 10.1038/s41467-020-19169-y

Claros, M. G., & Vincens, P. (1996). Computational method to predict mitochondrially imported proteins and their targeting sequences. *European Journal of Biochemistry*, *241*(3), 779–786.

Cleves, P. A., Krediet, C. J., Lehnert, E. M., Onishi, M., & Pringle, J. R. (2020). Insights into coral bleaching under heat stress from analysis of gene expression in a sea anemone model system. *Proceedings of the National Academy of Sciences*, *117*(46), 28906–28917. doi: 10.1073/pnas.2015737117

Cleves, P. A., Shumaker, A., Lee, J., Putnam, H. M., & Bhattacharya, D. (2020). Unknown to Known: Advancing Knowledge of Coral Gene Function. *Trends in Genetics*, *36*(2), 93–104. doi: 10.1016/j.tig.2019.11.001

Davies, S. W., Treml, E. A., Kenkel, C. D., & Matz, M. V. (2015). Exploring the role of Micronesian islands in the maintenance of coral genetic diversity in the Pacific Ocean. *Molecular Ecology*, *24*(1), 70–82. doi: 10.1111/mec.13005

Davies, Sarah W., Marchetti, A., Ries, J. B., & Castillo, K. D. (2016). Thermal and pCO2 Stress Elicit Divergent Transcriptomic Responses in a Resilient Coral. *Frontiers in Marine Science*, *3*. doi: 10.3389/fmars.2016.00112

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell Biology of Cnidarian-Dinoflagellate Symbiosis. *Microbiology and Molecular Biology Reviews*, *76*(2), 229–261. doi: 10.1128/MMBR.05014-11

De Bie, T., Cristianini, N., Demuth, J. P., & Hahn, M. W. (2006). CAFE: A computational tool for the study of gene family evolution. *Bioinformatics*, *22*(10), 1269–1271. doi: 10.1093/bioinformatics/btl097

De Smet, R., Sabaghian, E., Li, Z., Saeys, Y., & Van de Peer, Y. (2017). Coordinated Functional Divergence of Genes after Genome Duplication in *Arabidopsis thaliana*. *The Plant Cell*, *29*(11), 2786–2800. doi: 10.1105/tpc.17.00531

De'ath, G., Fabricius, K. E., Sweatman, H., & Puotinen, M. (2012). The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proceedings of the National Academy of Sciences*, *109*(44), 17995–17999. doi: 10.1073/pnas.1208909109

Deng, C., Cheng, C.-H. C., Ye, H., He, X., & Chen, L. (2010). Evolution of an antifreeze protein by neofunctionalization under escape from adaptive conflict. *Proceedings of the National Academy of Sciences*, *107*(50), 21593–21598. doi: 10.1073/pnas.1007883107

Des Marais, D. L., & Rausher, M. D. (2008). Escape from adaptive conflict after duplication in an anthocyanin pathway gene. *Nature*, *454*(7205), 762–765. doi: 10.1038/nature07092

Desalvo, M. K., Voolstra, C. R., Sunagawa, S., Schwarz, J. A., Stillman, J. H., Coffroth, M. A., … Medina, M. (2008). Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. *Molecular Ecology*, *17*(17), 3952–3971. doi: 10.1111/j.1365-294X.2008.03879.x

Diaz, J. M., Hansel, C. M., Apprill, A., Brighi, C., Zhang, T., Weber, L., … Xun, L. (2016). Species-specific control of external superoxide levels by the coral holobiont during a natural bleaching event. *Nature Communications*, *7*(1), 13801. doi: 10.1038/ncomms13801

Díaz-Almeyda, E. M., Prada, C., Ohdera, A. H., Moran, H., Civitello, D. J., Iglesias-Prieto, R., … Medina, M. (2017). Intraspecific and interspecific variation in thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1868), 20171767. doi: 10.1098/rspb.2017.1767

Dimos, B. A., Butler, C. C., Ricci, C. A., MacKnight, N. J., & Mydlarz, L. D. (2019). Responding to Threats Both Foreign and Domestic: NOD-Like Receptors in Corals. *Integrative and Comparative Biology*, *59*(4), 819–829. doi: 10.1093/icb/icz111

Dimos, B. A., Mahmud, S. A., Fuess, L. E., Mydlarz, L. D., & Pellegrino, M. W. (2019). Uncovering a mitochondrial unfolded protein response in corals and its role in adapting

to a changing world. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1905), 20190470. doi: 10.1098/rspb.2019.0470

Dixon, G., Abbott, E., & Matz, M. (2020). Meta-analysis of the coral environmental stress response: *Acropora* corals show opposing responses depending on stress intensity. *Molecular Ecology*, *29*(15), 2855–2870. doi: 10.1111/mec.15535

Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., & Tschopp, J. (2008). Innate Immune Activation Through Nalp3 Inflammasome Sensing of Asbestos and Silica. *Science*, *320*(5876), 674–677. doi: 10.1126/science.1156995

Downs, C. A., Mueller, E., Phillips, S., Fauth, J. E., & Woodley, C. M. (2000). A Molecular Biomarker System for Assessing the Health of Coral (Montastraea faveolata) During Heat Stress. *Marine Biotechnology*, *2*(6), 533–544. doi: 10.1007/s101260000038

Duarte, J. M., Cui, L., Wall, P. K., Zhang, Q., Zhang, X., Leebens-Mack, J., … dePamphilis, C. W. (2006). Expression Pattern Shifts Following Duplication Indicative of Subfunctionalization and Neofunctionalization in Regulatory Genes of Arabidopsis. *Molecular Biology and Evolution*, *23*(2), 469–478. doi: 10.1093/molbev/msj051

Duncan, J. A., & Canna, S. W. (2018). The NLRC4 Inflammasome. *Immunological Reviews*, *281*(1), 115–123. doi: 10.1111/imr.12607

Dunn, S. R, Schnitzler, C. E., & Weis, V. M. (2007). Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: Every which way you lose. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1629), 3079–3085. doi: 10.1098/rspb.2007.0711

Dunn, Simon R., Pernice, M., Green, K., Hoegh-Guldberg, O., & Dove, S. G. (2012). Thermal Stress Promotes Host Mitochondrial Degradation in Symbiotic Cnidarians: Are the

Batteries of the Reef Going to Run Out? *PLoS ONE*, *7*(7), e39024. doi: 10.1371/journal.pone.0039024

Eddy, S. R. (2011). Accelerated Profile HMM Searches. *PLoS Computational Biology*, *7*(10), e1002195. doi: 10.1371/journal.pcbi.1002195

Edmunds, P. J., & Carpenter, R. C. (2001). Recovery of Diadema antillarum reduces macroalgal cover and increases abundance of juvenile corals on a Caribbean reef. *Proceedings of the National Academy of Sciences*, *98*(9), 5067–5071. doi: 10.1073/pnas.071524598

Edmunds, P., Pochon, X., Levitan, D., Yost, D., Belcaid, M., Putnam, H., & Gates, R. (2014). Long-term changes in Symbiodinium communities in Orbicella annularis in St. John, US Virgin Islands. *Marine Ecology Progress Series*, *506*, 129–144. doi: 10.3354/meps10808

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., … Finn, R. D. (2019). The Pfam protein families database in 2019. *Nucleic Acids Research*, *47*(D1), D427–D432. doi: 10.1093/nar/gky995

Elinav, E., Strowig, T., Henao-Mejia, J., & Flavell, R. A. (2011). Regulation of the Antimicrobial Response by NLR Proteins. *Immunity*, *34*(5), 665–679. doi: 10.1016/j.immuni.2011.05.007

Emery, M. A., Dimos, B. A., & Mydlarz, L. D. (2021). Cnidarian Pattern Recognition Receptor Repertoires Reflect Both Phylogeny and Life History Traits. *Frontiers in Immunology*, *12*, 689463. doi: 10.3389/fimmu.2021.689463

Emms, David M., & Kelly, S. (2015). OrthoFinder: Solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biology*, *16*(1), 157. doi: 10.1186/s13059-015-0721-2

Emms, David M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, *20*(1), 238. doi: 10.1186/s13059-019-1832-y

Emms, D.M., & Kelly, S. (2018). *STAG: Species Tree Inference from All Genes* [Preprint]. Evolutionary Biology. doi: 10.1101/267914

Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J.-L., Jiang, H., … Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect Molecular Biology*, *15*(5), 645–656. doi: 10.1111/j.1365-2583.2006.00682.x

Fang, L., Huang, S., & Lin, K. (1997). High temperature induces the synthesis of heat-shock proteins and the elevation of intracellular calcium in the coral Acropora grandis. *Coral Reefs*, *16*(2), 127–131. doi: 10.1007/s003380050066

Ferwerda, G., Kramer, M., de Jong, D., Piccini, A., Joosten, L. A., Devesaginer, I., … Netea, M. G. (2008). Engagement of NOD2 has a dual effect on proIL-1beta mRNA transcription and secretion of bioactive IL-1beta. *European Journal of Immunology*, *38*(1), 184–191. doi: 10.1002/eji.200737103

Fiorese, C. J., Schulz, A. M., Lin, Y.-F., Rosin, N., Pellegrino, M. W., & Haynes, C. M. (2016). The Transcription Factor ATF5 Mediates a Mammalian Mitochondrial UPR. *Current Biology*, *26*(15), 2037–2043. doi: 10.1016/j.cub.2016.06.002

Foissac, S., Djebali, S., Munyard, K., Vialaneix, N., Rau, A., Muret, K., … Giuffra, E. (2019). Multi-species annotation of transcriptome and chromatin structure in domesticated animals. *BMC Biology*, *17*(1), 108. doi: 10.1186/s12915-019-0726-5

Force, A., Lynch, M., Pickett, F. B., Amores, A., Yan, Y. L., & Postlethwait, J. (1999). Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, *151*(4), 1531–1545.

Frias-Lopez, J., Bonheyo, G. T., Jin, Q., & Fouke, B. W. (2003). Cyanobacteria Associated with Coral Black Band Disease in Caribbean and Indo-Pacific Reefs. *Applied and Environmental Microbiology*, *69*(4), 2409–2413. doi: 10.1128/AEM.69.4.2409-2413.2003

Fritz, J. H., Ferrero, R. L., Philpott, D. J., & Girardin, S. E. (2006). Nod-like proteins in immunity, inflammation and disease. *Nature Immunology*, *7*(12), 1250–1257. doi: 10.1038/ni1412

Fuess, L. E., Pinzón C, J. H., Weil, E., Grinshpon, R. D., & Mydlarz, L. D. (2017). Life or death: Disease-tolerant coral species activate autophagy following immune challenge. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1856), 20170771. doi: 10.1098/rspb.2017.0771

Gardner, T. A. (2003). Long-Term Region-Wide Declines in Caribbean Corals. *Science*, *301*(5635), 958–960. doi: 10.1126/science.1086050

Gates, R. D., Baghdasarian, G., & Muscatine, L. (1992). Temperature Stress Causes Host Cell Detachment in Symbiotic Cnidarians: Implications for Coral Bleaching. *The Biological Bulletin*, *182*(3), 324–332. doi: 10.2307/1542252

Gillard, G. B., Grønvold, L., Røsæg, L. L., Holen, M. M., Monsen, Ø., Koop, B. F., … Hvidsten, T. R. (2021). Comparative regulomics supports pervasive selection on gene dosage following whole genome duplication. *Genome Biology*, *22*(1), 103. doi: 10.1186/s13059-021-02323-0

Gochfeld, D., & Aeby, G. (2008). Antibacterial chemical defenses in Hawaiian corals provide possible protection from disease. *Marine Ecology Progress Series*, *362*, 119–128. doi: 10.3354/meps07418

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, *29*(7), 644–652. doi: 10.1038/nbt.1883

Grant, C. E., Bailey, T. L., & Noble, W. S. (2011). FIMO: Scanning for occurrences of a given motif. *Bioinformatics*, *27*(7), 1017–1018. doi: 10.1093/bioinformatics/btr064

Green, D., Edmunds, P., & Carpenter, R. (2008). Increasing relative abundance of Porites astreoides on Caribbean reefs mediated by an overall decline in coral cover. *Marine Ecology Progress Series*, *359*, 1–10. doi: 10.3354/meps07454

Greenway, R., Barts, N., Henpita, C., Brown, A. P., Arias Rodriguez, L., Rodríguez Peña, C. M., … Tobler, M. (2020). Convergent evolution of conserved mitochondrial pathways underlies repeated adaptation to extreme environments. *Proceedings of the National Academy of Sciences*, *117*(28), 16424–16430. doi: 10.1073/pnas.2004223117

Guschanski, K., Warnefors, M., & Kaessmann, H. (2017). The evolution of duplicate gene expression in mammalian organs. *Genome Research*, *27*(9), 1461–1474. doi: 10.1101/gr.215566.116

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, *8*(8), 1494–1512. doi: 10.1038/nprot.2013.084

Hahn, M. W. (2005). Estimating the tempo and mode of gene family evolution from comparative genomic data. *Genome Research*, *15*(8), 1153–1160. doi: 10.1101/gr.3567505

Hahn, Matthew W., Demuth, J. P., & Han, S.-G. (2007). Accelerated Rate of Gene Gain and Loss in Primates. *Genetics*, *177*(3), 1941–1949. doi: 10.1534/genetics.107.080077

Hamada, M., Shoguchi, E., Shinzato, C., Kawashima, T., Miller, D. J., & Satoh, N. (2013). The Complex NOD-Like Receptor Repertoire of the Coral Acropora digitifera Includes Novel Domain Combinations. *Molecular Biology and Evolution*, *30*(1), 167–176. doi: 10.1093/molbev/mss213

Han, M. V., Thomas, G. W. C., Lugo-Martinez, J., & Hahn, M. W. (2013). Estimating Gene Gain and Loss Rates in the Presence of Error in Genome Assembly and Annotation Using CAFE 3. *Molecular Biology and Evolution*, *30*(8), 1987–1997. doi: 10.1093/molbev/mst100

Hao, Y., Xiong, Y., Cheng, Y., Song, G., Jia, C., Qu, Y., & Lei, F. (2019). Comparative transcriptomics of 3 high-altitude passerine birds and their low-altitude relatives. *Proceedings of the National Academy of Sciences*, 201819657. doi: 10.1073/pnas.1819657116

Hargreaves, D. C., & Medzhitov, R. (2005). Innate Sensors of Microbial Infection. *Journal of Clinical Immunology*, *25*(6), 503–510. doi: 10.1007/s10875-005-8065-4

Harvell, C. D. (1999). Emerging Marine Diseases—Climate Links and Anthropogenic Factors. *Science*, *285*(5433), 1505–1510. doi: 10.1126/science.285.5433.1505

Harvell, D., Jordán-Dahlgren, E., Merkel, S., Rosenberg, E., Raymundo, L., Smith, G., … Willis, B. (2007). Coral Disease, Environmental Drivers, and the Balance Between Coral and Microbial Associates. *Oceanography*, *20*(1), 172–195. doi: 10.5670/oceanog.2007.91

Haynes, C. M., & Ron, D. (2010). The mitochondrial UPR - protecting organelle protein homeostasis. *Journal of Cell Science*, *123*(22), 3849–3855. doi: 10.1242/jcs.075119

He, Y., Hara, H., & Núñez, G. (2016). Mechanism and Regulation of NLRP3 Inflammasome Activation. *Trends in Biochemical Sciences*, *41*(12), 1012–1021. doi: 10.1016/j.tibs.2016.09.002

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The Impact of Climate Change on the World's Marine Ecosystems. *Science*, *328*(5985), 1523–1528. doi: 10.1126/science.1189930

Hoegh-Guldberg, Ove. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, *50*(8), 839. doi: 10.1071/MF99078

Hofmann, D. K., Fitt, W. K., & Fleck, J. (1996). Checkpoints in the life-cycle of Cassiopea spp.: Control of metagenesis and metamorphosis in a tropical jellyfish. *The International Journal of Developmental Biology*, *40*(1), 331–338.

Hofmann, D. K., Neumann, R., & Henne, K. (1978). Strobilation, budding and initiation of scyphistoma morphogenesis in the rhizostome Cassiopea andromeda (Cnidaria: Scyphozoa). *Marine Biology*, *47*(2), 161–176. doi: 10.1007/BF00395637

Horvath, G. L., Schrum, J. E., De Nardo, C. M., & Latz, E. (2011). Intracellular sensing of microbes and danger signals by the inflammasomes. *Immunological Reviews*, *243*(1), 119–135. doi: 10.1111/j.1600-065X.2011.01050.x

Hsu, L.-C., Ali, S. R., McGillivray, S., Tseng, P.-H., Mariathasan, S., Humke, E. W., … Karin, M. (2008). A NOD2-NALP1 complex mediates caspase-1-dependent IL-1 secretion in response to Bacillus anthracis infection and muramyl dipeptide. *Proceedings of the National Academy of Sciences*, *105*(22), 7803–7808. doi: 10.1073/pnas.0802726105

Huang, K. M., & Chain, F. J. J. (2021). Copy number variations and young duplicate genes have high methylation levels in sticklebacks. *Evolution*, *75*(3), 706–718. doi: 10.1111/evo.14184

Huang, Y., Niu, B., Gao, Y., Fu, L., & Li, W. (2010). CD-HIT Suite: A web server for clustering and comparing biological sequences. *Bioinformatics*, *26*(5), 680–682. doi: 10.1093/bioinformatics/btq003

Huerlimann, R., Maes, G. E., Maxwell, M. J., Mobli, M., Launikonis, B. S., Jerry, D. R., & Wade, N. M. (2020). Multi-species transcriptomics reveals evolutionary diversity in the mechanisms regulating shrimp tail muscle excitation-contraction coupling. *Gene*, *752*, 144765. doi: 10.1016/j.gene.2020.144765

Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., von Mering, C., & Bork, P. (2017). Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. *Molecular Biology and Evolution*, *34*(8), 2115–2122. doi: 10.1093/molbev/msx148

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., … Bork, P. (2019). eggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research*, *47*(D1), D309–D314. doi: 10.1093/nar/gky1085

Hughes, T. P., Anderson, K. D., Connolly, S. R., Heron, S. F., Kerry, J. T., Lough, J. M., … Wilson, S. K. (2018). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science*, *359*(6371), 80–83. doi: 10.1126/science.aan8048

Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B. C., … Scheffer, M. (2017). Coral reefs in the Anthropocene. *Nature*, *546*(7656), 82–90. doi: 10.1038/nature22901

Inohara, N., & Nuñez, G. (2001). The NOD: A signaling module that regulates apoptosis and host defense against pathogens. *Oncogene*, *20*(44), 6473–6481. doi: 10.1038/sj.onc.1204787

Inohara, N., & Nuñez, G. (2003). NODs: Intracellular proteins involved in inflammation and apoptosis. *Nature Reviews. Immunology*, *3*(5), 371–382. doi: 10.1038/nri1086

Jacobovitz, M. R., Rupp, S., Voss, P. A., Maegele, I., Gornik, S. G., & Guse, A. (2021). Dinoflagellate symbionts escape vomocytosis by host cell immune suppression. *Nature Microbiology*, *6*(6), 769–782. doi: 10.1038/s41564-021-00897-w

Jiang, X., & Assis, R. (2019). Rapid functional divergence after small-scale gene duplication in grasses. *BMC Evolutionary Biology*, *19*(1), 97. doi: 10.1186/s12862-019-1415-2

Jones, J. D. G., Vance, R. E., & Dangl, J. L. (2016). Intracellular innate immune surveillance devices in plants and animals. *Science*, *354*(6316), aaf6395–aaf6395. doi: 10.1126/science.aaf6395

Jovaisaite, V., Mouchiroud, L., & Auwerx, J. (2014). The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. *Journal of Experimental Biology*, *217*(1), 137–143. doi: 10.1242/jeb.090738

Kamath, R. S., & Ahringer, J. (2003). Genome-wide RNAi screening in Caenorhabditis elegans. *Methods (San Diego, Calif.)*, *30*(4), 313–321.

Kayagaki, N., Warming, S., Lamkanfi, M., Walle, L. V., Louie, S., Dong, J., … Dixit, V. M. (2011). Non-canonical inflammasome activation targets caspase-11. *Nature*, *479*(7371), 117–121. doi: 10.1038/nature10558

Kayal, E., Bentlage, B., Sabrina Pankey, M., Ohdera, A. H., Medina, M., Plachetzki, D. C., … Ryan, J. F. (2018). Phylogenomics provides a robust topology of the major cnidarian

lineages and insights on the origins of key organismal traits. *BMC Evolutionary Biology*, *18*(1), 68. doi: 10.1186/s12862-018-1142-0

Kenkel, C. D., Goodbody-Gringley, G., Caillaud, D., Davies, S. W., Bartels, E., & Matz, M. V. (2013). Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. *Molecular Ecology*, *22*(16), 4335–4348. doi: 10.1111/mec.12391

Kenkel, C. D., Sheridan, C., Leal, M. C., Bhagooli, R., Castillo, K. D., Kurata, N., … Matz, M. V. (2014). Diagnostic gene expression biomarkers of coral thermal stress. *Molecular Ecology Resources*, *14*(4), 667–678. doi: 10.1111/1755-0998.12218

Kenkel, Carly D., Aglyamova, G., Alamaru, A., Bhagooli, R., Capper, R., Cunning, R., … Matz, M. V. (2011). Development of Gene Expression Markers of Acute Heat-Light Stress in Reef-Building Corals of the Genus Porites. *PLoS ONE*, *6*(10), e26914. doi: 10.1371/journal.pone.0026914

Kersse, K., Verspurten, J., Berghe, T. V., & Vandenabeele, P. (2011). The death-fold superfamily of homotypic interaction motifs. *Trends in Biochemical Sciences*, *36*(10), 541–552. doi: 10.1016/j.tibs.2011.06.006

Khan, Z., Ford, M. J., Cusanovich, D. A., Mitrano, A., Pritchard, J. K., & Gilad, Y. (2013). Primate Transcript and Protein Expression Levels Evolve Under Compensatory Selection Pressures. *Science*, *342*(6162), 1100–1104. doi: 10.1126/science.1242379

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., & Salzberg, S. L. (2013). TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology*, *14*(4), R36. doi: 10.1186/gb-2013-14-4-r36

Kirk, N. L., Howells, E. J., Abrego, D., Burt, J. A., & Meyer, E. (2017). *Genomic and transcriptomic signals of thermal tolerance in heat-tolerant corals (Platygyra daedalea) of the Arabian/Persian Gulf*. doi: 10.1101/185579

Koonin, E. V., & Aravind, L. (2000). The NACHT family – a new group of predicted NTPases implicated in apoptosis and MHC transcription activation. *Trends in Biochemical Sciences*, *25*(5), 223–224. doi: 10.1016/S0968-0004(00)01577-2

Kroemer, G., Dallaporta, B., & Resche-Rigon, M. (1998). THE MITOCHONDRIAL DEATH/LIFE REGULATOR IN APOPTOSIS AND NECROSIS. *Annual Review of Physiology*, *60*(1), 619–642. doi: 10.1146/annurev.physiol.60.1.619

Kulakovskiy, I. V., Medvedeva, Y. A., Schaefer, U., Kasianov, A. S., Vorontsov, I. E., Bajic, V. B., & Makeev, V. J. (2013). HOCOMOCO: A comprehensive collection of human transcription factor binding sites models. *Nucleic Acids Research*, *41*(D1), D195–D202. doi: 10.1093/nar/gks1089

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. doi: 10.1093/molbev/msw054

Kuwabara, T., & Imajoh-Ohmi, S. (2004). LPS-induced apoptosis is dependent upon mitochondrial dysfunction. *Apoptosis*, *9*(4), 467–474. doi: 10.1023/B:APPT.0000031453.90821.6a

Kvennefors, E. C. E., Leggat, W., Hoegh-Guldberg, O., Degnan, B. M., & Barnes, A. C. (2008). An ancient and variable mannose-binding lectin from the coral Acropora millepora binds both pathogens and symbionts. *Developmental & Comparative Immunology*, *32*(12), 1582–1592. doi: 10.1016/j.dci.2008.05.010

Kvennefors, E. C. E., Leggat, W., Kerr, C. C., Ainsworth, T. D., Hoegh-Guldberg, O., & Barnes, A. C. (2010). Analysis of evolutionarily conserved innate immune components in coral links immunity and symbiosis. *Developmental & Comparative Immunology*, *34*(11), 1219–1229. doi: 10.1016/j.dci.2010.06.016

Lage, S. L., Longo, C., Branco, L. M., da Costa, T. B., Buzzo, C. de L., & Bortoluci, K. R. (2014). Emerging Concepts about NAIP/NLRC4 Inflammasomes. *Frontiers in Immunology*, *5*. doi: 10.3389/fimmu.2014.00309

Lange, C., Hemmrich, G., Klostermeier, U. C., Lopez-Quintero, J. A., Miller, D. J., Rahn, T., … Rosenstiel, P. (2011). Defining the Origins of the NOD-Like Receptor System at the Base of Animal Evolution. *Molecular Biology and Evolution*, *28*(5), 1687–1702. doi: 10.1093/molbev/msq349

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(1), 559. doi: 10.1186/1471-2105-9-559

Latz, E., Xiao, T. S., & Stutz, A. (2013). Activation and regulation of the inflammasomes. *Nature Reviews Immunology*, *13*(6), 397–411. doi: 10.1038/nri3452

Lazzaro, B. P., & Clark, A. G. (2012). Rapid evolution of innate immune response genes. In R. S. Singh, J. Xu, & R. J. Kulathinal (Eds.), *Rapidly Evolving Genes and Genetic Systems* (pp. 203–210). Oxford University Press. doi: 10.1093/acprof:oso/9780199642274.003.0020

Lech, M., Avila-Ferrufino, A., Skuginna, V., Susanti, H. E., & Anders, H.-J. (2010). Quantitative expression of RIG-like helicase, NOD-like receptor and inflammasome-related mRNAs in humans and mice. *International Immunology*, *22*(9), 717–728. doi: 10.1093/intimm/dxq058

Lee, G.-S., Subramanian, N., Kim, A. I., Aksentijevich, I., Goldbach-Mansky, R., Sacks, D. B., … Chae, J. J. (2012). The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP. *Nature*, *492*(7427), 123–127. doi: 10.1038/nature11588

Lesser, M. P. (1997). Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs*, *16*(3), 187–192. doi: 10.1007/s003380050073

Lesser, Michael P. (2004). Experimental biology of coral reef ecosystems. *Journal of Experimental Marine Biology and Ecology*, *300*(1–2), 217–252. doi: 10.1016/j.jembe.2003.12.027

Levy, S., Elek, A., Grau-Bové, X., Menéndez-Bravo, S., Iglesias, M., Tanay, A., … Sebé-Pedrós, A. (2021). A stony coral cell atlas illuminates the molecular and cellular basis of coral symbiosis, calcification, and immunity. *Cell*, S0092867421004402. doi: 10.1016/j.cell.2021.04.005

Libro, S., Kaluziak, S. T., & Vollmer, S. V. (2013). RNA-seq Profiles of Immune Related Genes in the Staghorn Coral Acropora cervicornis Infected with White Band Disease. *PLoS ONE*, *8*(11), e81821. doi: 10.1371/journal.pone.0081821

Liu, H., Stephens, T. G., González-Pech, R. A., Beltran, V. H., Lapeyre, B., Bongaerts, P., … Chan, C. X. (2018). Symbiodinium genomes reveal adaptive evolution of functions related to coral-dinoflagellate symbiosis. *Communications Biology*, *1*(1), 95. doi: 10.1038/s42003-018-0098-3

Liu, Q., Zhang, D., Hu, D., Zhou, X., & Zhou, Y. (2018). The role of mitochondria in NLRP3 inflammasome activation. *Molecular Immunology*, *103*, 115–124. doi: 10.1016/j.molimm.2018.09.010

Liu, Y., Samuel, B. S., Breen, P. C., & Ruvkun, G. (2014). Caenorhabditis elegans pathways that surveil and defend mitochondria. *Nature*, *508*(7496), 406–410. doi: 10.1038/nature13204

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12). doi: 10.1186/s13059-014-0550-8

Lutz, A., Raina, J.-B., Motti, C. A., Miller, D. J., & van Oppen, M. J. H. (2015). Host Coenzyme Q Redox State Is an Early Biomarker of Thermal Stress in the Coral Acropora millepora. *PLOS ONE*, *10*(10), e0139290. doi: 10.1371/journal.pone.0139290

Lynch, M. (2000). The Evolutionary Fate and Consequences of Duplicate Genes. *Science*, *290*(5494), 1151–1155. doi: 10.1126/science.290.5494.1151

Lyndby, N. H., Rädecker, N., Bessette, S., Søgaard Jensen, L. H., Escrig, S., Trampe, E., … Meibom, A. (2020). Amoebocytes facilitate efficient carbon and nitrogen assimilation in the *Cassiopea* -Symbiodiniaceae symbiosis. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1941), 20202393. doi: 10.1098/rspb.2020.2393

Machado, L. R., & Ottolini, B. (2015). An Evolutionary History of Defensins: A Role for Copy Number Variation in Maximizing Host Innate and Adaptive Immune Responses. *Frontiers in Immunology*, *6*. doi: 10.3389/fimmu.2015.00115

MacKnight, N. J., Cobleigh, K., Lasseigne, D., Chaves-Fonnegra, A., Gutting, A., Dimos, B., … Brandt, M. (2021a). Microbial dysbiosis reflects disease resistance in diverse coral species. *Communications Biology*, *4*(1), 679. doi: 10.1038/s42003-021-02163-5

MacKnight, N. J., Cobleigh, K., Lasseigne, D., Chaves-Fonnegra, A., Gutting, A., Dimos, B., … Brandt, M. (2021b). Microbial dysbiosis reflects disease resistance in diverse coral species. *Communications Biology*, *4*(1), 679. doi: 10.1038/s42003-021-02163-5

Madin, J. S., Anderson, K. D., Andreasen, M. H., Bridge, T. C. L., Cairns, S. D., Connolly, S. R., … Baird, A. H. (2016). The Coral Trait Database, a curated database of trait information for coral species from the global oceans. *Scientific Data*, *3*(1), 160017. doi: 10.1038/sdata.2016.17

Malmstrøm, M., Matschiner, M., Tørresen, O. K., Star, B., Snipen, L. G., Hansen, T. F., … Jentoft, S. (2016). Evolution of the immune system influences speciation rates in teleost fishes. *Nature Genetics*, *48*(10), 1204–1210. doi: 10.1038/ng.3645

Mansfield, K. M., Carter, N. M., Nguyen, L., Cleves, P. A., Alshanbayeva, A., Williams, L. M., … Gilmore, T. D. (2017). Transcription factor NF-κB is modulated by symbiotic status in a sea anemone model of cnidarian bleaching. *Scientific Reports*, *7*(1). doi: 10.1038/s41598-017-16168-w

Mansfield, K. M., & Gilmore, T. D. (2019). Innate immunity and cnidarian-Symbiodiniaceae mutualism. *Developmental & Comparative Immunology*, *90*, 199–209. doi: 10.1016/j.dci.2018.09.020

Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., … Dixit, V. M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature*, *440*(7081), 228–232. doi: 10.1038/nature04515

Martindale, J. L., & Holbrook, N. J. (2002). Cellular response to oxidative stress: Signaling for suicide and survival. *Journal of Cellular Physiology*, *192*(1), 1–15. doi: 10.1002/jcp.10119

Martinon, F., Mayor, A., & Tschopp, J. (2009). The Inflammasomes: Guardians of the Body. *Annual Review of Immunology*, *27*(1), 229–265. doi: 10.1146/annurev.immunol.021908.132715

Masters, S. L., Dunne, A., Subramanian, S. L., Hull, R. L., Tannahill, G. M., Sharp, F. A., … O'Neill, L. A. J. (2010). Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. *Nature Immunology*, *11*(10), 897–904. doi: 10.1038/ni.1935

Matthews, J. L., Crowder, C. M., Oakley, C. A., Lutz, A., Roessner, U., Meyer, E., … Davy, S. K. (2017). Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences*, *114*(50), 13194–13199. doi: 10.1073/pnas.1710733114

Matys, V., Fricke, E., Geffers, R., Gössling, E., Haubrock, M., Hehl, R., … Wingender, E. (2003). TRANSFAC: Transcriptional regulation, from patterns to profiles. *Nucleic Acids Research*, *31*(1), 374–378.

Matz, M. V., Treml, E. A., Aglyamova, G. V., & Bay, L. K. (2018). Potential and limits for rapid genetic adaptation to warming in a Great Barrier Reef coral. *PLOS Genetics*, *14*(4), e1007220. doi: 10.1371/journal.pgen.1007220

Maynard, J., van Hooidonk, R., Eakin, C. M., Puotinen, M., Garren, M., Williams, G., … Harvell, C. D. (2015). Projections of climate conditions that increase coral disease susceptibility and pathogen abundance and virulence. *Nature Climate Change*, *5*(7), 688–694. doi: 10.1038/nclimate2625

McWilliam, M., Pratchett, M. S., Hoogenboom, M. O., & Hughes, T. P. (2020). Deficits in functional trait diversity following recovery on coral reefs. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192628. doi: 10.1098/rspb.2019.2628

Meiling, S. S., Muller, E. M., Lasseigne, D., Rossin, A., Veglia, A. J., MacKnight, N., … Brandt, M. E. (2021). Variable Species Responses to Experimental Stony Coral Tissue Loss

Disease (SCTLD) Exposure. *Frontiers in Marine Science*, *8*, 670829. doi: 10.3389/fmars.2021.670829

Meunier, E., & Broz, P. (2017). Evolutionary Convergence and Divergence in NLR Function and Structure. *Trends in Immunology*, *38*(10), 744–757. doi: 10.1016/j.it.2017.04.005

Miao, E. A., Rajan, J. V., & Aderem, A. (2011). Caspase-1-induced pyroptotic cell death: Caspase-1-induced pyroptotic cell death. *Immunological Reviews*, *243*(1), 206–214. doi: 10.1111/j.1600-065X.2011.01044.x

Mies, M., Sumida, P. Y. G., Rädecker, N., & Voolstra, C. R. (2017). Marine Invertebrate Larvae Associated with Symbiodinium: A Mutualism from the Start? *Frontiers in Ecology and Evolution*, *5*. doi: 10.3389/fevo.2017.00056

Miller, A. W., & Richardson, L. L. (2015). Emerging coral diseases: A temperature-driven process? *Marine Ecology*, *36*(3), 278–291. doi: 10.1111/maec.12142

Miller, D. J., Hemmrich, G., Ball, E. E., Hayward, D. C., Khalturin, K., Funayama, N., … Bosch, T. C. (2007). The innate immune repertoire in Cnidaria—Ancestral complexity and stochastic gene loss. *Genome Biology*, *8*(4), R59. doi: 10.1186/gb-2007-8-4-r59

Miller, J., Muller, E., Rogers, C., Waara, R., Atkinson, A., Whelan, K. R. T., … Witcher, B. (2009). Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. *Coral Reefs*, *28*(4), 925–937. doi: 10.1007/s00338-009-0531-7

Mills, E. L., Kelly, B., & O'Neill, L. A. J. (2017). Mitochondria are the powerhouses of immunity. *Nature Immunology*, *18*(5), 488–498. doi: 10.1038/ni.3704

Moya, A., Huisman, L., Ball, E. E., Hayward, D. C., Grasso, L. C., Chua, C. M., … Miller, D. J. (2012). Whole Transcriptome Analysis of the Coral *Acropora millepora* Reveals

Complex Responses to CO_2 -driven Acidification during the Initiation of Calcification: TRANSCRIPTOMIC RESPONSE OF CORALS TO ACIDIFICATION. *Molecular Ecology*, *21*(10), 2440–2454. doi: 10.1111/j.1365-294X.2012.05554.x

Muscatine, L., Pool, R. R., & Trench, R. K. (1975). Symbiosis of algae and invertebrates: Aspects of the symbiont surface and the host-symbiont interface. *Transactions of the American Microscopical Society*, *94*(4), 450–469.

Mydlarz, L., Couch, C., Weil, E., Smith, G., & Harvell, C. (2009). Immune defenses of healthy, bleached and diseased Montastraea faveolata during a natural bleaching event. *Diseases of Aquatic Organisms*, *87*, 67–78. doi: 10.3354/dao02088

Mydlarz, L. D., Jones, L. E., & Harvell, C. D. (2006). Innate Immunity, Environmental Drivers, and Disease Ecology of Marine and Freshwater Invertebrates. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 251–288. doi: 10.1146/annurev.ecolsys.37.091305.110103

Nargund, A. M., Pellegrino, M. W., Fiorese, C. J., Baker, B. M., & Haynes, C. M. (2012). Mitochondrial Import Efficiency of ATFS-1 Regulates Mitochondrial UPR Activation. *Science*, *337*(6094), 587–590. doi: 10.1126/science.1223560

Nargund, Amrita M., Fiorese, C. J., Pellegrino, M. W., Deng, P., & Haynes, C. M. (2015). Mitochondrial and Nuclear Accumulation of the Transcription Factor ATFS-1 Promotes OXPHOS Recovery during the UPRmt. *Molecular Cell*, *58*(1), 123–133. doi: 10.1016/j.molcel.2015.02.008

Neff, E. P. (2020). The quest for an animal model of coral health and disease. *Lab Animal*, *49*(2), 37–41. doi: 10.1038/s41684-019-0467-7

Neubauer, E. F., Poole, A. Z., Weis, V. M., & Davy, S. K. (2016). The scavenger receptor repertoire in six cnidarian species and its putative role in cnidarian-dinoflagellate symbiosis. *PeerJ*, *4*, e2692. doi: 10.7717/peerj.2692

Neubauer, E.-F., Poole, A. Z., Neubauer, P., Detournay, O., Tan, K., Davy, S. K., & Weis, V. M. (2017). A diverse host thrombospondin-type-1 repeat protein repertoire promotes symbiont colonization during establishment of cnidarian-dinoflagellate symbiosis. *ELife*, *6*, e24494. doi: 10.7554/eLife.24494

Newkirk, C. R., Frazer, T. K., & Martindale, M. Q. (2018). Acquisition and proliferation of algal symbionts in bleached polyps of the upside-down jellyfish, Cassiopea xamachana. *Journal of Experimental Marine Biology and Ecology*, *508*, 44–51. doi: 10.1016/j.jembe.2018.08.010

Newton, K., & Dixit, V. M. (2012). Signaling in Innate Immunity and Inflammation. *Cold Spring Harbor Perspectives in Biology*, *4*(3), a006049–a006049. doi: 10.1101/cshperspect.a006049

Nielsen, D. A., Petrou, K., & Gates, R. D. (2018). Coral bleaching from a single cell perspective. *The ISME Journal*, *12*(6), 1558–1567. doi: 10.1038/s41396-018-0080-6

Notredame, C., Higgins, D. G., & Heringa, J. (2000). T-coffee: A novel method for fast and accurate multiple sequence alignment 1 1Edited by J. Thornton. *Journal of Molecular Biology*, *302*(1), 205–217. doi: 10.1006/jmbi.2000.4042

Oakley, C. A., Durand, E., Wilkinson, S. P., Peng, L., Weis, V. M., Grossman, A. R., & Davy, S. K. (2017). Thermal Shock Induces Host Proteostasis Disruption and Endoplasmic Reticulum Stress in the Model Symbiotic Cnidarian *Aiptasia*. *Journal of Proteome Research*, *16*(6), 2121–2134. doi: 10.1021/acs.jproteome.6b00797

Ogura, Y., Inohara, N., Benito, A., Chen, F. F., Yamaoka, S., & Nunez, G. (2001). Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *The Journal of Biological Chemistry*, *276*(7), 4812–4818. doi: 10.1074/jbc.M008072200

Ohdera, A., Ames, C. L., Dikow, R. B., Kayal, E., Chiodin, M., Busby, B., … Ryan, J. F. (2019). Box, stalked, and upside-down? Draft genomes from diverse jellyfish (Cnidaria, Acraspeda) lineages: Alatina alata (Cubozoa), Calvadosia cruxmelitensis (Staurozoa), and Cassiopea xamachana (Scyphozoa). *GigaScience*, *8*(7), giz069. doi: 10.1093/gigascience/giz069

Ohdera, A. H., Abrams, M. J., Ames, C. L., Baker, D. M., Suescún-Bolívar, L. P., Collins, A. G., … Medina, M. (2018). Upside-Down but Headed in the Right Direction: Review of the Highly Versatile Cassiopea xamachana System. *Frontiers in Ecology and Evolution*, *6*. doi: 10.3389/fevo.2018.00035

Ohno, Susumu. (1970). *Evolution by Gene Duplication*. New York: Springer.

Ohtsubo, K., & Marth, J. D. (2006). Glycosylation in Cellular Mechanisms of Health and Disease. *Cell*, *126*(5), 855–867. doi: 10.1016/j.cell.2006.08.019

Oppen, M. J. H., Gates, R. D., Blackall, L. L., Cantin, N., Chakravarti, L. J., Chan, W. Y., … Putnam, H. M. (2017). Shifting paradigms in restoration of the world's coral reefs. *Global Change Biology*, *23*(9), 3437–3448. doi: 10.1111/gcb.13647

Palmer, C. V., McGinty, E. S., Cummings, D. J., Smith, S. M., Bartels, E., & Mydlarz, L. D. (2011). Patterns of coral ecological immunology: Variation in the responses of Caribbean corals to elevated temperature and a pathogen elicitor. *Journal of Experimental Biology*, *214*(24), 4240–4249. doi: 10.1242/jeb.061267

Palmer, Caroline V. (2018). Immunity and the coral crisis. *Communications Biology*, *1*(1). doi: 10.1038/s42003-018-0097-4

Palmer, Caroline V., & Traylor-Knowles, N. G. (2018). Cnidaria: Anthozoans in the Hot Seat. In E. L. Cooper (Ed.), *Advances in Comparative Immunology* (pp. 51–93). Cham: Springer International Publishing. doi: 10.1007/978-3-319-76768-0_3

Parkinson, J. E., Baumgarten, S., Michell, C. T., Baums, I. B., LaJeunesse, T. C., & Voolstra, C. R. (2016). Gene Expression Variation Resolves Species and Individual Strains among Coral-Associated Dinoflagellates within the Genus *Symbiodinium*. *Genome Biology and Evolution*, *8*(3), 665–680. doi: 10.1093/gbe/evw019

Pasquier, L. D. (2006). Germline and somatic diversification of immune recognition elements in Metazoa. *Immunology Letters*, *104*(1–2), 2–17. doi: 10.1016/j.imlet.2005.11.022

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*, *14*(4), 417–419. doi: 10.1038/nmeth.4197

Pellegrino, M. W., Nargund, A. M., & Haynes, C. M. (2013). Signaling the mitochondrial unfolded protein response. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1833*(2), 410–416. doi: 10.1016/j.bbamcr.2012.02.019

Pellegrino, M. W., Nargund, A. M., Kirienko, N. V., Gillis, R., Fiorese, C. J., & Haynes, C. M. (2014). Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. *Nature*, *516*(7531), 414–417. doi: 10.1038/nature13818

Pinzón C., J. H., Beach-Letendre, J., Weil, E., & Mydlarz, L. D. (2014). Relationship between Phylogeny and Immunity Suggests Older Caribbean Coral Lineages Are More Resistant to Disease. *PLoS ONE*, *9*(8), e104787. doi: 10.1371/journal.pone.0104787

Pinzon, J. H., Kamel, B., Burge, C. A., Harvell, C. D., Medina, M., Weil, E., & Mydlarz, L. D. (2015). Whole transcriptome analysis reveals changes in expression of immune-related genes during and after bleaching in a reef-building coral. *Royal Society Open Science*, *2*(4), 140214–140214. doi: 10.1098/rsos.140214

Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., … Zaneveld, J. R. (2018). Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nature Communications*, *9*(1), 4921. doi: 10.1038/s41467-018-07275-x

Poole, A. Z., Kitchen, S. A., & Weis, V. M. (2016). The Role of Complement in Cnidarian-Dinoflagellate Symbiosis and Immune Challenge in the Sea Anemone Aiptasia pallida. *Frontiers in Microbiology*, *7*. doi: 10.3389/fmicb.2016.00519

Poole, A. Z., & Weis, V. M. (2014). TIR-domain-containing protein repertoire of nine anthozoan species reveals coral–specific expansions and uncharacterized proteins. *Developmental & Comparative Immunology*, *46*(2), 480–488. doi: 10.1016/j.dci.2014.06.002

Prada, C., Hanna, B., Budd, A. F., Woodley, C. M., Schmutz, J., Grimwood, J., … Medina, M. (2016). Empty Niches after Extinctions Increase Population Sizes of Modern Corals. *Current Biology: CB*, *26*(23), 3190–3194. doi: 10.1016/j.cub.2016.09.039

Prunier, J., Caron, S., & MacKay, J. (2017). CNVs into the wild: Screening the genomes of conifer trees (Picea spp.) reveals fewer gene copy number variations in hybrids and links to adaptation. *BMC Genomics*, *18*(1), 97. doi: 10.1186/s12864-016-3458-8

Putnam, H. M., Barott, K. L., Ainsworth, T. D., & Gates, R. D. (2017). The Vulnerability and Resilience of Reef-Building Corals. *Current Biology*, *27*(11), R528–R540. doi: 10.1016/j.cub.2017.04.047

Qiu, Q., Zhang, G., Ma, T., Qian, W., Wang, J., Ye, Z., … Liu, J. (2012). The yak genome and adaptation to life at high altitude. *Nature Genetics*, *44*(8), 946–949. doi: 10.1038/ng.2343

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics (Oxford, England)*, *26*(6), 841–842. doi: 10.1093/bioinformatics/btq033

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. (n.d.).

Rast, J. P., Smith, L. C., Loza-Coll, M., Hibino, T., & Litman, G. W. (2006). Genomic Insights into the Immune System of the Sea Urchin. *Science*, *314*(5801), 952–956. doi: 10.1126/science.1134301

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things): phytools: R package. *Methods in Ecology and Evolution*, *3*(2), 217–223. doi: 10.1111/j.2041-210X.2011.00169.x

Rey, C., Veber, P., Boussau, B., & Sémon, M. (2019). CAARS: Comparative assembly and annotation of RNA-Seq data. *Bioinformatics*, *35*(13), 2199–2207. doi: 10.1093/bioinformatics/bty903

Richier, S., Sabourault, C., Courtiade, J., Zucchini, N., Allemand, D., & Furla, P. (2006). Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, Anemonia viridis. *FEBS Journal*, *273*(18), 4186–4198. doi: 10.1111/j.1742-4658.2006.05414.x

Riegl, B. M., Purkis, S. J., Al-Cibahy, A. S., Abdel-Moati, M. A., & Hoegh-Guldberg, O. (2011). Present Limits to Heat-Adaptability in Corals and Population-Level Responses to Climate Extremes. *PLoS ONE*, *6*(9), e24802. doi: 10.1371/journal.pone.0024802

Rippe, J. P., Dixon, G., Fuller, Z. L., Liao, Y., & Matz, M. (2021). Environmental specialization and cryptic genetic divergence in two massive coral species from the Florida Keys Reef Tract. *Molecular Ecology*, mec.15931. doi: 10.1111/mec.15931

Rivera, H. E., Aichelman, H. E., Fifer, J. E., Kriefall, N. G., Wuitchik, D. M., Wuitchik, S. J. S., & Davies, S. W. (2021). A framework for understanding gene expression plasticity and its influence on stress tolerance. *Molecular Ecology*, *30*(6), 1381–1397. doi: 10.1111/mec.15820

Roach, T. N. F., Dilworth, J., H., C. M., Jones, A. D., Quinn, R. A., & Drury, C. (2021). Metabolomic signatures of coral bleaching history. *Nature Ecology & Evolution*, *5*(4), 495–503. doi: 10.1038/s41559-020-01388-7

Rohlfs, R. V., & Nielsen, R. (2015). Phylogenetic ANOVA: The Expression Variance and Evolution Model for Quantitative Trait Evolution. *Systematic Biology*, *64*(5), 695–708. doi: 10.1093/sysbio/syv042

Ronco, F., Matschiner, M., Böhne, A., Boila, A., Büscher, H. H., El Taher, A., … Salzburger, W. (2021). Drivers and dynamics of a massive adaptive radiation in cichlid fishes. *Nature*, *589*(7840), 76–81. doi: 10.1038/s41586-020-2930-4

Rosales, S. M., Clark, A. S., Huebner, L. K., Ruzicka, R. R., & Muller, E. M. (2020). Rhodobacterales and Rhizobiales Are Associated With Stony Coral Tissue Loss Disease and Its Suspected Sources of Transmission. *Frontiers in Microbiology*, *11*, 681. doi: 10.3389/fmicb.2020.00681

Rose, N. H., Seneca, F. O., & Palumbi, S. R. (2016). Gene Networks in the Wild: Identifying Transcriptional Modules that Mediate Coral Resistance to Experimental Heat Stress. *Genome Biology and Evolution*, *8*(1), 243–252. doi: 10.1093/gbe/evv258

Rosenstiel, P., Jacobs, G., Till, A., & Schreiber, S. (2008). NOD-like receptors: Ancient sentinels of the innate immune system. *Cellular and Molecular Life Sciences*, *65*(9), 1361–1377. doi: 10.1007/s00018-008-7502-y

Rosenstiel, Philip, Philipp, E. E. R., Schreiber, S., & Bosch, T. C. G. (2009). Evolution and Function of Innate Immune Receptors – Insights from Marine Invertebrates. *Journal of Innate Immunity*, *1*(4), 291–300. doi: 10.1159/000211193

Rosin, D. L., & Okusa, M. D. (2011). Dangers Within: DAMP Responses to Damage and Cell Death in Kidney Disease. *Journal of the American Society of Nephrology*, *22*(3), 416–425. doi: 10.1681/ASN.2010040430

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, *18*(1), 529. doi: 10.1186/s12859-017-1934-z

Ruiz-Jones, L. J., & Palumbi, S. R. (2017). Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, *3*(3), e1601298. doi: 10.1126/sciadv.1601298

Sackton, T. B., Lazzaro, B. P., & Clark, A. G. (2017). Rapid expansion of immune-related gene families in the house fly, *Musca domestica*. *Molecular Biology and Evolution*, msw285. doi: 10.1093/molbev/msw285

Sackton, T. B., Lazzaro, B. P., Schlenke, T. A., Evans, J. D., Hultmark, D., & Clark, A. G. (2007). Dynamic evolution of the innate immune system in Drosophila. *Nature Genetics*, *39*(12), 1461–1468. doi: 10.1038/ng.2007.60

Shinzato, C., Khalturin, K., Inoue, J., Zayasu, Y., Kanda, M., Kawamitsu, M., … Satoh, N. (2020). Eighteen Coral Genomes Reveal the Evolutionary Origin of Acropora Strategies

to Accommodate Environmental Changes. *Molecular Biology and Evolution*, msaa216. doi: 10.1093/molbev/msaa216

Shinzato, C., Shoguchi, E., Kawashima, T., Hamada, M., Hisata, K., Tanaka, M., … Satoh, N. (2011). Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, *476*(7360), 320–323. doi: 10.1038/nature10249

Shoguchi, E., Beedessee, G., Hisata, K., Tada, I., Narisoko, H., Satoh, N., … Shinzato, C. (2021). A New Dinoflagellate Genome Illuminates a Conserved Gene Cluster Involved in Sunscreen Biosynthesis. *Genome Biology and Evolution*, *13*(2), evaa235. doi: 10.1093/gbe/evaa235

Shore, A., & Caldwell, J. M. (2019). Modes of coral disease transmission: How do diseases spread between individuals and among populations? *Marine Biology*, *166*(4), 45. doi: 10.1007/s00227-019-3490-8

Shpilka, T., & Haynes, C. M. (2017). The mitochondrial UPR: Mechanisms, physiological functions and implications in ageing. *Nature Reviews Molecular Cell Biology*, *19*(2), 109–120. doi: 10.1038/nrm.2017.110

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210–3212. doi: 10.1093/bioinformatics/btv351

Smith, T. B., Brandt, M. E., Calnan, J. M., Nemeth, R. S., Blondeau, J., Kadison, E., … Rothenberger, P. (2013a). Convergent mortality responses of Caribbean coral species to seawater warming. *Ecosphere*, *4*(7), art87. doi: 10.1890/ES13-00107.1

Smith, T. B., Brandt, M. E., Calnan, J. M., Nemeth, R. S., Blondeau, J., Kadison, E., … Rothenberger, P. (2013b). Convergent mortality responses of Caribbean coral species to seawater warming. *Ecosphere*, *4*(7), art87. doi: 10.1890/ES13-00107.1

Soneson, C., Love, M. I., & Robinson, M. D. (2015). Differential analyses for RNA-seq: Transcript-level estimates improve gene-level inferences. *F1000Research*, *4*, 1521. doi: 10.12688/f1000research.7563.1

Song, M. J., Potter, B. I., Doyle, J. J., & Coate, J. E. (2020). Gene Balance Predicts Transcriptional Responses Immediately Following Ploidy Change in *Arabidopsis thaliana*. *The Plant Cell*, *32*(5), 1434–1448. doi: 10.1105/tpc.19.00832

Spady, T. C., Parry, J. W. L., Robinson, P. R., Hunt, D. M., Bowmaker, J. K., & Carleton, K. L. (2006). Evolution of the Cichlid Visual Palette through Ontogenetic Subfunctionalization of the Opsin Gene Arrays. *Molecular Biology and Evolution*, *23*(8), 1538–1547. doi: 10.1093/molbev/msl014

Stearns, F. W. (2010). One Hundred Years of Pleiotropy: A Retrospective. *Genetics*, *186*(3), 767–773. doi: 10.1534/genetics.110.122549

Stuart-Smith, R. D., Brown, C. J., Ceccarelli, D. M., & Edgar, G. J. (2018). Ecosystem restructuring along the Great Barrier Reef following mass coral bleaching. *Nature*, *560*(7716), 92–96. doi: 10.1038/s41586-018-0359-9

Stutz, A., Horvath, G. L., Monks, B. G., & Latz, E. (2013). ASC Speck Formation as a Readout for Inflammasome Activation. In C. M. De Nardo & E. Latz (Eds.), *The Inflammasome* (Vol. 1040, pp. 91–101). Totowa, NJ: Humana Press. doi: 10.1007/978-1-62703-523-1_8

Sutterwala, F. S., Haasken, S., & Cassel, S. L. (2014). Mechanism of NLRP3 inflammasome activation: Mechanism of NLRP3 inflammasome activation. *Annals of the New York Academy of Sciences*, *1319*(1), 82–95. doi: 10.1111/nyas.12458

Taylor, J. S., & Raes, J. (2004). Duplication and Divergence: The Evolution of New Genes and Old Ideas. *Annual Review of Genetics*, *38*(1), 615–643. doi: 10.1146/annurev.genet.38.072902.092831

Tchernov, D., Kvitt, H., Haramaty, L., Bibby, T. S., Gorbunov, M. Y., Rosenfeld, H., & Falkowski, P. G. (2011). Apoptosis and the selective survival of host animals following thermal bleaching in zooxanthellate corals. *Proceedings of the National Academy of Sciences*, *108*(24), 9905–9909. doi: 10.1073/pnas.1106924108

Tejada-Martinez, D., de Magalhães, J. P., & Opazo, J. C. (2021). Positive selection and gene duplications in tumour suppressor genes reveal clues about how cetaceans resist cancer. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1945), 20202592. doi: 10.1098/rspb.2020.2592

Tekaia, F. (2016). Inferring Orthologs: Open Questions and Perspectives. *Genomics Insights*, *9*, GEI.S37925. doi: 10.4137/GEI.S37925

Tetreau, G., Pinaud, S., Portet, A., Galinier, R., Gourbal, B., & Duval, D. (2017). Specific Pathogen Recognition by Multiple Innate Immune Sensors in an Invertebrate. *Frontiers in Immunology*, *8*, 1249. doi: 10.3389/fimmu.2017.01249

Thornhill, D. J., LaJeunesse, T. C., Kemp, D. W., Fitt, W. K., & Schmidt, G. W. (2006). Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Marine Biology*, *148*(4), 711–722. doi: 10.1007/s00227-005-0114-2

Ting, J. P.-Y., Lovering, R. C., Alnemri, E. S., Bertin, J., Boss, J. M., Davis, B. K., … Ward, P. A. (2008). The NLR Gene Family: A Standard Nomenclature. *Immunity*, *28*(3), 285–287. doi: 10.1016/j.immuni.2008.02.005

Ting, J., & Williams, K. (2005). The CATERPILLER family: An ancient family of immune/apoptotic proteins. *Clinical Immunology*, *115*(1), 33–37. doi: 10.1016/j.clim.2005.02.007

Tivey, T. R., Parkinson, J. E., & Weis, V. M. (2020). Host and Symbiont Cell Cycle Coordination Is Mediated by Symbiotic State, Nutrition, and Partner Identity in a Model Cnidarian-Dinoflagellate Symbiosis. *MBio*, *11*(2). doi: 10.1128/mBio.02626-19

Tortorelli, G., Belderok, R., Davy, S. K., McFadden, G. I., & van Oppen, M. J. H. (2020). Host Genotypic Effect on Algal Symbiosis Establishment in the Coral Model, the Anemone Exaiptasia diaphana, From the Great Barrier Reef. *Frontiers in Marine Science*, *6*, 833. doi: 10.3389/fmars.2019.00833

Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M. J., … Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology*, *28*(5), 511–515. doi: 10.1038/nbt.1621

Traylor-Knowles, N., Connelly, M. T., Young, B. D., Eaton, K., Muller, E. M., Paul, V. J., … Rodriguez, K. (2021). Gene Expression Response to Stony Coral Tissue Loss Disease Transmission in M. cavernosa and O. faveolata From Florida. *Frontiers in Marine Science*, *8*, 681563. doi: 10.3389/fmars.2021.681563

Trench, Robert. (1983). Selectivity in phagocytosis and persistence of symbiotic algae by the scyphistoma stage of the jellyfish *Cassiopeia xamachana*. *Proceedings of the Royal*

Society of London. Series B. Biological Sciences, *219*(1214), 61–82. doi: 10.1098/rspb.1983.0059

Underhill, D. M., & Ozinsky, A. (2002). P HAGOCYTOSIS OF M ICROBES: Complexity in Action. *Annual Review of Immunology*, *20*(1), 825–852. doi: 10.1146/annurev.immunol.20.103001.114744

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115–e115. doi: 10.1093/nar/gks596

Vajjhala, P. R., Mirams, R. E., & Hill, J. M. (2012). Multiple Binding Sites on the Pyrin Domain of ASC Protein Allow Self-association and Interaction with NLRP3 Protein. *Journal of Biological Chemistry*, *287*(50), 41732–41743. doi: 10.1074/jbc.M112.381228

Valmiki, M. G., & Ramos, J. W. (2009). Death Effector Domain-Containing Proteins. *Cellular and Molecular Life Sciences*, *66*(5), 814–830. doi: 10.1007/s00018-008-8489-0

van der Burg, C. A., Prentis, P. J., Surm, J. M., & Pavasovic, A. (2016). Insights into the innate immunome of actiniarians using a comparative genomic approach. *BMC Genomics*, *17*(1). doi: 10.1186/s12864-016-3204-2

Van Opdenbosch, N., Gurung, P., Vande Walle, L., Fossoul, A., Kanneganti, T.-D., & Lamkanfi, M. (2014). Activation of the NLRP1b inflammasome independently of ASC-mediated caspase-1 autoproteolysis and speck formation. *Nature Communications*, *5*(1). doi: 10.1038/ncomms4209

van Oppen, M. J. H., & Blackall, L. L. (2019). Coral microbiome dynamics, functions and design in a changing world. *Nature Reviews Microbiology*, *17*(9), 557–567. doi: 10.1038/s41579-019-0223-4

van Woesik, R., & Randall, C. J. (2017). Coral disease hotspots in the Caribbean. *Ecosphere*, *8*(5), e01814. doi: 10.1002/ecs2.1814

Vega Thurber, R., Mydlarz, L. D., Brandt, M., Harvell, D., Weil, E., Raymundo, L., … Lamb, J. (2020). Deciphering Coral Disease Dynamics: Integrating Host, Microbiome, and the Changing Environment. *Frontiers in Ecology and Evolution*, *8*, 575927. doi: 10.3389/fevo.2020.575927

Veitia, R. A., & Birchler, J. A. (2021). Gene-dosage issues: A recurrent theme in whole genome duplication events. *Trends in Genetics*, S0168952521001475. doi: 10.1016/j.tig.2021.06.006

Voolstra, C. R., Schnetzer, J., Peshkin, L., Randall, C. J., Szmant, A. M., & Medina, M. (2009). Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC Genomics*, *10*(1), 627. doi: 10.1186/1471-2164-10-627

Warner, M. E., Fitt, W. K., & Schmidt, G. W. (1999). Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *Proceedings of the National Academy of Sciences*, *96*(14), 8007–8012. doi: 10.1073/pnas.96.14.8007

Water, J. A. J. M., Chaib De Mares, M., Dixon, G. B., Raina, J., Willis, B. L., Bourne, D. G., & Oppen, M. J. H. (2018). Antimicrobial and stress responses to increased temperature and bacterial pathogen challenge in the holobiont of a reef-building coral. *Molecular Ecology*, *27*(4), 1065–1080. doi: 10.1111/mec.14489

Waterhouse, R. M., Kriventseva, E. V., Meister, S., Xi, Z., Alvarez, K. S., Bartholomay, L. C., … Christophides, G. K. (2007). Evolutionary Dynamics of Immune-Related Genes and Pathways in Disease-Vector Mosquitoes. *Science*, *316*(5832), 1738–1743. doi: 10.1126/science.1139862

Weil, E., & Rogers, C. S. (2011). Coral Reef Diseases in the Atlantic-Caribbean. In Z. Dubinsky & N. Stambler (Eds.), *Coral Reefs: An Ecosystem in Transition* (pp. 465–491). Dordrecht: Springer Netherlands. doi: 10.1007/978-94-007-0114-4_27

Weirauch, M. T., Yang, A., Albu, M., Cote, A. G., Montenegro-Montero, A., Drewe, P., … Hughes, T. R. (2014). Determination and inference of eukaryotic transcription factor sequence specificity. *Cell*, *158*(6), 1431–1443. doi: 10.1016/j.cell.2014.08.009

Weis, V. M., Davy, S. K., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., & Pringle, J. R. (2008). Cell biology in model systems as the key to understanding corals. *Trends in Ecology & Evolution*, *23*(7), 369–376. doi: 10.1016/j.tree.2008.03.004

Williams, L. M., Fuess, L. E., Brennan, J. J., Mansfield, K. M., Salas-Rodriguez, E., Welsh, J., … Gilmore, T. D. (2018). A conserved Toll-like receptor-to-NF-κB signaling pathway in the endangered coral Orbicella faveolata. *Developmental & Comparative Immunology*, *79*, 128–136. doi: 10.1016/j.dci.2017.10.016

Williams, S. D., & Patterson, M. R. (2020). Resistance and robustness of the global coral–symbiont network. *Ecology*, *101*(5). doi: 10.1002/ecy.2990

Wilmanski, J. M., Petnicki-Ocwieja, T., & Kobayashi, K. S. (2008). NLR proteins: Integral members of innate immunity and mediators of inflammatory diseases. *Journal of Leukocyte Biology*, *83*(1), 13–30. doi: 10.1189/jlb.0607402

Wolenski, F. S., Garbati, M. R., Lubinski, T. J., Traylor-Knowles, N., Dresselhaus, E., Stefanik, D. J., … Gilmore, T. D. (2011). Characterization of the Core Elements of the NF- B Signaling Pathway of the Sea Anemone Nematostella vectensis. *Molecular and Cellular Biology*, *31*(5), 1076–1087. doi: 10.1128/MCB.00927-10

Wright, G., Terada, K., Yano, M., Sergeev, I., & Mori, M. (2001). Oxidative stress inhibits the mitochondrial import of preproteins and leads to their degradation. *Experimental Cell Research*, *263*(1), 107–117. doi: 10.1006/excr.2000.5096

Wright, R. M., Aglyamova, G. V., Meyer, E., & Matz, M. V. (2015). Gene expression associated with white syndromes in a reef building coral, Acropora hyacinthus. *BMC Genomics*, *16*(1). doi: 10.1186/s12864-015-1540-2

Wright, R. M., Kenkel, C. D., Dunn, C. E., Shilling, E. N., Bay, L. K., & Matz, M. V. (2017). Intraspecific differences in molecular stress responses and coral pathobiome contribute to mortality under bacterial challenge in Acropora millepora. *Scientific Reports*, *7*(1). doi: 10.1038/s41598-017-02685-1

Wu, Y., Zhou, Z., Wang, J., Luo, J., Wang, L., & Zhang, Y. (2019). Temperature regulates the recognition activities of a galectin to pathogen and symbiont in the scleractinian coral Pocillopora damicornis. *Developmental & Comparative Immunology*, *96*, 103–110. doi: 10.1016/j.dci.2019.03.003

Yoneda, T. (2004). Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. *Journal of Cell Science*, *117*(18), 4055–4066. doi: 10.1242/jcs.01275

Yudowski, G., Roberson, L., & Marty-Rivera, M. (2018). *Mitigation of coral bleaching by antioxidants*. doi: 10.1101/281014

Zhang, J. (2003). Evolution by gene duplication: An update. *Trends in Ecology & Evolution*, *18*(6), 292–298. doi: 10.1016/S0169-5347(03)00033-8

Zhang, Q., Zmasek, C. M., & Godzik, A. (2010). Domain architecture evolution of pattern-recognition receptors. *Immunogenetics*, *62*(5), 263–272. doi: 10.1007/s00251-010-0428-1

Zhang, X., Blair, D., Wolinska, J., Ma, X., Yang, W., Hu, W., & Yin, M. (2021). Genomic regions associated with adaptation to predation in *Daphnia* often include members of expanded gene families. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1955), 20210803. doi: 10.1098/rspb.2021.0803

Zhou, R., Tardivel, A., Thorens, B., Choi, I., & Tschopp, J. (2010). Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nature Immunology*, *11*(2), 136–140. doi: 10.1038/ni.1831

Ziegler, M., Grupstra, C. G. B., Barreto, M. M., Eaton, M., BaOmar, J., Zubier, K., … Voolstra, C. R. (2019). Coral bacterial community structure responds to environmental change in a host-specific manner. *Nature Communications*, *10*(1), 3092. doi: 10.1038/s41467-019-10969-5

Zimmer, C. T., Garrood, W. T., Singh, K. S., Randall, E., Lueke, B., Gutbrod, O., … Bass, C. (2018). Neofunctionalization of Duplicated P450 Genes Drives the Evolution of Insecticide Resistance in the Brown Planthopper. *Current Biology*, *28*(2), 268-274.e5. doi: 10.1016/j.cub.2017.11.060